Ecology of red scale (*Aonidiella aurantii* (Maskell) [Hemiptera: Sternorrhyncha: Diaspididae]) in citrus orchards on the Central Coast of New South Wales

Student:  
Dao Thi Hang

Principal Supervisor:  
Professor George Andrew Charles Beattie, University of Western Sydney

Co-supervisors:  
Associate Professor Paul Holford, University of Western Sydney  
Associate Professor Robert Spooner-Hart, University of Western Sydney  
Dr Markus Riegler, University of Western Sydney  
Dr Alan Meats, University of Sydney  
Dr Lester Burgess, University of Sydney

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Declaration

The work reported in this thesis is the result of my own experiments and has not been submitted in any form for another degree or diploma at any university or institute of tertiary education.

Dao Thi Hang

2 February 2012
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Summary

I studied the ecology of red scale (*Aonidiella aurantii*) in citrus orchards on the Central Coast of New South Wales over three seasons, from winter 2008 to winter 2011. Red scale is an occasionally important pest of citrus in the region, where average annual rainfall where citrus is grown ranges from about 700 mm to 1200 mm. My research focused on determining scale phenology, and the incidence and effectiveness of its natural enemies. In doing so, I used molecular techniques to confirm the identities of the scale and its parasitoids and entomopathogens. I also used molecular techniques to confirm the identity of yellow scale, *Aonidiella citrina*, which was also present in the orchards, and *Iridomyrmex rufoniger*, the most common native ant in the orchards.

I used pheromone traps over the three seasons to show that the scale has three annual generations in the region, with spring peaks of male flights occurring between 20 September and 20 October, summer peaks between 20 December and 20 January, and autumn peaks between 20 February and 30 March. Cold weather in winter, particularly ambient temperatures at sunset and sunrise, influenced scale phenology and scale mortality. I derived a positive correlation between numbers of male scale trapped in summer generations with those trapped in the preceding winter/spring generation.

I recorded five parasitoids, several predators and six entomopathogens of the scale. A native coccinellid beetle, *Halmus chalybeus*, appeared to be the most important natural enemy in the region. There was no evidence of competitive displacement among parasitoid species. I found no evidence of density-dependency relationships between the scale and its parasitoids, or between the scale and *Halmus chalybeus*. I rarely observed intraguild predation of *Aphytis* species on *Encarsia* species. In a field experiment in which I prevented *Iridomyrmex rufoniger* from foraging on honeydew produced by black scale (*Saissetia oleae*), I showed that the ant disrupted the activities of five red scale parasitoids (*Aphytis chrysomphali*, *Aphytis melinus*, *Encarsia citrina*, *Encarsia perniciosi* and *Comperiella bifasciata*) and two predators (*Halmus chalybeus* and *Rhyzobius lophanthae*, another native coccinellid). I also observed self-asphyxiation of black scale by its honeydew in the absence of *Iridomyrmex rufoniger*. The ant did not disrupt predation on red scale by *Orcus australasiae*, also a native coccinellid.
My molecular studies on yellow scale confirmed a recent (2006–2007) record of it being present in an important inland citrus producing region of the state where it had not been previously recorded. My molecular results for species of *Aphytis* species showed that *Aphytis chrysomphali* and *Aphytis melinus* occur in citrus orchards on the Central Coast of New South Wales: *Aphytis lingnanensis*, which occurs in Queensland, was not recorded. I recorded molecular differences between specimens of *Aphytis*. These differences, which were associated with variation in pigmentation of pupae, suggested that *Aphytis melinus* may have been introduced on more occasions than officially recorded, and from two sources, not one. My molecular results for the *Encarsia* species suggested that the form of *Encarsia citrina* parasitising armoured scales in coastal citrus orchards of New South Wales may be a native or Australasian strain, or from Southeast Asia. There are no formal records of *Encarsia citrina* being successfully introduced to Australia, and the form I recorded was genetically different to a University of California, Riverside accession.

The six species of entomopathogens comprised *Microcera coccophila*, *Microcera larvarum*, *Tetracrium coccicolum*, *Tetracrium novae-zealandiae*, *Clonostachys coccicola* and *Myriangium citri*. I obtained, for the first time, pure cultures from single spores of each species. My field observations indicated that they all play roles in the biological control of armoured scale in the region. I confirmed, for the first time, that *Microcera coccophila* and *Microcera larvarum* are entomopathogens of armoured scale, by fulfilling the Koch’s postulates in laboratory bioassays. Published records I reviewed during my studies suggested the presence of *Microcera coccophila*, *Tetracrium coccicolum* and two unidentified species in eastern Australia. One of these may have been *Myriangium citri*, specimens of which are in the Plant Pathology Herbarium, Orange, New South Wales. *Microcera larvarum* may have been recorded as another species, but I found no records of *Tetracrium novae-zealandiae* and *Clonostachys coccicola* being previously recorded in Australia. My study also represents the first occasion on which the anamorph of *Tetracrium novae-zealandiae* has been recorded and described.
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Chapter 1. Introduction

Red scale\(^1\) (*Aonidiella aurantii* (Maskell) [Hemiptera: Sternorrhyncha: Diaspididae]) is the most important pest of citrus in Australia (Hely 1968, Smith et al. 1997). It is the principle pest of citrus in California, South Africa, and New Zealand, and the most important citrus pest in some regions of Mexico, Chile, Argentina, Brazil, Palestine, and islands of the eastern Mediterranean (Quayle 1911a, Ebeling 1959, Rosen & DeBach 1978, Bedford et al. 1998, Pekas et al. 2010). Within Australia, red scale is a major pest of citrus in the citrus producing regions of Queensland, Western Australia, South Australia, Victoria, and inland New South Wales. It is occasionally important in coastal New South Wales and a relatively minor pest near Darwin in the Northern Territory (Smith et al. 1997).

\[\text{Figure 1.1. A larva of } Rhzobius lophanthae \text{ feeding on red scale.}\]

\(^1\) Red scale is the official common name of *Aonidiella aurantii* in Australia. It is used to avoid use of misleading common names such as 'California red scale' and 'la cochinilla roja australiana' that do not reflect the origins of the species (Beattie, pers. comm., 2009).
The scale can infest all above ground parts of trees but prefers outer parts of canopies (Quayle 1911a, 1938, Ebeling 1959, Hely 1982, Smith et al. 1997). Heavy infestations can occur on fruit, leaves and branches. During feeding, the scale injects a ‘toxic substance’ into its host plant with excreta in its saliva (Quayle 1911a, Ebeling 1950, Ebeling 1959, Hely et al. 1982, Banks 1990). The ‘toxin’ leads to chlorosis of leaf and young fruit. Severe infestations of the scale cause leaf drop, defoliation, and dieback of twigs and branches (Fig. 1.2). Young trees may be killed in the absence of effective controls (Quayle 1911a, 1938, Ebeling 1950, Hely et al. 1982, Smith et al. 1997, Bedford et al. 1998). However, the economic importance of red scale is mostly related to infestations that reduce the market value of fruit (Quayle 1911a, Ebeling 1959, Hely et al. 1982, Sawyer 1996, Bedford et al. 1998). Red scale is very similar in appearance to a congeneric species, yellow scale (*Aonidiella citrina* (Coquillett)), with which it may be easily confused. However, yellow scale has a narrower distribution within the citrus growing regions of Australia. According to Hely et al. (1982) and Smith et al. (1997), it occurs as a minor pest in coastal New South Wales and in the Murray River citrus districts of inland New South Wales, Victoria and South Australia. It prefers feeding on the leaves, particularly old inner leaves of infested canopies of mature trees, and less frequently on fruit (Hely 1955, Hely et al. 1982, Smith et al. 1997).

![Die-back of a mature tree caused by a severe infestation of red scale in the 1950s in the inland New South Wales](image)

*Figure 1.2.* Die-back of a mature tree caused by a severe infestation of red scale in the 1950s in the inland New South Wales (from Hely et al. 1982)*
Four other diaspidids, circular black scale (*Chrysomphalus aonidium* (Linnaeus)), white louse scale (*Unaspis citri* (Comstock)), purple scale (*Lepidosaphes beckii* (Newman)) and Glover’s scale (*Lepidosaphes gloverii* (Parkard)), also occur in citrus orchards in coastal regions of New South Wales and Queensland. Cottony cushion scale (*Icerya purchasi* Maskell [Hemiptera: Sternorrhyncha: Margarodidae]) and five species of soft scales, soft brown scale (*Coccus hesperidum* (L.)), black scale (*Saissetia oleae* (Olivier)), white wax scale (*Ceroplastes destructor* (Newstead)), pink wax scale (*Ceroplastes rubens* (Maskell)) and hard wax scale (*Ceroplastes sinensis* (del Guercio)) [Hemiptera: Sternorrhyncha: Coccidae], also occur in the orchards on the Central Coast of New South Wales, where they are tended by ants that forage for their honeydew (Hely et al. 1982, Smith et al. 1997). Ants are known to disrupt biological control of red scale and other armoured scales (DeBach et al. 1951a,b, Flanders 1951a, Steyn 1954a,b, Samways 1981, Samways et al. 1982, Murdoch et al. 1995, Martinez-Ferrer et al. 2002, Pekas et al. 2010). Studies in Australia have been limited to recording the abundance of red scale and soft brown scale in the presence or absence of ants in a citrus orchard at Leeton in the Riverina district of inland New South Wales (James et al. 1987, 1999).

From 1900 to 1970, control of red scale in Australia involved heavy use of pesticides (Hely et al. 1982, Furness et al. 1983, Smith et al. 1997). Fumigation with hydrogen cyanide (HCN) (Fig. 1.3) was introduced into Australia in the late 1890s and was a highly effective but labour intensive method in New South Wales for over 50 years (Hely et al. 1982), and kerosene emulsions were used in the late 1800s (Tryon 1889). Mineral oils have been used in sprays since the 1920s (Hely et al. 1982). High quality agricultural and horticultural mineral oils have been used since the 1970s (Hely et al. 1982, Furness et al. 1983, Beattie & Hardy 2005). Organophosphate insecticides were widely applied as sprays for control of red scale from the late 1940s until the 1970s (Hely et al. 1982). These insecticides had adverse effects on natural enemies (Hely 1968, Hely et al. 1982, Papacek & Smith 1992, Smith et al. 1997). Successful biological control and IPM programs based on native predators and introduced parasitoids since 1970, dramatically reduced uses of synthetic insecticides and control costs (McLaren & Buchanan 1972, Furness et al. 1983, Papacek & Smith 1992, Smith et al. 1997).
Despite of the importance of red scale as a pest of citrus, few Australian studies have focused on its biology and ecology. Ward & Johnston (1937) recorded its life history in an insectary. Willard (1971) and Abdelrahman (1974a) recorded development durations under laboratory conditions. Zhao (1990) studied the impacts of temperature on development of red scale under laboratory conditions in South Australia. Ward & Johnston (1937) recorded proportions of scale stages and stage mortalities in the field in Bamawm and Mildura (Victoria), and noted that no crawlers were produced at these locations between May and November. Hely (1955) recorded that the average period from birth of females until the reproduction of the next generation crawlers in New South Wales was about 3 months in warm weather and longer in winter. Hely et al. (1982) noted that, in the weather conditions prevalent in most of New South Wales, crawler production continued in winter at a slow rate. Yan & Isman (1986) reported that, in the field at Adelaide, South Australia, flight activity of red scale males occurred shortly after 14:00 to about 21:00 and peaked at about 18:00, at about 3498–5489 lux and 25–26°C.

According to Smith et al. (1997), there are 2–5 generations per year in South Australia, Victoria, New South Wales and Western Australia, 5–6 generations in Queensland and the Northern Territory. These estimates were based Bureau of Meteorology records and degree-day (°D) accumulations estimated by Watson & Beattie (1996) (Beattie. pers. comm., 2008). No studies have used pheromone traps and °D accumulations to accurately determine generations within a region.
Biological control of red scale in Australia has focused on its parasitoids, particularly three species of *Aphytis*, *Aphytis chrysomphali* (Mercet), *Aphytis lingnanensis* Compere, *Aphytis melinus* DeBach [Hymenoptera: Aphelinidae] (Hely 1968, Hely et al. 1982, Smith et al. 1997). *Aphytis melinus* is widely regarded as the most important parasitoid of red scale in the inland regions of New South Wales, Victoria and South Australia (Campbell 1976, Furness et al. 1983, Smith et al. 1997). It also occurs in regions of the Central Coast of New South Wales, where it was released near Richmond in 1991 (Spooner-Hart, University of Western Sydney, pers. comm., 2009). *Aphytis lingnanensis* occurs widely in Queensland (Smith 1978, Papacek & Smith 1992, Papacek 2006). It is not known to occur on the Central Coast of New South Wales, where *Aphytis chrysomphali* has been regarded as the most common species of *Aphytis* since its introduction to the region in 1925–1926 (Hely 1968, Hely et al. 1982). Smith & Maelzer (1986) found no density-dependent relationship between parasitism by *Aphytis melinus* and scale density on fruit in the orchards in an orchard at Waite Agriculture Research Institute, Adelaide, South Australia. Abdelrahman (1974b) studied oviposition behaviour and control of sex for *Aphytis melinus* in the laboratory.

Three endoparasitoids of red scale, *Encarsia citrina* (Craw), *Encarsia perniciosi* (Tower) [Hymenoptera: Aphelinidae] and *Comperiella bifasciata* (Howard) [Hymenoptera: Encyrtidae] also occur in Australia. *Comperiella bifasciata* is widely distributed in all mainland states including Queensland, New South Wales, South Australia, Victoria, and Western Australia and plays an important role in biological control of the scale in these states (Smith 1978, Furness et al. 1983, Smith et al. 1997, Broughton 2006). *Encarsia citrina* occurs in coastal New South Wales and Queensland and *Encarsia perniciosi* occurs in the Sunraysia region of Victoria, the Riverland region of South Australia and on the central coast of New South Wales (Furness et al. 1983, Smith et al. 1997).

Surveys of red scale populations in several regions of New South Wales, and parasitism of the scale by *Aphytis* species and *Comperiella bifasciata* in these regions, including the Central Coast of New South Wales (Beattie, unpublished data) are summarised in Chapter 2. Impacts of the two *Encarsia* species on red scale populations in Australia have not been determined.

Several predators are known to prey on red scale in Australia. The most important are native ladybirds, *Halmus chalybeus* (Boisduval), *Orcus australasiae* (Boisduval),
*Rhyzobius lophanthae* (Blaisdell) and *Rhyzobius hirtellus* Crotch [Coleoptera: Coccinellidae] (Hely et al. 1982, Smith et al. 1997, Waterhouse & Sands 2001, Ślipiński 2007). However, little is known about the biology of these ladybirds or their roles in biological control of red scale under field conditions.

Fungi that are assumed to be entomopathogens of the scale are also known to occur in coastal orchards in eastern Australia, two species in coastal New South Wales and up to four in Queensland (Koebele 1892, Tryon 1894, McAlpine 1899, Summerville 1934, Hely et al. 1982, Smith et al. 1997). The most commonly mentioned of these species is a red-headed fungus, *Microcera coccophila* Desm. However, its impacts on red scale populations, and the roles of the other diaspidid species, have not been recorded and factors influencing their occurrence have not been determined.

Most studies on biological control of red scale have been undertaken in California (DeBach & Fleschner 1959, Luck & Podoler 1985, Yu et al. 1990, Murdoch et al. 1995) and in Mediterranean countries (Sorribas et al. 2010, Sorribas & Garcia-Marí 2010, Pekas et al. 2010). These studies have been largely focused on *Aphytis melinus* and, to a less extent, *Encarsia perniciosi* under relatively dry ‘Mediterranean’ conditions. Other parasitoid species and coccinellid predators have not been studied. Several studies, though not intensive, have been undertaken in South Africa, where the climate is similar to New South Wales (Samways 1971, Atkinson 1977, Grout 1987, Grout & Richards 1989, Bedford et al. 1998). Moreover, the presence of Argentine ant (*Linepithema humile* [Mayr] [Hymentoptera: Formicidae]) and other ant species in association with soft scales in citrus orchards in California, Mediterranean countries and South Africa has influenced the success of biological control and integrated pest management programs for red scale (Flanders 1945, DeBach & Bartlett 1951, Bartlett 1961, Samways 1982, Moreno et al. 1987, Murdoch et al. 1995, Martinez-Ferrer et al. 2002). No comprehensive studies, other than one undertaken in China by Beattie (1984), have been undertaken in humid temperate regions of the world.

In order to address discrepancies in historical records related to the introduction of red scale parasitoids to Australia, I reviewed records related to *Aphytis lingnanensis*, *Aphytis chrysomphali*, *Encarsia citrina*, *Encarsia perniciosi* and *Comperiella bifasciata* (Chapter 2). I also review literature on the occurrence and impacts of predators and entomopathogens of the scale.
In order to determine the influences of climate and the roles of natural enemies in the regulation of red scale populations in a humid temperate region of Australia, I undertook a comprehensive study of the ecology of red scale in orchards on the Central Coast of New South Wales in which I:

- determined the phenology of red scale in the region (Chapter 4);
- determined seasonal abundance of predators and parasitoids in relation to the scale densities in the region and their roles as natural enemies of the scale (Chapter 5);
- determined relationships between scale densities and percent parasitism and predation (Chapter 6); and
- assessed impacts of black scale-ant mutualism on biological control of red scale (Chapter 7).

In laboratory studies, I confirmed:

- the identities of red scale and yellow scale, their parasitoids, and the ant associated with black scale in my study orchards (Chapter 8);
- the identities and pathogenicity of two red-headed fungi (Chapter 9); and
- the identities of four additional species of fungi, each regarded as a putative entomopathogens of armoured scales (Chapter 10).

In order to avoid confusion related to the large number of taxa mentioned in my thesis, I did not, with the exception of some tables and figures, abbreviate their scientific names.
Chapter 2: Literature Review

2.1. Red Scale

2.1.1. Taxonomic status

Red scale was first described as *Aspidiotus aurantii* in 1878 by William Maskell who based his description, and an illustration (Fig. 2.1), on scale present on orange and lemon fruit imported to Auckland, New Zealand, from Sydney, Australia (Maskell 1878\(^2\), Quayle 1911a, Stofberg 1937, Compere 1961, Charles & Henderson 2002). Maskell (1878) said of the scale ‘This is not an indigenous species, being found in immense numbers upon the oranges and lemons in our shops, imported from Sydney.’ He cited a report\(^3\) that mentioned an insect attacking orange (*Citrus × aurantium* L.) trees in Sydney and commented that it appeared to be a species of *Aspidiotus*. He concluded that it was his *Aspidiotus aurantii*. It was transferred to the genus *Aonidiella* by Antonio Berlese in 1895 (Berlese 1895), then to *Chrysomphalus* by Theodore Cockerell\(^4\) in 1899 (Cockerell 1899) before being placed, once again, in *Aonidiella* by Howard Lester McKenzie\(^5\) in 1937 (McKenzie 1937, 1938).

![Figure 2.1](image)

*Figure 2.1.* William Miles Maskell (left) and his illustrations (Maskell 1878) of red scale: (a) third instar female, (b) pygidium and (c) adult male.

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\(^2\)William Miles Maskell (1840–1898) was a farmer and entomologist. He described 300 species of Hemiptera, mostly scale insects (Deitz & Tocker 1980).

\(^3\)Schrader M. 1868. Proceedings of the Zoological-Botanical Society of Vienna.
2.1.2. Origin and first use of common name

Red scale is native to Southeast Asia (Compere 1961, Hely et al. 1982, Bedford et al. 1998) and was introduced to Australia before 1840 (Koebele 1892, Compere 1961). First use of ‘red scale’ as a common name for the scale appears to have been by James Pye, an orchardist, of Rocky Hall near Parramatta in metropolitan Sydney (Pye 1871a,b). Compere (1961) cited a report by Comstock (1881) as an example of the injuriousness of red scale in Australia, in which Comstock told of a grove of 13.4 ha (33 acres) there that nine years earlier had rented for £1800 and for which only £150 could be obtained six years later (Compere 1961).

2.1.3. Distribution and economic importance

The scale is widely distributed in all tropical and subtropical countries of the world (Quayle 1911a, Ebeling 1959, Clausen 1978, Rosen & DeBach 1978). As a major pest of citrus, it occurs mainly in subtropical areas between latitude 25° and 40° N and S (Rosen & DeBach 1978). Until the recent spread of the Asiatic citrus psyllid (Diaphorina citri Kuwayama [Hemiptera: Sternorrhyncha: Liviidae]) (Halbert & Manjunath 2004, Halbert & Núñez, 2004, Weinert et al. 2004, OEPP/EPPO 2005b, Villalobos, Conant et al. 2007, Poe 2007, Poe & Shea 2007), the most widely distributed vector of pathogens that cause huanglongbing, the most serious disease of citrus (Bové 2006, Beattie & Barkley 2009, Duan et al. 2009, Donovan et al. 2011), red scale, was considered to be the most destructive pest of citrus in the world (Quayle 1938, Ebeling 1951, 1959, Compere 1961, Hely et al. 1982, Smith et al. 1997, Bedford et al. 1998, OEPP/EPPO 2004). In terms of direct damage it still is. It is a major pest, and the most important armoured scale pest, of citrus in California, southern Africa, New Zealand, South America, the Mediterranean region, and China (Quayle 1911a, 1938, Ebeling 1959, Rosen & DeBach 1978, Bedford et al. 1998, Pekas et al. 2010). It is, in the absence of huanglongbing and it vectors, the most important pest of citrus in all citrus growing areas in Australia (Smith et al. 1997, Beattie & Barkley 2009). In New South Wales, it is a more serious pest in inland citrus areas than in coastal areas (Hely et al. 1982, Smith et al. 1997). The major citrus producing regions in Australia are illustrated in Fig. 2.2. The most important inland region of New South Wales is the Riverina district (formerly known as the Murrumbidgee Irrigation Areas) (Fig. 2.3). Less important regions include part of the lower Murray River Sunraysia district, the mid and upper Murray River districts, Hillston on the Lachlan River, and Narromine on
Figure 2.2. Commercial citrus producing regions in Australia (Smith et al. 1997).

Figure 2.3. Commercial citrus localities in New South Wales: Somersby and Richmond on the Central Coast/Sydney region, Narromine on the Macquarie River, Hillston on the Lachlan River, Griffith, Leeton and Narrenderra in the Riverina (Murrumbidgee and Coleamabilly Irrigation Areas), and Tocumwal, Barham and Dareton on the upper, mid and lower Murray River: adapted from Hely et al. (1992). The relatively low rainfall inland region is separated from the higher rainfall coastal region by the Great Dividing Range.
the Macquarie River. The main coastal region is the Central Coast, where the area planted to commercial citrus production fell from 1,200 ha in 1975 to 523 ha in 2005 and has continued to decline since then (Sandra Hardy, Agriculture New South Wales, pers. comm., 2010).

### 2.1.4. Hosts

Major host plants of *Aonidiella aurantii* include all species and varieties of *Citrus* [Sapindales: Rutaceae: Aurantioideae: Aurantieae], pear (*Pyrus* spp. [Rosales: Rosaceae]), rose (*Rosa* spp. [Rosales: Rosaceae]), palms [Arecales: Areaceae], apple (*Malus* spp. [Rosales: Rosaceae]), mulberry (*Morus* spp. [Rosales: Moraceae]) and nightshade (*Solanum nigrum* L. [Solanales: Solanaceae]). It also attacks other plants such as mango (*Mangifera* spp. [Sapindales: Anacardiceae]), plum (*Prunus × domestica* L. [Rosales: Rosaceae]), avocado (*Persea americana* Mill. [Laurales: Lauraceae]), olive (*Olea europaea* L. [Lamiales: Oleaceae]), fig (*Ficus* spp. [Rosales: Moraceae]) and passionfruit (*Passiflora* spp. [Malpighiales: Passifloraceae]) (Quayle 1911a, Ebeling 1959, Hely et al. 1982, Smith et al. 1997). Quayle (1938) listed 77 food plant species and 8 weed species as host plants. According to ScaleNet[^6], there are 256 host plants.

### 2.1.5. Host substrates and feeding

Red scale attacks all above ground parts of citrus trees. It prefers feeding on fruit, leaves, and green twigs and branches; it can settle on older, corky, branches and trunks but does not do so readily (Quayle 1911a, 1938, Ebeling 1950, 1959). It feeds by inserting its mouthparts into plant tissues and sucking the sap. Stylet track paths are intracellular with most paths terminating in parenchyma cells (palisade and spongy mesophyll of leaves, cortex of twigs, and flavedo of fruit), suggesting that the insect prefers to feed from this tissue (Washington & Walker 1990). The mouthparts consist of three important elements: the inner framework, a 4 mm-long piercing-sucking stylet fascicle or rostralis, and the labium. The inner framework has a strong sclerotised structure that functions as a support, protects various head organs, and attaches to the mandibular-maxillary muscles. The stylet fascicle comprises four long, fine, flexible setae-like stylets or bristles, representing pairs of modified mandibles and maxillae. The two mandibular stylets form the outer, lateral elements of the fascicle, enclosing the interlocking maxillary stylets between them. The inner faces of the maxillary stylets are

so formed that their juxtaposition creates two tubular canals that extend the entire length of the fascicle: a larger dorsal food channel and a smaller ventral salivary channel (Beardsley & Gonzalez 1975). The citrus leaf is so thin in relation to the length of the stylet (rostralis), that the latter must be extended laterally through leaf tissues (Ebeling 1959).

There is no direct connection between the stomach and hind intestine in diaspidids; they do not produce honeydew. Excess food is re-injected into the plant with salivary fluids. This leads to characteristic yellow chlorotic spots surrounding the scale on leaves and green fruit. Any fluid that is excreted from the anus appears to interact with the fibres secreted from the pygidial glands to form the armoured scale cover (Banks 1990). Yust & Fulton (1943) observed that after red scale were removed from a lemon fruit, a small droplet of fluid appeared where each scale had been feeding. They also observed that the exudation came from broken-off rostralis in the lemon and that droplets from mature scale were larger than from immature scale (Yust & Fulton 1943). The analysis of the exudation showed it to be practically pure sucrose; fungi grew readily in the exudation under humid conditions (Yust & Fulton 1943).

Heavily infested branches and fruit can be completely encrusted with all life cycle stages. Severe infestations cause leaf drop, defoliation, and dieback of twigs and branches. The market value of fruit is reduced by infestations. Young trees may be killed in the absence of effective controls (Quayle 1911a, Ebeling 1959, Hely et al. 1982, Bedford et al. 1998). Branches suffer the most injurious infestations, followed by leaves, and fruit (Quayle 1911a). This may be due to high scale populations being related to rates of parasitism lower than those that occur on leaves and fruit (Walde et al. 1989, Murdoch et al. 1995). In some instances, this can be related to accumulative populations of scale from season to season and to disruption of natural enemy activity by ants (DeBach et al. 1951b, Flanders 1951a, Steyn 1954, Samways 1981, Samways et al. 1982, Murdoch et al. 1995, Martinez-Ferrer et al. 2003, Pekas et al. 2010).

The economic importance of red scale in Australia is mostly related to the impact of infestations on fruit grown for domestic and export fresh fruit markets. Such blemishing occurs at lower levels of infestations than those that cause dieback and loss of yield. Even though scale can be removed from fruit during washing, brushing and
water-jetting\textsuperscript{7} in the packing shed, fruit may still not be acceptable for sale as fresh fruit because of scale or scale damage. Scaly Valencia orange fruit may only be suitable for juicing (Furness et al. 1983).

In 2008, the value of export quality navel and Valencia orange fruit in southern Australia ranged from $350–500 t\textsuperscript{-1} and $250–350 t\textsuperscript{-1}, respectively. In contrast, the value of navel oranges sold for juice was $200 t\textsuperscript{-1}, and 200–250 t\textsuperscript{-1} for domestic Valencia fruit (Andrew Green, Citrus Board of South Australia, pers. comm., 9 October 2008). The current average cost for spraying red scale infested trees with mineral oil or some insecticides is about $280, including diesel and labour costs (James Altmann, Fruit Doctors, South Australia, pers. comm., 25 September 2008; Ross Hitchcock, Kulnura, New South Wales, pers. comm., 21 October 2008).

The full cost of an armoured scale spray in the San Joaquin Valley of California in 2001 averaged US $400 ha\textsuperscript{-1}; in situations where resistance was a serious problem, growers were applying insecticides 2–3 times per season. Thus, scale control alone was costing US $800–1,200 ha\textsuperscript{-1}, and these growers suffered losses because a percentage of the fruit was still encrusted with scale (Grafton-Cardwell 2001)\textsuperscript{8}.

In the early 1970s, approximately 70\% of citrus growers in the Sunraysia region of Victoria and New South Wales benefited financially from introduction of parasitoids to control red scale and yellow scale (\textit{Aonidiella citrina} (Coquillett)) (McLaren 1971). In the citrus growing areas in Queensland, the application of biological control, based on the ectoparasitoid \textit{Aphytis lingnanensis} and endoparasitoid \textit{Comperiella bifasciata}, resulted in infestations being reduced to less than 0.05 adult females per fruit, a level of infestation equivalent to the best efficacy obtained by chemical control (Smith 1978). In the early 1980s, Furness et al. (1983) estimated the benefits of biological control in the Sunraysia-Riverland\textsuperscript{9} regions of Victoria and South Australia to be in the vicinity of $5 to $15 million. In comparison with chemical control, integrated pest management programs reduced costs by 68–81\% (Papacek & Smith 1992)\textsuperscript{10}.

\textsuperscript{7} A system, with series of high pressure water jets, developed by LJK Theron, Letaba Estates, South Africa, was first operated in commercial packing houses in 1977 (Bedford 1990). The jets, in combination with brushing, remove scales and increase packouts of exportable fruit (Honiball et al. 1979). Similar machines have been used in California, Australia and Israel but live scales are slightly more difficult to dislodge than dead scales (Furness et al. 1983, Walker et al. 1996, Walker et al. 1999).

\textsuperscript{8} http://citrusent.uckac.edu/resistan.htm

\textsuperscript{9} An area of approximately 14,000 ha in 1983.

\textsuperscript{10} Approximately $13 to $38 million in 2008 based on the Reserve Bank of Australia inflation calculator.
2.1.5. Life cycle stages: their morphology and general biology

Maskell (1878) wrote that 'The puparium of *Aspidiotus aurantii* is round, yellowish, flat. The insect, in the centre, is curiously shaped. It has a generally spherical out-line, but looks as if, from rich feeding, rolls of fat were produced, making the corrugations of the body very largely overlap the abdomen. It is yellow, the abdomen being the deepest coloured. The curve of the body and head is regular and smooth; the rudimentary antennae are absent; the abdominal region, very small in comparison with the rest, ends in six lobes of which the two middle ones are the largest. There are no groups of spinnerets.

The young insect (second stage) is somewhat different, being of a nearly regular oval shape, without the rolls of fat.

The male is very small, brown in colour; the antennae have ten joints. The two first joints are very small, round and smooth; the third, fourth, fifth and sixth equal in length, the seventh, eighth and ninth half as long, the tenth somewhat shorter still and pointed. All the last eight joints show numerous hairs. The thorax is short and thick, the thoracic band occupying more than one-half the width; the abdomen short, the double spike of some length. The wings are oval, about as long as the body. The legs are hairy, femora thick, tibiae longer, thicker at the end next the tarsus than at the other end; tarsi broad at the top, tapering gradually down to the usual single claw. The hairs on the femora are much fewer than those on the tibiae and tarsi.'


Reproduction is amphimictic, and adult females and males exhibit sexual dimorphism. Females retain the appearance of larval stages and remain sessile, while the males develop wings and fly in order to find a mate. Female red scale have three instars comprising a total of eight stages: first instar comprising a motile crawler and two sedentary stages; first moult; second instar; second moult; and third instar virgin female; and third instar mated female. Male red scale have eight life cycle stages: first instar comprising a motile crawler and two sedentary stages; first moult; second instar; prepupal; pupa; and winged adult.
For all stages (Fig. 2.4), with the exception of the crawler and male adult, the soft scale body is protected by semitransparent scale cover formed from filaments of proteinaceous material and wax, both produced by integumentary glands essentially located on the pygidium, and exuviae (cast larval skins) combined and cemented together by an anal liquid. The number of these glands is large, almost 50. The filaments come out through ducts that open on the dorsal surface at the end of the pygidium. The pygidium is lifted or arched to rub the under surface of the scale cover with its smooth dorsal surface that contains duct openings. The pygidium is moved slowly so that the covering material can adhere to the scale cover. The filament is 45% wax, 47% proteinaceous material and 8% exuviae.

Figure 2.4. Life cycle stages of red scale: 1 crawler; 2 second instar female; 3 virgin female; 4 mated female; 5 white-cap; 6 scale cover of second instar; 7 scale cover of adult female; 8 scale cover of male; 9 citrus foliage and fruit infested with scales (Agriculture New South Wales).

The pygidium (Figs 2.5 & 2.6), a key morphological feature for identifying diaspидid scales (Beardsley & Gonzalez 1975), is composed of the more or less fused and sclerotised, posterior abdominal segments (Ebeling 1959, Beardsley & Gonzalez 1975). In the first instar, the second instar female, and in adult females it is broad and round.
There are three pairs of conspicuous lobes: median lobes, second lobes, and third lobes, each narrowed at about one half their respective lengths. There is a spine closely located to the lobes on the dorsal surface. On each side (dorsal and ventral), there are 17 tubular ducts, with their openings arranged more or less in three lines. On the pygidial fringe, there are three, well-developed lobes. The two inner pairs are distinctly notched. The deeply fringed plates are located between the lobes and the lateral of the outer lobes. Two internal sclerotised structures are visible on the ventral side of pygidium.

Pygidial structures and associated markings can be used to distinguish between red scale, yellow scale (Ferris 1938, McKenzie 1938, OEPP/EPPO 2005a) and *Aonidiella eremocitri* (McKenzie) (McKenzie 1937, OEPP/EPPO 2005a), a species of uncertain origin that Flanders (1934) collected from Australian desert lime, *Citrus* (syn. *Eremocitrus* glauca (Lindl.) Burkill, in coastal Queensland in 1931, and from which he reared *Comperiella bifasciata* (Flanders 1934). In red scale (Fig. 2.5, left, and right, illustrations 1–8), small scleroses are present on the ventral side of the pygidium, just anterior to the vulva. These scleroses each comprise a pair of quite heavily sclerotised bodies having usually an inverted V or U shape, surrounding an apparently invaginated point and thus being of the nature of an apophysis. Ferris (1938) said that these structures are constant in form and occurrence. Accompanying them are one or two small areas of weaker sclerotisation, which are quite variable in form and extent, but of which there is always at least a trace. In yellow scale (Fig 2.5, right, G & H), on the other hand, only the sclerotised apophyses are present in this region, and these are normally of a very slender and acute V shape. There is never any indication of the other scleroses, but slight folds or irregularities of the derm may be present. However, these differences between red scale and yellow scale are still subjected to about 5% margin of errors (DeBach et al. 1978). In contrast to red scale and yellow scale (Fig. 2.5), no scleroses are visible in *Aonidiella eremocitri* (Fig. 2.6) (McKenzie 1937, 1938, Ferris 1938). Colour photographs of cleared and stained pygidia of *Aonidiella aurantii* and *Aonidiella citrina* are presented in OEPP/EPPO Bulletin PM7/51 (OEPP/EPPO 2005a). Literature on yellow scale is reviewed later in this chapter.

11 There appear to be no records of *Aonidiella eremocitri* on introduced species and varieties of *Citrus*.

12 However, DeBach et al. (1978) mentioned that even with these characters, about 5% of scales cannot be confidently separated.
**Figure 2.5.** Morphology and structure of pygidium of red scale (left, A–H), scleroses of red scale (right, 1–8) and pygidium and scleroses of yellow scale (right G–H) (from Ferris 1938).

**Figure 2.6.** Morphology and structure of pygidium of *Aonidiella eremocitri* (from McKenzie 1937): in contrast to red scale and yellow scale (Fig. 2.5), no scleroses are visible.
During the first and second instar male and female stages, and the third instar virgin female stage, the scale body is covered by the scale cover, but not attached to it. In contrast, the bodies of first moult males and females, second moult females, and third instar mated females are attached to their scale covers.

Physiological time (degree-days: °D) required for development of each instar and stage at a developmental threshold of 11.6°C is summarised in Table 2.1, and mentioned elsewhere within the text. This threshold is based on Yu (1986) and Murdoch et al. (1995). Requirements for males and females at threshold temperatures ranging from 10.2 to 13.8°C are summarised in Table 2.4\(^{13}\). However, factors other than temperature influence development. For example, scale size varies with host and host substrate (Ebeling 1950, Luck & Podoler 1985), and development is more rapid upon fruit than stem and leaves (Bliss et al. 1931).

### 2.1.5.1. First instar

The first instar passes through three stages: the motile crawler, and the sessile whitecap and nipple stage. It is the only stage in the life cycle when the scale cover has no orange pigmentation (Forster et al. 1995). The crawler has two antennae, each with four-segments, two eyes, and six well-developed legs. The body of the settled stage is about 0.24 mm long and 0.15 mm wide. The pygidium has two well-developed and conspicuous central lobes (Quayle 1911a).

After birth, the crawler, the dispersal and most vulnerable phase in the life cycle, remains underneath its mother’s scale cover near her pygidium for 1–2 d. They usually emerge in the afternoon (Willard 1973). Low temperatures, or high temperatures and low humidity, are unfavourable for its survival. For example, at 25°C, the survival periods recorded by Willard (1973) were 10 h at 70% RH and 6.8 h at 20% RH. Emergence from under the mother’s cover can be delayed during such unfavourable conditions (Parry-Jones 1935). The optimum conditions for settlement of the crawler recorded by Parry-Jones (1935) were approximately 20–23°C and 65–80% RH. Survival decreases with increase in rate of air flow (Willard 1973). Bodenheimer (1951) summarised crawler activity at several temperatures. Cold torpor occurred at

\(^{13}\) In this thesis, unless otherwise stated, 11.6°C is used for °D accumulations and estimates related to scale and natural enemy phenology.
10.5°C, weak movements of antennae at 12.8°C, crawling with interruptions at 13.8°C, high activity at 39.2°C, and instantaneous death at 43°C.

As the crawler settles, it inserts its stylets into plant tissue and then starts to produce white, cottony material for its scale cover, thus becoming the whitecap stage. As the cover continues to increase in diameter, the centre, the first part of the cover, becomes raised. At this point, the first instar becomes the nipple stage. Settlement usually occurs within several hours; a few may search for 24 h. The majority settle within a short distance of their mother, only a few further than 500 mm, unless dispersed more widely within and between trees, and between orchards, by wind, birds and movement of orchard equipment (Stofberg 1937, Ebeling 1950, 1959, Bodenheimer 1951, Willard 1974). Willard (1974) showed that they may be carried passively by wind over distances up to 312 m, but distance of dispersal fell rapidly over 19.5 m, then more slowly. Most settle on the adaxial (upper) surface of leaves, beside the midrib and main veins on the leaves and in the tiny depressions marking the location of the oil glands of the fruit (Quayle 1911a, Nel 1933, Ebeling 1959).

Studies have indicated that the crawlers are positively phototactic and negatively geotactic (Flanders 1951b, DeBach & White 1960), positively thigmotactic (DeBach & White 1960), and positively thermodactic and chemotactic (Bodenheimer 1951). Positive phototactism and thigmotactism appear to be the most important factors (Beardsley & Gonzalez 1975, Rodrigo et al. 2004), but Orphanides (1984) suggested that the key factor affecting intra-tree distribution is passive dispersal of crawlers, and that this overshadows other factors. Nevertheless, crawlers move preferentially outward to new growth and young fruits, and most second generation crawlers on fruit originate from reproducing females on fruit (Rodrigo et al. 2004). Most settle on exposed fruit surfaces rather than under calyxes (Rodrigo et al. 2004). Parry-Jones (1935) observed that more crawlers settled on the Valencia orange and lemon (*Citrus × limon* (L.) Osbeck) fruit than on leaves in summer, while in winter, a greater percentage of settlement occurred on leaves. Hely (1968) commented that red scale is a light-loving species found mostly on the outside of the tree canopy.

Bodenheimer (1951), Carroll (1979), Orphanides (1984) and Murdoch et al. (1989) studied scale distributions in relation to crawler dispersal in orchards in the northern hemisphere. Bodenheimer (1951) concluded from studies on the distribution of red scale in trees that the vertical distribution of the scale in trees is related to the density of
Percentages of scale present in the upper, middle and lower parts of the tree, 29, 48 and 23%, respectively, corresponded to the percentage of leaves, 30, 47 and 23%, respectively, in each sector (Bodenheimer 1951). Carroll (1979) observed that scale density was lowest in the warmest south-facing upper halves of trees, particularly in summer, on mature navel orange trees, each about 4.4 m high, in the San Joaquin Valley of California. He noted that scale numbers began at zero on new fruit in spring and increased rapidly over the other substrates as the fruit matured. However, throughout the year, scale numbers were divided more or less evenly throughout the year. He (Carroll 1979) also noted that the extremely high surface area of the leaves compensated for the very low scale density there, so that the leaf-inhabiting population was about equal to the wood-inhabiting population, and that the proportion of the total that inhabited wood increased as each generation progressed to maturity. Orphanides (1984) observed no differences between scale populations in the four cardinal sectors of mature 7-year-old lemon trees in Cyprus, but noted that populations, due to passive dispersal of crawlers, were higher in the central parts than on the periphery. Murdoch et al. (1989) recorded significantly higher population densities on the interior wood of trees than on exterior substrates: scale densities increased with of age of substrate.

Total physiological time required for development of the first instar is 106.5°D at 11.6°C (Table 2.1).

<table>
<thead>
<tr>
<th>Stage</th>
<th>Female (♀)</th>
<th>Male (♂)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration (°D)</td>
<td>Cumulative (°D)</td>
<td>Duration (°D)</td>
</tr>
<tr>
<td>First instar</td>
<td>106.5</td>
<td>106.5</td>
</tr>
<tr>
<td>First moult</td>
<td>69.5</td>
<td>176.0</td>
</tr>
<tr>
<td>Second instar</td>
<td>75.8</td>
<td>251.8</td>
</tr>
<tr>
<td>Second moult</td>
<td>92.5</td>
<td>344.3</td>
</tr>
<tr>
<td>Virgin adult</td>
<td>103.8</td>
<td>448.1</td>
</tr>
<tr>
<td>First crawler</td>
<td>202.8</td>
<td>650.9</td>
</tr>
<tr>
<td>produced</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2.1.5.2. First moult

During moulting, the stylets are withdrawn from plant tissues and the scale body becomes attached to the scale cover. The cast skin is incorporated within the scale cover. The body wall is hard and brittle and well distended, and the body content becomes more fluid and watery. The skin is split around the lateral margin. The cast dorsal skin can be seen as clearly as the insect itself, including pygidial characters. Physiological time required for development of the first moult is 69.5°D at 11.6°C (Table 2.1).

2.1.5.3. Second instar

After moulting to the second instar, the scale reinserts its stylets into the host plant to feed. Its body rotates inside the scale cover and produces wax material to form the circular armour surrounding the orange ring formed by combination of first instar cast skin and scale cover. The new band of armour is translucent, and the yellow-orange scale body, which is about twice the size of the first instar scale body, is visible through it. All the characters of the pygidium are distinct, and the legs and the antennae are lost. The pygidium comprises three conspicuous lobes with a spine arising from the base of each. Sexual differentiation, manifested by shapes and sizes of male and female scale covers occurs at this point. Scale covers of second instar females remain circular when viewed from above, while second instar males become elongated and slender. Physiological time required for development of the second instar male is 50.5°D, and the duration of second instar female is 75.8°D at 11.6°C (Table 2.1). The late second instar male body is about 0.7 mm long and 0.4 mm wide. It has two pairs of conspicuous eyes, one pair on the ventral anterior margin, the other more dorsal. Brown pigmentation of the pygidium, which is V-shaped, allows them to be distinguished from females.

2.1.5.4. Second moult female

The second moult of the female scale is similar to first moult, but the scale body is larger and the scale cover is wider due to an additional ring of wax. Physiological time required for development of this moult is 92.5°D at 11.6°C (Table 2.1).
2.1.5.5. Virgin adult female

After the second moult, the third instar virgin female body becomes separated from its scale cover, and a new band of wax appears as a grey ring around reddish bands of wax produced by the earlier stages. At this point, development of lateral thoracic lobes occurs. Segmentation of the soft body of the scale is obscure. The head and thorax are fused, and segments two to eight of the abdomen are fused to form the pygidium. The pygidium is round. The anus is on the dorsal side and genital aperture is on the ventral side. Physiological time required for development of this stage is 103.8°D at 11.6°C (Table 2.1) if mating occurs; virgin females do not mature unless they mate, and they can live for about 6 months without fertilisation (Quayle 1911a, 1938). The period of embryonic development, which is completed early in the life of third instar virgin female, is preceded by completion of growth (Parry-Jones 1935).

Virgin females produce a sex pheromone that attracts conspecific males (Tashiro & Chambers 1967). Tashiro & Moffitt (1968) observed that virgin females became attractive to males when the grey margins of their scale covers start growing, and that attractiveness peaked 4–5 weeks from the crawler stage. Identification and synthesis of the sex pheromone was reported by Roelofs (1978). A synthetic compound, 3-methyl-6-isopropenyl-3,9-decadien-1-yl acetate, has since been widely used in traps for detecting scale populations, monitoring scale phenology and for scale management of red scale (Rice & Moreno 1970, Shaw et al. 1971, Flint et al. 1984).

2.1.5.6. Mated adult female

The scale cover is reddish, almost circular, flatly convex, and about 0.78 mm wide and 1 mm long. Segmentation of the soft body of the scale is obscure. Prior to mating, the pygidia of virgin females protrude from the thoracic lobes of their scale bodies. After mating, the pygidium retracts inwards to a position between the lateral thoracic lobes of the scale body so that mating with other males will not be possible. At this point, the scale body becomes firmly attached to the scale cover, which becomes more evenly red. Physiological time from mating to beginning of crawler production is 202.8°D at 11.6°C (Table 2.1). Crawlers can be produced in as short a time as 42 d after the parent had settled when the mean minimum temperature was 22°C and maximum temperature was 33°C.
Mated females are viviparous\(^4\), and each female can produce about 150 to 200 young crawlers over an interval from one to two months or longer depending on climate. Fecundity and reproductive period vary and are influenced by several factors including, climate, host plant and host plant substrate (Bliss et al. 1931, Nel 1933, Stofberg 1937, Yust 1943, Tashiro & Beavers 1968, Perez 1972, Rosen & DeBach 1978) (Table 2.2). Carroll (1979) observed that scale on fruit were most prolific, and scale on wood least, but he recorded no significant difference in the reproductive duration on different substrates (leaf, fruit and wood). The duration of the reproductive phase in days, mean number of crawlers produced and average numbers of crawlers produced per day reported in the literature are summarised in Table 2.2. Atkinson (1977) reported that crawler production declined with reproduction age, and that temperature influenced both day-to-day crawler production and the pattern of production over reproduction span of females. Quayle (1911a) and Willard (1972) reported that unfertilised females live much longer than mated females. McLaren (1971) reported that delayed insemination influenced the productivity of red scale, with an initial increase in reproductive rate and a rapid decline in subsequent periods. He (McLaren 1971) also reported that delayed mating could cause a high initial natality. Reports are summarised in Table 2.2.

2.1.5.7. **Prepupa, pupa and adult male**

Prepupal, pupal and adult males do not become attached to the scale cover. The prepupa is orange-yellow with the dark red or brown eyes and is about 0.7 mm long and 0.35 mm wide. The posterior end is truncated with the button at the tip. The light yellow adults have a dark band on their dorsal thorax. They remain under their scale covers for a few days until their single pair of wings expand and dry. The antennae have 10 segments; the first two are shorter and thicker than the others. The head is more or less triangular in outline. It is largely membranous with conspicuous eyes and ridges. Several ridges support head capsule. There are broken lines located in the dorsal structure of the head. There are two pairs of accessory eyes and one pair of primary eyes. The ventral and dorsal accessory eyes are similar in structure. The ventral eyes are large, ovoid, situated on each side of the median line in the caudal haft of the ventral aspect of the head. The dorsal eyes are circular and dark-brown, situated just lateral of the antennae. The prothorax is mostly soft and fleshly. The mesothorax

\(^4\) There are no eggs: the young are born alive.
is the principle wing-bearing segment, well developed with sclerites and ridges. The legs are glassy white with light yellow tarsi except coxae.

The adult emerges from under its scale cover by pushing its way backward under the cover. It walks a short distance before flying in search of a mate. This provides them with a chance to mate with a female some little distance away, with dispersal sometimes assisted by wind, in order to prevent degeneration through in-breeding with a female of the same parents (Quayle 1911a). Nel (1933) reported that adult males started emerging at 09:45 and died at 10:45, but Quayle (1911a) reported that flight peaked just before sunset during spring and autumn, and during twilight in summer. Bodenheimer (1951) stated that males are delicate, certainly nocturnal, positively phototactic (at least in not to strong a light), negatively geotactic, and positively chemotactic towards females, in a varying degree. Tashiro & Beavers (1968) observed that most males emerged between 17:30 to 18:30 at light intensities of 3767 lux to 5651 lux, and Rice & Moreno (1970) reported that the optimum temperature for flight activity was 26.7°C. Copulation may occur within half to one hour after emergence (a 1911). Quayle (1911a) reported that adult males can live from one to five days, but Tashiro & Beavers (1968) observed that they die the morning after flight. Yan & Isman (1986) noted impacts of temperature, light intensity and humidity on male emergence and longevity. Constant temperatures in the range of 15–35°C or light intensities in the range of 267–10,763 lux shifted emergence toward mid afternoon from early evening. Optimum relative humidity for male emergence was 60–65%, and extreme low and high humidity delayed the emergence (Yan & Isman 1986). At 40°C, 80% and 100% mortality were recorded after 3 and 7 h, respectively. Some males lived for 29 h at 15°C, others for 31 h at low light intensity of 10.8 lux. Some males lived for 20 h at 80–85% RH (Yan & Isman 1986).

2.1.6. Sex ratio

Information presented by Quayle (1911a) suggests that males are more common in spring than females than in other summer, autumn and winter; in southern California the sex ratio ($\frac{♂}{♀}$) was 1:0.6 from January to July (mid winter to mid summer) in contrast to 1:1 from mid summer to mid winter (mid summer to mid winter). For populations of the scale reared on orange seedlings, Nel (1933) reported ratios of 1:0.4 for the first half of 1930 and 1:1.6 for the second half. Ebeling (1959) reported ratios of 1:2.6 on mature Valencia and 1:2.57 on mature Eureka lemon.
2.1.7. Population dynamics

Red scale is multivoltine (Bodenheimer 1951, Luck 1995). Numbers of red scale generations that occur annually, the duration of each generation, scale density and the impact of populations on hosts, are influenced by foliar nitrogen levels, host substrate, and a range of climatic conditions, including temperature, rainfall and humidity.

Bodenheimer (1951) stated that under rather harsh conditions of the experiments he reported, a prolonged deficiency diet, including nitrogen, did not influence the duration of development, but depressed reproduction slightly. In contrast, under ‘luxuriance’ conditions, development was slightly slackened and reproduction increased when nitrates were in excess. McClure (1980) stated that physiological responses of a host plant to changing edaphic conditions or to application of fertilisers often alter its susceptibility to attack by diaspids. He noted that Salama et al. (1972) concluded from their studies that fertilisation of seedlings with excess amounts of nitrogen, potassium, and phosphorus increased the resistance to red scale. However, Habib et al. (1972) stated that the total amount of nitrogen, phosphorus and carbohydrates in the leaves of different varieties showed no correlation with their susceptibility to infestations.

Bliss et al. (1931) reported that for all instars, development was more rapid upon fruit than upon the stems and leaves of potted seedlings, and leaves seemed to be somewhat more favourable than stems. Carroll (1979) mentioned that scale density was strongly dependent on host plant substrates. Scale density was relatively low on leaves and twigs and high on wood and fruit. However, leaf surface was > 90% of crown area. Thus, leaf-, wood-, and fruit-dwelling scale each contributed substantially to the scale population (Carroll 1979). However, Carroll’s (1979) studies were conducted in California where Argentine ant, *Linepithema humile* (Mayr) [Hymenoptera: Formicidae], disrupts natural enemies of the scale (DeBach et al. 1951b, Flanders 1951a, Murdoch et al. 1995).
Table 2.2. Summary of reports on the reproductive productivity of red scale.

<table>
<thead>
<tr>
<th>Author</th>
<th>Location</th>
<th>Latitude &amp; longitude</th>
<th>Condition</th>
<th>Host plant/ substrate</th>
<th>Mean number of crawlers per female (range)</th>
<th>Reproductive phase (days)</th>
<th>Reproductive rate (average number of crawlers per day)</th>
<th>Maximum number of crawlers per day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stofberg (1937)</td>
<td>Nelspruit (South Africa)</td>
<td>25° 28' N 30° 58' E</td>
<td>open insectary summer</td>
<td>sweet orange seedling</td>
<td>143</td>
<td>98.3</td>
<td>1.5</td>
<td>8.2</td>
</tr>
<tr>
<td>Quayle (1911a)</td>
<td>Whittier (California, USA)</td>
<td>33° 58' N 117° 19' E</td>
<td>open insectary winter field, autumn</td>
<td>citrus leaves</td>
<td>69</td>
<td>114.8</td>
<td>0.6</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>Whittier (California, USA)</td>
<td>33° 58' N 117° 19' E</td>
<td>insectary, summer field, from 26 April to 14 June 1935</td>
<td>lemon fruit</td>
<td>72.6 (18–176)</td>
<td>18.3</td>
<td>4.0</td>
<td>14</td>
</tr>
<tr>
<td>Yust et al. (1931)</td>
<td>near Corona, California</td>
<td>37° 52' N 125° 43' E</td>
<td>lemon fruit</td>
<td>lemon fruit</td>
<td>153 (5–300)</td>
<td>64.5 (7–154)</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>Yust (1943)</td>
<td>near Corona, California</td>
<td>37° 52' N 125° 43' E</td>
<td>lemon fruit</td>
<td>lemon fruit</td>
<td>158.5 (13–294)</td>
<td>85.3 (7–245)</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>Yust (1943)</td>
<td>near Corona, California</td>
<td>37° 52' N 125° 43' E</td>
<td>lemon fruit</td>
<td>lemon fruit</td>
<td>99.5 (8–192)</td>
<td>154.3 (7–287)</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>Yust (1943)</td>
<td>near Corona, California</td>
<td>37° 52' N 125° 43' E</td>
<td>lemon fruit</td>
<td>lemon fruit</td>
<td>65.6 (2–171)</td>
<td>145.1 (14–245)</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>Yust (1943)</td>
<td>near Corona, California</td>
<td>37° 52' N 125° 43' E</td>
<td>lemon fruit</td>
<td>lemon fruit</td>
<td>93.2 (7–221)</td>
<td>77 (35–119)</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>Willard (1972)</td>
<td>Glen Osmond, South Australia</td>
<td>34° 47' S, 137° 33' E</td>
<td>laboratory at 25°C</td>
<td>lemon leaf disks</td>
<td>267.0 (79–438)</td>
<td>106.4 (35–168)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Willard (1972)</td>
<td>Glen Osmond, South Australia</td>
<td>34° 47' S, 137° 33' E</td>
<td>laboratory at 25°C</td>
<td>lemon leaf disks</td>
<td>174 (69–318)</td>
<td>123.3 (35–175)</td>
<td>1.43</td>
<td>2.39</td>
</tr>
<tr>
<td>Perez (1972)</td>
<td>Philippines</td>
<td>12° 52' N, 121° 48' E</td>
<td>five-month old pomelo (Citrus maxima)</td>
<td>195 (101–312)</td>
<td>94.25 (73–120)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Bliss et al. (1931) and Carroll (1979) reported more rapid development occurring on fruit, followed by leaves, and stems. Smith (1957) observed that the length of the red scale life cycle on six host plants varied, and that it was shorter on yucca (*Yucca elata* Engelm. [Asparagales: Agavaceae]) and agave (*Agave decipiens* Baker [Asparagales: Agavaceae]) than on lemon, sago palm (*Cycas revoluta* Thunb. [Cycadales: Cycadaceae]), orange, and grapefruit, respectively. He recorded the shortest life cycle on castor bean (*Ricinus communis* L. [Malpighiales: Euphorbiaceae]) and increasingly longer life cycles on yucca, lemon, agave, grapefruit (*Citrus × aurantium* L.) and orange. Grout et al. (1989) also reported more rapid development on lemon trees than on orange and grapefruit trees. Furthermore, Perez (1972) concluded that development was more rapid on grapefruit than on oranges.

Extreme temperatures have an impact on red scale development (Nel 1933, Ward & Johnston 1937, Willard 1973, Abdelrahman 1974a, Zhao 1990), and these impacts differ with life-cycle stage. According to Abdelrahman (1974a), the growing stages were more tolerant to extreme temperatures than moulting stages, and prepupal and pupal stages of males were the most susceptible. Abdelrahman (1974a) found that, prepupal and pupal non-motile male stages were the most susceptible of all stages to extreme low temperature under laboratory conditions. The LD$_{50}$ temperatures for survival of these stages were about 10°C compared to -2.49, -2.46, -3.31 and -2.13°C for the first instar, second instar, third instar virgin female and early mated female, respectively, and 5.84, 6.06 and 3.09°C for the first moult, second moult female and crawler producing female stages, respectively (Abdelrahman 1974a).

Bliss et al. (1931) reported experiments in which the duration of the first instar was longer than the second instar, and that the duration of the third instar exceeded that of the other two combined. A change in temperature had the least-effect upon the first instar, greater effect upon the second stage, and the greatest effect upon the combined third instar. Stofberg (1937) also reported that temperature had the greatest effect on third instar, then the second instar, and least on the first instar. Tashiro & Beavers (1968), Rice & Moreno (1970) and Yan & Isman (1986) showed that male stage is short-lived, and not able to survive in direct sunlight and at high temperatures.

Extreme temperatures kill red scale. For example, a record heat wave in 1939, with a maximum shade temperature of 48°C, caused widespread mortality of red scale in the
Riverina region of New South Wales (Hely et al. 1982). Martin & Black (1960), in trials in which scale infested Washington navel fruit were placed in a dehydrator, observed 100% mortality after 2.25 h at 50°C and 12% RH. An observation at Yorba Linda (33° 53’ N, 117° 48’ W), in Orange County 45 km southeast of Los Angeles and 16 km northeast of Santa Ana in one of the intermediate zones of southern California (Fig. 2.7), also indicated that mortality of scale populations in the elevated parts of the grove where the highest temperature occurred was more severe than in other parts of the grove (Ebeling 1933).

Long-term average annual maximum and minimum temperatures and annual average rainfall for localities in the citrus producing regions of New South Wales (Fig. 2.3) are presented in Table 2.3. For comparative purposes, long-term, average, annual maximum and minimum temperatures and annual, average rainfall records are presented in Table 2.3 for locations in southern Africa (Fig. 2.8), the coastal, intermediate and interior citrus producing regions of southern California (sensu DeBach et al. 1955) (Fig 2. 8), and Visalia (270 km north of Los Angeles) in the San Joaquin Valley of California (not shown in Fig. 2.7), where the bulk of the state’s citrus is now grown.

Figure 2.7. General climatic zones in southern California citrus areas in the 1950s, showing the coastal, intermediate interior and desert zones: adapted from DeBach et al. (1955).
Figure 2.8. Citrus growing regions of South Africa in the mid 1990s: adapted from Bedford et al. (1998).

In South Africa, Stofberg (1937) recorded 4 generations of red scale per year under insulated roofing at Nelspruit, but Grout et al. (1989) reported 4 to 6 generations in orange orchards and 5 to 7 in lemon orchards in six climatically different regions. In Mazoe in Zimbabwe, Parry-Jones (1935) recorded 5 generations under shaded conditions and 6 to 7 in direct sunlight. Bliss et al. (1931) noted 3 generations under shaded conditions and commented that radiant heat (from orchard floors) and sunlight might shorten development sufficiently to allow a fourth or partial fourth generation at Whittier (33° 58' N, 117° 19' W) in one of the intermediate zones (see Fig. 2.7) for growing citrus in southern California. Kennett & Hoffmann (1985) and Murdoch et al. (1989) reported 3 to 4 generations in California, at Lindcove (36° 23' N, 118° 55' W), near Visalia, in the San Joaquin Valley, and at Fillmore (34° 23' N, 118° 55' W), in an intermediate zone on southern California, respectively. In Australia, there are 2–5 generations in South Australia, Victoria, New South Wales and southern Western
Australia, and 5–6 in Queensland, the Northern Territory and northern Western Australia (Smith et al. 1997).

Field generations are not clearly defined and considerable overlapping occurs. According to Parry-Jones (1935), reasons for overlapping generations include different rates of development in exposed and unexposed parts of the tree, and on leaves and fruits. Overlapping generations also result from the prolonged reproductive periods of the scale. Grout & Richards (1989) reported that overlapping generations are also related to levels of parasitism.

Most data in Table 2.4, though from studies undertaken in several geographical regions, were recorded under controlled conditions. First instar durations from crawler establishment to the end of first moult in these studies ranged from about 11 to 16 d. The larval period took longer than the moult stage, occupying about 70% of the total duration of first instar. The developmental durations of first instar given by studies were slightly different. The duration of different stages given by Perez (1972) was shorter than those studied by others. This rapid development was thought to be attributed to the host plant on which the scales were reared (Perez 1972). Development on fruit was shorter than that on leaves (Parry-Jones 1935).

Reported female second instar durations in Table 2.4 varied from about 11 to 30 d. The longest duration for the second instar recorded by Quayle (1911a) at Whittier in California occurred in the field during the coldest months when the average temperature was 0.3°C, whereas the other studies summarised in Table 2.4 were conducted under partially controlled conditions. The short durations observed by Perez (1972) in the Philippines may have been due to the tropical conditions under which the observations were made. Development durations given by Ward & Johnston (1937), Parry-Jones (1935), Nel (1933) were less variable and close to estimates obtained by Willard (1972) at 25°C. The duration of the second larval stage is approximately 60% of total duration of the second instar stage (Table 2.4).
Table 2.3. Average long-term annual maximum and minimum temperatures and rainfall records for citrus producing regions of New South Wales, southern Africa and California.

<table>
<thead>
<tr>
<th>Location (region)</th>
<th>Latitude &amp; longitude</th>
<th>Temperature</th>
<th>Rainfall</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>New South Wales, Australia</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Narromine (Macquarie River, based on Trangie)</td>
<td>32° 14' S, 148° 14' E</td>
<td>10.8</td>
<td>24.5</td>
</tr>
<tr>
<td>Peats Ridge (Somersby Plateau, Central Coast)</td>
<td>33° 19' S, 151° 14' E</td>
<td>11.3</td>
<td>21.5</td>
</tr>
<tr>
<td>Richmond (Hawkesbury Valley, Central Coast)</td>
<td>33° 36' S, 150° 44' E</td>
<td>11.0</td>
<td>23.7</td>
</tr>
<tr>
<td>Dareton (Sunraysia, lower Murray River, based on Mildura, Victoria)</td>
<td>34° 05' S, 142° 02' E</td>
<td>10.3</td>
<td>23.7</td>
</tr>
<tr>
<td>Griffith (Riverina)</td>
<td>34° 17' S, 146° 02' E</td>
<td>10.0</td>
<td>23.8</td>
</tr>
<tr>
<td>Narrandera (Riverina)</td>
<td>34° 45' S, 146° 33' E</td>
<td>10.0</td>
<td>23.3</td>
</tr>
<tr>
<td>Barham (mid Murray River, based on Kerang, Victoria)</td>
<td>35° 37' S, 145° 07' E</td>
<td>9.4</td>
<td>22.8</td>
</tr>
<tr>
<td>Tocumwal (upper Murray River)</td>
<td>35° 48' S, 145° 34' E</td>
<td>9.6</td>
<td>22.9</td>
</tr>
<tr>
<td>Bamawm (Victoria)</td>
<td>36° 15' S, 144° 33' E</td>
<td>9.3</td>
<td>19.6</td>
</tr>
<tr>
<td><strong>Southern Africa</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mazoe Citrus Estate (Bindura) Zimbabwe</td>
<td>17° 30' S, 30° 58' E</td>
<td>12.5</td>
<td>27.2</td>
</tr>
<tr>
<td>Polokwane (Pietersburg), Limpopo (Norther Province/Northern Transvaal), South Africa</td>
<td>23° 54' S, 29° 27' E</td>
<td>12</td>
<td>25</td>
</tr>
<tr>
<td>Nelspruit, Mpumalanga (Eastern Transvaal), South Africa</td>
<td>25° 29' S, 30° 59' E</td>
<td>14.0</td>
<td>24.5</td>
</tr>
<tr>
<td>Rustenberg, North West Province, South Africa</td>
<td>25° 40' S, 27° 14' E</td>
<td>12</td>
<td>28</td>
</tr>
<tr>
<td>Tshaneni, Swaziland</td>
<td>25° 59' S, 31° 45' E</td>
<td>19</td>
<td>28.5</td>
</tr>
<tr>
<td>Cape Town, Western Cape, South Africa</td>
<td>33° 55' S, 18° 22' E</td>
<td>11.4</td>
<td>22.0</td>
</tr>
<tr>
<td>Port Elizabeth, Eastern Cape, South Africa</td>
<td>33° 58' S, 25° 36' E</td>
<td>14</td>
<td>22</td>
</tr>
<tr>
<td><strong>California, United States of America</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visalia (San Joaquin Valley)</td>
<td>36° 32' N 119° 03' W</td>
<td>10.2</td>
<td>23.7</td>
</tr>
<tr>
<td>San Buenaventura (Ventura) (southern California, coastal)</td>
<td>34° 29' N 119° 29' W</td>
<td>11.2</td>
<td>19.2</td>
</tr>
<tr>
<td>Yorba Linda (southern California, intermediate)</td>
<td>34° 23' N, 118° 55' W</td>
<td>10.8</td>
<td>25.8</td>
</tr>
<tr>
<td>Location (region)</td>
<td>Latitude &amp; longitude</td>
<td>Temperature</td>
<td>Rainfall</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>----------------------</td>
<td>-------------</td>
<td>----------</td>
</tr>
<tr>
<td>Fillmore (southern California, intermediate)</td>
<td>34° 23' N, 119° 04' W:</td>
<td>7.3</td>
<td>24.2</td>
</tr>
<tr>
<td>Whittier (southern California, intermediate)</td>
<td>33° 58' N 117° 19' W</td>
<td>13.2</td>
<td>26.1</td>
</tr>
<tr>
<td>Riverside (southern California, interior)</td>
<td>33° 36' N, 117° 23' W</td>
<td>12.7</td>
<td>26.0</td>
</tr>
<tr>
<td>Mecca (southern California, desert)</td>
<td>33° 34' N, 117° 55' W</td>
<td>13.7</td>
<td>32.5</td>
</tr>
<tr>
<td>La Mesa (southern California, intermediate)</td>
<td>32° 46' N, 118° 58' W</td>
<td>12.7</td>
<td>24.6</td>
</tr>
<tr>
<td>San Diego (southern California, coastal)</td>
<td>32° 44' N, 117° 10' W</td>
<td>14.5</td>
<td>21.5</td>
</tr>
</tbody>
</table>
Table 2.4. Summary of duration (d) of the life cycle of red scale.

<table>
<thead>
<tr>
<th>Author</th>
<th>Location</th>
<th>Latitude &amp; longitude</th>
<th>Conditions</th>
<th>Host substrate</th>
<th>Female (♀)</th>
<th>Male (♂)</th>
<th>To first crawler production</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quayle (1911a)</td>
<td>Whittier, California, USA</td>
<td>33° 58' N 117° 19' W</td>
<td>field</td>
<td>citrus trees</td>
<td>16</td>
<td>10</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Riverside, California, USA</td>
<td>33° 57' N 118° 02' W</td>
<td>field</td>
<td>citrus trees</td>
<td>30</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>Willard (1972)</td>
<td>Glen Osmond, South Australia</td>
<td>34° 47' S, 137° 33' E</td>
<td>laboratory at 25°C</td>
<td>lemon leaves</td>
<td>44</td>
<td>35</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>dishes</td>
<td>90</td>
<td>35</td>
<td>12</td>
</tr>
<tr>
<td>Ward &amp; Johnston (1937)</td>
<td>Burnley (Victoria, Australia)</td>
<td>37° 49' S, 144° 59' E</td>
<td>insectary (15.5°C - 23.8°C)</td>
<td>young trees</td>
<td>16 (29–44)</td>
<td>17</td>
<td>10</td>
</tr>
<tr>
<td>Parry-Jones (1935)</td>
<td>Mazoe (Zimbabwe)</td>
<td>17° 30' S, 30° 58' E</td>
<td>screened &amp; roofed insectary controlled*</td>
<td>fruit leaves</td>
<td>13</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td>Nel (1933)</td>
<td>California</td>
<td>36° 47' N, 119° 10' W</td>
<td>young Valencia seedlings five-month old pomelo seedlings</td>
<td>14</td>
<td>10</td>
<td>11</td>
<td>32</td>
</tr>
<tr>
<td>Perez (1972)</td>
<td>Philippines</td>
<td>12° 52' N, 121° 48' E</td>
<td></td>
<td></td>
<td>32</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>49.9</td>
<td>60.26</td>
<td>60.26</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11.8</td>
<td>7.6</td>
<td>7.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11.1</td>
<td>7.6</td>
<td>7.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11.1</td>
<td>7.6</td>
<td>7.6</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.8</td>
<td>2.9</td>
<td>2.9</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>23.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

I*: instar stage including larval and moult stages
Third instar duration from end of second moult to production of first crawler by mated females ranged from about 27 to 44 d. It includes the time required for embryonic development of egg within adult mated females and is similar or longer than the combined duration of the first and second instars.

The time taken from crawler establishment to production of first crawler ranges from about 50 to 90 d, depending upon environment and host substrate. The shortest duration, recorded in the Philippines, was about 50 d. Quayle (1911a) reported that the minimum period from birth to the appearance of crawler was 73 d at Riverside (California), while at Whittier (California), it was from 83 to 120 d (90 d on average). Willard (1972) reported about 60 d at 25°C.

Male development is also highly dependent on temperature and host plant (Bodenheimer 1951). In the studies summarised in Table 2.4, male development ranged from 23.5 d in the Philippines, to 33.5 d at 25°C in South Australia, and up to 60 d at Whittier (California). Male development on fruit varied from 26 d in summer to 76 d in winter (30 d on average), and on leaves from 27 d to 86 d at Mazoe Citrus Estate in Rhodesia (now Zimbabwe) (Parry-Jones 1935) (Table 2.4). The duration of the first instar was longer than the second instar, prepupal and pupal stages. The total developmental time for males is generally coincident with the maturation of females. Development of the grey rim on the female scale cover required 3.2 d. At this point, female scale becomes fully developed and receptive (Perez 1972). According to Quayle (1911), this latter interval was 10 to 20 d.

Kennett & Hoffmann (1985) reported that phenologies of field populations of red scale at Lindcove, in California, were well described by physiological time (°D): °D accumulated between onsets of stage-specific increases were close to the thermal constant. Furthermore, peaks of male adults coincided with the peaks of virgin females. For lower and upper developmental thresholds of 11.7 and 37.8°C, respectively, Grout et al. (1989) calculated mean cumulative °D of 498, 577, and 609°D between population peaks on lemon, orange and grapefruit trees, respectively. They (Grout et al. 1989) did not detect peaks for second generation males and females. Thus, they (Grout et al. 1989) found it difficult to predict fruit infestation levels based on flight peaks of red scale males in South Africa, because populations of the scale comprised three-cohorts that were possibly related to oviposition over extended periods.
Developmental rates and °D accumulations reported in Table 2.4 differ between field studies and studies reported under laboratory or shaded conditions. Total development time in the field may be longer (Murdoch et al. 1995). Bliss et al. (1931) and Parry-Jones (1935) observed that scale development was more rapid under exposed conditions than in the insectary or under shaded conditions. Studies reported by Bialoglowski (1935), Bodenheimer (1951), Grout et al. (1989) and Zhao (1990) indicate that this more rapid development in sunlight is due to heliothermy (Bodenheimer 1951), where absorption of radiation by the scale leads to its body temperature rising above the temperature of its host substrate, and/or absorption of radiation of the host substrate leading to the increase in temperature of the substrate, and therefore the body temperature of the scale, rising above ambient shade temperatures (Bialoglowski 1935, Grout et al. 1989, Zhao 1990). Bodenheimer (1951) reported that the temperature difference between scale body temperatures on the leaves under shady and sunny conditions were up to 13.4°C on average. Bialoglowski (1935) observed that citrus leaf temperatures under sunlight were from 3 to 10°C above ambient air temperatures. Munger (1948), Atkinson (1977) and Grout et al. (1989) stated that temperatures experienced by red scale in direct sunlight were higher than ambient. Zhao (1990) measured temperatures above the rind of fruit and used them as approximate the body temperature of red scale in his studies. He noted that under sunlight, the air temperature immediately above the rind in sunlight could be much higher than ambient air temperatures under shade. The difference fluctuated with time of day and could be as great as 15 to 17°C. Zhao’s (1990) observations suggest that heliothermy may be less important that the the natural temperature of the host substrate in sunlight.

°D have been widely used in the studies on red scale phenology (Parry-Jones 1935, Bodenheimer 1951, Kennett & Hoffmann 1985, Hoffmann & Kennett 1985, Grout et al. 1989, Murdoch et al. 1995). Under laboratory conditions, Murdoch et al. (1995) reported that the total developmental duration from the time a female crawler settles to the time it matures and produces crawlers is approximately 650°D. Under the field conditions, Atkinson (1977) and Kennett & Hoffmann (1985) estimated the total development constant of 597 and 589°D at lower thresholds of 12 and 11.7°C, respectively. °D required for development from third instar virgin females to production of first crawler is almost equal to the total °D required for completion of the first and second instars (Table 2.5).
2.1.8. Natural enemies

Red scale has a numerous predators and parasitoids (Rosen & DeBach 1978, Hely et al. 1982, Forster et al. 1995), the range and number of which vary with location, in relation to prevailing climate conditions and host stage requirements (Forster et al. 1995). Parasitoids are considered to be more effective than predators (DeBach et al. 1953, Ebeling 1959, Hely et al. 1982, Murdoch et al. 1995, Sorribas & García-Marí 2010). In some regions, the scale is also parasitised by one or more entomopathogens (Koebele 1892, Tryon 1894, Rolfs & Fawcett 1908, Watson & Berger 1932, Searle 1964, Bedford et al. 1998, Hely et al. 1982, Smith et al. 1997).

2.1.8.1. Parasitoids

The most important primary parasitoids of red scale in Australia are *Aphytis chrysomphali*, *Aphytis lingnanensis*, *Aphytis melinus*, *Encarsia citrina*, *Encarsia perniciosi* [Hymenoptera: Aphelinidae] and the red scale strain of *Comperiella bifasciata* [Hymenoptera: Encyrtidae] (Wilson 1960, Hely et al. 1982, Furness et al. 1983, Smith et al. 1997). Species of *Aphytis* are ectoparasitoids and females lay their eggs externally on the body of their host. The two species of *Encarsia* and *Comperiella bifasciata* are endoparasitoids and females lay their eggs within the body of their host. The instars and stages in or on which eggs are laid, and from which adult parasitoids emerge, are summarised in Table 2.6. The biologies of these parasitoids, all considered to have been either inadvertently or formally introduced to Australia, are discussed below.

2.1.8.1.1. The ectoparasitoids: *Aphytis chrysomphali*, *Aphytis lingnanensis* and *Aphytis melinus*

Ectoparasitoids are idiobionts: they permanently paralyse their hosts; have long adult lifespans; have low fecundity, they are synovigenic (females emerge with at most only a fraction of their total egg complement); and have ‘fast’ larvae (relatively rapidly developing) and ‘slow’ (relatively long-lived) adults (Jervis & Kidd 1986, Traynor & Mayhew 2005). In contrast, endoparasitoids are koinobionts with ‘slow’ larvae and ‘fast’ adults (see below for the endoparasitoids). Species of *Aphytis* are primary, external parasitoids of armoured scales (Rosen & DeBach 1978). They are the most common parasitoids of these scales (Compere 1955) and play important roles in regulating red scale populations in California, Israel, South Africa and Australia (Quednau 1964a,b, Rosen & DeBach 1978, Smith et al. 1997, Bedford et al. 1998).
Table 2.5. The summary of development thresholds (°C) and thermal constants (cumulative °D) for red scale.

<table>
<thead>
<tr>
<th>Author</th>
<th>Location</th>
<th>Latitude &amp; longitude</th>
<th>Condition</th>
<th>Host substrate</th>
<th>Threshold</th>
<th>Female (♀)</th>
<th>Total</th>
<th>Male (♂)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bodenheimer (1951) from Parry-Jones (1937)</td>
<td>Mazoe (Zimbabwe)</td>
<td>17° 30’ S, 30° 58’ E</td>
<td>modelled field data for equal exposure to sunshine</td>
<td>fruit</td>
<td>development threshold (°C)</td>
<td>10.2</td>
<td>12.7</td>
<td>12.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>thermal constant (°D)</td>
<td>182</td>
<td>110</td>
<td>408</td>
<td>690</td>
</tr>
<tr>
<td>Kennett &amp; Hoffmann (1985)</td>
<td>Lindcove (California)</td>
<td>36° 23’ S, 118° 55’ W</td>
<td>field</td>
<td>leaves</td>
<td>development threshold (°C)</td>
<td>11.3</td>
<td>13.8</td>
<td>13.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>thermal constant (°D)</td>
<td>182</td>
<td>110</td>
<td>408</td>
<td>690</td>
</tr>
<tr>
<td>Atkinson (1977)</td>
<td>Tshaneni (Swaziland)</td>
<td>25° 59’ S, 31° 45’ E</td>
<td>field</td>
<td>mature navel orange trees</td>
<td>leaves and twigs</td>
<td>137.5</td>
<td>191.95</td>
<td>259.75</td>
<td>589.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>fruit</td>
<td>139.4</td>
<td>153.9</td>
<td>313.3</td>
<td>597.71</td>
</tr>
<tr>
<td>Murdoch et al. (1995)</td>
<td>averaged from experiments at 20°, 25°, 26.7° and 30°C in the laboratory at Riverside (California)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>176.0</td>
<td>178.3</td>
<td>306.6</td>
<td>650.9</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>128.5</td>
<td>20.9</td>
<td>49.3</td>
<td>397</td>
</tr>
<tr>
<td>Tashiro &amp; Beavers (1968)</td>
<td>laboratory at 25°C and 70%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>176.0</td>
<td>178.3</td>
<td>306.6</td>
<td>650.9</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>128.5</td>
<td>20.9</td>
<td>49.3</td>
<td>397</td>
</tr>
</tbody>
</table>

* includes larval and moult stages; ** virgin female to production of first crawler
The life cycle stages include the egg, three larval instars, the pupa and the adult. Adults are small, about 1 mm long, and yellowish with largely hyaline wings. The antennae have 6 segments, with the basal segments being much smaller than the distal segment (Rosen & DeBach 1978). The adult emerges through a small, round exit hole in the scale cover or by raising the scale cover (Rosen & DeBach 1979).

Adult females insert their ovipositors through the scale covers of their hosts and lay their eggs beneath the cover, on the outside of the scale body. The eggs are teardrop-shaped, translucent, whitish, stalked and adhere to the surface of the scale insect (Rosen & DeBach 1978, Forster et al. 1995). Therefore, they only parasitise host stages in which the scale cover is not attached to the scale body (Rosen & DeBach 1978, 1979).

### Table 2.6. Summary of scale instars and stages in or on which parasitoid eggs laid, and from which adult parasitoids emerge.

<table>
<thead>
<tr>
<th>Parasitoid species</th>
<th>Host instar and stage</th>
<th>In or on which eggs are laid</th>
<th>From which adults emerge</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ectoparasitoid</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aphytis chrysomphali</em></td>
<td>second instar, virgin female</td>
<td>second instar, prepupae, pupae</td>
<td>virgin female, second instar, prepupae, pupae</td>
</tr>
<tr>
<td><em>Aphytis lingnanensis</em></td>
<td>second instar, virgin female</td>
<td>second instar, prepupae, pupae</td>
<td>virgin female, second instar, prepupae, pupae</td>
</tr>
<tr>
<td><em>Aphytis melinus</em></td>
<td>second instar, virgin female</td>
<td>second instar, prepupae, pupae</td>
<td>virgin female, second instar, prepupae, pupae</td>
</tr>
<tr>
<td><strong>Endoparasitoid</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Comperiella bifasciata</em></td>
<td>from first instar to virgin second instar</td>
<td>second instar, prepupae, pupae</td>
<td>mated female, second instar, Prepupae, pupae</td>
</tr>
<tr>
<td><em>Encarsia citrina</em></td>
<td>first moult, second instar</td>
<td>first moult, second instar, prepupae</td>
<td>second instar, second moult, virgin female, prepupae, pupae</td>
</tr>
<tr>
<td><em>Encarsia perniciosa</em></td>
<td>first moult, second instar</td>
<td>first moult, second instar, prepupae</td>
<td>second instar, second moult, virgin female, prepupae, pupae</td>
</tr>
</tbody>
</table>

For red scale, Richardson (1978), based on the biology of *Aphytis melinus*, concluded that *Aphytis* attacking red scale lay 40% of their eggs on second instar (males and females) and prepupal male scales and 60% of eggs on virgin female scales. Abdelrahman (1974c) stated that in the absence of preferred host stages, female *Aphytis melinus* laid readily on unpreferred stages. Yu et al. (1990) noted an observation by Yu (1986) and Opp & Luck (1986) that *Aphytis* adults never parasitise first instar red scale,
and seldom deposit eggs on scales less than 0.15 mm$^2$ (i.e., smaller second instar). *Aphytis* females prefer to oviposit on third instar, virgin red scale, because they are larger food source for their progeny to develop on than second instars, and more offspring can be produced per host (Forster et al. 1995). However, the size of the grey margins of second instar and third instar virgin females also influences oviposition behaviour by *Aphytis* species. The longer second instar and third instar, virgin females feed, the wider their grey skirt becomes. Therefore, *Aphytis* may prefer a large, second instar scale with a wide grey skirt, over a young third instar with a narrow grey skirt. This may be more important than host size in the host selection process (Forster et al. 1995). Reeve (1987) mentioned that, in the field, second instar and prepupal were attacked at a rate equal to or greater than that for third instars, whereas Abdelrahman (1974c), based on laboratory experiment, noted that the order of preferred hosts for *Aphytis melinus* was third instar, second instar and prepupa. He (Abdelrahman 1974c) also stated that not only host size but also host quality influences host preference by *Aphytis melinus*.

Host size and host stages affect *Aphytis* behaviour, size, fecundity and sex ratio (Opp & Luck 1986, Reeve 1987). Luck et al. (1982) reported that numbers of eggs laid per host by *Aphytis melinus* and *Aphytis lingnanensis*, facultatively gregarious species, depends on host size. For instance, on red scale 0.46 to 0.60 mm$^2$ in size, 33% of the hosts received two *Aphytis melinus* eggs, while the remainder received one. The same size class of host received only one *Aphytis lingnanensis* egg. For scales 0.61 to 0.75 mm$^2$, *Aphytis lingnanensis* began to express gregariousness as 33% of the hosts in this size range received two eggs, and the remainder received one. Small hosts, such as the second instar, produced small *Aphytis* with a male-biased sex ratio. The third instar, a larger host, produced much larger *Aphytis* and a more balanced sex ratio (Reeve 1987).

The pre-ovipositional behaviour of *Aphytis* includes five phase: drumming, turning, drilling, probing and ovipositing. After locating the host, she walks forward to drum the scale cover with her antennae until they encounter the margin. Then she walks backward and rotates right and left. She drills her ovipositor through the scale cover into the scale body and inserts it into scale body. The probing inside the body of the host prior to oviposition apparently serves not only to determine the accessibility of the host but also to paralyse the host in order to prevent it from becoming unsuitable for the development of her progeny (Rosen & DeBach 1979). Oviposition on the host
commences after the wasp withdraws her ovipositor from the scale body (Luck et al. 1982). The durations of these activities vary among _Aphytis_ species (van Lenteren 1994).

After hatching, larvae feed externally on the scale body and eventually pupate under the scale cover (Quednau 1964b). The first instar larva is ovoid, segmentation is usually not clearly visible, but in some specimens the head and 12 body segments are evident. It has 4 pairs of spiracles, one in the mesocephalic skeleton and one in each of the first 3 abdominal segments. The mandibles are minute and triangular. The second instar larva has 8 pairs of functioning spiracles, one in the mesocephalic skeleton and one in each of the first 7 abdominal segments. Segmentation is much more visible than in the first instar. The mandibles are more developed and more acutely pointed. The third instar is considerable larger than the second, elongate, rounded anteriorly and somewhat narrower posteriorly (Rosen & DeBach 1979). The prepupal stage is similar to the larval stages but with no colouration in the gut. The larva excretes meconial pellets when it transforms into the prepupal stage. These pellets remain under the scale cover after the adult emerges (Forster et al. 1997). The pupa is exarate, distinctly flattened dorso-ventrally, considerable wider than thick. It lies on its dorsum, with ventral aspect and mouthparts facing the scale cover of its host (Rosen & DeBach 1979). During the pupal stage, eye pigmentation changes. Initially, the eyes are white. They then become red, then dark red, then blackish and finally green (Forster et al. 1995). The green-eyed pupal stage lasts only a day before the adult emerges (Abdelrahman 1974b). During this stage, the pigmentation of thoracic and abdominal tergites can be used to distinguish species of _Aphytis_: _Aphytis lingnanensis_ pupae have dark thoracic and abdominal tergites; _Aphytis melinus_ pupae have dark thoracic tergites; and _Aphytis chrysomphali_ pupae have no pigmentation of either thoracic and abdominal tergites (Rosen & DeBach 1978), except for a thin but conspicuous longitudinal black line on the mesoscutum (Prinsloo 1984).

Murdoch et al. (1989) recorded significantly higher levels of _Aphytis melinus_ parasitism of red scale in the exterior parts of tree canopies than on the interior wood of a tree. The ‘instantaneous parasitism’ rate (rates at which scale are parasitised per unit time) on twigs was 15 times higher than on interior wood (Murdoch et al. 1989). Walde et al. (1989) found that the population of scale was high, but parasitism by _Aphytis melinus_ was low, on trunks and woody branches compared to external branches and leaves.
They attributed the low level of parasitism to the small size of scales on wood relative to other substrates; 49% of third instar scales on wood were > 0.39 mm², the smallest size acceptable for successful reproduction by *Aphytis melinus*, 56% on stems, 66% on leaves, and 76% on fruits. However, by calculating expected levels of parasitism on wood, Walde et al. (1989) pointed out that the difference of scale size could only account for ≈10% of the observed difference and was not the primary explanation for the presence of the refuge. Subsequently, Hare & Morgan (2000) showed that parasitism was related to lower levels of o-caffeoyltyrosine in scale covers of the small scales on wood, than in large scales on fruit and leaves. They considered the lower levels of O-caffeoyltyrosine were probably a consequence of reduced nutritional quality of bark as a substrate for scale survival and growth (Hare & Morgan 2000).

Studies have focused on the relationships between parasitism by *Aphytis* species, particularly *Aphytis melinus*, and host scale densities. Outcomes of such relationships vary among studies. Atkinson (1977) reported that the relationship between *Aphytis* species and red scale were density-dependent. Reeve & Murdoch (1985), Smith & Maelzer (1986), Reeve (1987), Murdoch et al. (1995) reported that there were no density-dependent or aggregation relationships between *Aphytis melinus* and red scale. Reeve (1987) showed that under laboratory conditions, *Aphytis melinus* spent the same length of time for host-searching regardless of scale density. Murdoch (1994) and Murdoch et al. (1995) did not detect density dependency between red scale and *Aphytis melinus* and claimed that the mechanism for stabilisation of host-parasitoids lay in the parasitoid-host interaction itself, such as the size-structured interactions.

Murdoch et al. (1996) attributed rapid displacement of *Aphytis lingnanensis* by *Aphytis melinus* in inland valleys of California in part to the ability of *Aphytis melinus* to lay female eggs on smaller-size scales than that of *Aphytis lingnanensis* and, therefore, to produce more female progeny per unit area searched. They (Murdoch et al. 1996) noted that DeBach & Sundby (1963) reported that *Aphytis lingnanensis* required slightly more host meals per offspring than *Aphytis melinus*, thus raising the density of hosts needed to sustain *Aphytis lingnanensis* at equilibrium and, hence, to make it easier for *Aphytis melinus* to invade and out-compete *Aphytis lingnanensis*.

Ovarian development in *Aphytis* is synovigenic: females require proteinaceous nutrition for continuous ovigens. Rosen & DeBach (1978). Host scales are major sources of this protein which females acquire by ‘host-feeding’. This results in mutilation of the
scale body and scale mortality (Quednau 1964b, Rosen & DeBach 1978, Forster et al. 1995) that may exceed mortality caused by parasitism (Rosen & DeBach 1978). The scales develop large, brown, necrotic spots and die after several hours due to venom produced by the female wasp (Rosen & DeBach 1979, Forster et al. 1995). During host-feeding, females use their ovipositors to drill a hole through the scale cover and into the scale body. A highly viscous fluid exudes from the scale body as the female moves her ovipositor up and down within the scale body. When the female withdraws her ovipositor, a tiny straw-like feeding tube remains. She then turns around and inserts her mouthparts into the tube and feeds on the fluid. Abdelrahman (1974b) studied rates of mutilation by *Aphytis melinus* in the laboratory. The percentage of individuals mutilated were: first instar, 31.3%; first moult, 78.1%; second instar, 58.9%; third instar virgin females, 26.6%; prepupal male, 13.8%; pupal male, 9.4%. Second moult females, and third instar mated females were not mutilated. Walde et al. (1989) also reported higher levels of host-feeding on small, rather than large, scales. Heimpel et al. (1997) noted that nectar or honey influenced the longevity and fecundity of females *Aphytis*, and Zhao (1990) noted that when honey was provided, *Aphytis melinus* wasps lived longer and caused higher percentages of parasitism and host-feeding. Murdoch (1994) mentioned that *Aphytis melinus* is cannibalistic under laboratory conditions. Adult females were observed to stab and kill eggs previously laid in a scale. This behaviour was suspected to be potentially stabilising host-parasitoid relationship (Murdoch 1994).

*Aphytis chrysomphali* was originally described as *Aphelinus chrysomphali* Mercet in 1912 (Rosen & DeBach 1978). Quayle (1911a) incorrectly referred to it as *Aphenilus diaspidis* Howard (Compere 1961), a species originally described in 1881 and now known as *Aphytis diaspidis* (Howard) (Rosen & DeBach 1978), a name used along with *Aphelinus fuscipennis* Howard in early Australian literature for the same species (Wilson 1960). It was apparently introduced into Western Australia from China in November 1905 by George Compere (Wilson 1960), but some evidence suggests it was *Aphytis lingnanensis*, not *Aphytis chrysomphali*, that was introduced (Wilson 1960, Furness et al. 1983, Smith et al. 1997). However, *Aphytis chrysomphali* was introduced into New South Wales from Western Australia in 1925–1926, and it became established in the Gosford area and in inland districts (Hely 1968, Smith et al. 1997). It was still common in upper Murray River citrus orchards near Tocumwal (33° 51' S, 145°, 31' E, 114 m asl) in the early 1980s (Smith et al. 1997, Beattie, pers. comm., 2009). It was apparently present in Queensland in the early 1930s (Summerville 1934), but this
record, in which Summerville cited the species as *Aphelinus chrysomphali*, has not been verified. It was reintroduced into Victoria from California and New South Wales in 1954 (Smith et al. 1997), and was recorded in South Australia in the 1940s where it was well established in the 1960s and 1970s (Furness et al. 1983, Smith et al. 1997). The identity of the species recorded in South Australia was confirmed by Harold Compere, but there is no record of an official introduction to the state (Furness et al. 1983, Smith et al. 1997). If the species that was introduced by George Compere into Western Australia was *Aphytis lingnanensis*, then *Aphytis chrysomphali* must have been inadvertently introduced, presumably to Western Australia, before imports of citrus trees were prohibited by the Commonwealth Government in June 1915 (Hill 1918).

The host range of *Aphytis chrysomphali* includes yellow scale, dictyosperum scale (*Chrysomphalus dictyospermi* (Morgan)), circular black scale (*Chrysomphalus aonidum* (L.), syn *Chrysomphalus ficus* Ashmead), *Chrysomphalus* sp., coconut scale (*Aspidiotus destructor* Signoret), oleander scale (*Aspidiotus nerii* Bouché), *Pseudoaonidia trilobitiformis* (Green), cactus scale (*Diaspis echinocacti* (Bouché)), *Fulaspis* sp., rufous scale (*Selenaspidus articulatus* (Morgan)) and latania scale (*Hemiberlesia lataniae* (Signoret)) (Rosen & DeBach 1978, Malipatil et al. 2000).

*Aphytis chrysomphali* is both a solitary (Rosen & DeBach 1978) and gregarious (Abdelrahman 1974b, Luck et al. 1982) thelytokous (uniparental) species\(^{15}\); the male is absent altogether or extremely rare (Rosen & DeBach 1978). The adult wasp is lemon yellow, the thoracic sterna are dusky, the thoracic setae are slender and pale and the mandibles are brown. The forewings have a faint, dark cloud beneath the stigma and five rows of hairs basolateral of the speculum (Compere 1955). The antennae are slender with the club usually well over three times as long as wide. The pupa is entirely yellow with a distinctive, longitudinal, black line on the mesosternum (Rosen & DeBach 1979, Prinsloo 1984). The life cycle lasts 45, 22 and 14 d, respectively, at 15.6, 21 and 26.7°C. It is susceptible to extreme heat (all eggs die at 32°C), but less susceptible to extreme cold temperature than the respective stages of *Aphytis melinus* (Abdelrahman 1974a). The lower thermal threshold for development of *Aphytis chrysomphali* is 8.5°C (Abdelrahman 1974a). The optimum temperature for reproduction is 27°C (Rosen & DeBach 1979). Numbers of eggs/female were 25.1 at 25°C, compared to 21.3 at 20°C, and 6.6 at 30°C (Abdelrahman 1974a).

\(^{15}\) During preparation of this document I observed three pupae under the cover of an adult virgin red scale on an immature navel orange fruit collected from my Block 2 study site at Somersby on 18 February 2009.
*Aphytis lingnanensis* may have been, as noted above, introduced to Western Australia by George Compere in 1905 (Wilson 1960, Furness et al. 1983, Smith et al. 1997). It was introduced to Victoria from the University of California, Riverside by CJR Johnston in 1962, but it did not establish; it was introduced into California from Lingnan University\(^ {16} \), Guangzhou, Guangdong, by John Linsley Gressitt in November 1947 (Compere 1955, Rosen & DeBach 1979). It was present in Queensland before 1930 (Rosen & DeBach 1978). Specimens identified by Alexander Arsene Girault as *Aphytis chrysomphali* as early as 1930 were actually *Aphytis lingnanensis* (Rosen & DeBach 1978). DeBach collected *Aphytis lingnanensis* in Queensland in 1971 (Rosen & DeBach 1978), but there is no record of it being formally introduced, and Smith (1978) reported that it was the main parasitoid of red scale in Queensland. It may have been introduced from Western Australia as *Aphytis chrysomphali* in the 1920s (Wilson 1960) or before imports of citrus trees were prohibited in 1915 (Hill 1918). A commercial insectary for mass-producing *Aphytis lingnanensis* was established at Mundubbera in Queensland by Daniel Papacek in 1978 (Smith et al. 1997). It is now the predominant ectoparasitoid of red scale in Queensland (Smith 1978, Smith et al. 1997).

*Aphytis lingnanensis* attacks purple scale (*Lepidosaphes beckii* (Newman)), yellow scale, circular black scale, dictyosperum scale, latania scale, cactus scale, laurel scale (*Aonidia lauri* (Bouché)), rufous scale, coconut scale, oleander scale, Glover’s scale (*Lepidosaphes gloverii* (Packard)), and white louse (*Unaspis citri* (Comstock)) (Malipatil et al. 2000). It is a gregarious, biparental, arrhenotokous oriental species (Rosen & DeBach 1979, Luck et al. 1982). If two eggs are deposited during one host visit, the higher probability (79% for *Aphytis lingnanensis* and 93% for *Aphytis melinus*) would be that one would be male and other female (Luck et al. 1982).

The adult is yellow, with hyaline wings and a long propodeum. The head, wing pads and appendages are yellow to yellow-brown, and the dorsal surface is yellow (Rosen & DeBach 1978). The characteristic, dark, well-defined pigmentation on the mid-thoracic sterna and on the mid ventral abdominal plates of the pupal stage can be used to distinguish it from other *Aphytis* species on the same host (Rosen & DeBach 1978, Prinsloo 1984, Malipatil et al. 2000). Females start producing eggs within 2 to 6.5 h of emergence, and oviposition can occur daily until death (Fernando & Walter 1999).

\(^{16}\) Now the site of Zhongshan (Sun Yatsen) University.
Fernando & Walter (1999) also observed that *Aphytis lingnanensis* did not produce eggs at night due to insufficient light intensity. After each deposition, the adult female stands immobile while waiting for her next egg to mature. Up to 191 eggs, with maximum of 13 eggs per day, can be produced per female (Fernando & Walter 1999).

*Aphytis melinus* was first described in 1959 by Paul DeBach from specimens from red scale collected by GW Angale on rose (*Rosa* sp. [Rosales: Rosaceae]) in New Delhi (28° 38' N, 77° 13' E) and Gurgaon (28° 27' N, 77° 01' E) in India and Lahore (31° 32' N, 74° 20' E) in Pakistan in 1956 (DeBach 1959). It was introduced into Australia in 1961 from the University of California, Riverside by CJR Johnston (Furness et al. 1983, Malipatil et al. 2000). Establishment was achieved at Boundary Bend (34° 43' S, 143° 09' E) in Victoria in 1963 (Furness et al. 1983). Mass rearing was carried out at Mildura (34° 12' S, 142° 08') in the Sunraysia district of Victoria and New South Wales in 1967 and at Loxton in the Riverland district of South Australia in 1968. Widespread establishment was achieved in Sunraysia by MJ Byrne in 1968. Cultures in the Riverland were facilitated by Richardson, and establishment was achieved there in 1966 (Furness et al. 1983). Australia's first commercial insectary, Biological Services Inc., was established by Ron George at Loxton in 1971 to mass rear and release *Aphytis melinus* (Furness et al. 1983, George 1984). This resulted in establishment of *Aphytis melinus* in all Riverland orchards by about 1975. By 1983, the parasitoid was widely distributed in the Murray River citrus districts of Victoria, South Australia and New South Wales (Furness et al. 1983). It is now widely distributed in the citrus districts of Victoria, South Australia, Western Australia and inland New South Wales citrus districts, and in Alice Springs in the Northern Territory (Smith et al. 1997).

*Aphytis melinus* attacks both red scale and yellow scale. This species also attacks yellow scale, dictyospernum scale, oleander scale, latania scale, coconut scale, walnut scale (*Quadraspidiotus juglansregiae* (Comstock)), San José scale (*Diaspidiotus perniciosus* (Comstock)) and cactus scale (Rosen & DeBach 1978, 1979).

The green-eye pupa has dark pigmentation on the midthoracic sterna, the abdomen is clear yellowish ventrally, and the head, wing pads and appendages are usually yellow, and the dorsal surface yellow. These are the distinct morphological characteristics that distinguish it from the pupae of all other known species (Rosen & DeBach 1979).
It is a gregarious, biparental, arrhenotokous species; males are commonly found (DeBach 1959, Rosen & DeBach 1979). Fertilised eggs give rise to females, unfertilized eggs only to males (Rosen & DeBach 1979). However, fertilised females lay eggs that produce both males and females, which suggests that only some of their eggs are fertilised (Abdelrahman 1974b). Abdelrahman (1974b) also observed that the large, old, females of *Aphytis melinus* produced only males at the end of their lives, and those females mated only once. The degree of gregariousness depends on host size and quality (Abdelrahman 1974b, Luck et al. 1982). The larger the host, the greater the likelihood that several eggs will be deposited (Luck et al. 1982). Quednau (1964b) recorded a maximum of 9 eggs per host and observed that when more than one egg was laid on a host, female eggs were laid before male eggs. Parasitoid progeny in the same host scale develop synchronically (Abdelrahman 1974b).

The life cycle of *Aphytis melinus* on oleander scale on lemon fruit takes 12–13 d at 26.7°C and 50% RH. Each oviposition requires about 12 min, and females produce an average of 24 progeny, laying an average of 2.8 eggs per scale (DeBach & Sundby 1963, Rosen & DeBach 1978, 1979). In a test involving oleander scale and 30 female *Aphytis melinus*, DeBach & Sundby (1963) reported that the highest progeny production per wasp was 55, and the lowest 5. On red scale, the fecundity of *Aphytis melinus* is relatively high, averaging 51.2 progeny at 20°C, 67.4 at 25°C, and 55.5 at 30°C. Each female destroys an average of 54.2, 61.6 and 45 host scale by oviposition, and 41.5, 50.6 and 35.2 host scales by mutilation and host feeding, at 20, 25 and 30°C, respectively (Rosen & DeBach 1979).

Host identification in *Aphytis melinus* is mediated by a non-volatile kairomone, O-caffeoyltyrosine, in scale covers. Variation in concentrations of this kairomone in scale covers is thought to contribute to differences in levels of parasitism that occur on substrates such as fruit, leaf and bark (Hare & Morgan 2000). Variation in scale size on different substrates within a citrus tree (the largest scales occur on fruits, smallest on wood, and intermediate sized scales on leaves) may also contribute to the differences in parasitism rate (Luck & Podoler 1985). Murdoch et al. (1989) observed in ‘unmanipulated’ grapefruit trees in a grove near Fillmore in California that the fraction parasitised by *Aphytis melinus* was markedly and significantly greater on the exterior substrates than on wood. This pattern was consistent in all three vulnerable stages of scale (second instar females, virgin females, and second instar males). The parasitism
rate in the exterior (twigs) exceeded that in the interior by 6-fold (in virgin females) to 27-fold (in second instar). Low parasitism rates in the interior may have been caused by the parasitoid’s response to the bark substrate.

Abdelrahman (1974b) observed the partitioning behaviour of *Aphytis melinus*. Females sometimes partition egg clutches into two hosts, particularly when host density is high. For example, Abdelrahman (1974b) observed a female that attended a large host for 6 min, left it and went to a neighbouring small host for 4.5 min, and then returned to the first host for 4.5 min. The result was two male and two female progeny in the first host, and one male and one female in the second.

The lower thermal threshold for development of *Aphytis melinus* and *Aphytis chrysomphali* is 11°C (Abdelrahman 1974b). DeBach & Sisojevic (1960) who mentioned that reproduction for *Aphytis chrysomphali* was best at low temperatures and poorest at high temperatures, whereas it was vice-versa for *Aphytis lingnanensis*. DeBach & Argyriou (1966) noted that at 15.6°C, *Aphytis melinus* and *Aphytis lingnanensis* are quite sluggish and inactive, and that average fecundity between 15.6 to 20°C ranged from 11 to 17 progeny per female, compared to 28.2 at 26.7°C. Abdelrahman (1974a) mentioned that all stages of *Aphytis chrysomphali* were more tolerant to extreme cold and less tolerant to extreme heat than the respective stages of *Aphytis melinus*. The LD$_{50}$ values for *Aphytis chrysomphali* in extreme heat were 38.17, 47.98, 46.28 and 40.16°C for adult, pupal, larval and egg stages, respectively. The respective figures for *Aphytis melinus* were 38.74, 48.45, 47.23 and 42.52°C. Extreme temperatures had more severe impacts on parasitoids than on red scale (Abdelrahman 1974a). Abdelrahman (1974a) reported that, in laboratory studies, *Aphytis chrysomphali* was more effective at low temperatures than *Aphytis melinus*; LD$_{50}$ temperatures for survival in extreme cold conditions for *Aphytis chrysomphali* were -2.34, -2.88, 1.21 and -2.64 °C for adult, larva, pupa and egg stages, respectively and for *Aphytis melinus* were -1.10, -2.47, 1.58 and -0.53°C. DeBach et al. (1955) reported that temperatures from -1 to 10°C had adverse impacts on *Aphytis lingnanensis*, particularly to adult stage. Effects of cold temperatures on *Aphytis lingnanensis* populations varied with years and areas. Mortality closed to 100% in winter in inland areas (DeBach et al. 1955). They (DeBach et al. 1955) also reported that low humidity also affected adult stage more than immature stages which are normally protected. The longevity of adults at 20% RH was less than one-third as long
as at 80% RH (DeBach et al. 1955). Moreover, combination of high temperatures and low humidities produced extreme effects: longevity at 32°C and 20% RH was only one-twentieth of that at 21°C and 80% RH (DeBach et al. 1955).

Similar results were also reported by Sorribas et al. (2010), who concluded that parasitism by Aphytis chrysomphali in Spain increased from south (warmer conditions) to north (cooler conditions) and peaked during the colder months of the year. In contrast to the tolerance of Aphytis chrysomphali of low temperatures, both Aphytis menilus and Aphytis lingnanensis are more susceptible to low temperatures. According to DeBach & Sisojevic (1960) and DeBach & Argyriou (1966), low temperatures adversely affect the gender ratios and progeny production of Aphytis lingnanensis and Aphytis melinus: more so for Aphytis lingnanensis (Kfir & Luck 1979). Exposure of Aphytis lingnanensis adults to -1°C for 8 h or more would cause 100% mortality of sperm in males and in female spermathecae (DeBach & Rao 1968). Even though both Aphytis lingnanensis and Aphytis melinus were more effective than Aphytis chrysomphali at high temperatures (DeBach & Sisojevic 1960, Abdelrahman 1974b), Aphytis melinus can be superior to Aphytis lingnanensis at high temperatures. Podoler (1981) reported that a temperature regime of 29 to 35°C had a negative impact on responses of Aphytis lingnanensis and positive impacts on Aphytis melinus.

Abdelrahman (1974b) stated that Aphytis chrysomphali is more adapted to cold and less to heat than Aphytis melinus, and considered this as the explanation for differences for seasonal and annual fluctuations in their relative abundance in southern Australia, where he considered them to complement each other in controlling red scale. He stated that the data he presented suggested that Aphytis species in Australia may have evolved into more efficient control agents of red scale than elsewhere. In California, coastal regions were more favourable for Aphytis species than interior regions. Low temperatures adversely affect Aphytis development, especially during the adult and pupal stages. Dry conditions and high temperatures reduce adult longevity of Aphytis lingnanensis (DeBach et al. 1955).

2.1.8.1.2. Endoparasitoids: Encarsia perniciosi, Encarsia citrina, Comperiella bifasciata and indigenous species

Endoparasitoids tend to be koinobionts that allow their host to continue to develop after parasitisation, have long development times, short adult lifespans, high fecundity, lay
small eggs, emerge with many eggs matured, and have narrow host ranges. Koinobionts tend to have ‘slow’ developing larvae and ‘fast’ (short-lived) adults (Traynor & Mayhew 2005).

Species of *Encarsia* Förster are found worldwide. It is the largest genus within the Aphelinidae, with a total of 343 described species (Heraty et al. 2008). Many species have a wide or even cosmopolitan distribution, complicating taxonomic revisions on a local scale. However, three-quarters of the species seem to be restricted in their distribution to Australia, indicating a high level of endemism (Schmidt & Polaszek 2007). Species richness of *Encarsia* in Australia is highest in Queensland with 65 species (70%), followed by Western Australia with 31 species (33%) and New South Wales with 15 species (16%) (Schmidt & Polaszek 2007). The number of species occurring in Australia may be two to three times higher than currently known (Schmidt & Polaszek 2007). They are solitary.

The genus *Comperiella* comprises 9 species: 6 from Southeast Asia and Australia and 3 from southern Africa (Prinsloo 1996). They are economically-important, primary endoparasitoids of armoured scale insects, and the genus has been extensively studied since the description of the type species, *Comperiella bifasciata* from China, by Leland Ossian Howard in 1906 (Howard 1906). It is now a well-known and important natural enemy of red scale, throughout the world (Prinsloo 1996).

*Encarsia perniciosi* (= *Encarsia aurantii argentina* (De Santis), *Encarsia pernicioci* (Tower), *Prospaltella aurantii argentina* De Santis, *Prospaltella perniciosi* Tower) is a member of the *Encarsia-aurantii* group and parasitises scale insects and whiteflies (Forster et al. 1995, Schmidt & Polaszek 2007, Heraty et al. 2008). It was described as *Prospaltella perniciosi* by Daniel G Tower in 1913 (Tower 1913). Adults emerge from mummified second moult, third instar virgin female, and occasionally mated third instar female red scale (Forster et al. 1995). It belongs to the *Encarsia-aurantii*–’group’ species of which are parasitoids of armoured scales and whiteflies (Heraty et al. 2008). Schmidt & Polaszek (2007) examined of specimens from Kulnura in New South Wales in February 1967 by David Paul Annecke (one female) and by Geoffrey J Snowball (12 females) in *Aonidiella aurantii* and *Aonidiella citri* (possibly *Aonidiella citrina*) on citrus, and specimens from Queensland collected in 1998 at Palmwoods (26° 41' S, 153° 00' E) by Daniel Smith (one female) and Mundubbera (25° 35' S, 151° 17' E) by Daniel Papacek (one female).
Encarsia perniciosi has been recorded from Aonidiella aurantii, Aonidiella sp., San José scale, poplar scale (Diaspidiotus gigas (Thiem and Gerneck)), Lepidosaphes pallidula (Williams), Lepidosaphes ulmi (L.), Parlatoria acalcarata Maskell, Pseudaulacaspis pentagona (Targioni Tozzetti) and Unaspis citri (Universal Chalcidoidea Database\textsuperscript{17}, ScaleNet\textsuperscript{18}, Schmidt & Polaszek 2007).

Three morphologically indistinguishable forms of Encarsia perniciosi have been recognised (Rosen & DeBach 1978, Stouthamer & Luck 1991). A thelytokous\textsuperscript{19} form, possibly native to China, parasitises red scale (Flanders 1950), and thelytokous and arrhenotokous\textsuperscript{20} forms parasitise San José scale (Stouthamer & Luck 1991). The arrhenotokous strain is a heteronomous hyperparasitoid: males develop as secondary parasitoids, either on their own species, usually a female, or that of some other species (Stouthamer & Luck 1991).

The adult female is 0.61 mm long and the male 0.56 mm (Tower 1913). The egg is oval (0.04 mm wide and 0.085 mm long) with a micropyle at the smaller end. The chorion is smooth and hyaline, and the opaque granules with which the egg is filled show through (Tower 1914). Potential fecundity has been estimated as 120 to 130 eggs per female, but actual fecundity has been variously reported as 35 to 90 eggs per female, and may differ with strain with arrhenotokous strains having higher fecundity than thelytokous strains (Rosen & DeBach 1978). If more than one egg is deposited in a single red scale, only one parasitoid emerges (Rosen & DeBach 1978). The egg is usually located medially in the scale, slightly towards the pygidium. There are two larval stages (Tower 1914).

Tower (1914) observed development of the arrhenotokous San José scale strain of Encarsia perniciosi in San José scale. The first instar larva of the parasitoid initially feeds on the blood and smaller fat globules, avoiding the vital parts, as the growth of the second instar scale is not arrested until the first instar parasitoid reaches maturity. At this point it apparently attacks the vital parts of the scale, thus interfering with its normal functions and preventing the second moult of the scale from taking place. The

\textsuperscript{17} http://www.nhm.ac.uk/jdsml/research-curation/research/projects/chalcidoideas/details.ds: two synonyms, Aspidiotus perniciosus and Quadraspidiotus perniciosus, of Diaspidiotus perniciosus, and two synonyms, Insulaspis pallidula and Lepidosaphes pallida, of Lepidosaphes pallidula are listed as hosts (associates). See ScaleNet (http://www.sel.usda.gov/SCALENET/SCALENET.HTM) for details.

\textsuperscript{18} http://www.sel.usda.gov/catalogs/diaspidi/Lepidosaphespallidula.htm.

\textsuperscript{19} Parthenogenesis, in which females are produced from unfertilised eggs.

\textsuperscript{20} Unfertilised eggs develop into haploid males, and fertilised eggs develop into diploid females.
scale body becomes swollen and distended, and turns from its normal lemon yellow to a light orange. The first moult of the parasitoid usually takes place at approximately the same time that the scale body becomes light orange. No waste is produced by the first instar.

Feeding by the second instar results in destruction of the internal organs of the host and ends with the inside of the scale body scraped clean. Waste accumulated in the stomach during the life of the two larval instars is passed and usually deposited along the lateral margins of the skin of the scale body or at the ends. The larva, following the passing of its waste, is usually found lying on its back with its head at the head end of the swollen skin of its host, which has dried and become a mummified case in which the parasitoid pupates. At the end of its development, the pupa becomes nearly black.

Both male and female parasites emerge from the empty skins of second-stage and early third-stage female scales, but by far the largest number emerge from second-stage scales. Prior to eclosion, the larval skin is kicked off by the tarsi of the emerging adult. The adult emerges by making a hole in the mummified scale body through which the head is thrust before it pulls and pushes itself out of the host. After eclosion the adult walks a few steps, cleans itself, and straightens its wings. The insect spends some time in this way and then starts crawling about and is ready for copulation (Tower 1914).

Flanders (1953) reported that the thelytokous strain of Encarsia perniciosi oviposits in all nymphal stages of its host. He observed that the life cycle took 18 to 21 d at 27°C when eggs were laid in hosts in the first half of their development, and 26 to 28 d when eggs were laid in host in the last half of their development.

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In southern California, Encarsia perniciosi has almost two generations per red scale generation (Murdoch et al. 1989, Yu et al. 1990). Murdoch et al. (1989) and Yu et al. (1990) reported that levels of parasitism in the exterior parts on mature grapefruit trees in this region were twice that on interior wood. Murdoch et al. (1996) found no convincing evidence that parasitism by Encarsia perniciosi was density-dependent. Yu et al. (1990) reported that development of Encarsia perniciosi was most rapid when it parasitised an early second instar red scale and slowest when it parasitised the mated third instar female scale. However, on early second instar scale it was about 80% as fecund as a wasp that emerged from a mature female scale. They (Yu et al. 1990) also reported that Encarsia perniciosi:
- did not distinguish between unparasitised hosts and those previously parasitised by *Aphytis melinus*;
- it was always outcompeted by *Aphytis* when both species parasitised the same host;
- it preferred scale on stems whereas *Aphytis* prefers scale on leaves and fruits; and that
- partitioning of the scale resource by the two species explains why they coexist in coastal southern California, but it did not explain why *Encarsia perniciosi* disappeared from citrus groves in the inland valleys coincident with the introduction of *Aphytis melinus* into southern California.

DeBach & Sundby (1963) observed that, in the laboratory, females searched for scale as soon as they came into contact with red scale infested lemon fruit, and found scales within a few minutes. After examination, pre-ovipositional probing usually occurred near the centre of the scale cover. The time spent in penetrating the scale cover (< 1 min to 15 min) depended on the age of the host: the older the scale, the longer the interval. However, not all attacks resulted in oviposition. Sometimes, the host was abandoned after a few seconds of probing. Oviposition in male and younger scales usually took 2 to 3 min, whereas oviposition in mature females usually took 5 to 10 min. After oviposition, the female sometimes returned to the same recently attacked scale. No evidence of host feeding was observed by the authors.

One egg was usually deposited in each host but, under high parasitoid densities, more eggs are laid. As many as 3 eggs and 6 larvae were found in a host, but only one parasitoid per host completed development. Supernumerary eggs may have hatched and the excess larvae may have died shortly afterwards (DeBach & Sundby 1963, Rosen & DeBach 1978). In their study, DeBach & Sundby (1963) observed that at 27°C and 50% RH, the larval period lasted 10 to 11 d, and that the pupal period lasted 4 to 5 d. After oviposition, the host scale showed no sign of paralysis and continued to grow, turning yellow after 7 d. The edge of the scale body then turned brown and the whole scale became darker, except for a lighter zone in the centre, where the larva was located. The total development of *Encarsia perniciosi* varied depending on the age and condition of the host at oviposition. The shortest developmental period, 19 d, took place in the male scale. The average was about 20 d in optimum-size hosts. About half of the parasitoids developed in male scales. Of these, more than half emerged 20 to 22
d after oviposition. Most of the parasitoids from third instar virgin female and third instar mated female scales emerged 22 to 24 d after oviposition. *Encarsia perniciosi* seems to be able to oviposit throughout its life. Fecundity, based on progeny that reached the pupal stages averaged 46, ranging from 13 to 77. When the third instar scales (25 d or older) were used the number of progeny decreased.


articulatus (Morgan), Silvestraspis sp., and Tsugaspiodiotus tsugae (Marlatt) (Universal Chalcidoidea Database).  

The red scale race of Encarsia citrina was apparently introduced unsuccessfully to California from China in 1900 and then successfully from the same region in 1948 (Flanders 1950, Compere 1961). It was reported from South Africa in 1902 (Flanders 1950, Compere 1961). Evidence suggests that Encarsia citrina was the first parasitoid species of red scale to be introduced to Australia, to Western Australia, in 1902. In one report, Anon (1906) noted ‘It is pleasing to know that we have the assurance of our orchard inspectors that the red scale parasite has thoroughly established itself in this State, and can be seen on infested trees in and around Perth, where it is multiplying with great rapidity. This is a chalcid fly that was found in China, and is an internal parasite. An enlarged illustration of this insect will be found on p. 573 of the Handbook of Horticulture and Viticulture published by this Department’. The parasitoid illustrated on page 573 of the handbook (Despeissis 1903a) is unmistakeably Encarsia citrina: the illustration is reproduced in Fig. 2.9. George Compere (Fig. 2.10) wrote in Despeissis (1903a) that ‘During a recent visit of the writer to China, only a few scattering specimens of the red scale was to be found; these were sent to this State and from them a few specimens of the parasite were bred and liberated, but if it should happen that these few were all females or all males, then no results may be looked for.’ Compere was not aware that Encarsia citrina is uniparental, thereby enhancing prospects for successful establishment. It seems, however, that the introduction was unsuccessful, as Jean Marie Adrian Despeissis reported (Despeissis 1903b) that, with respect to shipment of parasites by George Compere from Hong Kong, ‘The red scale parasites, of which very few specimens were sent, did not come out at all, and I doubt whether we will breed any out of the specimens sent.’ Wilson (1960) mentioned the introduction of ‘Parasite sp.’ from southern China in 1902 but did not comment on whether it established.

**Figure 2.9.** The ‘internal parasite’ introduced to Western Australia for control of red scale by George Compere in 1902 (from Despeissis 1903a).

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22 [http://www.nhm.ac.uk/jdsml/research-curation/research/projects/chalcidoideas/details.ds](http://www.nhm.ac.uk/jdsml/research-curation/research/projects/chalcidoideas/details.ds)
Records suggest that *E. citrina* was present in Queensland before 1910. Alexandre Arsène Girault (Girault 1913) recorded it as *Aspidiotiphagus citrinus* Craw, from a female specimen captured from the foliage of an imported citron growing near jungle at Babinda (17° 19' S, 145° 57' E) in northern Queensland. He also collected a number of specimens from *'Diaspis and Chionospis'* on orange at Nelson near Cairns (16° 57' S, 145° 45' E) in July/August 1912. The species of *'Chionospis'* (*Chinoaspis*) may have been *Unaspis citri*. There are no other records of *Diaspis* on citrus in Australia.

Girault (1913) also described *Aspidiotiphagus australiensis* Girault, collected at Nelson, as a new species. Norman Scott Noble (Department of Agriculture, New South Wales) reared *Aspidiotiphagus australiensis* from batches of red scale infested leaves, twigs and fruit collected from the Sydney Botanic Gardens between June 1931 and October 1933 (Compere 1961). In a report to William Butler Gurney (Department of Agriculture, New South Wales), Noble wrote: ‘As far as I can determine *Aspidiotiphagus australiensis* Gir. appears to be identical with *Aspidiotiphagus citrinus* Craw.’ and ‘In view of the confusion regarding the various scales, slide mounts were made of the red scale from which I bred As. *australiensis* abundantly. These were a very deep brownish red and the pygidium was typical of red scale.’ The report was included with a letter that Gurney wrote to Harold Compere in June 1934 (Flanders 1950; Compere 1961). Flanders (1950) and Compere (1961)
questioned Girault’s identifications. Hayat (1989) resolved the uncertainty in 1989 when he made *Aspidiotiphagus australiensis* a synonym of *Encarsia citrina*. There is no record of *Encarsia citrina* being introduced intentionally from overseas or from Western Australia to Queensland or New South Wales. It may have been present in New South Wales before 1900 (Smith et al. 1997).

Little is known about its biology as a parasitoid of red scale, and its biology as a parasitoid of other armoured scales is difficult to ascertain. Reports by Taylor (1935) and Chumakova (1965) appear to be related to a species of *Pteroptrix* (=*Casca*), possibly *Pteroptrix parvipennis* (Gahan), parasitising coconut scale. In these reports, the parasitoid, referred to as ‘*Aspidiotiphagus citrina*’, appears to be a gregarious endoparasitoid. Flanders (1966) commented on this in relation to Taylor (1935), and concluded that some of the observations reported by Taylor applied to ‘*Casca parvipennis*’, not ‘*Aspidiotiphagus citrina*’.

DeBach & Argyriou (1967) wrote ‘*Aspidiotiphagus citrinus* attacks many species of armored scales and is often an abundant parasite at high scale population densities, but we know of no observations or records claiming that it is effective in regulating populations of armored scale insects at low levels. It was the only parasite reared from *Lepidosaphes beckii*.’

It contrast, Soares et al. (1997) concluded, on the basis of observation spanning two years in two citrus orchards on São Miguel Island (Azores) (37° 47’ N, 25° 30’ W), that *Encarsia citrina* contributed considerably to the biological control of *Unaspis citri* and *Lepidosaphes beckii*, lowering the proportion of larvae and males in the populations of both species. The highest flight activity and oviposition occurred in spring and summer with parasitism on *Unaspis citri* lower in summer and relatively high in the remaining seasons. Summer and spring were seasons of high parasitic activity in *Lepidosaphes beckii*. Parasitism and abundance of *Encarsia citrina* depended on the presence of susceptible stages for parasitism, and on the abiotic conditions (mainly temperature).

Matadha et al. (2004) studied its development on San José scale at constant temperatures of 15, 17.5, 20, 25, 27.5 and 30°C at 60–70% RH and a photoperiod of 16:8 (L:D). Larval development did not occur at 15° and 30°C. Total developmental time varied from 22.7 d at 27.5° to 47.4 d at 17.5°C. The lower development thresholds for larval, pupal, and egg to adult periods were 10.4, 6.3 and 9.0°C, respectively, and estimated complete development required 434.8°D based on the development thresholds
for each stage. The average longevity of adults ranged from 34.3 d at 15° to 8.0 d at 30°C. The average fecundity of females ranged from 96.3 eggs at 20°C to 40 eggs at 17.5°C. Life table parameters were also determined at four constant temperatures (17.5–27.5°C). Intrinsic rate of increase values for Encarsia citrina ranged from 0.074 at 17.5° to 0.176 at 27.5°C. The highest net reproductive rate of 93.7 offspring/female was estimated at 20°C. The mean generation time ranged from 49.3 d at 17.5° to 23.4 d at 27.5°C.

Studies have been undertaken to determine density dependency between Encarsia citrina and its hosts. Outcomes of those studies vary. Flanders (1971) considered ectoparasitoids to be density-dependent and endoparasitoids to be inversely density-dependent. McClure (1977) reported that Encarsia citrina was density-dependent to elongate hemlock scale, Fiorinia externa Ferris [Diaspididae], on Eastern hemlock, Tsuga canadensis (L.) Carrière [Pinales: Pinaceae], in Connecticut in the United States of America. Matadha et al. (2004) reported that Encarsia citrina was positively dependent on the density of scale Unaspis euonymi. Rebek et al. (2006) reported that Encarsia citrina was inversely density-dependent on on some occasions but usually density-independent to euonymus scale, Unaspis euonymus (Comstock) [Hemiptera: Diaspididae] on potted Euonymus fortunei (Turcz.) var. coloratus [Celastrales: Celastraceae].

Comperiella bifasciata (= Habrolepistia cerapterocera Mercet and Habrolepistia eugeniae Risbec) is a solitary endoparasitoid of diaspine scales. It was described by Howard (1906) from the specimen reared by George Compere on red scale (Smith HS 1942) and has been recorded from 6 species of Aonidiella, 5 species of Aspidiotus and Aspidiotus sp., 4 species of Chrysomphalus and Chrysomphalus sp., Clavaspis sp., Diaspidiotus gigas, Diaspis echinocacti (Bouché), Dynaspis abietis (Schrank), 2 species of Hemiberlesia, Lindingaspis fusca McKenzie, Morganella longispina (Morgan), Pseudaulacaspis pentagona (Targioni-Tozzetti) and Pseudaulacaspis sp. (Universal Chalcidoidea Database23). Some records for Comperiella bifasciata on Aonidiella orientalis in India relate to Comperiella lemniscata Compere & Annecke, which does not parasitise red scale (Compere 1961, Hayat 1977).

23 http://www.nhm.ac.uk/dsmi/research- curation/research/projects/chalcidoideas/details.ds: some of the listed host names (‘associates’) are invalid according to ScaleNet (http://www.sel.barc.usda.gov/scalenet/scalenet.htm), with synonyms of five listed valid species (Aonidiella aurantii (Maskell), Chrysomphalus aonidum (L), Diaspidiotus gigas (Thiem & Gerneck), Dynaspis abietis (Schrank), Hemiberlesia rapax (Comstock), also listed, and the name for San José scale given as Quadraspidiotus perniciosus (Comstock) and not as the valid name Diaspidiotus perniciosus (Comstock).
Biparental arrhenotokous strains of *Comperiella bifasciata* parasitise red scale, yellow scale and presumably most other hosts (Compere 1961, Rosen & DeBach 1978). Though solitary, it prefers to lay multiple-egg clusters from which only one offspring per host emerges (Blumberg & Luck 1990, Ode & Rosenheim 1998). A uniparental and deuterotokous or ampherotokus strain attacks circular black scale in South Africa (Cilliers 1971, Rosen & DeBach 1978). The adults are about 1.5 mm long and shiny black with greenish-golden reflections on the mesoscutum, and bluish metallic reflections on the mesoscutellum. Both sexes have two characteristic dark stripes on the forewings. The head has a central longitudinal occipital band that extends to pronotum, with a yellowish longitudinal band either side. The antennae are uniformly dark brown, nearly black, somewhat lighter at the tip of the club (Howard 1906, Compere 1926).

The strains that parasitise red scale and yellow scale differ in the ability of their eggs to hatch when deposited in the haemolymph of red scale (Flanders 1943b). The red scale strain can develop freely on both red scale and yellow scale, but the yellow scale strain, obtained by Curtis Paul Clausen from Asiatic red scale (*Aonidiella taxus* Leonardi) in Japan, and introduced to California in 1916-1917, does not develop on red scale (Compere 1961, Rosen & DeBach 1978). The eggs die (Compere & Smith 1927, Smith 1942, Flanders 1943b, Rosen & DeBach 1978). Host preference and suitability of the two strains is related to a single gene pair (DeBach & Rosen 1976). The introduction of the strain from Japan resulted in successful control of yellow scale in California (Smith 1942, Flanders 1944a), but delayed successful control of red scale because it was thought that *Comperiella bifasciata* was not an effective parasitoid of red scale (Rosen & DeBach 1978).

Jenkins (1946) reported the introduction of presumably the yellow scale strain to Western Australia from Japan by Compere in 1909 for control of red scale, but it failed to establish. There is no record to indicate whether yellow scale was present in Western Australia at the time, but information provided by Jenkins (1946) suggests that it was not.

The red scale strain of *Comperiella bifasciata* is assumed to be native to China. It was formally introduced into California from China in 1941 after four attempts over almost 40 years (Smith HS 1942, Flanders 1943a, DeBach 1948). It was introduced to

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24 *A. taxus* has been recorded on twigs and leaves of evergreen species belonging to the genera *Cephalotaxus* [Pinales: Cephalotaxaceae], *Podocarpus* [Pinales: Podocarpaceae] and *Taxus* [Pinales: Taxaceae] (Watson GW. Diaspididae: [http://nlbif.eti.uva.nl/bis/diaspididae.php?menuentry=soorten&id=86]).

Hely et al. (1982) reported that liberations of *Comperiella bifasciata* were made in the Murrumbidgee Irrigation Areas in the 1940s, but noted that it only became common there 20 years later. Smith et al. (1997) commented that *Comperiella bifasciata* may have been inadvertently introduced to Australia before the 1940s. This view stemmed from erroneous conclusions (Beattie, pers. comm., 2009) reached by Sands & Snowball (1980) about observations by Stanley Elsworth Flanders. Flanders, while at Marmor in Queensland during a visit from the University of California, Riverside, to Australia in 1931, reared *Comperiella bifasciata* from what he thought was yellow scale on the desert lime, *Citrus (=Eremocitrus) glauca*, a species of *Citrus* indigenous to Australia (Flanders 1934). The scale was subsequently described as *Aonidiella eremocitri* (McKenzie 1937). Sands & Snowball (1980) concluded that Flanders mistook yellow scale for circular black scale, and that the parasitoid was *Comperiella pia* (Girault). Such an error by Flanders is regarded by Beattie (pers. comm., 2008) as improbable given his knowledge, and it appears that Sands & Snowball (1980) were not aware that Flanders’ observations were related to *Aonidiella eremocitri*. In a report of a survey of natural enemies of diaspidid scales of citrus in eastern Australia, Snowball & Sands (1971) mentioned that ‘in the 1971 survey in Queensland, numerous *C. bifasciata* were reared from circular black scale’ and that ‘*C. bifasciata* was in fact recorded from *Chrysomphalus* sp. on *Eremocitrus glauca* in Queensland in 1930, before the importation of the red scale race, so the two races appear to have a different host range.’ In the same report, Snowball & Sands (1971) reported that ‘in the vicinity of Sydney, some eggs and larvae of the wasp *Comperiella bifasciata* inside red scales, *Aonidiella aurantii*, are killed by becoming encapsulated by host tissue, and similar encapsulation has been recorded by workers in South Australia. This phenomenon is virtually unknown in California, whence the red scale race of *C. bifasciata* was originally imported into Australia, so that there seems to be a different relationship between the parasite and the red scale in the two countries.’ There are no records for *Aonidiella citrina* in Queensland.
Flanders (1934) recorded eight diaspine species, seven in addition to Aonidiella eremocitri, on Citrus glauca. Their identities are not known and it is not known if Comperiella bifasciata evolved with Aonidiella eremocitri or whether it was introduced to Australia with another host. There is no record of the red scale strain parasitising Aonidiella eremocitri, or of the Aonidiella eremocitri strain parasitising red scale or yellow scale. In addition to Citrus glauca, Aonidiella eremocitri has been recorded on Barringtonia sp. [Ericales: Lecythidaceae] in Fiji, Cocos nucifera L. [Arecales: Arecaceae] in Papua New Guinea and Solomon Island, Cocos nucifera and Citrus in Vanuatu, Malaysia and the United States of America (Williams & Watson 1988). The Australian Faunal Directory lists 10 species of diaspine scales that have been recorded on Citrus glauca: Aonidiella aurantii (Maskell), Aonidiella eremocitri McKenzie, Clavaspis subfervens (Green), Lepidosaphes pallida (Green), Parlatoria fulleri Morrison, Poliaspis exocarpi Maskell, Poliaspis syringae Laing, and Remotaspidiotus reconditus Brimblecombe.

Tritrophic interactions between red scale, its hosts and Comperiella bifasciata have, in addition to the impact of host-specific strains, influenced successful biological control of red scale by the parasitoid. Successful introduction of Comperiella bifasciata to California for control of red scale was delayed by use of sago palm (Cycas revoluta Thunb. [Cycadales: Cycadaceae]) as a host for transporting and rearing the parasitoid in lieu of citrus, due to quarantine restrictions to prevent spread of citrus canker (Xanthomonas citri subsp. citri) (Schaad et al. 2006) [Pseudomonadales: Pseudomonadaceae]). It was several years before it was realised that the red scale strain of Comperiella bifasciata develops on red scale on citrus trees, but not on sago palm (Flanders 1942b, Smith HS 1942, Smith 1957). Host plants also indirectly influence the size, longevity, sex ratio, and mortality of Comperiella bifasciata (Smith 1957). Smith (1957) observed that Comperiella bifasciata that developed on red scale on yucca ‘Yucca filipendula’ [Aspargaceae: Agavaceae], were larger, greater longevity and more prolific than on citrus trees and sago palm.

Adult Comperiella bifasciata females parasitise all stages of red scale except second moult males, male pupae and adult males. A female is capable of ovipositing the day she emerges from the host. Females assess the suitability of potential hosts by tapping

26 Possibly Yucca recurvifolia Salisb.: there appears to be no other record of Yucca filipendula.
scale covers with their antennae. If the host is suitable for oviposition, the female *Comperiella bifasciata* probes the scale body with her ovipositor and selects the proper position. The host scale is not paralysed by the female wasp as she lays her eggs, usually one per host, by inserting her ovipositor into the scale body through the scale cover. Each female lays 50 eggs on average (Flanders 1944a). Flanders (1944a) reported 136 offspring per female when second instar scales were exposed for parasitism over an interval of 20 d. The eggs are white, twice as long as wide, and attached to the derm of the scale body by an anterior stalk in the form of slender collapsed tube. Second instar and third instar virgin female hosts are preferred for oviposition: Richardson (1978) reported that 28% of eggs are laid in second instar hosts and 42% in third instar virgin hosts. The larvae consume the fluid content of scale body as they pass through 5 instars.

Based on observations at summer temperatures in a laboratory at the University of California, Riverside, Compere & Smith (1927) observed that the larva voids the contents of the alimentary canal on about the ninth or tenth day of hatching. The meconium is brownish yellow and the pellets are pressed on either side parallel to the longitudinal axis of the larva, and voiding of meconium probably occurs about a day or so prior to the final larval moult. Each larva usually completes its development in a third instar mated female host (rarely in third instar virgin females) (Richardson 1978, Forster et al. 1995). The mummified body of the host becomes dry and brittle, and usually only one larva will complete its development if two eggs are laid in the scale body (Compere & Smith 1927, Forster et al. 1995).

Development the red scale strain of *Comperiella bifasciata* on red scale takes 20 to 40 d at 26.7°C. At this temperature, red scale reaches maturity in about 38 d (Flanders 1944a). Development of the parasitoid is most rapid when the host is in the early third instar when it is parasitised, and slowest when the host is in the early first instar. In the minimum life cycle of 21 d at 26.7°C, eggs hatch in 4.5 d, the first instar duration is 3 d, the second, third and fourth instars together take 3 d, the fifth instar takes 4 d27, and the pupa 7 d. According to Flanders (1944a), the duration of the first instar during the maximum duration life cycle at 26.7°C may be 15 d or more. The second, third, and fourth instars are very similar; there is the usual increase in body size, with the head of each successive instar proportionately smaller, the mandibles successively larger, and a

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27 This suggests that the average temperature in the laboratory in which Compere & Smith (1927) made their observations on the development of *Comperiella bifasciata* was about 26.7°C.
corresponding development of the tracheal system, so that in the fourth instar larvae possess nine pair of spiracles. Up to the fourth instar, the number of moults can be easily determined by the number of cast mandibles found in the skins which adhere to the larva (Compere & Smith 1927). The pupa is initially whitish or slightly yellow and opaque before turning to black (Compere & Smith 1927, Forster et al. 1995). The parasitoid emerges from its host through an exit hole it chews in the scale cover, near the nipple, over 1 to 2 h (Azim 1961, Heraty & Schauff 1998). The life cycle stages of Comperiella bifasciata are illustrated in Fig. 2.11.

Figure 2.11. Eggs, larval stages, mandibles of instars, head of mature larva, and oviposting female Comperiella bifasciata, as illustrated in Compere & Smith (1927).

*Comperiella bifasciata* eggs may be encapsulated within the host scale body. Encapsulation is a defence process of insects against endoparasitoids and other foreign organisms. In the process of encapsulation of *Comperiella bifasciata*, the blood cells of haemocytes of red scale collect and adhere to the surface of invading object forming a dense covering, which is called a capsule, around the parasitoids or foreign body (Rosen 1990, Blumberg 1997). Sands & van Driesche (2004) stated that ‘there are two biotypes of *Comperiella bifasciata* Howard, each adapted to parasitize only one of two closely related scales. The yellow scale biotype of *C. bifasciata* successfully parasitizes yellow scale, *Aonidiella citrina* (Coquillet), but it does not develop on red scale, *Aonidiella aurantii* (Maskell). The yellow scale biotype will oviposit in red scales, but many of the parasitoid eggs and some larvae become encapsulated, and no parasitoids develop (Brewer, 1971). By contrast, the red scale biotype of *C. bifasciata* successfully parasitizes up to 80% of adult females of *A. aurantii* (Smith et al., 1997).’ Brewer (1971) observed 59% to 85% encapsulation of immature stages of *Comperiella bifasciata* in mature red scale females in laboratory studies. His study was based on field-collected parasitoids in what he
considered to be the red scale strain of *Comperiella bifasciata*. The percentage of encapsulated parasitoids second instar scales was twice as high as in mature scales (Brewer 1971). The encapsulation rates of *Comperiella bifasciata* in yellow scale are significantly lower than that in red scale (Brewer 1971). Blumberg & Luck (1990) observed that only 28% of solitary eggs of red scale strain *Comperiella bifasciata* were encapsulated in studies in California. The difference in the encapsulation rates between the red scale strain of *Comperiella bifasciata* in two locations was unlikely due to the so-called California strain and Australian strain since *Comperiella bifasciata* was introduced into Australia from California. Levels of encapsulation reported by Brewer (1971) have never been observed in New South Wales in studies spanning more than 30 years: levels were rare to low, less than 5% (Beattie, pers. comm., 2009). This, and the study reported by Blumberg & Luck (1990), suggests that Brewer (1971) may have inadvertently used the yellow scale strain of *Comperiella bifasciata* in his studies. Which species was observed by Snowball & Sands (1971) is uncertain, but it would seem, given the absence of any record for *Aonidiella citrina* in Queensland (see above), that the observations were based solely on *Aonidiella aurantii*.

Blumberg & Luck (1990) observed that *Comperiella bifasciata* prefers to lay multiple-egg clusters so as to increase the probability that one offspring will avoid the host's encapsulation response and complete its development successfully. Ode & Rosenheim (1998) observed that if *Comperiella bifasciata* females produce more than one egg per host, the sex allocation was female biased. The heavy production of all-female clutches suggested that females benefit more from developing in multiple egg clutches than do males. They presented clear evidence that this is the case (Ode & Rosenheim 1998): female offspring were 3.1 times more likely to emerge when clutch size was two as compared to when clutch size was one; males were only 1.9 times as likely to develop successfully in two-egg clutches compared to single-egg clutches. They concluded that such allocation of male and female eggs facilitates the transition from solitary to gregarious development (Ode & Rosenheim 1998).

DeBach & Sundby (1963) mentioned that *Comperiella bifasciata* was better adapted to interior or intermediate climatic areas in California (see Fig. 2.7). Forster et al. (1995) mentioned that it was was common mainly in arid regions of California such as in the Central Valley (not shown in Fig. 2.7) and inland southern California. Flanders (1971) considered endoparasitoids to be inversely density dependent. Atkinson (1977) reported
that *Comperiella bifasciata* peaks at Tshaneni (25° 59' S, 31° 43' E) in Swaziland (see Fig. 2.8) coincided with adult female scale peaks and, and in contrast to Flanders (1971) considered the parasitoid to be density independent.

**Indigenous primary endoparasitoids.** Six indigenous, primary endoparasitoids, *Coccophagus scutellaris* (Dalman), *Coccophagus gurneyi* Compere, *Encarsia aurantii* (Howard) and *Encarsia brimblecombei* (Girault) from the *Encarsia-aurantii* group, *Encarsia lounsburyi* (Berlese & Paoli) from the *Encarsia-citrina* group, and *Encarsia iris* (Girault) from the *Encarsia-opulenta* group, have been recorded in Australia (Summerville 1934, Wilson 1960, Waterhouse & Sands 2001, Schmidt & Polaszek 2007). Host records listed by Heraty et al. (2008) suggest that *Encarsia aurantii* may parasitise red scale and, although they mention no hosts of *Encarsia iris*, comments by Summerville (1934) suggest that it may also parasitise the scale. *Coccophagus gurneyi* is a parasitoid of pseudococcids and soft brown scale (*Coccus hesperidum* L.) (Ebeling 1959), and *Coccophagus scutellaris* is a parasitoid of soft brown scale, black scale (*Saissetia oleae* (Olivier) [Hemiptera: Coccidae]) and citricola scale (*Coccus pseudomagnoliarum* (Kuwana)) (Ebeling 1950, Wilson 1960, Hely et al. 1982).

Molecular studies involving red scale parasitoids have recently been undertaken, most to confirm the validity of morphological classifications, and the evolution of the species (Dowton & Austin 1994, Babcook et al. 2001, Gillespia et al. 2005). Most phylogenetic studies have been based on the 28S ribosomal RNA, internal transcribed spacer region and mitochondrial cytochrome oxydase subunit I gene.

Interest has also focused on the bacterial endosymbionts of the parasitoids, *Wolbachia* (α-Proteobacteria) and ‘*Candidatus* Cardinium’ species (Sphingobacteria). The latter are intracellular endosymbionts known to influence the mode of reproduction in some parasitoids and mites, and parthenogenesis of oleander scale (Zchori-Fein et al. 2001, Zchori-Fein & Perlman 2004, Gruwell & Normark 2009). The putative species associated with species of *Encarsia* is ‘*Candidatus* Cardinium hertigii’ (Zchori-Fein et al. 2001) that was originally referred to as the ‘*Encarsia* bacterium’. It was initially detected in populations of *Encarsia* species, including parthenogenetic *Encarsia citrina* and *Encarsia perniciosi* parasitising armoured scales. *Wolbachia* has been widely studied and is known to cause cytoplasmic incompatibility, parthenogenesis and feminisation of wide range of arthropods (Zhou et al. 1998). Gottlieb et al. (1998) detected *Wolbachia* in all parthenogenetic species or forms of *Aphytis: Aphytis chilensis*
Howard, *Aphytis chrysomphali*, *Aphytis diaspidis* and *Aphytis lingnanensis*. Vasquez et al. (2011) discovered that *Wolbachia* induced cytoplasmic incompatibility in *Aphytis melinus*. They (Vasquez et al. 2011) reported that *Wolbachia* interfered with the fitness of biological control costs as *Wolbachia*-infected host had shorter longevity and lower fecundity.

2.1.8.2. Predators

The major predators of red scale in Australia are the larvae and adults of ladybirds [Coleoptera: Coccinellidae] and lacewings [Neuroptera: Chrysopidae & Hemerobiidae]. The most important ladybirds are the scale-eating ladybird (*Rhyzobius lophanthae* (Blaisdell)), the steelblue ladybird (*Halimus chalybeus* (Boisduval)), chilocorus (*Chilocorus circumdatus* Gyllenhal) and the orange-spotted ladybird (*Orcus australasiae* (Boisduval)) (Hely et al. 1982, Smith et al. 1997). The most important lacewings are the brown lacewing (*Micromus tasmaniae* (Walker) and green lacewings (*Mallada* sp.) (Hely et al. 1982, Smith et al. 1997). Other predators include the prostigmatid mite, *Eupalopsis jamesi* Gerson (Acari: Prostigmata), the phytoseiid mites *Euseius elinae* (Schicha) and *Euseius victoriensis* (Womersley) [Acari: Phytoseiidae] (Smith et al. 1997), other *Euseius* species (Muma 1971, Nelson 1973, Schausberger 1998, Juan-Blasco et al. 2008) and some predatory caterpillars e.g., *Batrachedra arenosella* (Walker) [Lepidoptera: Batrachedridae] (Hely et al. 1982, Smith et al. 1995). The scale cover of *Aonidiella aurantii* exhibits a physical defense against natural enemies (Beardsley & Gonzalez 1975). It is complemented by the sclerotised integument of the gravid and parturient scale body and by a ventral sheath beneath the body that strongly attaches the body to the substrate (Dickson 1951).

2.1.8.2.1. Coccinellids

*Rhyzobius lophanthae* is native to Australia but was first described in California as *Scymnus lophanthae* by Frank Ellsworth Blaisdell in 1892. Blaisdell wrote ‘My attention was first attracted to this very interesting species by Wm. Vortriede, gardener in charge of the parks at Coronado.’ Mr Vortriede had observed them feeding upon San José scale infesting *Acacia lophantha* Willd. [Fabales: Leguminosae], a synonym of *Paraserianthes lophantha* (Willd.) IC Nielsen. Blaisdell (1892) noted that ‘The scale was exceedingly abundant, as was also the beetle in all stages of its development.’ Synonyms, in addition to

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28 Coronado is near San Diego.
Scymnus lophanthae, include Lindorus lophanthae (Blaisdell) and Rhizobius toowoomba Blackburn (Blackburn 1892, Ślipiński 2007). It is a generalist predator and an important natural enemy of most armoured scale species, including red scale, yellow scale, oleander scale and San José scale, and soft scales including black scale, soft brown scale and citricola scale (Ebeling 1959, Rosen 1990, Smith et al. 1997). It is a common predator of red scale (Flanders 1930, Ebeling 1951, Smith et al. 1997) may have been introduced to California by Koebele in 1888–89 (Compere 1961, Rosen & DeBach 1978), or unintentionally before 1892 (Issac 1906, Compere 1961, Rosen & DeBach 1978). However, according to Rosen & DeBach (1979), it was not able to suppress Aonidiella aurantii populations successfully in California following its introduction in 1889. It was introduced to South Africa in 1900, Italy in 1908, and has since been introduced intentionally, or otherwise, to many other countries (Greathead 1973).

Albert Koebele found Rhizobius lophanthae preying on red scale and other armoured scales introduced to Australia in the Sydney region in 1888 (Compere 1961, Rosen & DeBach 1978), not 1898 as cited by Honda & Luck (1995). It was more common, according to Albert Koebele, near Toowoomba (27° 33’ S, 151° 57’ E) in Queensland than near Sydney (Compere 1961). Based on his observations of Rhizobius hirtellus and Rhizobius satelles Blackburn as predators of red scale in the Sydney region Koebele estimated that Rhizobius species had six broods per year and that, in the presence of an unlimited supply of food in an orchard, 15,000,000,000 beetles could be breed in one season under favourable conditions. In cages outdoors in Athens (37° 58’ N, 23° 46’ E) in Greece, Statthas (2000b) recorded five complete overlapping generations per year from late spring to mid autumn (May to October) and a sixth partial overlapping generation during late winter and early spring (February and March), and noted that adults of the fourth and fifth generation that survived winter conditions gave rise to the first generation of the following year. Females were reproductively active throughout the year, and no diapause was observed (Statthas 2000b). Koebele (1892) commented that Rhizobius were much faster in breeding than ‘Orcus chalybeus’ and ‘O. australasiae’ and found Rhizobius larvae, although not numerous at the time, in midwinter.

29 Halmus chalybeus and Orcus australasiae.
Koebele (1892) observed other species of *Rhyzobius* feeding on red scale. These were *Rhyzobius caecus* Blackburn (= *Rhyzobius caecus* Blackburn), *Rhyzobius debilis* Blackburn, *Rhyzobius hirtellus* Crotch (= *Rhyzobius ruficollis* Blackburn), *Rhyzobius plebeius* Blackburn, *Rhyzobius satelles* Blackburn (= *Scymnus minusculus* Korschefsky), and *Rhyzobius ventralis* (Erichson) (= *Scymnus ventralis* Erichson and *Scymnus restitutor* Sharp) (Compere 1961).

Quayle (1911b) said of *Rhyzobius lophanthae* 'This Coccinelid is probably the commonest and most abundant one feeding upon the red scale. While it is not restricted in its feeding to this scale exclusively it has been found more often associated with it than any of the other scales, unless it be the purple. It has been called the "Purple Scale Rhizobius" but this name is no more justifiable than "Red Scale Rhizobius." In fact, the latter would be an appropriate common name just as the "Black Scale Rhizobius" would be similarly appropriate for *Rhizobius ventralis*. Where both the red and black scales occur in the same orchard, or even on the same tree, *ventralis* will be found with the black and *lophanthae* with the red.' Quayle (1938) noted it also attacks yellow scale, greedy scale (*Hemiberlesia rapax* (Comstock)) and *Aspidiotus nerii*.

Atkinson (1977) reported that *Rhyzobius lophanthae* was the most common predator of red scale in Tshaneni in Swaziland in 1972–1975. He claimed that the type of predator damage probably reflected mostly *Rhyzobius lophanthae* because other predators levered-up scale covers. Samways (1985) reported that *Rhyzobius lophanthae* fed on various stages from first instar to virgin female scale, but not on mated females. He (Samways 1985) found that *Rhyzobius lophanthae* and red scale were density dependent and recorded up to 88% young scale eaten by *Rhyzobius lophanthae* in an orchard at Komatipoort (25° 25’ S, 31° 56’ E) in South Africa.

*Rhyzobius lophanthae* is considered to be the most important predator of red scale in the inland area citrus growing regions of southern Australia (Wilson 1960, Hely et al. 1982). It develops well in harsh, hot conditions and is unconcerned with dust deposits. It occurs commonly in the autumn. It colonises heavily infested trees very effectively and can reduce a gross infestation rapidly under heavy infestation of scale, but it was not effective against low-density scale populations (Hely 1968, Hely et al. 1982). James et al. (1999) recorded 134 adult *Rhyzobius lophanthae* in studies at Leeton, New South Wales, in which samples of motile arthropods were taken by tapping each of 4 major branches within canopies of 16 mature Valencia orange trees monthly (excluding

The adult of *Rhyzobius lophanthae* is convex-oval shaped and range between 2 to 3 mm in length. It has metallic black elytra etched with greyish or light-brown hairs and a brown prothorax (Ebeling 1959). The mandible is bifid apically, and resembles the mandibles of aphid-feeding coccinellids. The bifid tip is used for piercing and sucking while feeding (Samways 1997, Ślipiński 2007). This mandible type appears to be unsuitable for preying on gravid red, and other armoured, scales (Honda & Luck 1995). The outer surface of the adult mandible is simple, strongly curved, and concave at the base. The sickle-shaped medial surface forms an incisor at a bifid apex. The wide base of the mandible has acanthae and 2 condyles. The basal tooth is positioned on the medial surface of the molar region. Ventrally, a membranous prostheca extends between the incisor and molar area with a continuous row of bristles, which are probably used for grooming or straining food (Hodek 1973). Some species have a mandibular groove which is used for introducing mesenteronic juices into the victim and then sucking up the digested liquids (Samways et al. 1997).

Honda & Luck (1995) observed that *Rhyzobius lophanthae* develops and reproduces readily on second instar male and female, and third instar female *Aonidiella aurantii*, and from the second instar to all further stages of *Aspidiotus nerii*. Morphologically, the mandibles of *Rhyzobius lophanthae* are similar to those of the aphid-feeding coccinellids, which appear ill suited to prey on gravid *Aonidiella aurantii* or other scale species with hardened covers. The adult beetle requires long periods to penetrate the hard scale cover. Once having penetrated the cover, it is then faced with the sclerotised scale body, which further impedes its feeding. However, *Rhyzobius lophanthae* mandibles appear capable of penetrating the smaller, softer covers of immature stages of both scale species and the covers of the gravid and parturient stages of *Aspidiotus nerii* lacking cementing material (Honda & Luck 1995). It can feed on the gravid *Aonidiella aurantii* either by chewing through the cover and penetrating the sclerotised integument or, alternatively, by separating the scale from its substrate and feeding on the softer ventral surface of the body. However, these processes are time consuming and failure rates are high. Furthermore, the larva of *Rhyzobius lophanthae* usually failed to survive
when offered only gravid *Aonidiella aurantii* as food (Honda & Luck 1995). Honda & Luck (1995) reported that the second instar male *Aonidiella aurantii* was particularly attractive to the adult beetles as, at that stage, the scale became thinly covered and loosely attached to the fruit surface. The adult does not feed on the first instar scale, but the early larval stages do so. The late larval stages prefer more advanced scale stages.

Honda & Luck (1995) noted that differences in feeding damage observed on the scale covers of *Aonidiella aurantii* and *Aspidiotus nerii* appeared to be related with differences in scale cover morphology. They observed that feeding damage on a gravid *Aonidiella aurantii* cover was characteristically limited to a small area on the cover margin peripheral to the exuviae. The central section containing the exuviae remained undamaged: the larval and adult beetles could not penetrate this central section because their mandibles slid off when they attempted to bite. Moreover, once the beetles penetrated the cover margin they were confronted with a sclerotised scale body strongly attached to the host plant substrate. Given the difficulty of penetrating the sclerotised scale body, the predator often quit attempts to feed on the scale, and resumed searching. In contrast, the entire cover of gravid *Aspidiotus nerii* with its soft, unsclerotised body could sustain damage and was easily shredded, torn, and consumed by the predator (Honda & Luck 1995). Hodek (1973) reported that food consumption was greatest during ovipositing period, less in non-ovipositing females and least in males.

Sorribas & Garcia-Marí (2010) observed both adults and immature stages of *Rhyzobius lophanthae* preying on almost all life cycle stages of red scale. *Rhyzobius lophanthae* adults and larvae were able to break scale covers with mandibles and feed on scale body, leaving irregular holes on the covers. The larvae often took refuge under the calyx of the fruit and were observed preying on *Aphytis* pupae (sometimes eating only the head) (Sorribas & Garcia-Marí 2010).

Stathas (2000a) studied the prey consumption of *Rhyzobius lophanthae* on larvae and on virgin adults, and the fecundity on mated females of *Aspidiotus nerii*. He recorded that for the development of first instar larvae of *Rhyzobius lophanthae* at 25°C, 1.2 adult female *Aspidiotus nerii* were consumed; for the development of the second, third and forth instar larvae, prey consumption was 2.7, 7.5 and 24.6 adult female *Aspidiotus nerii*. *Rhyzobius lophanthae* male and female adults consumed in their lifetime 390.6 and 672.3 adult female *Aspidiotus nerii*, respectively. Stathas (2001) concluded that *Aonidiella aurantii* did not seem to be a suitable prey for *Rhyzobius lophanthae*.  

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Flanders (1930) observed that when black scale eggs were glued to fruit, third and fourth instar *Rhyzobius lophanthae* larvae seemed to prefer them to red scale. However, although the beetle exhibited a fondness for black scale eggs, it could be reared successfully on them only after the second ecdysis. Quayle (1911a) observed that one or two eggs are deposited beneath the cover of the host scale, and that the eggs are small, oval, and white with iridescence. Flanders (1930) reported that oviposition started on the third day after emergence and could last for 20 d, that maximum daily egg deposition for one female was 25, and noted that eggs hatch in 5–6 days. Flanders (1930) observed that the instinct of the adult female was to insert its eggs in crevices, as illustrated by the fact that it occasionally filled the space between the abdomen and elytra of dead adults with a mass of eggs. Stathas (2001) noted that the egg is elliptic with a narrowing front edge, and that its colour varies depending on the host species on which the ovipositing females feed. The female reared on *Aspidiotus nerii* produced yellow eggs, whereas the eggs laid in nature by females which had consumed chaff scale (*Parlatoria pergandei* Comstock) were rose-coloured (Stathas 2001). Stathas (2000a) found that 98.3% eggs were laid under the scale cover of *Aspidiotus nerii*, some others were found on the surface of scale cover. More than one-third of the eggs which were deposited under the scale covers, were found in groups of three; the percentage of eggs found in groups of 2, 4, 1 and 5 decreased progressively. All the eggs on the upper surface of *Aspidiotus nerii* scale covers were laid singly (Stathas 2001).

In contrast to Flanders (1930), who reported an average of 144 eggs per female when female *Rhyzobius lophanthae* were allowed on black scale eggs, Stathas et al. (2005) found that, at 25°C, females laid about 633.7 eggs when feeding on *Aspidiotus nerii*: daily fecundity ranged from 18 to 25 eggs. Average longevity in this study was 63.4 d for mated adult females and 119.4 d for unmated females. Stathas (2000a) cited other studies, one (Sezer 1969) in which *Rhyzobius lophanthae* females reared and fed on circular black scale laid 600–700 eggs in their lifetimes, with a maximum of 1200, and another (Rubstov 1952) that reported that females fed on diaspidid scales had an average fecundity of 500 eggs, with a maximum of 800 or more.

There are four larval instars. The body colour of larvae after each ecdysis changes from a dark grey to light yellow. The young larvae usually seek a crevice such as under a loose fruit button in which to undergo ecdysis. The newly emerged last stage larva is approximately 3 mm in length. A characteristic mark on the larva is a yellow dorsal
streak due to the colour of the middle third of each of the first four abdominal segments (Flanders 1930). The larva consists of a strongly sclerotised head, 3 less sclerotised thoracic segments. Larvae of all instars and pupa have the same number and distribution of spiracles. There are two parallel longitudinal lines of 9 spiracles along lateral each side of the body of larvae and pupa (Stathas 2001). Newly-hatched larvae placed on black scale eggs suffered a heavy mortality and the length of the first instar was three times that of those feeding on red scale. Moreover, all of the second stage larvae died with an abundance of black scale present (Flanders 1930). Quayle (1938) observed that larvae feed on the scale on which the eggs from which they hatched were laid and attacked many other scales before reaching maturity. The larva eats out an irregular hole, rectangular usually, in the scale covering and most commonly just beyond the insect which is lying beneath (Quayle 1911a, 1938). The adult also feeds on the scale (Quayle 1938).

Two to three days prior to pupation, the larva ties itself up after it attaches its terminal abdominal segment to the plant surface. The pupa is broad, oval with a narrow end. Like the egg, the colour of pupae depends on the body colour of the host scale larvae. Pupae of larvae fed on *Aspidiotus nerii* were yellow, and rose when fed on *Parlatoria pergandei* (Stathas 2001).

The development of *Rhyzobius lophanthae* fed on *Aspidiotus nerii* at the different temperatures on controlled laboratory conditions was reported by Stathas (2000b). The life cycle from egg to oviposition lasted 78.7, 43.6, 32.1 and 23.9 d at 15, 20, 25 and 30ºC, respectively. Average adult longevity at these temperatures was 257.6, 171.4, 121.3 and 88.5 d, respectively. In another study undertaken at 25ºC and 65% RH, Stathas (2001) reported that total developmental period from egg to adult was 27.1 d, and no mortality was observed (0%) when *Aspidiotus nerii* was used as prey. The respective numbers when the prey was *Aonidiella aurantii* were 48.8 d and 84% (Stathas 2001). Stathas (2000b) reported the low temperature threshold of *Rhyzobius lophanthae* immature life stages ranged from 7.6 to 9.3ºC, and the thermal constant for the development of *Rhyzobius lophanthae* from egg to adult on *Aspidiotus nerii* was 443.5ºD. On circular black scale at 30ºC, the duration of the life cycle reported by Stathas (2002) was 27.2 compared to 86.6 d at 15ºC, and the thermal constant for the completion of the life cycle was 588ºD. For the immature stages of the predator
feeding on the scale, the highest scale mortality was recorded for pupae and the lowest for first instars (Stathas 2002).

_Halmus chalybeus_ was described as _Coccinella chalybea_ by Jean Baptiste Boisduval in 1835 from specimens in Australia (Ślipiński 2007). It is widely distributed naturally along the east coast of Australia from Victoria, to New South Wales and northern Queensland. It was introduced to Western Australia from New South Wales in 1902 (Wilson 1960). It is now widely distributed in Australia and has been introduced to New Zealand, California and Hawaii (Ślipiński 2007). The beetle is a well known and common predator of scale insects, including the armoured scales, red scale, yellow scale, circular black scale and purple scale, the soft scales, black scale, green coffee scale (_Coccus viridis_ (Green)) and _Pulvinaria_ spp., the wax scales, _Ceroplastes destructor_ (Newstead)\(^30\), _Ceroplastes sinensis_ del Guercio and _Ceroplastes rubens_ (Maskell), and fluted scale _Icerya_ spp. [Hemiptera: Margarodidae], (Quayle 1911a, Hely et al 1982, Beattie et al. 1991, Smith et al. 1997, Lo & Chapman 2001, Ślipiński 2007), and mites, including citrus red mite _Panonychus citri_ (McGregor) [Acarina: Tetranychidae] (Jamieson et al. 2005). Flynn (1995) listed 21 host species of _Halmus chalybeus_ including Hemiptera (16 species), Coleoptera (1 species), Lepidoptera (1 species), Acari (2 species) and Hymenoptera (1 species). Among those, Flynn (1995) regarded red scale, San José scale, latania scale, oleander scale, black scale, _Clenochiton piperis_ Maskell [Hemiptera: Coccidae] and _Pittosporum_ psyllids [Hemiptera: Triozidae] as essential prey. Froggatt (1902) wrote: ‘Coquillet and Koebele, reporting upon the species that had been introduced in California from Australia and placed in the orchards in the summer 1892, in one place found that where 540 specimens had been liberated it was hard to find 100 two years later. Coquillet said that those placed on plants infested with red scale had done well, but others among olive scale (_Lecanium oleae_ ) had all died out or gone away.’ Koebele (1892) did not regard black scale as a host of _Halmus chalybeus_. He said ‘I have never found this insect feeding upon _Lecanium_ in Australia and did not expect it would feed upon _L. oleæ_.’ At this point black scale was known as _Lecanium oleae_, not _Saissetia oleae_.

On his second historic visit to Australia in search of natural enemies for biological control, Albert Koebele (Koebele 1892) wrote ‘red scale ….. in fact is at present the most injurious to citrus trees in Australia; but its progress is checked by its natural enemies. Australia is in possession of more than enough natural enemies to keep this coccid in check with ease, although nearly all these are preyed upon by parasites. To spray or fumigate to kill the red scale would also mean the

\(^{30}\) Cited as _Gascardia destructor_ (Newstead) in some publications.
destruction of the numerous beneficial insects, and those that were not killed outright would mostly leave the orchard in search of other food, and the consequence would be that in a few months the trees would again become infested, with but few enemies present, and the scales would do great damage unless the spray is again applied.‘ During this visit to Australia, Koebele (Fig. 2.12) observed ‘O. chalybeus and O. australasia’ in very large numbers and noted that ‘in regard to the two species of Orcus, they were found, if my observations in the field are correct, to be two-brooded in Australia, the mature insects hibernating during winter.’ and ‘On a day when the temperature reached above 100°F. in the shade in Australia, the number of Orcus chalybeus upon each orange tree could be estimated, as all the beetles came down on the stems near the ground, which was a beautiful sight for an enthusiastic bug-hunter, and from 175 to 300 beetles were collected on each stem; but the larvae of the same upon the trees were probably ten times as many. It should be understood that these trees are never sprayed.’ Hely (1968) and Hely et al. (1982) mentioned that under hot and dry conditions, the beetles congregate around the base of tree trunks, or leave orchards for more salubrious situations on creek banks or in thick vegetation. It was one of the predators (Fig. 2.13) that Koebele introduced from Australia to California in 1888 (Koebele 1892).

![Albert Koebele](image)

**Figure 2.12.** Albert Koebele.
Figure 2.13. Beneficial insects collected in Australia by Albert Koebele in 1888, including *Halmus chalybeus* and *Orcus australasiae* (Koebele 1892).

*Halmus chalybeus* adults are 3–4 mm long, broadly oval and convex. There is a considerable colour variation on dorsal surfaces, from dark bluish to almost green, with interspaces between punctures mostly polished, but sometimes showing some degree of cuticular reticulation. The dorsal surface of the male is metallic bluish to almost green except for most of the head and anterior corners of pronotum, which are yellowish. The head in males is always bicoloured with upper surface darker but the extent of darkness varying from upper 1/5 to half the length of the head. The female is similar to male but monochromatic (Ślipiński 2007). The head is transverse, and the eyes are not emarginated. The antennae have 7 segments. The clypeus is expanded laterally, and the labrum is entirely exposed and transverse. The mandible is unidentate, which is an adaptation for lifting the scale cover by cutting it open in a tin-opener type of way (Samways et al. 1997, Ślipiński 2007). The mandibles are rather delicate, the outer margins strongly curved, apices acute. The larvae are 3–5 mm long, yellow-grey, nearly cylindrical, and broadly fusiform. The larval head capsule is transverse, the antennae are very short, with one segment (Hely et al. 1982, Ślipiński 2007).
Tryon (1889) recorded the beetle feeding on scale insects in Queensland. Froggatt (1902) reported that it was recorded from Queensland, New South Wales, Victoria and Tasmania. Crichton (1893a) mentioned that it was exceedingly voracious in New South Wales and Victoria, and Summerville (1934) noted that it was the most useful ladybird predator of red scale in Queensland. Froggatt (1902) described it as a beautiful little ladybird. He considered this one of States most useful scale-devouring species because it attacked so many scales and was also very common. Froggatt (1902) noted that ‘it is the commonest species in this group in our citrus orchards, where, especially neglected ones, by shaking over a sheet, one can soon collect a few hundred anywhere near in the early parts of the summer.’

Prey recorded by (Froggatt 1902) included red scale and white louse scale. Froggatt (1902) also mentioned that it in its native state it destroyed many indigenous species. *Halmus chalybeus* is a common and useful predator in New South Wales coastal orchards in late spring and early summer, and overwinters as an adult, but inland areas of the State are unfavourable due to its susceptibility to hot and dry conditions (Hely 1968, Hely 1982). Beattie et al. (1991) observed spring and autumn peaks, and winter and summer troughs, in adult populations in mature orange orchards at Somersby, New South Wales. Hely (1968) reported that it is very allergic to inert dusts and deposits of Bordeaux sprays.

Lo (2000) reported that adults were active throughout the year at Kerikeri (35° 13’ S, 173° 56’ E) and Whangarei (35° 43’ S, 174° 19’ E) in Northland, New Zealand, while larvae were most abundant in early summer when they comprised the majority of populations. Lo & Chapman (2001) observed small clusters of inactive adults sheltering in orchards during winter at Kerikeri and Whangarei. They noted that Flynn (1995) had shown that this dormancy was not a true diapause, but facultative oligopause (delayed direct response to deteriorating conditions), because activity can resume immediately once temperatures rise (Lo & Chapman 2001).

Charanasri & Nishida (1975) observed that adults were more abundant at the beginning of the year than later. They recorded up to about 70 adults/tree on unsprayed in 3 to 4 m high and frangipani (*Plumeria obtusa* L. [Gentianales: Apocynaceae]) trees at Oahu (21° 28’ N, 157° 58’ W) in the tropical Hawaiian Islands. Meteorological data suggests that the lowest populations occurred during the warmest and driest months.

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31 At this point the orchards in New South Wales were predominantly located in coastal districts.
Flynn (1995) reported that the number of D (at 6.9°C) for Halmus chalybeus to develop from egg to adult were 49.4 (645), 36.9 (667) and 28.2 (651) at 20, 25 and 30°C, respectively, and recorded two annual generations of the beetle in Auckland (36° 45' S, 174° 46' E) in New Zealand in 1993–1994. He reported that, in Auckland, dormancy of Halmus chalybeus was not true diapause as feeding activity still occurred. Females developed their reproductive functions rapidly in spring (Flynn 1995). Eggs occurred in early November, larvae in December and January (mid-summer). Peaks of newly emerged adults occurred in January and March/April. Lo & Chapman (2001) reported that adults and larvae preyed on first instar and second instar Ceroplastes destructor and Ceroplastes sinensis: rarely, if ever, on third instars. During many hours of observations the predator was never observed preying on adult Ceroplastes sinensis (Lo 1995). Lo & Chapman (2001) reported that when Halmus chalybeus adults and larvae were allowed to prey on settled first and second instar of the wax scales on bagged branches, each beetle consumed an average 15.6 Ceroplastes destructor and 13.3 Ceroplastes sinensis per day, and larvae consumed 9.7 Ceroplastes destructor per day.

Orcus australasiae was described as Coccinella australasiae by Boisduval in 1835. Synonyms include Parapriasus australasiae (Boisduval), Coccinella australasiae (Boisduval) and Orcus australasiae var. quadrinotatus Lea (Ślipiński 2007). It is commonly known as the orange-spotted ladybird and is native to eastern Australia. It was introduced to Western Australia to control red scale (Wilson 1960) and into California for control of scale insects by Koebele (Koebele 1892, Isaac 1906).

Crichton (1893a) reported that Orcus australasiae was one of the most common useful insects in New South Wales and Victoria. Hely et al. (1982) said it was common in coastal areas of New South Wales, and sometimes common under mild wet conditions in inland regions of the state. Summerville (1934) said that Orcus australasiae accomplished very little in the way of effective reduction in numbers, even when many were working. However, it was one of three species mentioned in 1922 as being plentiful and doing ‘much good work’ (Compere 1961) and Koebele (1892) recorded that Orcus australasiae, Rhyzobius hirtellus and other species were always present in large numbers in association with black scale in New South Wales.

The adult is 5 mm long, broadly oval, convex, hemispherical. Its dorsum is glabrous, the elytra are dark blue with yellow blotches. The head is transverse and is exposed dorsally, and the eyes are distinctly emarginated (Ślipiński 2007). The male is similarly
marked, but it is smaller (Isaac 1906). The antennae are short, with nine segments (Ślipiński 2007). It has unidentate mandibles (Ślipiński 2007). The mandible is stout, with the apex short and acute, and outer margin subangulate at middle length. The terminal segment of maxillary palp is subsecuriform, the apex oblique, and lacinia with a patch of three or four short and stout bristles, in addition to the row of long setae. Terminal segment of labial palp is cylindroacuminate, rounded at apex. The prosternal lobe is moderate, convex, margined laterally, the bead prolonged part way around the coxal cavity (Chapin 1965). The abdomen has six visible sternites with the fifth broadly truncate in male and five with the fifth broadly rounded in female. The legs are moderately stout, tibiae are slightly widened toward apices, tibia I with a row of small denticles along outer margin, tarsal claw stout, with a subquadrate, translucent tooth in basal half (Chapin 1965).

The larva is 6–8 mm long, nearly cylindrical, broadly fusiform (Hely et al. 1982, Ślipiński 2007). The dorsal and lateral surface is armed with setose seti. It is creamy grey in the dorsal surface with partially or entirely dark brown to black in legs, dorsal plates and most dorsal setose (Ślipiński 2007). Adults and larvae feed on various scale insects (Ślipiński 2007). It is a more general feeder than Halmus chalybeus (Isaac 1906). Its prey include red scale, San José scale, circular black scale, softbrown scale, citricola scale, white wax scale, black scale (Isaac 1906, Wilson 1960, Waterhouse & Sands 2001) and woolly apple aphid (Eriosoma lanigerum (Hausmann) [Hemiptera: Aphididae]) (Asante 1995).

Little is known about the biology and ecology of Orcus australasiae. Issac (1906) observed that it prefers sunshine and is more numerous in the top and the outside parts trees. Asante (1995) observed that an adult could consume relatively larger numbers of early instars than older instars of woolly apple aphid. James et al. (1999) recorded 120 adult Orcus australasiae in studies at Leeton, New South Wales, in which samples of motile arthropods were taken by tapping each of 4 major branches within canopies of 16 mature Valencia orange trees monthly (excluding May and June 1995) from September 1994 to August 1996 on each tree for 22 months between 1994 and 1996.

Serangium maculigerum Blackburn (= Serangium bicolor Blackburn) [Coleoptera: Coccinellidae] is another native Australian coccinellid. Swezey (1925) reported that it feeds on diaspine scales. Beattie et al. (1991) noted, on the basis of personal communication with Jennifer Merciana Elizabeth Anderson, that Serangium bicolor was
a facultative predator of mites and scale insects. Ślipiński (2007) stated that all Serangium species are probably predators of whiteflies.

The adults are 1 to 2.5 mm long. The adult body is hemispherical, strongly convex, with head head in repose closely fitting ventrally against prominent prosternal lobe. The dorsum is apparently glabrous with sparse setae on pronotum and elytra. The mandibles are unidentate, small, triangular with single apical tooth and reduced mola (Ślipiński 2007). Beattie et al. (1991) observed that population peaked in autumn and early spring at Somersby, and troughs occurred in late-spring/summer and in winter.

2.1.8.2.2. Lacewings

Drea (1990) lists Neuroptera reported as predators of armoured scale in the North America, Asia, Europe and Africa, but the role of lacewings in biological control of these and other prey in Australia has been overshadowed substantially by interest in the predatory values of Coccinellidae (New 2002). By contrast with other parts of the world where Neuroptera are important biological control agents, Australia lacks members of the ‘Chrysoperla carnea group’ of species so predominant in such activities (New 2002). The only lacewing known to prey on red scale in Australia is the indigenous green lacewing, Mallada signatus (Schneider) (= Chrysopa signata Schneider) [Chrysopidae] (Hely et al. 1982, Smith et al. 1997). The green lacewing, Plesiochrysa ramburi (Schneider) [Chrysopidae], is known to prey on citricola scale in Australia, but there is no record of it preying on red scale. Clausen (1940) included diaspidids as occasional prey of brown lacewings [Hemerobiidae], but there are no records of them preying on red scale, or other diaspids in Australia.

Nearly all neuropteran species have the long curved mandibles that act like forceps to grasp and pierce the prey and suck out the body fluid (Rosen & DeBach 1974). Most research with chrysopids was focused on their association with prey other than armoured scale insects (Drea 1990). Green lacewings can also have an adverse impact on other beneficial predators such as coccinellids, including Chilocorus and Rhyzobius (Essig 1915, Smirnoff 1957, Yinon 1969, Drea 1990).

Mallada signatus (Schneider) is widespread in Australia. It is one of the most effective generalist predators available on a commercial scale. The voracious, debris-carrying larvae are polyphagous and feed on aphids, two-spotted mite (Tetranychus urticae),
greenhouse whitefly, scales, mealybugs, moth eggs and small caterpillars (Papacek et al. 1995, Horne et al. 2001, Llewellyn 2002, New 2002). Adults are green with long antennae, prominent eyes and four wings folded in an inverted V over the back. The clear wings have numerous veins giving a lacy appearance (Queensland Department of Primary Industries and Fisheries). They may discharge a disagreeable odour if disturbed (Drea 1990). They feed on nectar and pollen. Each female is capable of producing up to 600 eggs over a life-span of three to four weeks, following a preoviposition period of about 7 d (Llewellyn 2002, Bugs for Bugs). Each egg sits on the end of a slender stalk, which elevates it from the plant or other substrates on which it is laid, generally in groups. This reduces the chance of predation by ants. The eggs take approximately 4 d to hatch (Llewellyn 2002). The larvae were described by Boros (1984), but many details of their biology have not been clarified, and there are few records of their prey or feeding activity (Horne et al. 2001). They have small spines on their backs upon which they impale the remains of prey. This provides a form of camouflage and allows the larvae to appear inconspicuous amongst the prey (Llewellyn 2002, Bugs for Bugs). They pass through three moults over a period of about 12 d before pupating in a silken cocoon. Adults emerge after 9 d (Bugs for Bugs). The larvae are also cannibalistic (Horne et al. 2001).

The first instar is 1.8–3.0 mm long with a humped abdomen. Head markings are similar to other instars (see below) but oblique epicranial markings are less distinct posteriorly, or are markings obscured by general black pigment. The second instar is 3.0–3.8 mm long. Head markings are usually as in the third instar (see below), or the dorsum is completely black. The thoracic pronotal sclerites are either as in third instar or completely darkened, and the metanotum has 4 stout and 6 fine major setae. The third instar is 4.50–8.25 mm long and the abdomen is humped and globular. The dorsal head is creamy with 3 pairs of light brown to dark grey markings (Boros 1984).

Anderson et al. (2003) studied influence of a dorsal trash-package on interactions between larvae of Mallada signatus. They observed that when third instar larvae were fed on eggs of angoumois grain moth (Sitotroga cerealella (Olivier) [Lepidoptera: Gelechiidae]) and crawlers of citrus mealybug (Planococcus citri (Risso) [Hemiptera: Pseudococcidae]), in an environment that also contained rice hulls, larvae that carried

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trash experienced lower rates of cannibalism. They attributed this to camouflage conferred by the debris. They also observed that larvae preferred physically hard material over normal dietary items when constructing their trash-package and suggested that the inclusion of these materials in the package may provide both physical and chemical camouflage from predator and prey alike. When encounter rates between the larvae were low, those that carried trash increased their activity rates as they aged but trash-denuded larvae decreased activity rates as they aged. Among first-instar larvae, as the density of larvae increased and encounters became more frequent, those with trash moved further than those without. Larvae with trash packages exhibited lower cannibalism and higher activity rates, which may subsequently enhance foraging capacity (Anderson et al. 2003).

Canard (2001) described predation behaviour of green lacewing larvae. When searching for prey, most green lacewing larvae are frequently active, scrambling up and down, mainly by night, either slowly or very quickly depending on the species. When a larva is hungry, its activity increases and, as it seeks to find prey, this activity increases in step-wise function with the duration of starvation, but decreases later until a comatose state is reached if no prey is encountered. The discovery of potential prey happens at random, independent of light but slightly stimulated by the presence of honeydew of sap-sucking insects or lepidopteran scales. Discovery of food is conditioned by contact by the palpi and/or the antennae, followed by probing by the jaws, achieving chemosensory positive recognition. Taking prey is the outcome of fixed phases: a slow approach to the target; stopping; opening the jaw; sudden attack by throwing the head forward and simultaneous jaw nipping, most often induced by moving of the prey; the catch is sometimes followed by lifting the prey. Salivary secretions are then injected into the prey body while the internal tissues are lacerated in order to pound and to liquefy the content and to make available the nutritive juice which is then drawn up. Feeding on the prey ends when a sufficient quantity of food is ingested, leading to satiety. The larva then cleans its mouthparts by rubbing them together and on the substrate. Resting posture is maintained until activity is resumed.

2.1.8.2.3. Moths

They feed under fine, white webbing and are most commonly associated with heavy infestations of the scale (Hely et al. 1982). Waterhouse & Sands (2001) stated that *Batrachedra arenosella* is one of the most important native predators of red scale in Australia. The moth also attacks oriental scale (*Aonidiella orientalis*), soft brown scale, black scale, white louse scale (Smith et al. 1997, Elder et al. 1998, Waterhouse & Sands 2001).

Meyrick (1888) described that adult male and females as ‘11–14 mm. Head and thorax whitish-ochreous. Palpi whitish-ochreous, apex of second joint blackish, scales slightly projecting, terminal joint more whitish, with sub-median and apical black rings. Antennae whitish-ochreous, indistinctly ringed with fuscous, towards apex with three or four broader fuscous bands. Abdomen ochreous-grey-whitish. Legs dark fuscous, ringed with, whitish, posterior tibiae ochreous-whitish irrorated with dark fuscous. Forewings elongate, very narrow, long-pointed; veins 6 and 7 separate; pale yellow-ochreous, finely sprinkled with dark fuscous; a dark fuscous dot in disc before ⅜, and another at ⅜, occasionally obsolete: cilia pale grey, on costa whitish-ochreous, sharply divided at apex. Hindwings with veins 2, 3, and 5 absent; pale grey; cilia pale grey.’

### 2.1.8.3. Entomopathogens

#### 2.1.8.3.1. *Microcera coccophila* and *Podonectria coccicola*

Entomopathogenic fungi of armoured scale insects in Australia were first recorded in the late 1800s (Cooke 1887, Koebel 1892, Tryon 1894, McAlpine 1899). *Microcera coccophila* and *Podonectria coccicola* have been the most commonly mentioned species (Cooke 1887, Koebel 1892, Tryon 1894, McAlpine 1899, Summerville 1934, Searle 1964, Hely et al. 1982, Evans & Prior 1990, Smith et al. 1997, Waterhouse & Sands 2001). Tryon (1894) recorded four entomopathogens of armoured scale in orange orchards in, and near, Maryborough in the Wide Bay region of Queensland. He referred to two of them as *Microcera coccophila* and *Microcera rectispora* (now *Podonectria coccicola*). McAlpine (1899) recorded three species, *Microcera coccophila*, *Microcera rectispora* and *Fusarium epicoccum*. Fig. 2.14 features illustrations from Petch (1921b) of entomopathogenic fungi of armoured scales.

*Microcera coccophila* Desms. *Microcera coccophila* is the anamorph (asexual stage) of an entomopathogen commonly known as the red-headed fungus. It was first
described by Jean Baptiste Henri Joseph Desmazières in 1848 from scale insects on willow (Salix sp. [Malpighiales: Salicaceae]) and ash (Fraxinus sp. [Lamiales: Oleaceae]) in Normandy, France (Desmazières 1848, Petch 1921a,b). For several decades until recently, it has been referred to as Fusarium coccophilum (Desms.) (Searle 1964, Booth 1971, Joffe 1974, Smith et al. 1997). The teleomorph (sexual stage), Sphaerostilbe coccophila Tul., was first described by Louis René Tulasne and Charles Tulasne in 1861 from specimens collected in Florence, Italy (Tulasne & Tulasne 1861, Petch 1921a,b). Dingley (1950) named the teleomorph as Nectria flammea comb. nov. based on Desmazières (1848) but also referred to Sphaerostilbe coccophila as a synonym of Nectria aurantiicola Berkeley & Broome and referred to the anamorph of the latter as Microcera aurantiicola Petch. Synonyms of the anamorph and the teleomorph are summarised in Appendix II. Microcera coccophila is now regarded as the ‘unitary’ name (Gräfenhan et al. 2011).

Microcera coccophila is the most commonly recorded as an entomopathogen of armoured scale in Australian literature. It was first recorded in Australia by Tryon (1889) in citrus orchards near Toowoomba in Queensland. Koebele (1892) wrote: ‘Aside from the numerous parasites and predaceous insects destructive to scale insects in Australia, there exist several species of fungoids detrimental to various coccids. Microcera coccophila, if once started upon a tree infested with the red scale, will keep on spreading until all the scales are destroyed’. Tryon (1894) reported that red scale, purple scale and circular black scale were frequently killed by Microcera coccophila in orange orchards in and near Maryborough in the Wide Bay region of Queensland. McAlpine (1899) recorded it on red scale on orange and pomelo (shaddock: Citrus maxima (Burm.) Merr.) in Sydney and lemon in Queensland. Summerville (1934) stated that of the ‘fungous enemies’ of scale insects in Queensland, the red-headed fungus ‘as Sphaerostilbe coccophila Tul. is often styled’, is by far the most commonly found. Though present in all the major citrus-producing districts, it was ‘usually of moment’ only in the more humid coastal parts. He observed more than 70% infection of large colonies of red scale, but less than 1% infection was encountered in normal times, and considered it to be an unimportant factor in scale control. Red scale was the commonly infected scale, with other hosts including pink wax scale, Ceroplastes rubens. This latter record seems unlikely given current knowledge. Hely et al. (1982) and Smith et al. (1997) mentioned that it is effective under humid conditions.
Microcera coccophila has also been commonly recorded on armoured scale insects in Florida since the late 1800s (Rolfs 1897, Rolfs & Fawcett 1908, Watson 1915). Rolfs & Fawcett (1908) noted that Florida climate conditions were the most favourable for entomopathogenic fungi to attacked scale insects. They (Rolfs & Fawcett 1908) wrote: ‘Many species of scale insects and similar insects in Florida suffer great annual diminution from fungus diseases. This can be readily proved by simply treating a scale-infested plant with a fungicide, such as Bordeaux mixture, which will then destroy the fungi that kill scale insects, and a great increase of the latter will ensue’. Fawcett (1908) mentioned ‘a simple method of infection developed in Florida by one of the peachgrowers, Mr FP Henderson, by tying into the top of an infested tree a short piece of wood whose bark bears a good supply of the fungus.’ This was proved to be a very effective way of distributing this fungus, and saved thousands of dollars to the peach and orange growers of the State (Fawcett 1908).

Fuller (1901) recorded Microcera coccophila (cited as Sphaerostilbe coccophila) on red scale and purple scale in Natal, South Africa. Searle (1964) recorded entomopathogenic fungi attacking armoured scale of citrus in South Africa including, Microcera coccophila (cited as Fusarium coccophilum and Fusarium coccinellum), Microcera larvarum (cited as Sphaerostilbe aurantiicola) and Podonectria coccicola (cited as Tetracrium rectisporum). However, there is some uncertainty about the records from South Africa. Bedford et al. (1998) mentioned Microcera larvarum (cited as Fusarium larvarum Fuckel (teleomorph Cosmospora aurantiicola (Berk. & Broome) Rossman & Samuels) could be factor in control of red scale in the KwaZulu Natal region on the east coast of South Africa. They (Bedford et al. 1998) did not mention Microcera coccophila but noted that Microcera larvarum had also been reported from circular black scale, pine needle scale (Chionaspis heterophyllae Cooley), oystershell scale (Diaspidiotus ostreaeformis (Curtis)), latania scale, greedy scale, Hemiberlesia sp., purple scale, Glover’s scale, Leucaspis sp., obscure scale, camellia scale, almond scale (Suturaspis archangelskyae (Lindinger)), black oak scale (Targionia vitis (Signoret)) and white louse scale. Moore (2002), in his review of entomopathogens of citrus pests in South Africa, reported that Microcera larvarum (cited as Sphaerostilbe aurantiicola), Podonectria sp., Microcera coccophilum (cited as Fusarium coccinellum) and ‘Fusarium lateritium’ (teleomorph Gibberella sp.) as being associated with red scale, Fusarium larvarum with circular black scale and purple scale, and Myiophagus sp. with purple scale. Microcera larvarum has been reported as a pathogen of black oak scale by

Berger (1926) mentioned another species, the pink-headed scale fungus or pink scale fungus *Cosmospora diploa* (Berk. & M.A. Curtis) Rossman & Samuels (syn. *Nectria diploa* Berk. & M.A. Curtis: anamorph *Fusarium coccidicola* P. Henn., syn. *Fusarium juruanum* P. Henn.) as a pathogen of several armoured scales in Florida: red scale, circular black scale and purple scale on citrus, and of water oak or obscure scale *Melanaspis obscura* (Comstock) (syn. *Chrysomphalus obscurus* Leonardi) on water oak (*Quercus nigra* L. [Fagales: Fagaceae], and gloomy or red maple scale *Melanaspis tenebricosa* (Comstock) (*Chrysomphalus tenebricosus* Fernald) on red or swamp maple (*Acer rubrum* L. [Sapindales: Sapindaceae]). He (Berger 1926) said that it had undoubtedly been present a long time in Florida, was less common than the red-headed scale-fungus and, to the casual observer, indistinguishable from than the latter. He described its heads as pink rather than red, with a pink or reddish border surrounding scales it has killed. However, Muma & Clancy (1961) mentioned that later investigations by Holloway & Young (1943), Fisher et al. (1949), Ziegler (1949), and Fisher (1950a, b) indicated that ‘*Sphaerostilbe aurantiicola*’ and ‘*Nectria diploa*’ were saprophytic. In contrast, Evans & Prior (1990) stated that they were ‘in no doubt that armoured scale insects, particularly in the humid tropics, are subject to periodic and often devastating attacks by highly adapted fungal pathogens. These fungi, therefore, are thought to significantly influence scale insect populations and have probably evolved with their hosts over a considerable time span.’ Furthermore, data ‘strongly support this hypothesis, especially the rigid host specificity or specialisation, which is the hallmark of an ancient coevolution.’ Evans & Prior (1990). Fawcett (1944) noted that well-controlled experiments had been done on a few scale-inhabiting fungi and he cited work by Watson (1915) in which the latter dipped branches of small citrus trees infested with circular black scale in spore suspensions of ‘*Nectria diploa*’ in April at Gainsville, Florida: by November, every scale had been killed.

Among the numerous hypocreaceous parasites of scale insects, Booth (1971) stated that ‘only three species have been found which have a conidial state referable to the genus *Fusarium.*’ Although Evans & Prior (1990) cited confirmed records of insect-pathogenic *Fusarium* species infecting Diaspididae, Evans & Hywel-Jones (1997) could find no reliable records of an insect pathogenic *Fusarium* associated with Coccidae. Evans & Hywel-Jones (1997) also said, based on their experience, that except for the *Fusarium coccophilum* group on Diaspididae (Booth 1971) and despite numerous records
describing *Fusarium* from scale insects, most of these species are present as opportunistic necrotrophs or saprobes. However, *Fusarium stilboides* Wollenw. (teleomorph *Gibberella stilboides* Gordon ex Booth), and *Fusarium moniliforme* Sheldon var. *subglutinans* Wollenw. & Reink. (teleomorph *Gibberella fujikuroi* var. *subglutinans* E.T. Edwards) were identified, by New South Wales Agriculture mycologists, from egg masses under the wax scales *Ceroplastes destructor* and *Ceroplastes sinensis* collected from orchards at Somersby in the 1980s (Smith et al. 1997, Beattie, pers. comm., 2008). More recently, Tyson et al. (2005) found *Fusarium coccophilum* on *Eriococcus cavellii* (Maskell) [Hemipreta: Eriococcidae] during surveys in New Zealand in 2002–2003, and Ferguson & Fletcher (1991) recorded *Fusarium stilboides* on *Hemiberlesia rapax* and San José scale in New Zealand. Evans & Prior (1990) mentioned a record for *Nectria barbata* Petch on *Lepidosaphes beckii*, but commented that the identity of the pathogen required validation: there appears to be no record of an anamorph.

Booth (1971) stated that fusaria that are parasitic on scale insects ‘show adaptations to this habitat both in their perithecial and conidial fructifications. The significance of these adaptations has been over emphasised and this has resulted in these species being placed in numerous different genera.’

In nature, presumably as a response to their rather exposed habitate adjacent to or on the surface of scale insects, the stromatic sporodochia are covered with protective hyphal strands during the early stages of development. These strands fuse together in varying degrees in a ladder-like manner by the formation of lateral protrusions. Later as the conidia develop this overlying veil becomes ruptured and assumes the form of an open cup with a ragged edge. As the conidia continue to develop this becomes filled with the slimy, white to orange coloured mass of conidia which in dry weather has a horny appearance and texture. Under moist conditions the mucilage sweels and the conidia are dispersed by rain. If sporodochia develop under very moist conditions or in culture, then this protective sheath is not formed. In nature also the sporodochia tend to be produced on a marked stromatic cushion which often becomes so pronounced as to form a stalk; this is also absent in culture. The perithecial states of the entomogenous *Nectria* species are characterized by their thick-walled perithecia which are heavily pigmented. This is also presumably a response to their environment although at the present time we have not succeeded in producing perithecia in culture and are therefore unable to describe the contrast. In culture the species are typified by the fine white arachnioid mycelium and the presence of a single conidial state which is not scattered over the mycelium but is confined to sporodochia.’

In nature, white mycelia surround and extend to cover the scale body, following by development of sporodochia on this. Later, perithecia (fruiting bodies) begin to grow
and surround the edge of scale covers. Some 12 perithecia may develop on each scale. The perithecia are globose with a small ostiolar papilla and a finely roughened wall that is composed of outer (6–8 layers) and inner (3–4 layers) regions (Booth 1971).

Devnath (1987) described *Microcera coccophila* growing on *Hemiberlesia rapax* on tea (*Camellia sinensis* (L.) Kuntze [Ericales: Theaceae]) in Darjeeling, India. The sporodochia varied considerably in their size and shape, and rose as a small globular to slender club shaped to wedge shaped body. Fructifications of this fungus on scale bodies were pink to deep rose that rose from the body of the scale insect. The number of sporodochia arising from the scale body varied considerably and was occasionally branched in dichotomous pattern.

Joffe (1974) reported that cultures of *Microcera coccophila* on potato carrot agar are white, rose or orange. Booth (1971) reported that they grow slowly on this medium. It has two types of conidia, macroconidia and microconidia. Macroconidia are hyaline, cylindrical, multiseptate, long and sickle-shaped. The basal part of the spore is broad and basal cell weakly develops. Microconidia are uniseptate (occasionally biseptate), globose and pear-shaped. The mass of macroconidia are born on sporodochia. The sporodochia are formed of a loose sparsely branched mass of somewhat elongated conidiophores. Each branch is terminated in one or two phialides (Booth 1971). Chlamydospores are absent (Booth 1971, Joffe 1974). Microconidia are scattered throughout the aerial mycelium only (Teetor-Barsch & Roberts 1983). The length and width of *Microcera coccophila* macroconidia with varying numbers of septa of were given by Joffe (1974): 3-sept. 38–54 × 3.6–4.8 µm, 5-sept. 49–72 × 4.4–6.4 µm, 7-sept. 66–84 × 4.8–7.2 µm, 9-sept. 77–96 × 5.0–7.4 µm, 10-sept. 86–102 × 5.2–7.5 µm, 12-sept. 91–114 ×5.3–7.7 µm.

*Podonectria coccicola* (Ellis & Everh.) Petch. *Podonectria coccicola* is the teleomorph of an entomopathogen known as the white-headed fungus. It was first described by Job Bicknell Ellis and Benjamin Matlock Everhart in April 1886 as *Nectria coccicola* from specimens on scale insects on back of living orange trees in Florida (Ellis & Everhart 1886). The anamorph, *Tetracrium coccicolum* Höhn., was first described by Cooke in 1887 as *Microcera rectispora* Cooke and Mass. on *Coccus* of the orange in Brisbane, Queensland (Cooke 1887). Cooke (1887) listed it as one of several new Australian fungi. Synonyms and related information are listed in Appendix III.
Tryon (1894) reported it under *Microcera rectispora* on white louse scale on orange trees in Wide Bay (Maryborough) region, Queensland. He (Tryon 1894) reported that white louse scale was frequently killed by *Podonectria*, being rendered quite invisible by brown rounded granule-like bodies (presumably teleomorph) or little white tufts composed of large elongated radiating spores (anamorph). McAlpine (1899) described it as *Microcera rectispora* from specimens also from on white louse scale on orange trees in Wide Bay (Maryborough) region, Queensland. Summerville (1934) mentioned that a species of *Podonectria* was sometime found on purple scale, but the level of control scale insects rarely resulted. Hely (1968) stated that, in New South Wales ‘...some Entomogenous fungi such as *Sphaerostilbe* sp. and *Podonectria* sp. exercise a measure of control on some scale insects and mites’.

This fungus was first recorded in Florida by Hubbard in 1885 (Watson & Berger 1932). Hubbard (1885) referred it as a bark fungus and not parasitic. He wrote: ‘Of these fungi the one most readily mistaken for Scale-insects commonly appears upon the trunk and branches as little hard excrescences of gray colour, which, in wet weather, burst, disclosing a white cottony interior, from which they are often confounded with the "Mealy Bug," (*Dactylopius*). The resemblance to the Coccid is increased when the white spicules, a bundle of which fills each little fungus cup, are beaten out by rains, and felted upon the bark in a mold-like coating. The fungus is confined to the surface of the bark, and appears to germinate exclusively among the débris of Scale-insects. It is always found upon trunks that have long been coated with Chaff Scale (*Parlatoria pergandii*). It may also be found upon the leaves when they have become infested with this scale, and is easily removed by gentle friction between the fingers, coming off with the scales, and showing no close attachment to the surface of the leaf.’ Rolfs (1897), Watson & Berger (1915) regarded it was an important factor for the control of purple scale in Florida. It has been recorded as entomopathogenic fungus of scale insects in Florida (Watson 1927), in southeast China (Gao & Ouyang 1981), North America, West Indies, Ceylon, Java, Formosa, South Africa, New Zealand (Dingley 1954), in India (Rao & Sohi 1979), in Brazil (Arantes & Correia 1999). Seaver (1909) recorded the fungus in association with dead scale on the bark of living orange trees. As for ‘*Sphaerostilbe aurantiicola*’ and ‘*Nectria diploa*’, Muma & Clancy (1961) mentioned that Holloway & Young (1943), Fisher et al. (1949), Ziegler (1949) and Fisher (1950a, b) considered it to be a saprophyte.

Recorded hosts include Glover’s scale, purple scale, *Parlatoria pergandii, Parlatoria zizyphi, Aspidiotus ficus* (Miyabe & Sawada 1913, Berger 1927, Arantes & Correia 1999). Rao & Sohi (1979) stated that it was an interesting entomogenous fungus of
armoured scales feeding on different citrus species such as mandarins, grapefruit, sweet orange and trifoliate orange.

*Podonectria coccicola* is common in the tropics and subtropics (Rossman 1977). It occurred nearly everywhere in Florida (Watson & Berger 1932) and Berger (1926) stated that it occurs wherever there is the long (Glover’s) scale or purple scale. Hence, ‘it is an open question whether this fungus or, the red-headed scale-fungus is the most common’. It has also been recorded in the West Indies, Ceylon, Java, Japan, and South America: in Japan on the purple scale, and in Japan and Argentina on Glover's scale; in Java, Queensland, and Brazil on unidentified scales (Fawcett 1948). Rao & Sohi (1979) recorded that it as abundant at Coorgarea, Karnataka, India, where high rainfall and moderate temperatures prevail. It was used to control scale insects on citrus in Florida until about the 1920s (Rossman, pers. comm., May 2010). Arantes & Correia (1999) monitored its seasonal abundance in association with *Parlatoria ziziphi* in São Paulo, Brazil, and reported that in can occur there at high levels in autumn and winter.

Stromata are rounded, more or less prominent and whitish. Conidia are born in clusters of 3–5, are large, broad at the base, taper into a bristle-like apex, have 15–20-septa, are 100–150 × 7–7.5 µm in size and have a distinct, stem-like base. Perithecia are in cespitose clusters, nearly globose or a little longer than broad, reddish becoming dark brownish, minutely roughened, at first clothed with a few hyaline hairs, then naked and 300–500 µm in diameter. Asci are cylindrical, tapering below into a stem-like base and 150–200 × 20 µm in size. Spores are clavate or subcylindrical, 100–120 × 6–7 µm at the base, contain 15–20-septa and are hyaline (Seaver 1909).

### 2.1.8.3.2. Fungal infection, dispersal and epizootics

Evans & Hywel-Jones (1997) described how entomopathogenic fungi infect their hosts. Infection occurs directly through the exoskeleton. Spores produced by entomopathogenic fungi are adapted for both dispersal and infection. Because of the complexity of the insect cuticle, these spores possess a unique range of properties, which enable them to attach to, and penetrate the cuticle. ‘Essentially, there are two main forms of propagules: dry and ‘wet’ (slime) spores. The wet spores bind to the cuticle using the mucilaginous matrix surrounding them, whilst the dry employ a combination of electrostatic forces and chemical bonding agents (e.g., lipoproteins), which facilitate attachment to the hydrophobic, lipophilic epicuticle; further attachment-enhancing structures, acting like suction pads (appressoria) may be formed in some groups. Typically, spore germination is induced by high relative humidity and the
resultant germ-tube bores through the cuticular layers by a combination of enzymes (chitinases, lipases, proteinases) and physical pressure. Once penetrating through the exoskeleton, the fungus encounters specific cellular resistance mechanisms which differentiate between self and non-self. It is this battery of physical, chemical and cellular barriers which filter out the opportunistic or secondary pathogens, as well as primary but non-coevolved pathogens. Once inside the host, the fungus multiplies rapidly in a yeast-like phase and spreads throughout the haemocoel, eventually killing the insect, probably following toxin production. The water content of the host tissues becomes exhausted because of growth of the true fungal mycelium. Consequently, the cadaver becomes mummified. The life cycle is completed when the stroma produces its sporulating structures. It occurs invariably following prolonged periods of high humidity.

The wet or slime spores are efficiently dispersed over plant tissues, and thus come into contact with their hosts in run-off rainwater, and possibly by rainsplash, accounting for the rapid and almost complete elimination of living coccid colonies within a host plant once the fungus becomes established. Lateral movement to coccid colonies on other plants, however, is probably much more uncertain and less efficient, and is mainly facilitated by the production of dry, aerially-dispersed spores. The chances of actually hitting their insect target are, of course, remote and the fungi have evolved insurance mechanisms to overcome this by forming secondary sticky spores after landing. These probably serve to make contact with the crawling stages of the insect host.’ (Evans & Hywel-Jones 1997).

Evans & Prior (1990) stated that ascospores are usually violently released from the perithecia, almost certainly to lift them from the laminar boundary layer at the leaf surface and into air currents, and noted that ‘These must function for long-distance or horizontal dispersal of the fungus between diaspidid colonies. The mechanisms of adhesion to the host of these dry spores are probably more subtle, involving both chemostatic and electrostatic forces.’ ‘The fruitbodies are equally well-adapted to the exposed, somewhat precarious, habitat which diaspidid scale insects occupy. The ascospores are formed within thick-walled, often fleshy perithecia, whilst the conidia are similarly protected.’

Dispersal of fungal conidia by ants and other motile insects was documented by Gracia-Garza et al. (1997). Gracia-Garza et al. (1997) observed that ants, Pheidole spp., and Pheidole megacephala (Fabricius) [Hymenoptera: Formicidae], passively carried propagules of Fusarium oxysporum Schlechtend: Fr. f. sp. erythroxyli, applied as herbicide against a narcotic plant, outside their bodies, as well as either closely adhering to the outside or within their bodies. Spores may also be dispersed by parasitoids. Lacey & Mesquita (2002) summarised studies on dispersal of fungal spores by parasitoids. They stated that a potential benefit to fungal interaction of parasitoids with fungus-infected hosts is the transmission of spores or hyphal bodies after probing by
ovipositing parasitoids on insect hosts. Generally, epizootics of entomogenous fungi are influenced by both environmental (temperature, humidity, light) and biological factors (host density, host immunity innate or acquired, and stress which host suffers) (MacLeod et al. 1966).

2.1.9. Control of red scale in Australia

The earliest forms of chemical control of red scale in Australia involved the use of dilute kerosene-soft soap and resin soda washes (made by boiling resin with caustic soda and either fish or whale oil or soft soap) in the late 1800s (Hely et al. 1982). Koebele (1890) mentioned use of kerosene-tar soft soap washes as a trunk and major branch treatment for purple scale and commented that a grower claimed that the treatment was not required again for 7–8 years. He commented that the treatment probably reduced red scale infestations (Koebele 1890). When the New South Wales Department of Agriculture was established in 1890, use of ‘Gishurst compound’, soft soap and kerosene emulsions for scale insects were noted in the proceedings of a conference that included delegates from the fruit growing industry (Hely 1968).

Control of red scale was the subject of a great deal of investigation within Australia and overseas and principally involved spraying and fumigation. In the early 1900s, kerosene emulsion and resin soda washes were the main materials used and several applications were needed to provide the moderate results achieved. Following the development of hydrogen cyanide (HCN) fumigation in California as an effective and practicable method, the practice was introduced into New South Wales by WJ Allen at the end of the 1880s (Hely 1968). This was regarded as the the first great break through in scale control and typical of grower reaction was reflected in a comment by a well known orchardist who had tried the new method. Hely (1968) said ‘spraying was not in the same paddock as fumigation’. The practice was used more widely in inland districts than in coastal districts (Hely 1968).

Hely (1968) reported that HCN fumigation during second half of February was the most effective single treatment for red scale control. It was was a costly and messy treatment. Briefly, each tree was covered with a tent into which HCN gas was released. The tent was then removed from the tree after about 40 min. The practice was almost completely abandoned a few years after World War II. The reason for its decline in popularity was not because of poor scale kill, but largely on account of the high cost of
labour for night work and the development of more efficient spray machines and the introduction of more efficient scleridal sprays (Hely 1968). It may have been last used at Barham on the mid-Murray River in NSW in about 1965 (Beattie, pers. comm., 2008). Use of HCN fumigation also declined as the efficacy of spray equipment improved and as organophosphates and carbamates became available after World War II. This led to a period of heavy reliance on these synthetic pesticides and widespread disruption of control by natural enemies as a result of their use (Hely 1968, Hely et al. 1982, Smith et al. 1997).

Kerosene emulsions ceased to be used in the early 1900s when they were replaced by crude petroleum distillates and engine oils that became available as more or less standardised proprietary formulations around 1920. These unrefined lubricating oils were emulsified with carbolic or cresylic acid and were known generally as red spraying oils and the risk of pytotoxicity was high. In the late 1920s and early 1930s, highly refined lubricating oil with low levels of unsaturated hydrocarbons known to cause acute phytotoxicity became available. These oils met standards developed in California. In the 1940s and 1950s, sprays were applied thoroughly at high volume hand or by drive past oscillating boom and airblast sprayers. In the late 1960s, further improvement in oil quality led to use narrow-range petroleum spray oils (the equivalent of some contemporary horticultural mineral oils: HMOs) with low risk of phytotoxicity. These oils were commonly formulated as mayonnaise emulsions and recommended for use in aqueous emulsions at 2% (v/v). Contemporary products, both HMOs and agricultural mineral oils (AMOs: broad-range petroleum spray oils), are now recommended for use at 1% (v/v) for control of red scale, and the risk of phytotoxicity is low (Beattie & Hardy 2005). The highest quality products are based on medicinal paraffins with ≥ 99.8% unsulfonated residue (UR) values and formulated as clear miscible emulsive products (Beattie & Hardy 2005). The glossary in Beattie et al. (2002) defines these terms.

HMOs and AMOs offer many benefits over broad spectrum pesticides:

- they are as effective or more effective than broad-spectrum synthetic pesticides for a wide range of pests and diseases;
- many pests can be controlled simultaneously;
- they have less harmful effects on the natural enemies of citrus pests;
- they do not stimulate other pest outbreaks;
• pests are not known to develop resistance to them;
• the oil deposits are broken down within weeks to form simple, harmless molecules;
• when using oils only minimum protective clothing needs to be worn;
• they are suitable, depending on the emulsifiers and additives used to formulate products, for use in organic farming; and
• they are not toxic to humans or other animals (Beattie & Hardy 2005).

Interest in biological control of red scale in Australia by its natural enemies commenced in the late 1800s with visits made by Albert Koebele and George Compere to Australia on the assumption that the scale, based on Maskell’s description of it on lemon fruit imported from Sydney to Auckland in 1878, originated in Australia. Success was not immediate, and was not achieved in Australia until the 1970s following the introduction of parasitoids, as mentioned above, directly from Asia or from Asia via the University of California, Riverside, and reduced the use of HCN fumigation and synthetic pesticides, mostly organophosphates (Smith et al. 1997).

Koebele (1892) noted that ‘In one of such orchards, of several acres in extent, but a few living twigs covered with red scale were found, yet not a single one of the many predaceous insects preying upon them could be noticed. In another instance, an orchard of some eight or ten acres and about thirty-five years old, the proprietor of which always supplied sufficient manure and kept the ground cultivated, during the whole time of its existence had been infested with red as well as other scales, and yet but a very few trees along the border of one side could be found that showed any traces of such. The whole orchard during the thirty-five years had never been pruned or sprayed, nor even had the trunks ever been washed. Numerous dead limbs were present, the stems and limbs partly covered with lichens, and yet I did not meet with a finer lot of trees in Australia such glossy, deep-green foliage, abundance of fruit, and so free from scale.’

In addition to information provided in this review, aspects of the history of biological control of red scale in Australia have been reported by Wilson (1960), Hely (1968), Furness et al. (1983) and Smith et al. (1997). It, and use of mineral oils when required, formed the foundation for highly successful integrated pest management (IPM) programs that have reduced pest management cost incurred by Australian citrus growers by more than 70% since 1970 (McLaren 1971). The background of this success, and the natural enemies on which this success was based, is summarised above in this review.
2.2. Yellow scale

*Aonidiella citrina* is an occasionally important pest of citrus in Australia (Hely et al. 1982, Smith et al. 1997). Ebeling (1950) reported that yellow scale was not widely distributed over the world as the red scale, and it was recorded from Australia, Japan, India, Russia, Iran, California, Texas, and very sparingly in Florida. It was introduced to Australia from Asia and more commonly occurred in coastal areas and in the Lower Murray district (Hely et al. 1982, Smith et al. 1997), but Hely et al. (1982) and Smith et al. (1997) did not record it as being present in the Murrumbidgee Irrigation Areas, now the Riverina region, of New South Wales. Yellow scale causes less damage to trees than red scale, and a less obvious blemish on mature fruit (Hely et al. 1982).

It was described as a variety of the red scale by Daniel William Coquillett in 1891 (Ebeling 1951). Maskell (1894) wrote: ‘In the United States "Agricultural Bulletin," No. 28, 1891, Mr. Coquillett mentions, under the name of "the yellow, scale, Aspidiotus citrinus," a form which, from careful examination of specimens sent to me by Mr. Ehrhorn, of Santa Clara County, California, I find are clearly only *A. auranti*ii. Mr. Ehrhorn informs me that he and Professor Comstock and several others have come to the same conclusion. I am sorry that a distinction founded on so slight a character as mere-colour should be so often suggested. No scientific description of this form has yet appeared, that I know of.’

Quayle (1911a) mentioned that yellow scale is ‘very similar’ to the red scale and regarded it as a variety of red scale. He stated that red scale and yellow had the same morphological characteristics under a microscope, but that the differences in their appearance on the trees were not difficult to determine with yellow scale being much lighter in colour, less convex and slighter larger in diameter, and not found on the twigs. Also, red scale is commonly found on the upper and lower sides of the leaves, yellow scale commonly found under side of the leaves (Quayle 1911a). Quayle (1911a) wrote: ‘Where occurrence of yellow scale is severe, all parts of the tree may be attacked. But it cannot be said that the yellow doesn't like heat, for it is most important in the warmest part of the southern citrus belt, and occurs exclusively in the large interior valleys of the north, where the summers are hotter than any part of the southern belt.’

Nel (1933) was the first to regard them as distinct species, based on morphological, biological and ecological differences. Life histories of red scale and yellow scale as recorded by Nel 1933 are summarized in Table 2.7.
Table 2.7. Average life history (number of days) of red scale and yellow scale conducted in a latch house during warmer months and in an insectary in winter (average temperature of 25.5°C) (Nel 1933).

<table>
<thead>
<tr>
<th>Scale</th>
<th>First moult</th>
<th>Second moult</th>
<th>First instar</th>
<th>Second instar</th>
<th>Third instar</th>
<th>Total from first instar to start of reproduction</th>
<th>Sex ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red scale</td>
<td>3.4</td>
<td>3.6</td>
<td>13</td>
<td>8.8</td>
<td>32.5</td>
<td>60.3</td>
<td>1:1.3</td>
</tr>
<tr>
<td>Yellow scale</td>
<td>3.1</td>
<td>3.7</td>
<td>13.1</td>
<td>9.1</td>
<td>35.6</td>
<td>64.3</td>
<td>1:0.7</td>
</tr>
</tbody>
</table>

McKenzie (1938) observed the presence of pygidial prevulvar scleroses in red scale and their absence in yellow scale that the two could be readily distinguished, albeit with a small, about 5%, margin of error (DeBach et al. 1978) (as presented earlier). Differences in pygidial prevulvar scleroses are evident in colour photographs in OEPP/EPPO Bulletin PM 7/51 (OEPP/EPPO 2005a).

Hely (1955) reported that yellow scale was common in coastal orchards and in Lower Murray citrus groves in south-eastern Australia and that where it occurred in these orchards it was usually the dominant scale pest on older trees, though pure infestations of red scale and yellow scale could sometimes be seen on different trees in the same orchard. Yellow scale preferred situations protected from strong sunlight on trees in these orchards and was commonly found on leaves and fruit, rarely on twigs and branches where it caused disfigurement of fruit and fall of leaves. Infestations were more common on undersides of the leaves and on the lower branches inside trees (Hely 1955).

DeBach et al. (1978) reported that the competitive displacement of yellow scale by red scale in Southern California was caused by its ecology homologue as both scales lived on fruit and leaves, but red scale was able to survive and reproduce also on the woody parts of trees. Hely (1968) recorded that yellow scale replaced red scale as the dominant pest in some lower Murray districts and is less serious in its effects on the trees. McLaren (1971) reported that the upper thermal death point was similar for both species, yellow scale reproduced more rapidly than red scale over the median range of temperatures, and lower temperature threshold of red scale and yellow scale were 15 and 18°C, respectively.

Molecular studies have been widely used for identification. Morse & Normark (2006) reported phylogenetic results of 89 species of armoured scale insects in the subfamilies
Diaspididae and Aspidiotinae based on two regions of the nuclear protein-coding gene, elongation factor 1α and the large subunit 28S ribosomal RNA gene.

2.3. Black scale

Black scale (*Saissetia oleae* (Olivier) [Hemiptera: Coccidae]), also known as brown olive scale, was first described 1791 as *Coccus oleae* by Guillaume-Antoine Olivier from specimens taken on olive, myrtle (*Myrtus communis* L. [Myrtales: Myrtaceae]) and *Phillyrea* sp. [Santalales: Oleaceae] in 1782 (De Lotto 1971). Multiple synonyms are listed in ScaleNet. Smith & Compere (1928) mentioned that recorded on olive trees in Nice, Italy in 1743. It is native to Africa and is now cosmopolitan (Ebeling 1950, Smith & Compere 1928, Bartlett 1978, Smith et al. 1997). It has a wide range of hosts, including all commercial varieties of citrus, olive [Lamiales: Oleaceae], apple [Rosales: Rosaceae], gardenia [Gentianales: Rubiaceae], oleander (*Nerium oleander* L. [Gentianales: Apocynaceae]), passionfruit [Malpighiales: Passifloraceae], hibiscus [Malvales: Malvaceae], jacaranda [Lamiales: Bignoniaceae], pepper (*Piper nigrum* L. [Piperales: Piperaceae]) (Quayle 1938, Hely et al. 1982, Smith et al. 1997). Larval and adult stages, particularly the young adult, produce honeydew that leads to growth of sooty mould fungi36 (Quayle 1911b, Ebeling 1951, Hely et al. 1982). Heavy infestations of sooty mould reduce photosynthesis and respiration of trees. Trees with such heavy infestations lose vigour and may shed leaves. Fruit blemished with the sooty mould may be unsuitable for sale unless washed (Quayle 1911b, Quayle 1938, Ebeling 1950, 1959, Hely et al. 1982, Argov 1993, Rössler 1993).

2.3.1. Life cycle

The female scale has seven stages: egg, crawler, 3 larval instars, pupa, and adult. The adult stage can be divided into three substages: young female, rubber-stage female, and ovipositing female (Bartlett 1978, Podoler et al. 1979). The male scale has 7 stages: egg, crawler, first instar, second instar, prepupae, pupa and adult. Males are rarely seen (Ebeling 1959, Hely et al. 1982) and are not necessary for reproduction (Barlett 1978)

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36 It is widely assumed that sooty mould growth on *Citrus* and other horticultural plants is related to a single species, *Capnodium citri* Berk. & Desm. [Capnodiales: Capnodiaceae] (Reynolds 1999). However, Reynolds (1999) showed that the taxon *Capnodium citri* is invalid and that in Florida sooty mould fungi on *Citrus* and ornamental plants comprise four species: *Polychaeton citri* (Persoon) Léveillé (syn. *Capnodium citri* (Persoon) Berkeley & Desmazières), *Antennariella californica* Batista & Ciferri and *Chaetobolus falcata* Miller & Bonar with spherical fruitbodies and *Calderiamoites fumago* Woronochin and *Polychaeton citri* (Persoon) Léveillé with elongate fruitbodies. The species composition of sooty mould fungi on species and hybrids of *Citrus* in Australia has not been determined.
The upper surface of adult female scale is dark brown or black, tough, leathery and smooth. It is 3–5 mm long, 2 mm wide, and 2 mm high, and nearly circular and hemispherical. There are two ridges forming the letter ‘H’ on the dorsum. The eggs are oval, 0.3 mm long, white, changing to cream and pinkish as they mature. Single females produce an average of 2000 eggs under their bodies (varying from few hundred to 4000 eggs). They take from two in summer to six weeks in winter to hatch (Quayle 1911b, Quayle 1938, Ebeling 1959, Smith et al. 1997). First instar crawlers are light brown, 0.34 mm long and 0.2 mm wide. The body is flat and oval, the eyes are black, and antennae have six segments. After hatching, crawlers remain under scale shell for 1 to 2 days, then, they emerge from mother scale shell through the arch which consists of the slightly raised portion at the posterior tip of the scale. They move and settle a day or two after hatching, on leaves or young shoots. The majority of crawlers settle on the leaves, preferably on clean upper surfaces not exposed to full sunlight (Quayle 1911b, Quayle 1938, Ebeling 1959, Hely et al. 1982, Smith et al. 1997).

The first moult occurs from a month to six weeks after birth during summer, and two months during winter. After the second moult, female scales remain in the same position for several weeks before moving to the twigs to take up a permanent position. This migration is due to two reasons. Firstly, leaves are more likely to abscise and drop from trees. Secondly, there may be food supply for more mature scale in the twigs than in the leaves. Therefore, mature scales are very rare on leaves and fruit. At this point, the young females are referred as the ‘rubber’ stage (Quayle 1938, Hely et al. 1982).

The distribution of black scale on citrus trees in Israel was also studied by Podoler et al. (1979). The population density on leaves was higher in the lower parts of trees than upper parts. Scales tended to migrate from leaves to twigs within trees. The highest population density was observed in the northern part of the tree (equivalent to the southern sectors of trees in Australia), and lowest in the western part (Podoler et al. 1979).

The rare male scale is distinctly differentiated after the first moult. It becomes elongated and the shell is narrow and light brown. Its eyes are visible eyes as dark areas on the front margin. The second instar is capable of movement over about four weeks, before becoming pupal stage. The puparium is a glassy-like covering formed from the secretion of numerous pores on the body surface. Under this cover, the male develops through propupal, pupal stages and adult. The propupa is light brown with scattered red
pigmentation, particularly at the posterior end. The head is reddish with dark red or brown eyes. It takes 5 to 8 days to pupate in the warm condition. The pupa is 1.2 mm long, and 0.4 mm wide, and lasts for 8 to 12 days. Its colour is similar to the propupal stage, but more pigment at the anterior end, and with red head and black eyes. The wing pads are conspicuous and extend to the third abdominal segment. The adult male remains beneath the puparium for about 1 to 3 days before emerging. It has long, white caudal filaments (8 mm) that project from the tip of posterior end. The antennae are whitish, 10 jointed and 5 mm long. Males are 1 mm long, brown, and have a pair of wings (Ebeling 1959, Hely et al. 1982). The wings are honey yellow, 1 mm long and 0.5 mm wide. The head is dark yellow (Quayle 1911b, Quayle 1938, Hely et al. 1982).

The occurrence of a yellow or ‘brightly-coloured form’ of the female was reported by Mendel et al. (1982). They observed that a certain proportion of females were bright yellow up to rubber stage, and becoming brown at ovipositing stage. These scales were observed some two to four weeks after second moult.


Black scale has one to two generations or more per year depending on host plant and climatic conditions (Argov & Rössler 1993). It prefers temperate climates with fairly high humidity (Hely et al. 1982). There are two generations in southern Australia, and three to four in northern Australia (Smith et al. 1997). In southern areas, hatching occurs in summer (December-January) and and again in autumn (Hely et al. 1982, Smith et al. 1997). The second generation produces fewer eggs (Smith et al. 1997). Hely et al. (1982) commented that *Saissetia oleae* is found in both coastal and inland regions of New South Wales, where it is and is most often important as pest in citrus nurseries. They noted that widespread infestations of mature trees were seen most
commonly in lower Murray River orchards with severe infestations resulting in extensive growth of sooty mould on trees and fruit, and sometimes to leaf-fall.

In California and on the central coast of Israel, where one complete generation occurs annually (Quayle 1938, Podoler et al. 1979), female scale mature in early spring, and eggs are laid during May and June (late spring and early summer). First, second and third instar larvae peaked during a first half of June, the second half of June and mid July, respectively. Populations of the young females peaked during August (late summer) and occur at almost constant density until January (mid winter). The rubber stage reached the highest densities during October, and maintained at constant density until February. Ovipositing females peaks during October and November (Podoler et al. 1979).

2.3.2. Natural enemies

2.3.2.1. Parasitoids

There were over sixty parasitoids of black scale, with the highest proportion belonging to the genus *Coccophagus* (Compere 1940, Ebeling 1959) and, in its native southern Africa, the scale is under excellent biological control (Bedford et al. 1998) unless parasitoid activity is impeded by ants (Compere 1940). The most important species in Australia are *Metaphycus helvolus* (Compere) (= *Aphycus helvolus* Compere and *Euaphycus helvolus* Compere) and *Metaphycus lounsburyi* (Howard) (= *Aphycus lounsburyi* Howard and *Metaphycus bartletti* Annecke & Mynhardt) [Hymenoptera: Encyrtidae], and *Scutellista caerulea* (Fonscolombe) (= *Scutellista cyanea* (Motschulsky)) [Hymenoptera: Pteromalidae]. All three are native to southern Africa. Other species recorded in New South Wales include *Tomocera californica* Howard [Pteromalidae], *Myiocnema comperei* Ashmead (= *Euryischia aleurodis* Dodd and *Euryischia shakespearei* Girault) [Encyrtidae], *Moranila comperei* (Ashmead) (= *Aphobetoideus comperei* Ashmead, *Tomocera io* Girault, *Tomocera saissetiae* Girault and *Tomocera transversifasciata* Girault) [Pteromalidae], *Coccophagus scutellaris* (Dalman) (=*Entedon scutellaris* Dalman and *Coccophagus australiensis* Girault), *Coccophagus ceroplastae* (Howard) (= *Aneristus ceroplastae* Howard, *Aneristus fumosipennis* Girault, *Coccophagus orientalis* Howard and *Prococcophagus orientalis* Howard) (Aphelinidae) (Wilson 1960).
Scutellista caerulea was described by Etienne Laurent Joseph Hippolyte Boyer de Fonscolombe in 1832 (Fonscolombe 1832). It was introduced to Australia on several occasions in the 1900s, initially from South Africa in 1902–04 (Wilson 1960). Wilson (1960) noted that the establishment of Scutellista caerulea and Metaphycus lounsburyi from South Africa, Tomocera californica from New South Wales, and several other natural enemies, provided a level of control of black scale that was one of the outstanding successes of biological control in Western Australia. Forms of Scutellista caerulea were also introduced for control of white wax scale from Kenya by William Butler Gurney, an entomologist with New South Wales Agriculture, and from South Africa by CSIRO personnel (Gurney 1936, Wilson 1960, Hely 1968, Sands et al. 1986). Hely (1968) commented that it was present in New South Wales before the introduction of the African strain by Gurney.

Scutellista caerulea is regarded as an egg predator, as larvae complete their development, most commonly their entire development, by preying on host eggs (Quayle 1911b, 1938, Sands et al. 1986). The female wasp inserts her ovipositor through the posterior scale shell, and lays her eggs under the body margins of immature or mature female scale. If host eggs are present, the wasp larvae consume them and pupate. If host eggs are not present, the wasp eggs hatch and larvae enter diapause at the end of the first instar (Sands et al. 1986). Sands et al. (1986) observed that a slight increase in size (up to 1.64 × length at hatching) that occurred in some first instar Scutellista caerulea larvae on non-gravid hosts indicated that feeding had occurred. They (Sands et al. 1986) stated that these larvae did not feed on the body tissues of the host, and that the nature of the body tissues on which the larvae fed could not be identified. In a three-year study in the 1980s, Beattie (pers. comm., 2009) observed first instar Scutellista caerulea larvae under immature adult white wax scale and hard wax scale over several intervals of 3–4 months for each scale. He attributed their increase in size and the appearance of their gut contents to feeding on non-gravid female scales, and observed that females eclosing from one host species readily oviposited under immature females of the other species (Beattie, pers. comm., 2009). There was no indication that separate biotypes of Scutellista caerulea (see Sands et al. 1986, Waterhouse & Sands 2001) attacked the scales (Beattie, pers. comm., 2009). Sands et al. (1986) stated that ‘the biotype of S. caerulea adapted to C. destructor appeared to complete development only if eggs of its host were present. Otherwise, newly-hatched parasitoid larvae survived beneath the scale for up 89 days without feeding. However, in South Africa the parasitoid larvae were
also recorded feeding on the body tissues of the scale insect (Cilliers 1967).’ This review suggests that the biotype(s) currently associated with wax scales and black scale on citrus in Australia need to be determined by host specificity and molecular studies.

*Scutellista caerulea* eggs are pearly white with a long stalk. Its larvae are white, curved and grub-like (Quayle 1938, Mendel et al. 1984), but no detailed information on larval instar morphology, or the number of instars, possibly three as for other pteromalids (see Tormos et al. 2009), appears to have been published. Each larva consumes some 200–400 eggs of the host and unless more than one larva develops on a host many eggs of the host may hatch (Quayle 1938, Mendel et al. 1984). Ehler (1989) regarded *Scutellista caerulea* as having limited effectiveness in regulating black scale populations due to the host density independence and the limited consumption of host eggs by individual larvae. Mendel et al. (1984) showed that in the winter in Israel, 90% of about 370 eggs per female scale were eaten by each larva, while during spring, only 50% of total eggs (approximately 700–1200) were consumed. The abundance and effectiveness of *Scutellista caerulea* may be influenced by the presence and phenology of its alternative hosts (Beattie, pers. comm., March 2009). Alternative hosts include *Saissetia hemisphaerica* (Targ.), *Ceroplastes cyanea* (Linnaeus), *Ceroplastes rubens*, *Ceroplastes destructor*, *Ceroplastes sinensis*, *Phenacoccus artemisae* (Ehrh.), *Saissetia nigra* (Nietn.), *Coccus hesperidum* (Linnaeus) (Smith & Compere 1928, Smith et al. 1997). Quayle (1911b) mentioned that number of generations per year can vary, ranging from 4–5 generations to 6–7 generations, depending on the weather and favourable conditions for its hosts.

Adult *Scutellista caerulea* emerge through a large circular exit hole they make in the dorsal scale shell. Sometimes two or three, very rarely four, larvae may develop on a single host, each emerging from separated exit holes. Adult females start producing eggs within 24 hours of emergence. They are metallic blue, the antennae and the tarsi except the last joint are usually light brown. The scutellum is long and extends to the tip of the body. The head is broad and connects closely to the thorax. It is bent under forming with scutellum a rounded arch. They are frequently seen walking slowly over scale infested twigs (Quayle 1911b, Quayle 1938).

*Metaphycus helvolus* is a solitary endoparasitoid (Flanders 1942a). It was described by Harold Compere in 1926 (Compere 1926) and introduced into Australia from California in 1942–44 (Wilson 1960, Bartlett 1978). The adults are small and 1 mm long.
Females are orange-yellow, males are dark brown (Flanders 1942a, Ebeling 1959, Tena et al. 2008). Mating occurs immediately after emergence and females start producing eggs in the following day (Flanders 1942a). Females prefer larger-sized scales in which to oviposit (Quayle 1938). The eggs are laid in the host body fluids of second and third instar nymph stages, and connected to the scale by the egg stalk (Bartlett 1978). Females produce 400 eggs on average (Flanders 1942b, Ebeling 1959). Eggs hatch in two days, and there are four larval instars. It has short life cycle that it completes in 13–14 days at 24ºC. The adult female has long-life up to 2–3 months and destroys hosts by host feeding (Flanders 1942b, Bartlett 1978). Host-feed scales turn light brown (Compere 1940). Alternative hosts include Saissetia nigra (Nietn.), Lecanium corni Bouché, Coccus pseudomagnoliarum (Kuw.) and C. hesperidum (Flanders 1942a).

*Metaphycus lounsburyi* is native to South Africa and was first described by Leland Ossian Howard in 1898 from specimens reared by Charles P Lounsbury from black scale (Smith & Compere 1928, Quayle 1938). It was introduced into Australia in 1902 from South Africa and became one of the effective parasitoids of black scale (Bartlett 1978, Malipatil et al. 2000). It is a gregarious endoparasitoid with an average of three progeny per scale. Its development is similar to *Metaphycus helvolus*, but slightly longer and, like *Metaphycus helvolus*, it host-feeds on black scale (Barzman & Daane 2001). Argov & Rössler (1993) mentioned that *Metaphycus lounsburyi* females prefer the ‘rubber’ stage of the scale in which to oviposit, but noted that they also parasitise young and ovipositing females. However, Mendel et al. (1984) showed that in Israel, *Metaphycus lounsburyi* mainly emerged from ovipositing females, and that only small proportion emerged from the rubber stage. According to Quayle (1938), the advanced stage of scale is preferred. If they parasitise the egg laying stage of the host, some 75% of host eggs still hatch (Mendel et al. 1984). *Metaphycus lounsburyi* is most abundant in mid summer when more suitable size scales are available. Its life cycle takes 1 month to develop from eggs to adult emergence during summer time, whereas in the colder months, it is about three months (Smith & Compere 1928). Alternative hosts include Ceroplastes floridensis, soft brown scale, citricola scale and hemispherical scale (Saissetia coffeae (Walker)) (Malipatil et al. 2000).

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37 Charles P Lounsbury was a Government Entomologist for the Union of South Africa.
2.3.2.2. Predators


*Rhizobius ventralis* females lay their eggs under the scale shell, eggs are pearly white and oval, 0.8 mm long, and 0.4 mm wide. The larvae live under scale shell, and feed on eggs, or young crawlers. The larva is 5–6 mm long, its upper surface is black and ventral surface is grey. The adult is broadly oval, 3 mm long with shiny black thorax and elytra. The adult is covered with grey hairs (Quayle 1911b, Quayle 1938).

2.3.2.3. Entomopathogens

*Lecanicillium lecanii* (Zimm.) Zare & W. Gams [Hypocreales: incertae sedis] (=*Cephalosporium lecanii* Zimm., *Verticillium lecanii* (Zimm.) Viégas, *Acrostalagmus coccidicola* Guég., *Cephalosporium coccidicola* (Guég.) Petch, as ‘*coccidicolum*’, *Hyalopus yvonis* Dop, *Cephalosporium coccorum* Petch, *Hirsutella confragosa* Mains) is a entomopathogen of a wide spectrum of insect hosts (Russo et al. 1988, Zare & Gams 2003). Hosts of include members of the family Coccidae, particularly green coffee scale (*Coccus viridis* (Green)) (Evans & Hywel-Jones 1997, Zare & Gams 2003). Other hosts include soft brown scale (*Coccus hesperidum* L.), *Coccus* spp., *Ceroplastes* spp., *Parthenolecanium* spp., *Philephedra*, *Planococcus* spp., and *Saissetia* (Kenneth & Olmert 1975, Mendel et al. 1984, Zare & Gams 2003). *Cephalosporium coccorum* was recorded on the diaspidids *Chionaspis* sp. and *Lepidosaphes* sp. (Petch 1925, Zare & Gams 2003). The teleomorph is *Torrubiella confragosa* Mains (Zare & Gams 2003). Hyphae penetrate bodies of hosts, sporulate, and eventually kill the host (Zare & Games 2003). Conidia are formed in heads at the apex of phialides. The conidia are typically short-ellipsoidal, 2.5–3.5(–4·2) × 1–1·5 μm, and homogeneous in size and shape (Zare & Gams 2003).
Epizootics are common in autumn in orchards on the central coast of New South Wales, where entire groves of young trees can be, though not commonly, heavily infested with black scale to the extent that the soil surface beneath trees can be stained black with sooty mould shed from canopies (Beattie, pers. comm., 2009). The optimal temperatures for growth are 21 to 24°C. Growth does not occur at or above 33°C (Petch 1925, Barson 1976, Evans & Hywel-Jones 1997). Mendel et al. (1984) stated that *Lecanicillium lecanii* requires high humidity (approaching the saturation point) and mild temperatures (23°C) for spore germination. Therefore, high infectivity was observed during wet winters. He cited research in Israel that showed that overhead irrigation prolonged the activity of the pathogen.

### 2.4. Impacts of ants on biological control of soft and armoured scales


Flanders (1943c) mentioned that Argentine ant, while engaging in gathering the honeydew excreted by mealybugs, soft scale and aphids, performed two important services: a sanitary service to prevent accumulation of honeydew and military service to protect scales from natural enemies. Flanders (1943, 1951) mentioned that many natural enemies were not subjected to interference by ants. Flanders (1943c) noted that Argentine ant was able to interfere the activity of *Metaphycus helvolus* as it needs to host-feed on black scale, and oviposts. In contrast, *Coccophagus* species and
Metaphycus stanleyi were not readily disturbed by the ant as they do not require host feeding and can lay their eggs more quickly (Flanders 1951).

A number of studies have claimed detrimental impacts of honewdew-feeding ants on natural enemies of red scale (Flanders 1945, DeBach 1951, Steyn 1954a,b, DeBach 1959, Bedford 1968, Samways 1982, Moreno et al. 1987, Murdoch et al. 1995, James et al. 1997, 1999, Martinez-Ferrer et al. 2003, Pekas et al. 2010). Flanders (1945) reported that Argentine ant disrupted activity of Comperiella bifasciata on yellow scale in California. DeBach (1951) reported that Argentine ant reduced parasitism by Aphytis chrysomphali, but not Rhyzobius lophanthae activity. DeBach (1959) stated that heavy infestations of red scale in California were related to Argentine ant. Steyn (1954a, b) reported negative impacts of the cosmopolitan brown house ant, Pheidole megacephala F, and pugnacious ant, Anoplepis custodiens Smith, respectively, on Aphytis species and coccinellid predators of red scale in Letaba, South Africa. Martinez-Ferrer et al. (2003) reported the relative influence of southern fire ant (Solenopsis xyloni McCook), Argentine ant and native gray ant (Formica aerata (Francoeur)) on parasitism of red scale by Comperiella bifasciata and Aphytis melinus in laboratory studies. All three species reduced parasitism by Comperiella bifasciata and parasitism and host mutilation by Aphytis melinus. Southern fire ant was the least disruptive and native gray ant the most disruptive. Southern fire ant removed 12% of scale from the lemons, presumably to feed on them, while the other ant species did not exhibit significant removal of scale compared to the controls. Parasitism by Comperiella bifasciata was more than twice the level exhibited by Aphytis melinus. Percentage mutilation of the scale, including probing and host feeding, was nearly 5-fold higher for Aphytis melinus than for Comperiella bifasciata. Martinez-Ferrer et al. (2003) concluded that because Aphytis melinus required a longer total host examination oviposition period in the absence of ants than Comperiella bifasciata, and because oviposition occurs as the last act in a sequence of behaviours, disruption by the ants had a more significant negative effect on oviposition by Aphytis melinus.

Hely et al. (1982) referred to small black ants, Iridomyrmex sp., being attracted to honeydew produced by Saissetia oleae on citrus orchards in New South Wales, and noted that they were more notably attracted and active during the nymph stages. However, only limited studies have been undertaken their seasonal activity in these orchards and their impacts on natural enemies of soft and armoured scales.
Stevens et al. (1998) used pitfall traps to monitor seasonal activity of ants in two citrus orchards in the Murrumbidgee Irrigation Areas of New South Wales for 22–24 months in 1992–1994. The orchards were at Yanco (Farm 1139/40, 34° 36' S, 146° 25' E, on red clay/loam soil) and Cudgel (Farm 1559, 34° 39' S, 146° 26' E on sandhills). *Iridomyrmex rufoniger* (Lowne) group of species were the most dominant of 24 species of ants recorded. Total ant captures showed a positive correlation with mean daily temperatures. Whilst all taxa were less active during winter, some species, including *Iridomyrmex rufoniger* group species 2, *Pheidole* sp. and *Rhytidoponera metallica* (F. Smith) continued to forage at reduced levels. Other species of *Iridomyrmex*, including *Iridomyrmex rufoniger* group species 1 and *Iridomyrmex purpureus* ceased foraging almost entirely during June-September.

In another study, Stevens et al. (2007) used pitfall traps to assess the abundance of ants in 20 orange groves, 10 in MIA within a 15-km radius of Leeton (34° 32' S, 146° 24' E) in New South Wales, and 10 in the Sunraysia districts of New South Wales and Victoria within a 20-km radius of Mildura (34° 11' S, 142° 09' E). The most predominant ant species on trees were ants within the *Iridomyrmex rufoniger* species group that accounted for 74% of trapped fauna. *Iriomyrmex purpureus* (F. Smith) represented only 0.9% of fauna (Stevens et al. 2007).

James et al. (1997) evaluated the impact of *Iridomyrmex rufoniger* group species on citrus canopy populations of soft brown scale insect (*Coccus hesperidum* L.) and red scale during two seasons (spring-autumn) from October 1993 to April 1995 in a grove 7 km east of Leeton. Numbers of both scales were substantially greater (3–12 times) in trees containing foraging ants than in trees from which ants were excluded. This was attributed to disruption of normal activities of natural enemies of both scales.

Studies concerning ants as vectors of entomopathogenic fungi have largely been neglected (Bird et al. 2004). Bird et al. (2004) considered this surprising given reports that ants can vector *Fusarium* wilt of cocoa on their cuticles (Gracia-Garza et al. 1997) and *Lecanicillium longisporum* (Zimmerman) Zare & Gams to the black bean aphid, *Aphis fabae* Scopoli, resulting in significant decline of populations of the aphid due to fungus infection (Flower 2002). In laboratory studies, Bird et al. (2004) observed *Lecanicillium longisporum* conidia on the tarsi, antennae and mandibles of *Lasius niger* L. The ant was not susceptible to infection and workers artificially contaminated with conidia caused 68.3, 30.8 and 3.7% infection of aphids under laboratory, semi-field and field conditions, respectively. In a more recent study, Douglas Jackson (University of Michigan), recorded dispersal of *Lecanicillium lecanii* by an arboreal-nesting ant,
*Azteca instabilis* F. Smith, which has a mutualistic relationship with green coffee scale. Although laboratory experiments demonstrated that *Azteca instabilis* is capable of transporting *Lecanicillium lecanii* conidia, field experiments suggested that the ant’s role as a vector of the entomopathogen is negligible under field conditions (Jackson, pers. comm., December 2011).
Chapter 3. General materials and methods

3.1. Experimental locations and orchard descriptions

Field observations and assessments were conducted in seven citrus orchards at six locations on the Central Coast of New South Wales (Fig. 3.1: from north to south):

- an orchard at Kulnura (33° 13’ S, 151° 13’ E, 351 m asl) with Eureka lemon (*Citrus × limon* (L.) Osbeck), and Washington navel and Valencia orange (*Citrus × aurantium* L.) trees (Lister’s);
- an orchard at Kulnura (33° 14’ S, 151° 13’ E, 332 m asl) with Eureka lemon, Washington navel and Valencia trees (Hitchcock’s);
- an orchard at Somersby (33° 22’ S, 151° 16’ E, 252 m asl) with Eureka lemon, Washington navel and Valencia trees (Britten’s);
- an orchard at Lower Portland (33° 26’ S, 150° 52’ E, 6–12 m asl) with Washington navel and Valencia trees (Wallis’);
- an orchard at Cornwallis (33° 35’ S, 150° 29’ E, 12 m asl) with Washington navel and Valencia trees (Gardiner’s);
- an orchard on the Hawkesbury campus of the University of Western Sydney at Richmond (33° 36’ S, 150° 44’ E, 23 m asl) with Washington navel and Valencia orange trees; and
- an orchard at Castlereagh (33° 40’ S, 150° 40’ E, 23 m asl) with Washington navel and Valencia trees, and some grapefruit (*Citrus × aurantium* L.) and blood orange trees (Hartog’s).

The orchards were chosen on the basis of accessibility by bicycle, bus, train and car from the University of Western Sydney at Richmond, and for the presence of red scale and/or black scale. Choice was limited by the number of commercial orchards. Six orchards were used to determine the phenology of the scale. The orchards at Somersby, Lower Portland, Cornwallis and Castlereagh were used for general assessments of scale densities, percent parasitism, incidence of predators, and predation. Lister’s orchard at Kulnura was used to determine the impact of an ant, *Iridomyrmex rufoniger*, on parasitoids of red scale and predators of red scale and black scale (Chapter 7).
Figure 3.1. Location of study orchards (north to south on the Central Coast of New South Wales): Kulnura and Somersby on Somersby Plateau, and Lower Portland, Richmond, Cornwallis, and Castlereagh in the Hawkesbury Valley.
3.1.1. Kulnura

The study blocks on Lister’s orchard at Kulnura comprised, among a total of 9 blocks, three Hamlin orange blocks (blocks 1, 2 and 3 in Fig. 3.2) used to study the impact of *Iridomyrmex rufoniger* on parasitoids and predators of red scale, and predators of black scale, a block of Eureka lemon trees, and four mature Washington navel orange blocks and two Valencia orange blocks that were used to survey impacts of the ant on natural enemies of red scale. Further details are given in Chapter 7.

![Study blocks 1–3 used to determine the impact of *Iridomyrmex rufoniger* on red scale populations in the Lister’s orchard at Kulnura.](image)

Hitchcock’s orchard at Kulnura comprised one block of Eureka lemon trees and 3 blocks of Valencia orange trees, with rows in each block running north-south (Fig. 3.3). These four blocks were used to monitor the phenology of red scale with male pheromone traps. Trees in the lemon block (about 260 trees) were planted on a 3.5 × 6 m grid initially and were 3–4 m high when studies commenced in October 2008. In spring 2010 they were heavily pruned to 1.5–2 m-high. By the end of the study (December 2011), they were 3 m-high. Valencia orange blocs 2 comprised some 260 mature 3–4 m-high trees planted on a 4 × 4 m grid. Valencia block 3 comprised some
1,130 mature 3–4 m-high trees planted on a 4 × 4 m grid. Valencia orange block 4 comprised about 850 young 2 m-high trees planted on 4 × 4 m grid. The blocks were surrounded by native vegetation and adjacent blocks of lemon and orange trees.

![Figure 3.3. Study blocks 1–4 in Hitchcock’s orchard at Kulnura: block 1, Eureka lemon, blocks 2-4, Valencia orange.](image)

### 3.1.2. Somersby

Study blocks in Britten’s orchard at Somersby initially comprised three blocks of Washington navel orange trees (blocks 1, 2 & 4) and one blocks of Valencia orange trees (block 3) (Fig. 3.4).

- block 1 comprised about 1,600 mature 3–4 m-high Washington navel trees planted on 3.7 × 6.5 m grid in single rows running southwest to north-east in the southern sector, then curving north in the northwest corner. The trees were removed in winter 2009 and the block planted to avocado. Block 5 was then used in its place.

- block 2 comprised about 2,000 mature 2.5–4 m-high Washington navel trees planted in double-planted rows running approximately north-south. Trees within rows were planted on a 3 × 3.2 m grid. The distance between the outer trees in each row was 4.3 m.

- block 3 comprised about 1,300 mature 2.5–4 m-high Valencia trees planted in double-planted, rows running approximately east-west. Tree within rows were
planted on a $3 \times 3.2$ m grid. The distance between the outer trees in each row was 4.3 m.

- block 4 comprised about 750 mature 3–4 m-high Washington navel trees planted on 3–4 m grid in double rows running approximately east-west. Tree within rows were planted on a $3 \times 3.2$ m grid. The distance between the outer trees in each row was 4.3 m.

- block 5 comprised about 1,140 mature 2.5–4 m-high Washington navel trees planted in double-planted rows running approximately north-south. Tree within rows were planted on a $3 \times 3.2$ m grid. The distance between the outer trees in each row was 4.3 m. Trees in this block were removed in winter 2010 and the block planted to avocado. Block 6 was then used in its place.

- block 6 comprise about 300 mature 2–3 m-high Eureka lemon trees, with trees planted in 10 rows on a $3 \times 3.2$ m grid.

Blocks 1–4 were used to monitor adult red scale phenology with pheromone traps from date to date. Blocks 2, 3, 4 and 5 were then used for this purpose until date. Block 6 was used for the purpose after trees in block 5 were removed. Blocks 1 & 2 were used for assessments of scale population densities, parasitism, incidence of predators, and predation from December 2008 to June 2009. Blocks 2 and 3 were then used for these purposes from November 2009 to July 2011.

![Study blocks 1–6 in Britten’s orchard at Somersby: blocks 1, 2, 4 & 5, Washington navel orange; block 3, Valencia orange; block 6, Eureka lemon.](image)
3.1.3. Lower Portland

Study blocks in Wallis’ orchard at Lower Portland, on the left bank of the Hawkesbury River, comprised a small Valencia orange block (block 1) and a larger Washington navel orange block (block 2) to the east (Fig. 3.5). The Valencia orange block comprised 60, 1.5–3 m-high trees planted on a 4 × 6 m grid, with rows running north-south. Block 2 comprised about 550, 3–4 m high trees planted on a 4 × 6 m grid, with rows running north-south. The orchard is shadowed in the early morning by hills rising to 100 m in the east, and by similar hills to the west in the afternoon.

![Figure 3.5. Study blocks 1 and 2 in Wallis' orchard at Lower Portland: block 1, Valencia orange; block 2 Washington navel orange.]

3.1.4. Richmond

Study sites on the Hawkesbury campus of the University of Western Sydney at Richmond comprised two blocks, one smaller than the other (Fig. 3.6). The smallest block (block 1) comprised a mixture of 30 Washington navel orange and 21 Valencia orange trees planted in two rows on a 3.2 × 6.3 grid, with trees ranging in height from of 3 to 4.5 m, and rows running northeast to southwest to. The larger block (block 2) comprised 140, 3–4 m-high Valencia orange trees, planted on a 3.3 × 4.4 m grid in 7 rows running northwest to southeast.
3.1.5. Cornwallis

Study blocks in Gardiner’s orchard at Cornwallis comprised two mixed Washington navel and Valencia orange blocks (Fig. 3.7). Block 1 comprised 2,000 trees in 30 rows of Washington navel orange trees and 10 rows of Valencia orange trees at the eastern end. Block 2 comprised 2,500 trees in 25 rows of Washington navel orange trees and 25 rows of Valencia trees at the western end. Of these, 1,800 trees in the 44 rows in the eastern end were used for scale densities, parasitism and footprints assessments. Of these, 1,125 trees in 25 rows were Washington navel and 755 trees in the 17 rows were Valencia orange. All trees were 4 to 5 m-high and planted on a $3 \times 6$ m grid, with rows running northeast to southwest. Canopies tended to overlap across inter-row spaces.
3.1.6. Castlereagh

Study sites in Hartog’s orchard at Castlereagh comprised a mixed-age and interplanted Valencia orange and mandarin block (block 1) with about 180 trees and an adjoining block of about 200 mixed-age trees (mostly Valencia), two Washington navel orange blocks (block 2 with about 400 trees and block 3 with about 350 trees), a mixed block of 60 blood orange and 20 grapefruit trees (block 4), and Valencia orange block (block 5). Blocks 1–3 and 5 were used to assess adult male phenology for the course of the study. Blocks 2 and 3 were used for general assessments of scale densities, percent parasitism, incidence of predators, and predation from December 2008 to June 2009. Block 2 was as horse paddock from August 2009. Blocks 3 and 5 were then used for general assessments of scale densities, percent parasitism, incidence of predators, and predation from November 2009 to July 2011. Block 5 comprised of 110 mature Valencia orange trees. Tree sizes and planting distances varied but most trees in blocks 2, 3 and, to less extent, 5 were 4–5 m high and planted on a 3.2 × 6.5 m grid. Tree health declined, particularly in block 3, due to neglect over the term of the study. All rows ran north-south. Blocks 1 and 2 were used from November 2008 to May 2009 to assess the phenology of life cycle stages of red scale, and parasitism. From November 2009 to the end of the study blocks 1, 3 and 5 were used for these purposes. Incidence of predators was based on counts on blocks 3 and 5. Predation was assessed in block 3.
3.2. Degree-day (°D) accumulations

Degree-day (°D) accumulations were based on temperatures recorded by Gemini Tinytag Plus Data Loggers (Hastings Data Loggers, Port Macquarie, NSW) placed at fixed locations in each of the six study orchards, Bureau of Meteorology records when data-loggers malfunction (rarely), and on equations given by Watson & Beattie (1995). Two dataloggers were placed in each orchard, one recorded ambient temperatures every 2 h, the other relative humidity every 2 h. Loggers at Somersby, Lower Portland and Richmond, the loggers were placed, 1.2 m above ground level, in a Stevenson screen (Fig. 3.9). Loggers at Kulnura, Cornwallis and Castlereagh were housed in ventilated 150 mm diameter and 300 mm long lengths of white polypropylene pipe suspended within a tree canopies and positioned so that each datalogger was 1.2 m above ground level (Fig. 3.10).

°D accumulations were based on a lower developmental threshold of 11.6°C and a log interval of 2 h. Upper developmental thresholds are not considered, so that the term 'threshold' refers exclusively to the lower developmental threshold. Degree-days were calculated thus:

![Figure 3.8. Study blocks 1–5 in Hartog’s orchard at Castlereagh.](image)
\[ \theta D = \left[ \sum_{i=1}^{n} (T_i - LT) \right] / n(T_i > LT) \]

where \( T_i \) is temperature at time \( i \), \( LT \) is lower threshold, and \( n \) is number of log intervals per day.

Annual \( \theta D \) accumulations were based on records from the winter solstice (21 June) of each year during the study.

**Figure 3.9.** A Stevenson screen in block 2 in Britten’s orchard at Somersby: Andrew Beattie (left) & Hang Thi Dao (right).
Figure 3.10. A white 150 mm diameter, 300 mm long, polypropylene pipe housing a Tinytag datalogger within a Washington navel orange canopy in block 1 in the orchard at Cornwallis: the open base and four 3 cm diameter holes near the top of each pipe allowed air to move freely.
Table 3.1. Summary of orchard management practices (based on grower records).

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<thead>
<tr>
<th>Practice</th>
<th>Lister’s</th>
<th>Hitchcock’s</th>
<th>Britten’s</th>
<th>Wallis’</th>
<th>Gardiner’s</th>
<th>UWS</th>
<th>Hartog’s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fertilisers</td>
<td>Nitrophoska Blue Special (Incitec Pivot Limited): 12.0% N, 5.2% P, 14.1% K, 6.0% S at approximately 150 g per tree once annually</td>
<td>Nitrophoska Blue Special at approximately 1 kg per tree twice annually</td>
<td>chicken manure once annually at about 6 t/ha</td>
<td>not applied</td>
<td>chicken manure, and foliar applications of K-Forte</td>
<td>Incitec Pivot Grower 12 in 2009</td>
<td>not applied</td>
</tr>
<tr>
<td>Irrigation</td>
<td>no irrigation</td>
<td>overhead, rarely</td>
<td>under-tree microjet</td>
<td>overhead, rarely</td>
<td>overhead, frequently in summer and when cold and dry in winter</td>
<td>overhead, rarely</td>
<td>under-tree microjet, rarely in 2008–2009; not thereafter</td>
</tr>
<tr>
<td>Pest &amp; disease management</td>
<td>copper oxide (Nordox 75 WG – Nordox As) in November 2010</td>
<td>Nordox at petal fall with 0.5% (v/v) nC24 mineral oil</td>
<td>copper at petal fall and 6 and 16 weeks thereafter: Dupont Kocide Ultra Blue for Washington navel trees, and Champ Dry Prill for Valencia trees</td>
<td>copper at petal fall with 0.5% (v/v) nC24 agricultural mineral oil</td>
<td>zineb for rust mites, May 2010; dimethoate for control of fruit fly in Washington navel trees, in May, and Valencia trees in, November, 2010; copper at petal fall and 6 and 16 weeks thereafter; 0.5% (v/v) nC24 agricultural mineral oil with copper if citrus leafminer present</td>
<td>not applied</td>
<td>1% copper oxychloride with 0.5% (v/v) nC24 mineral oil in October 2008; no sprays thereafter</td>
</tr>
<tr>
<td>Weed management</td>
<td>mowing and skirt applications of glyphosate</td>
<td>mowing and skirt applications of glyphosate</td>
<td>mowing and skirt applications of glyphosate</td>
<td>mowing (rarely): orchard floor often covered with thick long grass</td>
<td>mowing and skirt applications of glyphosate, for control of ‘</td>
<td>mowing and under skirt applications of glyphosate monthly from late</td>
<td>mowing (rarely): orchard floor often covered with thick long grass</td>
</tr>
<tr>
<td>Other practices</td>
<td>lemon trees pruned in late 2010</td>
<td>irregular on-going hedging of rows of trees in block 2, never both sides or all trees simultaneously</td>
<td>interrow spaces ploughed twice between November 2009 and January 2010</td>
<td>spring to early autumn and every second month from late autumn to mid spring</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Chapter 4. Red scale phenology on the Central Coast of New South Wales

4.1. Introduction

Knowledge of the phenology of an insect pest is fundamental to understanding its ecology (Schwartz 2003). Red scale has 2–5 annual generations in New South Wales (Smith et al. 1997). Based on Bureau of Meteorology records for Richmond Airport (33º 36' S, 151º 45' E, 19 m asl, on the Hawkesbury River) and Mangrove Mountain (33º 18' S, 151º 11' E, 305 m asl, on the Somersby Plateau) and a generation time of 650ºD at a lower development threshold of 11.6ºC (Kennett & Hoffman 1985, Murdoch et al. 1995), I estimated that 2–3 generations may occur annually on the Central Coast of New South Wales. These calculations also indicated that the potential for 3 annual generations was greater in riparian orchards along the Hawkesbury River than in orchards on the Somersby Plateau.

In California and South Africa, red scale male (Fig. 4.1) traps based on the use of synthetic female pheromone have been used since the early 1980s to determine red scale phenology (Moreno & Kennett 1985, Morse et al. 1985, Grout et al. 1989, Flint 1984). There is no published record of such use in Australia. In this chapter, I used the pheromone traps to determine, as accurately as possible, the number of generations of the scale in four riparian orchards along the Hawkesbury River and two orchards on the Somersby Plateau. I compared these results with estimates based on the abundance of life cycle stages of the scale on fruit sampled for studies on levels of parasitism and predation (see Chapter 5). I also derived linear regressions for relationships between spring and summer generation male flight peaks, and summer and autumn generation male flight peaks. Because extreme temperatures are known to affect red scale mortality and flight activity of adult males (Abdelrahman 1974a, Tashiro & Beavers 1968, Yan & Isman 1986), I also assessed the impact of ambient temperatures, particularly at sunset during winter and during a record heat wave in early 2011, on the phenology of the scale.
4.2. Material and methods

4.2.1. Ambient temperature and °D calculations

Ambient temperatures were measured, and °D accumulation calculated, according to procedures outlined in Chapter 3. Annual °D accumulations were based on records from the winter solstice (21 June). Numbers of days on which minimum temperatures ≤ 2°C occurred were also recorded: an observed temperature of 2.2°C in a Stevenson screen 1.2 m above the ground indicates frost.\(^{38}\)

Daily temperatures at sunset, 1 h and 45 min before and 45 min after sunset, were extracted from temperature records obtained from Australian Bureau of Meteorology for the Richmond Airport and Mangrove Mountain weather stations. Sunset and twilight

times were estimated from Geoscience Australia\textsuperscript{39} based on Australian Eastern Standard Time (AEST).

\subsection*{4.2.2. Male flight phenology}

Adult males were captured in traps housing a lure – a slow release septum impregnated with a red scale female-specific pheromone, racemic 3-methyl-6-isopropenyl-9-deceny acetate (3-R,S- 6-R,S) (Scentry Biologicals Inc., 610 Central Avenue, Billings, Montana 59102, United States of America\textsuperscript{40}). A single lure was located at the apex of each trap (Fig. 4.2), which was made from laminated white paper (110 mm × 300 mm) marked with a 10–20 mm grid pattern, folded centrally and acutely at approximately 40°. The inner marked surface of each card was coated thinly with polybutene (Tanglefoot\textsuperscript{®}, Australian Entomological Supplies, Sydney, Australia), and hung, apex upward, from a wire hanger suspended from a branch 1.8 to 2.4 m above ground level, within the canopy of a randomly selected tree in near the centre of each block (see Flint et al. 1984). Septa were replaced every 4 to 6 weeks. One trap was placed in each of four blocks of trees at:

- Kulnura (one Eureka lemon block and three Valencia orange blocks in Hitchcock’s orchard);
- Somersby (initially, three Washington navel orange blocks and one Valencia orange block; then, from August 2010, two navel orange blocks, one Valencia block, and one Eureka lemon block in Britten’s orchard);
- Castlereagh (two Valencia orange blocks, one Washington navel orange block and one block comprising a mixed planting of grapefruit, lemon and blood orange trees in Hartog’s orchard).

Two traps were used in each of two blocks at:

- Cornwallis (both mostly Washington navel orange with several rows of Valencia orange in Gardiner’s orchard); and
- Richmond (one Washington navel and one a mixture of Washington navel and Valencia orange at the University of Western Sydney, Hawkesbury).

\textsuperscript{39}http://www.ga.gov.au/geodesy/astro/sunrise.jsp
\textsuperscript{40}http://www.scentry.com/images/ScentryCatalog%20Regular.pdf
Two traps were placed in a Washington navel orange block, and one trap was placed in a Valencia orange block in Wallis’ orchard at Lower Portland.

![Figure 4.2. A red scale pheromone trap comprising a pheromone lure (red septa) and tent-like sticky trap made from laminated paper coated on the inner surface with polybutene.](image)

The details of numbers of trees in each block were given in Chapter 3. Traps were replaced every 7–20 d, depending on the time of the year. The number of males on each trap was counted under a Wild M7S stereomicroscope (Leica Microsystems Pty Ltd., North Ryde, Australia).

4.2.3. Proportions of red scale life cycle stages on fruit

Samples of scale-infested fruit were collected from my study orchards at Somersby, Lower Portland, Cornwallis, and Castlereagh every 15 to 25 d (at approximately 200–300ºD intervals) from November 2009 to July 2010. Numbers of fruit sampled on each occasion depended on scale densities. Fruit were picked evenly but otherwise randomly from trees within each block, with the exception of perimeter trees (except those in the Valencia block at Lower Portland) and trees used to assess scale density. Sampled fruit were placed in an insulated bag for a few hours and then in a cool room at 7 to 8°C for up to 7 d. My objective was to obtain and examine at least 100 live adult females on each occasion. However, on several occasions, when scale population densities on fruit
were very low, as in early season samples from November to early January, numbers of adult female assessed were less than 100. The fruit were used in this instance to determine proportions of life cycle stages of live red scale on the fruit. All fruit were examined with a Wild M7S stereomicroscope. I used the same fruit to assess parasitism and predation of the scale (see Chapter 5).

4.2.4. Data analysis

Average numbers of adult males trapped per sampling interval in each orchard were divided by the number of days each set of four traps was in the field. For each season, these data were then plotted against °D accumulations from the winter solstice at the beginning of the season to the winter solstice at the end of the season. Log-transformed (log (x + 1)) data were plotted in a similar manner. Proportions of second instar, second moult, virgin, and mated female scale recorded during laboratory assessments of parasitism and predation on scale-infested fruit sampled in 2009–2010 (see Chapter 5) were plotted against °D from the winter solstice on 21 June 2009. Sigmaplot® 11.0 Version (Systat Software Inc, San Jose, California, United States of America) was used to graph the data. In order to relate the size of populations in late winter/early spring to the size of summer populations in all orchards, I regressed (using the statistical program in Microsoft Excel 2007), for each orchard, average numbers of males trapped during late winter/early spring against average numbers of males trapped during summer.

4.3. Results

4.3.1. Ambient temperatures

Ambient temperatures at Richmond Airport and Mangrove Mountain at the time sunset occurred daily in winter and spring in 2008, 2009 and 2010 are presented in Appendix I. It was colder at sunset during winter (92 d) and during winter and spring (183 d) at Mangrove Mountain than it was at Richmond Airport (Table 4.1). During winter, average numbers of days over four years (2008–2011) on which winter sunset temperatures were ≤ 16°C at the two locations were 85 d and 75.5 d, respectively (Table 4.1). During winter and spring, the average number of days over three years (2008–2010) on which winter sunset temperatures were ≤ 16°C at the two locations was 126 d and 98 d, respectively (Table 4.1). Other records from the two weather stations also indicated that temperatures during twilight, including at sunset, were below ≤ 16°C on
most days from late May (i.e., before the winter solstice), to early September. Winter, and winter and spring sunset temperatures ≤ 16°C were less frequent in 2009 than in 2008 and 2010; the average number of days in winter and spring on which sunset temperatures recorded at both weather stations in 2009 were ≤ 16°C was 103 d compared averages of 116.5 d for combined records in both 2008 and 2010.

The number of days on which minimum ambient temperatures ≤ 2°C were recorded in my study orchards during 2009, 2010 and 2011 are presented, with the exception of Kulnura, in Table 4.2. These records, and Bureau of Meteorology records summarised in Table 4.1, indicate that the coldest winter during my studies from January 2008 to December 2011 occurred in 2010. They indicate that fewer frosts would have occurred at Somersby, Lower Portland, Cornwallis, Richmond and Castlereagh in 2009 and 2011 than in 2010, most noticeably at Somersby and Lower Portland. Among the Hawkesbury Valley orchards, the least number of frosts occurred at Cornwallis. I attribute this to the size of trees in the orchard being larger than those in the other orchards and, as a result, also to their canopies touching across rows. Moreover, overhead irrigation was used in this orchard in all seasons; in winter, in part, to reduce the impact of frosts: the other orchards in my study were not irrigated or occasionally irrigated in summer.

Table 4.1. Number of days when the ambient temperature at sunset was ≤ 16°C at Richmond Airport and Mangrove Mountain in winter (92 d), and winter and spring (183 d) during 2008, 2009 and 2011: based on Australian Bureau of Meteorology records.

<table>
<thead>
<tr>
<th>Location</th>
<th>2008 winter</th>
<th>2009 winter &amp; spring</th>
<th>2010 winter</th>
<th>2010 winter &amp; spring</th>
<th>2011 winter</th>
<th>2011 winter &amp; spring</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mangrove Mountain</td>
<td>87</td>
<td>130</td>
<td>78</td>
<td>116</td>
<td>88</td>
<td>133</td>
</tr>
<tr>
<td>Richmond Airport</td>
<td>78</td>
<td>103</td>
<td>56</td>
<td>90</td>
<td>81</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 4.2. Number of days when daily minimum temperatures in my study orchards on were ≤ 2°C 2009, 2010 and 2011: based on temperatures recorded within each orchard.

<table>
<thead>
<tr>
<th>Location</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kulnura</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Somersby</td>
<td>3</td>
<td>17</td>
<td>8</td>
</tr>
<tr>
<td>Lower Portland</td>
<td>23</td>
<td>49</td>
<td>38</td>
</tr>
<tr>
<td>Cornwallis</td>
<td>22</td>
<td>25</td>
<td>28</td>
</tr>
<tr>
<td>Richmond</td>
<td>41</td>
<td>45</td>
<td>41</td>
</tr>
<tr>
<td>Castlereagh</td>
<td>41</td>
<td>47</td>
<td>44</td>
</tr>
</tbody>
</table>
4.3.2. Male flight phenology

Trap counts indicated that male flights peaked three times annually in citrus orchards on the Central Coast of New South Wales and that the timing of these peaks varied with season. Spring peaks occurred between 20 September and 20 October, summer peaks between 20 December and 20 January and autumn peaks between 20 February and 30 March (Figs. 4.3–4.8). Spring troughs occurred between 20 October and 15 November, summer troughs occurred between 20 January and 20 February and winter troughs occurred between 16 May and 25 August.

Very few or no males were trapped from mid June to mid September (Figs. 4.3–4.8), when temperatures during sunset were \( \leq 16^\circ\text{C} \). There was a positive correlation between numbers of days on which no males were trapped and number of days when temperatures at sunset were \( \leq 16^\circ\text{C} \) from 1 July to 31 August over three seasons from 2009–2011 \( (r^2 = 0.28; P = 0.031) \) (Fig. 4.9). The shortest intervals over which no males were trapped occurred at Castlereagh and Richmond, followed by Cornwallis and Lower Portland. The longest intervals were at Somersby and Kulnura.

Spring flight activity (from the winter trough to spring trough) generally commenced in last week of August and ended in the first week of November. Summer flight activity (from the spring trough to the summer trough) generally commenced in the first week of November and ended in the second week of February. Autumn flight activity (from the summer trough to the late autumn/early winter trough) generally commenced in the second last week of February and ended in the second last week of May (Table 4.5).

Significant positive regression correlations were derived for total numbers of males trapped during intervals over which male flight activity occurred in winter/spring and summer \( (r^2 = 0.367; P = 0.047) \), and total numbers of males trapped during intervals over which activity occurred in summer and autumn \( (r^2 = 0.48; P = 0.017) \), respectively (Figs. 4.10–4.12).
Figure 4.3. Average number ± SE (above) and log number ± SE (below) of red scale males caught in traps (n = 4) in Hitchcock’s orchard at Kulnura.
Figure 4.4. Average number ± SE (above) and log number ± SE (below) of red scale males caught in traps (n = 4) in Britten’s orchard at Somersby.
Figure 4.5. Average number ± SE (above) and log number ± SE (below) of red scale males caught in traps (n = 3) in Wallis’ orchard at Lower Portland.
Figure 4.6. Average number ± SE (above) and log number ± SE (below) of red scale males caught in traps (n = 4) in Gardiner’s orchard at Cornwallis.
Figure 4.7. Average number ± SE (above) and log number ± SE (below) of red scale males caught in traps (n = 4) at the University of Western Sydney, Richmond.
Figure 4.8. Average number ± SE (above) and log number ± SE (below) of red scale males caught in traps (n = 4) in Hartog’s orchard at Castlereagh.
Figure 4.9. Regression relationship between numbers of days on which no males were trapped in all orchards and numbers of days from 1 July to 31 August in 2009, 2010 and 2011 when temperatures at sunset were ≤ 16°C ($r^2 = 0.24; P = 0.031$). Data were log transformed.

Figure 4.10. Regression relationship between numbers of males trapped during spring and summer peaks of male flight activity in all study orchards over two seasons 2009–2010 and 2010–2011 ($r^2 = 0.367; P = 0.047$). Data were log transformed.
Figure 4.11. Regression relationship \( r^2 = 0.48; P = 0.017 \) between red scale males trapped during summer and autumn peaks male flight activity in all study orchards over two seasons 2009–2010 and 2010–2011. Data were log transformed.

Figure 4.12. Regression relationship \( r^2 = 0.64; P = 0.0094 \) between numbers of red scale males trapped during summer and autumn peaks of male flight activity in citrus orchards at Richmond, Cornwallis and Lower Portland between 2009–2011. Data from Somersby, where the highest levels of parasitism occurred, and data from Castlereagh, where the highest incidence of predation occurred among the study orchards, were excluded in order to determine if high natural enemy activity influenced the regression of summer and autumn trap data. The regression based on data from all orchards was presented in Fig. 4.11. Data were log transformed.
Average °D accumulations between the spring peak and the summer peak, the summer peak and the autumn peak, and the autumn peak to the following spring peak over two seasons were 778.9 ± 36.8, 682.8 ± 36.6 and 688.8 ± 44.5°D, respectively (Table 4.4). °D accumulations between generation peaks in male flight activity varied amongst orchards and seasons (Table 4.4). In 2009–2010, °D accumulations from the spring peaks to the summer peaks were greater in the orchards at Somersby, Richmond and Castlereagh than at Lower Portland and Cornwallis. In contrast, °D accumulations from the summer peaks to the autumn peaks were greater in the orchards at Lower Portland and Cornwallis than at Somersby, Richmond and Castlereagh. There was less variation in accumulations from the autumn peaks to the following spring peaks. In 2010–2011, °D accumulations between the summer and the autumn peaks were greater than between the spring and the summer peaks in all orchards. This was attributed to the impact of a record heatwave in January/February 2011.

°D accumulations between generation troughs and number of days related to seasonal flight activity are presented in Table 4.5. Average °D accumulations from the winter solstice to the spring trough, the spring trough to the summer trough and the summer trough to the autumn trough were 419 ± 17.3, 999.6 ± 24.5 and 794.6 ± 33 d, respectively, over the three seasons for all orchards, except the orchard at Kulnura where records for 2010–2011 were affected by management practices. Average numbers of days related to seasonal flight activity were approximately 60, 80 and 120 d in spring, summer and autumn, respectively (Table 4.5). The longer duration of male flight activity in summer and autumn reflected the occurrence of several cohorts in one generation. The extending duration in autumn also indicated that low temperatures slowed the development and emergence of adult males.

The results indicated that flight activity in the spring generation generally occurred earlier in the orchards at Richmond and Castlereagh than in other study orchards. Data for 2010–2011 showed clear differences in timing of male flight activity in the spring generation. Flight activity occurred in early September at Castlereagh and Richmond, late September at Cornwallis and Lower Portland, and in early October at Somersby. Differences between Somersby Plateau orchards and the Hawkesbury Valley orchards were attributed to the differences in numbers of days when temperatures were ≤ 16°C at sunset (Table 4.1).
Variation also occurred for °D accumulations between intergeneration troughs in male flight activity and the number of days within each generation from the date when traps in which males were trapped were placed in each orchard to the date of the subsequent generation trough (Table 4.5). In all orchards, °D accumulations from the winter solstice to the spring trough were lower than those from the spring trough to summer trough. Spring flight activity commenced later in the 2010–2011 season (September) than in 2009–2010 season (August).

**Table 4.4.** Estimated °D accumulations at 11.6°C between peaks in male red scale flight activity in 2009–2010 and 2010–2011 at Kulnura, Somersby, Lower Portland, Cornwallis and Castlereagh on the Central Coast of New South Wales.

<table>
<thead>
<tr>
<th>Orchard</th>
<th>spring to summer peak °D</th>
<th>summer to autumn peak °D</th>
<th>autumn to spring peak °D</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>2009-2010</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kulnura</td>
<td>908</td>
<td>625</td>
<td>766</td>
</tr>
<tr>
<td>Somersby</td>
<td>923</td>
<td>507</td>
<td>782</td>
</tr>
<tr>
<td>Lower Portland</td>
<td>738</td>
<td>595</td>
<td>720</td>
</tr>
<tr>
<td>Cornwallis</td>
<td>734</td>
<td>607</td>
<td>766</td>
</tr>
<tr>
<td>Richmond</td>
<td>981</td>
<td>601</td>
<td>904</td>
</tr>
<tr>
<td>Castlereagh</td>
<td>914</td>
<td>593</td>
<td>924</td>
</tr>
<tr>
<td>mean</td>
<td>866.3</td>
<td>588.0</td>
<td>810.3</td>
</tr>
<tr>
<td>SE</td>
<td>42.5</td>
<td>16.7</td>
<td>33.9</td>
</tr>
<tr>
<td><strong>2010-2011</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kulnura</td>
<td>793</td>
<td>642</td>
<td></td>
</tr>
<tr>
<td>Somersby</td>
<td>769</td>
<td>662</td>
<td>505</td>
</tr>
<tr>
<td>Lower Portland</td>
<td>685</td>
<td>901</td>
<td>520</td>
</tr>
<tr>
<td>Cornwallis</td>
<td>629</td>
<td>850</td>
<td>516</td>
</tr>
<tr>
<td>Richmond</td>
<td>694</td>
<td>750</td>
<td>555</td>
</tr>
<tr>
<td>Castlereagh</td>
<td>579</td>
<td>861</td>
<td>619</td>
</tr>
<tr>
<td>mean</td>
<td>691.5</td>
<td>777.7</td>
<td>543.0</td>
</tr>
<tr>
<td>SE</td>
<td>33.1</td>
<td>44.7</td>
<td>19.0</td>
</tr>
<tr>
<td><strong>2009-2011</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean</td>
<td>778.9</td>
<td>682.8</td>
<td>688.8</td>
</tr>
<tr>
<td>SE</td>
<td>36.8</td>
<td>36.6</td>
<td>44.5</td>
</tr>
</tbody>
</table>

*spring peak of the following season
Table 4.5. *°D accumulations within seasons, and number of days within each generation from the date when traps in which males were trapped were placed in each orchard and the date of the subsequent generation trough, in five citrus orchards on the Central Coast of New South Wales in the 2009-2010 and 2010-2011.*

<table>
<thead>
<tr>
<th>Orchard</th>
<th>winter solstice to spring trough</th>
<th>spring trough to summer trough</th>
<th>summer trough to winter trough</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>°D  Number of days</td>
<td>°D  Number of days</td>
<td>°D  Number of days</td>
</tr>
<tr>
<td><strong>2009-2010</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hitchcock’s Kulnura</td>
<td>359 66 from 28 August to 2 November</td>
<td>973 70 from 20 November to 29 January</td>
<td>794 103 from 29 January to 12 May</td>
</tr>
<tr>
<td>Britten’s Somersby</td>
<td>301 49 from 22 August to 10 October</td>
<td>1018 79 from 12 November to 30 January</td>
<td>815 105 from 30 January to 15 May</td>
</tr>
<tr>
<td>Wallis’ Lower Portland</td>
<td>416 70 from 12 August to 21 October</td>
<td>916 93 from 21 October to 22 January</td>
<td>946 128 from 22 January to 30 May</td>
</tr>
<tr>
<td>Hartogs’s Cornwallis</td>
<td>447 62 from 26 August to 27 October</td>
<td>1053 94 from 27 October to 29 January</td>
<td>860 118 from 29 January to 27 May</td>
</tr>
<tr>
<td>UWS Richmond</td>
<td>491 79 from 17 August to 4 November</td>
<td>970 86 from 4 November to 29 January</td>
<td>864 131 from 29 January to 9 June</td>
</tr>
<tr>
<td>Gardiner’s Castlereagh</td>
<td>373 64 20 August to 23 October</td>
<td>1058 98 from 23 October to 29 January</td>
<td>922 131 from 29 January to 9 June</td>
</tr>
<tr>
<td><strong>mean</strong></td>
<td>397.8 65.0</td>
<td>998 86.7</td>
<td>886.8 119.3</td>
</tr>
<tr>
<td><strong>SE</strong></td>
<td>27.1 4</td>
<td>22.0 4.3</td>
<td>23.5 5.1</td>
</tr>
<tr>
<td><strong>2010-2011</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hitchcock’s Kulnura</td>
<td>375 60 from 26 August to 25 October</td>
<td>813 51 from 23 December to 12 February</td>
<td>581 125 from 12 February to 17 June</td>
</tr>
<tr>
<td>Britten’s Somersby</td>
<td>458 36 from 13 October to 18 November</td>
<td>821 83 from 18 November to 9 February</td>
<td>661 118 from 9 February to 7 June</td>
</tr>
<tr>
<td>Wallis’ Lower Portland</td>
<td>386 39 from 24 September to 2 November</td>
<td>1065 75 from 28 November to 11 February</td>
<td>791 125 from 11 February to 16 June</td>
</tr>
<tr>
<td>Hartogs’s Cornwallis</td>
<td>459 47 from 26 September to 12 November</td>
<td>1026 84 from 12 November to 4 February</td>
<td>657 126 from 4 February to 10 June</td>
</tr>
<tr>
<td>UWS Richmond</td>
<td>425 66 from 3 September to 8 November</td>
<td>1031 95 from 8 November to 11 February</td>
<td>715 119 from 11 February to 10 June</td>
</tr>
<tr>
<td>Gardiner’s Castlereagh</td>
<td>442 68 from 3 September to 10 November</td>
<td>1038 93 from 10 November to 11 February</td>
<td>715 108 from 11 February to 30 May</td>
</tr>
<tr>
<td><strong>mean</strong></td>
<td>434.0 52.6</td>
<td>996.2 80.2</td>
<td>707.8 120.2</td>
</tr>
<tr>
<td><strong>SE</strong></td>
<td>13.5 5.7</td>
<td>44.3 6.5</td>
<td>24.3 2.8</td>
</tr>
<tr>
<td><strong>2009-2011</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean</td>
<td>419.8 58.8 (August to November)</td>
<td>999.6 83.4 (October to February)</td>
<td>794.6 119.0 (January to June)</td>
</tr>
<tr>
<td><strong>SE</strong></td>
<td>17.3 3.8</td>
<td>24.5 3.9</td>
<td>33.0 2.8</td>
</tr>
</tbody>
</table>
4.3.2. Phenology of other life cycle stages

Trends in proportions of adult virgin red scale females in populations in the orchards at Somersby, Cornwallis, and Castlereagh followed the trends for males caught in pheromone traps placed in these orchards (Figs. 4.13–4.16). The high proportions of second instar male scale recorded in July suggested that populations in winter comprised mature females of the preceding autumn generation, and second instar male scale that developed from crawlers produced in autumn (Figs 4.13–4.16).

Figure 4.13. Proportions of second instar male and virgin female scale, and numbers of adult males/trap from October 2009 to June 2010 at Somersby.
Figure 4.14. Proportion of second instar male and virgin female scale, and numbers of adult males/trap from October 2009 to June 2010 at Lower Portland.

Figure 4.15. Proportion of second instar male and virgin female scale, and numbers of adult males/trap from October 2009 to October 2010 at Cornwallis.
4.3. Discussion

My results show that three distinct generations of red scale occur annually in citrus orchards on the Central Coast of New South Wales. Peaks in male flight activity in spring, summer and autumn represented winter/spring, summer and autumn generations, respectively. The results confirmed my hypothesis, based on Bureau of Meteorology records and °D requirements, for the number of annual generations of red scale in citrus orchards in the region. The number of annual generations I recorded in the region was therefore similar to the number cited by Smith et al. (1997).

4.3.1. The winter-early spring generation

Autumn generation females produced crawlers that developed to the second instar before mid winter. Some of the progeny may also have developed to virgin females before mid winter. Some of these virgin females then mated with late autumn peak males. These mated females produced some crawlers during winter and in early spring. However, the majority of autumn generation scales remained as second instar and virgin female scale during winter. Very few or no males were trapped from mid June to mid
September, when sunset temperatures were $\leq 16^\circ$C (Figs 4.5–4.10). During this interval, sunset temperatures and minimum temperatures could also have affected the survival of the second moult, prepupal and pupal stages, and emergence, and flights, of males. Therefore, scale that developed to the virgin female stage after mid June remained unmated until late September when the autumn and winter second instar males completed their development from September to early November. Adult male emergence peaked in late September 2009 and 2011, or early October in 2010. These males would have mated with virgin females from the winter generation and with those that developed from second instar female scale present in early winter. Therefore, early spring populations of the scale comprised mostly winter and spring mated females.

### 4.3.2. The summer generation

This generation comprised progeny of mated females produced during winter and females that mated in early spring. Crawlers produced in winter colonised branches, leaves, sepals and peduncles in late September and October. Crawlers produced by females that mated in spring would also have colonised young fruit. The adult male peak for this generation occurred between late December and early January.

### 4.3.3. The autumn generation

This generation comprised progeny of the summer generation, which commenced between late January and early February. Timing of peak male emergence varied between seasons, but occurred from late February to late March.

Ward & Johnston (1937) estimated two complete generations and a partial third generation of red scale in Bamawm and Mildura, Victoria where winters are colder than on the Central Coast of New South Wales (Table 2.6, Chapter 2).

Several authors have reported on the annual number of generations of red scale that occurred in southern Africa. Stofberg (1937) recorded four generations per year under insulated roofing at Nelspruit in South Africa. Atkinson (1977) recorded five to six generations annually at Tshaneni in Swaziland. He also reported that red scale was active in winter. Grout et al. (1989) reported four to six generations in orange orchards and five to seven in lemon orchards in six climatically different regions of South Africa. They recorded male flight activity in winter and three peaks of male scale from 19 April
to 6 October. At Mazoe, Zimbabwe, Parry-Jones (1935) recorded five generations annually under shaded conditions, and six to seven in direct sunlight.

Bliss et al. (1931) noted four generations, or a possible partial fourth, under sunlight, and three under shaded conditions at Whittier, near Los Angeles, in southern California (see Chapter 2). Kennett & Hoffmann (1985) reported three to four generations in orchards at Lindcove in the San Joaquin Valley. Moreno et al. (1985) recorded four male flights in the San Joaquin Valley, in April-May (overwintering male flights in spring), June-July (first summer generation), August (second summer generation flights) and September/October (autumn generation flights). Murdoch et al. (1989) noted 3.5 generations in orchards near Fillmore in another intermediate zone of southern California.

I attribute differences in numbers of generations I recorded and those recorded in the above mentioned studies to differences in climate. The locations where the number of annual generations were determined by authors cited above generally have higher annual minimum and maximum temperatures that those that occur in the Hawkesbury Valley and on the Somersby Plateau (see Table 2.3). In instances where more than 3 generations have been reported from locations with generally similar temperatures, e.g. the 3.5 generations reported by Murdoch et al. (1989) at Fillmore in California, greater hours of sunshine (as indicated in Table 2.3 by annual rainfall records) may have raised body temperatures of exposed scale, as has been demonstrated (Bliss et al. 1931, Munger 1948, Bodenheimer 1951, Zhao 1990). Bliss et al. (1931) noted that radiant heat from direct sunshine may shorten development of the scale sufficiently to allow a fourth or partial fourth generation at Whittier in southern California.

Reflection of heat from bare orchard floors may have raised orchard temperatures above those recorded in official weather records. Both of these factors have also enhanced the potential for the scale to complete, partially or fully, generations above that predicted on the basis of official weather records. Use of sod-culture, growth of cover crops, grass or weeds in interrow spaces, has been recommended in New South Wales since the late 1970s/early 1980s as a means of reducing orchard temperatures in order to limit the number of annual generations (and the size of populations) of red scale, and to enhance the effectiveness of natural enemies (Beattie, pers. comm., 2011). All of the orchards in my study used sod culture, and where it was least practiced on occasions, at Cornwallis, the orchard floor was well shaded by tree canopies. Ground covers are not typically
maintained in orchards in California, and use of herbicides in interrow spaces is common (Flint 1991, Anon 2003).

My results indicate that cold temperatures in winter have marked impacts on size of red scale populations in late autumn and early winter, before most Washington navel oranges are harvested. Very low numbers of males were trapped from late June to early September. Male flight activity in late winter and early spring commenced between August and September when temperatures at sunset were generally > 16°C. The results indicate that the severity of low winter temperatures (minima and the duration over which sunset temperatures \( \leq 16\text{°C} \) occurred during winter and spring) restricted male development, leading to male scale remaining in the second instar stage during winter, and low levels of flight activity. My study is the first to relate numbers of days on which no males were trapped and number of days when temperatures at sunset were \( \leq 16\text{°C} \).

My conclusions are supported by observations made by Abdelrahman (1974a) under laboratory conditions, Rice & Moreno (1970) under laboratory conditions, and Yan & Isman (1986) in laboratory and field studies. According to Abdelrahman (1974a), cold is more critical for red scale than heat, and appeared to be the main factor determining the distribution and abundance of the scale. He (Abdelrahman 1974a) found that prepupal and pupal male stages were the most susceptible stages to extreme low temperature. The LD_{50} temperatures for survival of these stages were about 10°C compared to -2.49, -2.46, -3.31 and -2.13°C for the first instar, second instar, third instar virgin female and early mated female, respectively, and 5.84, 6.06 and 3.09°C for the first moult, second moult female and crawler producing female stages, respectively. According to Rice & Moreno (1970), mature red scale males emerge between 16° and 30°C and between 20 and 65% RH, before or after sunset, as light intensities decline from 8611 to 1 lux. Yan & Isman (1986) reported that male emergence occurred in late afternoon and peaked at about 3498–5489 lux and 25–26°C in the field. Response of males to light was negligible at temperatures \( \leq 10\text{°C} \).

The durations of the intervals over which no males were trapped in my study would also have influenced the age at which virgin female scale mated, with longer intervals delaying opportunities for them to mate. McLaren (1971) reported that delayed mating led to increased natality and rapid reproduction in early spring. Although I did not record natality or crawler mortality, I commonly observed dead embryos inside mated
female scale in winter (Fig. 4.17) during my assessments of parasitism in each of the three seasons (Chapter 5). Beattie (pers. comm. 2011) observed similar phenomena during his assessments of red scale population in New South Wales from 1979–1984. These observations suggest that cold weather in winter causes mortality of red scale embryos on the Central Coast of New South Wales.

I attribute the low numbers of males that were trapped in the spring and summer generations of the 2010–2011 season, in contrast to those trapped during the 2008–2009 and 2009–2010 seasons, to the relatively cold winter of 2010 and a heatwave that occurred during late January and early February 2011. During the heatwave, maximum daily ambient temperatures $\geq 36^\circ C$ were recorded from 30 January to 5 February in the Hawkesbury Valley ($\geq 36$ and $38^\circ C$ over 7 and 6 consecutive days, respectively) and from 31 January to 3 February on the Somersby Plateau ($\geq 36^\circ C$ over 4 consecutive days). I also attribute the relatively late occurrence of the summer peak of male flight activity in 2011 to this heatwave. According to Abdelrahman (1974a), the thermal death $LD_{50}$ for red scale in extreme hot conditions under laboratory conditions ranges from 46.3–48.6°C at about 75% RH. In the field, impacts of the heatwave may have been more detrimental, particularly on crawlers, the most vulnerable stage in the scale’s life cycle (Abdelrahman 1974a). The majority of red scale stages that occurred in my orchards from 26 January and 9 February 2011 were virgin and mated females (see Chapter 5). The heatwave would most probably have caused high mortality of crawlers produced by mated females during this interval. In contrast to red scale, impacts of heat
on its natural enemies are more severe than is cold. Abdelrahman (1974a) reported that the thermal death $\text{LD}_{50}$ for (a) adult *Aphytis chrysomphali* (females only) and *Aphytis melinus* (males and females) were 38.2 and 38.7°C, respectively, and (b) for adult male and female *Comperiella bifasciata*, 39.2 and 43.6°C, respectively. Abdelrahman (1974a) noted that impacts of the heat on adult stages of *Aphytis chrysomphali* and *Aphytis melinus* were more detrimental than to their other life cycle stages. Although I did not record levels of parasitism during January-February 2011, the heatwave may have contributed to the low levels of parasitism that I recorded in late summer and autumn 2011, as *Aphytis chrysomphali*, *Aphytis melinus* and *Comperiella bifasciata* females would most probably have been actively seeking to oviposit in virgin (*Aphytis* and *Comperiella*) and mated (*Comperiella*) females that were present in relatively high proportions at that time. I have discussed the impacts of the heatwave on parasitism in greater detail in Chapter 5.

I attribute the higher °D intervals between peak flights of males in my study compared to observations reported by Hoffmann & Kennett (1985) and Grout et al. (1989) to impacts of extreme temperatures, such as the heatwave and the cold weather in winter. Mean °D accumulations between the summer and the autumn peaks in 2009–2010 were similar with those recorded by Hoffmann & Kennett (1985) and Grout et al. (1989).

I attribute occurrence of dual cohorts in the summer generation to the age structure of the late winter and early spring generation of scale and the host substrates. The first cohort represented progeny of overwintering mated females; the second cohort represented progeny of the overwintering virgin and second instar females. This result was similar to observations reported by Morse et al. (1985) in the San Joaquin Valley of California. In my studies, the cohorts were also related to substrates on which the scale occurred. The first cohort represented male scale flights of scale that colonised substrates other than fruit, while the second cohort represented male scale flights of scale that colonised fruit. I attribute the cohorts in autumn generations to overlapping generations and impacts of natural enemies. The occurrence of triple cohorts in the orchards at Somersby and Castlereagh in 2009–2010 was related to relatively low levels of male scale being trapped as a result of natural enemy activity. This is similar to conclusions reported by Grout et al. (1989) who noted that a triple-cohort structure occurred when number of male flight peaks did not exceed 800 males per card, usually in the presence of *Aphytis* species. In my studies, the low numbers of males trapped in
summer and autumn appeared to be related to impacts of *Aphytis* species, *Comperiella bifasciata* and *Encarsia* species in all orchards and *Halmus chalybeus*, particularly at Castlereagh.

### 4.5. Conclusions

My study is the first to determine red scale phenology in citrus orchards on the Central Coast of New South Wales and the first to show that climate and natural enemies play major roles in limiting the abundance of the scale. The most important factor affecting scale phenology during the course of my study was the number of days in winter and spring on which sunset temperatures $\leq 16^\circ\text{C}$ occurred. Heatwaves during summer also influenced scale phenology directly, and indirectly through impacts on natural enemies. My study is also the first to demonstrate positive correlations between (a) total numbers of males trapped during intervals over which male flight activity occurred in winter/spring and then in summer, and (b) total numbers of males trapped during intervals over which activity occurred in summer and then in autumn. My results over the three seasons of the study suggest that the potential severity of red scale populations in summer can be predicted on the basis of numbers of scale male trapped in late winter/early spring, and the number of days on which temperatures are $\leq 16^\circ\text{C}$ at sunset.

If climate change leads, as widely predicted (Department of Environment, Climate Change and Water New South Wales, 2010), to increasing seasonal and annual temperatures as a results of human activities, temperatures in orchards on the Central Coast of New South Wales in 2050 will be warmer: minimum temperatures by 1.5–3°C and maximum temperatures by 2–3°C (Department of Environment, Climate Change and Water New South Wales, 2010). Based on mean minimum and mean maximum winter temperatures that occurred in my study orchards from 2009–2011 (inclusive) (Table 4.6), the temperatures in the Hawkesbury Valley in 2050 will be similar to those that currently occur on the Somersby Plateau.
Table 4.6. Mean minimum and mean maximum winter temperatures in six orchards used for my studies on the Central Coast of New South Wales in 2009, 2010 and 2011.

<table>
<thead>
<tr>
<th>Location</th>
<th>Mean minimum temperature (°C)</th>
<th>Mean maximum temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kulnura</td>
<td>6.5</td>
<td></td>
</tr>
<tr>
<td>Somersby</td>
<td>6.39</td>
<td>5.5</td>
</tr>
<tr>
<td>Lower Portland</td>
<td>4.5</td>
<td>3.7</td>
</tr>
<tr>
<td>Cornwallis</td>
<td>5.5</td>
<td>4.9</td>
</tr>
<tr>
<td>Richmond</td>
<td>3.7</td>
<td>4.1</td>
</tr>
<tr>
<td>Castlereagh</td>
<td>3.6</td>
<td>3.7</td>
</tr>
</tbody>
</table>

If temperatures do increase as predicted, red scale and other armoured scales may not be more abundant than at present and impacts on natural enemies may not be significant. If this occurs, biological control of armoured scales should be as effective as it is now. However, it would be of interest to study the roles of natural enemies of red scale in orchards on the north-east coast of New South Wales and in coastal southern Queensland in order to understand impacts of climate change on red scale phenology and natural enemies of the scale.
5.1. Introduction


Despite the success of the IPM programs, there are only limited reports on the abundance of these natural enemies and their impacts on red scale populations in citrus orchards in coastal New South Wales near Sydney, where *Aphytis* species and *Comperiella bifasciata* are regarded as the most important parasitoids, and *Halmus chalybeus* the most important predator, of the scale (Hely et al. 1982, Smith et al. 1997). Hely (1968) said, ‘So effective is the combination of *Aphytis* and *Orcus* in coastal areas’, in this instance *Aphytis chrysomphali* and *Halmus chalybeus*, that ‘this scale is only of consequence in very hot, dry seasons and in special situations’. During a visit to Australia in 1891, Koebele (1892) made the following observations in an orchard in the Sydney region: ‘On a day when the temperature reached above 100°F. in the shade in Australia, the number of *Orcus chalybeus* upon each orange tree could be estimated, as all the beetles came down on the stems near the ground, which was a beautiful sight for an enthusiastic bug-hunter, and from 175 to 300 beetles were collected on each stem; but the larvae of the same upon the trees were probably ten times as many. It should be understood that these trees are never sprayed.’
In Chapter 4, I summarised records in the region over three seasons (2008–2010) and impacts of climate on red scale phenology. In this Chapter, I will discuss impacts of climate on the seasonal abundance of parasitoids and predators, and the impacts of the parasitoids and predators on red scale populations in three sweet orange orchards on the Hawkesbury River, and in one sweet orange orchard on the Somersby Plateau.

Based on the broad similarities in microclimates, geographical distribution, and general orchard practices I hypothesised that scale population densities and natural enemy activity in the region would be similar. In order to test these hypotheses I (a) monitored and compared scale densities on fruit and leaves, (b) determined levels of parasitism by *Aphytis species, Encarsia citrina, Encarsia perniciosi* and *Comperiella bifasciata*, (c) the incidence of ladybirds and lacewings, and (d) predation by *Halmus chalybeus*. I also made general observations on the incidence and activities of other natural enemies.

5.2. Materials and methods

5.2.1. Orchards and blocks

Assessments of red scale populations, levels of parasitism and footprints were undertaken in each of two blocks in each of four orchards at:

- Somersby (two Washington navel orange blocks from December 2008 to June 2008, and one Valencia orange block, one navel orange block from November 2009 to May 2011);
- Lower Portland (one Washington navel orange block and one Valencia orange block);
- Cornwallis (both mostly Washington navel orange with several rows of Valencia orange); and
- Castlereagh (initially two navel orange blocks from December 2008 to June 2009, one Washington navel orange block and one Valencia block from November 2009 to May 2011).

Details were presented in Chapter 3.
5.2.2. Scale densities

Densities of live adult female (virgin and mated) red scale were assessed in two seasons, 2009–2010 and 2010–2011. In 2009–2010, assessments were conducted every two to four weeks from November 2009 to July 2010. In 2010–2011, assessments were conducted every fortnight from February 2011 to June 2011. With the exception of the Valencia orange block at Lower Portland, scale densities were assessed on each of 40 trees chosen randomly within each block at the beginning of the study. Assessments in the Valencia orange block at Lower Portland were based on 20 of 60 trees. All sample trees were numbered and labelled. On each sampling occasion, 40 leaves and 40 fruit were selected randomly, 5 of each between knee-height and eye-level at each of 8 evenly spaced points around each tree. In each season, terminal mature growth flush leaves were used: mature spring flush leaves until autumn flush leaves matured and then mature autumn flush leaves until spring flush leaves matured. Both sides of each leaf were examined. The entire surface of each fruit was examined. These assessments led to average numbers of adult females per 40 leaves and 40 fruit on each tree for the entire study. In order to compare levels of infestation with the economic thresholds for red scale on fresh fruit market (Smith et al. 1997), numbers of adult females per fruit were also recorded on each of 40 fruit per tree and of 40 trees in each block in May and June 2011. The economic thresholds are regarded as 3 or more adult females scale on 10% or more fruit on early season varieties (Washington navel orange), and on 15 to 20% or more fruit on late season varieties (generally Valencia orange) (Smith et. al. 1997).

5.2.3. Parasitism

5.2.3.1. Sampling procedures

Samples of scale-infested fruit were collected from each block every 15 to 25 d (at approximately 200–300°D intervals) from November 2009 to June 2010, and from February 2011 to May 2011, as described in Chapter 4.
5.2.3.2. Assessment procedures

5.3.3.2.1. Parasitoids

Scales were examined under a Wild M7S stereomicroscope (Leica Microsystems Pty Ltd., North Ryde, Australia) and stage specific parasitism recorded for second instar male (2I♂) and second instar female (2I♀), second moult female (2M♀), prepupal male (PP♂) and pupal male (P♂), third instar virgin female (3V♀) and third instar mated female (3M♀) stages. Unparasitised scales were also recorded. Data were recorded separately for each fruit. Parasitism by each parasitoid species was based on the characteristics of pupal and adult stages, and/or meconia.

5.2.3.2.1.1. Aphytis species

Proportions of 2I♂, 2I♀, P♂, PP♂, and 3V♀ parasitised by Aphytis species were recorded. Identification of Aphytis species (Aphytis chrysomphali, Aphytis lingnanensis and/or Aphytis melinus) was based on pigmentation of sternites (thoracic and abdominal) in the ‘green-eye’ pupal stage (Compere 1955, DeBach 1959, Rosen & DeBach 1979). During this stage, Aphytis lingnanensis has a large dark area on the mid-thoracic sternum, Aphytis melinus has a small dark patch, and Aphytis chrysomphali has a longitudinal black line on the mesosternum. The egg and larval stages were recorded as Aphytis sp.

5.2.3.2.1.2. Encarsia citrina and Encarsia perniciosi

Proportions of 2I♂, 2I♀, 2M♀, and 3V♀ parasitised by Encarsia perniciosi and Encarsia citrina were based on identification of adult wasps emerging from parasitised scales kept separately in a labelled gelatine capsules at room temperature. Adults of the two species were easily distinguished by the length of their wing fringes: those of Encarsia citrina being distinctly longer than those of Encarsia perniciosi (Craw 1891, Tower 1913, Woodworth 1913, Flanders 1950, Malipatil et al. 2000) (see Chapter 2). I distinguished between scale parasitised by Encarsia perniciosi and Encarsia citrina on the basis of the appearance of mummies, meconia and size of exit holes from which the parasitoids emerged. Meconia of Encarsia perniciosi were darker and more irregularly distributed within exuviae of parasitised scale than those of Encarsia citrina. Meconia of Encarsia citrina usually lined the sides of mummies from the mid region to the
posterior end of the remains of the parasitised scale body. Exit holes of *Encarsia perniciosa* were slightly larger than those by *Encarsia citrina*.

5.2.3.2.1.3. *Comperiella bifasciata*

*Comperiella bifasciata* emerges mostly from adult mated females (3M♀) and it is the only primary parasitoid of red scale in Australia that emerges from this stage. Meconial pellets line the sides of mummified scales: they are not scattered freely as with *Aphytis* species. Numbers of mated females parasitised by *Comperiella bifasciata* were recorded. Scales in which *Comperiella bifasciata* larval stages were not readily recognised were squashed gently with a needle and body contents examined for the presence of larvae, generally one. Numbers of mummies from which *Comperiella bifasciata* adults had emerged were also recorded but these records were not used to calculate parasitism.

5.2.4. Predators

5.2.4.1. Seasonal incidence

Numbers of eggs, larvae and adults of ladybirds and lacewings were recorded when scale densities were assessed. These assessments, of fruit and leaves on 40 trees in each block, took approximately 4 h on each occasion.

5.2.4.2. Predation by *Halmus chalybeus*

As a consequence of scale predation by *Halmus chalybeus* in the four orchards, transient pale scale-sized marks, that I refer to as ‘footprints’, remained for more than 2 weeks on host plant substrates on which the scale were feeding. Fig. 5.1 shows a footprint marking the position where a mated adult female red scale was feeding on an immature orange fruit shortly before it was preyed on by a *Halmus chalybeus* larva. I therefore based my assessments of predation by *Halmus chalybeus* on the incidence of these footprints on the fruit used to assess parasitism.
5.2.5. Data analysis

Average numbers of adult virgin and mated female red scale per 40 fruit and 40 leaves were plotted against °D using Sigmaplot® (11.0 Version, Systat Software Inc., San Jose, California, United States of America). Standard errors were calculated as: SE = std/(n^0.5), where n = 40 except in the Valencia orange block at Lower Portland, where n = 20, and std = standard deviation.

Total numbers of *Halmus chalybeus* larvae and adults recorded during scale density assessments of red scale populations on 40 trees in each study block were plotted against °D.

Percentage parasitism and predation (footprints) were calculated as follows:

\[
\% \text{parasitism by } Aphytis \text{ spp.} = \frac{\text{W_1} + 2 \text{W_2} + \text{V}_1 + 2 \text{V}_2 + \text{P}_1 + \text{P}_2 + \text{PP}_1}{(2 \text{V}_1 + 2 \text{W}_2 + \text{V}_1 + \text{P}_1 + \text{PP}_1) \text{parasitised by } Aphytis \text{ spp.} + \text{unparasitised (2W_1 + 2W_2 + V_1 + V_2 + M_1 + P_1 + PP_1)}}
\]

\[
\% \text{parasitism by } Encarsia \text{ spp.} = \frac{(2 \text{V}_1 + 2 \text{W}_2 + \text{V}_1 + \text{P}_1 + \text{PP}_1) \text{parasitised by } Encarsia \text{ spp.} + \text{unparasitised (2W_1 + 2W_2 + V_1 + V_2 + M_1 + P_1 + PP_1)}}{(2 \text{V}_1 + 2 \text{W}_2 + \text{V}_1 + \text{P}_1 + \text{PP}_1) \text{parasitised by } Encarsia \text{ spp.} + \text{unparasitised (2W_1 + 2W_2 + V_1 + V_2 + M_1 + P_1 + PP_1)}}
\]

\[
\% \text{parasitism by } C. \text{ bifasciata} = \frac{M_2 \text{parasitised by } C. \text{ bifasciata}}{M_2 \text{parasitised by } C. \text{ bifasciata} + \text{unparasitised } M_2}
\]

\[
% \text{footprints} = \frac{\text{number of footprints}}{\text{number of footprint + number of live scale}}
\]

The data were plotted against °D using either Sigmaplot® (11.0 Version) or Microsoft Excel 2007. Standard errors for percent parasitism and percent predation were calculated as SE = ((p * q))/n^0.5, where p = proportion of parasitism or predation and q = 1 – p.
Calculations of proportions of *Aphytis melinus* and *Aphytis chrysomphali* observed on parasitised scales from each study orchard were based on identification of *Aphytis* pupae observed during the assessments. Calculations of proportions of *Encarsia citrina* and *Encarsia perniciosi* observed in parasitised scales from each study orchard were based on identification of adults that emerged from parasitised scale.

### 5.3. Results

Variation occurred in densities of adult female red scale, levels of parasitism, incidence of footprints orchards and between or among blocks within an orchard. However, there were no obvious differences between Washington navel and Valencia orange groves. Results and general observations for each orchard are presented in the following order: Somersby, Lower Portland, Cornwallis, and Castlereagh. In 2009–2010, assessments were undertaken from November 2009 (approximately 800°D after the winter solstice) to June 2010. Assessments in 2010–2011 were based on time-limitations that led me to focus on scale phenology and densities, and natural enemy activity, from February 2011 (approximately 1200°D after the preceding winter solstice) to June. Comparisons between seasons, and blocks within orchards, were also made. At the end of sections dealing with each orchard, I summarise the incidence of each parasitoid. Photographs of infested fruit are presented in Figs 5.2 & 5.3.

*Figure 5.2.* Red scale-pitted young orange fruit.
In order to confirm the accuracy of scale population counts in the field, I performed a regression analysis for numbers of adult females per 40 fruit versus percent infested fruit, using data from each block in my study orchards at Somersby, Lower Portland and Cornwallis (Fig. 5.4). The regression showed a strong correlation between scale density and percent infested fruit ($P < 0.001, r^2 = 0.87$).

**Figure 5.3.** Mixed stages of red scale on an immature orange fruit.

**Figure 5.4.** Correlation between number of adult female red scale per 40 fruit and percent infested fruit from each blocks in the study orchards at Somersby, Lower Portland and Cornwallis in the 2010–2011. Data were log-transformed. $P < 0.001, r^2 = 0.87$. 
5.3.1. Somersby

Adult female red scale densities in the Washington navel block were low in the summer generation in 2009–2010, ranging from 2.7 to 5.1 adult females per 40 fruit (Fig.5.5). Scale numbers then increased during the autumn generation, reaching 20.1 adult females per 40 fruit, before declining to low levels (< 6.5 per 40 fruit) in early winter (Fig. 5.5). In 2010–2011, scale densities were low until mid-summer but then increased dramatically to 22.6 adult females per 40 fruit in autumn. In contrast to 2009–2010, densities in 2010–2011 remained above 15 adult females per 40 fruit until early June. Levels of parasitism in 2009–2010 were noticeably higher than in other orchards, particularly parasitism by Aphytis species and Comperiella bifasciata, with levels reaching 43.1 and 48.1%, respectively, during the autumn generation of the scale in 2010. Parasitism by Encarsia species reached 26.4 and 25.6% in the summer and autumn generations, respectively (Fig. 5.6). In contrast to 2009–2010, levels of parasitism in 2010–2011 were much lower. Levels of parasitism by Aphytis species, Encarsia species and Comperiella bifasciata reached 20.5 ± 1.8, 9.9 ± 2.2 and 7.7 ± 4.0% in autumn 2011, respectively. The incidence of predation by Halmus chalybeus was low on most occasions (Fig. 5.7): the percentage of footprints peaked at 16.7% in autumn in 2009–2010. In 2010–2011, Halmus chalybeus adults and larvae were recorded on two occasions, on 16 February (2 individuals per 40 trees) and 2 March 2011 (9 individuals per 40 trees). They were not recorded thereafter. The percentage of footprints was low, ranging from 0 to 2.2% (Fig. 5.6). Mallada signatus (Fig 5.8) was not observed.

In the Valencia orange block, scale populations occurred at low levels throughout 2009–2010 (Fig.5.5). In contrast to all other groves in the study, scale densities on the leaves in this block were higher than on fruit. Populations in the block 2010–2011 were higher than in 2009–2010 (Fig. 5.5). Levels of parasitism by Aphytis species, Encarsia species and Comperiella bifasciata reached 14.4, 29.9 and 34.3% in autumn 2010, respectively. Levels of parasitism by Aphytis species, Encarsia species and Comperiella bifasciata reached 4.6 ± 1.2, 13.7 ± 1.6 and 2.6 ± 1.2% in autumn 2011, respectively (Fig. 5.6). Halmus chalybeus was active, with 11 adults and larvae recorded on 30 January 2010 when the ambient temperature reached 27ºC. The percentage of footprints reached 51.8% in autumn 2010 (Fig. 5.7). In contrast, the percentage of footprints reached only 2% in autumn 2011. In contrast to the
Washington navel block, *Mallada signatus* was present in this block. Its eggs and larvae were most abundant from late October to early December in 2009 (Table 5.1).

Proportions of *Aphytis chrysomphali* and *Aphytis melinus* were 78.9 and 21.1%, respectively (n = 194), over the two seasons (2009–2010 and 2010–2011) (Fig. 5.9). However, only *Aphytis chrysomphali* was recorded in both blocks in 2010–2011 (n = 64). Proportions of *Encarsia citrina* and *Encarsia perniciosi* were 91.2 and 8.8%, respectively (n = 182), over the two seasons (Fig 5.10).

<table>
<thead>
<tr>
<th>Date</th>
<th>Egg</th>
<th>Larvae</th>
<th>Pupae</th>
<th>Adult</th>
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<tbody>
<tr>
<td>17 October 2009</td>
<td>0</td>
<td>0</td>
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<td>12 December 2009</td>
<td>54</td>
<td>15</td>
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</tr>
<tr>
<td>9 January 2010</td>
<td>2</td>
<td>1</td>
<td>0</td>
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</tr>
</tbody>
</table>

**Table 5.1.** Numbers of eggs, larvae, pupae and adults of *Mallada signatus* recorded on 15 randomly-selected trees in the Valencia orange block at Somersby during 10 min observation on each tree during late spring and early summer 2009–2010.

**Figure 5.5.** Average number of virgin and mated female scales per 40 fruit and 40 leaves per tree (n = 40 trees) at Somersby in Washington navel ‘block 2’ in 2009–2010 (top left) and 2010–2011 (top right) and Valencia orange ‘block 3’ in 2009–2010 (bottom left) and 2010–2011 (bottom right).
Figure 5.6. Percent parasitism of red scale by *Encarsia* species, *Aphytis* species and *Comperiella bifasciata* at Somersby in Washington navel ‘block 2’ in 2009–2010 (top left) and 2010–2011 (top right) and Valencia orange ‘block 3’ in 2009–2010 (bottom left) and 2010–2011 (bottom right).
Figure 5.7. Numbers of *Halmus chalybeus* adults and larvae observed within 4 h, and incidence of footprints of *Halmus chalybeus*-predated scale expressed as a percentage of total numbers of predated and live scale on fruit sampled at Somersby in Washington navel ‘block 2’ in 2009–2010 (top left) and 2010–2011 (top right) and Valencia orange ‘block 3’ in 2009–2010 (bottom left) and 2010–2011 (bottom right).
Figure 5.8. Green lacewing, *Mallada signatus*: (left) eggs, (middle above) a pupal shell, (middle, below) a larva with predated scale, (right) an adult.
Figure 5.9. Proportions of *Aphytis chrysomphali* and *Aphytis melinus* in four orchards in 2009–2010 and 2010–2011, based on pupae observed during assessments of parasitism of red scale.

Figure 5.10. Proportions of *Encarsia citrina* and *Encarsia perniciosi* in four orchards in 2009–2010 and 2010–2011, based on adults emerging from parasitised red scale kept separately in gelatine capsules.
5.3.2. Lower Portland

In the 2009–2010 summer generation, scale densities in the Washington navel and Valencia orange blocks ranged from 0.2 to 5 females per 40 fruit, levels lower than in my other study orchards (Fig. 5.11). Densities increased rapidly in autumn 2010, reaching 37.2 and 31.2 females per 40 fruit, on average, in the Washington navel and Valencia blocks, respectively. The populations, that were higher than at this point than in my other study orchards, then declined in early winter. In 2010–2011, populations were also low in mid-summer in both blocks before increasing in autumn. However, in contrast to 2009–2010, populations in 2010–2011 reached, on average, only 21.4 and 13.8 females per 40 fruit in the Washington navel and Valencia orange blocks, respectively, but remained at relatively high levels of about 15 and 10 females per 40 fruit in the Washington navel and Valencia orange blocks, respectively, until the end of the season (Fig. 5.11).

Levels of parasitism in the Washington navel block in 2009–2010 were considerably lower than in other orchards. Comperiella bifasciata was not recorded until late autumn when 3.2 ± 1.4% of mated female. Levels of parasitism by Encarsia species, which were higher than levels of parasitism by Aphytis species and Comperiella bifasciata, did not exceed 10% in the summer generation of the scale, and only reached 23.3 ± 1.5% in the autumn generation of the scale (Fig. 5.10). Levels of parasitism in 2010–2011 were similar to levels in 2009–2010: in autumn 2011, 4.3 ± 1.1, 20.7 ± 2.0 and 5.5 ± 1.3% for Aphytis species, Encarsia species and Comperiella bifasciata, respectively.

Levels of parasitism in the Valencia orange block in 2009–2010 were slightly higher than in the Washington navel block, and also lower than in the my other study orchards. Parasitism by Aphytis species, Encarsia species and Comperiella bifasciata, in autumn reached 28.8 ± 2.0, 18.8 ± 2.2 and 16.9 ± 4.6%, respectively (Fig. 5.12). Levels of parasitism in the block in the 2010–2011 were also low: in autumn 2011, 12.1 ± 1.9, 16.1 ± 2.1 and 6.1 ± 1.2 % for Aphytis species, Encarsia species and Comperiella bifasciata, respectively.

In 2009–2010 and also in 2010–2011, Aphytis chrysomphali was the only species of Aphytis recorded in both blocks in this orchard (n = 195) (Fig. 5.9): Aphytis lingnanensis and Aphytis melinus were not recorded in the orchard during the course of
my study. Proportions of *Encarsia perniciosi* and *Encarsia citrina* were 86.3 and 13.7%, respectively (n = 227), over the two seasons (2009–2010 and 2010–2011) (Fig. 5.10).

Numbers of *Halmus chalybeus* in 2009–2010 were higher in both the Washington navel and Valencia orange blocks of this orchard than in the other study orchards. Maximum numbers of adults and larvae in blocks in autumn 2010 reached 33 (on 40 trees) and 18 (on 20 trees), respectively (Fig. 5.13). In 2010–2011, numbers were lower than in 2009–2010, but still more frequently observed than in other orchards in the same season. In the Washington navel block, the beetle was observed on 7 of 8 occasions, including in winter, with maximum of 9 adults and larvae on 40 trees on 10 May 2011, when the maximum temperature was 18.4°C. In the Valencia block, adults and larvae were observed on 4 of 6 occasions, with a maximum of 4 recorded on 20 trees (Fig. 5.13).

On the 3 February 2011, when the ambient temperature was 40 to 41°C and red scale densities low, I observed 70 adult *Halmus chalybeus* on the trunks of a Valencia orange tree. Some were sheltering in shallow natural crevices of the trunk. The temperature within one of these crevices was 10°C lower than the ambient air temperature. On 10 February 2011, when the ambient temperature was 32°C, no beetles were present on the tree. In shade on fine winter days I observed adults sheltering between touching leaves and on fruit surfaces covered by leaves. I observed them actively searching for scale on warm and sunny days, irrespective of season, when ambient temperatures ranged from 17 to 25°C.

I did not observe *Mallada signatus* in this orchard.
Figure 5.11. Average number of virgin and mated female scales per 40 fruit and 40 leaves per tree (n = 40 trees) at Lower Portland in Washington navel ‘block 2’ in 2009–2010 (top left) and 2010–2011 (top right) and (n = 20) in the Valencia orange ‘block 3’ in 2009–2010 (bottom left) and 2010–2011 (bottom right).
Figure 5.12. Percent parasitism of red scale by *Encarsia* species, *Aphytis* species and *Comperiella bifasciata* at Lower Portland in Washington navel ‘block 2’ in 2009–2010 (top left) and 2010–2011 (top right) and in the Valencia orange ‘block 3’ in 2009–2010 (bottom left) and 2010–2011 (bottom right).
Figure 5.13. Numbers of *Halmus chalybeus* adults and larvae observed within 4 h at Lower Portland in Washington navel ‘block 2’ in 2009–2010 (top left) and 2010–2011 (top right) and 2 h in the Valencia orange ‘block 3’ in 2009–2010 (bottom left) and 2010–2011 (bottom right) and incidence of footprints of *Halmus chalybeus*-predated scale expressed as a percentage of total numbers of predated and live scale on fruit sampled from the each block.
5.3.3. Cornwallis

Densities of adult female red scale in Washington navel/Valencia block 1 in summer 2009–2010 were 4.5 and 7.1 females per 40 fruit and 40 leaves, respectively. Populations then increased, reaching 27.2 females per 40 fruit in autumn 2010 (Fig. 5.114). In contrast to 2009–2010, scale populations in 2010–2011 were much lower: densities reached 5.2 ± 0.1 females per 40 fruit by mid-autumn and declined to 2.3 ± 0.4 in early winter (Fig. 5.14).

Parasitism by Encarsia species was more pronounced than parasitism by Aphytis species and Comperiella bifasciata. Two distinct peaks of parasitism by Encarsia species, the first occurred between 1300º and 1400ºD and the second at about 1900ºD, after the winter solstice at the beginning of each season. These peaks occurred approximately 200ºD after the peaks of second instar and second moult stages of red scale. Parasitism by Aphytis species, Encarsia species and Comperiella bifasciata in autumn 2010 reached 15 ± 1.9, 49.1 ± 2.1 and 12.9 ± 2.9%, respectively (Fig. 5.15). In autumn 2011, parasitism by Aphytis species, Encarsia species and Comperiella bifasciata reached 26.7 ± 3.0, 39.6 ± 2.9 and 2.8 ± 2.7%, respectively (Fig. 5.15).

The maximum number of Halmus chalybeus adults and larvae observed in 2009–2010 was 5 on 40 trees: footprints peaked at 39.8% in autumn 2010 (Fig. 5.16). In 2010–2011, 14 Halmus chalybeus adults were recorded per 40 trees on 17 February 2011. On 4 March 2011, 5 adults were recorded but my counts were influenced by application of 0.5% v/v oil spray for control of citrus leafminer (Phyllocnistis citrella Stainton [Lepidoptera: Gracillariidae]) in this block as I was counting. Halmus chalybeus were not recorded in this block thereafter until the end of the season. The spray appeared to cause adult beetles to disperse.

Densities of adult female red scale in the Washington navel/Valencia block 2 in 2009–2010 reached 7.7 ± 0.4 and 10.6 ± 6.6 females per 40 fruit in the summer and autumn generations, respectively (Fig. 5.14). In 2010–2011, densities reached 4.1 ± 0.6 females per 40 fruit in mid autumn before declining to 2.1 ± 0.5 per 40 fruit in early winter.

Seasonal trends for parasitism in 2009–2010 resembled those in the Washington navel/Valencia block 1. Parasitism by Aphytis species, and Encarsia species Comperiella bifasciata reached 25.1, 47 and 15%, respectively, in autumn 2010 (Fig.
Parasitism trends in 2010–2011 were similar to trends in 2009–2010, though levels in the former were lower than in the latter. Parasitism by *Aphytis* species, *Encarsia* species and *Comperiella bifasciata* reached $23.1 \pm 3.3$, $42.7 \pm 3.9$ and $2 \pm 1.9\%$, respectively, in autumn 2011.

Proportions of *Aphytis chrysomphali* and *Aphytis melinus* in the orchard (blocks 1 and 2) were 90 and 10%, respectively ($n = 70$), over the two seasons (2009–2010 and 2010–2011) (Fig. 5.9). *Aphytis lingnanensis* was not recorded (Fig. 5.9). Proportions of *Encarsia citrina* and *Encarsia perniciosi* were 94.4 and 5.6%, respectively ($n = 337$), over the two seasons (Fig. 5.19).

The maximum number of *Halmus chalybeus* adults and larvae recorded in 2009–2010 was 7 per 40 trees in winter: footprints peaked at 65% (Fig. 5.16). In 2010–2011, *Halmus chalybeus* was recorded on three occasions up to 10 March: a maximum of 5 adults on 40 trees was recorded on 20 February. They were not recorded thereafter until the end of the season. I attributed this, as noted above, to application of an oil spray for control of citrus leafminer on 5 March 2011.

In late spring and early summer 2009 I observed *Mallada signatus* in the orchard. I recorded a total of 39 eggs and larvae on 28 October 2009 when the ambient temperature was about 25ºC.
Figure 5.14. Average number of virgin and mated female scales per 40 fruit and 40 leaves per tree (n = 40 trees) at Cornwallis in Washington navel and Valencia orange ‘block 1’ in 2009–2010 (top left) and 2010–2011 (top right) and Washington navel and Valencia orange ‘block 2’ in 2009–2010 (bottom left) and 2010–2011 (bottom right).
Figure 5.15. Percent parasitism of red scale by *Encarsia* species, *Aphytis* species and *Comperiella bifasciata* at Cornwallis in Washington navel and Valencia orange ‘block 1’ in 2009–2010 (top left) and 2010–2011 (top right) and Washington navel and Valencia orange ‘block 2’ in 2009–2010 (bottom left) and 2010–2011 (bottom right).
Figure 5.16. Numbers of *Halmus chalybeus* adults and larvae observed within 4 h and incidence of footprints of *Halmus chalybeus*-predated scale expressed as a percentage of total numbers of predated and live scale on fruit sampled at Cornwallis in Washington navel and Valencia orange ‘block 1’ in 2009–2010 (top left) and 2010–2011 (top right) and Washington navel and Valencia orange ‘block 2’ in 2009–2010 (bottom left) and 2010–2011 (bottom right).
5.3.4. Castlereagh

This orchard was not maintained, aside from occasional mowing, from mid 2009. Trees remained in reasonably good conditions in 2009–2010 but in 2010–2011 they lost vigour: canopies thinned and numbers of fruit declined dramatically. Therefore, in the Washington navel block, I was only able to assess scale densities on 20 fruit on each of 20 trees.

Densities of adult female red scale in the Washington navel block in 2009–2010 increased to 13.9 ± 2.0 females per 40 fruit in early March 2010. They then declined to 0.2 ± 0.1 in late March and early April 2010 (Fig. 5.17). In 2010–2011, populations reached 6 ± 1.3 females per 20 fruit in April and then declined to 3.7 ± 0.8 females per 20 fruit, and then remained relatively stable until the end of the season (Fig 5.17).

Levels of parasitism by Encarsia species in 2009–2010 reached 16.0 ± 1.7% in summer before declining to 1.8 ± 0.8% in late autumn. Levels of parasitism by Comperiella bifasciata also declined from late March. No scales parasitised by Comperiella bifasciata were recorded on 29 April 2010 (Fig. 5.18), the final assessment for 2009–2010.

In 2010–2011, parasitism by Encarsia species was noticeably higher than by Aphytis species and Comperiella bifasciata. Levels of parasitism by Aphytis species, Encarsia species and Comperiella bifasciata reached 11.8 ± 5.3, 37.2 ± 7.6 and 7.4 ± 4.4%, respectively, in autumn 2011.

In 2009–2010, 10 Halmus chalybeus adults and larvae were recorded per 40 trees in the Washington navel block at Castlereagh on 29 December 2009. The percentage of footprints increased dramatically from 8.9% in early February to 88.5% in late March (Fig. 5.19). In 2010–2011, a maximum of 2 Halmus chalybeus adults and larvae were recorded in February and March. In contrast to 2009–2010, in 2010–2011, the percentages of footprints were much lower, reaching only 12.5 ± 5.2% in autumn 2011.

In the Valencia orange block, population densities in 2009–2010 were low in the summer generation and then increased to 16.2 females per 40 fruit in the early autumn, before declining to 2.3 females per 40 fruit in early winter 2010 (Fig. 5.17). Levels of parasitism by Encarsia species in 2009–2010 were higher than levels of parasitism by
Aphytis species and Comperiella bifasciata on most occasions. Aphytis species were less abundant in this block. In 2010–2011, scale populations were low throughout the season, reaching only 2.4 ± 0.5 females per 40 fruit in autumn, before declining to 1.7 ± 0.8 females per 40 fruit in early winter (Fig. 5.18). Parasitism by Encarsia species was consistently high and more noticeable than parasitism by Aphytis species and Comperiella bifasciata on all occasions. Parasitism by Encarsia species ranged from 46.7 ± 6.8 to 58.4 ± 6.8% in late summer and autumn 2011. The incidence of footprints reached 52% in autumn 2010 and 16.7 ± 4.9% in autumn 2011. Halmus chalybeus (Fig. 5.20) was relatively active in late summer and autumn in both seasons, with maximum 5 and 9 adult and larvae per 40 trees in autumn 2010 and 2011, respectively (Fig. 5.19).

On 22 April 2010, when the weather was fine and the maximum ambient temperature 28ºC, I observed a Halmus chalybeus adult moving intensely, searching the entire surface of a fruit in the Valencia orange block in this orchard. It consumed consuming 4 to 5 mixed stages of the scale over an interval of about 10 min, rather like a vacuum cleaner sucking dust. At this point there were about 20 footprints on the fruit and no live scale. I assumed initially that more than one beetle had been active on the fruit, this did not appear to be so.

Proportions of Aphytis chrysomphali, and Aphytis melinus, from both blocks, were 12.9 and 87.1%, respectively (n = 93), over the two seasons (2009–2010 and 2010–2011) (Fig. 5.9). Proportions of Encarsia perniciosi and Encarsia citrina were 85.6 and 16.8, respectively (n = 171), over the two seasons (Fig. 5.10).

In late spring and early summer 2009 I observed Mallada signatus in the orchard. I recorded a total of 32 eggs and larvae in the in the Valencia orange block on 23 October 2009 when the ambient temperature was about 26ºC.
Figure 5.17. Average number of virgin and mated female scale per 40 fruit and 40 leaves per tree (n = 40) at Castlereagh in the Washington navel ‘block 3’ in 2009–2010 (top left), 2010–2011 (top right) and in the Valencia orange ‘block 4’ in 2009–2010 (bottom left) and 2010–2011 (bottom right).
Figure 5.18. Percent parasitism of red scale by *Encarsia* species, *Aphytis* species and *Comperiella bifasciata* at Castlereagh in the Washington navel ‘block 3’ in 2009–2010 (top left), 2010–2011 (top right) and in the Valencia orange ‘block 4’in 2009–2010 (bottom left) and 2010–2011 (bottom right).
Figure 5.19. Numbers of *Halimus chalybeus* adults and larvae observed within 4 h and incidence of footprints of *Halimus chalybeus*-predated scale expressed as a percentage of total numbers of predated and live scale on fruit sampled from the orchard at Castlereagh in the Washington navel ‘block 3’ in 2009–2010 (top left), 2010–2011 (top right) and in the Valencia orange ‘block 4’in 2009–2010 (bottom left) and 2010–2011 (bottom right).
5.3.5. General observations on other natural enemies

5.3.5.1. *Orcus australasiae*

I did not observe *Orcus australasiae* in my orchard at Somersby, or in my study orchards in the Hawkesbury Valley. However, I did observe it in a block of orange trees at New South Wales Industry & Investment’s Somersby Research Station (33° 22’ S, 151°18’ E, 208 m asl, about 1.8 km east of Britten’s orchard at Somersby), in Kemp’s orchard at Peats Ridge, and in two of my study orchards at Kulnura (Hitchcock’s and Lister’s). At the Research Station, where tree canopies were open and thin, I observed 5 larvae and 12 adults on one Valencia tree on 5 March 2009, when the weather was fine and the ambient temperature about 23°C. The tree was heavily infested with red scale that had caused several branches on the tree to dieback: no soft scales or ants were present. In the orchards at Peats Ridge and Kulnura and the one at Peats Ridge, larvae and adults were abundant on trees heavily infested with red scale and black scale in the presence of high ant activity.
I also observed *Orcus australasiae* larvae and adults feeding readily on red scale on five-year old Hamlin orange trees at Lister’s orchard at Kulnura, during summer and autumn 2011 (the orchard is described in Chapter 3). The trees were in three blocks that I used for studies reported in Chapter 7 on the impact of common ant on natural enemies of red scale. Many of the trees were heavily infested with red scale and black scale. The adult beetles preferred to prey on virgin and mature female red scale. They consumed the scale cover and body of virgin females. When preying on mated females, the adult beetles lifted the scale to expose the soft ventral surface of the scale body. The beetle then consumed the ventral surface of the scale and its body contents, leaving the scale cover and dorsal scale exoskeleton partially attached to the plant surface. I recorded 165 larvae, 75 pupae and 32 adult *Orcus australasiae* on one of the Hamlin orange trees (170 cm high) on 19 January 2011, when the weather was fine and the maximum temperature 29°C. During additional observations in this orchard on 30 March 2011, I observed that the beetle was patchily distributed within and between the blocks. The distributions suggested a preference for sunlight. The ambient air temperature was about 24°C, with short intervals of bright sunlight between rain showers. The adults and larvae activity sheltered during rain, becoming less easy to observe than when observations were made during full sunlight. The beetles also appeared to disperse from trees they had colonised to adjacent heavily red scale and black scale-infested trees only after exploiting the red scale population on the colonised tree.

During September 2011, I found *Orcus australasiae* eggs on one of the Hamlin orange trees. Where the beetle laid its eggs had puzzled me for several months as there are no records in the literature and I assumed that its eggs would be laid in clusters on leaves or fruit in a manner similar to eggs laid by *Halmus chalybeus*. My assumption was incorrect. Eggs, in this instance, were laid under integuments of dead black scale, more commonly those of late instars and adults with parasitoid exit holes (Fig. 5.21–5.22). There were about 3–10 eggs under each of 10 scales that I observed. The eggs were cylindrical, approximately 0.4 × 2 mm. When I collected the eggs they were off-white. They turned light yellow, then black (Fig. 5.23), before hatching. Newly hatched larvae were black and 3–5 mm long. They were active and started to search for food shortly after eclosion. Under laboratory conditions at 25°C and 70% RH, the eggs hatched 10 d after being collected from the field. Under the same conditions, and with access to oleander scale maintained on pumpkin, the larvae of each of four instars took about 6–8
d to complete their development. The larval stages are illustrated in Figs. 5.24–5.25. The pupal stage took about 7 d. A pupa, an adult eclosing from a pupa, and adults are featured in Fig. 5.26.

Figure 5.21. Eggs of *Orcus australasiae* under black scale integuments.

Figure 5.22. *Orcus australasiae* eggs under a black scale integument with parasitoid exist holes: (a) ventral view, (b) dorsal view.
Figure 5.23. Full developed embryos (left) and egg shells (right) of Orcus australasiae.

Figure 5.24. First instar (left) and second instar (right) larvae of Orcus australasiae.
Figure 5.25. Third instar (left), forth instar (right) larvae of *Orcus australasiae*

Figure 5.26. *Orcus australasiae* pupa (left), newly emerged adult (mid), mating adults (right).
5.3.5.2. *Rhyzobius lophanthae*

I did not observe *Rhyzobius lophanthae* on the randomly selected trees in the blocks/orchards on which I assessed population densities and natural enemy activity during my regular assessments of scale populations in my study orchards. However, I observed adults, larvae, and/or eggs occasionally on scaly fruit and leaves of heavily red scale infested trees on the edge of the Washington navel block at Somersby, and in the two Valencia blocks at Castlereagh, in autumn 2009 and 2010. Life cycle stages of *Rhyzobius lophanthae* are illustrated in Fig. 5.27.

![Figure 5.27. *Rhyzobius lophanthae* eggs (top left), larva (top right), adult (bottom left) and pupa (bottom right).](image-url)
5.3.5.3. *Chilocorus circumdatus* and *Hemisarcoptes cooremani*

I observed, though very rarely, *Chilocorus circumdatus* adults (Fig. 5.28) in the both study blocks at Cornwallis, and in the Washington navel block at Lower Portland, in late summer and autumn 2011. I also observed, again very rarely, *Hemisarcoptes cooremani* under a few crawler producing females on the fruit collected from the both study blocks at Cornwallis in autumn 2011. They were not observed on other occasions in these orchards or in my other study orchards.

![Image of Chilocorus circumdatus](image)

**Figure 5.28.** *Chilocorus circumdatus* adult resting on an orange leaf.

5.3.5.4. *Euseius elinae*

I observed *Euseius elinae* on fruit samples, most under calyces in all the study orchards, most commonly at Cornwallis, during summer and winter. Although *Euseius elinae* is reported to feed on diaspidid crawlers (Smith et al. 1997), it feeds predominantly on mites and pollen (Beattie et al. 1991, McMurtry & Croft 1997).
5.3.5.5. *Karnyothrips flavipes*

I occasionally observed *Karnyothrips flavipes* nymphs and adults under calyces of fruit samples from both blocks at Somersby and Valencia orange block at Lower Portland: generally one to two nymphs and/or adults occurred per fruit, on a few fruit in each sample of about 50 fruit. They were most common in autumn.

5.3.5.6. *Mataeomera dubia* and *Batrachedra arenosella*

*Mataeomera dubia* was observed in the orchard at Kulnura and Peats Ridge, where the population densities of black scale and red scale were high (Fig. 5.29 right). It was abundant in summer and autumn. I found up to 5 larvae/pupae on one Hamlin tree on the Lister’s orchard at Kulnura during a 1-min observation of the tree in January 2011. It readily developed and completed its life cycle on oleander scale in the laboratory conditions. I also observed *Batrachedra arenosella* feeding on heavily red scale-infested fruit in the same orchard at Kulnura in June 2010 (Fig. 5.29 left). I also observed it on heavily red scale-infested mature Valencia orange fruit in orchards at Griffith in September 2010.

![Figure 5.29. A Batrachedra arenosella caterpillar preying on red scale on a Valencia orange fruit (left) and a Mataeomera dubia caterpillar foraging for black scale on an orange leaf](image)

5.3.5.7. *Aphelinus* sp.

I recorded, though very rarely, pupae and adults of one aphelinid species on red scale in autumn 2011 in the orchards at Somersby and Kulnura. The pupae of this species were distinctly different from *Aphytis chrysomphali*, *Aphytis lingnanensis* and *Aphytis*...
menilus. In Chapter 9, I used molecular methods in order to identify this species. The results showed that it was a species of Aphelinus.

5.4. Discussion

The results rejected my hypothesis for limited variation in scale population densities, levels of parasitism, and the incidence of predation among orchards and among blocks within an orchard. General trends of scale population densities, and levels of parasitism and predation, during 2009–2010 and 2010–2011 are compared and discussed.

5.4.1. Scale population densities

In both seasons, scale populations on fruit were low in the summer generation before increasing to higher levels in the autumn generation and then declining to low levels in late autumn and early winter. I attributed the seasonally high populations of the scale on fruit in autumn to (a) more rapid development of individuals on fruits than on other substrates (Carroll 1979, Carroll & Luck 1984), (b) limited scope for second generation crawlers to disperse from fruit to twigs, branches and leaves, as dispersal by walking is limited, in most instances, to movement from fruit via peduncles, and (c) to the majority of crawlers settling within a short distance of their mother (Stofberg 1937, Ebeling 1951, Meats & Wheeler 2011). I attributed the reduction of scale populations in late autumn, early winter to the impacts of climate and natural enemy activity and intrinsic age-related mortality.

Scale populations in summer and early autumn in 2009–2010 were higher than in the same seasons in 2010–2011. In contrast, scale populations in late winter and early autumn in 2009–2010 were lower than in the same seasons in 2010–2011. The exception was in the orchard at Cornwallis in which scale populations in 2010–2011 were consistently lower than in 2009–2010. I attributed these differences to climate. I attributed low scale populations in 2010–2011 to prolonged impacts of cold weather in winter and early spring 2010 and the heatwave occurred from 30 January to the 5 February 2011 (see Chapter 4). I attributed the high populations in late autumn and early winter in 2010–2011 to detrimental impacts of climate on parasitoids, and consequently the scale.
With the exception of the heavily red scale-infested trees on perimeters of blocks and trees with soft scales tended by native ants, the scale was rarely present on wood of citrus trees in my study orchards. Most scale colonised fruit and leaves on the outer parts of trees. In contrast to the low levels of red scale infestations on wood in my study orchards (and in most citrus orchards in Australia; Beattie. pers. comm., 2009), high density infestations appear to be common on wood in California (Carroll 1979, Carroll & Luck 1984, Murdoch et al. 1995). Carroll & Luck (1984) reported that although wood only represented 5% of available substrates, populations on wood represented, depending on the season, 20–60% of all scale. These high levels of the scale on wood in California are related to impacts of Argentine ant on natural enemies (Flanders 1945, DeBach & Bartlett 1951a, DeBach 1959, Bartlett 1961, Musgrove & Carman 1965, Moreno et al. 1987, Murdoch et al. 1995). Murdoch et al. (1989) reported that scales on wood were also less acceptable as hosts for *Aphytis melinus* and *Encarsia perniciosi*. They (Murdoch et al. 1989) recorded a 27-fold reduction of *Aphytis melinus* parasitism between interior and exterior parts of trees, and a two-fold reduction for *Encarsia perniciosi*. Argentine ant does not, with the exception of Western Australia, occur in orchards in the major citrus producing regions of Australia (Smith et al. 1997).

Although the general trends were similar among orchards and blocks, scale densities differed from block to block. The most noticeable difference was recorded between two blocks in the orchard at Somersby, where higher populations occurred in the Washington navel block than in the Valencia block. I attributed this to the duration fruit were present on trees. Fruit in the Washington navel block were usually harvested between late June and September, some 8–11 months after fruit set, whilst Valencia fruit were present on trees for up to 18 months. Therefore, some winter generation progeny would have colonised both immature and mature Valencia fruit.

The most noticeable differences between the two seasons were an increase in yellow scale populations to levels not observed for more than 30 years (Beattie & Meats, pers. comm.). The highest incidence of the scale occurred at Cornwallis where there was a marked decline in red scale populations between two seasons. I attributed the increase in yellow scale regionally, but particularly in the Hawkesbury Valley, to higher than average annual numbers of rainy days and higher than average annual rainfall from 2007, in contrast to the 7 preceding years (Table 5.2), leading to yellow scale colonising outer canopy surfaces that it would normally not colonise in years with more sunny days and less rainfall.
Table 5.2. Numbers of rainy days and average annual rainfall from 2000–2011 recorded by the Australian Bureau of Meteorology at Richmond Airport and Mangrove Mountain, New South Wales.

<table>
<thead>
<tr>
<th>Year</th>
<th>Richmond Airport</th>
<th>Mangrove Mountain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of rainy days</td>
<td>Average annual rainfall (mm)</td>
</tr>
<tr>
<td>2000</td>
<td>136</td>
<td>675.2</td>
</tr>
<tr>
<td>2001</td>
<td>114</td>
<td>745</td>
</tr>
<tr>
<td>2002</td>
<td>97</td>
<td>582.4</td>
</tr>
<tr>
<td>2003</td>
<td>116</td>
<td>693.8</td>
</tr>
<tr>
<td>2004</td>
<td>97</td>
<td>694</td>
</tr>
<tr>
<td>2005</td>
<td>121</td>
<td>688.4</td>
</tr>
<tr>
<td>2006</td>
<td>102</td>
<td>490.6</td>
</tr>
<tr>
<td>2007</td>
<td>147</td>
<td>1051.2</td>
</tr>
<tr>
<td>2008</td>
<td>148</td>
<td>800</td>
</tr>
<tr>
<td>2009</td>
<td>115</td>
<td>578.2</td>
</tr>
<tr>
<td>2010</td>
<td>134</td>
<td>713</td>
</tr>
<tr>
<td>2011</td>
<td>127</td>
<td>663.6</td>
</tr>
<tr>
<td>Average</td>
<td>121.2</td>
<td>698.0</td>
</tr>
</tbody>
</table>

The highest levels of yellow scale in 2010–2011 were recorded at Cornwallis. I attributed this to the relatively large trees in this orchard providing more shade in contrast to my other study orchards, where trees were smaller and where canopies overlapped less frequently across rows, and to frequent use of overhead irrigation in the orchard, also in contrast to my other study orchards.

5.4.2. Parasitism

I recorded and confirmed (Chapter 8) the presence of five primary parasitoids of red scale in the region: *Aphytis chrysomphali*, *Aphytis melinus*, *Encarsia citrina*, *Encarsia perniciosi* and *Comperiella bifasciata*. I also screened these parasitoid species for the presence of bacterial symbionts ‘*Candidatus Cardinium*’ and *Wolbachia* (Chapter 8).

Marked variation in levels of parasitism occurred between the seasons. The most noticeable variation occurred in levels of parasitism by *Aphytis* species and *Comperiella bifasciata*. I attributed this to impacts of a record heatwave during late January and early February 2011 (see Chapter 4) on heat prone (see Abdelrahman 1974a, Rosen & DeBach 1979) *Aphytis* and *Comperiella bifasciata* adults searching for virgin (*Aphytis* and *Comperiella*) and mated (*Comperiella*) hosts that were present at the time (Chapter
4). In contrast to the *Aphytis* species and *Comperiella bifasciata*, levels of parasitism by the *Encarsia* species were relatively high in 2010–2011, particularly at Cornwallis and Castlereagh. I attributed this to the likelihood that relatively low numbers of *Encarsia* adults would have been searching for their second moult and second instar hosts when the heatwave occurred.

Marked variation in levels of parasitism also occurred among orchards and blocks within orchards. This appeared to be related to environmental differences. Most noticeably, the *Aphytis* species and *Comperiella bifasciata* were more common in the orchards on the Somersby Plateau than in the Hawkesbury Valley, and the *Encarsia* species were more common in the orchards in the Hawkesbury Valley than in the orchards on the Somersby Plateau.

Levels of parasitism in 2009–2010 suggested that the parasitoids preferred the relatively mild climate of the Somersby Plateau in contrast to the relatively harsher climate of the Hawkesbury Valley. Temperatures were less extreme at Somersby than along the Hawkesbury River (see Chapter 4). Very low levels of parasitism occurred at Lower Portland in the summer generation. It is possible that this could have been related to the very low densities of scale in the orchard and the low incidence of stages suitable for parasitism stemming from low twilight and night temperature during the preceding winter and early spring. However, in Chapter 6, I showed that relationships between the scale and its parasitoids are not density dependent. The most plausible explanation may be that the low levels of parasitism were related to high summer temperatures, which were more extreme than in the other study orchards.

The seasonal trends of parasitoids suggested that higher levels of parasitism by *Encarsia* species were recorded in the early season because they parasitise and emerge from early stages of the scale (Rosen & DeBach 1978, Yu et al. 1990, Forster et al. 1995). In contrast, *Aphytis* species, which predominantly parasitise and kill third instar virgin female scale, and *Comperiella bifasciata*, which predominantly parasitises third instar mated female scale (Richardson 1978, Forster et al. 1995), were more common later in the season, particularly from mid autumn. Similarly, Sorribas & Garcia-Marí (2010) reported that the highest levels of parasitism by *Aphytis chrysomphali* and *Aphytis melinus* in sweet orange orchards in Valencia in Spain, occurred in autumn (from August and November, peaking in September), and lowest in late winter and early spring (February and March).
Most studies have focused on the parasitoid complexes in which only two or three parasitoid species have been present particularly, *Aphytis melinus* and *Encarsia perniciosi* (Murdoch et al. 1989, Yu et al. 1990, Borer et al. 2004, Sorribas et al. 2010, Sorribas & Garcia-Marí 2010). As noted above, I recorded five parasitoid species in my study. In southern China, part of the regions where the red scale is thought to be native, Beattie (1984) noted that at least 11 primary parasitoid species of red scale have been recorded, including, *Pteroptrix chinensis* (Howard) [Aphelinidae] a gregarious endoparasitoid that Flanders & Gressitt (1958) and Flanders (1966) regarded as the most important parasitoid of red scale. In the following section, I discuss the seasonal incidence of parasitoid species in my study orchards and compare my observations with those reported in other studies.

5.4.2.1. *Aphytis melinus*, *Aphytis chrysomphali* and *Aphytis lingnanensis*

Most work on species of *Aphytis* as parasitoids of red scale has focused on *Aphytis melinus* in California where it is considered to be the most common and effective parasitoid of red scale (Reeve & Murdoch 1985, Murdoch 1989, Borer et al. 2004). During the course of my study, I recorded *Aphytis chrysomphali* and *Aphytis melinus* attacking red scale. The former was generally more abundant than the latter. I did not record *Aphytis lingnanensis* in during my studies. Fig. 5.30 (left) features an *Aphytis* egg on a virgin female red scale; Fig. 5.30 (right) features *Aphytis* larvae on a virgin female.

*Aphytis chrysomphali* occurred in all four orchards in both seasons and was more common than *Aphytis melinus* in the orchards at Somersby in 2009–2010 and at Cornwallis in 2009–2010 and 2010–2011. *Aphytis chrysomphali* was the only species of *Aphytis* present in the orchard at Lower Portland in both seasons, and at Somersby in 2010–2011. *Aphytis melinus* was more common than *Aphytis chrysomphali* in the orchard at Castlereagh in both seasons. I attributed the variation in the occurrence and abundance of *Aphytis* species to their susceptibility to extreme high (*Aphytis chrysomphali*) or low (*Aphytis melinus*) temperatures. The orchard at Somersby had the

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41 It was not observed parasitising red scale on citrus during extensive studies undertaken from June 1983 and to May 1984 in the southern Chinese provinces of Hunan, Guangdong and Fujian by Beattie (1984). He did observe it. He attributed its absence on citrus to possible competition from *Encarsia citrina* and *Comperiella bifasciata*, both of which occurred in red scale on *Cycas revolute* [Cycadales: Cycadaceae], in all orchards sampled, and its presence on cycad to absence of *Comperiella bifasciata* on cycad. During preparations for travelling to Australia to undertake my PhD I collected *Pteroptrix chinensis* parasitised red scale on citrus near Ha Noi in 2006.
The orchard at Lower Portland had the lowest temperatures among my study orchards in winter 2009–2010. Temperatures at Cornwallis were also milder than in my other study orchards in the Hawkesbury Valley. Mean maximum temperatures at Castlereagh were slightly higher than those in the other orchards (Chapter 4). I attributed the absence of *Aphytis melinus* in the orchard at Lower Portland in both seasons and at Somersby in 2010–2011, to its inability to survive the lowest temperatures that occurred at these locations in winter (Chapter 4 and Appendix I). I attributed the high incidence of *Aphytis melinus* at Castlereagh to its tolerance of high temperatures.

My results and conclusions are similar to those reported by other authors. DeBach & Sisojevic (1960) mentioned that *Aphytis chrysomphali* reproduction was best at low temperatures and poorest at high temperatures, whereas, the opposite applied for *Aphytis lingnanensis*. Beattie (1984) also recorded *Aphytis lingnanensis*, most commonly in warmer coastal regions of Guangdong, China, with hot, humid summers and cool winters, and *Aphytis chrysomphali*, in inland areas of the province with cold winters. Sorribas (2007) concluded that parasitism by *Aphytis chrysomphali* in Spain increased from south (warmer) to north (cooler) and peaked during the colder months of the year. Abdelrahman (1974a) reported that, *Aphytis chrysomphali* was less susceptible to low temperatures than *Aphytis melinus*: in laboratory studies, LD$_{50}$ temperatures for survival of adults, larvae, pupae and eggs were 2.34°, -2.88°, 1.21°, -2.64 °C, respectively for *Aphytis chrysomphali*, and -1.10°, -2.47°, 1.58°, -0.53°C for

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**Figure 5.30.** Egg (left) and larvae (right) of *Aphytis* species on third instar virgin female red scale.
Aphytis melinus. According to DeBach & Sisojevic (1960) and DeBach & Argyriou (1966), low temperatures adversely affect the gender ratios and progeny production of Aphytis lingnanensis and Aphytis melinus: more so for Aphytis lingnanensis (Kfir & Luck 1979). Exposure of Aphytis lingnanensis adults to -1°C for 8 h or more would cause 100% mortality of sperm in males and in female spermathecae (DeBach & Rao 1968).

I consider such impacts as the most likely cause the absence of Aphytis lingnanensis in all orchards, and the absence of Aphytis melinus at Lower Portland (about 30 km from the location where it was released in 1991) even though both Aphytis melinus and Aphytis lingnanensis are more effective than Aphytis chrysomphali at high temperatures. The impact of low temperatures on the gender ratios of Aphytis lingnanensis and Aphytis melinus suggests that Aphytis chrysomphali is an effective parasitoid at low temperatures because the red scale race of Aphytis chrysomphali is a parthenogenetic thelytokous ectoparasitoid. My molecular results (Chapter 9) also indicated that presence of Wolbachia in Aphytis lingnanensis and Aphytis melinus may have contributed to their susceptibility to extreme low and high temperatures in my study orchards.

Aphytis species are synovigenic: they feed on their hosts in order to produce eggs. Rosen & DeBach (1979) considered such host-feeding to be an important cause of scale mortality caused by Aphytis females. They presented a relationship in which scale mortality rose rapidly from very low levels of parasitism before levelling off at approximately 65% mortality when parasitism of reach about 5% (Rosen & DeBach 1979: Figure 56 on page 51). However, during my study I rarely observed scale killed by host feeding. The percent of unexplained deaths of second and virgin female stages reached about 20% of total scale of these two stages (7% of total live scale) in autumn 2010 in the Washington navel block at Somersby (in which the levels of parasitism were highest among my study orchards in autumn). Low levels of host-feeding on red scale by Aphytis melinus have also been recorded in Spain where Sorribas & Garcia-Mari (2010) recorded only 2.1% of host-feeding per 250 live, mostly virgin, scale collected from the field. However, they commented that this host feeding was probably underestimated as scale died rapidly after being fed on by Aphytis; the incidence of host feeding was, therefore, not always evident. When I replotted data from Figure 56 in Rosen & DeBach (1979) I obtained a linear correlation between percent scale mortality
and percent parasitism which suggested that 55% unexplained mortality occurred in the absence of parasitism and that this level of unexplained mortality increased by about 10% when parasitism reached approximately 25%.

I attributed the low levels of host feeding in my study to possible use of unidentified non-host foods by *Aphytis* adults. Rosenheim et al. (2000) mentioned that adult female *Aphytis aonidiae* (Mercet) feed on host and (unspecified) non-host foods, and can use the nutrients obtained thereby to continue to mature eggs. This is presumably similar for other *Aphytis* species. Additionally, *Aphytis* larvae can store proteins and other nutrients while they develop. These proteins and other nutrients can subsequently be used during the adult stage to mature approximately 15 eggs without any additional protein meals (Rosenheim et al. 2000).

Host feeding and scale-size preference could probably be related to the low levels of parasitism by *Aphytis* in spring and summer. Low levels of scale density for host feeding in spring and summer may reduce the reproductive rate. Moreover, *Aphytis* species prefer large-size scales to small ones and scales on fruit and the leaves to those on the wood (Yu & Luck 1988, Forster et al. 1995, Hare & Morgan 2000). Therefore, the winter scale populations, particularly on the wood, could be relatively unsuitable for parasitism by *Aphytis*.

Beattie et al. (unpublished data) recorded an average of 28.2% parasitism by *Aphytis* species of red scale on scale infested fruit sampled on 17 occasions during autumn months from 1981 to 1984 on the Somersby Plateau. The incidence of host feeding by *Aphytis* adults on scale in these samples was either very low (Beattie et al., unpublished data).

I could not determine if *Aphytis melinus* has dispersed over 50–60 km (directly), to reach orchards on the Somersby Plateau from Richmond naturally or in association with human activities (from Richmond in a direct line) since it was released at Richmond in 1991. However, my morphological and molecular studies in Chapter 8 detected differences between *Aphytis melinus* populations in at Castlereagh and Somersby. These differences and their consequences are discussed in Chapter 8. Furthermore, I did not record *Aphytis melinus* from my orchard at Lower Portland (about 20 km from Richmond) during my study. This may have been a consequence of orchards along the Hawkesbury River often being separated by hilly areas of native vegetation.
5.4.2.2. *Encarsia citrina* and *Encarsia perniciosi*

*Encarsia citrina* was the predominant species of *Encarsia* at Somersby and Cornwallis: it accounted for > 90% parasitism by the two species, and average parasitism for all samples from the two locations over the two seasons was about 20%. Beattie (1984) recorded similar levels (17%) of parasitism by *Encarsia citrina* during his studies in southern China. There are no reports of parasitism from other locations with similar environments.

*Encarsia perniciosi* was predominant at Lower Portland and Castlereagh: it accounted for 85 and 63% of parasitism by the two species at these two locations, respectively, and average parasitism for all samples from the two locations over two seasons was about 19%. Fig. 5.31 features scale parasitised by the *Encarsia* species.

![Figure 5.29. Red scale parasitised by *Encarsia* species: (top left) second instar red scale, (top right) second moult, (bottom left) third instar virgin, and (bottom right) second instar male.](image)
The seasonal abundance of the species at Somersby and Cornwallis suggested a tendency towards at least three generations from spring 2009 to autumn 2010. These corresponded to three peaks of the second instar stage of the scale.

Matadha et al. (2004) reported that, under laboratory conditions, *Encarsia citrina* required 434.8°D at lower development thresholds of 10.4°, 6.3° and 9°C for larvae, pupae and adults, respectively. The total development time was 22.7 d at 27.5°C and 47.4 d at 17.5°C (Matadha et al. 2004). McClure (1978) recorded two generations of *Encarsia citrina* on elongate hemlock scale (*Fiorinia externa* Ferris) and shortneedle conifer scale (*Dynaspidiotus* (syn. *Tsugaspidiotus*) *tsugae* (Marlatt)) annually from 1975–1978 in Fairfield County in Connecticut, where the average temperature in July was 23°C (16.1 to 29°C) and -2.3°C (-7.3 to 2.7°C) in January (McClure 1978). Based on the monthly average temperature records from Westport (41.1° N, 73.3° W) in Connecticut and Richmond Airport in New South Wales, I estimated that °D accumulations at lower development threshold of 10°C at the two locations were approximately 1400° and 2289°D, respectively. Therefore, I attributed the differences in the number of annual generations of *Encarsia citrina* in my study and those reported by McClure (1978) to differences in °D accumulations.

Levels of parasitism by *Encarsia perniciosi* were lower in the summer generation than in the autumn generation. These trends were similar to those reported by Yu et al. (1990), who mentioned that parasitism of red scale by the red scale strain of *Encarsia perniciosi* peaked in autumn (August & September) and troughed during the remainder of the year in California. My results differed to those of Sorribas & Garcia-Marí (2010) who in their studies observed the highest rates of parasitism by *Encarsia perniciosi* in spring, with levels up to 21% on twigs and 10% on fruit.

McClain et al. (1990) recorded six flights of a uniparental race of *Encarsia perniciosi* parasitising San José scale in an apple orchard at Clayton (35° 39' N, 78 ° 32' W) in North Carolina. These flights probably corresponded with peaks of different lifecycle stages of scale from which the parasitoids emerged. On some occasions, numbers of parasitoids caught were very low (McClain et al. 1990). In my study, the majority of parasitism by the *Encarsia* species was recorded in the second instar and second moult stages of red scale. I estimated, based on the monthly average temperature records from

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42 http://www.homefacts.com/weather/Connecticut/Fairfield-County/Westport.html
43 http://weather.yahoo.com/climo/USNC0132_f.html?woeid=2381103
Clayton and from Richmond Airport, that °D accumulations at lower development threshold of 10°C at these locations were approximately 2355° and 2289 °D, respectively. Due to the small difference in °D accumulations between these two locations, the number of generations in these two locations would probably be similar.

My results for stages of red scale parasitised by *Encarsia perniciosa* (presented in Chapter 6) were similar to observations reported by Yu et al. (1990) and information summarised by Forster et al. (1995). Forster et al. (1995) noted that *Encarsia* species prefer to lay their eggs in second instar scale male and female, and that the adults emerge from second moult or mummified third instar. Similarly, Yu et al. (1990) reported that in grapefruit orchards in Ventura in California in 1984–1985, *Encarsia perniciosa* preferred small scales and that 86% of adults emerged from second moult and second instar male, 11% from third instar virgin female, and only 3% from mated female scale. In contrast to my results, the highest levels of parasitism by *Encarsia perniciosa* reported by Sorribas & Garcia-Marí (2010) were in virgin and mated females in spring and summer. I assume the differences between my observations and those of Sorribas & Garcia-Marí (2010) could be related to environmental factors influencing host suitability or to differences in strains of the parasitoid. Sorribas & Garcia-Marí (2010) attributed, at least in part, an intense reduction in the presence of *Encarsia perniciosa* during summer and autumn to predation by *Aphytis* larvae on *Encarsia* parasitised scale that were more abundant in these periods of the year. Yu et al. (1990) reported that *Encarsia perniciosa* was outcompeted by *Aphytis melinus* on the same host under laboratory conditions. They showed differences in host stage and substrate preferences between two species and concluded that these differences were a result of interspecific competition. During the course of my study, I very rarely observed *Aphytis* larvae parasitising scales initially parasitised by *Encarsia*. I attributed the differences between my observations related to *Aphytis* species parasitising scales initially parasitised by *Encarsia* and those reported by Sorribas & Garcia-Marí (2010) to differences in the host stages parasitised by *Encarsia perniciosa* in the studies. The high predation of *Encarsia perniciosa* by *Aphytis* species reported by Sorribas & Garcia-Marí (2010) may have been due to *Encarsia perniciosa* and *Aphytis melinus* both preferring to parasitise virgin female scale.
5.4.2.3. *Comperiella bifasciata*

My results showed that levels of parasitism by *Comperiella bifasciata* on orchards on the Central Coast of New South Wales were relatively low in the summer generation and then increased to peak in autumn. It was more common at Somersby in 2009–2010, where winter levels of parasitism averaged 42%, than in the Hawkesbury Valley orchards where winter parasitism averaged 16%. I attributed this to the parasitoid preferring milder and more humid conditions than those that prevailed in the Hawkesbury River orchards. The levels of parasitism I recorded at Somersby were similar to those observed by Beattie et al. (unpublished data), who recorded an average of 46.4% parasitism for 18 monthly samples of scale infested fruit sampled in winter months from 1981 to 1984 at Peats Ridge and Kulnura. Other records (Beattie et al. unpublished) for inland New South Wales also indicated a preference by *Comperiella bifasciata* for milder conditions. In assessments based on large numbers of scale-infested fruit sampled in the late 1970s and early 1980s, winter parasitism of mated female scale by *Comperiella bifasciata* averaged 60.1% at Barham (35° 37' S, 144° 07' E) and Tocumwal (35° 48' S, 145° 34' E) on the Murray River under a slightly milder conditions than at Yanco (34° 36' S, 146° 25' E) and Griffith (35° 17' S, 146° 03' E) in the Riverina (Table 2.3 in Chapter 2, and Table 5.3) where the average was 22.4%. Beattie (1984) recorded an average of 38% parasitism by *Comperiella bifasciata* of mated red scale females (n = 2165) during his studies in southern China.

<table>
<thead>
<tr>
<th>Location</th>
<th>Average days annually with maximum ambient temperatures ≥ 40 °C</th>
<th>Relative humidity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>09:00</td>
</tr>
<tr>
<td>Peats Ridge</td>
<td>0.6</td>
<td>75</td>
</tr>
<tr>
<td>Barham (based on Kerang)</td>
<td>4.7</td>
<td>69</td>
</tr>
<tr>
<td>Tocumwal</td>
<td>4.7</td>
<td>73</td>
</tr>
<tr>
<td>Griffith</td>
<td>5.2</td>
<td>66</td>
</tr>
<tr>
<td>Yanco (based on Narranderra)</td>
<td>4.9</td>
<td>68</td>
</tr>
</tbody>
</table>

Table 5.3. Annual average days with maximum ambient temperatures ≥ 40 °C, and relative humidity (%) at 09:00 and 15:00 (based on long-term Bureau of Meteorology records) at Peats Ridge (Central Coast), Barham, Tocumwal (Murray River), and Griffith and Yanco (Riverina).
Figure 5.32. Comperiella bifasciata larvae in mated female red scale (top and bottom left), exit hole in a mated female red scale (bottom right), and an adult (top right).

My results also showed that levels of parasitism by Comperiella bifasciata were lower in 2010–2011 than in 2009–2010. I attributed this to impacts of the record heatwave in late January and early February 2011, and rainfall and numbers of rainy days in autumn 2011, particularly on the Somersby Plateau. As mentioned above, the heatwave occurred during summer peaks of virgin and mated female red scale.

In contrast to my records and studies by Beattie (1984) and Beattie et al. (unpublished), studies on Comperiella bifasciata in the coastal, intermediate and interior regions of southern California (Fig. 2.7, Chapter 2) suggests that it is less effective in California in relatively mild coastal regions than it is in relatively harsher interior regions (weather records for the citrus producing regions of California and New South Wales were summarised Chapter 2, Table 2.6). DeBach & Sundby (1963) also mentioned that Comperiella bifasciata was better adapted to interior or intermediate zones of southern California, than to coastal zones. Forster et al. (1995) mentioned that Comperiella bifasciata occurred more commonly in arid interior and similar regions of southern
California than in nearby coastal areas. Yu (1986) reported that parasitism of mated female scales on lemon, grapefruit and navel orange branches by *Comperiella bifasciata* in orchards at Corona and Riverside during 1983–1984 in the ‘interior’ citrus region of southern California ranged from 3 to 33%. Carroll & Luck (1984) recorded levels of 60–80% (‘high’), 20–60% (‘moderate’), 20–45% (‘low’) and 5–35% (‘very low’) on fruit, leaves, twigs and branches sampled from mature navel orange trees near Exeter (with hotter summers and colder winters that coastal southern California) in the San Joaquin Valley. These levels of parasitism were regarded as being inadequate to control the scale (Carroll & Luck 1984).

During the course of my study, I did not observe scale containing encapsulated eggs of *Comperiella bifasciata*. Beattie (unpublished data, pers. comm., 2008), who examined hundreds of mated female red scale from New South Wales Central Coast orchards during the late 1970s and early 1980s, very rarely observed encapsulation of *Comperiella bifasciata*. In contrast, Brewer (1971) and Blumberg & Luck (1990) reported 84.7 and 28% encapsulation, respectively, under laboratory conditions. Both of these studies were based on the red scale strain of the parasitoid reported that only of solitary eggs of red scale strain *Comperiella bifasciata* were encapsulated under the laboratory conditions. I attributed the differences between my observations and those of Brewer (1971) and Blumberg & Luck (1990) to the conditions in which the observations were made. I also consider it possible that the Brewer (1971) may have inadvertently based his studies on the yellow scale race of *Comperiella bifasciata*. Alternatively, susceptible forms of the red scale strain parasitoids may have gradually become extinct or very rare, at least in Australia. My molecular studies (Chapter 9) indicated that specimens of *Comperiella bifasciata* parasitising red scale and yellow scale on citrus on the Central Coast and Griffith in New South Wales, and near Perth in Western Australia, were genetically identical.

Fig. 5.32 features adult female red scale parasitised by *Comperiella bifasciata*, an exit hole of the parasitoid in a mated female red scale, and a *Comperiella bifasciata* adult.

### 5.4.3. Predation

*Halmus chalybeus* was the most commonly observed predator in all of my study orchards. It appeared to be effective at both low and high host densities. The incidence
of *Halmus chalybeus* and levels of footprints differed from block to block. The highest incidence of footprints was recorded in the Washington navel block at Castlereagh and the lowest in the Washington navel block at Somersby: both in autumn 2010. In 2010–2011, the incidence of footprints was lower than in 2009–2010 in all orchards. I attributed this to: (a) low scale population densities in summer and early autumn leading to insufficient food for populations of the beetle to increase in late autumn; (b) and impacts of rainfall and numbers of rainy days on the oviposition and searching efficiency. Low incidence of adults, larvae and percent footprints in the Washington navel block at Castlereagh, in which I recorded 88% footprints in 2009–2010, may have been relate to canopy densities, which declined during the course of my study due to lack of interest by the owner of the orchard in maintaining the trees. Low incidence of footprints at Cornwallis may have been due to the application, as mentioned above, of mineral oil sprays for control of citrus leafminer. The seasonal incidence of *Halmus chalybeus* adults and larvae was not consistent among blocks and orchards. The reasons for the variation were not apparent. Number of adults and larvae were highest during January, February and March.

Anderson (1981, 1982) reported the summer and winter dormancy of *Apolinus (Scymnodes) lividigaster* (Mulsant) and breeding in spring and late summer-autumn. However, she mentioned that this was related to the abundance of the prey (species of aphids). In contrast, *Halmus chalybeus* can prey on a wide range of hosts, particularly armoured scale insects that are abundant throughout late spring to early winter. Flynn (1995) reported that in Auckland (36° 51' S 174° 46' E) in New Zealand in 1993–1994, when mean minimum temperatures in June, July and August were 6–8°C, *Halmus chalybeus* clustered in curled leaves or firmly overlapping leaves from the end of May. From late June to late July, 80% or more of the sampled populations were found in clusters. Clustering was less evident in August and was no longer observed in late September (Flynn 1995). He (Flynn 1995) recorded that winter dormancy of *Halmus chalybeus* was not true dormancy and regarded it as ‘Facultative Oligopause’, based on elytral shade and hardness, low incidence of sperm in female spermathecae (despite production of sperm by males), low incidence of food particles in adults crops (10–15% of adults), and a decline in fat body content and development in winter.

Although I did not study dormancy of *Halmus chalybeus*, my observations in my study orchards suggested that its adults did not experience dormancy. I did not observe them
in clusters in winter but they did shelter between touching leaf surfaces. Two of my colleagues, Mr Goran Lopaticki and Dr Tara Murray (University of Western Sydney, pers. comm., 2011), also observed *Halmus chalybeus* occasionally between eucalyptus leaves at Richmond in winter 2011. I assume that seasonal trends of *Halmus chalybeus* populations may therefore be related to seasonal variation in temperature and humidity. The abundance of eggs in spring and autumn suggested that the heat and dryness in summer may reduce predatory activities and reproduction and thereby lead to a decline in beetle populations.

I observed that *Halmus chalybeus* eggs (Fig. 5.33) were laid randomly as clusters of 6 to 14 eggs and choice of oviposition site did not appear to be related to the host density. There are no records in the literature with which I could compare my observations. However, Seagraves (2009) reported that the selection of oviposition sites by some coccinellid species in proximity to food for young larvae must be weighed against the risk of cannibalism and intraguild predation. This oviposition behaviour may be a defence mechanism by which the beetle may minimise the probability of the eggs being cannibalised by conspecific adults, or preyed by other predators. Michaud & Grant (2004) reported sibling egg cannibalism by newly hatched larvae of three coccinellid, *Cycloneda sanguine* L., *Harmonia axyridis* (Pallas), and *Olla v-nigrum* Mulsant. Hales (1976) also reported sibling cannibalism for the Australian coccinellid, *Coelophora inaequalis* (F.). Based on these studies and my observations, I assume that newly hatched larvae *Halmus chalybeus* may also cannibalise their sibling eggs. If it occurs, this behaviour may also be related to the fact eggs are deposited randomly and the newly hatched larvae can access food in situ before they search for scale hosts.

In 2010–2011, the incidence of *Halmus chalybeus* adults peaked from late December to early February. This followed peaks in the abundance of the eggs and young larvae (Fig. 5.33) of the predator in late October and early November. The eggs were again observed from mid February and became relatively abundant in March. My observations suggested that there were at least two generations of *Halmus chalybeus* on Central Coast of New South Wales. At least two annual generations were recorded by Flynn (1995) in Auckland, where the mean maximum temperature in summer months was about 24°C, seldom exceed 30°C, and mean minimum temperatures in winter month were about 6–8°C.
However, the seasonal incidence of the predator recorded by Lo (2000) in Northland in northern New Zealand differed to observations by Beattie et al. (1991) on the Central Coast of New South Wales. Beattie et al. (1991) mentioned that population peaks in late-summer/autumn and in spring/early-summer coincided with seasonal rises and falls in temperature and evaporation. Population troughs in late-spring/summer and winter coincided with peaks in temperature and evaporation in summer and troughs in temperature and humidity in winter (Beattie et al. 1991). Differences between the two studies may have been related to impacts of the weather conditions when observations were undertaken, and to the frequency of the observations and host phenologies.
Weather conditions when the observations were made in my study had a considerable impact on the numbers of *Halmus chalybeus* adults and larvae I recorded. The beetle congregated inside tree canopies and on the tree trunks in the hot and dry days (ambient temperature ≥ 40°C). Likewise, it appeared to be less active in cold weather and during rain.

Little is known about the biology of *Halmus chalybeus* in Australia, even though it is indigenous ladybird and was reported to be an important and abundant predator in New South Wales by Koebele (1892) and Froggatt (1902). It is possible that the use of lime-sulphur or Bordeaux since the late 1800s (Crichton 1893a) to control white louse scale and diseases such as citrus scab (*Elsinoë fawcettii* Bitancourt & Jenkins), melanose (*Diaporthe citri* F. A. Wolf) and black spot (*Guignardia citricarpa* Kiely) (Hely et al. 1982, Scott 1982) may have adversely affected *Halmus chalybeus* in the region. However, there are no records to verify such impacts.

I did not observe *Halmus chalybeus* feeding on black scale during studies reported in this chapter or in my studies (Chapter 7) on the impact of honeydew-foraging ants on the activity of *Halmus chalybeus*. In contrast, Valentine (1974) recorded *Halmus chalybeus* as a predator of black scale in New Zealand, where it was apparently introduced for control of the scale (Flynn 1995). However, Valentine (1974) did not mentioned impacts of honeydew-foraging ants on the activity of *Halmus chalybeus*. Lo (2000) and Lo & Chapman (2001) reported that *Halmus chalybeus* is an important predator of *Ceroplastes destructor* and *Ceroplastes sinensis* in northern New Zealand. However, during the course of my study, I did not observe *Halmus chalybeus* in association with *Ceroplastes destructor* or *Ceroplastes sinensis*.

My observations confirmed that *Orcus australasiae* larvae and adults prey on red scale and other armoured scales. This supports observations in the late 1800s and early 1900s by Tryon (1889), Koebele (1892), Crichton (1893a), Froggatt (1902). It is native to Australia where it has been recorded in Queensland, New South Wales, Victoria, Tasmania and Western Australia (Froggatt 1902, Łączyński & Tomaszewska 2009). Hely et al. (1982) mentioned that it it as common in coastal districts of New South Wales, and sometimes in mild wet conditions in inland regions of the state. James et al. (1999) recorded it in irrigated citrus orchards at Leeton in inland regions of the state.
Koebele (1892) observed, during visit to Australian in 1891, that several coccinellid species including, *Orcus australasiae*, were always present where black scale was abundant in New South Wales. In the same publication, he noted that ‘A large number of predaceous insects were found preying upon the red scale in Australia. Of the most numerous were *Orcus chalybeus, Orcus australasia, and Rhizobius satellus*. My observations suggested that adults and larvae prefer feeding on the scale in trees with open and thin canopies. Similar to observations were reported by Issac (1906). He reported that the beetle was more numerous in the top and the outside parts of the tree and that it preferred surfaces exposed to sunshine than shaded surfaces. The abundance of *Orcus australasiae* in the orchards at New South Wales Industry & Investment’s Somersby Research Station and at Kulnura and Peats Ridge, but not in my other study orchards in the region suggested that, in contrast to *Halmus chalybeus*, this species is associated with high-density red scale populations in citrus orchards, particularly in the presence of soft scales such as black scale.

I commonly observed *Orcus australasiae* feeding on red scale, but not on black scale in my study orchards. However, they may feed on first and second instar black scale as Broughton (2011) mentioned it as the most important predator of the scale in olive groves in Western Australia, and Koebele (1892) mentioned that it was commonly associated with it. There is no mention in the literature of its natural hosts but its host include aphids (Crichton 1893a, Waterhouse & Sands 2001, Sandanayaka et al. 2003). Boisduval (1835) did not mention the prey associated with the specimens on which he based his description of the species.

My study was the first in which eggs of *Orcus australasiae* have been observed or reported, in this instance under the integuments of dead black scale, particularly those with parasitoid exit holes of *Metaphycus* spp. and *Scutellista caerulea*. I also observed the beetle in association with white wax scale and hard wax scale, and I assume females may also lay their eggs under the integuments of dead wax scale females, particularly those with parasitoid exit holes. My observations suggest that *Orcus australasiae* may be associated with native coccids such as *Eriococcus, Lecanium (=Coccus), Spharococcus* and *Rhizococcus* species (Maskell 1892), and associated armoured scales.

*Rhyzobius lophanthae*, which has been widely introduced to other countries from Australia (Koebele 1892, Compere 1961, Greathead 1973, Rosen & DeBach 1978), has been more commonly mentioned in the literature as a predator of red scale than *Halmus*
*chalybeus* and *Orcus australasiae*. Abdelrahman (1974a) reported that the thermal death temperature (LD$_{50}$) for the cold and the heat were approximately 0 and 41.7°C. Atkinson (1983) reported that *Rhyzobius lophanthae* was the most common predator of red scale in Swaziland lowlands between 1972 and 1975 and that the relationship between it and red scale was density-dependent. Samways (1985) reported that red scale predated by *Rhyzobius lophanthae* were 8–9% and 15–8% of red scale in the tops and bottoms of the trees in the orchards at Nelspruit (South Africa) in 1982–83 and 88% at Komatipoort on the 12 April 1983. Sorribas & Garcia-Marí (2010) reported that it was one of the most abundant predators in Valencia, eastern Spain, during 2006–2008. They (Sorribas & Garcia-Marí 2010) recorded 109 adults and larvae of *Rhyzobius lophanthae* from 19 groves. My observations indicated that *Rhyzobius lophanthae* occurred under high scale density populations. I attributed this to the oviposition characteristics of the adult which required scale cover to protect their eggs. The number of prey required to complete life cycle of *Rhyzobius lophanthae* male and female were 390.6 and 672.3 adult female *Aspidiotus nerii*, respectively (Stathas 2000). The high consumption of *Rhyzobius lophanthae* may also related to its occurrence in high scale densities. The high scale densities may also provide potential oviposition sites for of *Rhyzobius* adults.

Koebele (1892) estimated that *Rhyzobius lophanthae* has about 6 broods per year in coastal New South Wales. He also estimated that one female could lead to 15,000,000,000 beetles in one year. When I reared *Rhyzobius hirtellus* (Fig. 5.34) on oleander scale in the laboratory I obtained hundreds of adults after about one month. My observations and those of Koebele suggest potential for mass rearing and augmentative release of these highly voracious beetles in orchards, particularly in inland orchards where *Rhyzobius* species are more effective than *Halmus chalybeus* (Hely et al. 1982).

![Figure 5.34](image)

*Figure 5.34. Rhyzobius hirtellis* cultured on oleander scale.
5.5. Conclusions

My study is the first that I know of in which the ecology of red scale has been studied in the presence of five of its primarily parasitoids, four coccinellid predators, and six entomopathogens (see Chapters (9 & 10). In spite of the variation in scale densities, levels of parasitism and incidence of predation, scale numbers on fruit in my study decreased in autumn and early winter and did not exceed economic thresholds. Cold weather in winter and natural enemy activities were important factors limiting the potential for the scale to be a serious pest in the region. My results showed that variation in levels of parasitism by each of the parasitoids I recorded was related to weather conditions, particularly temperature extremes. Levels of parasitism by *Aphytis* species and *Comperiella bifasciata* on the Somersby Plateau were consistent with records from the 1980s, but my study was the first to highlight the impact of variation in climate on the incidence of these parasitoids within the region. My study was also the first to show that the *Encarsia citrina* and *Encarsia perniciosi* are important, previously ignored or under-rated, parasitoids of red scale in citrus orchards on the Central Coast of New South Wales. During the course of my study, I was able to assess impacts of a record heatwave on scale populations and natural enemy activity.

My study is the first to confirm to observations made by Koebele (1892) and Froggatt (1902) about the role of coccinellid predators, particularly *Halmus chalybeus* in the control of red scale. It was clear from my results that predators, particularly *Halmus chalybeus*, play major roles in regulating scale populations in the region. The biology and ecology of this beetle warrants further study. The following should be addressed in orchards with introduced hosts and in natural ecosystem with native prey:

- seasonal incidence;
- prey consumption of adult and larval stages on red scale;
- oviposition behaviour;
- preferred habitats;
- alternative habitats during harsh conditions, such as extreme temperatures and rain;
- seasonal development and reproduction, including dormancy and breeding status;
- effects of extreme temperatures on the development and survival; and
- cannibalism and dispersal of the beetle.
Scale densities were maintained at levels below economic thresholds by both abiotic and biotic factors. These factors are listed below in order of importance.

- °D limitations to the number of annual generations of red scale (Chapter 4);
- impacts of limitations on the number of annual generations of the scale posed by cold evening and night temperatures in winter and spring on survival of prepupal and pupal male stages and subsequently, delayed mating of virgin females and mated female longevity (Chapter 4);
- suppression of scale populations by predators, particularly *Halmus chalybeus*; and
- suppression of scale populations by the ecto- and endoparasitoids.

My study was the first to report the numerous abundance of *Orcus australasiae* and location of eggs of *Orcus australasiae*. This finding provided fundamentals for the potential future research on this native ladybird in Australia.

Climate changes might influence the biological control of red scale in the region. As a native species, *Halmus chalybeus* would be able to avoid the future increased temperatures by congregating in the cooler areas. *Aphytis melinus* might become more common than *Aphytis chrysomphali*. With short life cycle and high fecundity, *Encarsia* species could readily recover after heat waves. Environmental factors such as weather in winter would still contribute to reduce scale populations in winter.
Chapter 6. Density and other interactions between red scale, its parasitoids, and the coccinellid predator, *Halmus chalybeus*

6.1. Introduction

Reported interactions between insects and their natural enemies include relationships between host densities and levels of parasitism and predation, host size and host plant substrates, and intraguild predation. Views on relationships between host densities and parasitoids and predators and whether, or not, density dependency exists have been debated for several decades (Varley 1941, Comins & Hassell 1979, Hassell 1980, 2000, Lessells 1985, Reeve 1987, Stiling 1987, Walde & Murdoch 1988, Matsumoto et al. 2004). Of 171 relationships reviewed and comprehensively summarised by Stiling (1987), 25% were density-dependent, 23% inversely density-dependent and 52% density-independent. Of 201 examples in reviews cited by Hassell (2000), 28% showed direct density-dependent patterns of parasitism, 26% exhibited inverse patterns and 44% showed patterns uncorrelated with host density (i.e., density-independent).

Reports for, and views of, such relationships between armoured scales and their parasitoids and predators have also varied. Flanders (1971) considered ectoparasitoids to be density dependent and endoparasitoids to be inversely density-dependent. McClure (1977) derived density dependent relationships between *Encarsia citrina* and elongate hemlock scale, *Fiorinia externa* (Ferris). Rebek et al. (2006) reported that the relationship between *Encarsia citrina* and euonymus scale, *Unaspis euonymi* (Comstock) was on some occasions inversely density-dependent, but usually density-independent. Matsumoto et al. (2004) found that *Aphytis yanonensis* DeBach & Rosen parasitism of arrowhead scale, *Unaspis yanonensis* Kuwana, was temporally density-dependent at the whole orchard level, while parasitism by *Coccobius fulvus* Compere & Annecke [Hymenoptera: Aphelinidae] was not. Parasitism by both species was rarely positive to scale density at the single tree level (Matsumoto et al. 2004).

For red scale in Swaziland in southern Africa, Atkinson (1977) found that proportions of predation, largely by *Rhyzobius lophanthae*, and parasitism by *Aphytis* species and
scale density appeared to be density-dependent, whereas proportions of parasitism by *Comperiella bifasciata* and *Habrolepis rouxi* Compere [Hymenoptera: Encyrtidae] were density-independent, or perhaps inversely density-dependent. In South Africa, Samways (1985) found that the behaviour of *Aphytis* species (including *Aphytis melinus*) was inversely density-dependent at relatively high density populations of the scale.

In California, Reeve & Murdoch (1986) and Reeve (1987) found no evidence of a dependent relationship between red scale density and parasitism by *Aphytis melinus*. Murdoch et al. (1985) and Reeve & Murdoch (1986) reported that, in such regions, *Aphytis melinus* can persist at low stable equilibriums. Murdoch et al. (1995) also recorded no direct density-dependency between parasitism rate, host mutilation and predation by *Aphytis melinus* and red scale density. They also found that no density-dependent relationship between parasitism by *Encarsia perniciosi* and scale density. In South Australia, Smith & Maelzer (1986) also found no evidence of a dependent relationship between red scale density and parasitism by *Aphytis melinus*. The studies in Africa, California and Australia were undertaken in regions where *Aphytis melinus* is regarded as the most important parasitoid of red scale.

Mechanisms for coexistence of *Aphytis melinus* and *Encarsia perniciosi* on red scale have been described by Yu et al. (1990) and Borer (2002) under laboratory conditions, and Borer et al. (2004) and Sorribas & Garcia-Marí (2010) under field conditions in California, United States of America, and in Valencia, Spain, respectively. These mechanisms relate to differences in scale stages and size, and host substrates (Yu et al. 1990, Borer 2002, Sorribas & Garcia-Marí 2010). Borer (2002) and Borer et al. (2003) reported that *Aphytis melinus* and *Encarsia perniciosi* can coexist via intraguild predation, defined by Polis et al. (1989) as a combination of competition and predator/parasitism interaction among members of the same guild or exploitation of the same resource in a similar way. Flanders (1966) assumed that the abortive attempts to maintain a mixed culture of *Aphytis lingnanensis* and *Encarsia perniciosi* on red scale, as reported by DeBach & Sundby (1963), may have been related to mutilation of latter by the former. In contrast to these reports (Flanders 1966, Borer 2002, Borer et al. 2003, Sorribas & Garcia-Marí 2010), I rarely observed *Aphytis* predation on *Encarsia* species during the course of my study. Likewise, Beattie (pers. comm., 2011) does not
recollect observing *Aphytis* predation on *Encarsia* when he examined red scale on fruit sampled from citrus orchards on Central Coast of New South Wales in 1980s.

My data sets permitted opportunities for me to assess host-natural enemy density relationships for red scale and its natural enemies in complex ecosystems within orchards on the Central Coast of New South Wales: for five parasitoid species, *Aphytis chrysomphali, Aphytis melinus, Encarsia citrina, Encarsia perniciosi* and *Comperiella bifasciata*, and the most important native coccinellid predator, *Halmus chalybeus*.

I analysed data from my studies in order to determine if such interactions occurred in my study orchards based on the following theory: that, evidence for effective interference between parasitoid species would be revealed if the proportion of total parasitism on a given stage of host by a given parasitoid species changed with the abundance of unparasitised potential hosts at the census. This presupposes that a superior competitor would increase its share of hosts if there were not enough hosts for all parasitoids or hosts were difficult to locate (i.e., when there were few or zero hosts remaining unparasitised). The reverse would be the case for an inferior competitor.

In Chapter 4, I based calculations of percent parasitism by each parasitoid on numbers of parasitised scale of the relevant stage as function of total numbers of scale of the relevant stage plus older stages that were not parasitised. In this chapter, I use total numbers of the scale stage parasitised as the denominator. Although the host stage preferences for each parasitoid species have been widely recorded, my records provided me with an opportunity to determine seasonal trends for each parasitoid in relation to available hosts. The records also provided me with an opportunity to determine if differences occurred among my study orchards.

**6.2. Materials and methods**

**6.2.1. Sampling procedures and parasitism assessments**

Sampling procedures and parasitism assessments were as presented in Chapter 5.
6.2.2. Statistical analysis

6.2.2.1. Parasitism related to date and stage of host

Percent parasitism by *Aphytis* species, *Encarsia* species and *Comperiella bifasciata* recorded at each scale stage assessed on one sampling occasion was calculated as follows:

\[
C. \text{ bifasciata of 3M}^O = \frac{3M^O \text{ parasitised by } C. \text{ bifasciata}}{3M^O \text{ parasitised by } C. \text{ bifasciata} - \text{unparasitised} 3M^O - 3M^O \text{ parasitised by Encarsia spp.}}
\]

\[
\text{Aphytis spp. of 3M}^F = \frac{3M^F \text{ parasitised by Aphytis spp.}}{3M^F \text{ parasitised by Aphytis spp.} - \text{unparasitised} 3M^F - 3M^F \text{ parasitised by Encarsia spp.}}
\]

\[
\text{Encarsia spp. of 3M}^F = \frac{3M^F \text{ parasitised by Encarsia spp.}}{3M^F \text{ parasitised by Encarsia spp.} - \text{unparasitised} 3M^F - 3M^F \text{ parasitised by Aphytis spp.}}
\]

\[
\text{Aphytis spp. of 2M}^F = \frac{2M^F \text{ parasitised by Aphytis spp.}}{2M^F \text{ parasitised by Aphytis spp.} - \text{unparasitised} 2M^F - 2M^F \text{ parasitised by Encarsia spp.}}
\]

\[
\text{Encarsia spp. of 2M}^F = \frac{2M^F \text{ parasitised by Encarsia spp.}}{2M^F \text{ parasitised by Encarsia spp.} - \text{unparasitised} 2M^F - 2M^F \text{ parasitised by C. bifasciata}}
\]

\[
\text{Encarsia spp. of 2M}^F = \frac{2M^F \text{ parasitised by Encarsia spp.}}{2M^F \text{ parasitised by Encarsia spp.} - \text{unparasitised} 2M^F - 2M^F \text{ parasitised by Aphytis spp.}}
\]

\[
\text{Aphytis spp. of 2M}^F = \frac{2M^F \text{ parasitised by Aphytis spp.}}{2M^F \text{ parasitised by Aphytis spp.} - \text{unparasitised} 2M^F - 2M^F \text{ parasitised by Encarsia spp.}}
\]

\[
\text{Encarsia spp. of PP & RF} = \frac{\text{PP & RF parasitised by Encarsia spp.}}{\text{PP & RF parasitised by Encarsia spp.} - \text{unparasitised PP & RF} - \text{PP & RF parasitised by Aphytis spp.}}
\]

\[
\text{Aphytis spp. of PP & RF} = \frac{\text{PP & RF parasitised by Aphytis spp.}}{\text{PP & RF parasitised by Aphytis spp.} - \text{unparasitised PP & RF} - \text{PP & RF parasitised by Encarsia spp.}}
\]

\[
\text{Footprints by } H. \text{ chaetica} = \frac{\text{number of footprints}}{\text{number of footprint} - \text{unparasitised scale} - \text{parasitised scale}}
\]
Standard errors were estimated using the formula $SE = (((p \times q)/n)^{0.5}) \times 100$, where $p =$ proportions of parasitism at each stage, $q = (1 - p)$ and $n =$ the total number of scale counted of the assessed stage.

Data from each of two blocks in each of the four orchards at Somersby, Lower Portland, Cornwallis, and Castlereagh in the 2009–2010 season were used. Percentages of parasitism were plotted against °D using SigmaPlot® 10.0 (Systat Software Inc., San Jose, California). Error bars were standard errors as calculated above.

6.2.2.2. Tests for density-related responses

Where sufficient data were available from sampling occasions in 2009–2010 and 2010–2011, linear regressions of proportions of parasitised scale of a particular stage of host against the number of available hosts of that stage were tested for significance and the plots were also related to the expectations of the binomial distribution. Regression between proportions of parasitism and total number of available stages were also determined. Regression of proportion of footprints (see Chapter 5 for definition of footprints) against total number of parasitised and unparasitised scale was also performed.

6.2.2.3. Tests for interference between parasitoid species

Interactions between two parasitoid species on a certain host stage were tested using linear regression analysis. The analysis was performed between two variables, $a/(a+b)$ on $u/(a+b+u)$, where:

- $a =$ the number of hosts parasitised by most common parasitoid,
- $b =$ the number parasitised by the other parasitoid species, and
- $u =$ the number of hosts unparasitised.

Significant slopes should be negative as the proportion of species A should decline relative to that of species B as the proportion of unparasitised hosts increased (i.e., as the competition pressure on species B gets less). Such a test would require a situation where two species of parasitoid were present in sufficient numbers for analysis on a given stage of host and on sufficient fruit on one sampling occasion. The absence of a particular parasitoid on any particular stage cannot be assumed to be unambiguous proof of interference because that species may have been absent or a very low density at the time or was not compatible in some way with the host stage in question. Within a
particular census, data from a particular fruit were only used if at least one individual was parasitised as interference cannot be inferred if no parasitoids are present. This reduced the number of fruits available for regression tests but in 12 cases, the number was in the range 23–36 and only 5 in the range 16–22.

6.3. Results

6.3.1. Parasitism in relation to life cycle stages of red scale

**Second instar female** scale were killed at low levels, up to 20%, by *Aphytis* and *Encarsia* species.

**Second instar male** scale were attacked by *Aphytis* and *Encarsia* species. The highest levels of parasitism by *Encarsia* species on second instar male were recorded in the Washington navel block at Somersby, and the Washington navel and Valencia orange blocks 1 and 2 at Cornwallis, with maxima of 96.5 ± 3.4, 94.5 ± 2.4 and 85.2 ± 4.5%, respectively. The lowest levels were recorded in Washington navel block and Valencia orange block at Lower Portland, with maxima of 44.8 ± 4.8 and 39.6 ± 6.4%, respectively. Highest levels of parasitism by *Aphytis* species on second instar male scale were recorded in the Washington navel block at Somersby and the Valencia orange block at Lower Portland, with maxima of 41.4 ± 4.2 and 42.9 ± 2.9%, respectively. Parasitism by *Aphytis* on second instar male scale in the other blocks was relatively similar, reaching a maximum of about 20%.

**Second moult** scale were predominantly killed by *Encarsia* species. On two occasions they were killed by *Comperiella bifasciata*. Highest levels of parasitism by *Encarsia* species on second moult stage were recorded in both Washington navel/Valencia orange blocks at Cornwallis, with maxima of 87.3 ± 3 and 83.6 ± 5%, respectively (Figs 6.5 & 6.6). The lowest levels were recorded in the Valencia orange block at Lower Portland and in the Washington navel block at Castlereagh, with maxima of 33.3 ± 9.6 and 34.3 ± 4.7%, respectively (Figs 6.4 & 6.8).

**Virgin female** scale were predominantly killed by *Aphytis* species in all orchards. The highest levels of parasitism were recorded in the Washington navel block at Somersby (Fig. 6.1) and Washington navel/Valencia orange block 1 at Cornwallis (Fig. 6.5), with
maxima of 82.1 ± 7.2 and 77.4 ± 2.9% in autumn 2010, respectively. There was also variation within orchards. At Somersby, percent parasitism in the Washington navel and Valencia orange blocks peaked at 77.4 ± 2.9% and 22.5 ± 4%, respectively (Figs 6.1 & 6.2). Similarly, at Cornwallis, maximum percent parasitism in Washington navel/Valencia orange blocks 1 and 2 peaked at 82.1 ± 7.2 and 25.8 ± 7.8%, respectively (Figs 6.5 & 6.6). Parasitism also peaked in April, and then declined in winter.

Encarsia species, particularly Encarsia perniciosi, also emerged from virgin female red scale. The highest levels of parasitism by Encarsia species, primarily Encarsia perniciosi, were recorded in the Washington navel block at Lower Portland and in the Washington navel block at Castlereagh, with maxima of 22.2 ± 3.8 and 32.1 ± 6.4%, respectively (Figs 6.3 & 6.8). The lowest levels were recorded in the Washington navel block at Somersby and both blocks at Castlereagh, with maxima of 7.8 ± 7.3, 4.8 ± 4.6 and 12.5 ± 8.8%, respectively (Figs 6.1, 6.7 & 6.8). Parasitism varied among blocks.

In all orchards, except at Cornwallis, parasitism by Encarsia species on second instar male scale was more predominant than parasitism by Aphytis species early in season, but parasitism by Aphytis tended to increase late in the season. In Washington navel/Valencia orange blocks 1 and 2 at Cornwallis, parasitism by Encarsia species were noticeably higher on all occasions than parasitism by Aphytis species.

Prepupal and pupal male scale were predominantly attacked by Aphytis but suffered no attacks early in the season. Due to limited numbers of prepupal and pupal stages on most occasions data for parasitism on these stages was highly variable. Nevertheless, they were recorded and the seasonal trends were similar in all orchards.

Mated female scale were predominantly killed by Comperiella bifasciata (Figs.6.1–6.8). The highest levels of parasitism were recorded at Somersby (maximum of 51.4 ± 3.8%) in 2009–2010 (Figs 6.1 & 6.2) and the lowest at Lower Portland (16.9 ± 4.6%) in the same season (Figs 6.3 & 6.4). In all orchards, except at Lower Portland, parasitism by Comperiella bifasciata peaked in April and remained at relatively high levels from early winter until the fruit were harvested in late June 2011.
Figure 6.1. Parasitism of male and female red scale by *Aphytis* species (second instar males and females, virgin females) and *Encarsia* species (second instar males and females, second moult females, virgin females), and *Comperiella bifasciata* (mated females) in the Washington navel block at Somersby in 2009–2010.
Figure 6.2. Parasitism of male and female red scale by *Aphytis* species (second instar males and females, virgin females) and *Encarsia* species (second instar males and females, second moult females, virgin females), and *Comperiella bifasciata* (mated females) in the Valencia orange block at Somersby in 2009–2010.
Figure 6.3. Parasitism of male and female red scale by Aphytis species (second instar males and females, virgin females) and Encarsia species (second instar males and females, second moult females, virgin females), and Comperiella bifasciata (mated females) in the Washington navel block at Lower Portland in 2009–2010.
Figure 6.4. Parasitism of male and female red scale by *Aphytis* species (second instar males and females, virgin females) and *Encarsia* species (second instar males and females, second moult females, virgin females), and *Comperiella bifasciata* (mated females) in the Valencia orange block at Lower Portland in 2009–2010.
Figure 6.5. Parasitism of male and female red scale by *Aphytis* species (second instar males and females, virgin females) and *Encarsia* species (second instar males and females, second moult females, virgin females), and *Comperiella bifasciata* (mated females) in the Washington navel block 1 at Cornwallis in 2009–2010.
Figure 6.6. Parasitism of male and female red scale by *Aphytis* species (second instar males and females, virgin females) and *Encarsia* species (second instar males and females, second moult females, virgin females), and *Comperiella bifasciata* (mated females) in the Washington navel block 2 at Cornwallis in 2009–2010.
Figure 6.7. Parasitism of male and female red scale by Aphytis species (second instar males and females, virgin females) and Encarsia species (second instar males and females, second moult females, virgin females), and Comperiella bifasciata (mated females) in the Valencia orange block at Castlereagh in 2009–2010.
Figure 6.8. Parasitism of male and female red scale by *Aphytis* species (second instar males and females, virgin females) and *Encarsia* species (second instar males and females, second moult females, virgin females), and *Comperiella bifasciata* (mated females) in the Washington navel block at Castlereagh in 2009–2010.
6.3.2. Tests for density-related responses

The tests were based on relatively high and consistent levels of parasitism. Linear regression analyses were performed for: (1) proportions of second instar male and second moult females parasitised by *Encarsia* species versus the numbers of available stages; (2) proportions of virgin female scale and second instar male scale parasitised by *Aphytis* species versus the number of available stages; (3) proportions of mated female scale parasitised by *Comperiella bifasciata* versus numbers of mated female. Due to the number of data sets analysed, particularly for *Encarsia* species, one figure for each relationship is presented for each parasitoid. Statistical values for all results are summarised in Tables 6.1–6.4.

6.3.2.1. *Aphytis* species

Regression analysis results for proportions of *Aphytis* species parasitising second instar male versus virgin female scale and available second instar male (see Fig. 6.9) and virgin female scale in populations are presented in Table 6.2. Numbers of observation ranged from 30 to 43. Regressions were not significant for all 14 data sets (P < 0.05). Examples were shown in Figs 6.10 & 6.11 for *Aphytis* on second instar male and virgin female scale, respectively.

![Image of a second instar male red scale parasitised by a larva of *Aphytis*](image)

**Figure 6.9.** A second instar male red scale parasitised by a larva of *Aphytis*. 
Table 6.1. Results for linear regression of proportions of second instar male (2I♂), third instar virgin females (3V♀) and total (second instar male, second instar females, third instar virgin females) parasitised by Aphytis species versus number of available of second instar male, third instar virgin females and total (second instar male, second instar females, third instar virgin females).

<table>
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<th>P &gt;</th>
<th>r²</th>
<th>n</th>
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Figure 6.10. Proportions of virgin female scale parasitised by Aphytis species versus number of available virgin female scale based on data from the Somersby orchard on 15 May 2010: purple, red and green curves represent the mean upper and lower 95% confidence limits, respectively, of the binomial distribution pertinent to the plotted data. P = 0.95, r² = 0.0001.
6.3.2.2. *Encarsia* species

A total of 46 datasets were analysed. On all occasions except one, P values were $> 0.05$ (Table 6.2). Thus, there was no evidence of density dependency for the *Encarsia* species. For *Encarsia citrina* (Fig. 6.12), examples of relationships between parasitism by *Encarsia citrina* on second instar male and second moult scales are shown in Figs 6.13 & 6.14, respectively. Examples for *Encarsia perniciosi* and its hosts are shown in Figs. 6.15 & 6.16.

![Figure 6.12. An Encarsia citrina adult](image)

*Figure 6.11.* Proportions of second instar male scale parasitised by *Aphytis chrysomphali* versus number of available second instar male scale based on data from the Lower Portland orchard on 18 May 2010: purple, red and green curves represent the mean upper and lower 95% confidence limits, respectively, of the binomial distribution pertinent to the plotted data. $P = 0.73$, $r^2 = 0.004$. 

![Graph](image)
Table 6.2. Results for linear regression of proportion of scale stages parasitised by Encarsia species: (a) parasitised second moult females (2M♀) versus available second moult females; (b) parasitised second instar males (2I♂) versus available second instar males; and (c) parasitised second instar males and females, second moult females (2M♀) and third instar virgin females (3V♀) versus total available scale (second instar males and females, second moult females and virgin females).

<table>
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Figure 6.13. Proportions of second instar male scale parasitised by *Encarsia* species (mostly *Encarsia citrina*) versus number of available second instar male scale based on data from the Cornwallis orchard on 10 March 2010: red and green curves represent the mean upper and lower 95% confidence limits, respectively, of the binomial distribution pertinent to the plotted data. $P = 0.43$, $r^2 = 0.015$.

Figure 6.14. Proportions of second moult male scale parasitised by *Encarsia* species (mostly *Encarsia citrina*) versus number of available second moult male scale based on data from the Cornwallis orchard on 10 March 2010: purple and green curves represent the mean upper and lower 95% confidence limits, respectively, of the binomial distribution pertinent to the plotted data. $P = 0.7$, $r^2 = 0.004$. 
Figure 6.15. Proportions of second instar male scale parasitised by Encarsia species (mostly Encarsia perniciosi) versus number of available second instar male scale based on data from the Lower Portland orchard on 1 April 2010: red and green curves represent the mean upper and lower 95% confidence limits, respectively, of the binomial distribution pertinent to the plotted data. \( P = 0.1, r^2 = 0.08 \).

Figure 6.16. Proportions of second moult female scale parasitised by Encarsia species (mostly Encarsia perniciosi) versus number of available second moult female scale based on data from the Lower Portland orchard on 20 April 2010: red and green curves represent the mean upper and lower 95% confidence limits, respectively, of the binomial distribution pertinent to the plotted data. \( P = 0.15, r^2 = 0.08 \).
6.2.2.3. *Comperiella bifasciata*

Regression analysis results of 8 data sets for relationships between parasitism by *Comperiella bifasciata* versus number of available mated female are presented in Table 6.3. The number of observations ranged from 19 to 39. The regression relationships were not significant ($P < 0.05$) (Table 6.3). An example of a regression plot is shown in Fig 6.17. In this instance, data were well fitted to a binominal distribution.

<table>
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<td>0.25</td>
<td>0.06</td>
<td>23</td>
</tr>
<tr>
<td>3 April 2010</td>
<td>Somersby</td>
<td>2</td>
<td>0.67</td>
<td>0.006</td>
<td>33</td>
</tr>
<tr>
<td>15 May 2010</td>
<td>Somersby</td>
<td>2</td>
<td>0.4</td>
<td>0.02</td>
<td>40</td>
</tr>
<tr>
<td>20 June 2009</td>
<td>Somersby</td>
<td>2</td>
<td>0.51</td>
<td>0.02</td>
<td>23</td>
</tr>
<tr>
<td>5 June 2009</td>
<td>Somersby</td>
<td>1</td>
<td>0.64</td>
<td>0.006</td>
<td>34</td>
</tr>
<tr>
<td>18 July 2009</td>
<td>Lower Portland</td>
<td>1</td>
<td>0.51</td>
<td>0.02</td>
<td>24</td>
</tr>
<tr>
<td>15 May 2010</td>
<td>Lower Portland</td>
<td>2</td>
<td>0.48</td>
<td>0.029</td>
<td>19</td>
</tr>
<tr>
<td>10 March 2010</td>
<td>Cornwallis</td>
<td>1</td>
<td>0.94</td>
<td>0.00018</td>
<td>35</td>
</tr>
</tbody>
</table>

Table 6.3. Results for linear regression of proportions of third instar mated female (3M♀) scale parasitised by *Comperiella bifasciata* versus number of available mated female scale.

Figure 6.17. Proportions of mated female scale parasitised by *Comperiella bifasciata* versus number of available mated female scale based on data from the Somersby orchard on 15 May 2010, red and green curves are respectively the mean upper and lower 95% confidence limits of the binomial distribution pertinent to the plotted data $P = 0.32$, $r^2 = 0.024$. 

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**Figure 6.17.** Proportions of mated female scale parasitised by *Comperiella bifasciata* versus number of available mated female scale based on data from the Somersby orchard on 15 May 2010, red and green curves are respectively the mean upper and lower 95% confidence limits of the binomial distribution pertinent to the plotted data $P = 0.32$, $r^2 = 0.024$. 

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All tests except one for the parasitoids revealed no significant trends for parasitism versus scale density on any date. The only exception, for parasitism of second instar males by *Encarsia* species at Lower Portland on 20 April 2010, had a significant negative slope (although the slope was very slight). The total data for each source of mortality was compared to binomial expectations (Figs 6.10–6.13 & Figs 6.15–6.17). Although there was a general similarity with such expectations, the data were clearly over-dispersed relative to the binomial (i.e., many more than expected higher values and many more than expected low (especially zero) values). This is consistent with the fact that the distribution of scale mortality among fruit from each source had much higher variation than normal expectations, coefficients of variation being well in excess of unity.

### 6.3.3. Scale density and incidence of footprints *Halmus chalybeus* predated scales

Data from Castlereagh in 2010–2011 were used for this analysis. On all five occasions, 31–59 fruit were examined. Correlations for proportion of footprints versus available scale were negative (P > 0.05), except for data recorded on 29 April 2010 (Table 6.4). A *Halmus chalybeus* adult and *Halmus chalybeus* larva, and footprints of red scale preyed upon by the beetle are featured in Fig. 6.18.

<table>
<thead>
<tr>
<th>Date</th>
<th>Orchard</th>
<th>Block</th>
<th>P</th>
<th>r²</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 February 2010</td>
<td>Castlereagh</td>
<td>3</td>
<td>0.36</td>
<td>0.02</td>
<td>46</td>
</tr>
<tr>
<td>4 March 2010</td>
<td>Castlereagh</td>
<td>3</td>
<td>0.27</td>
<td>0.04</td>
<td>31</td>
</tr>
<tr>
<td>22 March 2010</td>
<td>Castlereagh</td>
<td>3</td>
<td>0.87</td>
<td>0.0005</td>
<td>59</td>
</tr>
<tr>
<td>8 April 2010</td>
<td>Castlereagh</td>
<td>3</td>
<td>0.6</td>
<td>0.005</td>
<td>58</td>
</tr>
<tr>
<td>29 April 2010</td>
<td>Castlereagh</td>
<td>3</td>
<td>0.015</td>
<td>0.14</td>
<td>42</td>
</tr>
</tbody>
</table>
Figure 6.18. *Halmus chalybeus* adult and larvae (above), and footprints on an orange fruit.

Figure 6.19. Relationship between proportions of footprints and numbers of available scale in the Washington navel ‘block 3’ in the orchard at Castlereagh on 22 March 2010 ($P = 0.87, r^2 < 0.001$).
In order to determine relationship between levels of footprints and total number of scale per fruit, I regressed mean number of scale per fruit against levels of footprints recorded during assessments of the in the preceding sample. The result is shown in Fig. 6.20. The regression was negative ($P = 0.002$, $r^2 = 0.8$). This suggested that predation by *Halmus chalybeus* was the major factor to the rapid decline of scale populations in autumn 2010 in the Washington navel block at Castlereagh in autumn 2010.

**Figure 6.20.** Regression between percentage of footprints and mean number of live scale per fruit for 8 consecutive observations from January 2010 to April 2010 in the Washington navel block in the orchard at Castlereagh, $P = 0.002$, $r^2 = 0.8$. 
6.3.4. *Intraguild relationships*

Data for parasitism of second instar male scale was used to determine potential impacts of *Aphytis* species on *Encarsia* species. No significant interaction was detected (Table 6.5). Such instances (Fig. 6.21) were rare.

**Table 6.5.** Results for linear regression of interaction of *Encarsia* spp. on *Aphytis* spp. on second instar male scale

<table>
<thead>
<tr>
<th>Date</th>
<th>Orchard</th>
<th>Block</th>
<th>P &gt;</th>
<th>r²</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 April 2010</td>
<td>Lower Portland</td>
<td>2</td>
<td>0.78</td>
<td>0.0029</td>
<td>29</td>
</tr>
<tr>
<td>20 April 2010</td>
<td>Lower Portland</td>
<td>2</td>
<td>0.84</td>
<td>0.001</td>
<td>29</td>
</tr>
<tr>
<td>19 May 2010</td>
<td>Lower Portland</td>
<td>2</td>
<td>0.42</td>
<td>0.029</td>
<td>24</td>
</tr>
<tr>
<td>10 May 2009</td>
<td>Cornwallis</td>
<td>2</td>
<td>0.51</td>
<td>0.00012</td>
<td>26</td>
</tr>
<tr>
<td>21 June 2010</td>
<td>Lower Portland</td>
<td>2</td>
<td>0.68</td>
<td>0.009</td>
<td>20</td>
</tr>
</tbody>
</table>

Data for parasitism by *Aphytis* species on virgin female scale and *Comperiella bifasciata* in mated female scale was used to determine potential impacts of *Aphytis* species on *Comperiella bifasciata* species. No significant interaction was detected (Table 6.6).

**Figure 6.21.** *Aphytis* larva on scale parasitised by *Encarsia* species.
Table 6.6. Results for linear regression of interaction of Aphytis species on virgin female and Comperiella bifasciata in mated female.

<table>
<thead>
<tr>
<th>Date</th>
<th>Orchard</th>
<th>Block</th>
<th>P &gt;</th>
<th>$r^2$</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 March 2010</td>
<td>Somersby 2</td>
<td>0.15</td>
<td>0.08</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>17 April 2010</td>
<td>Somersby 2</td>
<td>0.87</td>
<td>0.001</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>30 April 2010</td>
<td>Somersby 2</td>
<td>0.34</td>
<td>0.03</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>15 May 2011</td>
<td>Somersby 2</td>
<td>0.02</td>
<td>0.15</td>
<td>32</td>
<td></td>
</tr>
</tbody>
</table>

6.4. Discussion

My records for parasitism of the life cycle stages of red scale indicated that: the two Encarsia species usually emerged from second instar male, second instar female, second moult female, and virgin female scale; the two Aphytis species usually emerged from second instar male, second instar female and third instar virgin female scale; Comperiella bifasciata emerged predominantly from third instar mated females. General trends were similar to those outlined by Yu et al. (1990) and Forster et al. (1995). Variation in levels of parasitism occurred among orchards and among blocks within orchards. For example, Aphytis species were more common on third instar virgin female scale than on second instar male and second instar female scale in the orchard at Somersby whereas, they were more common on second instar male scale in the orchard at Lower Portland. I attributed these differences to influences of local microclimates on the phenology of the parasitoids. I attributed higher levels of Encarsia parasitism of third instar virgin female scale at Lower Portland and Castlereagh to higher proportions of Encarsia perniciosi relative to Encarsia citrina. Most Encarsia adults that emerged from virgin female red scale were Encarsia perniciosi (Fig. 6.22). I assume that the difference could be related to the size of the parasitoids: the wing span of Encarsia perniciosi was 1.73 mm, compared to 1 mm for Encarsia citrina, although their body lengths were similar (0.6 mm) (Craw 1891, Tower 1906). In some instances levels of parasitism by Aphytis and Encarsia species of prepupal and pupal stages of scale were based on low numbers of available scale on a few occasions. Therefore, the data presented in Figs 6.1–6.8 for prepupal and pupal parasitism should be treated with caution).

High levels of parasitism by Aphytis of second instar male scale towards the end of the season, particularly in the orchards at Lower Portland and Castlereagh, were similar to trends in levels of parasitism recorded by Reeve (1987) at Fillmore (34° 24’ N, 118° 55’
E) in California in 1982–83. Reeve (1987) attributed this to the possibility that *Aphytis*
may not be able to distinguish this stage from the third instar virgin females due to
overlapping of body lengths of these two stages, variation in vulnerability of hosts, and
impacts of Argentine ant on parasite activity in relation to location of hosts within trees.
I attributed the high levels of parasitism in my study to cold weather in late autumn and
winter delaying development of second instar male scale thereby, exposing them to
higher levels of parasitism. This conclusion is supported by observations reported by
Abdelrahman (1974c), who suggested that amount of available food for *Aphytis*
larvae in second instar male prepupae is less than that in second instar males and third instar
virgin females, as pupal stage scales are more sclerotised.

![Figure 6.22. A virgin female red scale parasitised by *Encarsia perniciosi* on an orange leaf.](image)

My results clearly showed that parasitism by all parasitoid species of red scale occurring
in my study orchards was density-independent. The results for *Aphytis* species were
similar to results reported by Smith & Maelzer (1986), Reeve (1987) and Murdoch et al.
(1995). In contrast, my results differed from the views of Flanders (1971), who stated
that ectoparasitoids and their hosts were density-dependent, and analysis of field results
by Samways (1985) in South Africa that were significant in some instances (but all $r^2$
ranged from 0.127 to 0.231) and related on some occasions to the month samples were
collected in autumn, and the number of sampled fruit with a single scale. In my studies,
I randomly sampled red scale-infested fruit. Therefore, fruit with a single scale were rare. He concluded that the relationships between species of *Aphytis* and red scale were density-dependent. However, he based this conclusion on seasonal abundance of the scale and the parasitoids, not on regressions of parasitism versus scale densities within seasons. On the same basis he concluded that the relationship between two endoparasitoids, *Comperiella bifasciata* and *Habrolepis rouxi* Compere and red scale were also density-dependent. These conclusions led him to view species of *Aphytis* as regulators of red scale populations and the endoparasitoids as ineffective from the point of view of host regulation.

My results for *Encarsia* species, as for species of *Aphytis*, were once again similar to those of Murdoch et al. (1995), but differed from McClure (1977) who derived strong positive density-dependent relationships between parasitism by *Encarsia citrina* and elongate hemlock scale in Connecticut, and Rebek et al. (2006) who reported a density-dependent relationship between the parasitoid and euonymus scale, *Unaspis euonymi* (Comstock) [Diapsididae], in Indiana. In both studies, *Encarsia citrina* was the only parasitoid species present.

As mentioned in Chapter 2, *Aphytis* females emerge with, at most, only a fraction of their total egg complement and need to source protein in order for oogenesis to continue, whereas *Encarsia* species emerge with many mature eggs and do not need to host feed in order to lay their eggs (Jervis & Kidd 1986, Traynor & Mayhew 2005). Similarly, Reeve (1987) reported that *Aphytis melinus* encountered the same fraction of hosts, but the fraction parasitised declined at high densities, at least for third instar virgin females. He (Reeve 1987) attributed the decline to the inability of the parasite to produce and lay more than four eggs per day, as suggested by Abdelrahman (1974c). The tendency for *Aphytis melinus* to lay more than one egg per third instar virgin female scale (Rosen & DeBach 1978, Forster et al. 1995) may also have contributed to the decline. In my study, I commonly observed 2–3 *Aphytis* eggs, larvae, or pupae per third instar virgin female. Such differences in oogenesis and oviposition behaviour may partially explain variation in reports for density dependency for species of *Aphytis* and a possible higher probability of density-dependency occurring for species of *Encarsia*. Density-dependency has been frequently reported for *Encarsia* species parasitising non-diaspidid hosts (Summy et al. 1985, Meagher & French 2004).
I did not study the foraging behaviour of adult parasitoids. Lessells (1985) tested whether foraging behaviour shifted density-dependency to inverse density-dependency. He concluded that neither rejecting time nor interference was sufficient to provide explain of inverse density-dependency, and that egg and time limitations may affect density relationships if the parasitoids enter, depart and re-enter host patches randomly. In the case of Aphytis species, adults do not parasitise scales parasitised by conspecifics (Abdelrahman 1974c, Rosen & DeBach 1978). Umbanhowar et al. (2002) examined several foraging behaviours that have been hypothesised to create density-dependent variation in parasitism rates, including spatial aggregation of parasitoids to high host density, mutual interference among searching parasitoids and decelerating functional responses of the parasitoid. Their studies were based on parasitism of Tachinomyia similis [Diptera: Tachinidae] attacking western tussock moth (Orgyia vetusta (Boisduval) [Lepidoptera: Lymantriidae]) on lupine bushes (Lupinus arboreus Sims. [Fabales: Leguminosae]) at Bodega Bay in California. Most of the variation in parasitism was not correlated with density of Orgyia vetusta.

I did not determine mechanism for stability of the parasitoid-host relationship. Hassell & Waage (1984) reported that density-dependence on a spatial scale within a generation is a powerful stabilising mechanism. Murdoch et al. (1992), Murdoch et al (1996), Murdoch et al (2005) reported the stability between Aphytis melinus and red scale but Murdoch (1994) failed to detect density-dependency or any evidence among the hypotheses for stability in the absence of density-dependent systems. Murdoch (2005) reported that neither refuge dynamics nor metapopulation dynamics contributed to population stability. Reeve & Murdoch (1985) suggested that host size and host selection may be involved in stabilising scale populations. DeBach (1955) claimed that most studies regarding interactions between hosts and natural enemies developed by biomathematicians had ignored abiotic variations in the environment.

I did not detect intraguild relationships among and between the parasitoid species I recorded in my study. My results differed from those reported by Flanders (1971), Borer (2002) and Sorribas & Garcia-Marí (2010). Flanders (1971) reported that Aphytis ectoparasitoids were active at high population densities of host scale, whereas endoparasitoids were more effective at low scale population densities. Sorribas & Garcia-Marí (2010) reported that reduced parasitism by Encarsia perniciosi during summer and autumn was, at least in part, due to predation by Aphytis larvae that are
more abundant in these seasons. During the course of my study, I rarely observed *Aphytis* predation on *Encarsia* species.

My study is the first to demonstrate relationship between levels of predated scale and declining scale populations. The strong correlation presented in Fig 6.17 for percent footprints versus scale density clearly showed that *Halmus chalybeus* played a major role in reducing scale populations in the Washington navel block in the orchard at Castlereagh in autumn 2010. Other observations, reported in Chapter 5, showed that choice of oviposition site by *Halmus chalybeus* is not related to densities of red scale.

A number of studies have focused on the response of coccinellids to their prey, particularly for aphids (e.g., Anderson 1981, Schellhorn & Andow 2005, Conway & Kring 2010). Schellhorn & Andow (2005) studied response of four species of coccinellids, *Coleomegilla maculata* (DeGeer), *Hippodamia tredecimpunctata* (Say), *Hippodamia convergens* (Guerin) and *Adalia bipunctata* (L.) to corn leaf aphid, *Rhopalosiphum maidis* (Fitch) in maize (*Zea mays* L. [Poales: Gramineae]) fields. Density-dependence was infrequently detected. Schellhorn & Andow (2005) attributed the absence of density-dependence to different observation scale, behavioural, and habitat features among species. In my study, I obtained no evidence of a density-relationship between *Halmus chalybeus* and red scale. However, results of studies reported in Chapter 5 indicate that, due to their habit of laying eggs under scale covers or integuments, that *Orcus australasiae* and species of *Rhyzobius* may be density dependent. Seagraves (2009) stated that the food available to coccinellid larvae and their exposure to predation was influenced by where the egg was oviposited. The need to place eggs in proximity to food for offspring to feed on must be weighed against the risk of cannibalism and intraguild predation (Seagraves 2009). Therefore, laying eggs under scale covers or integuments could be means by which *Orcus australasiae* and species of *Rhyzobius* optimise their survival.

In my studies, the scale populations were regulated by climate and natural enemies. Scale populations were low in the winter-spring generation, increased to higher levels in the summer generation, reached the highest levels in the autumn generation, and then declined to relatively low levels in winter. Climate was the major factor leading to the decline of populations in winter and also limited the size of both the spring and summer generations of the scale. Natural enemies had their greatest impacts in late summer and autumn and, given numerous reports of detrimental impact of pesticides on natural
enemies leading to high and damaging scale populations (DeBach 1955, 1958, Grout 87, Hely et al. 1982, Smith et al. 1997), there is no doubt that natural enemies (parasitoids, predators and entomopathogens) of red scale on the central coast of New South Wales play significant roles in suppressing and maintaining ‘stability’ of scale populations. However, the latter still fluctuate widely. The reasons for this type of population behaviour are not known but may fall under the class of dynamics that has been called ‘density vague’ (Strong 1986).
Chapter 7. Impacts of ant-black scale mutualism on the biological control of red scale

7.1. Introduction

Detrimental impacts of ants, particularly species associated with soft scales, on natural enemies of red scale have been widely recognised for several decades (DeBach et al. 1951a, Flanders 1951a, Steyn 1954a,b, Samways 1981, Samways et al. 1982, Murdoch et al. 1995, Martinez-Ferrer et al. 2002, Pekas et al. 2010). Impacts of ants on red scale predators were reported by Flanders (1943c), DeBach et al. (1951a), Bartlett (1961) and Hill & Blackmore (1980). However, most studies have focused on their impacts on parasitoids (Flanders 1943c, DeBach et al. 1951a, Steyn 1954a,b, Samways 1981, Samways et al. 1982, Murdoch et al. 1995, Martinez-Ferrer et al. 2002, Pekas et al. 2010). Hely et al. (1982) and Smith et al. (1997) mentioned disruptive effects of ants on red scale in Australian citrus orchards but neither cited publications showing negative impacts on natural enemies of the scale. James et al. (1997, 1999) studied the impact of honeydew-seeking ants, cited as *Iridomyrmex rufoniger* group spp., on red scale, and the incidence of beneficial arthropods in a citrus orchard at Leeton in the Riverina district of inland New South Wales. However, no studies have quantified impacts of ants on levels of parasitism and incidence of predators in the citrus orchards in coastal areas of eastern Australia, where red scale is an occasionally important pest, or elsewhere in Australia, where it is a major pest (Smith et al. 1997).

Black scale is also an occasionally important citrus pest in citrus orchards on the Central Coast of New South Wales (Hely et al. 1982, Smith et al. 1997). It commonly occurs at high levels on young citrus trees in orchards in the presence of honeydew-seeking ants (Hely et al. 1982, Smith et al. 1997). Heavy infestations of the scale lead to high levels of red scale, and red scale-induced dieback of branches and whole young trees (Beattie, pers. comm., 2008). The most common ant species associated with black scale in my study orchards was identified by Dr Steve Shattuck (CSIRO Ecosystem Sciences, Canberra, Australia) as *Iridomyrmex rufoniger* (Lowne). My molecular results (Chapter 8) verified this identification. The ant also tends lycaenid caterpillars, protecting them

I hypothesised that *Iridomyrmex rufoniger* activity associated with black scale reduces the effectiveness of biological control of red scale by reducing the effectiveness of the scale’s parasitoids and predators. I also hypothesised, based on general observations during studies reported in Chapter 4, that differences between treatments could be as significant, or more significant, for parasitism than for predation. Moreover, I hypothesised that impacts of *Iridomyrmex rufoniger* on activities of different species of predators would differ significantly. In this chapter, I assessed these potential impacts in the presence and/or absence of black scale and *Iridomyrmex rufoniger*, initially in a survey of mature and immature trees, and then in an experiment in which the ant was excluded from foraging in tree canopies. I dissected sampled scales to assess parasitism, counted predators in canopies, and assessed levels of predation. In this Chapter, I also recorded the incidence of *Microcera coccophila* on trees. In Chapter 9, I show, in a laboratory test, that *Iridomyrmex rufoniger* can passively spread *Microcera coccophila* conidia, the most common entomopathogen of red scale on the Central Coast of New South Wales.

### 7.2. Materials and methods

#### 7.2.1. Orchard and trees

The experiments were conducted in Lister’s orchard (Fig. 7.1) at Kulnura (33° 13′ S, 151° 13′ E, 386 m asl) on the Somersby Plateau on the Central Coast of New South Wales. The orchard was chosen on the basis of black scale infestations and ant activity. The initial survey of sweet orange trees (*Citrus × aurantium*) included three blocks of mature Valencia orange trees and three blocks of Hamlin orange trees that were four years old in April 2010. The subsequent ant exclusion experiment was confined to the Hamlin orange trees that were 1 to 1.5 m–high and planted on 4 × 2.5 m grid, with rows running east to west.

No insecticides were applied on experimental trees during the period of the study. Trees that were not used in the experiment were sprayed by hand with copper oxide (Nordox 75 WG, Nordox As, IMCD Australia Limited, Mulgrave, Victoria, Australia).
in November 2010. The trees were not irrigated but were fertilised twice annually with Nitrophoska Blue Special (Incitec Pivot Limited, Southbank Victoria, Australia): 12.0% N, 5.2% P, 14.1% K, 6.0% S) applied at approximately 150 g per tree.

### 7.2.2. Survey

Trees sampled for the survey comprised immature trees in the three Hamlin orange blocks that were heavily infested with black scale and red scale, and with high ant activity, and mature Valencia orange trees in three adjacent blocks in which black scale was absent, red scale present, and ant activity low. Red scale-infested fruit were sampled on 6 April 2010 when 60 fruit, 20 fruit per block, were sampled randomly from black scale-infested Hamlin orange trees. Variable numbers of fruit were sampled from the Valencia orange trees in order to obtain at least 100 virgin and mated female red scale per block.

### 7.3.3. Ant exclusion experiment

The location, within the orchard, of the Hamlin orange three blocks (sites) used for the experiment is shown in Fig. 7.1. Sites 1 and 2 each comprised 126 and 133 trees, respectively, in 7 rows. Site 3 comprised 90 trees in 5 rows. Trees in sites 1 and 2 were slightly larger than those in site 3. Site 3 was more shaded than sites 1 and 2.

The experiment initially comprised three treatments:

- unbanded trees with black scale, red scale and ants present;
- banded trees with black scale and red scale present and ants absent; and
- control trees with red scale present and black scale and ants at low levels.

Within each site, 12 trees were selected on the basis of high and similar levels of black scale, red scale and ants. Of these, 6 trees were randomly selected and banded. The remaining 6 trees served as the ‘unbanded’ treatment. Another 6 trees on which red scale was present, and black scale and ants either absent or at low levels, were selected in each site as ‘control’ trees.

Death of black scale from self-induced asphyxiation on the banded trees due to accumulation of its honeydew in the absence of ants led me to include an additional banded treatment (late-banded) from 16 February 2011.
Figure 7.1. Map of Lister’s orchard at Kulnura and sites used for the experiment (from Google Earth).

Ants were excluded from tree canopies by wrapping a 50 mm-wide strip of black gaffer cloth tape around each tree trunk, 15–20 cm above ground level. The band was then coated, relatively thickly, with polybutene (Tangletrap®, Australian Entomological Supplies, Sydney, Australia). To prevent the polybutene damaging trees, another strip of tape was wrapped around the trunk beneath the polybutene-coated band. Bands were replaced monthly. Their positions on trunks varied in order to minimise potential damage to the trees (Fig. 7.2).

Due to the high incidence of ladybird larvae and adults, particularly Rhyzobius spp. larvae, being trapped on the sticky surface of the bands, coarse (10 × 10 mm) 150 mm-wide black plastic mesh (‘gutter guard’) was wrapped around trunks above each band in order to reduce entrapment of the ladybirds (the impact of the mesh bands was not assessed).
Ant activity was assessed monthly from July 2010 to June 2011 by counting the number of ants moving downwards past a point on the trunk of each tree during 4 minute-long observations (2 min on the northern side and 2 min on the southern side). Observations were conducted on fine sunny days when ambient temperatures ranged from 20 to 35°C from August 2010 to April 2011 and 13 to 16°C in July 2010 in May and June 2011.

The incidence of predators was assessed during visual inspections of each canopy for 1 min per tree on the same days that ant activity was assessed.

Parasitism of red scale was assessed on three occasions: 29 June 2010 (pre-treatment) and 7 April and early June 2011. At least two to 10 infested fruit were randomly picked from each tree. Fruit from each tree were placed in a paper bag and stored in a cool...
room at 7 to 8°C within a few hours after picking. Assessments were conducted within 4 d.

Assessments of levels of predation were based on the fruit that were used to assess levels of parasitism. Methods were described in Chapter 5 and were based on the tell-tale evidence of predation. *Halmus chalybeus* and *Orcus australasiae* leave remnants (‘footprints’) of scale that they eat on branches, twigs, leaves and fruit (see Chapter 5). *Rhizobius* species adults and larvae prey on a scale body through a hole they puncture in the scale cover, and leave most of the scale cover on the host substrate.

The incidence of *Microcera coccophila* on each tree was assessed in autumn 2011. Incidence levels were ranked as: common (++); less common (+) and none (-).

Ambient temperatures were recorded in Hitchcock’s orchard, approximately 1.25 km to the southwest, in which I studied scale phenology. Details were given in Chapter 3.

### 7.2.4. Data analysis

Levels of parasitism (%) and predation (%) recorded in the survey, and the experiment, were calculated as described in Chapter 5. Data from the experiment were tested for homogeneity of variances using Levene’s test of equality of error variances (SPSS Statistics 18: SPSS Inc. Chicago, United States of America). Differences between treatments, sites and treatment × site interactions were analysed using Univariate Analysis of Variance (ANOVA) (SPSS 18). Means were separated using least significant differences (LSD) method ($\alpha = 0.05$) (SPSS 18).

### 7.3. Results

#### 7.3.1. Survey

The preliminary results indicated that *Iridomyrmex rufoniger* activity markedly reduced percent parasitism and incidence of footprints. Percent parasitism by *Encarsia* species, *Aphytis* species, *Comperiella bifasciata* and incidence of footprints in the presence of black scale and *Iridomyrmex rufoniger* was 2.3, 6.4, 3.3 and 0.5%, respectively, on the immature Hamlin orange trees compared to 54.8, 21.8, 43.4 and 27.8%, respectively, on the mature Valencia trees in the absence of black scale and the ant (Fig. 7.3). Combined
parasitism of red scale by *Aphytis chrysomphali* and *Aphytis melinus* on the immature and mature trees was 8.3 and 91.7%, respectively (n = 36). Parasitism of the scale by *Encarsia citrina* and *Encarsia perniciosi* on the immature and mature trees was 61.9 and 38.1%, respectively (n = 72). *Encarsia perniciosi* was the only species of *Encarsia* recorded in on the immature trees (n = 18).

![Graph showing percent parasitism and percentage of predated scales](image)

**Figure 7.3.** Percent parasitism and percentage of predated scales in the presence and absence of black scale and *Iridomyrmex rufoniger* activity in autumn 2010 (mean ± SE).

### 7.3.2. Ant exclusion experiment

Black scale populations on the banded trees declined dramatically after *Iridomyrmex rufoniger* was excluded. Black scale population densities assessed on the 26 November 2010 (5 months after the trees were banded) were 12.3 nymphs and adults on unbanded trees, compared to 2.2 nymphs and adults on banded trees ($F_{1, 34} = 62.44$, $P < 0.001$). I did not record black scale population densities before banding the trees on the 29 June 2010, when the trees were selected to be banded or left unbanded, but the scale was far more abundant then than in November, and levels of infestation were visually similar. By 26 November 2010, sooty mould fungi on the initially heavily infested banded trees had also virtually disappeared and most leaves were clean, due to dislodgement of the sooty mould fungi by wind and rain.
The following predators and parasitoids of red scale were recorded during the experiment:

- 6 coccinellid species: *Halmus chalybeus*, *Orcus australasiae*, *Rhyzobius hirtellus*, *Rhyzobius lophanthae* (all common), *Harmonia testudinaria* (first record and uncommon) and *Chilocorus circumdatus* (observed occasionally);
- 5 parasitoid species: *Encarsia perniciosi*, *Encarsia citrina*, *Comperiella bifasciata*, *Aphytis chrysomphali* and *Aphytis lingnanensis*; and
- a moth, *Batrachedra arenosella*.

Predators of black scale included:

- *Orcus australasiae*, *Rhyzobius ventralis*, *Rhyzobius* spp., *Cryptolaemus montrouzieri* (all common) and *Diomus* sp. (observed occasionally); and
- a moth, *Mataeomera dubia*.

Common entomopathogenic fungi associated with red scale included *Microcera coccophila* and *Podonectria coccicola*44. *Lecanillium lecanii* was associated with black scale. *Microcera coccophila*, the most common entomopathogen of red scale, was observed in all three sites and was more common on unbanded trees.

In this study, I focused on the incidence of four coccinellids (*Halmus chalybeus*, *Orcus australasiae*, *Rhyzobius hirtellus* and *Rhyzobius lophanthae*) and parasitism by all 5 parasitoid species.

The only ant species observed on unbanded trees was *Iridomyrmex rufoniger* (Fig. 7.4). Two other species, *Rhytidoponera confusa* [Hymenoptera: Formicidae: Ectatomminae] and a small unidentified species, were only observed on control trees.

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44 See Chapters 2, 9 and 10.
Observations, except in July and August 2010, and May and June 2011, were made when ambient temperatures ranged from 20 to 30°C. The lowest average number of downward ant movements recorded per 4 min count was 39.6 in June 2011. Activity increased as average daily ambient temperatures rose (Fig. 7.5) and reached the highest average number of 271.1 downward movements per 4 min. in December 2010. Activity then fell to 185 per 4 min in January 2011 and remained at similar levels in February before declining to an average of about 100 individuals per 4 min in March and April 2011.

Numbers of ant movements were significantly higher on unbanded trees than on control trees (P < 0.001). No *Iridomyrmex rufoniger* were observed on the banded trees over the 12 months. There were no significant differences in ant numbers among the 3 sites for each of the 12 months (P > 0.05). The high incidence of *Orcus australasiae* on two trees in site 2 during January 2011 reduced black scale populations on these trees to very low levels. This led to very low numbers of *Iridomyrmex rufoniger* on these 2 trees and, therefore, they were excluded from analysis.

No ants were observed on the banded trees. Average numbers of *Iridomyrmex rufoniger* on control trees were very low from July to November 2010, ranging from 4.5 to 11.1 individuals per tree per 4 min assessment. They increased to 26.2 individuals in
December 2010 and peaked at 47.9 in February 2011. Average numbers of *Iridomyrmex rufoniger* on control trees were higher in site 2 than in sites 1 and 3.  

![Graph showing seasonal activity (mean ± SE number of ants observed in 4 min counts) of *Iridomyrmex rufoniger* on control trees (n = 18) on which black scale was absent, and banded (n = 18) and unbanded trees (n = 18) on which heavy black scale infestations were present at the beginning of ant exclusion experiment. Also presented are average ambient temperatures when the observations were made.]

**Figure 7.5.** Seasonal activity (mean ± SE number of ants observed in 4 min counts) of *Iridomyrmex rufoniger* on control trees (n = 18) on which black scale was absent, and banded (n = 18) and unbanded trees (n = 18) on which heavy black scale infestations were present at the beginning of ant exclusion experiment. Also presented are average ambient temperatures when the observations were made.

### 7.3.2.1. Pre-treatment levels of parasitism and predation

On the trees selected for the control treatment, average percent parasitism of red scale by *Aphytis* species, *Comperiella bifasciata* and *Encarsia* species was 3.3, 22.8 and 7.5%, respectively. The percentage of footprints in this treatment was 9.2%. On the trees selected for the unbanded treatment, average percent parasitism of red scale by *Aphytis* species, *Comperiella bifasciata* and *Encarsia* species was 5.8, 10.2 and 10.2%, respectively. No footprints were recorded in this treatment. On the trees selected for the banded treatment, average percent parasitism of red scale by *Aphytis* species, *Comperiella bifasciata* and *Encarsia* species was 7.3, 2.7 and 4.3%, respectively. The percentage of footprints was 0.1%. Differences for each parasitoid among the unbanded, banded and control treatments were not significant (F$_{2,6}$ = 1.58, P = 0.28 for *Encarsia* species; F$_{2,6}$ = 0.91, P = 0.45 for *Aphytis* species; F$_{2,6}$ = 0.51, P = 0.62 for *Comperiella bifasciata*).
7.3.2.2. Impact of *Iridomyrmex rufoniger* on predators

*Iridomyrmex rufoniger* significantly reduced the incidence of *Halmus chalybeus*. Average numbers of *Halmus chalybeus* adults and larvae per tree over the 12 months for all sites were 0.1, 1.7 and 0.3 in the unbanded, banded, and control treatments, respectively. Differences in numbers of *Halmus chalybeus* over the 12 months between treatments were significant (F\(_{2,45} = 12.1, P < 0.001\)). Numbers of *Halmus chalybeus* in the banded treatment were significantly higher than those in the unbanded and control treatments (\(\alpha = 0.05, P < 0.001\)). Numbers of *Halmus chalybeus* on unbanded and control trees were not significantly different. Numbers of *Halmus chalybeus* adults and larvae were evenly distributed among three sites. Differences in number of *Halmus chalybeus* over 12 months among sites were not significant (F\(_{2,45} = 0.087, P = 0.917\)). Interactions between site and treatment were not significant (F\(_{4,45} = 0.34, P = 0.845\)) (Fig 7.6).

![Figure 7.6. Average numbers of *Halmus chalybeus* adults and larvae per tree during 1 min observations per tree (mean ± SE) in the ant exclusion experiment.](image_url)

*Iridomyrmex rufoniger* disrupted the activity of *Rhyzobius* species. Average numbers of *Rhyzobius* adults and larvae per tree over 12 months from September 2010 to February 2011 of all sites were 0.3, 0.9 and 0.4 in the unbanded, banded and control treatments, respectively. Differences among the unbanded, banded and control treatments when data were analysed for all sites over the 12 months were not significant (F\(_{2,45} = 4.29, P = 0.02\)). Numbers of adults and larvae were significantly higher on banded trees and unbanded (\(\alpha = 0.05, \alpha = 0.018\)) and control trees (\(\alpha = 0.05, P = 0.08\)) (Fig. 7.7).
Differences among sites were significant ($F_{2,45} = 6.69$, $P = 0.003$). Number of adults and larvae were significantly higher in the site 3 than sites 1 and 2. Interactions between site and treatment were not significant ($F_{4,45} = 1.37$, $P = 0.258$).

Figure 7.7. Average numbers of *Rhyzobius* spp. adults and larvae per tree during 1 min observations in the ant exclusion experiment.

In contrast to *Halmus chalybeus* and *Rhyzobius* species, *Iridomyrmex rufoniger* did not suppress the activity of *Orcus australasiae* (Fig. 7.8 & 7.9). Average numbers of *Orcus australasiae* adults and larvae per tree over the 12 months were 6, 0.9 and 0.3 in the unbanded, banded and control treatments, respectively. Differences among unbanded, banded and control treatments over the 12 months were significant ($F_{2,45} = 8.08$, $P = 0.001$). Numbers of *Orcus australasiae* adult and larvae on unbanded trees were significantly higher than on the banded ($\alpha = 0.05$, $P = 0.002$) and control trees ($\alpha = 0.05$, $P = 0.001$). There were no significant differences between banded and control trees ($\alpha = 0.05$, $P = 0.69$) (Fig. 7.5). Differences among sites were significant ($F_{2,45} = 7.12$, $P = 0.002$). *Orcus australasiae* was significantly more abundant in site 2 than in sites 1 ($\alpha = 0.05$, $P = 0.007$) and 3 ($\alpha = 0.05$, $P = 0.001$). Differences in site and treatment interactions were also significant ($F_{4,45} = 4.7$, $P = 0.003$).
Figure 7.8. *Orcus australasiae* larva feeding on red scale, and footprints of predated scale.

Figure 7.9. Average numbers of *Orcus australasiae* adults and larvae per tree during 1 min observations per tree (mean ± SE) in the ant exclusion experiment.

Average percent predated scale was 4.6, 29.1, 11.8 and 28.6% on unbanded, banded, control and late-banded trees, respectively. Differences among treatments were significant ($F_{3, 57} = 9.767, P < 0.001$). Percent predated scale on late-banded trees was significantly higher than on unbanded, banded and control trees. There were no significant difference among sites ($F_{2, 57} = 0.88, P = 0.42$). Interactions between treatments and sites were not significant ($F_{6, 57} = 1.191, P = 0.324$) (Fig. 7.10).
Figure 7.10. Percent predated scale in the ant exclusion experiment in April 2011 (mean ± SE). 

*Iridomyrmex rufoniger* did not suppress the activity of *Mataeomera dubia* (Fig. 7.11). Average numbers of *Mataeomera dubia* larvae and pupae per tree over the 12 months were 2.8, 0.3 and 0 in the unbanded, banded and control treatments, respectively. Differences in numbers of *Mataeomera dubia* over the 12 months among the unbanded, banded and control treatments were significant ($F_{2, 45} = 5.479$, $P = 0.007$). Numbers on unbanded trees were significantly higher than on banded ($\alpha = 0.05$, $P < 0.001$) and control trees ($\alpha = 0.05$, $P < 0.001$). *Mataeomera dubia* was more common in site 1 than in sites 2 and 3 ($\alpha = 0.05$, $P = 0.002$). Differences among sites were significant ($F_{2, 45} = 26.12$, $P < 0.001$). Interactions between site and treatment were significant ($F_{4, 45} = 3.849$, $P = 0.009$) (Fig. 7.12).
Figure 7.11. The black scale predator, *Mataeomera dubia*: caterpillars under black scale integuments (upper left and right), a pupa (lower left) and adult (lower right).

Figure 7.12. Numbers of *Mataeomera dubia* pupae and larvae per tree during 1 min observations of trees assessed in the ant exclusion experiment.
Average percent parasitism by *Encarsia* species was 2.5, 14.8, 18.2 and 2.6% in the unbanded, banded, control and late-banded treatments, respectively. Differences among treatments were significant ($F_{3, 57} = 20.7$, $P < 0.001$). Percent parasitism on banded trees was significantly higher than on unbanded ($\alpha = 0.05$, $P < 0.001$) and late-banded ($\alpha = 0.05$, $P < 0.001$), but not significantly different from control trees ($\alpha = 0.05$, $P = 0.23$). Differences among sites were also significant ($F_{2, 57} = 14.96$, $P < 0.001$). Interactions between treatment and site were not significant ($F_{6, 57} = 1.6$, $P = 0.17$) (Fig. 7.14).

![Figure 7.14](image-url)  
*Figure 7.14.* Percent parasitism by *Encarsia* species in the ant-exclusion experiment in April 2011.

My observations indicated that *Microcera coccophila* was noticeably more abundant on unbanded trees than on banded trees. The fungus was observed on all above ground parts of trees including the main tree trunk. As I reported in Chapter 9, the fungus was also observed in summer. Although it was less abundant than in autumn, winter and spring, was still more noticeably than in other orchards where no soft scales were present.

### 7.4. Discussion

#### 7.2.1. Ant activity

*Iridomyrmex rufoniger* was the most common ant species on soft scale infested trees in citrus orchards on the Central Coast of New South Wales during my field studies from 2008 to 2012. Synonyms of *Iridomyrmex rufoniger* include *Formica rufonigra* Lowne,
Acantholepis mamillatus Lowne, Iridomyrmex rufoniger septentrionalis Forel and Iridomyrmex rufoniger domesticus Forel (Heterick & Shattuck 2011). Smith et al. (1997) noted that ants in the Iridomyrmex rufoniger species-group occurred in over 80% of samples collected historically in citrus orchards in New South Wales, Victoria, South Australia and southern Queensland. They are associated with soft brown scale in citrus orchard in Leeton, inland New South Wales (James et al. 1997).

Iridomyrmex rufoniger was the only species associated with black scale in my experiments, and was far more common than Rhytidoponera confusa, a member of Rhytidoponera impressa group (Ward 1981), which I also observed. I did not observe Rhytidoponera confusa feeding on honeydew. It only appeared to be present in the absence of black scale. However, two species in this group, Rhytidoponera chalybaea and Rhytidoponera purpurea, have been recorded feeding on honeydew in forests in Australia and New Guinea (Ward 1981), and Andersen (1986) reported that Rhytidoponera species were cryptic and opportunistic.

My results indicate that seasonal trends of ant activity were generally related to prevailing ambient temperatures. The numbers of ants peaked in summer and troughed in winter. Seasonal incidence of activity was similar to observations made by Markin (1970) for Argentine ant (Linepithema humile) in California citrus groves, Briese & Macauley (1980) for several ant species, including Iridomyrmex, in semi-arid Australia, and by Sanders (1972) for carpenter ants (Camponotus spp moving along underground trails in north-western Ontario (Canada). Briese & Macauley (1980) reported that an Iridomyrmex sp. was active at high ambient temperatures in contrast to some other species that are active at low ambient temperatures. They also reported that activity of the ant was diurnal, though not strictly.

The seasonal activity of the ants on the unbanded trees was not only related to ambient temperatures but also to the levels of black scale populations. I attributed the reduction of ant numbers between December 2010 and January 2011 to the reduction of black scale populations on unbanded trees due to predation by Orcus australasiae, particularly in site 2, and predation by Scutellista caerulea and Lecanicillium lecanii epizootics in all three sites.
7.4.2. Survey

My survey results indicated that high activity of *Iridomyrmex rufoniger* markedly reduced levels of parasitism by *Aphytis* species, *Comperiella bifasciata* and *Encarsia* species. Similar effects on parasitism of red scale were observed by Flanders (1945), DeBach et al. (1951a), Steyn (1954a,b), Samways (1981), Samways et al. (1982), Moreno (1987), Murdoch et al. 1995, Martinez-Ferrer et al. (2002) and Pekas et al. (2010). Flanders (1945) observed that co-occurrence between soft brown scale and yellow scale resulted from the activities of Argentine ant. DeBach (1951) reported that Argentine ant reduced levels of parasitism by *Aphytis* species. Martinez-Ferrer et al. (2002) reported, under laboratory conditions, that three ant species, *Solonopsis xyloni* McCook, Argentine ant, and gray ant (*Formica aerate* (Framcoeur)) had negative impacts on parasitism levels of *Aphytis melinus* and *Comperiella bifasciata*, and on host mutilation by *Aphytis melinus*. Pekas et al. (2010) reported that high activity of three native Mediterranean ants that occurred throughout the year increased red scale populations in citrus orchards in Valencia in Spain, but they did not detect significant differences in levels of parasitism by *Aphytis chrysomphali* and *Aphytis melinus* on the fruit in the presence or absence of ants. My study is the first to report such impacts on five parasitoid species in the field. Moreover, my results also indicate that predation by ladybirds (as evidenced by footprints) was also reduced by ant activity, in this instance *Iridomyrmex rufoniger*. These differences have not been previously reported. However, this preliminary experiment was based on trees of different ages and this may have influenced the outcome. I therefore used trees of the same age in the ant exclusion experiment.

7.4.3. Ant exclusion experiment

7.4.3.1. Black scale self-asphyxiation

My results showed that high black scale mortality occurred in high density populations of the scale through self-asphyxiation by its honeydew in the absence of *Iridomyrmex rufoniger* (Figs 7.14–7.16). Similar results were reported by Way (1954)\(^{45}\) for *Saissetia zanzabiensis* Williams and the African weaver ant, *Oecophylla longinoda* (Latreille), on clove (*Jambosa caryophyllus* Nied.) [Myrtales: Myrtaceae] trees under field-like

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\(^{45}\) Professor Michael James Way, born in 1922, passed away in England on 18 January 2011, shortly after I read his papers on *Saissetia zanzabiensis* and *Oecophylla longinoda.*
conditions in Zanzibar (now part of Tanzania). Way (1954) undertook several experiments to determine if *Saissetia zanzabiensis* could be self-asphyxiated by its honeydew in the absence of *Oecophylla longinoda*. Here, I summarise the results of two of those experiments. In the first, he assessed the impact of the ant on populations of the scale on scale-infested branches of clove trees banded with grease with populations of the scale on scale-infested, unbanded branches on which the ant was abundant. On the banded trees, total numbers of *Saissetia zanzabiensis* on six unbanded branches (*Oecophylla longinoda* present) were 3120 individuals at the beginning and 2280 individuals four weeks later. In contrast, total numbers of the scale on six banded branches (*Oecophylla longinoda* absent) were 2820 at the beginning and 162 after four weeks. In the second experiment, Way (1954) determined the impact of honeydew removal by *Oecophylla longinoda* from trees. He compared three treatments: (1) *Oecophylla longinoda* present, (2) *Oecophylla longinoda* absent and honeydew removed by watering, and (3) *Oecophylla longinoda* absent, honeydew not removed. Total numbers of *Saissetia zanzabiensis* per five plants in each of these treatments were approximately 400–500 individuals before the experiment commenced. After 132 days, scale populations in the presence of *Oecophylla longinoda* increased rapidly to 3220 individuals per 5 plants. In the absence of the ant, and with honeydew removed by watering, scale populations increased to 1080 individuals per 5 plants. In the absence of the ant, and with no watering, the scale populations declined to some 100–150 individuals per five plants.

In my experiment, black scale did not re-establish on the banded trees, whereas Way (1954) recorded survival and re-establishment, though at very low levels, of *Saissetia zanzabiensis* in the absence of ants and no rain or applications of water. Differences, between my results and those of Way (1954), could be related to differences in prevailing weather conditions in the two studies, the scale and ant species, and natural enemies.
Figure 7.14. A Hamlin orange tree heavily infested with black scale, and with heavy growth of sooty mould fungi, Lister’s orchard at Kulnura before the trunk was banded in late June 2010 to exclude *Iridomyrmex rufoniger* workers from foraging for honeydew in the canopy.
Figure 7.15. Branch of unbanded Hamilin orange tree in Lister’s orchard at Kulnura in December 2010, six months after the experiment to assess the impact of *Iridomyrmex rufoniger* on natural enemies of red scale began. The branch is heavily infested with black scale and heavey growth of sooty mould fungi is evident.
Figure 7.16. Branch of banded Hamlin orange tree in Lister’s orchard at Kulnura in December 2010, six months after the experiment to assess the impact of *Iridomyrmex rufoniger* on natural enemies of red scale began. Black scale infestations, and growth of sooty mould fungi, present at the beginning of the experiment have almost disappeared.
Ants stimulate excretion of honeydew by caressing honeydew-producing insects with their antennae (Darwin 1859, Andrews 1930, Collins & Scott 1982, Malumphy 1997), and interactions between ants, honeydew and survival of honeydew-producing insects have been widely recognised (Andrews 1930, Smith 1942, Flanders 1942a, Flanders 1943c, Flanders 1951a, Nixon 1951, Way 1954, 1963, Bess 1958, Bedford et al. 1968, Williams & Williams 1980, Jutsum et al. 1981, Bristow 1984, Hanks & Sadof 1990, Bach 1991, Itioka & Inoue 1996, Gullan 1997, Malumphy 1997). These relationships included direct harm in which the insects were trapped and self-asphyxiated by syrupy deposits, and indirect harm due to sooty mould contamination of host plants. Additionally, Andrews (1930) mentioned that dextrins (low-molecular-weight carbohydrates produced by the hydrolysis of starch or glycogen) contributed to the sticky nature of aphid honeydew that was harmful to aphids when it fell upon, and dried on them. Such impacts have only been demonstrated for coccids experimentally by Way (1954) with *Saissetia zanzibarensis*, as above, and by Bach (1991) with *Coccus viridis* (Malumphy 1997).

Gullan (1997) mentioned that in some coccids, such as *Saissetia zanzibarensis*, the mechanism for ejection of honeydew droplets is inefficient and that rapid body contamination occurs when ants are excluded. My results for *Saissetia oleae*, and those of Way (1954) for *Saissetia zanzibarensis*, suggest that the mechanism for excreting honeydew within the tribe Saissetiini may be similar. Gullan (1997) was uncertain whether insect mortalities were due to asphyxiation or the effect of fungal growth on honeydew contamination. However, the rapid mortality of black scale I observed in my experiment suggested that mortality of the scale was due to asphyxiation by its honeydew. This view is supported by observations made by Flanders (1942a) who noted that black scale cultures on potato (*Solanum tuberosum* L. [Solanaceae]) sprouts were subject to asphyxiation from excess deposits of honeydew and that washing scale-infested potato tubers weekly kept scale healthy. Furthermore, during the interval over which black scale populations declined rapidly on banded trees in my experiment, growth of sooty mould was low and *Lecanicillium lecanii* was not evident.

There are no records on asphyxiation of aphids, membracids and soft scales within tribes Ceroplastini, Coccini and Pulvinariini by their honeydew. In contrast to black scale and *Saissetia zanzibarensis*, these insects are capable of projecting honeydew
away from their bodies with great muscular effort (Andrews 1930, Nixon 1951, Williams & Williams 1980, Gullan 1997, Malumphy 1997). Collins & Scott (1982) reported that in the absence of ants and bees, scale can project honeydew about 5 mm from their bodies by the active expulsion of droplets. They (Collins & Scott 1982) attributed mortality of cottony pigface scale, Pulvinariella mesembryanthemi (Vallot) (Pulvinariini), on Carpobrotus edulis [Caryophyllales: Aizoaceae] in Western Australia in the absence of ants to sooty mould fungi, not honeydew. Bach (1991) attributed increased mortality of Coccus viridis (Coccini) in the absence of ants to increased honeydew build-up and resultant colonisation by sooty mould fungi, and high levels of parasitism and predation, not solely honeydew. Gullan (1997) mentioned that some soft scale species, in the absence of ants, can expel honeydew droplets away from their body, for example, 6–12 mm for the third instar nymphs of Ceroplastes sinoiae Hall (Ceroplastini) (Bedford et al. 1968, cited by Gullan 1997). Ceroplastes sinensis is capable of projecting its honeydew longer distances (Beattie, pers. comm., 2010). Therefore, mortalities of species of Ceroplastes and Coccus, and species within the Pulvinariini, at high population densities in the absence of ants and may be more likely to caused by growth of sooty mould fungi than directly by honeydew contamination.

Absence of ants in mutualistic associations may also lead to self-asphyxiation of mealybugs and whiteflies. Moreno et al. (1987) reported that in the absence of ants, populations of citrus mealybug (Plannococcus citri (Risso)), whitefly (Aleurothrixus flocosus (Maskell)) and red scale were effectively reduced by their respective natural enemies. They also reported that impacts of ants on decline of populations of honeydew producers occurred sooner than on non-honeydew producers, and that black scale did not increase sufficiently to measure effects in either orchard (Moreno et al. 1987). In this instance, low levels of black scale populations in their experiments could also have been related to the self- asphyxiation by honeydew.

Results of my experiment, and results of studies published by authors cited above, led me to conclude that the mutualism between ants and honeydew-producing insects is essential for survival of black scale. Darwin (1859) regarded aphids as voluntarily yielding honeydew for ants and the ant-aphid relationship as a strong example of an ‘animal apparently performing an action for the sole good of another’. However, honeydew-producing insects tended by ants also benefit from the removal of honeydew that may
lead to self-asphyxiation of the trophobiont, and growth of sooty mould fungi that may also pose a danger to the survival of these insects.

There were no records of lycaenid species self-asphyxiation by their honeydew in the absence of the ants. However, oviposition behaviour, aggregation and distribution patterns of lycaenids (Atsatt 1981, Smiley 1988, Axén & Pierce 1998) suggest that occurrence of lycaenid is ant-dependent. Cushman et al. (1994) reported that in the field lycaenids only persisted on host plants that ants subsequently colonised.

The contributions of other factors to the decline of black scale populations, such as natural enemies and natural mortalities, could be very limited. I discuss these relationships in the following section.

### 7.4.3.2. Impacts of *Iridomyrmex rufoniger* on predators

My results indicate that *Iridomyrmex rufoniger* disrupted predation by *Halmus chalybeus* and species of *Rhyzobius*. Previous reports of such impacts have only been related to general observations unsupported by empirical data.\(^{46}\)

The relatively even distribution of *Halmus chalybeus* among the three Hamlin orange sites and observations of its behaviour elsewhere in the orchard, and observations in my other study orchards (Chapter 5), led me to conclude that this beetle readily disperses in search of prey, predominantly armoured scales, particularly red scale. I rarely observed it feeding on black scale during the course of my study. In Chapter 2, I cited Koebel (1892) and Froggatt (1902), who stated that *Halmus chalybeus* was not a predator of black scale. Hely et al. (1982) and Smith et al. (1997) did not regard it as a predator of black scale, soft brown scale or long soft scale.

In contrast, the Valentine (1967) listed *Halmus chalybeus* as a predator of black scale in New Zealand. Flynn (1995) mentioned that *Halmus chalybeus* was introduced to New Zealand to control black scale on citrus in 1889 and gum tree scale, *Eriococcus coriaceus* Maskell, on *Eucalyptus* sp. in 1905. Waterhouse & Sands (2001), citing Wilson (1960), listed it as a predator of black scale. Wilson (1960) mentioned it as predator of coccids. However, these records did not mention impacts of honeydew-seeking ants on *Halmus chalybeus* activity. Lo & Blank (1992), Lo (2000), Lo &

\(^{46}\) (http://www.brisbaneinsects.com/brisbane_ladybirds/Orange-spotted.htm)
Chapman (2001) regarded *Halmus chalybeus* as an important predator of wax scale in New Zealand. Similarly, Hely et al. (1982), Smith et al. (1997) and Lo & Chapman (2001) mentioned it as predator of pink wax scale, white wax scale and hard wax scale, all species of *Ceroplastes*.

However, these records suggest that predation by *Halmus chalybeus* on soft scales may be related to the manner in which the scales excrete honeydew, as discussed above. Due to these differences, I assume that ants foraging on black scale honeydew disrupt the activity of *Halmus chalybeus*, whereas the predator may prey upon *Ceroplastes* species in the absence high accumulation of honeydew, and therefore relatively low levels of ant activity. Disruption might occur in some circumstances e.g., where very high populations of wax scales lead to heavy deposits of sooty mould fungi. However, during the course of my study, I did not observe *Halmus chalybeus* in association with *Ceroplastes* species, despite high populations of *Ceroplastes sinensis* and *Ceroplastes destructor* on some trees in Lister’s orchard. Lo & Chapman (2001) reported that *Halmus chalybeus* feeds on first and second instar larvae of scales in New Zealand but Beattie (pers. comm., 2011) cannot recollect seeing it feed on wax scales during observations in citrus orchards in New South Wales from 1975 to the present. Moreover, there is no evidence of high populations of wax scales leading to high populations of *Halmus chalybeus* or any other coccinellid species (Beattie, pers. comm., 31 July 2011). I assume that the wax scales may not be a host of choice and that the short duration over which wax scale stages are suitable as prey may be too short for the beetle to complete its development.

In my experiment, the high incidence of *Rhyzobius* spp., particularly *Rhyzobius lophanthae*, adults and larvae and percentage of predated scale on banded and late-banded trees in site 3 suggested that ant activity reduced predation by *Rhyzobius* spp. My observations are similar to those reported by Bartlett (1961) who, in contrast to DeBach et al. (1951), reported that Argentine ant was highly aggressive towards *Rhyzobius lophanthae* and that the beetle had a tendency to avoid it.

*Rhyzobius lophanthae* is an important predator of most diaspidid species (Statas 2000). It was first described by Frank Ellsworth Blaisdell who found all developmental stages were exceedingly abundant on San José scale infesting *Paraserianthes lophantha* (Willd.) I.C. Nielsen (syn. *Acacia lophantha* Willd.) [Fabales: Leguminosae] in Coronado Park, California (Blaisdell 1892). There were no reports of *Rhyzobius*
lophanthae feeding on soft scales. My results and general observations suggested to me that the occurrence of coccinellids in citrus orchards in coastal New South Wales is cryptic. Therefore, assessments of proportions of scales predated by each species may more accurately reflect their activity, rather than observations based solely on the incidence of larvae, pupae and adults.

The high incidence of predated scale in the assessments made in late autumn suggested that: (1) remnant, *Rhyzobius*–predated scales remain longer on scale-infested branches, twigs, leaves and fruit than do footprints, the tell-tale evidence of predation by *Halmus chalybeus* and *Orcus australasiae*; and (2) *Rhyzobius* species were more common in autumn. I did not observe *Rhyzobius* in December 2010 and January and February 2011 in site 3 where both *Rhyzobius hirtellus* and *Rhyzobius lophanthae* were more common than in the other two sites. Hely (1968) and Hely et al. (1982) mentioned that *Rhyzobius lophanthae* was a common predator of red scale in inland New South Wales, particularly in autumn and winter. Stathas (2000) reported that *Rhyzobius lophanthae* does not diapauses. He observed that females reproduced actively throughout the year in plexiglass containers located outdoors at Kifissia (37° 59’ N, 23° 43’ E) in Greece. In this instance, the plexiglass containers were made of cylinders covered with muslin at each end (Iperti & Burn 1969). Therefore, the conditions of the experiments should have been similar to field conditions.

Koebele (1892) mentioned that in New South Wales (presumably near Sydney, possibly Gordon or Parramatta) *Rhyzobius* species had 6 ‘broods’ per year. This is similar to observations reported by Stathas (2000b) in Greece. He (Stathas 2000b) recorded 5 overlapping generations from late spring (May) to mid autumn (October) and a partial sixth generation in late winter and early spring on oleander scale nymphs reared on pumpkin (*Cucurbita maxima* Duchesne [Violales: Cucurbitaceae]) and potato tubers and sprouts under outdoor controlled conditions. Therefore, I assume that at least 5 generations of *Rhyzobius lophanthae* may occur on the Central Coast of New South Wales.

The abundance of *Rhyzobius lophanthae* in site 3 and of *Orcus australasiae* in site 2 may have been related to their reproductive characteristics and host scale densities. Trees in site 3 were slightly smaller and became shaded earlier in the afternoon than trees in sites 1 and 2. *Orcus australasiae* prefers sunshine, with a preference for feeding on outer parts of the tree (Issac 1906). These observations are similar to mine (see...
Chapter 5). Shadowing by clouds and onset of rain led adults and larvae to move to sheltered positions where they were difficult to see. In my experiment, the high incidence of *Orcus australasiae* on some trees with high host densities and not on adjacent trees with similar prey densities suggests that this beetle may colonise and exploit a resource before migrating in search of unexploited resources. This may explain the patchy distribution of this beetle within sites 1 and 2. High levels of predation by *Orcus australasiae* and *Halmus chalybeus* and removal of scale during feeding by these two predators would reduce the availability of prey for *Rhyzobius* species and also suitable sites for oviposition.

The variation in the responses of coccinellid species to *Iridomyrmex rufoniger* may be related to the size of predator species. Body lengths of adults of *Orcus australasiae*, *Halmus chalybeus* and *Rhyzobius lophanthae* species range from 2.8–5.8, 2.5–4.5 and 1.0–5.2 mm, respectively. Body lengths of larvae of *Orcus australasiae*, *Halmus chalybeus* and *Rhyzobius lophanthae* range from 6–8, 3–5 and 3–4 mm (Ślipiński 2007). The larger size of *Orcus australasiae* adults and larvae may simply make them less prone to having their activities disrupted by the ant.

My results indicated that *Iridomyrmex rufoniger* did not suppress the activities of *Orcus australasiae* and *Mataeomera dubia*, and there are no previous reports of negative impacts of ants on these two species. Several authors (Potin 1959, Eisner et al. 1978, Völkl 1995, Kanedo 2007) have shown that some ant species associated with honeydew producers do not disrupt natural enemies. Völkl (1995) reported that *Platynaspis luteorubra* Goeze [Coleoptera: Coccinellidae] larvae were significantly more common in aphid colonies attended by the black garden ant, *Lasius niger* L., than in unattended colonies. Larvae, pupae and adults of *Platynaspis luteorubra* have morphological adaptations to *Lasius niger*: larvae are protected by their unusual shape, inconspicuous movements and chemical camouflage; pupae are attacked but protected by their dense hair cover; adults are attacked but responded either by fleeing or by pressing their body tightly against the plant surface (Völkl 1995).

In my experiment, I recorded 165 larvae, 75 pupae and 32 adult *Orcus australasiae* on one unbanded tree in site 2 on 19 January 2011 when the weather was fine and the maximum temperature 29°C. I also observed *Iridomyrmex rufoniger* moving over and around *Orcus australasiae* adults, but they did not disturb the beetle. The beetle can rest and feed readily in the presence of high activity of *Iridomyrmex rufoniger*. My
observations indicated that *Iridomyrmex rufoniger* did not harass *Orcus australasiae* adults and larvae, nor did *Orcus australasiae* appear to take evasive action in order to avoid encounters with *Iridomyrmex rufoniger*. My observations suggested that the relatively slow movements of *Orcus australasiae* adults and larvae, and the long and dense hairs on the body of larvae, may contribute to its abundance in the presence of *Iridomyrmex rufoniger*.

The significantly higher incidence of *Mataeomera dubia* on unbanded trees suggested that its larvae and pupae are not affected by ant activity. I attributed this to the fact that the larvae and pupae are protected by black scale integuments (Fig. 7.11) that may mimic the scale physically and chemically. My results were similar to those of Way (1954) who reported that predation of *Saissetia zanzibarensis* by *Eublemma* spp. [Lepidoptera: Noctuidae] was not prevented by *Oecophylla longinoda*.

7.4.3.3. Impacts of *Iridomyrmex rufoniger* on parasitoids

Some parasitoids of ant-attended insect species have morphological adaptations for avoiding suppression of their activities by ants (Potin 1959, Völkl 1992, Völkl & Mackauer 1992, Liepert & Dettner 1996, Kaneko 2007). Interactions vary according to the ant and parasitoid species involved (Liepert & Dettner 1996, Kaneko 2007). In my study, I did not record parasitism of black scale. However, of several parasitoid species of black scale occur in the region (Hely et al. 1982, Smith et al. 1997, Waterhouse & Sands 2001) and it is known that *Metaphycus lounsburyi* is disrupted by ant activity (Flanders 1951a) and that *Coccophagus* spp. (Flanders 1951a) and *Scutellista caerulea* (Barzman & Daane 2001) are not.

*Iridomyrmex rufoniger* significantly reduced levels of parasitism by *Encarsia* species in autumn 2011. These results thus confirmed the impacts of the ant that I recorded in the survey I conducted in 2010. However, I could not confirm such impacts on species of *Aphytis* species and *Comperiella bifasciata* in the ant exclusion experiment due to low levels of parasitism by these species in autumn 2011 (see Chapter 5). The results thus strongly confirmed those of the survey that *Iridomyrmex rufoniger* activity reduced the parasitism by *Encarsia* species. There were no published studies regarding the impacts of ants on *Encarsia* species on red scale. Flanders reported negative effects of Argentine ant on parasitism by *Comperiella bifasciata* (1945) and Martinez-Ferrer et al. (2002) reported negative effects of three ant species, including Argentine ant, on
parasitism by *Comperiella bisfasciata* and parasitism and host-mutilation by *Aphytis melinus*: the impacts on *Aphytis melinus* were greater than on *Comperiella bisfasciata*.

### 7.4.3.4. Interactions of *Iridomyrmex rufoniger* with other insects


Cushman et al. (1994) reported that the Bathurst copper butterfly, *Paralucia aurifera* (Edwards & Common) only persisted on host plants that *Anonychomyrma itinerans* (cited as *Iridomyrmex nitidiceps*) subsequently colonised. Smiley et al. (1988) reported that local distribution of *Jalmenus evagoras* is dependent upon the distribution, patchiness and foraging behaviour of host ants. Atsatt (1981) observed that the oviposition of *Ogyris amaryllis* was induced by ants.

These reports and the results of my studies led me to hypothesise that associations between native ant species and native honey-dew producing hemipterans may be an important factor in lycaenids choosing oviposition sites. There are no reports of such associations in the literature. Research is required to determine such associations were beyond the scope of my studies. Nevertheless, it is of interest to note that application sugar solution (anticipated to attract *Anonychomyrma itinerans*) to potted *Bursaria spinosa subsp. lasiophylla* (E.M. Benn.) L.W. Cayzer et al. [Apiales: Pittosporaceae] plants apparently also fostered increased caterpillar occupation of treated plants.
7.4.3.5. Incidence of *Microcera coccophila*

My observations on the incidence of *Microcera coccophila* in the presence and absence of *Iridomyrmex rufoniger* suggest that the ant contributes passively to spread of fungal conidia on the trees. The distribution of *Microcera coccophila* on all above ground parts of trees, particularly on the main tree trunk on which ants move up and down supports this hypothesis. Sooty mould fungi, by slowing evaporation of water from plant surfaces, may also favour development of the pathogen. The high incidence of *Microcera coccophila* may be also be elated to high red scale density stemming from the impact of ant activity on natural enemies of the scale (see Chapter 9).
Chapter 8. Confirmation of taxonomic identities of red scale, yellow scale, their parasitoids and a honeydew-seeking ant

8.1. Introduction

Red scale and yellow scale are similar in appearance and difficult to distinguish (Quayle 1911, Quayle 1938, Hely 1955, Compere 1961, Hely et al. 1982, Smith et al. 1997). Quayle (1911a) noted that both scales share similar morphological characters and considered yellow scale a variety of red scale, as it is lighter in colour, less convex, larger and rarely found on twigs. Nel (1935) was the first to regard them as distinct species, based on morphological, biological and ecological differences, but it was not until McKenzie (1938) observed the presence of pygidial, prevulvar scleroses in red scale and their absence in yellow scale that the two could be readily distinguished, albeit with a small, about 5%, margin of error (DeBach et al. 1978). Nevertheless, the presence of scleroses has remained the most accurate means of separating the species until the advent of modern molecular techniques that have been used in several recent studies (Provencher et al. 2005, Morse & Normark 2006, Anderson et al. 2010, Park et al. 2010). The species comprise genetically distinct populations that do not intergrade and apparently do not hybridise (McKenzie 1938, OEPP/EPPO 2005a). Yellow scale closely resembles Aonidiella comperei and Aonidiella eremocitri, but the presence of perivulvar pores in the last two species precludes confusion (McKenzie 1938, OEPP/EPPO 2005a).

In order to minimise the risk of misidentification affecting interpretation of studies reported elsewhere in my thesis, I used morphological and molecular methods to confirm the presence or absence of red and yellow scale in my study orchards at Somersby, Kulnura, Lower Portland, Cornwallis, Richmond and Castlereagh. In doing so, I also studied molecular variation within each species. For this component of my studies, I also obtained samples of the scales from inland New South Wales and from Victoria, South Australia, Western Australia and the Northern Territory.
In Chapter 2, I noted there are discrepancies in historical records for the primary parasitoids of red scale in Australia and, in Chapter 5, I indicated that at least 5 parasitoid species occur in orchards on the Central Coast of New South Wales. The most difficult parasitoids to distinguish are the species of *Aphytis*, for which pupal pigmentation during the green-eyed pupal stage has been recommended as a useful method (Rosen & DeBach 1979). However, variation and low percentages of green-eyed pupae in samples mean that the method, despite its usefulness, is prone to error. I encountered difficulty in using the method to determine if *Aphytis lingnanensis* and *Aphytis melinus* occurred in my orchards. I, therefore, used molecular methods to verify the presence of these species in my study.

I also used molecular techniques to determine genetic variation among populations of *Encarsia citrina* and *Encarsia perniciosi* parasitising red scale, yellow scale and purple scale, and of *Comperiella bifasciata* parasitising red scale and yellow scale. Although adults of the two *Encarsia* species can be readily distinguished on the basis of the length of marginal wing hairs (Craw 1891, Tower 1913, Woodworth 1913, Flanders 1950, Malipatil et al. 2000), they have a wide range of hosts (Universal Chalcidoidea Database). Likewise, *Comperiella bifasciata* is readily identifiable, but it also contains two host-specific races, a yellow scale race and a red scale race (Flanders 1944a, 1950, Smith 1942). Brewer (1971) reported that encapsulation of almost 60% of eggs and larvae of the red scale race of *Comperiella bifasciata* by red scale limited the effectiveness of it as a parasitoid of this scale, whereas this limitation did not apply to yellow scale in which almost no parasitoid encapsulation occurred. Such encapsulation by red scale of the immature stages of the parasitoid has not been recorded or observed in more recent studies (Furness et al. 1983, Beattie, unpublished data). Moreover, Flanders (1934) reared *Comperiella bifasciata* from *Aonidiella eremocitri* (Flanders 1934, McKenzie 1937) on *Citrus glauca* in Marmor near Rockhampton on the east coast of Queensland in 1930 before the parasitoid was formally introduced to Australia in 1940s47. Although I could not obtain *Comperiella bifasciata* on *Aonidiella eremocitri* nor the host scale during my study, I used molecular techniques to determine if DNA sequences of red scale and yellow scale strains of *Comperiella bifasciata* are identical.

47Flanders (1934) mentioned that he reared the parasitoid from what appeared to him to be yellow scale, then known as *Chrysomphalus citrinus*. Sands & Snowball (1980) claimed that these observations were erroneous and proposed that the scale observed by Flanders was circular black scale (*Chrysomphalus aonidum*) and the parasitoid *Comperiella pia* (Girault). However, the scale was *Aonidiella eremocitri* (McKenzie 1937, 1938).
It was of interest to screen DNA specimens of scales and parasitoids for the presence of their bacterial symbionts, *Wolbachia* (α-Proteobacteria) and ‘*Candidatus* Cardinium’ (Bacteroidetes). *Wolbachia* infects a wide range of insects and associates with different reproductive types of hosts including cytoplasmic incompatibility in a wide range of insects, parthenogenesis in wasps and male feminisation (Werren 1995, Zhou et al. 1998). Vasquez et al. (2011) reported that cytoplasmic incompatibility-inducing *Wolbachia* was associated with fitness costs in biological control of *Aphytis melinus*. No studies have mentioned associations of *Wolbachia* with red scale or yellow scale. ‘*Candidatus* Cardinium’ has been reported to be associated with the parthenogenetic type of oleander scale and *Encarsia* species (Zchori-Fein et al. 2001, 2004, Provencher et al. 2005). ‘*Candidatus* Cardinium’ is associated with parthenogenesis in some parasitoid wasps and other insects including *Encarsia* species (Zchori-Fein et al. 2001, Gruwell & Normark 2009). Therefore, in this Chapter, I also screened the parasitoids for presence of these bacteria.

As red scale and yellow scale are amphimictic, bacterial symbionts, if present, may induce other biological modes. Furthermore, screening the host scale could help to determine whether bacterial symbionts of parasitoids are affected by bacterial symbionts of the hosts. Therefore, in this study, several individual specimens of both red scale and yellow scale from different locations were screened for the presence of *Wolbachia*.

In this chapter, I also verify the identity of the ant species associated with black scale in my study orchards, particularly at Lister’s orchard at Kulnura, where I undertook the experiments reported in Chapter 7.

### 8.2. Materials and methods

#### 8.2.1. Morphological identification

##### 8.2.1.1. Red scale and yellow scale

###### 8.2.1.1.1. General observations

Observations were based on distributions of red scale and yellow scale within tree canopies, among blocks within orchards, and among my study orchards at Kulnura, Somersby, Lower Portland, Cornwallis, Richmond and Castlereagh. Additional observations were made in the several Valencia orange, Washington navel orange and grapefruit orchards at Griffith (34° 19’ S, 146° 04’ E) in September 2010. Distributions
of scales on upper and lower surfaces of leaves, and on inner and outer surfaces of fruit, were recorded. Damage caused by each species was also recorded.

The general appearance of the scale was examined visually with or without use of Wild M7S and Leica MZ12 stereomicroscopes (Leica Microsystems Pty Ltd., North Ryde, Australia). Photographs were taken with a Leica MZ12 stereomicroscope (fitted with a KY F1030 digital camera (JVC, Expandore Electronics Pte Ltd., Shun Li Industrial Complex, Singapore).

8.2.1.1.2. Slide preparation and microscopy

Fresh or preserved, adult, female scale specimens were mounted on slides following the procedures described by Wirth & Marston (1968) or Sandlant (1978). The procedure described by Wirth & Marston (1968) was used initially. It comprised the following steps:

- placement of specimens in 10% potassium hydroxide in an excavated block (38 mm square, 30 mm diameter) with a cover glass (Australian Entomological Supplies Pty. Ltd., New South Wales, Australia) preheated to 93°C for 5–10 min;
- transfer of specimens to 75% alcohol for 30 min;
- transfer of specimens to a solution of phenol and 70% alcohol (1:1 v/v alcohol/phenol) for about 10 min and then to a phenol-100% alcohol solution for 3–4 h (1:1 v/v alcohol/phenol); and
- mounting of specimens on slides in Canada balsam.

This method proved inadequate as specimens could not be cleared perfectly; some fatty bodies remained. The following method, given by Sandlant (1978), yielded clearer specimens and was used for the rest of the work:

- placement of specimens in a excavated block with 50 drops of Essig’s solution;
- heating the solution in an oven at 60°C for 3 h;
- carefully puncturing the specimens with a fine needle and teasing out the body contents, including any embryos, without destroying the taxonomic features;
- transferring the specimens to 70% ethanol for 5–10 min;
- transferring the specimens to a solution comprising equal parts of 70% ethanol and chloroform for 5–10 min;
- transferring the specimens to 100% ethanol 5–10 min; and
- mounting the specimens on slides in Canada balsam.
Specimens were examined under an Olympus BX60 compound microscope (Olympus Corporation, Tokyo, Japan) to determine the presence or absence of pygidial, prevulvar scleroses as reported by (McKenzie 1938). The microscope was fitted with a ProgRes C14 digital camera.

### 8.2.1.2. Parasitoids

Specimens were collected from my study orchards (Chapter 3).

Specimens of *Aphytis* species were initially separated by the presence or absence of characteristic pigmentation of sternites (thoracic and abdominal) in the ‘green-eye’ pupal stage. During this stage, *Aphytis lingnanensis* has a large, dark area on the mid-thoracic sterna, *Aphytis melinus* has a small, dark patch, and *Aphytis chrysomphali* has no dark pigmentation but is readily identifiable by the presence of a longitudinal, black line on the mesosternum (Compere 1955, DeBach 1959, Rosen & DeBach 1979). Once identified, each specimen was photographed and then set aside in 100% ethanol for molecular studies.

For *Encarsia* species, parasitised red, yellow and purple scales were kept individually in separate gelatine capsules until the adults emerged. Adults were then identified and set aside in 100% ethanol for molecular studies.

*Comperiella bifasciata* adults were collected from parasitised red and yellow scales and stored in 100% ethanol for molecular studies.

### 8.2.2. Molecular identification

#### 8.2.2.1. Specimen origins and accession numbers

Adult female scale of given species were used for molecular studies. Details for each scale species and host plant, and locations from which specimens were collected are shown in Tables 8.1–8.3. Likewise, adult parasitoids were used for molecular studies except for *Aphytis* species, for which the mature pupae were used. Details for *Aphytis* species, *Comperiella bifasciata*, *Encarsia* species, host scales and plants, and locations from which specimens were collected are shown in Tables 8.4–8.6.

Single ant workers, mostly *Iridomyrmex rufoniger*, were used for DNA extraction. Details for the specimen, associated scale insects, and plants and locations from which specimens were collected are listed in Table 8.7.
Table 8.1. Host plants, host plant substrates and locations in New South Wales from which red scale (*Aonidiella aurantii*) was collected for molecular studies.

<table>
<thead>
<tr>
<th>Specimen code</th>
<th>Host plant</th>
<th>Substrate</th>
<th>Location</th>
<th>Latitude and longitude</th>
<th>Accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. aurantii</em> SNSW1</td>
<td>Washington navel orange</td>
<td>leaf</td>
<td>Somersby, New South Wales</td>
<td>33° 22' S, 151° 16' E</td>
<td></td>
</tr>
<tr>
<td><em>A. aurantii</em> RNSW5</td>
<td>Valencia orange</td>
<td>leaf</td>
<td>Richmond, New South Wales</td>
<td>33° 36' S, 150° 44' E</td>
<td>JQ434495 +</td>
</tr>
<tr>
<td><em>A. aurantii</em> RNSW7</td>
<td>Valencia orange</td>
<td>leaf</td>
<td>Richmond, New South Wales</td>
<td>33° 36' S, 150° 44' E</td>
<td>JQ434503 +</td>
</tr>
<tr>
<td><em>A. aurantii</em> RNSW1</td>
<td>Washington navel orange</td>
<td>fruit</td>
<td>Richmond, New South Wales</td>
<td>33° 36' S, 150° 44' E</td>
<td></td>
</tr>
<tr>
<td><em>A. aurantii</em> RNSW2</td>
<td>Valencia orange</td>
<td>leaf</td>
<td>Richmond, New South Wales</td>
<td>33° 36' S, 150° 44' E</td>
<td></td>
</tr>
<tr>
<td><em>A. aurantii</em> LNSW2</td>
<td>sweet orange</td>
<td>fruit</td>
<td>Lower Portland, New South Wales</td>
<td>33° 23' S, 150° 52' E</td>
<td>JQ434500</td>
</tr>
<tr>
<td><em>A. aurantii</em> GNSW2</td>
<td>Valencia orange</td>
<td>fruit</td>
<td>Griffith, New South Wales</td>
<td>34° 19' S, 146° 04' E</td>
<td>JQ434498</td>
</tr>
<tr>
<td><em>A. aurantii</em> GNSW1</td>
<td>Valencia orange</td>
<td>fruit</td>
<td>Griffith, New South Wales</td>
<td>34° 19' S, 146° 04' E</td>
<td></td>
</tr>
<tr>
<td><em>A. aurantii</em> GNSW</td>
<td>grapefruit</td>
<td>Griffith, New South Wales</td>
<td>34° 19' S, 146° 04' E</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. aurantii</em> BNSW</td>
<td>sweet orange</td>
<td>fruit</td>
<td>Barham, New South Wales</td>
<td>35° 31' S, 143° 56' E</td>
<td>JQ434497</td>
</tr>
<tr>
<td><em>A. aurantii</em> BNSW1</td>
<td>sweet orange</td>
<td>fruit</td>
<td>Barham, New South Wales</td>
<td>35° 31' S, 143° 56' E</td>
<td></td>
</tr>
<tr>
<td><em>A. aurantii</em> BNSW2</td>
<td>sweet orange</td>
<td>fruit</td>
<td>Barham, New South Wales</td>
<td>35° 31' S, 143° 56' E</td>
<td></td>
</tr>
</tbody>
</table>
Table 8.2. Host plants, host plant substrates and locations in Victoria, South Australia, Western Australia, Northern Territory and Indonesia from which red scale (*Aonidiella aurantii*) was collected for molecular studies.

<table>
<thead>
<tr>
<th>Specimen code</th>
<th>Host plant</th>
<th>Substrate</th>
<th>Location</th>
<th>Latitude and longitude</th>
<th>Accession number</th>
<th>28S</th>
<th>EF</th>
<th>COI</th>
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<tr>
<td><em>A. aurantii</em> MuVic</td>
<td>sweet orange</td>
<td>leaf</td>
<td>Murrabit, Victoria</td>
<td>35° 31' S, 143° 57' E</td>
<td>JQ434499</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. aurantii</em> SVIC</td>
<td>grapefruit</td>
<td>fruit</td>
<td>Sunraysia, Victoria</td>
<td>34° 12' S, 142° 08' E</td>
<td>JQ434494</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. aurantii</em> ToVic</td>
<td>Washington navel orange</td>
<td>leaf</td>
<td>Tocumwal, Victoria (AaA10)</td>
<td>35° 48' S, 145° 34' E</td>
<td>JQ434502</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><em>A. aurantii</em> LSW</td>
<td></td>
<td></td>
<td>Loxton, South Australia</td>
<td>34° 27' S, 140° 34' E</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>A. aurantii</em> GWA</td>
<td>Valencia orange</td>
<td>fruit</td>
<td>Gingin, Western Australia</td>
<td>31° 20' S, 115° 54' E</td>
<td>JQ434493</td>
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<td></td>
</tr>
<tr>
<td><em>A. aurantii</em> DNT</td>
<td>grapefruit</td>
<td>fruit</td>
<td>Darwin, Northern Territory</td>
<td>12° 28' S, 130° 50' E</td>
<td>JQ434501</td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>A. aurantii</em> IND1</td>
<td>sweet orange</td>
<td>fruit</td>
<td>Universitas Gadjah Mada, Yogyakarta, Indonesia</td>
<td></td>
<td>JQ434496</td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>A. aurantii</em> IND2</td>
<td>mandarin</td>
<td>fruit</td>
<td>Ngablak, Central Java, Indonesia</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>
Table 8.3. Host plants, host plant substrates and locations in New South Wales and Victoria from which yellow scale (*Aonidiella citrina*) was collected for molecular studies.

<table>
<thead>
<tr>
<th>Specimen code</th>
<th>Host plant</th>
<th>Substrate</th>
<th>Location</th>
<th>Latitude and longitude</th>
<th>Accession number</th>
<th>28S</th>
<th>EF</th>
<th>COI</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. citrina</em></td>
<td>CaNSW1</td>
<td>sweet orange</td>
<td>leaf</td>
<td>Castlereagh, New South Wales</td>
<td>33° 40' S, 150° 40' E</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>A. citrina</em></td>
<td>CaNSW2</td>
<td>Washington navel orange</td>
<td>leaf</td>
<td>Castlereagh, New South Wales</td>
<td>33° 40' S, 150° 40' E</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>A. citrina</em></td>
<td>CaNSW</td>
<td>Washington navel orange</td>
<td>leaf</td>
<td>Castlereagh, New South Wales</td>
<td>33° 40' S, 150° 40' E</td>
<td>JQ82398</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>A. citrina</em></td>
<td>CoNSW1</td>
<td>Washington navel orange</td>
<td>leaf</td>
<td>Cornwallis, New South Wales</td>
<td>33° 35' S, 150° 49' E</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>A. citrina</em></td>
<td>RNSW1</td>
<td>Valencia orange</td>
<td>leaf</td>
<td>Richmond, New South Wales</td>
<td>33° 36' S, 150° 44' E</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>A. citrina</em></td>
<td>RNSW7</td>
<td>sweet orange</td>
<td>leaf</td>
<td>Richmond, New South Wales</td>
<td>33° 36' S, 150° 44' E</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. citrina</em></td>
<td>RNSW2</td>
<td>sweet orange</td>
<td>fruit</td>
<td>Richmond, New South Wales</td>
<td>33° 36' S, 150° 44' E</td>
<td>JQ82396</td>
<td></td>
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<tr>
<td><em>A. citrina</em></td>
<td>RNSW</td>
<td>Valencia orange</td>
<td>leaf</td>
<td>Richmond, New South Wales</td>
<td>33° 36' S, 150° 44' E</td>
<td>JQ82399</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. citrina</em></td>
<td>LPNSW2</td>
<td>Washington navel orange</td>
<td>fruit</td>
<td>Lower Portland, New South Wales</td>
<td>33° 26' S, 150° 52' E</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. citrina</em></td>
<td>SNSW</td>
<td>Washington navel orange</td>
<td>leaf</td>
<td>Somersby, New South Wales</td>
<td>33° 22' S, 151° 16' E</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td><em>A. citrina</em></td>
<td>SNSW3</td>
<td>Washington navel orange</td>
<td>leaf</td>
<td>Somersby, New South Wales</td>
<td>33° 22' S, 151° 16' E</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td><em>A. citrina</em></td>
<td>SNSW6</td>
<td>Washington navel orange</td>
<td>leaf</td>
<td>Somersby, New South Wales</td>
<td>33° 22' S, 151° 16' E</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td><em>A. citrina</em></td>
<td>GNSW4</td>
<td>grapefruit</td>
<td>leaf</td>
<td>Griffith, New South Wales</td>
<td>34° 19' S, 146° 04' E</td>
<td>JQ82395</td>
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<tr>
<td><em>A. citrina</em></td>
<td>GNSW3</td>
<td>grapefruit</td>
<td>leaf</td>
<td>Griffith, New South Wales</td>
<td>34° 19' S, 146° 04' E</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td><em>A. citrina</em></td>
<td>GNSW1</td>
<td>grapefruit</td>
<td>leaf</td>
<td>Griffith, New South Wales</td>
<td>34° 19' S, 146° 04' E</td>
<td>JQ82397</td>
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<tr>
<td><em>A. citrina</em></td>
<td>MiVic</td>
<td>grapefruit</td>
<td>leaf</td>
<td>Mildura, Victoria</td>
<td>34° 12' S, 142° 08' E</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Species</td>
<td>Accession code</td>
<td>Host scale</td>
<td>Host plant</td>
<td>Location</td>
<td>Latitude and longitude</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------------</td>
<td>----------------</td>
<td>----------------</td>
<td>------------------</td>
<td>---------------</td>
<td>------------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aphytis melinus</em></td>
<td><em>A. melinus</em> KNSW1</td>
<td><em>Aonidiella aurantii</em></td>
<td>Eureka lemon</td>
<td>Kulnura, NSW</td>
<td>33° 14' S, 151° 13' E</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aphytis melinus</em></td>
<td><em>A. melinus</em> KNSW2</td>
<td><em>Aonidiella aurantii</em></td>
<td>Eureka lemon</td>
<td>Kulnura, NSW</td>
<td>33° 14' S, 151° 13' E</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aphytis melinus</em></td>
<td><em>A. melinus</em> KNSW3</td>
<td><em>Aonidiella aurantii</em></td>
<td>Valencia orange</td>
<td>Kulnura, NSW</td>
<td>33° 13' S, 151° 13' E</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aphytis melinus</em></td>
<td>unidentified aphelinus1</td>
<td><em>Aonidiella aurantii</em></td>
<td>Valencia orange</td>
<td>Kulnura, NSW</td>
<td>33° 13' S, 151° 13' E</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aphytis melinus</em></td>
<td>unidentified aphelinus2</td>
<td><em>Aonidiella aurantii</em></td>
<td>Valencia orange</td>
<td>Kulnura, NSW</td>
<td>33° 13' S, 151° 13' E</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aphytis chrysomphali</em></td>
<td><em>A. chrysomphali</em> SNSW</td>
<td><em>Aonidiella aurantii</em></td>
<td>Washington navel orange</td>
<td>Somersby, NSW</td>
<td>33° 22' S, 151° 16' E</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aphytis chrysomphali</em></td>
<td><em>A. chrysomphali</em> LNSW</td>
<td><em>Aonidiella citrina</em></td>
<td>Washington navel orange</td>
<td>Lower Portland, NSW</td>
<td>33° 26' S, 150° 52' E</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aphytis melinus</em></td>
<td><em>A. melinus</em> CaNSW</td>
<td><em>Aonidiella aurantii</em></td>
<td>sweet orange</td>
<td>Castlereagh, NSW</td>
<td>33° 40' S, 150° 40' E</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aphytis lingnanensis</em></td>
<td><em>A. lingnanensis</em> culture1</td>
<td><em>Aspidiotus nerii</em></td>
<td>pumpkin (culture)</td>
<td>commercial insectary</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aphytis lingnanensis</em></td>
<td><em>A. lingnanensis</em> culture2</td>
<td><em>Aspidiotus nerii</em></td>
<td>pumpkin (culture)</td>
<td>commercial insectary</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aphytis melinus</em></td>
<td><em>A. melinus</em> WA2</td>
<td><em>Aonidiella aurantii</em></td>
<td>Valencia orange</td>
<td>Perth, WA</td>
<td>31° 57' S, 115° 51' E</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aphytis melinus</em></td>
<td><em>A. melinus</em> WA1</td>
<td><em>Aonidiella aurantii</em></td>
<td>Valencia orange</td>
<td>Gingin, WA</td>
<td>31° 20' S, 115° 54' E</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aphytis melinus</em></td>
<td><em>A. melinus</em> WA3</td>
<td><em>Aonidiella aurantii</em></td>
<td>Valencia orange</td>
<td>Gingin, WA</td>
<td>31° 20' S, 115° 54' E</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aphytis melinus</em></td>
<td><em>A. melinus</em> culture1</td>
<td><em>Aspidiotus nerii</em></td>
<td>commercial insectary</td>
<td>commercial insectary</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aphytis melinus</em></td>
<td><em>A. melinus</em> culture2</td>
<td><em>Aspidiotus nerii</em></td>
<td>commercial insectary</td>
<td>commercial insectary</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 8.5. Host scale, host plants and locations from which *Comperiella bifasciata* was collected for molecular studies.

<table>
<thead>
<tr>
<th>Accession coder</th>
<th>Host scale</th>
<th>Host plant</th>
<th>Location</th>
<th>Latitude and longitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>CbS1</td>
<td><em>Aonidiella aurantii</em></td>
<td>Washington navel orange</td>
<td>Somersby, NSW</td>
<td>33° 22' S, 151° 16' E</td>
</tr>
<tr>
<td>CbG1</td>
<td><em>Aonidiella aurantii</em></td>
<td>Valencia orange</td>
<td>Griffith, NSW</td>
<td>34° 17' S, 146° 02' E</td>
</tr>
<tr>
<td>CbWA1</td>
<td><em>Aonidiella aurantii</em></td>
<td>Valencia orange</td>
<td>Gingin, WA</td>
<td>31° 20' S, 115° 54' E</td>
</tr>
<tr>
<td>CbL1</td>
<td><em>Aonidiella citrina</em></td>
<td>Washington navel orange</td>
<td>Lower Portland, NSW</td>
<td>33° 26' S, 150° 52' E</td>
</tr>
<tr>
<td>CbCa1</td>
<td><em>Aonidiella citrina</em></td>
<td>Washington navel orange</td>
<td>Castlereagh, NSW</td>
<td>33° 40' S, 150° 40' E</td>
</tr>
<tr>
<td>CbG2</td>
<td><em>Aonidiella citrina</em></td>
<td>Valencia orange</td>
<td>Griffith, NSW</td>
<td>34° 17' S, 146° 02' E</td>
</tr>
</tbody>
</table>
Table 8.6. Host scales, host plants and locations from which *Encarsia* species were collected for molecular studies.

<table>
<thead>
<tr>
<th>Species</th>
<th>Accession code</th>
<th>Host scale</th>
<th>Host plant</th>
<th>Location</th>
<th>Latitude and longitude</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Encarsia citrina</em></td>
<td><em>E. citrina</em> SNSW1</td>
<td><em>Aonidiella aurantii</em></td>
<td>Valencia orange</td>
<td>Somersby, NSW</td>
<td>33° 22' S, 151° 16' E</td>
</tr>
<tr>
<td><em>Encarsia citrina</em></td>
<td><em>E. citrina</em> CaNSW1</td>
<td><em>Aonidiella citrina</em></td>
<td>Washington navel orange</td>
<td>Lower Portland, NSW</td>
<td>33° 26' S, 150° 52' E</td>
</tr>
<tr>
<td><em>Encarsia citrina</em></td>
<td><em>E. citrina</em> LNSW1</td>
<td><em>Aonidiella citrina</em></td>
<td>Washington navel orange</td>
<td>Lower Portland, NSW</td>
<td>33° 26' S, 150° 52' E</td>
</tr>
<tr>
<td><em>Encarsia citrina</em></td>
<td><em>E. citrina</em> RNSW1EcR1</td>
<td><em>Aonidiella citrina</em></td>
<td>Washington navel orange</td>
<td>Richmond, NSW</td>
<td>33° 36' S, 150° 44' E</td>
</tr>
<tr>
<td><em>Encarsia citrina</em></td>
<td><em>E. citrina</em> CoNSW1</td>
<td><em>Aonidiella aurantii</em></td>
<td>Washington navel orange</td>
<td>Cornwallis, NSW</td>
<td>33° 35' S, 150° 49' E</td>
</tr>
<tr>
<td><em>Encarsia citrina</em></td>
<td><em>E. citrina</em> CoNSW2</td>
<td><em>Lepidosaphes beckii</em></td>
<td>Washington navel orange</td>
<td>Cornwallis, NSW</td>
<td>33° 35' S, 150° 49' E</td>
</tr>
<tr>
<td><em>Encarsia citrina</em></td>
<td><em>E. citrina</em> CoNSW2</td>
<td><em>Lepidosaphes beckii</em></td>
<td>Valencia orange</td>
<td>Castlereagh, NSW</td>
<td>33° 40' S, 150° 40' E</td>
</tr>
<tr>
<td><em>Encarsia perniciosi</em></td>
<td><em>E. perniciosi</em> CaNSW2</td>
<td><em>Aonidiella citrina</em></td>
<td>Washington navel orange</td>
<td>Castlereagh, NSW</td>
<td>33° 40' S, 150° 40' E</td>
</tr>
<tr>
<td><em>Encarsia perniciosi</em></td>
<td><em>E. perniciosi</em> CoNSW2</td>
<td><em>Aonidiella aurantii</em></td>
<td>Washington navel orange</td>
<td>Cornwallis, NSW</td>
<td>33° 35' S, 150° 49' E</td>
</tr>
<tr>
<td><em>Encarsia perniciosi</em></td>
<td><em>E. perniciosi</em> CoNSW 2</td>
<td><em>Unaspis citri</em></td>
<td>Washington navel orange</td>
<td>Cornwallis, NSW</td>
<td>33° 35' S, 150° 49' E</td>
</tr>
</tbody>
</table>
8.2.2.2. DNA extraction

8.2.2.2.1. Specimens

Specimens used for DNA extraction were either fresh or stored in 100% ethanol at 20°C. Single specimens were used for each extraction. Third instar virgin and mated female red scale and yellow scale were used. In order to compare the morphology and molecular results for individual specimens, some red scale and yellow scale specimens were photographed and their pygidium removed using a sterilised scalpel blade before the remaining body was used for DNA extraction. Pupae or adults of the *Aphytis* species, and adult *Comperiella bifasciata*, *Encarsia citrina* and *Encarsia perniciosi* were used. The ant workers were used. DNA extraction was carried out using QIAamp DNA Micro Kit (QIAGEN Pty Ltd, Australia, Doncaster, Victoria, Australia).

8.2.2.2.2. DNA amplification

The mitochondrial cytochrome oxidase subunit I (*COI*) gene, the nuclear, protein-coding gene, elongation factor (EF), and the 28S ribosomal RNA (28S) of the scales were amplified using the primers listed in Table 8.8. The 28S, internal transcribed spacer (ITS) regions of *Aphytis*, the 28S and *COI* of *Encarsia* species, and the 28S and *COI* genes of *Comperiella bifasciata* were amplified using primers listed in Table 8.9.
For the ant specimens, the COI gene was amplified using primers CO1f (LCO1490) and CO1r (HCO2198) as listed in Table 8.8.

**Table 8.8.** Primers used to amplify different gene regions for red scale and yellow scale in this study

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5’ → 3’)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO1f (LCO1490)</td>
<td>GGTCAAACAAATCATAAAGATATTGG</td>
<td>Folmer et al. (1994)</td>
</tr>
<tr>
<td>CO1r (HCO2198)</td>
<td>TAAACTTCAGGGTGACCAAAAAATCA</td>
<td>Folmer et al. (1994)</td>
</tr>
<tr>
<td>EF1 (EF1a)</td>
<td>GATGCTCCGGGACAYAGA</td>
<td>Morse &amp; Normark (2006)</td>
</tr>
<tr>
<td>EF2r (EF2)</td>
<td>ATGTGAGCGGTGTGGCAATCCAA</td>
<td>Morse &amp; Normark (2006)</td>
</tr>
<tr>
<td>28Sf rDNA (28B)</td>
<td>TCGGAAGGAACCAGCTACTA</td>
<td>Morse &amp; Normark (2006)</td>
</tr>
<tr>
<td>28Sr rDNA (S3660)</td>
<td>GAGAGTTMAASAGTACGTAAC</td>
<td>Morse &amp; Normark (2006)</td>
</tr>
</tbody>
</table>

**Table 8.9.** Primers used to amplify different gene regions for parasitoids of red scale and yellow scale, and their bacterial symbionts in this study.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5’ → 3’)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Encarsia spp. &amp; Aphytis spp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28S rDNA (28S D-2F)</td>
<td>C GTTGTCCTTGA TATG CAGG</td>
<td>Campbell et al. (1993)</td>
</tr>
<tr>
<td>28S rDNA (28S D-2R)</td>
<td>TGGGTCGGTGTTTCAAGACGG</td>
<td>Campbell et al. (1993)</td>
</tr>
<tr>
<td>CO1 (C1-J-1718)</td>
<td>GAGGATTGTGGAAATGGATTAGTCC</td>
<td>Simon et al. (1994)</td>
</tr>
<tr>
<td>CO1 (C1-N-2191)</td>
<td>CCCGGTAAAAATATAAATACCTTC</td>
<td>Simon et al. (1994)</td>
</tr>
<tr>
<td>ITS (5.8S-F)</td>
<td>TGTAAGCTGCAGGACACATGAAC</td>
<td>de León et al. (2010)</td>
</tr>
<tr>
<td>ITS (28S-R)</td>
<td>ATGCTTAATTTAGGGGTA</td>
<td>de León et al. (2010)</td>
</tr>
<tr>
<td>Comperiella bifasciata</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CO1 (C1-J-1718)</td>
<td>GAGGATTGTGGAAATGGATTAGTCC</td>
<td>de León et al. (2010)</td>
</tr>
<tr>
<td>CO1 (C1-N-2191)</td>
<td>CCCGGTAAAAATATAAATACCTTC</td>
<td>de León et al. (2010)</td>
</tr>
<tr>
<td>28S rDNA (D2-3551F)</td>
<td>CGTGTTGCTTGTAGTGCAGC</td>
<td>Gillespie et al. (2002)</td>
</tr>
<tr>
<td>28S rDNA (D2-4057R)</td>
<td>TCAAGACGGGTCCCTGAAA GT</td>
<td>Gillespie et al. (2002)</td>
</tr>
<tr>
<td>‘Candidatus’ Cardinium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16S rDNA (ChF)</td>
<td>TACTGTAAGAATAAGCACCACGT</td>
<td>Zchori-Fein &amp; Perlman (2004)</td>
</tr>
<tr>
<td>16S rDNA (ChR)</td>
<td>GTGGATACCTTAACGCATTTCG</td>
<td>Zchori-Fein &amp; Perlman (2004)</td>
</tr>
<tr>
<td>Wolbachia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>wspf (wsp 81F)</td>
<td>TGGTCCAATAAGTGATGAAGAAGA</td>
<td>Zhou et al. (1998)</td>
</tr>
<tr>
<td>waspr (wsp 691R)</td>
<td>AAAAATCAAACGCCTACTCCA</td>
<td>Zhou et al. (1998)</td>
</tr>
<tr>
<td>fts2f</td>
<td>GTTGTCGCAAAATACCGATGC</td>
<td>Zhou et al. (1998)</td>
</tr>
<tr>
<td>ftsZr</td>
<td>CTTAAGTAAGCTGGTATATC</td>
<td>Zhou et al. (1998)</td>
</tr>
</tbody>
</table>
8.2.2.2.3. PCR component

For the scales, the amplification reaction (20 µL) for COI contained 4 µL 5× reaction colour buffer (GoTaq® Flexi DNA Polymerase, Promega Corporation, Madison, Wisconsin, United States of America); 2.8 µL MgCl₂ (25 mM) (Promega), 0.4 µL dNTPs (1 mM) (Promega); 0.8 µL forward primer (20mM); 0.8 µL reverse primer (20 mM); 0.2 µL Taq polymerase (0.05 U/µL); 1 µL DNA template; 10 µL sterilised milliQ water. The amplification reaction (20 µL) for the EF and 28S genes of the scale insects contained 4 µL 5× reaction colour buffer (GoTaq® Flexi DNA Polymerase, Promega Corporation, Madison, Wisconsin, United States of America); 2 µL MgCl₂ (25 mM) (Promega), 0.4 µL dNTPs (1mM) (Promega); 0.8 µL forward primer (20 mM); 0.8 µL reverse primer (20 mM); 0.1 µL Taq polymerase (0.05 U/µL); 1 µL DNA template; 10.9 µL sterilised milliQ water.

For the parasitoids, the amplification reaction (25 µL) for COI contained 5 µL 5× reaction colour buffer (GoTaq® Flexi DNA Polymerase, Promega Corporation, Madison, Wisconsin, United States of America); 1.6 µL MgCl₂ (25 mM) (Promega), 0.5 µL dNTPs (1 mM) (Promega); 0.5 µL forward primer (20 mM); 0.5 µL reverse primer (20 mM); 0.1 µL Taq polymerase (0.05 U/µL); 1 µL DNA template; 18.4 µL sterilised milliQ water. The amplification reaction (20 µL) for the ITS region and 28S rDNA gene of the scale insects contained 3 µL 5× reaction colour buffer (GoTaq® Flexi DNA Polymerase, Promega Corporation, Madison, Wisconsin, United States of America); 1.6 µL MgCl₂ (25 mM) (Promega), 0.5 µL dNTPs (1 mM) (Promega); 1 µL forward primer (20 mM); 1 µL reverse primer (20 mM); 0.1 µL Taq polymerase (0.05 U/µL); 1 µL DNA template; 16.8 µL sterilised milliQ water.

The following PCR conditions were used for the bacterial symbionts. For the 16S rDNA gene of ‘Candidatus Cardinium’: an initial denaturation for 2 min at 95°C; 30 cycles of 30 s at 95°C for DNA denaturation, 30 s at 57°C for primer annealing, and 30 s at 72°C for primer extension; a final extension of 5 min at 72°C (Zchori-Fein & Perlman 2004). For the wsp, ftsZ gene regions from Wolbachia: an initial denaturation for 2 min at 94°C; 35 cycles of 1 min at 94°C for DNA denaturation, 1 min at 55°C for primer annealing, and 1 min at 72°C for primer extension; a final extension of 5 min at 72°C (Zhou et al. 1998).
8.2.2.2.4. PCR conditions

The following PCR conditions were used for the scale insects. For COI, the conditions were: an initial denaturation for 5 min at 95°C; 33 cycles of 45 s at 95°C for DNA denaturation, 90 s at 50°C for primer annealing, and 120 s at 72°C for primer extension; a final extension of 5 min at 72°C. For the EF and 28S genes a touch-down procedure was used in which an initial annealing temperature of 58°C was decreased by 2°C every three cycles until a final temperature of 42°C was reached and held for 18 cycles. The other steps included an initial denaturation for 5 min at 95°C; denaturation at 95°C for 30 s, extensions at 72°C for 2 min and a final extension of 5 min at 72°C (Morse & Normark 2006).

The following PCR conditions were used for the parasitoids. For COI: an initial denaturation for 5 min at 95°C; 33 cycles of 45 s at 95°C for DNA denaturation, 90 s at 58°C for primer annealing, and 120 s at 72°C for primer extension; a final extension of 5 min at 72°C (de León et al. 2010). For the ITS region: an initial denaturation for 3 min at 94°C; 45 cycles of 20 s at 95°C for DNA denaturation, 20 s at 45°C for primer annealing, and 60 s at 72°C for primer extension; a final extension of 5 min at 72°C (de León et al. 2010). For the 28S rRNA gene for Aphytis species and Encarsia species: an initial denaturation for 5 min at 95°C; 33 cycles of 45 s at 95°C for DNA denaturation, 90 s at 50°C for primer annealing, and 120 s at 72°C for primer extension; a final extension of 5 min at 72°C (Campbell 1993).

Thermocycling was performed using a Dyad Peltier thermal cycler (Bio-Rad Laboratories Inc., Berkeley, California, United States of America).

8.2.2.2.5. Gel electrophoresis and DNA sequencing

PCR products were loaded into 1% agarose gels containing 0.5 μg/mL ethidium bromide. A 100 base pair DNA ladder (Promega) was used as a size marker. Electrophoresis was conducted at 100 V for 40 to 45 min. Bands in gels were visualised and photographed using UV transillumination in a GelDoc 2000 (Bio-rad Laboratories Inc., Berkeley, California, United States of America).

DNA purification of amplicons was conducted using either the Wizard® Genomic DNA Purification Kit (Promega) following the manufacturer’s instructions or EXoSAP (Exonuclease I (New England Biolabs® Inc., Ipswich, Massachusetts, United States of America).
America) – shrimp alkaline phosphatase (Promega)). For the latter, a volume of 2 µL of 5× EXoSAP mix (containing 2.5 µL exonuclease I (20 U/µL), 25 µL shrimp alkaline phosphatase (1 U/µL) in 172.5 µL Milli-Q water) was added into each PCR product sample. The sample was then incubated at 37°C for 30 min and then 95°C for 5 min.

Purified PCR products were sequenced by Macrogen Inc., Seoul, Korea using an ABI 3700 DNA sequencer.

**8.2.2.2.6. Phylogenetic analysis**

The forward and reverse DNA sequences of both genes were assembled, edited and aligned using DNA Sequencher™ 4.8 (Gene Codes Corporation Michigan, United States of America).

Three phylogenetic analysis methods were used, maximum parsimony and maximum likelihood using PAUP® 4.0 (Phylogenetic Analysis Using Parsimony version 4.0 beta 10 WIN) (Swofford 2001), and Bayesian inference using MrBayes version 3.0 for Windows (Hall 2008). Parsimony analysis was performed using tree-bisection-reconnection branch swapping with a heuristic search with 1000 bootstrap replicates. For the maximum likelihood analyses, and the Bayesian inference, the evolutionary model was selected using MrModeltest 2.3 (Nylander 2004). Genetic distances between taxa were estimated using p-distances calculated using MEGA (Version 5) (Tamura et al. 2011).

**8.3. Results and discussion**

**8.3.1. Aonidiella auranti and Aonidiella citrina**

**8.3.1.1. Morphology and habitat**

The general appearance of the scales and the morphology of their pygidia (the posterior body region or caudal segment of certain insects and other invertebrates) are discussed. Habitats of the scales within orchards, blocks of trees and canopies are also compared and discussed.

My descriptions in Table 8.10 and photographs in Figs 8.1–8.3 indicated that differences in the shape and colour of the scale cover and habitat within trees can be used to distinguish the two species in the field. My observations were similar with
those reported by Quayle (1911a, 1938), Hely (1955), Hely et al. (1982) and Smith et al. (1997). Even though chlorosis resulting from feeding by yellow scale on green fruit was noticeable in contrast to chlorosis related to feeding by red scale on green fruit being uncommon, red scale is more destructive than yellow scale, because it colonises all above ground surfaces of citrus trees whereas yellow scale populations are restricted to leaves and, less commonly, fruit.

Table 8.10. Appearance and habitat differences between red scale and yellow scale in this study.

<table>
<thead>
<tr>
<th>Character</th>
<th>Red scale</th>
<th>Yellow scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour of scale cover</td>
<td>• red-brown</td>
<td>• pale yellow</td>
</tr>
<tr>
<td>Colour of scale body</td>
<td>• opaque white or dark yellow to brown</td>
<td>• lemon yellow</td>
</tr>
<tr>
<td>Shape</td>
<td>• convex</td>
<td>• relatively flat, slightly larger</td>
</tr>
<tr>
<td>Scale cover thickness</td>
<td>• thicker, relatively opaque</td>
<td>• thinner, relatively transparent, easily seen through</td>
</tr>
<tr>
<td>and transparency</td>
<td>• preference for sunlight, higher densities adjacent to windbreaks,</td>
<td>• more common in shaded and dense canopies within orchard; more</td>
</tr>
<tr>
<td>Habitat within orchards/blocks</td>
<td>common on both young and mature trees, more so on young trees</td>
<td>common on mature trees</td>
</tr>
<tr>
<td>Habitat within trees</td>
<td>• common on outer and upper parts of trees, more abundant on the recently</td>
<td>• commonly found in parts of trees not exposed to direct sunlight such as</td>
</tr>
<tr>
<td></td>
<td>mature leaves; also common, with respect to yellow scale, on twigs and</td>
<td>lower inner canopies, lower side of leaves; more abundant on old leaves</td>
</tr>
<tr>
<td></td>
<td>branches</td>
<td>• found on outer parts on trees adjoining trees</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• does not colonise twigs and branches</td>
</tr>
<tr>
<td>Distribution on fruit and leaves</td>
<td>• common on side of fruit exposed to sunlight, and on upper surfaces of leaves</td>
<td>• more commonly found between touching surfaces of fruit and/or leaves, and on</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• lower surfaces of leaves</td>
</tr>
<tr>
<td>Chlorosis</td>
<td>• chlorosis around scales on green fruit uncommon</td>
<td>• chlorosis around scales on green fruit noticeable</td>
</tr>
</tbody>
</table>
Figure 8.1. Mixed stages of red scale (left) and yellow scale (right) on immature sweet orange fruit.

Figure 8.2. Virgin female red scale (left) and virgin female yellow scale (right) on immature sweet orange fruit.

Figure 8.3. Mated female red scale (left) and mated female yellow scale (right) on an orange leaf and an immature sweet orange fruit, respectively.
I examined 50 third instar virgin and mated female red scale, and 70 third instar virgin and mated female yellow scale. The extent of differences in the pygidial, prevulvar scleroses of the scales is evident in Fig. 8.4. In red scale, the scleroses were visible, though variable in extent, in all specimens I cleared and examined. Variation in the extent of pygidial, prevulvar scleroses and apophyses is common in red scale (Ferris 1938), and instances where the scleroses are absent (Ferris 1938) probably account for uncertainty about the identity of the scale in some instances, as noted by DeBach et al. (1978). In yellow scale, the scleroses were absent in all specimens.

Figure 8.4. Red scale pygidium (left) and yellow scale pygidium (right). Prevulvar scleroses are evident anterior to the prevulva scleroses in red scale (lower left) but are absent in yellow scale (lower right).

8.3.1.2. Molecular studies

I successfully amplified sequences of the 28S rDNA, EF and COI genes of red scale and yellow scale and detected differences between sequences of the two scales. The sources of scale specimens are shown in Tables 8.1–8.3. The genetic analysis was undertaken using three methods: maximum likelihood, maximum parsimony and Bayesian inference. Analysis results are present as following order for each gene: (1) maximum
likelihood; (2) maximum parsimony; and (3) Bayesian inference. (For the Bayesian inference trees, names of taxa are not italicised.)

My 28S rDNA gene sequences comprised 665 base pairs. Among the taxa used as out-groups and the in-groups, 604 characters were constant, 24 characters were parsimony-uninformative and 37 characters were parsimony-informative. Phylogenetic trees derived from aligned sequences of this gene using the three methods are shown in Figs 8.5–8.7. The maximum likelihood and Bayesian inference analyses were performed using the best-fit model, HKY+I (Hasegawa-Kishino-Yano) (Hasegawa et al. 1985, Yang 1996) selected by AIC (Akaike’s information criterion) in MrModeltest 2.3. The settings for the HKY+I model in the maximum likelihood analysis were: Lset Base=(0.2042 0.2971 0.3045) Nst=2 TRatio=6.1064 Rates=equal Pinvar=0.1846. The the settings for the HKY+I model for use in MrBayes were: Lset nst=2 rates=propinv;Prset statefreqpr=dirichlet(1,1,1,1).

The phylogenetic trees derived from the maximum likelihood, maximum parsimony and Bayesian inference analyses were similar. With the exception of Aonidiella citrina MiVic, red scale and yellow scale accessions were in a clade separate from Chrysomphalus aonidium and Aspidiotus nerii. Within the clade, accessions of Aonidiella citrina formed an unresolved, basal polytomy, whereas accessions of Aonidiella aurantii formed a subclade with 86 and 87% bootstrap support for maximum likelihood and maximum parsimony, respectively; the posterior probability from the Bayesian inference was 100%. Within the subclade, further subdivision was evident, with a set of 3 and a set of 9 accessions forming two separate groups (Groups 1 and 2); the remainder of the Aonidiella aurantii accessions (Group 3) formed an unresolved, basal polytomy. In the maximum parsimony and maximum likelihood analyses, Aonidiella citrina MiVic was sister to the red and yellow scale clade. However, the separation from the other accessions only has weak bootstrap support and, in the Bayesian analysis, this accession formed part of the basal polytomy.

Within the groupings found in the red scale subclade, the associations were not related to the locations from which the specimens were collected. For example, the specimens in Group 1 sequenced in this study came from Universitas Gadjah Mada, Yogyakarta, Indonesia (A. aurantii IND1) and Richmond, New South Wales (A. aurantii RNSW1), and their sequences were identical with that of a red scale sequence DQ145289 from California taken from the molecular databases. Also, accessions of scale from New South Wales were found in all three groups.
Figure 8.5. 50% majority-rule bootstrap consensus tree of the 28S rDNA region of accessions of red scale and yellow scale derived from maximum likelihood analysis. *Parlatoria pergandii* was used as the out-group and bootstrap values are provided as percentages from 1000 replications.
Figure 8.6. 50% majority-rule bootstrap consensus tree of the 28S rDNA region of accessions of red scale and yellow scale derived from maximum parsimony analysis. *Parlatoria pergandii* was used as the out-group and bootstrap values are provided as percentages from 1000 replications.
Figure 8.7. Bayesian inference tree resulting from analysis of the 28S rDNA region of red scale and yellow scale. *Parlatoria pergandii* was used as the out-group and posterior probabilities are shown above and below each branch.
The 28S sequences of red scale and yellow scale were more closely related to *Chrysomphalus dictyospermi* than *Aspidiotus nerii*. My sequences of *Aspidiotus nerii* (parthenogenetic form) from cultures were identical with the molecular database sequence, GU213889, of *Aspidiotus nerii* from Italy (Andersen et al. 2010).

My elongation factor gene sequences comprised 828 base pairs. Among the taxa used as the out-group and in-groups, 625 characters were constant, 109 variable characters were parsimony-uninformative and 94 characters were parsimony-informative. Phylogenetic trees derived from maximum likelihood, maximum parsimony and Bayesian analysis methods are shown in Figs 8.8–8.10. The model of evolution SYM+G (Symmetrical Model plus Gamma) (Zharkikh 1994) selected by AIC (Akaike’s information criterion) in MrModeltest 2.3. The likelihood settings for the SYM+G model were: Lset Base=equal Nst=6 Rmat=(0.5141 5.8481 4.4106 0.8226 11.1106) Rates=gamma Shape=0.2139 and for MrBayes were Lset nst=6 rates=gamma; Prset statefreqpr=fixed (equal).

There was variation among the topologies of the results obtained from maximum likelihood, maximum parsimony and Bayesian analyses (Figs 8.8–8.10). In all three trees, the red scale accessions fell in a distinct clade with 82 and 93% bootstrap support for maximum likelihood and maximum parsimony, respectively, and an 88% posterior probability for the Bayesian inference. In the maximum likelihood and Bayesian trees, accessions of yellow scale were also in a distinct clade with 67% bootstrap support and posterior probability of 87%. In the maximum parsimony analysis, three accessions (*A. citrina* GNSW1, *A. citrina* RNSW and *A. citrina* RNSW1) formed an unresolved, basal polytomy and, separate from this, was a clade containing the remainder of the yellow scale accessions and a further clade containing the red scale accessions.

In the red scale clade, the elongation factor sequences of red scale from Universitas Gadjah Mada, Indonesia and GenBank accession DQ145401 from California were identical. The accession *A. aurantii* BNSW from Barham was more closely related to these two accessions, and all three formed a subclade in both maximum likelihood and Bayesian analyses. In the maximum likelihood analysis, the red scale accessions, *A. aurantii* RNSW2 and *A. aurantii* RNSW7, were identical and grouped with 53% bootstrap support. The remainder of the red scale clade formed an unresolved, basal polytomy. In Bayesian analysis, accession *A. aurantii* RNSW2 grouped with *A.
aurantii MuVic. This group was separated to the remainder of the red scale clade with a posterior probability of 79%.

The accession *A. aurantii* LSA from South Australia did not differ from *A. aurantii* RNSW5 from Richmond and *A. aurantii* BNSW1 from Barham. There was no variation among specimens from New South Wales. In the yellow scale clade, the Bayesian interference analysis showed the variation, though limited, between specimens from Richmond and Griffith, New South Wales and those from other locations (Fig. 8.10).

![Diagram](image_url)

**Figure 8.8.** 50% majority-rule bootstrap consensus tree of the elongation factor region of accessions of red scale and yellow scale derived from maximum likelihood analysis. *Parlatoria oleae* was used as the out-group and bootstrap values are provided as percentages from 1000 replications.
Figure 8.9. 50% majority-rule bootstrap consensus tree of the elongation factor region of accessions of red scale and yellow scale derived from maximum parsimony analysis. *Parlatoria oleae* was used as the out-group and bootstrap values are provided as percentages from 1000 replications.
Figure 8.10. Bayesian inference tree resulting from analysis of the elongation factor region of red scale and yellow scale. *Parlatoria oleae* was used as the out-group and posterior probabilities are shown below each branch.

The sequences of the red scale COI gene comprised 590 base pairs. Among the taxa used as the out-group and the in-groups, 449 characters were constant, 69 characters were parsimony-uninformative and 72 were parsimony-informative. Phylogenetic trees derived from maximum parsimony, likelihood and Bayesian inference analyses are shown in Figs 8.11–8.13. The maximum likelihood and Bayesian inference analysis was performed using the best-fit model, GTR+G (General Time Reversible plus Gamma) (Rogers 2001) selected by hLRTs (Hierarchical Likelihood Ratio Tests) in MrModeltest 2.3. Likelihood settings for the best-fit model (GTR+G) were: Lset Base=(0.4092 0.1278 0.0617) Nst=6 Rmat=(113370.1172 265158.2500 170059.1094 167936.7344 1996436.7500) Rates=gamma Shape=0.2386 Pinvar=0]. For MrBayes, the settings were: Lset nst=6 rates=gamma; Prset statefreqpr=dirichlet(1,1,1,1).

There were no available sequences of the COI gene for yellow scale in the molecular databases. Trees derived from maximum likelihood, maximum parsimony and
Bayesian analysis were similar. In the trees from all three types of analyses, the accessions of red scale formed a clade separate from those of yellow scale. The two sequences of red scale from Richmond were identical with molecular database sequence, HM474068, of red scale from California (Park et al. 2011). Red scale and yellowed scale clades were supported by 94–100% bootstrap values. My result showed that the variation COI sequences between red scale and yellow scale occurred in 36–37 base pairs. Sequences of the 12 yellow scale samples collected on the Central Coast of New South Wales, from inland New South Wales and from Mildura, Victoria were identical, with only minor variation occurring in the sequence of *A. citrina* CaNSW2 from Castlereagh. In the Bayesian analysis, all accessions of yellow scale formed a single clade. However, with the maximum likelihood and maximum parsimony analyses, accession CaNSW2 was sister to all other sequences of this scale, although the separation of this accession only has weak bootstrap support.

Figure 8.11. 50% majority-rule bootstrap consensus tree of the COI region of accessions of red scale and yellow scale derived from maximum likelihood analysis. *Parlatoria ziziphi* was used as the out-group and bootstrap values are provided as percentages from 1000 replications.
Figure 8.12. 50% majority-rule bootstrap consensus tree of the COI region of accessions of red scale and yellow scale derived from maximum parsimony analysis. *Parlatoria zizi phi* was used as the out-group and bootstrap values are provided as percentages from 1000 replications.
Figure 8.13. Bayesian inference tree resulting from analysis of the COI region of red scale and yellow scale. *Parlatoria ziziphi* was used as the out-group and posterior probabilities are shown above or below each branch.

My molecular results confirmed the identities of both red scale and yellow scale by showing that they are genetically distinct. The COI gene showed more genetic divergence between red scale and yellow scale (difference occurred within 36–37 base pairs) compared to the elongation factor (7–9 base pairs) and the 28S rDNA (3 base pairs) regions. Sequences of both the elongation factor and 28S regions showed no obvious association between their placement in clades or subclades and the locations within Australia from which they were collected. Specimens from Indonesia were identical with the molecular database sequences of red scale collected in California for both the elongation factor and 28S genes. There were no genetic differences among red scale specimens from Richmond and Barham and the red scale specimen from South Australia, where Brewer (1971) recorded high levels of encapsulation of immature stages the red scale race of *Comperiella bifasciata* by the scale.
My sequences of both the 28S and EF regions of red scale from Universitas Gadjah Mada, Indonesia, and from California (molecular database) were identical. The 28S sequence of one specimen from Richmond grouped with these two accessions.

My morphological and molecular results have allowed red and yellow scale accessions from Australia to be categorically identified. As a consequence, I have been able to show that that yellow scale occurred on both Valencia orange and Washington navel orange trees in my study orchards the on the Central Coast of New South Wales, in Griffith, New South Wales, and Mildura, Victoria. It was more common in 2010–2011 than in 2008–2009 and 2009–2010, and it was more common in the orchards in the Hawkesbury River area, particularly in the orchards at Cornwallis and Lower Portland than on the Somersby Plateau (see Chapter 5). According to Beattie (pers. comm., 2010), yellow scale appeared to be more common in orchards on the Central Coast of New South Wales in 2010-2011 than in any season since 1975. I also confirmed the presence of yellow scale on grapefruit leaves and fruit in an orchard Griffith, New South Wales, in spring 2006 or early 2007, where it was first noticed by Rob Wepppler (Riverina IPM, pers. comm., 2012). It has not been previously recorded in the Riverina (MIA) (Hely et al. 1982, Smith et al. 1997).

8.3.2. Parasitoids

8.3.2.1. Aphytis species

8.3.2.1.1. Morphology

The majority of Aphytis pupae in scale samples from my study orchards were readily distinguished (Fig. 8.14). However, as noted in the introduction to this chapter, variation in pupal pigmentation indicated the presence, in addition to Aphytis chrysomphali, of both Aphytis melinus and Aphytis lingnanensis. Fig. 8.15 shows the variation among the Aphytis melinus pupae. Pupae in Figs 8.15a & b appeared to be different to published accounts of pigmentation in Aphytis melinus pupae, whereas pigmentation of the pupa in Fig. 8.15c resembled published accounts (DeBach 1959, Rosen & DeBach 1979). Fig. 8.16 is representative of the pupae I occasionally observed under covers of red scale. These pupae, although resembling those of Aphytis melinus, appeared to belong to an unidentified and previously unrecorded aphelinid species. All photographs in Figs 8.15 & 8.16 were taken before DNA was extracted from the pupae for molecular analysis.
Figure 8.14. *Aphytis lingnanensis* pupa (top, left), adult (top, right); *Aphytis melinus* pupa (middle, left) adult (middle, right); *Aphytis chrysomphali* pupa (bottom left), adult (bottom, right).
Figure 8.15. Variation in pupal pigmentation in *Aphytis melinus*: *A. melinus* WA1 (above, left), *A. melinus* KNSW2 (top, right), *A. melinus* WA2 (bottom left), *A. melinus* KNSW3 (bottom, right).

Figure 8.16. Pigmentation in two unidentified aphelinid pupae parasitising red scale at Kulnura.
**8.3.2.1.2. Molecular studies**

I successfully amplified the 28S rDNA and ITS regions of nine *Aphytis melinus*, two *Aphytis lingnanensis* and three *Aphytis chrysomphali* pupae, and two pupae of the unidentified parasitoid. The sources of these pupae are listed in Table 8.5. The 28S rDNA sequences of each species comprised 443 base pairs. Among taxa used as in-groups and the out-group, 282 characters were constant, 23 variable characters were parsimony-uninformative and 138 were parsimony-informative.

The maximum likelihood and Bayesian analyses were performed using the GTR+G evolution model (General Time Reversible plus Gamma) selected by hLRTs in MrModeltest 2.3. The maximum likelihood settings for GTR+G were: Lset Base=(0.1502 0.2782 0.3210) Nst=6 Rmat=(0.3867 3.7283 4.6338 0.2402 5.2995) Rates=gamma Shape=0.5025 Pinvar=0;]. The settings for the Bayesian analysis were: Lset nst=6 rates=gamma; Prset statefreqpr=dirichlet (1,1,1,1).

The phylogenetic trees derived from maximum likelihood, maximum parsimony and Bayesian analysis of the 28S gene were identical. All accessions of *Aphytis* were in one clade and the unidentified *Aphelinus* accessions grouped in a second clade with the *Aphelinus* accessions found in molecular databases. Within the *Aphytis* clade, accessions of *Aphytis chrysomphali* formed a subclade as did accessions of *Aphytis lingnanensis*. The sequences of *Aphytis lingnanensis* and *Aphytis chrysomphali* were identical to molecular database accession, AY635333, for *Aphytis lingnanensis* from south China⁴⁸, and accession, AY635330, for *Aphytis chrysomphali* (origin was not mentioned) (Kim & Heraty, unpublished direct submissions), respectively.

Accessions of *Aphytis melinus*, however, formed two separate subclades (subclade 1 and subclade 2). Subclade 1 comprised four accessions: KNSW1, KNSW3, WA2 and AY635342 of *Aphytis melinus* from China⁴⁹. Subclade 2 comprised five accessions: culture 1, culture 2 (both derived from material imported from the Indian Subcontinent via California in 1961⁵⁰), KNSW2, WA1 and CaNSW1, the latter being sister to the other four. The separation of the two *Aphytis melinus* subclades had high bootstrap support in both types of analysis. Separation of the *Aphytis melinus* accessions into the

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⁴⁸ Sequence derived from material collected by Robert Luck in Guangdong, China (Gillispie et al. 2005, Supplementary Material 1: http://hymenoptera.tamu.edu/rna/models/cha1/data/sup/Supp_Mat_1_487.R1.pdf).

⁴⁹ Sequence derived from material collected by Robert Luck in Guangdong, China (Gillispie et al. 2005, Supplementary Material 1: http://hymenoptera.tamu.edu/rna/models/cha1/data/sup/Supp_Mat_1_487.R1.pdf).

⁵⁰ Furness et al. (1983).
two subclades in my results from 28S rDNA was not associated with their place of collection, as accessions collected from New South Wales and Western Australia were found in both major subclades (Figs 8.17–8.19). As shown in Fig. 8.15, it appeared to be an association between pupal pigmentation and phylogenetic placement of accessions WA1, WA2, KNSW2 and KNSW3. The specimens WA1 and KNSW2 that resembled Aphytis lingnanensis fell in subclade 1, and WA2 and KNSW3 fell in subclades 2. However, among pupae collected from my study orchards, pupae of Aphytis melinus from Castlereagh and Richmond, where the parasitoid was released in 1991 (see Chapter 2), closely resembled published accounts of pupal pigmentation, whereas those from the Somersby Plateau were more variable and less similar to published accounts. Accessions KNSW1 and KNSW3 from the Somersby Plateau were identical to molecular database accession, AY635342A, from China. In contrast, accession KNSW2 was identical to the culture 1 and 2 accessions, both of which were obtained from commercial insectary cultures (Biological Services, Loxton) of Aphytis melinus introduced from California from cultures derived from parasitoids collected in and India from Pakistan 1956 (Rosen & DeBach 1978, Furness et al. 1983).

The 28S rDNA results also showed that Aphytis melinus was more closely related to Aphytis lingnanensis than to Aphytis chrysomphali. These genetic relationships correspond with their taxonomy, in that Aphytis melinus and Aphytis lingnanensis are classified in the Lingnanensis group, while Aphytis chrysomphali belongs to the Chrysomphali group (Rosen & DeBach 1979).

The unidentified species grouped with Aphelinus species from molecular databases, with 100% bootstrap support, separating them from the Aphytis species (Figs 8.17–8.19).
**Figure 8.17.** 50% majority-rule bootstrap consensus tree of the 28S rDNA region of accessions of *Aphytis* species and unidentified species, red scale parasitoids, from maximum likelihood analysis. *Torymus bedeguaris* was used as the out-group and bootstrap values are provided as percentages from 1000 replications.
Figure 8.18. 50% majority-rule bootstrap consensus tree of the 28S rDNA region of accessions of *Aphytis* species and unidentified species, red scale parasitoids, from maximum parsimony analysis. *Torymus bedeguaris* was used as the out-group and bootstrap values are provided as percentages from 1000 replications.
Figure 8.19. Bayesian inference tree resulting from analysis of the 28S rDNA region of accessions of *Aphytis* species and unidentified species, red scale parasitoids. *Torymus bedeguaris* was used as the out-group and posterior probabilities are shown at the base of each branch.

The ITS region of the *Aphytis* species comprised 485 base pairs. The phylogenetic tree derived from maximum parsimony is shown in Fig. 8.20. In this instance, *Aphytis lingnanensis* was not sequenced perfectly; therefore, it was excluded from the analysis.

As for the 28S region, the ITS sequences placed the accessions of *Aphytis chrysomphali* and the two groups of *Aphytis melinus* accessions into two subclades. For the ITS region, the differences in phylogenetic placement of the accessions of *Aphytis melinus* was associated with the location from where they were sourced, with the two accessions (KNSW2 and KNSW3) from NSW being in one subclade and the two accessions (WA1 and WA3) from Western Australia being in the second subclade. My sequences of *Aphytis melinus* from Western Australia were identical with molecular database accession, EU561658, for *Aphytis melinus* from California (Zhu & Fang 2009). Once again, the unidentified aphelinid fell in the *Aphelinus* clade.
My 28S rDNA and ITS results indicated that only two species of *Aphytis*, *Aphytis chrysomphali* and *Aphytis melinus* parasitised scale in citrus orchards on the Central Coast of New South Wales. My molecular results indicated that pupae that resembled *Aphytis lingnanensis* pupae from my study orchards during the course of my studies were, in fact, pupae of *Aphytis melinus*. Likewise, pupae from the Perth and nearby locations in Western Australia that resembled *Aphytis lingnanensis* pupae also proved to pupae of *Aphytis melinus*, which was released at several locations in Western Australia between 1987 and the early 1990s (James Altmann, Biological Services, Loxton, South Australia, pers. comm., 4 October 2011). I did not observe *Aphytis chrysomphali* pupae among approximately 150 *Aphytis* pupae in red scale samples collected for me from locations in and around Perth by Jeremy Lindsey (Department of Agriculture and Forestry, South Perth, Western Australia) between April and August 2011. This was surprising, as the literature indicates that either *Aphytis lingnanensis* or *Aphytis chrysomphali* was the first parasitoid successfully introduced into Western Australia (Jenkins 1945, Wilson 1960, Compere 1961, Furness et al. 1983, Smith et al. 1997). *Aphytis chrysomphali* was introduced to New South Wales from Western Australia in 1925–1926 (Wilson 1960, Hely 1968, Smith et al. 1997). It is possible that extreme, cold winters or very hot summers in Western Australia may have eliminated
populations of *Aphytis lingnanensis* and *Aphytis chrysomphali*, respectively. *Aphytis lingnanensis* is an effective parasitoid of red scale in Queensland (Smith 1978, Papacek & Smith 1992) and possibly in northern New South Wales. It is also possible, given competitive displacement of *Aphytis chrysomphali* by *Aphytis lingnanensis* and eventually of *Aphytis lingnanensis* by *Aphytis melinus* in California (DeBach & Sundby 1963), that *Aphytis melinus* may have displaced one or both of the other species in Western Australia. According to Furness et al. (1983) and Smith et al. (1997), *Aphytis melinus* was introduced to Victoria in 1961 and *Aphytis lingnanensis* in 1962 (Furness et al. 1983). The former is widely established in the region, whereas the latter became established on one property for a brief period (Furness et al. 1983, Smith et al. 1997). However, my results indicate that *Aphytis melinus* has not replaced *Aphytis chrysomphali* in orchards on the Central Coast of New South Wales. It is not known to have displaced *Aphytis chrysomphali* in inland regions of the State, but there was no evidence that it had in the 1980s (Beattie, pers. comm., 2011).

The purpose of this section of work was to confirm the identities of the armoured scale insects and their parasitoids that I studied. The purpose was not to study the phylogenetics and origins of the taxa, otherwise the sampling protocols would have differed. However, separation of *Aphytis melinus* specimens into subclades for both the 28S rDNA and ITS regions, though not consistent, suggests that it may have been introduced to Australia on more than one occasion (see Chapter 11). This possibility of two introductions needs to be confirmed by sampling a larger number of specimens from a greater number of regions, including overseas. In addition to this, it would also be necessary to sequence larger portions of their genomes, including genes encoded within their mitochondria.

The unidentified aphelinid occurred at low levels in association with red scale in the orchards. The results from a BLAST search of molecular databases using the 28S rDNA sequence of the unidentified species as the query sequence showed that it was most closely related to *Aphelinus varipes* Förster (accession HQ599562) (93% similarity) with the next closest association being with *Aphelinus asychis* (DQ350482) (Walker) (92% similarity). *Aphelinus* species are parasitoids of aphids (Wilson 1960, Hely et al. 1982, Viggiani 1984, Smith et al. 1997). *Aphelinus asychis* is a parasitoid of spotted alfalfa aphid, *Therioaphis trifolii* f. *maculate* (Monell), that feeds on lucerne in Queensland (Franzmann et al. 1990). *Aphelinus abdominelis* (Dalman) has been...
commercially available for control of aphids from Biological Services, Loxton, South Australia since 2010. There are no sequences of *Aphelinus abdominelis* in molecular databases. According to Hayat & Fatima (1990), Girault described some 23 *Aphelinus* species from Australia in the early decades of last century but, of these, only four were valid, three with close affinity to *Aphelinus varipes*. Viggiani (1984) stated that for many aphelinid species, diploid eggs develop into primary endoparasitoid females while haploid eggs develop into ectoparasitoid males. My results suggest that specimens of the unidentified aphelinids I recorded as ectoparasitoids of red scale were probably male pupae of an unidentified species of *Aphelinus*. If so, these may be the first records of such associations with armoured scales. However, I assume such parasitism is rare and perhaps seasonal.

**8.3.2.2. Encarsia citrina and Encarsia perniciosi**

**8.3.2.2.1. Morphology**

During my studies, I also observed differences, as reported in literature (Rosen & DeBach 1979), between mummies of scale parasitised by *Encarsia citrina* and *Encarsia perniciosi* (Fig. 8.22), and differences in the length of forewing fringes (Craw 1891, Tower 1913, Woodward 1913, Flanders 1950, Malipatil et al. 2000) (Fig. 8.23). Mummified scales parasitised by *Encarsia perniciosi* are darker and contain more exuviae than those parasitised by *Encarsia citrina* (Rosen & DeBach 1979). *Encarsia perniciosi* exit holes were larger and more irregular than those of *Encarsia citrina*. Moreover, *Encarsia perniciosi* can parasitise larger hosts than *Encarsia citrina*, and only *Encarsia perniciosi* has been recorded in virgin female red scale (Figs 8.22 & 8.23).

![Figure 8.22. Encarsia citrina adult with long marginal wing hairs (left) and Encarsia perniciosi adult (right).](image)
**Figure 8.23.** Mummified scale parasitised by *Encarsia citrina* (left) and by *Encarsia perniciosi* (right). Meconia of *Encarsia citrina* normally line edges of mummified scale; in this case, they were removed during clearing of the specimen.

### 8.3.2.2.2. Molecular studies

I extracted DNA from seven *Encarsia citrina* and three *Encarsia perniciosi* adults. Sources of these specimens are listed in Table 8.7. The sequences of the 28S and COI genes for *Encarsia citrina* and *Encarsia perniciosi* were compared with each other and with data from molecular databases. Eight successfully amplified sequences of the 28S rDNA gene comprised 571 base pairs. The maximum parsimony analysis showed that the *Encarsia perniciosi* and *Encarsia citrina* clades were separated with 100% bootstrap support (Fig. 8.24). There was no variation in the 28S rDNA sequences of the five *Encarsia citrina* specimens from armoured scale species in my study orchards: *E. citrina* SNSW1 from red scale at Somersby, *E. citrina* RNSW1 from yellow scale at Richmond, *E. citrina* CaNSW1 from yellow scale at Castlereag, *E. citrina* LNSW1 from yellow scale at Lower Portland, and *E. citrina* CaNSW2 from purple scale from Castlereagh. There were differences in 25 base pairs between *Encarsia citrina* in my study and the accession AF254236 of *Encarsia citrina* from an armoured scale in Riverside, California (Babcock & Hearty 2001).

In contrast to *Encarsia citrina*, there was variation among the *Encarsia perniciosi* specimens from New South Wales; *E. perniciosi* CaNSW1 from red scale from Castlereagh and *E. perniciosi* CoNSW1 from red scale at Cornwallis were identical to each other and with molecular database accession AF254235 of *Encarsia perniciosi*.
from California (University of California, Riverside culture). The sequence of *E. perniciosi* CoNSW2 from white louse scale at Cornwallis differed in 8 bases and was sister to the above. As my data was based on a small number of specimens, I assume that the difference may be related to the different strains of *Encarsia perniciosi*.

Figure 8.24. 50% majority-rule bootstrap consensus tree of the 28S region of accessions of *Encarsia citrina* and *Encarsia perniciosi*, red scale parasitoids, from maximum parsimony analysis. *Encarsia aurantii* was used as the out-group and bootstrap values are provided as percentages from 1000 replications.

Figure 8.25. Bayesian inference tree resulting from analysis of the 28S rDNA region of accessions of *Encarsia* species, red scale parasitoids. *Encarsia aurantii* was used as the out-group and posterior probabilities are shown below each branch.

The COI sequences of five *Encarsia citrina* and two *Encarsia perniciosi* were amplified successfully. Sequences comprised 651 base pairs. Among the in-group and out-group
taxa, 272 characters were constant, 329 variable characters were parsimony uninformative and 50 characters were parsimony informative. There were no sequences of COI of Encarsia citrina or Encarsia perniciosi available from molecular databases. Parsimony analysis of the COI sequences (Fig. 8.26) indicated that there were two clades, Encarsia perniciosi and Encarsia citrina, with 100% bootstrap support. Within the Encarsia citrina clade, sequences of the four Encarsia citrina specimens from armoured scale species in my study orchards (E. citrina SNSW1 from red scale at Somersby, E. citrina RNSW1 from yellow scale at Richmond, E. citrina CaNSW1 from yellow scale at Castlereagh, and E. citrina LNSW1 from yellow scale at Lower Portland) were identical. The sequence of E. citrina CaNSW2 from purple scale at Castlereagh was sister to the above four specimens from red scale and yellow scale. The sequences of the two accessions of Encarsia perniciosi (both from red scale, at Castlereagh E. perniciosi CaNSW1 and at Cornwallis CoNSW1) were identical.

![Figure 8.26.](image)

**Figure 8.26.** 50% majority-rule bootstrap consensus tree of the COI region of accessions of Encarsia citrina and Encarsia perniciosi, red scale parasitoids, from maximum parsimony analysis. Encarsia iris was used as the out-group and bootstrap values are provided as percentages from 1000 replications.

My molecular results confirmed that both Encarsia citrina and Encarsia perniciosi occur on the Central Coast of New South Wales. The 28S sequences showed no variation among Encarsia citrina parasitising red scale, yellow scale or purple scale in my study orchards. However, my accessions differed from molecular database accession, AF254236, from Riverside. The COI sequences showed no variation between Encarsia citrina specimens parasitising red scale and yellow scale, but variation occurred between these specimens and the Encarsia citrina specimen parasitising purple scale. Unfortunately, there was no available of COI sequences of Encarsia citrina from California or elsewhere. Further studies are required to verify these differences in order to determine if the accessions from my study orchards may in fact represent a native species that is closely related to Encarsia citrina. This may, in
part, explain the relatively high levels of *Encarsia citrina* parasitism of red scale in my study orchards (Chapter 5) in contrast to low levels of parasitism of the scale in California reported by (Compere 1961, Rosen & DeBach 1978).

In contrast to *Encarsia citrina*, my 28S sequences of *Encarsia perniciosi* showed that my two accessions of the parasitoid from red scale were identical to the molecular database accession, AF254235, of *Encarsia perniciosi* from California, but they and AF254235 differed from my *Encarsia perniciosi* from white louse scale. This outcome suggests that the differences may be related to strains of *Encarsia perniciosi* parasitising red scale and white louse scale. However, my results were based on a relatively small number of specimens and further studies are required verify my observations.

### 8.3.2.3. *Comperiella bifasciata*

I successfully extracted DNA from six *Comperiella bifasciata* adults parasitising red scale and yellow scale. The sources of these specimens are shown in Table 8.6. The 28S rDNA and COI genes and the ITS region were successfully amplified. The sequences of the 28S rDNA gene comprised 600 base pairs. The six sequences were identical to each other and with that of molecular database accession, AY599317 (Gillespie et al. 2005). The sequence of the COI gene comprised 484 base pairs. Similar to the 28S gene, the six sequences of the COI gene were identical to each other. There was no available datasets in the databases for COI and the ITS region of *Comperiella bifasciata*. The most similar sequence (92% similarity) was that from *Trichogramma ostriniae* Pang & Chen Guangzhou strain (accession DG177914). The sequence of the ITS region comprised 484 base pairs. Likewise, the six sequences of the ITS gene were identical to each other and the most similar sequence (98% similarity) in molecular databases was from *Syrphophagus aphidivorus* (Mayr) [Hymenoptera: Encyrtidae] (accession HQ599576) (Gariepy & Messing 2011, in press). As no variation among sequences from different specimens for each of the three regions was found, no further analysis was undertaken.

My molecular results indicated that there was no genetic variation in the sequences of the three regions sequenced among *Comperiella bifasciata* specimens, regardless of the host scale or locations from which specimens were collected. My results in this chapter, and results reported in Chapter 5, including only rare instances of encapsulation of the parasitoid by red scale, indicate that the race of *Comperiella bifasciata* parasitising both
yellow scale and red scale in Australia is the red scale race of the species. My results also suggest that yellow scale race, which has been previously mentioned as the race attacking yellow scale in Australia (Smith et al. 1997), may now be rare or may no longer occur in Australia. The yellow scale race of Comperiella bifasciata is encapsulated by red scale (Compere & Smith 1927). Brewer’s (1971) reports of high levels of encapsulation by red scale of eggs and larvae of what he regarded as the red scale race of Comperiella bifasciata are in stark contrast to my observations and successful establishment of Comperiella bifasciata as a parasitoid of red scale in Australia in the 1970s (Furness et al. 1983, Smith et al. 1997). Snowball & Sands (1971) reported that some eggs and larvae of Comperiella bifasciata inside red scale in the vicinity of Sydney were killed by encapsulation, and similar encapsulation had been recorded in South Australia. They (Snowball & Sands 1971) noted the phenomenon was virtually unknown in California from where the red scale race of Comperiella bifasciata was originally imported into Australia, and that there seemed to be a different relationship between the parasite and the red scale in the two countries. It is no longer possible to verify which strain Brewer (1971) used in his studies or the strain on which Snowball & Sands (1971) based their observations in Sydney. Brewer’s (1971) laboratory studies were based on cultures of Comperiella bifasciata reared from material obtained from citrus orchards at Berri (34° 17’ S, 140° 36’ E) in South Australia. Thus, his laboratory study may not have been based on the red scale race of Comperiella bifasciata.

8.3.3. Bacterial symbionts

8.3.3.1. Wolbachia

I detected the surface protein gene, wsp, of Wolbachia from all three Aphytis species, Aphytis chrysomphali, Aphytis lingnanensis and Aphytis melinus. I did not detect the gene in red scale, yellow scale nor in the Encarsia species and Comperiella bifasciata. The wsp sequences of Wolbachia of Aphytis species comprised 546 base pairs. The unrooted phylogenetic tree derived from maximum parsimony analysis (Fig. 8.27) separated the sequences into three clades. The first clade comprised of Wolbachia from Aphytis lingnanensis and Aphytis menilus from my studies, molecular database wsp sequences of Wolbachia of the common house mosquito Culex pipiens complex and Drosophila pseudoanassae Bock [Diptera: Drosophilidae]. The second clade comprised Wolbachia from Aphytis chrysomphali from my study, and molecular
database wsp sequences of Wolbachia of the nematodes *Onchoceca cervicalis* Railliet & Henry, *Brugia malayi* Brug [Spirurida: Onchoercidae] and *Dactylopius tomentosus* (Lamark) [Hemiptera: Dactylopiidae]. The third clade comprised two accessions Wolbachia from *Trichogramma brassiae* (Bezdenko) [Hymenoptera: Trichogrammatidae]. The first, second and third clades were supported by 95, 100 and 100% bootstrap support, respectively. This suggests that the wsp gene of Wolbachia from *Aphytis lingnanensis* and *Aphytis melinus* (biparental) are genetically different to that of *Aphytis chrysomphali* (uniparental).

Within the first clade, Wolbachia from *A. lingnanensis* culture2 (from the commercial insectary), *A. melinus* KNSW1 and *A. melinus* KNSW2 from Kulnura were identical. These sequence accessions were closely related to Wolbachia from *A. melinus* WA2 from Western Australia and *A. melinus* culture2 from the commercial insectary. I also detected the ftsZ gene of Wolbachia from *Aphytis chrysomphali* but not from *Aphytis lingnanensis* or *Aphytis melinus*. Marcon et al. (2011) reported that the ftsZ gene had a poor ability to detect Wolbachia and generated false negatives in 44.9% of the samples.

![Figure 8.27. Unrooted 50% majority-rule bootstrap consensus tree of the wsp gene of Wolbachia, bacterial symbiont of Aphytis species from my studies and of molecular database of other insect species derived from maximum parsimony analysis. The wsp gene from Wolbachia from Dactylopius tomentosus was used as the out-group and bootstrap values are provided as percentages from 1000 replications.](image)

It is known from the literature that Wolbachia induces parthenogenetic reproduction in *Trichogramma* species (Stouthamer 1993, Vavre et al. 1999). Wolbachia are also known to induce cytoplasmic incompatibility in *Drosophila* species and *Culex pipiens*.
(O’Neill & Karr 1992, Veneti et al. 2003). My results, based on the wsp gene, indicate that *Wolbachia* was present in both uniparental *Aphytis chrysomphali* and biparental *Aphytis lingnanensis* and *Aphytis melinus*. My results differed from Zchori-Fein et al. (1995) and Gottlieb et al. (1998) who, based on 16S rDNA gene, only detected *Wolbachia* in the uniparental *Aphytis chrysomphali* and the uniparental form of *Aphytis lingnanensis* and not in biparental *Aphytis melinus* and the biparental form of *Aphytis lingnanensis*. They (Zchori-Fein et al. 1995) therefore regarded *Wolbachia* as a parthenogenesis-inducing bacterium. In contrast, my results were similar to those in a recent publication by Vasquez et al. (2011) who, also based on the 16S rDNA gene, recorded *Wolbachia* in *Aphytis melinus* in which it induced cytoplasmic incompatibility. I assume that *Wolbachia* may have induced cytoplasmic incompatibility in *Aphytis melinus* in my study orchards, but this needs to be confirmed in future studies.

Stouthamer (1993) reported that *Wolbachia* induced parthenogenesis in parasitoids enhancing potential efficacy. Vavre et al. (1999) reported that *Wolbachia* enhanced the fecundity of *Trichogramma bourarachae* Pintureau & Babault. This suggests that *Wolbachia*-induced parthenogenesis in *Aphytis chrysomphali* plays a role in the effectiveness of the parasitoids. However, high temperatures resulted in high levels of male offspring male produced by parthenogenetic females (Stouthamer et al. 1999). This suggests that low levels of parasitism by *Aphytis chrysomphali*, particularly at Somersby and Kulnura in 2011 may have been partially due to impacts of the heatwave in January-February 2011 on *Wolbachia* and the subsequent effect on its host. In contrast, if *Wolbachia* associated with *Aphytis lingnanensis* and *Aphytis melinus* in my study orchards induces cytoplasmic incompatibility then high temperatures during the heatwave may have killed the bacterium and led to higher reproduction of *Aphytis melinus* populations thereafter at Richmond and Castlereagh.

In my study, *Wolbachia* was detected in *Aphytis* specimens from the field and from both commercial insectaries (one for *Aphytis lingnanensis* and the other for *Aphytis melinus*). Similarly, Vasquez et al. (2011) recorded a high *Wolbachia*-infection frequency in *Aphytis melinus* from both field and insectary populations. They (Vasquez et al. 2011) also reported that *Wolbachia*-infected females had shorter life-spans than uninfected females, and that uninfected females had higher fecundities than infected females. Antibiotic treatment has been widely used to eliminate *Wolbachia* from insect hosts (Koukou et al. 2006, Dedeien et al. 2006, Pike & Kingcombe 2009). Such use of
antibiotics could lead to more efficient mass rearing of *Aphytis melinus* and *Aphytis lingnanensis*, but impacts of releases of ‘Wolbachia-free’ parasitoids on the incidence of the bacterium in field populations would probably be short-lived.

8.3.3.2. ‘*Candidatus Cardinium*’

My results of screening for the endosymbiont, ‘*Candidatus Cardinium*’, based on its 16S rDNA gene (Fig. 8.28), indicated that the bacterium was present in all my *Encarsia citrina* and *Encarsia perniciosi* specimens, and in oleander scale. However, I did not detect it in two *Aphytis chrysomphali*, one *Aphytis melinus*, five *Comperiella bifasciata* and two red scale specimens. The 16S rDNA sequences of the bacterium comprised 380 base pairs. Phylogenetic trees were derived from the 16S rDNA region using parsimony analysis. The 16S rDNA sequence of *Bemisia tabaci* (JF766342) was used as an out-group. The ‘*Candidatus Cardinium*’ endosymbionts of *Encarsia citrina* and *Encarsia perniciosi* were in one group with 91% bootstrap support.

There was no variation among sequences of ‘*Candidatus Cardinium*’ in the four *Encarsia citrina* specimens from New South Wales (‘*Candidatus Cardinium*’ in *E. citrina* RNSW1 from yellow scale from Richmond ‘*Candidatus Cardinium*’ in *E. citrina* CaNSW1, ‘*Candidatus Cardinium*’ in *E. citrina* CoNSW1 from yellow scale at Cornwallis and ‘*Candidatus Cardinium*’ in *E. citrina* CaNSW2 from purple scale from Castlereagh). These sequences were also identical with the sequence from California. These five sequences formed a 5-way basal polytomy that was sister to the three sequences of ‘*Candidatus Cardinium*’ from *Encarsia perniciosi*. The three ‘*Candidatus Cardinium*’ sequences from *Encarsia perniciosi* specimens were identical. For both hosts, the sequences of the endosymbionts were identical regardless of host scale and locations from which the parasitoids were collected. However, the 16S sequences of ‘*Candidatus Cardinium*’ in *Encarsia citrina* were closely related to those in *Encarsia perniciosi* and only differed in one base pair. The similarity of the 16S rDNA sequences of the ‘*Candidatus Cardinium*’ endosymbionts of *Encarsia citrina* and *Encarsia perniciosi* was similar to the results of Gruwell & Normark (2009) who reported a monophyletic lineage of ‘*Candidatus Cardinium*’ infecting multiple species of *Encarsia*. The sequences from *Candidatus Cardinium* from *Encarsia citrina* and *Encarsia perniciosi* differed to a molecular database sequence from of the endosymbiont in *Encarsia pergandiella* Howard (AY332002).
The 16S sequences of ‘Candidatus Cardinium’ in *Aspidiotus nerii* from my studies were close related to that from *Unaspis euonymi* (Comstock) (molecular database sequence GQ455412). The ‘Candidatus Cardinium’ endosymbiont of *Aspidiotus nerii* and *Unaspis euonymi* (GQ455412) were in one group with 68% bootstrap support. Provencher et al. (2005) and Gruwell & Normark (2009) found that ‘Candidatus Cardinium’ induces parthenogenesis in *Aspidiotus nerii*.

‘Candidatus Cardinium’ is widely known to induce parthenogenetic reproduction in some insect species (Zchori-Fein et al. 2001, Zchori-Fein & Perlman 2004, Groot & Breeuwer 2006, Giorgini et al. 2009). This bacterium may also interfere with other reproductive modes of its insect hosts in ways similar to those recorded for *Wolbachia* (Gotoh et al. 2007). Gotoh et al. (2007) reported that ‘Candidatus Cardinium’ was more tolerant to the heat than *Wolbachia*. They (Gotoh et al. 2007) reported that temperatures of 35°C for 3 or 7 d completely eliminated *Wolbachia* from cultures of *Tetranychus puercalica* Ehara & Gotoh [Acari: Tetranychidae], whereas ‘Candidatus Cardinium’ tolerated up to 40°C for 7 d. Such differences in the susceptibility of *Wolbachia* and ‘Candidatus Cardinium’ to heat may partially explain better survival of *Encarsia* species than *Aphytis* species and *Comperiella bifasciata* during the record 2011 heatwave in my studies (see Chapter 5).

Zchori-Fein et al. (2001) detected an ‘*Encarsia* bacterium’ that caused thelytokous parthenogenesis in 6 populations of *Encarsia*, including *Encarsia citrina* and *Encarsia perniciosi*. Stouthamer & Luck (1991) reported that *Encarsia perniciosi* parasitising red scale is thelytokous, and there was no transition from the arrhenotokous form to the thelytokous form in culture. This may be due to the presence of ‘Candidatus Cardinium’ inducing these reproduction modes in *Encarsia* species.
Figure 8.28. 50% majority-rule bootstrap consensus tree of the 16S rDNA region of ‘Candidatus Cardinium’, bacterial symbiont of Encarsia species and Aspidiotus nerii, from maximum parsimony analysis. ‘Candidatus Cardinium’ from Bemisia tabaci was used as the out-group and bootstrap values are provided as percentages from 1000 replications.

8.3.4. Ants

The COI gene sequences of ant specimens comprised 574 base pairs. The COI sequences of Iridomyrmex rufoniger associated with black scale in my study orchards at were identical with those of Iridomyrmex rufoniger (DQ249967, DQ249968) from molecular databases (Eastwood et al. 2006) (Fig. 8.29). This result verified the identification that was kindly undertaken by Dr Steve Shattuck (CSIRO Ecosystem Sciences, Canberra, Australia). Unidentified specimens, LNSW1 and LNSW2, were associated with aphids on Bursaria spinosa spp. lasiophylla (E. M. Benn) L. W. Cayzer et al. [Apiales: Pittosporaceae] near Lithgow, New South Wales. Unidentified specimen, RNSW AiR1, was associated with cottony cushion scale (Icerya purchasi Maskell [Hemiptera: Margarodidae]) on citrus at Richmond.

Figure 8.29. 50% majority-rule bootstrap consensus tree of the COI gene of ant species, from maximum parsimony analysis. Iridomyrmex mattirolai was used as the out-group and bootstrap values are provided as percentages from 1000 replications.
8.4. Conclusions

I confirmed that red scale and yellow scale are two distinct species based on their morphology, habitat and molecular differences. This is the first study to confirm the presence of yellow scale in the Riverina district in inland New South Wales since it was first noticed by Rob Weppler (Riverina IPM) in spring 2006 or early 2007. The genetic variation among the specimens collected from different locations within Australia, based on the elongation factor and 28S genes, suggests that strains of red scale were introduced from separate locations. My molecular results showed that the 28S region sequences of red scale from Indonesia, New South Wales and California were identical. This suggests that the strain of red scale introduced to California in the late 1800s from Australia may have been introduced from Indonesia.

I confirmed the identities of all five primary parasitoid species of red scale in coastal New South Wales. I found that *Aphytis lingnanensis* did not occur in my study orchards during the course of the study. Morphological characteristics of *Aphytis melinus* pupae and molecular results indicated that there were variation in both morphology and molecular aspects among these populations of *Aphytis melinus*. Any biological consequences of this variation, if they exist, require further investigation. As accessions visually identified as *Aphytis lingnanensis* were found to be *Aphytis melinus*, similarities and variation in pupal pigmentation should be taken into account in future investigations of *Aphytis* and in reviews of the literature.

In contrast to variation in *Aphytis melinus*, molecular studies of *Comperiella bifasciata* indicated that there was no genetic variation among specimens regardless of the host scale and locations from which they were collected. This suggests that populations at the locations where the specimens were collected may have stemmed from a single introduction or from a single source. It would be of interest to confirm this hypothesis and to compare specimens from armoured scales on native and introduced species and hybrids of *Citrus*. This could resolve the origin of *Comperiella bifasciata* that Flanders (1934) recorded from *Aonidiella eremocitri*, and provide an opportunity, if different strains are detected, to repeat Brewer’s (1971) research on encapsulation.

There was limited variation among *Encarsia citrina* from red scale, yellow scale and purple scale. Variation among *Encarsia perniciosi* populations from red scale and white louse scale was greater than for *Encarsia citrina.*
My results suggested that *Encarsia citrina* parasitising armoured scales on the Central Coast of New South Wales differed genetically to *Encarsia citrina* from California, where the parasitoid, in contrast to observations in my studies, does not parasitise red scale: material assumed to be a red scales strain of the parasitoid was imported to California from China in 1899–1900 but failed to establish (Compere 1961, Rosen & DeBach 1978). Notwithstanding this failure, Beattie (1984) and Zhang & Gu (1994) reported that *Encarsia citrina* was a common and effective parasitoid of red scale in China. My studies, and those Beattie (1984) and Zhang & Gu (1994, 1995), suggest that its role biological control red scale needs to be more thoroughly assessed. Molecular studies should be undertaken to determine whether the strain I recorded in my studies is identical to the strain(s) recorded in southern China by Beattie (1984) and Zhang et al. (1994, 1995). My results also suggest scope for re-introducing the parasitoid to California and for its introduction to other countries.

Specimens of *Aphytis melinus* parasitising red scale at Kulnura were genetically identical to specimens collected in ‘Fuht’, China, by Prof Robert Luck, University of California, probably in the early 1990s (Robert Luck, pers. comm., to Andrew Beattie, January 2011)51. Whether the specimens collected by Prof Luck were from naturally occurring populations in China or from populations stemming from the introduction of *Aphytis melinus* to Guangdong from the University of California, Riverside in 1988 (Zhang & Gu 1994) is not known, but the sequences were not identical to accessions from commercial insectary cultures derived from introductions of *Aphytis melinus* to Australia in the 1960s. Irrespective of the uncertain origins of accessions AY635333 and AY635342, my results and records of introductions of *Aphytis melinus* to Australia (Wilson 1960, Furness et al. 1983) suggest that populations of *Aphytis melinus* on the Somersby Plateau may be derived from parasitised red scale, or other armoured scale hosts, on fruit or plants imported to Australia at some point since the early 1800s. Further research is therefore required to compare differences among *Encarsia* and *Aphytis* populations occurring in Australia, California and in Asia. These studies should focus on both morphological and molecular differences in order to ensure accurate identification of taxa.

My results also indicated that species other than *Iridomyrmex rufoniger* associated with soft scales in Sydney suburban areas. Ants have been also recorded to tend honeydew

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51 http://hymenoptera.tamu.edu/rna/models/chal/data/sup/Supp_Mat_1_487.R1.pdf
produced by the native lycaenid species in Australia (Eastwood & Frazer 1999, Eastwood et al. 2006). It would be of interest for future studies to determine the identities of ants and honeydew-producing insects and their coincident relationships.
Chapter 9. The two red-headed fungi (*Microcera coccophila* Desm. and *Microcera larvarum* ( Fuckel) Gräfenhan, Seifert & Schroers): morphological description and identification, molecular verification and studies on pathogenicity and dispersal

9.1. Introduction

Numerous records indicate that *Microcera coccophila* and *Microcera larvarum*\(^5\) are widely distributed entomopathogens of armoured scale insects [Hemiptera: Diaspididae] in temperate, subtropical and tropical regions of the world (Tryon 1889, Koebele, 1892, Rolfs 1897, McAlpine 1899, Earle 1899, Reinking 1921, Petch 1921a,b, Dingley 1954, Annecke 1963, Booth 1971, Smith et al. 1997, Rossman et al. 1999, Gräfenhan et al. 2011). In Australia, *Microcera coccophila* has been recorded in Queensland (Tryon 1889, 1894, McAlpine 1899, Summerville 1934) and New South Wales (Koebele 1892, McAlpine 1899, Hely 1968, Hely et al. 1982). *Microcera larvarum* may have been recorded in Victoria as *Fusarium epicoccum* McAlpine on red scale on *Citrus reticulata* at Burnley in August 1899, but the records are not clear (McAlpine 1899, Petch 1921b).

Tryon (1889, 1894) and Koebele (1890) considered *Microcera coccophila* to be highly effective in minimising populations of various scale insects. Tryon (1894) regarded it as one of three or four entomopathogenic fungi parasitising armoured scale insects in orange orchards near Maryborough in the Wide Bay region of Queensland. He (Tryon 1894) noted that "The presence of this red scale insect could be detected in all the orchards visited.

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\(^5\) In order to avoid confusion, I refer to these fungi hereafter by their anamorph names, *Microcera coccophila* and *Microcera larvarum*. Synonyms of *Microcera coccophila* and *Microcera larvarum* and their teleomorphs, the host scale species attacked by them, and host plants on which scale hosts and fungi have been recorded are summarised in Appendix II, Table 1. Use of *Microcera* for these species and *Microcera diploa* (Berk. & M.A. Curtis) Gräfenhan & Seifert and *Microcera rubra* Gräfenhan & Seifert in lieu of teleomorphic *Cosmospora* also complies with use of unitary names for anamorphic and teleomorphic species of fungi under recent changes to the International Code of Nomenclature for fungi, plants and algae. Under these changes single-name nomenclature employing the oldest generic name in combination with the oldest species name should be used except where there are valid reasons for adopting an alternative unitary name (see Gräfenhan et al. 2011). *Microcera* was established by Desmazières (1848) and *Cosmospora* by Rabenhorst (1862). *Microcera* therefore has precedence (Gräfenhan et al. 2011) but not all species of *Cosmospora* as recognised by Gräfenhan et al. (2011) are congeneric with species of *Microcera*. 
As a rule, however, it did not appear to be occasioning much damage, being generally held in check by a minute hymenopterous parasite, and also by a small scarlet fungus, *Microcera coccophila*, which very generally preyed upon it. Smith et al. (1997) and Waterhouse & Sands (2001) also mention it as being present in Australia. It was the most common of six putative entomopathogens of red scale that I recorded in citrus orchards on the Central Coast of New South Wales.

Despite early records and interest (Tryon 1889, 1894, Koebele, 1890, McAlpine 1899, Reinking 1921, Petch 1921a,b), no thorough studies of the role of *Microcera coccophila* or *Microcera larvarum* in the biological control of armoured scales have been undertaken in Australia or elsewhere. There are no molecular records verifying the presence either species, or the presence of the other four entomopathogens I recorded in Australia (see Chapter 10). Moreover, although *Microcera coccophila* has been reported as an effective pathogen of armoured scales (Tryon 1889, Koebele 1890, Tryon 1897, Rolfs 1897, Fawcett 1908), some authors, particularly in Florida, have expressed uncertainty about its pathogenicity (Ziegler 1949, Fisher 1949, 1950b, Kono & Ferrer 1972). Indeed, Fisher (1949, 1950b) considered it to be a saprophyte.

Observations in Central Coast orchards over the past 38 years have suggested that *Microcera coccophila* is most evident in the presence of ants and soft scales, particularly black scale (Beattie, pers. comm., 2008). In Queensland, Tryon (1889) made similar observations, noting that ‘when the orange trees are infested with the “red scale,” and especially when the branches also are black with the *fumagine*\(^{53}\) consequent on the presence also of the *Lecanium oleae*, there may be observed small irregular rose-red bodies’.

I, therefore, hypothesised that ants associated with black scale may induce fungal epizootics of *Microcera coccophila* by passively dispersing fungal conidia over plant surfaces infested with armoured scales.

In this chapter, I report studies designed to confirm the identity of *Microcera coccophila* and *Microcera larvarum* on the basis of their morphological and molecular characteristics and determine their pathogenicity to oleander scale (*Aspidiotus nerii*). Field observations are also presented. I also describe the incidence of *Microcera coccophila* in the presence and absence of ants and report studies to determine if *Iridomyrmex rufoniger* workers move conidia.

\(^{53}\) Sooty mould fungi
9.2. Materials and methods

9.2.1. Morphological studies

Specimens of four armoured scale species infected by Microcera coccophila were collected in citrus orchards on the Central Coast of New South Wales from January 2009 to July 2010. Details are given in Table 9.1. Specimens of armoured scale insects infected with Microcera larvarum were collected from mature Washington navel orange trees at Somersby during 2010 and 2011 (Table 9.1).

Sporodochia typical of Microcera coccophila growing on scale cadavers were selected for isolation of the fungus. Macroconidia from sporodochia were used to produce pure cultures on potato dextrose agar (PDA) and carnation leaf agar (CLA) using the single-spore method (Burgess et al. 1994). Cultures of Microcera coccophila (isolate AaS2*, Table 9.1) and Microcera larvarum (Fl2*, Fl3*) were also established from a single ascospore. Cultures were incubated in a laboratory at 25°C under continuous fluorescent light.

After 20 d of incubation, cultures were examined for the following characteristics: shape, size and mode of formation of macroconidia, presence of microconidia and chlamydospores. The appearance, including pigmentation, of colonies on CLA and PDA was also recorded. The length and width of conidia and number of septa were assessed using an Olympus BX60 compound microscope (Olympus Corporation, Tokyo, Japan) fitted with a ProgRes C14 digital camera (JenOptik L.O.S., GmbH, Germany). Height and diameter of perithecia were measured using a stereomicroscope. Colony diameters were also measured. One isolate of Microcera coccophila, AaK, was deposited in the New South Wales Plant Pathology Herbarium (DAR AU 073), Orange Agricultural Institute, Orange, New South Wales, Australia.
Table 9.1. Geographic origin, host scales, and plant host of scales from which isolates of Microcera coccophila and Microcera larvarum were isolated.

<table>
<thead>
<tr>
<th>Accession code</th>
<th>Host scale</th>
<th>Host plant</th>
<th>Location</th>
<th>Latitude &amp; longitude</th>
<th>Accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Microcera coccophila</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AaCa1</td>
<td>A. aurantii</td>
<td>Valencia orange</td>
<td>Castlereagh</td>
<td>33° 40’ S, 150° 40’ E</td>
<td>JQ240473 KC413020</td>
</tr>
<tr>
<td>AaR</td>
<td>A. aurantii</td>
<td>Valencia orange</td>
<td>Richmond</td>
<td>33° 36’ S, 150° 44’ E</td>
<td>KC338994 KC312887</td>
</tr>
<tr>
<td>AaCo</td>
<td>A. aurantii</td>
<td>Washington navel orange</td>
<td>Cornwallis</td>
<td>33° 22’ S, 151° 16’ E</td>
<td>KC338996 KC312890</td>
</tr>
<tr>
<td>AaCa2</td>
<td>A. aurantii</td>
<td>Valencia orange</td>
<td>Castlereagh</td>
<td>33° 40’ S, 150° 40’ E</td>
<td>KC339000 KC338993</td>
</tr>
<tr>
<td>AaS1</td>
<td>A. aurantii</td>
<td>Washington navel orange</td>
<td>Somersby</td>
<td>33° 22’ S, 151° 16’ E</td>
<td>JQ240474 JQ341030</td>
</tr>
<tr>
<td>AaK</td>
<td>A. aurantii</td>
<td>Eureka Lemon</td>
<td>Kulnura</td>
<td>33° 14’ S, 151° 13’ E</td>
<td>KC338995 KC312886</td>
</tr>
<tr>
<td>AcLP</td>
<td>A. citrina</td>
<td>Washington navel orange</td>
<td>Lower Portland</td>
<td>33° 23’ S, 150° 52’ E</td>
<td>JQ582400 JQ341029</td>
</tr>
<tr>
<td>LbCa</td>
<td>L. beckii</td>
<td>Washington navel orange</td>
<td>Castlereagh</td>
<td>33° 40’ S, 150° 40’ E</td>
<td>KC338998 JQ303074</td>
</tr>
<tr>
<td>UcS</td>
<td>U. citri</td>
<td>Washington navel orange</td>
<td>Somersby</td>
<td>33° 22’ S, 151° 16’ E</td>
<td>KC338997 KC312889</td>
</tr>
<tr>
<td>AaS2*</td>
<td>A. aurantii</td>
<td>Washington navel orange</td>
<td>Somersby</td>
<td>33° 22’ S, 151° 16’ E</td>
<td>KC338999 KC312888</td>
</tr>
<tr>
<td><strong>Microcera larvarum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AaK4</td>
<td>A. aurantii</td>
<td>Eureka lemon</td>
<td>Kulnura</td>
<td>33° 14’ S, 151° 13’ E</td>
<td>KC354704 KC338992</td>
</tr>
<tr>
<td>AaK5*</td>
<td>A. aurantii</td>
<td>Eureka lemon</td>
<td>Kulnura</td>
<td>33° 14’ S, 151° 13’ E</td>
<td>KC354705 KC312891</td>
</tr>
<tr>
<td>As1*</td>
<td>armoured scale</td>
<td>Washington navel orange</td>
<td>Somersby</td>
<td>33° 22’ S, 151° 16’ E</td>
<td>KC344733 JQ266266</td>
</tr>
<tr>
<td>AsS2</td>
<td>armoured scale</td>
<td>Washington navel orange</td>
<td>Somersby</td>
<td>33° 22’ S, 151° 16’ E</td>
<td>KC349902 -</td>
</tr>
<tr>
<td>AsS3*</td>
<td>armoured scale</td>
<td>Washington navel orange</td>
<td>Somersby</td>
<td>33° 22’ S, 151° 16’ E</td>
<td>KC349903 KC413021</td>
</tr>
</tbody>
</table>

* Cultures obtained from single ascospores.
9.2.2. DNA extraction

Fungal mycelium and conidia from each *Microcera coccophila* and *Microcera larvarum* isolate were obtained from pure cultures grown on PDA for 14 d at 25°C in the laboratory under continuous fluorescent light. Genomic DNA was extracted following the procedure described in Ghajar (2004). Mycelium and conidia were scraped off and ground to obtain fine powder in liquid nitrogen using a mortar and pestle.

9.2.3. PCR

Amplifications using the PCR of the internal transcribed spacer (ITS) region of ribosomal DNA and 28S ribosomal RNA gene region from the isolates of *Microcera coccophila* and *Microcera larvarum* (Table 9.1) were undertaken using primer pairs ITS1F and ITS4 (ITS) and NL1 and NL4 (28S ribosomal RNA) (Table 9.2).

<table>
<thead>
<tr>
<th>Table 9.2.</th>
<th>Primers used in this chapter to amplify the ITS (White et al. 1990) and 28S ribosomal RNA regions (O'Donnell 1992).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primer</td>
<td>Sequence (5’ → 3’)</td>
</tr>
<tr>
<td>Internal transcribed spacer</td>
<td></td>
</tr>
<tr>
<td>ITS1F</td>
<td>CTTGGTCATTTAGAGGAAGTAA</td>
</tr>
<tr>
<td>ITS4</td>
<td>TCCTCCGCTTATTGATATGC</td>
</tr>
<tr>
<td>28S ribosomal RNA</td>
<td></td>
</tr>
<tr>
<td>NL1</td>
<td>GCATATCAATAAGCGGAGGAAAAG</td>
</tr>
<tr>
<td>NL4</td>
<td>GGTCGCTGTTCAGCAGG</td>
</tr>
</tbody>
</table>

The PCR reaction mixture consisted of: 0.1 µL Taq polymerase (GoTaq® Flexi DNA Polymerase, Promega Corporation, Madison, Wisconsin, United States of America) (0.05 U/µL); 2.5 µL 5x reaction buffer; 1.5 µL MgCl2; 1 µL forward primer (10 mM); 1 µL reverse primer (10 mM); 0.5 µL dNTPs (Promega) (10 mM); 1 µL DNA template (50–100 ng/µL); and sterilised milliQ water to obtain 25 µL. Primers were obtained from Invitrogen™ (Invitrogen Australia, Mulgrave, Victoria).

PCR conditions for 28S ribosomal RNA were: an initial denaturation for 2 min at 94°C; 40 cycles of 30 s at 94°C for DNA denaturation, 30 s at 52°C for primer annealing, and 90 s at 72°C for primer extension; a final extension of 10 min at 72°C (Voigh et al. 1999). PCR conditions for the ITS gene were: an initial denaturation for 2 min at 95°C; 30 cycles of 30 s at 95°C, 30 s at 50°C, 2 min at 72°C extension; and a final extension of 10 min at 72°C (White et al. 1990). Thermocycling was performed using a Dyad
Peltier thermal cycler (Bio-rad Laboratories, Berkeley, California, United States of America).

9.2.3.1. Gel electrophoresis

PCR product was mixed with blue-orange loading buffer (6×) (Promega) and loaded into 1% agarose gels containing 0.5 μg/mL ethidium bromide. A 100 base pair DNA ladder (Promega) was used as a size marker. Electrophoresis was conducted at 100 V for 40 to 45 min. Bands in gels were visualised and photographed using UV transillumination in a GelDoc 2000 (Bio-Rad Laboratories, Inc. Berkeley, California, United States of America).

DNA purification of amplicons was conducted using either the Wizard® Genomic DNA Purification Kit (Promega) following the manufacturer’s instructions or EXoSAP (Exonuclease I (New England Biolabs® Inc., Ipswich, Massachusetts, United States of America) – shrimp alkaline phosphatase (Promega). For the latter, a volume of 2 μL of 5× EXoSAP mix (containing 2.5 μL Exonuclease I (20 U/μL), 25 μL shrimp alkaline phosphatase (1 U/μL) in 172.5 μL Milli-Q water) was added into each PCR product sample. The sample was then incubated at 37°C for 30 min and then 95°C for 5 min.

9.2.3.2. DNA sequencing

Purified PCR products were sequenced by Macrogen Inc., Seoul, Korea, using an ABI 3700 DNA sequencer.

9.2.3.3. Phylogenetic analysis

The forward and reverse DNA sequences of both genes were edited and aligned and contigs assembled using DNA Sequencher™ 4.8. Sequences of the Microcera coccophila and Microcera larvarum isolates were aligned with sequences of related species from molecular databases (Tables 9.3 & 9.4). Sequences of two genes of the 10 isolates of Microcera coccophila used in this study were submitted to GenBank. Parsimony analysis was performed using tree-bisection-reconnection branch swapping with a heuristic search with 1000 bootstrap replicates using PAUP* (Phylogenetic Analysis Using Parsimony, Version 4.0 beta 10 WIN) (Swofford 2001).
Table 9.3. GenBank sequences of the ITS gene of Microcera spp. (as Fusarium, Microcera and Nectria) used in this study.

<table>
<thead>
<tr>
<th>Species</th>
<th>Cited as</th>
<th>Accession number</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microcera coccophila</td>
<td>Microcera coccophila</td>
<td>HQ897794</td>
<td>Gräfenhan et al. (2011)</td>
</tr>
<tr>
<td>Microcera larvarum</td>
<td>Fusarium larvarum</td>
<td>EU860063</td>
<td>Bills et al. (2009)</td>
</tr>
<tr>
<td>Cosmospora coccinea</td>
<td>Cosmospora coccinea</td>
<td>HQ897827</td>
<td>Gräfenhan et al. 2011</td>
</tr>
</tbody>
</table>

Table 9.4. Genebank sequences of the 28S ribosomal RNA gene of Microcera spp. (as Fusarium, Nectria and Tubercularia) used in this study.

<table>
<thead>
<tr>
<th>Species</th>
<th>Cited as</th>
<th>Accession number</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microcera coccophila</td>
<td>Nectria flammea</td>
<td>U88103</td>
<td>O'Donnell (1993)</td>
</tr>
<tr>
<td>Microcera larvarum</td>
<td>Fusarium larvarum</td>
<td>EU8700734</td>
<td>Bills et al. (2009)</td>
</tr>
<tr>
<td>Cosmospora coccinea</td>
<td>Cosmospora coccinea</td>
<td>GQ505990</td>
<td>Hirooka et al. (2010)</td>
</tr>
</tbody>
</table>

9.2.4. Field observations

The following features were recorded in the field: scale species, host plant of scale, appearance of infected hosts, and seasonal and spatial abundance in associations with other organisms.

9.2.5. Pathogenicity of Microcera coccophila and Microcera larvarum to oleander scale

9.2.5.1. Oleander scale cultures

Oleander scale was cultured on Eureka lemon fruit collected from an unsprayed orchard at Castlereagh. The fruit were washed thoroughly with tap water and partially wrapped in Parafilm (Laboratory Film, Pechiney Plastic Packaging, Menasha, Wisconsin, United States of America) in order to minimise water loss. Each lemon was held upright by placing it in a 30 mm diameter plastic cup (Fig. 9.1). Lemon fruit were infested with oleander scale crawlers by positioning them under pumpkins that were heavily infested with the scale. The lemons were then placed in the laboratory at 25°C for about 20 d before they were used for the experiment.
9.2.5.2. Inoculum preparation and inoculation methods

Fungal conidia were obtained from 20-day-old pure cultures on PDA, as described above. Conidia were scraped-off agar plates and suspended in sterile distilled water containing 0.01% Tween 80 in a 50 mL flask. Culture clumps were broken up by adding several 2.5 mm diameter glass beads to the flask (Biospec Products, Inc, Bartlesville, Oklahoma, United States of America) and shaking the flask vigorously on a platform mixer (Ratek Instruments Pty Ltd, Boronia, Victoria) (200 rpm) until the clumps were no longer visible. Each pathogenicity test comprised two treatments, oleander scale inoculated with Microcera coccophila or Microcera larvarum and the control comprising non-inoculated insects. For each treatment in each of the two trials, 10–15 scale-infested lemon fruit were sprayed with 15 mL of sterile distilled water (control) or with 15 mL of conidial suspension (10^6 conidia/mL) on days 1, 3 and 6. Each fruit was considered a replicate. The concentration of macroconidia was assessed using a haemocytometer (Neubauer improved counting chamber). On each occasion, sprays were applied thoroughly to the lemon fruit using a Paasche airbrush sprayer (Paasche Airbrush Company, Harwood Heights, Illinois, United States of America) operated at 100 kPa.

9.2.5.3. Incubation conditions

The fruit were incubated in a 400 × 300 × 150 mm plastic container at 25°C, 80 to 85% RH and 12:12 light: dark cycle in a constant temperature (CT) room. On each day after the first spray, all fruit in the container were sprayed with sterilised distilled water using
a 450 mL PlasPak Selecta Spray multi-purpose sprayer (PlasPak, Minto, New South Wales) at 17:00. The lid of each container was kept firmly closed after each application of water until 07:00 to 08:00 on the following morning when it was partially removed until the next application of water.

9.2.5.4. Assessments of pathogenicity

Numbers of live and dead scales on one quarter of the surface of each scale-infested fruit were counted under a Wild M7S stereomicroscope (Leica Microsystems, North Ryde, New South Wales) before the application of the first spray, and then at 6 d intervals; scale numbers on two other quarters were counted in order to determine the optimum time (15–20 d) for assessing mortality on the remaining quarter of the exposed fruit surface. A fine needle was used to lift scale covers in order to determine if scales were alive or dead. Morphological features of cadavers were also recorded. After assessing mortality, fruit were maintained under the same conditions and regularly checked for formation of sporodochia or perithecia. Conidia obtained from these sporodochia were re-cultured on PDA in order to fulfil Koch’s postulates of pathogenicity.

9.2.5.5. Management of fungal feeding mites

A fungal feeding mite (*Tyrophagus* nr. *putrescentiae* (Shrank) [Acari: Acaridae]) invaded fruit in the first two trials (results not shown). In an unsuccessful attempt to control these mites, fruit were washed in a warm water bath and then dipped in 5 g/5 L Pyranica (200 g/kg tebufenpyrad) prior to infestation with oleander scale. Subsequently, the predatory mite *Typhlodromips montdorensis* Schicha [Acari: Phytoseiidae], kindly supplied by Bugs for Bugs, Integrated Pest Management Pty Ltd, Munduberra, Queensland, was released several times during each experiment in order to suppress the mycetophagous mite.

9.2.6. Data analysis

Percent mortality was calculated as:

\[
\% \text{ mortality} = \frac{\text{number of dead scale}}{\text{number of dead scale} + \text{number of live scale}} \times 100
\]
Differences between inoculated treatment and control were analysed using a one-way analysis of variance (ANOVA) (Minitab 15, State College, Pennsylvania, United States of America).

### 9.2.7. Movement of macroconidia *Microcera coccophila* by *Iridomyrmex rufoniger*

The experiment was conducted using 150 mm diameter, 18 mm deep, plastic Petri dishes containing 2% water agar to a depth of 3 mm. A 62 mm diameter filter paper was placed in the middle of each dish before the agar was poured (Fig. 9.2). A ring of agar was removed from each dish to leave a central agar island 20 mm diameter surrounded by a 12 mm wide channel and an outer ring of agar 44 mm wide. The filter paper was used in order to reduce possible loss of conidia under the agar. On each occasion, 600–800 µL of conidial suspension from a pure culture was pipetted into the channel. *Iridomyrmex rufoniger* workers were then released onto the agar island and allowed to walk through the channel to the outer agar ring. Numbers of conidia moved from the channel to the outer ring were counted using an Olympus SZX 16 stereomicroscope (Olympus Corporation, Tokyo, Japan). Ant tracks on the outer ring were marked with a marking pen and distances over which ants moved conidia were measured.

![Figure 9.2. Design for fungal movement by ants experiment. *Iridomyrmex rufoniger* workers are visible on the central agar island in a 150 mm Petri dish. A conidial suspension of *Microcera coccophila* was placed in the channel surrounding the island and ants allowed to walk through it to the outer ring of agar.](image-url)
9.3. Results

9.3.1. Cultural characteristics

9.3.1.1. *Microcera coccophila*

*Microcera coccophila* grew slowly on PDA; from a single macroconidum, it took 14 d to attain a mean colony diameter of 20.2 ± 0.9 mm. The colonies were slimy, dark orange or red with whitish or light yellow mycelium on their edges and produced masses of macroconidia (Fig. 9.3). Colony shape varied from circular to slightly irregular, raised to flat. Pigmentation on the reverse side of the colony was light coral or salmon coloured. The fungus did not grow well on CLA, producing only a thin, hyaline layer of mycelium. Only small, sparse sporodochia were formed on CLA (Fig. 9.3). Cultures, established from single ascospores, produced colonies and macroconidia identical to those that developed from a single macroconidium.

In nature, sporodochia of *Microcera coccophila* usually form on the margin of the host scale (Fig 9.4). They can also be found growing on *Microcera coccophila* mycelium on plant substrates on which host scales are present. Their size and shape vary, and their colour ranges from orange to orange red\(^{54}\). The macroconidia are produced from phialides borne on conidiophores (Fig. 9.4b,c). A newly formed sporodochium containing macroconidia is illustrated in Fig 9.5a. An expanded sporodochium from which macroconidia are dispersing is illustrated in Fig. 9.5b.

![Figure 9.3](http://en.wikipedia.org/wiki/Web_colors#Color_table)

**Figure 9.3.** *Microcera coccophila* culture on PDA (left) and CLA (right).
Figure 9.4. Sporodochia of *Microcera coccophila* (a) on red scale, (b) on purple scale, (c) on yellow scale, and (d) on white louse scale.

Figure 9.5. Scanning electron micrographs of sporodochia of *Microcera coccophila*: (a) newly formed and (b) soft and expanded.
The macroconidia are hyaline, slightly curved, slender towards the ends with 7–9 septa, forming in sporodochia from short, cylindrical phialides. The foot and apical cells were difficult to distinguish (Fig. 9.6).

The shape of macroconidia formed in cultures did not differ from those formed on infected scales in nature (Fig. 9.7). The dimensions of macroconidia formed on infected scales in nature were 90–132 (mean = 105) µm long × 6–9 (mean = 7.2) µm wide (n = 50); on CLA, they were 84–114 (mean = 96) µm long × 6–8 (mean = 7.4) µm wide (n = 50) (Fig. 9.7). Microconidia and chlamydospores were not observed.

**Figure 9.6.** Scanning electron micrographs of macroconidia of *Microcera coccophila* (a & b) and macroconidia and conidiophore under a light microscope (c).

**Figure 9.7.** Macroconidia of *Microcera coccophila* from (a) a culture on CLA, and (b) infected scale in the field.

I did not observe perithecia in culture. However, perithecia were commonly formed in nature on infected scales. They were globose, with a small, ostiolar papilla. Immature perithecia are bright red, turning to dark red when mature. Their colour became slightly darker in 3% KOH and they turned light golden in 100% lactic acid. They are found in a group with an average of 12 (4–21) perithecia around the edge of each scale or
scattered on the surface of mycelium spreading over scales, and their host plant substrates. They are also found arising from the base of sporodochial stroma (Fig. 9.8). Perithecia were 194–387 (mean = 295) µm high and 194–355 (mean = 284) µm in diameter, and the perithecial wall consisted of two regions, the outer region being 24.4–42.1 (mean = 33.2) µm thick and the inner region being 9.3–13.7 (mean = 12.7) µm thick (Fig. 9.9d).

The asci were cylindrical, containing 8 ascospores (Fig. 9.9b), and the ascospores were hyaline, elliptical with rounded ends, constricted at septum. The ascospores consisted of two cells and were 14–19 (mean = 17.4) µm long × 6–10 (mean = 8.4) µm wide (Fig. 9.9c).

Figure 9.8.  (a) Mycelium, sporodochia and perithecia of Microcera coccophila on red scale, and (b) perithecia.
Figure 9.9. *Microcera coccophila*: (a) longitudinal section of a perithecium, (b) ascus, (c) germinating ascospore, and (d) perithecial wall.
9.3.1.2. *Microcera larvarum*

*Microcera larvarum* grew well on PDA and CLA from a single macroconidium or a single ascospore. On PDA, cultures derived from a single macroconidia and ascospores collected on scale insects in the field reached 30–35 mm after 15 d. The cultures contained off-white to light yellow, cottony mycelium with only a few small orange fruiting bodies formed after 15–20 d (Fig. 9.10b). There was variation in cultures on PDA (Fig. 9.10) that was possibly an effect of cultural degeneration: the cultures in Fig. 9.10c,d are subcultures. On CLA, a thin, transparent layer of mycelium spread on the agar plate and a few small pink fruiting bodies formed after about 15–20 d. The fungus also grew on SDA, on which colonies reached about 20 mm in diameter after 15 d. Colonies on SDA were irregular, raised, containing corn-silk-coloured, cottony mycelium and dark golden-red sporodochia.

![Figure 9.10](image_url)

*Figure 9.10.* *Microcera larvarum* cultured from field material (a, b), subcultures (c, d).
Macroconidia are hyaline, strongly curved with 3 septa. On CLA, macroconidia were 18.1–26.9 (mean = 22.2) μm long × 3.5–5.5 (mean = 4.4) μm wide (n = 30) (Fig. 9.11). The shape of macroconidia in culture was similar to that of macroconidia on infected scales in nature. Microconidia and chlamydospores were not observed.

![Figure 9.11. Microcera larvarum: macroconidia (left), conidiophores (right) cultured on CLA](image)

The perithecia and sporodochia of *Microcera larvarum* were commonly found in close association with the perithecia and sporodochia of *Microcera coccophila*. The perithecia of *Microcera larvarum* are globose, with a small, ostiolar papilla. Early stage perithecia are bright red, turning dark red when mature (Fig. 9.12). The perithecia are similar to those of *Microcera coccophila* and it is difficult to distinguish between them. However, in contrast to *Microcera coccophila*, both stages of *Microcera larvarum* were only found on bark of old branches. Perithecia of *Microcera larvarum* were sparsely distributed (Fig 9.12) while those of *Microcera coccophila* were normally in groups scale hosts and adjacent host plant tissues of the scales (Fig. 9.8b). Perithecia of *Microcera larvarum* were also found in close association with sporodochia of *Tubercularia coccicola* (Fig. 9.12).

*Microcera larvarum* asci are cylindrical, containing 8 ascospores. The asci were 88–120 (mean = 98) μm long × 8.4–13 (mean = 10.5) μm wide (n = 17) and the ascospores were 13.8–24.9 (mean = 17.7) μm long × 6.9–10.4 (mean = 8.4) μm wide (n = 30), hyaline, ovate, with one septum. The ascospores (Fig. 9.13) are longer and narrower than those of *Microcera coccophila* (Fig. 9.9).
Figure 9.12. Perithecia (red) of *Microcera larvarum* on a Washington navel orange tree branch in the presence of bright orange sporodochia of *Clonostachys coccicola* (see Chapter 10).

Figure 9.13. Asci and ascospores of *Microcera larvarum* collected from white louse scale on a branch of a Washington navel orange tree.
9.3.2. Field observations

During my studies in the citrus orchards, I observed *Microcera coccophila* on red scale, yellow scale, purple scale, Glover’s scale, and white louse scale (Fig. 9.4). The fungus killed all life cycle stages of the scales except crawlers, first instars and adult males. Mycelium developed around the edge of scale covers. Subsequently, scale bodies became soft, each containing mycelium, and were easily removed from host plant substrates, especially when dry. White mycelium that developed in and on scale bodies under the scale cover produced sporodochia. The sporodochia developed on the edge of the dead scale or on its scale cover and were bright orange-red, often forming conical upright masses on the surface of the host. The numbers of sporodochia varied from one to about ten. The size and shape of sporodochia also varied. The sporodochia became dry and shrivelled under the dry conditions. When conditions were moist and humid, the sporodochia became soft and expanded, and outer macroconidia became suspended in free water droplets around the scale. Scanning electron micrographs of sporodochia of *Microcera coccophila* and mycelium developing on the red scale are shown in Fig. 9.14.

![Figure 9.14.](image)

**Figure 9.14.** Sporodochia of *Microcera coccophila* on a mated female red scale (a); mycelium of *Microcera coccophila* underneath a red scale (b); and longitudinal section of a red scale and sporodochia growing on the scale (c).

Perithecia were usually more abundant during winter and under wet conditions. They were found more commonly on scales on the tree trunks, old branches, leaves and fruit than on young twigs, young leaves and immature fruit.
Microcera coccophila occurred in all of the study orchards, but the incidence was variable within and between blocks/orchards. In the orchards, where populations of soft scale were rare, such as at Somersby, Lower Portland, Cornwallis and Castlereagh, the sporodochia usually developed from mid to late autumn, increasing through winter and spring. The sporodochia rarely developed in summer and early autumn. In these orchards, the incidence of the fungus appeared to be distributed irregularly within blocks. It was more noticeable on the trees that were heavily infested with red scale or other armoured scales. Such trees were generally located on the edge of Washington navel orange blocks at Somersby and Lower Portland and the Valencia orange block at Castlereagh, or within both Washington navel/Valencia orange blocks at Cornwallis. On these trees that were heavily infested with red scale, Microcera coccophila occurred on scaly fruit, twigs and leaves in the outer parts of trees, particularly at Somersby and Castlereagh. In contrast, the fungus appeared to be more abundant on the old leaves and twigs, on which algae and moulds grew, in the lower part of trees. Whether the fungus prefers sunlight is not known and was not determined. In contrast to the orchards with low levels of soft scale, Microcera coccophila was present throughout the year in the orchards where levels of soft scale (particularly black scale) were high, such as at Kulnura in three 5 year-old blocks of Valencia orange block trees in Lister’s orchard at Kulnura and in one block of lemon trees in Hitchcock’s orchard. In these blocks, Microcera coccophila occurred on red scale that was associated with black scale infestations tended by ants (Iridomyrmex rufoniger). Fruiting bodies were observed on the branches, twigs, leaves and fruit with the highest incidence on the branches and twigs. The sporodochia tended to be evenly distributed on these branches and twigs regardless of their position in the canopy (Fig. 9.15 & 9.16).

Figure 9.15. Close-up of sporodochia of Microcera coccophila on a lemon branch heavily infested with red scale.
Figure 9.16. Sporodochia of *Microcera coccophila* on a lemon branch heavily infested with red scale. The infestation of red scale stemmed from ant activity associated with a heavy infestation of black scale that had at this point collapsed due to an epizootic of *Lecanicillium lecanii*. 
I observed conidia and perithecia of *Microcera larvarum* in the Washington navel orange block (block 2) in Britten’s orchard at Somersby, in the Eureka lemon block (block 1) in Hitchcock’s orchard, and in the immature Hamlin orange blocks in Lister’s orchard. It was common inside tree canopies on old, thick bark associated with algae, the putative entomopathogenic species recorded in Chapter 10, and dead organic matter. Perithecia tended to be concealed within fissures of old bark surface. The incidence of *Microcera larvarum* macroconidia in the field was closely associated with *Microcera coccophila*, and the other entomopathogens, but much less common.

### 9.3.3. Pathogenicity

#### 9.3.3.1. Pathogenicity of *Microcera coccophila* to oleander scale

Two trials were undertaken between 11 December 2009 and 4 February 2010, and 5 September and 15 October 2010. Average temperatures and relative humidity were 26.8°C and 83.8%, respectively. The majority (80%) of scale before treatment were second instar scale.

Percent scale mortality in inoculated treatments in the two trials was 90.3 ± 8.1 (n = 1997) and 94.6 ± 6.3% (n = 709). Infected scale bodies became brown and flimsy, dry and flat, and adhered on the fruit surface after death. Mycelium penetrated bodies of the scales before the insects died (Fig. 9.17). Percent mortality in the control was 11.6 ± 6.2 (n = 835) and 10.9 ± 6.3% (n = 462), respectively (Table 9.5). Live scales in the control treatment were turgid (Fig. 9.17) and produced eggs that developed to young stages. Dead scales in the control were brown; no mycelium or sporodochia was observed on dead scales in the control.

![Figure 9.17. Untreated scale (left), *Microcera coccophila*-treated oleander scale (centre) and fungal mycelium and fruiting bodies on treated oleander scale (right).](image-url)
Sporodochia were recorded approximately 25 d after the first spray in the 11 December 2009 to 4 February 2010 trial. These sporodochia were smaller and lighter in colour than observed on the armoured scale hosts in nature. However, the conidia were identical with those on scales in nature and in culture. Cultures obtained from these conidia were identical to those in the original cultures. Thus, Koch’s postulates were fulfilled.

Table 9.5. Pathogenicity of Microcera coccophila against Aspidiotus nerii.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Treatment</th>
<th>Control</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total number of scales</td>
<td>% mortality ± SE</td>
<td>Total number of scales</td>
</tr>
<tr>
<td>11 December 2009 to 4 February 2010</td>
<td>1997</td>
<td>90.3 ± 8.1</td>
<td>2678</td>
</tr>
<tr>
<td>5 September to 15 October 2010</td>
<td>709</td>
<td>94.6 ± 6.3</td>
<td>645</td>
</tr>
</tbody>
</table>

9.3.3.2. Pathogenicity of Microcera larvarum to oleander scale

A bioassay to determine if Microcera larvarum is an entomopathogen of armoured scales was undertaken from 21 September to 20 October 2011. Sprays were applied on three occasions: on 21, 24 and 28 of September. Two counts were undertaken, one 10 d (8 October) and the other 15 d (13 October), after the third spray. During the experiment the average temperature was 24.7°C and the average relative humidity 85.4%. The majority (circa 95%) of oleander scale on 21 September were immature third instar and gravid females.

Percent scale mortality on 8 October and 13 October was 38.4 (± 2.8) and 50.2% (± 2.9), respectively. Percent mortality in the control (water sprayed) was 3.2 (± 1) and 3.9% (± 0.6) (Table 9.6).

Table 9.6. Pathogenicity of Microcera larvarum against oleander scale, Aspidiotus nerii.

<table>
<thead>
<tr>
<th>Days after the third spray</th>
<th>Treatment</th>
<th>Control</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total number of scale</td>
<td>% mortality ± SE</td>
<td>Total number of scale</td>
</tr>
<tr>
<td>10</td>
<td>835</td>
<td>38.4 ± 2.8</td>
<td>895</td>
</tr>
<tr>
<td>15</td>
<td>462</td>
<td>50.2 ± 2.9</td>
<td>459</td>
</tr>
</tbody>
</table>
Dead scales in the *Microcera larvarum* treatment were observed after the second spray. Fruiting bodies were observed 5 d after the third spray. Dead scales in the *Microcera larvarum* treatment became dehydrated, hard, darkened and shrivelled and eventually turned black, but their scale covers appeared normal on most occasions when viewed from above. I observed pink sporodochia on the dead scale (Fig. 9.18a). Masses of *Microcera larvarum* macroconidia were expelled when scales killed by the fungus were placed in drop of water and gently crushed under a cover slip (Fig. 9.19) Live scales in the control treatment were turgid and producing eggs (Fig. 9.18b). Dead scales in this treatment were brown: no *Microcera larvarum* fruiting bodies or mycelium was observed. Cultures obtained from these conidia were identical to those in the original cultures. Thus, Koch’s postulates were fulfilled.

![Figure 9.18](image)

**Figure 9.18.** Fungal mycelium and sporodochia of *Microcera larvarum* on oleander scale sprayed with conidia of the fungus (a) and a scale sprayed with distilled water (b).

### 9.3.4. Movement of conidia of *Microcera coccophila* by *Iridomyrmex rufoniger*

*Iridomyrmex rufoniger* workers moved conidia from the channel containing a conidial suspension of *Microcera coccophila* to the outer agar ring in the Petri dishes in 14 of 16 observations. On average, each ant moved $29 \pm 8$ conidia from the channel to the outer ring of agar over an interval of a few seconds. The average maximum distance over which one or more conidia were moved by the ants was $31.4 \pm 5.2$ mm. In 10 of the 14 observations, the workers carried conidia on their legs, dragging the conidia over the agar surface. On the other four occasions, ant bodies were immersed in the conidial
suspension as they fell into the channel while attempting to move to the outer ring of agar. In these circumstances workers of *Iridomyrmex rufoniger* were observed to move tiny droplets of conidial suspensions.

In this experiment, the distance over which *Iridomyrmex rufoniger* moved conidia was restricted by the edge of Petri dishes. Some turned inwards when they came into contact with the rim of the dish; others ran over rims and out of the dishes. For the latter, further tracking was not possible.

![Figure 9.19. Macroconidia of *Microcera larvarum* being released from an oleander scale cadaver.](image)

### 9.3.5. Phylogenetic studies

Sequences of two genes (ITS and 28S rRNA) were successfully obtained from each of the 10 isolates of *Microcera coccophila* and five isolates of *Microcera larvarum*. Maximum parsimony analyses based on 1000 bootstraps are shown in Figs 9.20 (ITS) & 9.21 (28S rRNA). Sequences of the ITS gene comprised 570 base pairs of which 37 positions were parsimony informative. There were two clades (Fig. 9.20): *Microcera coccophila* and *Microcera larvarum*, supported by 100 and 94% bootstrap values, respectively. In the *Microcera coccophila* clade, variation only occurred in one base: in position 84, it was character T in group 1 and C in group 2. Based on this variation, these two groups were supported by 64 and 77% bootstraps values.
The GenBank accession sequence HQ897794 (Gräfenhan et al. 2011), was sister to the two *Microcera coccophila* groups, with 77% bootstrap support. However, bootstrap support for separation from other accessions was weak, with 64% bootstrap support. The first group (group 1) included isolates from Somersby, Cornwallis, Richmond and Castlereagh. The second group (group 2) included isolates from Somersby, Kulnura, Lower Portland and Castlereagh. The sequence of the isolate from white louse scale was identical with that of isolates on red scale in the first group. Sequences of the isolates from yellow scale and purple scale were identical with those on red scale in the second group. The sequence of the isolate cultured from a single ascospore (AaS2*) was identical with those from conidia. In the *Microcera larvarum* clade, there were two groups. The first group, which was separated from the second group by 53% bootstrap support, comprised three *Microcera larvarum* isolates, AsS2, AsK4 and AsK5*. In the second group, the *Microcera larvarum* isolates AsS1* and AsS3* were identical with the *Microcera larvarum* GenBank accession EU860063. The GenBank sequence of *Microcera larvarum* var. *rubrum*, EU860069 accession, was sister to the two *Microcera larvarum* groups. Variation between these two groups of *Microcera larvarum* occurred within 3 bases.

The 28S rRNA sequences of *Microcera coccophila* and *Microcera larvarum* was comprised of 552 base pairs of which 21 base pairs were parsimony informative. There were two clades representing *Microcera coccophila* and *Microcera larvarum*, each with 100% bootstrap support (Fig. 9.21). In *Microcera coccophila*, the variation among the 10 isolates occurred within 1 of these base pairs at position 4, where there was a T in group 1 and a C in group 2. Based on this variation, sequences were separated into two groups with only 62% bootstrap support. The arrangement of sequences of 10 isolates in these two groups were the same as for the ITS gene. Sequences of the group 2 obtained in this study were identical with that of *Microcera coccophila* (cited as *Nectria flammea*) from GenBank (accession U88103, O'Donnell 1993). I did not include this Genbank sequence data in the phylogenetic tree as it had only 481 base pairs and did not contain the positions in which the variation between two groups occurred. The 28S rRNA sequence of the isolate cultured from a single ascospore was identical with that from macroconidia. Likewise, the 28S rRNA sequence of the isolate cultured from a single ascospore was identical with that from macroconidia. In the *Microcera larvarum* clade, there were two groups. In the first group, two *Microcera larvarum* isolates, AsS1* and AsS3*, were identical with the *Microcera larvarum* GenBank accession
EU870064. The second group, that separated from the first group by 72% bootstrap support, comprised two isolates, AsK4 and AsK5*. The variation between these two groups occurred within 6 bases. The GenBank sequence of *Microcera larvarum* var. *rubrum*, accession EU860073, was sister to these two groups of *Microcera larvarum*. Sequences of the isolates cultured from single ascospores were identical with those from macroconidia.

**9.4. Discussion**

The morphological and molecular studies confirmed the identity of the anamorphic and teleomorphic states of *Microcera coccophila*, the most common of the two red-headed fungi observed in the field during these studies. The morphology of cultures and macroconidia produced from macroconidia were identical with those produced from a single ascospore. My descriptions of the asexual and sexual states of the fungus are similar to those of Tryon (1889), McAlpine (1899), Fawcett (1908), Petch (1921a,b), Dingley (1954), Booth (1971) and Joffe (1974). My study represents the first occasion that *Microcera coccophila* has been cultured from ascospores. Thus, I was able to resolve the uncertainty and confusion about the connection between the anamorph and teleomorph of *Microcera coccophila* that has prevailed since 1861 when *Sphaerostilbe coccophila* was first considered to be the teleomorph (Tulasne & Tulasne 1861, Petch 1921a,b, Gräfenhan et al. 2011) after Jean Baptiste Henri Joseph Desmazières described the anamorph as *Microcera coccophila* in 1848.

Although I did not record the incidence of *Microcera coccophila* in relation to scale densities, my observations indicate that it was more common on red scale in my study orchards than *Microcera larvarum* and the other four entomopathogens that I recorded (see Chapter 10). *Microcera larvarum* was far less common than *Microcera coccophila*. Parkin (1906) mentioned that *Microcera larvarum* (cited as *Nectria aurantiicola*) was common on scale insects in the tropics. Differences in climate could be the reason for its low incidence in my orchards, as all were located in the temperate coastal region of New South Wales. Differences in the frequency of occurrence may also have been due to earlier misidentifications, as the conidial states of the two species are more distinct on the specimens collected in tropical zones than those collected in temperate zones (Petch 1921a, b).
Figure 9.20. 50% majority-rule bootstrap consensus tree of the ITS region of accessions of Microcera coccophila and Microcera larvarum derived from maximum parsimony analysis. Cosmospora coccinea was used as the out-group and bootstrap values are provided as percentages from 1000 replications. * Cultured from a single ascospore.
Figure 9.21. 50% majority-rule bootstrap consensus tree of the 28S ribosomal RNA gene of accessions of *Microcera coccophila* and *Microcera larvarum* derived from maximum parsimony analysis. *Cosmospora coccinea* was used as the out-group and bootstrap values are provided as percentages from 1000 replications. * Cultured from a single ascospore.
Microcera coccophila was first recognised as a pathogen of red scale in Australia by Tryon (1889) in citrus orchards near Toowoomba (average annual rainfall about 950 mm) in Queensland. Shortly afterwards, Koebele (1892) reported it from orchards in suburbs in the Sydney area (average annual rainfall about 1200 mm) within 30 km of the city centre. Shortly afterwards, Tryon (1894) recorded it on red scale, circular black scale and Glover’s scale in citrus orchards near Maryborough (average annual rainfall about 1150 mm) in the Wide Bay area of Queensland in 1894, and McAlpine (1899) reported it from the Royal Botanic Gardens, Sydney, and also from Queensland (presumably Brisbane). Summerville (1934) and Hely et al. (1982) noted that it was confined to humid regions of Queensland and New South Wales and that it was more common in wet seasons than in dry seasons. Hely et al. (1982) mentioned that it seemed to attack mainly weakened scales. Tryon (1889) noted it was abundant when red scale occurred in the presence of black scale, but he did not mention ant activity associated with the latter. Annecke (1963) observed Microcera coccophila to be more abundant in the more damp and humid orchards, and in the presence of large numbers of red scale and circular black scale, in the area between Dondo (19° 36' S, 34° 44' E) and Manica (18° 56' S, 32° 52' E) in Mozambique. He commented that ants did not seem to affect the abundance of the fungus. However, he noted that the fungus was abundant on heavy infestations of red scale, on woody parts of the young tree including stems, twigs and fruit (Annecke 1963). This observation suggests that such abundance of the fungus could relate to the activity of ants that tend the soft scales on young trees. Tryon (1894) regarded it, together with a minute parasitoid, possibly Encarsia citrina, to hold red scale in check in citrus orchards in the Wide Bay area of Queensland.

My field observations indicate that factors such as climate, scale densities and presence of ants influence the distribution and abundance of Microcera coccophila and Microcera larvarum fungi, which were most common during warm wet and humid weather. Similar observations were reported by Hely et al. (1982) and Smith et al. (1997). It is not known to occur in the relatively dry, inland, citrus-producing regions. Rob Weppler (Riverina IPM, pers. comm., 2010) has not observed it in Griffith (34° 17' S, 146° 02' E: average annual rainfall 402 mm) in inland in New South Wales, and I did not observe it when I visited there in September 2010. Rainfall and dew appeared to increase spread of conidia on branches, leaves and fruit in my study orchards. Fruiting bodies expanded under such conditions. This observation was similar to that reported
by Petch (1921b) who said, of specimens examined from Sri Lanka, India, Taiwan, Madagascar, the West Indies, Georgia and Florida in the United States of America, and Italy, ‘The species of Microcera on scale insects are orange red and subtranslucent when fresh, and become brownish red, hard, and horny when dry. When placed in water, the head expands and the outer conidia float off.’

I also observed Microcera coccophila attacking second instar and pupal male stages of red scale. These stages have previously been considered non-hosts of the fungus. It was most common on red scale, moderately common on purple scale and Glover’s scale, and least common on yellow scale, on which I rarely observed it. These observations accord with comments by Hely et al. (1982) and Smith et al. (1997) who mentioned Microcera coccophila occurring on red scale, purple scale, Glover’s scale, but not on yellow scale. This could be due to the preferred habitats of scales. My observations indicate that the pathogen normally colonises scale on the outer parts of trees where red scale is most abundant within tree canopies. In contrast, the pathogen was less common in inner parts of tree canopies where yellow scale is more common, particularly on lower surfaces of leaves.

Microcera coccophila has been reported as an entomopathogen of purple scale elsewhere, including Florida (Rolfs 1897, Fawcett 1908, Rolfs & Fawcett 1908, Watson 1915, Petch 1921a,b, Berger 1942, Fisher et al. 1949, Fisher 1950, Gräfenhan et al. 2011). In addition to Australia and Florida, the fungus has been recorded in France, the United Kingdom, Philippines, Sri Lanka, South Africa, China, Malaysia, Zambia, New Zealand, Tanzania and Uganda, as listed in Appendix II. However, given the history of confusion and misidentifications, molecular studies are required to verify these records.

My pathogenicity tests provided further evidence that Microcera coccophila is an entomopathogen with high infectivity against armoured scales under favourable conditions, and Koch’s postulates were fulfilled. Although pathogenicity of Microcera coccophila was reported by Rolfs (1897), the fungus was subsequently regarded as a saprophyte by Thomson (1939), Fisher et al (1949), Fisher (1950b), Ziegler (1949) and Kuno & Ferrer (1973).

Rolfs (1897) conducted eight field experiments on San José scale-infested peach trees (Prunus persica (L.) Batsch)) to demonstrate the pathogenicity of the fungus. Under favourable conditions in four of the experiments, the fungus killed the scale. Rolfs
(1987) described artificial dissemination methods by means of diseased scales or spores cultured on slightly acid bread. Branches with diseased scales were tied onto scale-infested branches where the pathogen was either absent or present at low levels. In addition, conidial suspensions were applied to scale-infested plants with a sponge or cloth, or in sprays. Fawcett (1908), Rolfs & Fawcett (1908) and Watson & Berger (1932) also mentioned methods for applying the fungus in sprays and distributing it to scale infested trees in peach and orange groves in Florida by means of leaf-pinning or short pieces of wood. Rolfs & Fawcett (1908) mentioned that the latter was sold commercially by Mr FP Henderson of Gainsville in Florida.

In contrast to the above accounts, Ziegler (1949) found no evidence of a host-parasite relationship between the ‘red-headed scale fungus’ and the scales. Fisher et al. (1949) regarded the ‘red-headed’ fungus, *Microcera coccophila* (as *Sphaerostilbe aurantiicola*) and the ‘pink-headed’ fungus (cited as *Nectria diploa*) as saprophytes. In her review, Fisher (1950) of reports by numerous authors in Florida from the late 1890s to 1940s, she found no evidence to sustain or reject views that red-headed fungi were entomopathogens of armoured scales. Moreover, Fisher et al. (1949) reported that pathogenicity tests for both of these fungi were negative in tests in which spores from cultures and mature fruiting bodies were applied as sprays in water suspensions to first, second and third instar circular black scale and purple scale of grapefruit. However, they (Fisher et al. 1949) did not mention concentrations of conidia used in spray suspensions or the number of occasions on which sprays were applied to fruit infested with either red scale or purple scale. Furthermore, they did not mention the prevailing conditions under which they conducted field experiments in which sprays with conidial suspension were applied to purple scale-infested sweet orange trees. Fisher (1950), citing Earle (1899), stated that the fungus was entirely inefficient against San José scale on apple in Alabama. However, Earle (1899) noted that the fungus was common and grew abundantly on obscure scale, *Melanaspis obscura* (Comstock) (see Appendix II). When he tied bark of water oak (*Quercus nigra* L.[Fagales: Fagaceae]) infested with this fungus on some San José scale infested apple branches he attributed slow spread of this fungus to dry weather (Earle 1899). The apparent low level of effectiveness of the fungus against purple scale in Florida suggests that its spread within citrus canopies may be limited to dispersal of conidia by wind and rain, as none of the above authors mentioned ants associated with armoured scales, soft scales and *Microcera coccophila*. 
Kuno & Ferrer (1973) tested the pathogenicity of ‘Fusarium roseum’ and ‘Fusarium episphaeria’ (nomen confusa: Lester Burgess, University of Sydney, 1 December 2011) against rufous scale (Selenaspis articulates (Morgan)) by either spraying the scale with fungal suspension and also lifting scale cover and inoculating the scale body with small amount of the suspension. Kuno & Ferrer (1973) recorded no significant differences in mortality of adult and second instar between treatment and control and concluded that these two fungi are saprophytic in nature. Kuno & Ferrer (1973) regarded ‘Fusarium episphaeria’ as a synonym of Sphaerostilbe aurantiicola (possibly Microcera larvarum) and Nectria coccophila. However, they did not provide any evidence to confirm the identities of the fungi. According to these authors, a culture of ‘Fusarium episphaeria’ after 72 d and ‘Fusarium roseum’ after 32 d completely covered the surface of PDA in 200 mm × 1.5 mm Petri dishes. The fast growth of both fungi suggests that they were probably not Microcera coccophila. The low infectivity could also be related to application procedures. Moreover, Kuno & Ferrer (1973) did not mention any replication or the duration of the experiment. From my experiment, repeated spraying and subsequent application of water created resulted in conditions similar to those required in nature for Microcera coccophila to infect its hosts.

Impacts of fungicides on the incidence of Microcera coccophila were not considered in this study. However, Reinking (1921) reported that Bordeaux mixture or other copper sprays that killed Microcera coccophila resulted in increased severity of scale infestations. Miller (1937) and Thomson (1939) also reported that copper fungicides destroyed the ‘beneficial fungi’ or ‘scale fungi’ on citrus groves in Florida. Thomson (1939) concluded that heavy residues of copper and zinc sprays inhibited the growth of entomopathogenic fungi, thus resulting in favourable conditions for purple scale in Florida. Fisher (1949) conducted an experiment to examine the growth of red-headed fungi on purple scale sprayed with oil emulsion, copper-oil emulsion, copper, zinc, lime, wettable sulphur and control. Fisher (1949) recorded higher percentages of fungi on living scale on trees sprayed with an oil emulsion than on living scale on unsprayed trees, trees sprayed with copper-oil emulsion, and on trees sprayed with copper, zinc, lime and wettable sulphur. In contrast, Ziegler (1949) reported that the increase in scale populations after application of Bordeaux appeared to be due to the build-up of a residue that protected the scale, not to the sprays killing the fungus. Ziegler (1949) also reported that Bordeaux sprays with heavy residues of lime resulted in higher infestation of the scale than Coposil (a non residue-forming fungicide (copper
zinc hydroxide sulphate)) sprays and that application of mineral oil emulsions increased fungal abundance. This suggests that the oil may have enhanced the infectivity of the fungi by increasing the survival of fungal conidia perhaps by reducing desiccation. It is well known that mineral oils increase survival of fungal conidia and enhance their infectivity against targeted insects (Inglis et al. 2002). The high mortality of scale in the presence of oil also suggests that the oil may have weakened the scale making it more prone to infection by the fungi.

Watson & Berger (1932) also reported that fungicide sprays had detrimental impacts on entomopathogens in Florida, resulting in vast increases in purple scale populations. They noted that it was possible to kill a citrus tree in the course of a year or so by repeated sprays of Bordeaux and recommended application of an oil emulsion within two months of a Bordeaux spray in order to forestall increases in scale populations.

Based on the literature, I assumed that the use of Bordeaux to control citrus diseases and the use of lime sulphur for control of white louse scale in coastal orchards of New South Wales (see Hely et al. 1982) may have influenced the abundance of Microcera coccophila and Microcera larvarum. Use of zineb for control of citrus rust mites (Phyllocoptruta oleivora (Ashmead) and Tegolophus australis Keifer) [Acarina: Eriophyidae]) may have led to the low incidence of Microcera coccophila in the orchard I used for my studies at Cornwallis. It would be of interest to assess the influences of fungicides, including copper and oil formulations on the abundance of entomopathogenic fungi of armoured scale insects on the Central Coast of New South Wales. The ease with which I cultured Microcera coccophila and Microcera larvarum suggests that it should be feasible to compare impacts of fungicides and insecticides on these fungi, as was done in laboratory studies by Hall (1981) with Lecanicillium lecanii.

Ants are known to disperse fungal conidia. Gracia-Garza et al. (1997) observed that the ants, Pheidole spp. and Pheidole megacephala (Fabricius), passively carried propagules of the plant pathogen, Fusarium oxysporum Schlechtend: Fr. f. sp. erythroxyli, outside their bodies, as well as either closely adhering to the outside or within their bodies. Bird et al. (2004) reported that workers of the common black ant, Lasius niger L., were able to carry conidia of the entomopathogenic fungus, Lecanicillium longisporum, attacking rosy apple aphid, Dysaphis plantaginea (Passerini), on their tarsae, antennae and mandibles after they were inoculated with fungal conidia under laboratory and field conditions. Azteca instabilis (Smith), an arboreal-dwelling ant, is also capable of
transporting Lecanicillium lecanii spores on coffee bushes, but other mechanisms such as the wind or any of the sundry flying or crawling insects dominate (Doug Jackson, University of Michigan, pers. comm., 5 December 2011). During my field studies, I tested several methods for capturing ants to determine the presence of conidia of Microcera coccophila on their bodies. Workers of Iridomyrmex rufoniger collected from lemon trees with high incidence of red scale and Microcera coccophila were brought to the laboratory and placed on 2% water-agar plates that were subsequently examined for development of fungal cultures. These tests were not successful as fungal contaminants generally grew more quickly than entomopathogens. I finally used the method, as presented above, for which culturing of fungi was not required. These tests confirmed that ants moved conidia of Microcera coccophila in situ in the laboratory. My results suggest that in nature, in the presence of soft scales, wet plant surfaces as occur during and after rain and with heavy dew and high humidity, the movement of conidia by ants leads to fungal epizootics of Microcera coccophila.

My molecular studies were the first to be undertaken with isolates of Microcera coccophila and Microcera larvarum from Australia. Although based on a small number of isolates, my phylogenetic results indicated low variation of both gene regions among the isolates. This suggested that the sequences of the ITS and 28S rRNA regions were relatively similar regardless of the locations from which the isolates were collected, host scales, and forms of the fungus that were cultured. The variation between my ITS sequences and the Genbank dataset HQ897794 (Gräfenhan et al. 2011) suggested that variation occurs among specimens regarded morphologically as Microcera coccophila. This is consistent with the occurrence of several ‘phylogenetic species’ that Gräfenhan et al. (2008) detected among anamorph and teleomorph collections morphologically similar to Microcera coccophila, Microcera diploa and Microcera larvarum. My study was also the first to compare phylogenetic variation of Microcera coccophila and Microcera larvarum among different host scales in Australia, but more rigorous studies are required to verify these observations. Moreover, it would be of interest to determine phylogenetic variation among isolates of Microcera coccophila from scales from a wide range of host plants as listed in Appendix II and different bioclimatic regions where the plants were recorded as hosts. It would also be of interest to determine if Microcera species are endophytes in these plants. Summerell et al. (2011) stated that species of Fusarium (then including Microcera) can be recovered from plants, soils as pathogens, endophytes and saprobes. Fusarium oxysporum has been recognised as an endophyte
that is an effective entomopathogen of burrowing nematode, *Radopholus similis* (Cobb) Thorne [Tylrenchidae: Pratylenchidae], in banana (*Musa* spp. [Zingiberales: Musaceae]) (Athman et al. 2006). Results presented in Chapter 10 suggest that species of *Myriangium* are possibly endophytes in citrus trees. Based on reports for citrus and other plants, the known entomopathogenic endophytes that may occur on citrus are *Lecanicillium lecanii* that readily colonises cotton (*Gossypium hirsutum* L. [Malvales: Malvaceae]) and *Aphis gossypii* Glover [Hemiptera: Aphididae] (Gurulingappa et al. 2011).

My studies indicate the presence of *Microcera larvarum* attacking armoured scale insects in citrus orchards on Central Coast of New South Wales. The teleomorph of *Microcera larvarum* was first found on scale insects in Sri Lanka (cited as Ceylon) and described as *Nectria aurantiicola* by Berkeley & Broome in 1873 (Petch 1921a,b). The anamorph was subsequently described by Thomas Petch as *Microcera aurantiicola* (Petch 1921b). Synonyms of the teleomorph include *Sphaerostilbe aurantiicola* (Berk. & Br.) Petch, *Corallomyces aurantiicola* (Berk. & Br.) Höhn. (Booth 1971) and *Cosmospora larvarum* (Tyson et al. 2005). Synonyms of the anamorph include *Fusarium larvarum* Fuckel, *Microcera parlatoria* Trab., *Microcera curta* Sacc., *Microcera tonduzii* Pat. (Gräfenhan et al. 2011).

My morphological and molecular results confirm the identity of *Microcera larvarum*. My morphological descriptions of the anamorph and the teleomorph of *Microcera larvarum* were similar to those reported by Petch (1921b), Booth (1971) and Gräfenhan et al. (2011). My sequences of the ITS and 28S rDNA regions were identical with those of *Microcera larvarum* in GenBank (Bills et al. 2009, Gräfenhan et al. 2011).

My studies showed that the morphology of colonies and macroconidia of *Microcera larvarum* cultured from field-collected specimen anamorph and teleomorph states were identical. The 28S rDNA gene sequences of these cultures were also identical. As a result, I was able to confirm the relationship between the anamorph and teleomorph of *Microcera larvarum*.

My observations on the occurrence of *Microcera larvarum* in the field in association with *Microcera coccophila* and other fungi were similar to those reported by Petch (1921a) and Dingley (1950). Petch (1921a) noted that ‘One mounts the ordinary *Microcera* synnema, or an isolated perithecium, and finds the small curved conidium on the slide. It apparently
occurs on the slight weft of mycelium at the base of the perithecium’. Dingley (1950) described the conidial stage of ‘Nectria aurantiicola’ as ‘conidiophores united to form a stalklike or pulvinate stroma, coral or scarlet, conidia 6-9 septate, cylindrical, filiform, sometimes falcate, apex apiculate 60-90 x 4-5.5μ a few sporidia 3 septate, crescent shaped 12-13 x 4μ’. However, Petch (1921b) based his description of Microcera coccophila on both Microcera coccophila and Microcera larvarum (see Booth 1971), and Dingley (1950) based her descriptions of both species (cited Nectria aurantiicola and Nectria flammea) on mixtures of conidia of both species, the errors arising from the often close association of the two species in nature, particularly on mature bark.

In contrast to Microcera coccophila, Microcera larvarum appears to be a common species in the tropics, extending occasionally into temperate regions (Petch 1921a,b). McAlpine (1899) recorded Fusarium epicoccum McAlpine on red scale on branches of a mandarin tree in Burnley, a suburb of Melbourne, in Victoria. He noted that conidia of Microcera larvarum were long and twice as broad as those of Fusarium epicoccum. However, the taxonomic status of Fusarium epicoccum is uncertain. There are no published records of Fusarium epicoccum aside from McAlpine (1899) and Petch (1921b), and Petch (1921b) appeared to regard it as Microcera larvarum at some points but not at others. If the species described by McAlpine (1899) was not Microcera larvarum, then my study is the first to record Microcera larvarum in Australia.

My results for pathogenicity fulfilled the Koch’s postulates for Microcera larvarum. The fungus readily killed oleander scale and produced sporodochia. However, mortality caused by Microcera larvarum was not as high as those caused by Microcera coccophila. I attributed this to use of the late stage of oleander scale I used. The majority of scales were third instar scale when the trial was commenced. If I had used second instar scale, mortalities caused by the fungus may have been higher.

Cozzi et al. (2002) tested Microcera larvarum at 10^5, 10^6 and 10^7 CFU/20 mL against black scale on olive leaves in the field. They recorded 65–70% reductions of black scale populations, as compared with the control after 7 d, regardless of fungal concentration. Although I did not study entomopathogens of black scale, my general observations suggest that the form of Microcera larvarum tested by Cozzi et al. (2002) may not have been the same as mine. Smirnoff (1970) recorded Microcera larvarum attacking Adelges piceae (Ratz.) [Hemiptera: Adelgidae] on fir trees [Pinales: Pinaceae] on the Gaspé Peninsula in Canada. He tested the pathogenicity of Microcera larvarum
against six aphid species, but no mortality was caused by the fungus. In the test against *Adelges piceae*, only 20–30% of dead individuals were covered with mycelium. It was warm and dry when the test was conducted and thus was not possible to accurately estimate mortality caused by the pathogen due to a high level of natural mortality (Smirnoff 1970).

Thomson (1939), Fisher et al. (1949), Ziegler (1949) and Fisher (1950b) regarded *Microcera larvarum* (cited as *Sphaerostilbe aurantiicola* (Berk. & Br.) as saprophytic on citrus trees in Florida, though it is uncertain whether the fungus they referred to was *Microcera coccophila* or *Microcera larvarum*, or both. They did not record pathogenicity of the ‘red-headed’ fungus and concluded that it was saprophytic. Their conclusions about the pathogenicity of *Microcera coccophila* and/or *Microcera larvarum* were apparently based on inappropriate procedures. Their conclusions led to the neglect of research on entomopathogenic fungi of armoured scales in Florida since the late 1940s. It may be coincidental, but the views of Fisher and Ziegler appear to have coincided with a period of increasing promotion and use of fungicides for control of plant pathogens of citrus in Florida. Rossman (1978) mentioned that the role of entomopathogenic fungi in Florida has undoubtedly been usurped by chemical sprays. The success of the integrated pest management program for citrus pests and diseases in Australia in the past 30 years and reduced use of Bordeaux sprays may have contributed to the abundance of entomopathogens I observed in this study, as reported in this chapter and in Chapter 10.
Chapter 10. Studies on four other fungi associated with armoured scales on citrus: descriptions and molecular verifications

10.1. Introduction

During the field studies on *Microcera coccophila* and *Microcera larvarum* (Chapter 9), four other fungi, all putative entomopathogens, were observed in association with armoured scales. On the basis of morphological studies, these fungi were identified, on the basis of accepted names when the studies commenced, as:

- the teleomorph, *Podonectria coccicola* (Ellis & Everh.) Petch, and its anamorph, *Tetracrium coccicolum* Höhl. (Ellis & Everh.) [Pleosporales: Tubeufiaceae], known as the ‘white-headed’ fungus, based on the appearance of the anamorph;
- the teleomorph, *Podonectria novae-zealandiae* Dingley, and its previously undescribed anamorph, here given the common name ‘pin-cushion fungus’, based on the appearance of the anamorph;
- the teleomorph, *Nectria tuberculariae* Petch, and anamorph, *Tubercularia coccicola* Stevenson [Hypocreales: Nectriaceae], here given the common name ‘pink-headed’ fungus; and
- the teleomorph, *Myriangium citri* Henn. [Myriangiales: Myriangiaceae], of the ‘black-headed’ fungus: this species was identified by Dr Michael Priest, curator of the New South Wales Herbarium, Orange Agricultural Institute, Agriculture New South Wales.

The objectives of the studies reported here are to examine the morphology and molecular biology of these fungi, and to sequence appropriate markers in order to verify their identities. In addition to these objectives, I report on the appearance and distribution of these fungi and their abundance within tree canopies. Under recent changes to the International Code of Nomenclature for fungi, plants and algae, single-name nomenclature employing the oldest generic name in combination with the oldest species epithet should be used, irrespective of whether these names could be interpreted as teleomorphic or anamorphic (see Gräfenhan et al. 2011). To comply with this...
requirement and in order to minimise confusion, I therefore refer to the fungi by their oldest valid generic name throughout this chapter, aside from some instances where references are made to the literature. *Podonectria* was established by Petch (1921a,b) and *Tetracrium* by Hennings (1902); therefore, *Tetracrium* has precedence and *Podonectria coccicola* will be referred to as *Tetracrium coccicolum*, and *Podonectria novae-zealandiae* as *Tetracrium novae-zealandiae* despite the anamorph being described for the first time in this study. *Tubercularia* was established by Tode (1790) and *Nectria* by Fries (1849). Therefore, the ‘pink-headed’ fungus will be referred to as *Tubercularia coccicola*. *Myriangium* was established by Montagne & Berkeley in Berkeley (1845). Batista & Silva Maia (1957) described what they regarded as the anamorph of *Myriangium citri*, but they did not name it, and the description has not been verified by other authors. Therefore, the ‘black-headed’ fungus will be referred to as *Myriangium citri*.

### 10.2. Materials and methods

#### 10.2.1. Morphological studies

Samples of infected armoured scale insects were collected in citrus orchards on the Central Coast of New South Wales (Table 10.1).

The fungi were isolated directly into pure culture using conidia obtained from sporodochia of the anamorphs of *Tetracrium coccicolum*, *Tetracrium novae-zealandiae*, and *Tubercularia coccicola* and using ascospores obtained from perithecia of *Tetracrium novae-zealandiae*, and ascospores obtained from apothecia of *Myriangium citri* on scale cadavers. Pure cultures were obtained using the single-spore method on potato dextrose agar (PDA), carnation leaf agar (CLA) and/or on Sabouraud dextrose agar (SDA) (Oxoid Ltd., Basingstoke, Hampshire, England). All cultures were incubated in the laboratory at 25°C under fluorescent and filtered daylight for 10–14 h/day. *Tetracrium coccicolum* and *Tetracrium novae-zealandiae* were also cultured on small, thin, sterile strips of butternut pumpkin fruit peel infested with dead oleander scale (*Aspidiotus nerii*) placed on PDA.
Table 10.1. Geographic origin, host scales and plant hosts of scales from which four putative, entomopathogenic fungi were isolated.

<table>
<thead>
<tr>
<th>Accession number</th>
<th>Host scale</th>
<th>Host plant</th>
<th>Location</th>
<th>Latitude &amp; longitude</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tetracrium coccicolum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLbCo</td>
<td><em>L. beckii</em></td>
<td>Washington navel orange</td>
<td>Cornwallis</td>
<td>33° 35’ S, 150° 49’ E</td>
</tr>
<tr>
<td>PAaK</td>
<td><em>A. auranti</em>*</td>
<td>Hamlin orange</td>
<td>Kulnura</td>
<td>33° 14’ S, 151° 13’ E</td>
</tr>
<tr>
<td>PLbK</td>
<td><em>L. beckii</em></td>
<td>Valencia orange</td>
<td>Kulnura</td>
<td>33° 14’ S, 151° 13’ E</td>
</tr>
<tr>
<td>PUCs10</td>
<td><em>U. citri</em></td>
<td>Washington navel orange</td>
<td>Somersby</td>
<td>33° 22’ S, 151° 16’ E</td>
</tr>
<tr>
<td>PUCs15</td>
<td><em>U. citri</em></td>
<td>Washington navel orange</td>
<td>Somersby</td>
<td>33° 22’ S, 151° 16’ E</td>
</tr>
<tr>
<td><strong>Tetracrium novae-zealandiae</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAsS1</td>
<td>armoured scale</td>
<td>Washington navel orange</td>
<td>Somersby</td>
<td>33° 22’ S, 151° 16’ E</td>
</tr>
<tr>
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<td><em>A. auranti</em>*</td>
<td>Washington navel orange</td>
<td>Kulnura</td>
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<td><em>L. beckii</em></td>
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<td>Somersby</td>
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<td>Somersby</td>
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<td>Somersby</td>
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<td>PUCs14*</td>
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<td>Somersby</td>
<td>33° 22’ S, 151° 16’ E</td>
</tr>
<tr>
<td><strong>Tubercularia coccicola</strong></td>
<td></td>
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</tr>
<tr>
<td>BAAaS</td>
<td><em>A. auranti</em>*</td>
<td>Valencia orange</td>
<td>Somersby</td>
<td>33° 22’ S, 151° 16’ E</td>
</tr>
<tr>
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<td>Somersby</td>
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</tr>
<tr>
<td>BLbS</td>
<td><em>L. beckii</em></td>
<td>Washington navel orange</td>
<td>Somersby</td>
<td>33° 22’ S, 151° 16’ E</td>
</tr>
<tr>
<td><strong>Myriangium citri</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAsS1</td>
<td>armoured scale</td>
<td>Washington navel orange</td>
<td>Somersby</td>
<td>33° 22’ S, 151° 16’ E</td>
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<td>MAsS2</td>
<td>armoured scale</td>
<td>Washington navel orange</td>
<td>Somersby</td>
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<td>MAaK</td>
<td><em>A. auranti</em>*</td>
<td>Eureka lemon</td>
<td>Kulnura</td>
<td>33° 14’ S, 151° 13’ E</td>
</tr>
</tbody>
</table>

* Cultured from a single ascospore.

After incubation for 15–50 d (the duration depending on fungal species), cultures were examined for the following features: shape, size and mode formation of conidia. The appearance including pigmentation of colonies on CLA, PDA and SDA was also described. Dimensions of conidia, ascospores, asci and numbers of septa were assessed using an Olympus BX60 compound microscope (Olympus Corporation, Tokyo, Japan) fitted with a ProgRes C14 digital camera. Measurements of conidia and numbers of septa of *Tetracrium coccicolum* were based on field samples of sporodochia and on cultures on small, thin, sterile strips of butternut pumpkin fruit peel infested with oleander scale placed on PDA and from field samples of sporodochia. Measurements of conidia and numbers of septa of *Tetracrium novae-zealandiae* were based on field samples of sporodochia. Measurements of conidia of *Tubercularia coccicola* were
based on cultures of field samples of sporodochia and cultures on CLA. Measurements of ascospores, asci and number of septa of *Tetracrium coccicolum*, *Tetracrium novae-zealandiae* and *Myriangium citri* were based on field samples of perithecia. Heights and diameters of perithecia were measured using a stereomicroscope. Colony diameters were also assessed. Infected scales, fruiting bodies and conidia of the anamorphs of *Tetracrium coccicolum*, *Tetracrium novae-zealandiae* and *Tubercularia coccicola* were also photographed using a JSM-6512 scanning electron microscope. The thin sections of perithecia were taken using a Leica CM1510S microtome (Leica Microsystems Pty Ltd., North Ryde, Australia). One isolate of each species was deposited at the New South Wales Plant Pathology Herbarium (DAR AU 073), Orange Agricultural Institute, Orange, New South Wales.

### 10.2.2. Molecular studies

Extractions of DNA from cultures of *Tetracrium coccicolum*, *Tetracrium novae-zealandiae*, *Tubercularia coccicola* and *Myriangium citri* were based on the methods described in Chapter 9 for *Microcera coccophila* and *Microcera larvarum*. In each instance, pure cultures grown on PDA were used. A REDExtract-N-Amp™ Plant PCR Kit (Sigma-Aldrich Pty. Ltd., Sydney, Australia) was used for two isolates of *Tetracrium coccicolum* only. Procedures were based on manufacturer’s instructions. Briefly, about 100 mg of a fungal culture was placed in a 1.5 µL Eppendorf tube. A volume of 100 µL of extraction solution was added to the tube. The tube was vortexed briefly and incubated at 95°C for 10 min. A volume of 100 µL of dilution solution was then added to the tube. The mixture was vortexed and stored at 2–8°C.

#### 10.2.3.1. PCR

PCR amplifications of the internal transcribed spacer (ITS) and 28S rDNA regions from all four species were undertaken using primers ITS1F and ITS4 for the ITS region, and NL1 and NL4 for the 28S ribosomal RNA gene (Table 10.2).
Table 10.2. Primers used to amplify the ITS (White et al. 1990) and 28S ribosomal RNA (O'Donnell 1992).

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5’ → 3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Internal transcribed spacer</td>
<td></td>
</tr>
<tr>
<td>ITS1F</td>
<td>CTTGGTCATTTAGAGGAAGTAA</td>
</tr>
<tr>
<td>ITS4</td>
<td>TCCTCCGCTTATTGATATGC</td>
</tr>
<tr>
<td>28S rDNA</td>
<td></td>
</tr>
<tr>
<td>NL1</td>
<td>GCATATCAATAAGCGGAGGAAAAG</td>
</tr>
<tr>
<td>NL4</td>
<td>GGTCCGTGTTTCAAGACGG</td>
</tr>
</tbody>
</table>

PCR components, thermal conditions for the ITS region and 28S rDNA were the same as for *Microcera* species in Chapter 9, with the exception of amplification of 28S rDNA gene for *Myriangium citri* isolates, for which the annealing temperature was 58°C. DNA purification, DNA sequencing and sequence analyses for all isolates of four species were the same as for *Microcera* species (Chapter 9).

DNA samples of the two isolates of *Tetracrium coccicolum* that were extracted using the REDExtract-N-Amp™ Plant PCR Kit were amplified using Extract-N-Amp PCR ReadyMix. A 20 µL PCR reaction contained 10 µL Extract-N-Amp PCR ReadyMix, 1 µL forward primer, 1 µL reverse primer, 4 µL fungal extract and 4 µL sterilised Milli-Q water.

**10.2.3.1. Phylogenetic analysis**

Phylogenetic analysis was the same as in Chapter 9, except for *Tetracrium* species with which maximum likelihood (PAUP*, Phylogenetic Analysis Using Parsimony, Version 4.0 beta 10 WIN) (Swofford 2001) was used. The evolutionary model was selected using MrModeltest 2.3 (Nylander 2004).

**10.2.3. Field observations**

The following features were recorded in the field: host scale, host plant of scale, appearance of infected hosts, and seasonal and spatial abundance in association with other organisms.
10.3. Results

10.3.1. Morphology and cultural studies

10.3.1.1. *Tetracrium coccicolum*

*Tetracrium cocciculum* grew extremely slowly on PDA in comparison to *Microcera coccophila* and *Microcera larvarum* (Chapter 9), and *Tubercularia coccicola* and *Myriangium citri* (this chapter). Colonies only become obvious after 20 d and were 15 mm in diameter after 40 d on PDA. Colonies were cottony (powdery), with creamy white to light yellow mycelium, raised and with an irregular margin (Fig. 10.1 left). Conidia formed on small, sparsely distributed clumps, which I assumed to be stroma, on mycelium with small, thin strips of sterilised pumpkin fruit peel infested with oleander scale on PDA after 40–50 d. Conidia produced in culture were similar to those on the scale insect in the field (Figs. 10.2 & 10.3). Conidia commonly have 3 arms, rarely 4 arms. Each arm contains 13–24 septa. I also observed what I presumed to be secondary conidia with short 3-arm structures in both culture and in field samples (Figs. 10.2 & 10.4). The mature conidia presumably developed from these structures. Dimensions of each arm of conidia obtained from cultures were 89–155 (mean = 124) µm long × 7–9 (mean = 8.1) µm wide (n = 20).

![Figure 10.1](image-url)  
*Figure 10.1*. Cultures of *Tetracrium cocciculum* on PDA with pumpkin peel infested with oleander scale (left, 50 d after inoculation) and *Tetracrium novae-zeaelandiae* on PDA (right, 25 d after inoculation).
Figure 10.2. Conidia of *Tetracrium coccicolum* from a PDA culture.

Figure 10.3. Conidium of *Tetracrium coccicolum* from field-collected white louse scale.
In nature, conidia were produced from a white sporodochium on a cushion-shaped, hard stroma formed directly on host scales (Fig. 10.5). Conidia commonly had 3 arms, rarely 4. The arms of each conidium were joined at a basal globose cell (4.9–8.4 (mean = 6.8) \times 3.3–6.4 \, \mu m (mean = 4.8 \, \mu m), n = 30). The conidiophores were short and rarely observed (Fig. 10.4). The arms were arranged in wider angles (up to 90°) (Fig. 10.3) than those of conidia of *Tetracrium novae-zealandiae* (see Figs 10.8 and 10.9). The dimensions of each arm were 107–182 (mean = 147) \, \mu m long \times 5.8–8.3 (mean = 7.0) \, \mu m wide (n = 50). They were multi-septate with 13–24 septa. Each arm was narrower towards the apex. The middle arm was usually longer than the two side arms (Fig. 10.3). Conidia were hyaline, holoblastic staurospores as described by Rossman (1978).

Perithecia were commonly found in groups of about 10–30 on their armoured scale hosts. Perithecia formed on scale insects were globose, slightly oval, 346–500 (mean = 346) \, \mu m high and 217–475 (mean = 320) \, \mu m wide (n = 50). Immature perithecia were creamy white and became dark brown as they grew and matured. The ostiole was small and became noticeably lighter in colour as the perithecium matured. The surface of perithecia was smooth to granular and scurfy, appearing dirty and rough (Fig. 10.6) as described by Rossman (1978).

The asci are bitunicate, long and slender and have a rounded apex and narrow base. The asci are 191–419 (mean = 286) \, \mu m long \times 20–34 (mean = 24) \, \mu m wide (n = 30) (Fig. 10.6). Each ascus has eight parallel, long, slender ascospores, twisted at the base. Ascospores are rounded at the apex and multi-septate (13–28 septa). The dimensions of ascospores are 138–208 (mean = 169) \, \mu m long \times 5.6–10.5 (mean = 8.1) \, \mu m wide (n = 30) (Fig. 10.6).
Figure 10.4. An immature conidium and conidiophore (left) and a mature conidium and conidiophore (right) of *Tetracrium cocciculum* on white louse scale in the field.

Figure 10.5. *Tetracrium cocciculum*: sporodochia and perithecia on red scale (left), mycelium and perithecium (right) on white louse scale on Washington navel orange tree branches.
Figure 10.6. *Tetrarium cocciculum*: (a) mature perithecia on white louse scale on an orange branch; (b) a longitudinal section of a perithecium; (c) an ascus; and (d) an ascospore.
10.3.1.2. *Tetracrium novae-zealandiae*

Cultures from the anamorph and teleomorph states of this fungus grew more slowly than *Microcera coccicola* and *Microcera larvarum* (Chapter 9), and *Tubercularia coccicola* and *Myriangium citri* (this Chapter), but more rapidly than *Tetracrium coccicolum*. Colonies of *Tetracrium novae-zealandiae* reached 10 mm after 18 d on PDA. Colonies were initially white and cottony, but after about 15 d, the central ring of colonies gradually turned to a light lemon colour. Colony shape was circular to slightly irregular and raised (Fig. 10.1 right). The appearance of cultures established from a single ascospore were similar with those obtained from a single conidium in appearance.

In nature, conidia were produced from a white sporodochium (Fig. 10.10) on a cushion-shaped, hard stroma formed directly on scale hosts (Fig. 10.9). Conidia commonly have 4 arms, rarely 3 arms or 5 arms. In contrast to *Tetracrium coccicolum*, the arms in one conidium were arranged more closely to each other (Figs 10.7 & 10.8). The arms were 117.1–161.6 (mean = 143.8) μm long × 7.3–11.9 (mean = 10) μm wide (n = 30). Each arm has 16–30 septa. A scanning electron micrograph of the top view of a sporodochium of *Tetracrium novae-zealandiae* is shown in Fig. 10.7 (left).

![A sporodochium and conidia of *Tetracrium novae-zealandiae* under a scanning electron microscope.](image)

Figure 10.7. A sporodochium and conidia of *Tetracrium novae-zealandiae* under a scanning electron microscope.
Figure 10.8. Conidia of *Tetracrium novae-zealandiae* from colonised white louse scale collected in the field.

Figure 10.9. A longitudinal section of an adult female red scale bearing a sporodochium of *Tetracrium novae-zealandiae*. 
Perithecia are commonly observed scattered or clustered in groups of 10–20 on branches and tree trunks that were infested with armoured scales. They were light yellow-orange to light grey, globose to subglobose, covered with hyaline hairs and resembled a pin-cushion (Fig. 10.10 & 10.11). The perithecia were 373–562 (mean = 439) μm tall × 154–311 (mean = 213) μm wide (without hairs) (n = 20), 373–563 (mean = 439) μm tall × 154–311 (mean = 214) μm wide (with hairs) (n = 30). Ostioles were small and became darker and more obvious as the perithecia matured (Fig. 10.11).

**Figure 10.10.** Sporodochia and perithecia of *Tetracrium novae-zealandiae* on red scale on a Washinton navel orange tree leaf (left), and on white louse scale on a Washington navel orange tree branch (right).

Asci are cylindrical, bitunicate and hyaline, 221–283 (mean = 256) μm long × 21.5–27 (mean = 24.3) μm wide (n = 30). Each ascus has eight ascospores. The ascospores are long-clavate, rounded at their apex, tapering to narrowly-rounded base and contain 13–23 septa. The ascospores were shorter, but slightly wider, than those of *Tetracrium coccicolum*. The ascospores were 97.1–178 (mean = 143) μm long × 8.8–11.6 (mean = 10) μm wide (n = 20). Each ascospore has 13–21 septa (Fig. 10.12).
Figure 10.11. Perithecia of *Tetracrium novae-zealandiae* on white louse scale on a Washington navel orange tree branch (above), and perithecia on bark from a Washington navel orange tree branch (below).
Figure 10.12. *Tetracrium novae-zealandiae*: (a) longitudinally sectioned perithecium, (b) perithecia on an armoured scale insect in the field; (c) an ascus; and (d) an ascospore.
10.3.1.3. *Tubercularia coccicola*

*Tubercularia coccicola* grew slowly on PDA from a single conidium to attain a colony diameter of 14.2 ± 0.3 mm after 20 d. Colonies were raised and varied from circular to slightly irregular. The colonies were initially white and velvety. Sporodochia formed after about 30 d on PDA. They were pink or reddish-orange\(^5\), small, cup-shaped structures, sparsely located on the surface of colonies. In some instances, an orange-coloured layer of conidia was formed (Fig 10.3d). The fungus also grew slowly on CLA from single conidium to attain colony diameters of 10.6 ± 0.3 mm after 30 d. Colonies consisted of a thin, hyaline layer of mycelium. Sporodochia were observed after 15–20 d on CLA. They were small, pink to orange and were arranged in a circular pattern at the centre of the colony or were sparsely and irregularly distributed on the surface (Fig. 10.13b). Conidia produced on PDA and CLA, and from field specimens were identical in shape and size (Fig.10.14a & b). Conidia did not change colour in 3% KOH.

*Figure 10.13.* Cultures of *Tubercularia coccicola*: (a), (c) and (d) on PDA; and (b) on CLA (b).

\(^5\)http://en.wikipedia.org/wiki/Web_colors#Color_table

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One to five sporodochia were commonly found on the margins of armoured scale hosts (Fig. 10.16). Most were cushion-shaped, velvety and initially pink, at which point they contained masses of conidia; on some occasions, the sporodochia were formed on stalks (Fig. 10.16c). They become dark pink or orange when wet. Pigmentation gradually disappeared as conidia were released. Empty sporodochia resembled creamy white cups. The shape and size of sporodochia varied from round to oval, somewhat irregular, ranging from 427–913 (mean=599) µm long to 317–535 (mean = 448) µm wide (n = 20). Conidia were small, hyaline, oval, 3.2–5.5 (mean = 4) µm long and 2.1–3.3 (mean = 2.7) µm wide (n = 50) (Figs 10.14 & 10.15). The conidiophores are cylindrical, hyaline, long and branched. Conidia are produced on the conidiogenous cells.

I commonly observed larvae of mycetophagous mould beetles ([Coleoptera: Lathridiidae]) feeding on sporodochia of *Tubercularia coccicola*, with the activity resulting in cup-shaped structures (Figs 10.17 & 10.18).
**Figure 10.15.** Scanning electron micrographs of *Tuberculosis cocicola*: (a) and (b) conidia; (c) sporodochium.

**Figure 10.16.** Sporodochia of *Tuberculosis cocicola* on white louse scale (a, c & d); red scale (b). Fig. 10.11c shows long stalks on which the sporodochia were formed.
Figure 10.17. Mycetophagous beetle larva feeding on sporodochia of Tubercularia coccicola.

Figure 10.18. Cup-shaped sporodochia of Tubercularia coccicola, after feeding by a mycetophagous beetle larva.
10.3.1.4. *Myriangium citri*

Colonies on PDA and SDA are grey to black, rugose and velvety. They are raised, umbonate with an irregular margin. The colonies on both media split the agar, more so on SDA. Colonies reached 12–16 (mean = 13) mm after 15 d under fluorescent light. Only a thin white layer of mycelium grew on CLA. In contrast to pigmented colonies on PDA and SDA, the colony on CLA was not pigmented, reaching 18 mm after 15 d (Fig. 10.19).

![Figure 10.19. Colonies of Myriangium citri: (a) in PDA; (b) CLA; and (c) and SDA.](image)

In the field, apothecia of *Myriangium citri* resembled circular to irregularly shaped black scabs that varied in size being 417–4071 (mean = 2657) µm long × 402–3902 (mean = 1971) µm wide (n = 10) (Fig. 10.20). They were commonly found scattered over the bark and were hard and difficult to crush. They became gelatinous when wet. I commonly found mature ascospores during winter and spring under humid conditions. The asci were globose, occasionally ellipsoidal, stalked, 26.7–35.6 (mean = 31.4) µm in diameter and located on the apothecium. Each ascus contains eight ascospores. Ascii and ascospores of *Myriangium citri* are pigmented. Ascospores are oblong, occasionally round, multiseptate (about 6–9), 21.8–34.6 (mean = 26.9) µm long × 8.8–14 (mean = 11.7) µm wide (n = 30) (Fig. 10.21).
Figure 10.20. Apothecia of *Myriangium citri* on Washington navel orange tree branches.

Figure 10.21. *Myriangium citri*: field specimens of (a) asci and (b) ascospores.
The anamorph state was observed in specimens of the fungus sampled directly from Washington navel orange tree branches collected from the study orchards, and was visible on the sides, and near the surface of apothecia. Conidia were readily exposed after sectioning apothecia longitudinally. Conidia were small, one septate, oblong to cylindrical and hyaline. The conidia were 7.2–10.4 (mean = 8.5) μm long × 3.5–4.4 (mean = 3.9) μm wide (Fig. 10.22). They were formed on conidiophores.

Figure 10.22. Conidia of *Myriangium citri* from field specimens.

10.3.2. Distribution and incidence within tree canopies

10.3.2.1. *Tetracrium coccicolum* and *Tetracrium novae-zealandiae*

I observed *Tetracrium coccicolum* and *Tetracrium novae-zealandiae* on red scale, purple scale, Glover’s scale and white louse scale. They were more common on white louse scale and purple scale than on red scale, except in immature Hamlin orange blocks in Lister’s orchard at Kulnura where they were commonly found on red scale. This occurred in presence of abundant growth of sooty mould fungi associated with black scale honeydew. Sporodochia and perithecia of both species were commonly observed on the same substrates (Fig. 10.12).

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57 Batista & Silva Maia (1957) described the anamorph: ‘Externally the stroma are pseudo-parenchymatous, rough; in longitudinal section however the structure is truly prosenchymatous, hyphae with individual cells often acuminate, in the flat portions of the stromata, they appear to be fertile, producing conidia. These conidiophorous cells are conical or ‘lageniformes’ as phialides, 6-9 x 5-6 μm, light brown, differentiated in stromal (stromatous) hyphae, septate, with cells from 5 to 12.5 x 4-5 μm. Support acrogenous conidia, singly or in chain, subglobose, spiny, hyaline, 3-4 um diam’ (kindly translated from original Portuguese by Dr Silvio Lopes, Fundecitrus, Brazil).
*Tetracrium coccicolum* and *Tetracrium novae-zealandiae* were common within canopies on the leaves, young twigs and branches, particularly on old bark and old leaves on which dust and/or moulds were present. I occasionally observed *Tetracrium coccicolum* and *Tetracrium novae-zealandiae* on the leaves. *Tetracrium coccicolum* and *Tetracrium novae-zealandiae* occurred throughout the year, but were more noticeable under wet and humid conditions.

![Figure 10.23. Perithecia and conidia of *Tetracrium coccicolum* and *Tetracrium novae-zealandiae* occurring together on white louse scale on an orange branch.](image)

*Figure 10.23.* Perithecia and conidia of *Tetracrium coccicolum* and *Tetracrium novae-zealandiae* occurring together on white louse scale on an orange branch.

*Tetracrium coccicolum* and *Tetracrium novae-zealandiae* appeared to be more common on mature, upright branches than on horizontal branches. When present on the latter, they were more abundant on the lower side than on the upper side. This suggests that rainfall leading to run-off may play an important role in the dispersal of these fungi. My observations on the arrangement of conidia on substrates (Fig. 10.24) also indicated that the rain leads to the release and spread of conidia from sporodochia. In contrast to *Microcera coccophila*, the abundance of *Tetracrium coccicolum* and *Tetracrium novae-zealandiae* was not associated with the presence of ants and soft scales. *Tetracrium coccicolum* and *Tetracrium novae-zealandiae* were more common than *Microcera*
coccophila, particularly in the orchards at Somersby and Castlereagh, where white louse scale and purple scale were more abundant than in my study orchards at Kulnura, Lower Portland, Cornwallis and Richmond, locations where white louse scale and purple scale were less abundant.

Figure 10.24. Anamorph and teleomorph stages of Tetracrium coccicola and Tetracrium novae-zealandiae on an orange branch. The figure shows the spread of masses of fungal conidia on the substrate.

In nature, both fungi killed scales and developed to form a layer of white mycelium that partially or wholly covered host scale cadavers (Fig. 10.5b). Sporodochia then developed on stroma protruding from the edges or surface of scales. Perithecia also formed on surface of dead scales. However, perithecia of Tetracrium novae-zealandiae observed on old plant substrates were less obvious due to loss of hairs, and their colour was also darker due to dust, algae and other material on the bark. Their appearance appeared to be related to their age.

On various occasions, Tetracrium novae-zealandiae appeared to be more common than Tetracrium coccicolum especially on old, woody branches and twigs. On these occasions, this fungus could be regarded as the most abundant entomopathogen of the armoured scale insects, especially white louse scale. I also observed sporodochia and perithecia of the Tetracrium species in summer when the sporodochia and perithecia of Microcera coccophila were less abundant.

10.3.2.2. Tubercularia coccicola

I observed Tubercularia coccicola in all of my study orchards. It was more common on inner parts of canopies, particularly on old woody branches rather than on the leaves, fruit, twigs or young branches. It was associated with red scale, purple scale, Glover’s scale and white louse scale and was most common in late autumn, winter and early
spring under humid conditions after rain or heavy dew. Sporodochia were more obvious (larger, dark pink or orange and more velvety) under humid conditions. During dry weather, sporodochia tended to be smaller, shrivelled and pale and therefore less noticeable.

10.3.2.3. **Myriangium citri**

I observed *Myriangium citri* in all of my study orchards. Unlike other entomopathogens that I observed, *Myriangium citri* was only associated with armoured scales on the bark of woody branches. I did not observe apothecia of *Myriangium citri* on substrates other than bark. It was more common in the Washington navel block in Britten’s orchard at Somersby and in the Eureka lemon block in Hitchcock’s orchard at Kulnura than at the other sites. It occurred throughout the year. Accurate identification of the host in the field was difficult, as its apothecia developed and subsequently covered scales.

**Table 10.3.** Summary of distribution and incidence of four entomopathogenic fungi within tree canopies.

<table>
<thead>
<tr>
<th>Character</th>
<th><em>Tetracrium coccicolum</em></th>
<th><em>Tetracrium novae-zealandiae</em></th>
<th><em>Bionectria</em> sp.</th>
<th><em>Myriangium citri</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Habitat</td>
<td>branches, twigs, sometimes on leaves</td>
<td>branches, twigs, rarely on leaves</td>
<td>branches, leaves</td>
<td>branches only</td>
</tr>
<tr>
<td>Distribution within tree</td>
<td>mostly inside, sometimes outside</td>
<td>mostly inside</td>
<td>mostly inside, sometimes outside</td>
<td>inside tree canopies only</td>
</tr>
</tbody>
</table>

10.3.3. Molecular characterisation

10.3.3.1. *Tetracrium coccicolum* and *Tetracrium novae-zealandiae*

The sequences of the 28S rDNA gene of *Tetracrium coccicolum* and *Tetracrium novae-zealandiae* comprised 519 base pairs. The maximum likelihood analysis (Fig. 10.25) was performed using the SYM+G (Symmetrical Model plus Gamma) (Zharkikh 1994) model of evolution selected by AIC (Akaike’s information criterion) in MrModeltest 2.3. The likelihood settings for the SYM+G model were: Lset Base=equal Nst=6 Rmat=(0.4499 1.8983 0.6557 1.0057 5.0668) Rates=gamma Shape=0.2835 Pinvar=0.

There were no available DNA sequences of either species in GenBank. I chose GenBank sequences of Tubeufiaceae to which, according to Barr (1979), Rossman...
(1987) and Kodsueb et al. (2006b), ‘Podonectria’ species belong on the basis of their morphology. My results indicated that the 28S sequences of *Tetracrium coccicolum* and *Tetracrium novae-zealandiae* were more closely related to *Pyrenochaeta* sp. (HM595582) and *Pyrenochaeta nobilis* (GQ387615) in the family Pleosporaceae than to species of Tubeufiaceae (Fig. 10.25).

*Tetracrium coccicolum* and *Tetracrium novae-zealandiae* were separated in two distinct clades, both supported by 96% bootstrap values (Fig 10.25). Sequences of isolates within *Tetracrium coccicolum* were identical. Within the *Tetracrium novae-zealandiae* accessions, sequences of isolates cultured from single ascospores (*Tetracrium novae-zealandiae* PUcS13* and PUcS14*) were identical with those from conidia. There was variation between *Tetracrium novae-zealandiae* PLb9 with other sequences of *Tetracrium novae-zealandiae*, but bootstrap support was weak (64%).

The ITS sequences of *Tetracrium coccicolum* and *Tetracrium novae-zealandiae* comprised 517 base pairs. The maximum likelihood analysis was performed using the best-fit model HKY+I +G (Hasegawa-Kishino-Yano) (Hasegawa et al. 1985, Yang 1996) selected by hLRT in MrModeltest 2.3. The maximum likelihood settings for HKY+I +G were: Lset Base=(0.2274 0.2589 0.2156) Nst=2 TRatio=1.5688 Rates=gamma Shape=4.0246 Pinvar=0.3253.

As for the 28S region, there were no available DNA sequences of either species in GenBank. I chose GenBank sequences of Tubeufiaceae to which, according to Barr (1980), Rossman (1987) and Kodsueb et al. (2006b), ‘Podonectria’ species belong on the basis of their morphology. The maximum parsimony tree is shown in Fig 10.26. Sequences of *Tetracrium coccicolum* and *Tetracrium novae-zealandiae* fell in two distinct groups, both with 100% bootstrap support. The result clearly showed that *Tetracrium coccicolum* is distinctly different from *Tetracrium novae-zealandiae* with differences in 47 of 517 base pairs of the ITS region (Fig. 10.27). Within my *Tetracrium novae-zealandiae* accessions, sequences of the isolate cultured from single ascospore (*Tetracrium novae-zealandiae* PUcS13*) was identical to those from conidia.
Figure 10.25. The 50% majority-rule bootstrap consensus tree of the 28S rDNA region of accessions of *Tetracrium coccicolum* and *Tetracrium novae-zealandiae* derived from maximum likelihood analysis. *Letendraea hehiminthicola* (Berk. & Broome) Weese ex Petch was used as the out-group and bootstrap values are provided as percentages from 1000 replications. * Isolates of *Tetracrium coccicolum* and *Tetracrium novae-zealandiae* cultured from single ascospores. ** This study. Placement of taxa within order and family is based on literature cited in the text.
The analysis of the ITS and 28S regions indicates that *Tetracrium coccicolum* and *Tetracrium novae-zealandiae* do not belong to the Tubeufiaceae, a family within the order Pleosporales (Figs. 10.25 & 10.26). My sequences of *Tetracrium coccicolum* and *Tetracrium novae-zealandiae* were most closely related to species of *Pyrenochaeta*, another genus within the Pleosporales, but in the family *Pleosporaceae* (Kodsueb et al. 2006a). The family Tubeufiaceae was erected by Barr (1979) to accommodate a number of pleosporaceous fungi that are typically either hypersaprophytic on other fungi or on substrates previously colonised by other fungi, or hyperparasitic on foliicolous fungi, or parasitic on scale insects, occasionally parasitic on living leaves (Barr 1980, Kodsueb et al. 2006b). They possess bright-coloured, superficial ascomata and long, multisepate ascospores (Kodsueb et al. 2006b) such as I observed for *Tetracrium novae-zealandiae* (Figs 10.11 & 10.12). However, *Tetracrium coccicolum* and *Tetracrium novae-zealandiae*, particularly the latter (Figs 10.6, 10.11 & 10.12), do not resemble species within genera of the *Pleosporaceae*, which are characterised by perithecial ascomata that are immersed and become erumpent, are usually black and sometimes hairy or setose, cylindrical with cellular pseudoparaphyses, have fissitunicate asci and brown phragmosporous or dictyosporous ascospores (Dong et al. 1998, Kirk et al. 2001). These fungi are regarded as parasites or saprophytes on wood and dead herbaceous stems or leaves (Kodsueb et al. 2006a).
Figure 10.26. 50% majority-rule bootstrap consensus tree of the ITS region of accessions of *Tetracrium coccicolum* and *Tetracrium nova-zealandiae* derived from maximum likelihood analysis. *Letendraea helminthica* (Berk. & Broome) Weese ex Petch was used as the out-group and bootstrap values are provided as percentages from 1000 replications. * Isolates of *Tetracrium coccicolum* and *Tetracrium nova-zealandiae* cultured from single ascospores. ** This study Placement of taxa within order and family is based on literature cited in the text.
Figure 10.27. Sequences of the ITS gene of Tetracrium cocciculum (T. c) and Tetracrium novae-zealandiae (T. n) showing differences in 47 base pairs.

### 10.3.3.2. Tubercularia coccicola

The 28S rDNA region of each of the five isolates of Tubercularia coccicola in my studies comprised 493 base pairs. The sequences of Tubercularia coccicola isolates from red scale, purple scale and white louse scale were identical. There was no variation among isolates collected from Kulnura, Somersby, Lower Portland and Richmond. GenBank sequences for 28S rDNA that most closely resembled my sequences of Tubercularia coccicola included species named as belonging to Bionectria and Clonostachys. My sequences did not match species listed as belonging to Nectria or Tubercularia [Hypocreales: Nectriaceae], the latter being the genus in which Petch (1921a,b) placed the fungus. Parsimony analysis showed that members of the Bionectriaceae formed a clade within which there were three subclades forming a 3-fold polytomy. My sequences from Tubercularia coccicola formed one of the subclades; species of Clonostachys and Bionectria were distributed between the other subclades. Species of Tubercularia, a member of the Nectriaceae, formed separate clades that were sister to the Bionectriaceae clade (Fig. 10.28). Sequences of my isolates were most closely related to Bionectria sesquicillii (Samuels) Schroers (anamorph Clonostachys sesquicillii Schroers) (herbarium specimen CBS 180.88, Centraalbureau voor Schimmelcultures-Fungal Biodiversity Centre, Utrecht Netherlands) from bark of recently dead woody plants or decaying leaves (Schroers 2001).

Sequences of the ITS region of two isolates of this fungus on white louse scale from Somersby and Lower Portland were successfully obtained. They comprised 510 base pairs. Parsimony analysis clearly separated two clades with 100% bootstrap support,
one consisting of members of the Bionectriaceae and the other, the Nectriaceae (Fig 10.29). My sequences from the ITS region of *Tubercularia coccicola* fell within the Bionectriaceae clade. Once again, the results suggested that this fungus belongs to the Bionectriaceae, and its anamorph to be a species of *Clonostachys*. Within the Bionectriaceae clade, my ITS sequences were most closely related to *Bionectria* sp. (HQ022506), an endophyte of rubber trees (*Hevea brasiliensis* and *Hevea guianensis* [Malpighiales: Euphorbiaceae]) (Gazis et al. 2011). Another species in this clade, *Clonostachys rosea* (Link: Fries) Schroers et al. (Ascomycota: Hypocreales) (AJ876484) (teleomorph *Bionectria ochroleuca* (Schweinitz)), is an entomopathogen of two sharpshooters, *Oncometopia tucumana* (Schröder) and *Sonesimia grossa* (Signoret) [Hemiptera: Cicadellidae], in Argentina (Toledo et al. 2006).

I also combined two sequences of *Tubercularia coccicola* using the concatenate sequence tools in Geneious Trial 6.0.5 (Biomatters Ltd, Auckland, New Zealand). The following sequences were concatenated and subjected to parsimony analysis: the ITS region of *Tubercularia coccicola* SUcCo and 28S rDNA region of *Tubercularia coccicola* LbS to make up concatenation sequence 1, and the ITS region of *Tubercularia coccicola* BUcCo and 28S rDNA of *Tubercularia coccicola* BAa to make up concatenation sequence 2. The concatenated sequences were compared with GenBank sequences of species of *Tubercularia* [Nectriaceae] and *Bionectria* (including anamorphic *Clonostachys*) [Bionectriaceae], and *Stephanonectria keithii* (Berk. & Broome) Schroers & Samuels [Bionectriaceae]. These sequences contained gene regions that matched my concatenated sequences of *Tubercularia coccicola*. *Hypomyces chrysospermus* Tul. & C. Tul. [Hypocreaceae] was used as the outgroup.

The analysis placed the concatenated sequences of *Tubercularia coccicola* (Fig. 10.30) sequences within a Bionectriaceae clade that was separated from a Nectriaceae clade comprising *Tubercularia lateritia* (Berk.) Seifert (HM534899 and EU715616), *Tubercularia vulgaris* (HM534894) and *Microcera rubra* (as *Fusarium larvarum* var. *rubrum* EU860073) by 100% bootstrap support. The Bionectriaceae clade comprised a five-way polytomy comprising a subclade consisting of 10 *Bionectria/Clonostachys* accessions obtained from GenBank that was separated by 100% bootstrap support from the remaining four clades comprising: (a) the concatenated sequences of *Tubercularia coccicola*; (b) *Bionectria sesquicillii* (Samuels) Schroers (AF210666); (c) *Bionectria rossmaniae* Schroers (AF210665); and (d) monotypic *Stephanonectria keithii* (Berk. &
Broome) Schroers & Samuels (AF210671). As in the separate analyses for the 28S rDNA (Fig. 10.28) and ITS (Fig. 10.29) regions of my sequences of *Tubercularia coccicola*, the results of this analysis also suggested that this fungus belongs to the Bionectriaceae. The morphology of the anamorph (see section 10.3.1.3 above) is similar to the morphology of species in the genus *Clonostachys* as described by Schroers et al. (2001). Thus, *Tubercularia coccicola* may be an entomopathogenic species of *Bionectria/Clonostachys* within the Bionectriaceae, not a species, as currently circumscribed, of *Tubercularia* within the Nectriaceae.
Figure 10.28. 50% majority-rule bootstrap consensus tree of the 28S rDNA region of accessions of *Tubercularia coccicola* derived from maximum parsimony analysis. *Microcera coccophila* was used as the out-group and bootstrap values are provided as percentages from 1000 replications.
Figure 10.29. 50% majority-rule bootstrap consensus tree of the ITS region of accessions of *Tubercularia coccicola* derived from maximum parsimony analysis. *Microcera coccophila* was used as the out-group and bootstrap values are provided as percentages from 1000 replications.
Figure 10.30. 50% majority-rule bootstrap consensus tree of the concatenated sequences of ITS and 28S rDNA regions of *Tubercularia coccicola* and GenBank accessions of *Tubercularia lateritia*, *Tubercularia vulgaris*, *Microcera rubra* (as *Fusarium larvarum* var. *rubrum*) and species of *Bionectria/Clonostachys* and *Stephanonectria keithii*. *Hypomyces chrysospermus* was used as the outgroup. Bootstrap values are provided as percentages from 1000 replications.

### 10.3.3.3. *Myriangium citri*

The sequences of the 28S rDNA and ITS regions of three isolates of *Myriangium citri* comprised 500 and 510 base pairs, respectively; the sequences of the three isolates were identical for each of the two regions. The trees resulting from parsimony analysis of these regions are shown in Figs 10.31 & 10.32. In both analyses, the sequences from *Myriangium citri* occurred within a clade consisting of other species of *Myriangium*. My ITS sequences of *Myriangium citri* were grouped with *Myriangium* species, including *Myriangium* sp. (EF464586) isolated from elongate hemlock scale in Bedford (New York), New Jersey and Connecticut (United States of America) (Marcelino et al. 2007) and a *Myriangiaceae* sp. endophyte (HM595588) associated with conifer trees (*Abies beshanzuensis*) in Zhejiang province, China (Yuan et al. 2011). The ITS regions
were also closely related with an unidentified endophyte (EF419944) associated with healthy photosynthetic tissues of three closely related tree species in the Cupressaceae [Coniferales] (Hoffman & Arnold 2008). Most recently, Tan (2012) recorded unidentified endophytic Myriangiaceae in the orchid genus *Holcoglossum* [Asparagales: Orchidaceae].

For the 28S rDNA, sequences of *Myriangium citri* were more closely related to *Myriangium hispanicum* (90% similarity) (GU301854) (Schoch et al. 2009) than to *Myriangium duriae* (NG027579) (95% similarity) from molecular databases (Fig. 10.32).
Figure 10.31. 50% majority-rule bootstrap consensus tree of the ITS region of accessions of Myriangium citri derived from maximum parsimony analysis. Pleospora herbarum (Pers.) Rabenh. was used as the out-group and bootstrap values are provided as percentages from 1000 replications.
Figure 10.32. 50% majority-rule bootstrap consensus tree of the 28S rDNA region of accessions of *Myriangium citri* derived from maximum parsimony analysis. *Pleospora herbarum* (Pers.) Rabenh. was used as the out-group and bootstrap values are provided as percentages from 1000 replications.

<table>
<thead>
<tr>
<th>Order: Pleosporales</th>
<th>Family: Pleosporaceae</th>
<th>Host (this study)</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pleospora herbarum</em> DQ678049</td>
<td>Pleosporales: Pleosporaceae</td>
<td>armoured scale</td>
<td>Somersby</td>
</tr>
<tr>
<td><em>Myriangium hispanicum</em> GU301854</td>
<td>Myriangiales: Myriangiaceae</td>
<td>armoured scale</td>
<td>Somersby</td>
</tr>
<tr>
<td><em>Myriangium citri</em> MAsS1</td>
<td>Myriangiales: Myriangiaceae</td>
<td>armoured scale</td>
<td>Somersby</td>
</tr>
<tr>
<td><em>Myriangium citri</em> MAsS2</td>
<td>Myriangiales: Myriangiaceae</td>
<td>armoured scale</td>
<td>Somersby</td>
</tr>
<tr>
<td><em>Myriangium citri</em> MAaK</td>
<td>Myriangiales: Myriangiaceae</td>
<td>armoured scale</td>
<td>Somersby</td>
</tr>
<tr>
<td><em>Myriangium sp. uncultured</em> JF424297</td>
<td>Myriangiales: Myriangiaceae</td>
<td>Aonidiella aurantii</td>
<td>Kulnura</td>
</tr>
<tr>
<td><em>Myriangiaceae sp.</em> HM595588</td>
<td>Myriangiales: Myriangiaceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Myriangium duriae</em> AY016365</td>
<td>Myriangiales: Myriangiaceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Myriangium duriae</em> NG027579</td>
<td>Myriangiales: Myriangiaceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Elsinoë eucalyptorum</em> DQ923530</td>
<td>Myriangiales: Elsinoaceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ascomycota sp.</em> JN120347</td>
<td>Myriangiales: Elsinoaceae</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
10.4. Discussion

10.4.1. *Tetracrium coccicolum* and *Tetracrium novae-zealandiae*

*Tetracrium coccicolum* was first described as *Nectria coccicola* Ellis & Everhart from specimens on scale insects on orange trees in Florida in April 1886 (Ellis & Everhart, 1886). *Tetracrium coccicolum*, was first described by Franz Xaver Rudolf von Höhnel in 1911 (von Höhnel 1911). Synonyms of the anamorph and teleomorph of *Tetracrium coccicolum* and related information are listed in Appendix II. Petch (1921a,b) was not consistent regarding what he considered to be the anamorph of *Podonectria coccicola*. He regarded *Microcera rectispora* Cooke & Mass. described from specimens on ‘Coccus of orange’ collected by Frederick Manson Bailey in Brisbane, Australia in 1887 (Cooke 1887) as the anamorph on one occasion, and on another occasion stating ‘consequently it is uncertain to which species of *Podonectria* *Tetracrium rectisporum* should be assigned’.

Tryon (1894) regarded *Microcera rectispora* as one of the four entomopathogens of armoured scale insects in orange orchards in and near Maryborough in the Wide Bay region of Queensland. Summerville (1934) noted that an undetermined species of *Podonectria* was sometimes found on purple scale, but an appreciable degree of control of the insect rarely resulted in Queensland. Hely (1968) mentioned that a *Podonectria* sp., together with *Microcera* sp., exercised a measure of control on some scale insects and mites in New South Wales. The identity of these species has not been determined, but given my results they could have been either *Tetracrium coccicolum* or *Tetracrium novae-zealandiae*.

Cooke (1891) described another species, *Lasiospharia larvaespora* Cooke & Massee. It was subsequently described as *Ophionectria larvaespora* (Cooke & Massee) Hansf. by Hansford (1956), and as *Podonectria larvaespora* (Cooke & Massee) Rossman by Rossman (1978). The specimens were collected in association with scale insects and mites on bark of an unidentified tree at Mt Macedon, Victoria (Hansford 1956, Rossman 1978). *Podonectria larvaespora* was only known from Australia until 1981, when it was collected in a hardwood forest in Fiji (Rossman 1987). The morphology and dimensions of the asci and ascospores of my
specimens of *Tetracrium coccicolum* were not similar to those of *Podonectria larvaespora* as described by Rossman (1977, 1978) and *Podonectria novae-zealandiae* as described by Dingley (1954) and Rossman (1978). In contrast to the perithecia of my specimens of *Tetracrium coccicolum*, which were dark brown, smooth, and my specimens of *Tetracrium novae-zealandiae*, which were light yellow to creamy and covered with straight hairs, perithecia of *Podonectria larvaespora* are apparently light yellow-orange appearing bright-yellow due to a loose covering of hyphae encrusted with bright-yellow granules (Rossman 1977, 1978). I believe that *Podonectria larvaespora* does not occur in citrus orchards on the Central Coast of New South Wales, or occurs rarely. My descriptions were similar to those of *Tetracrium coccicolum* of (McAlpine 1899, Petch 1921a,b, Dingley 1954, Rossman 1977, 1978).

Although *Tetracrium coccicolum* has been reported as an effective entomopathogen of armoured scale insects (Tryon 1894, Fawcett 1908, Rolfs & Fawcett 1908), Koch’s postulates have not been proven and little is known about its ecology. Further studies are warranted, and these studies should also clarify the distribution of ‘*Podonectria larvaespora*’ in Australia, where it has not been recorded since it was first described by Cooke (1891).

*Tetracrium coccicolum* has been recorded as an entomopathogen of armoured scale insects in Queensland and New South Wales (Tryon 1894, McAlpine 1899, Summerville 1934, Hely 1968). *Tetracrium coccicolum* has been recorded from scale insects in tropical and subtropical regions (Rossman 1978). Rossman (1987) stated that it may be the important entomopathogen of scale insects. Descriptions have been reported by Rolf & Fawcett (1908), Petch (1921a,b,), Dingley (1954) and Rossman (1977, 1978, 1987). All of these records require molecular verification.

My description of *Tetracrium novae-zealandiae* closely resembles teleomorphic *Podonectria novae-zealandiae* as described by Dingley (1954) and Rossman (1978) (Table 10.4). The original description was based on specimens collected on scale insects, *Leucaspis* sp. (host plant not stated) in Auckland, New Zealand. Rossman’s description (1978) was based on the original specimens. The fungus has only been recorded in New Zealand. Thus, this is the first report of *Tetracrium novae-zealandiae*

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58 Rossman (1978) referred to it as *Podonectria novae-zealandica*, but the correct orthographical name is, as described by Dingley (1953), *Tetracrium novae-zealandiae* (Pennycook 2008).
zealandiae in Australia or elsewhere other than New Zealand and the first occasion on which the anamorph has been described. Descriptions of the sexual stage of Tetracrium novae-zealandiae by Dingley (1954) and Rossman (1978) and my description of teleomorph and anamorph are summarised in Table 10.4. The similar appearance of cultures and identical molecular sequences confirmed the connection between the teleomorph and anamorph (for the first time) of Tetracrium novae-zealandiae.

I did not seek to fulfil Koch’s postulates for Tetracrium coccicolum and Tetracrium novae-zealandiae due to time constraints, and I did not assess levels of infection by them on scale in the field. However, my field observations suggested that these two species were the most common entomopathogens of armoured scales in all my study orchards, particularly on white louse scale. Tetracrium coccicolum and Tetracrium novae-zealandiae also occurred over a more prolonged period than Microcera species. My observations suggested that Tetracrium coccicolum and Tetracrium novae-zealandiae prefer shaded regions inside tree canopies, on old bark where white louse scale and purple scale are common.

This is the first report of descriptions of cultures of these fungi and of the induction of conidium formation in culture. This is also the first study on DNA sequences and phylogenetic aspects of Tetracrium species. The genus Tetracrium currently belongs to the family Tubeufiaceae (see above). However, my molecular results indicate that the genus Tetracrium does not belong to this family. My molecular results suggest that it may belong to the Pleosporaceae, but the morphologies of the two species do not conform to this family (Barr 1979, 1980, Kodsueb et al. 2006b).

As mentioned above, the abundance of Tetracrium coccicolum on immature Hamlin orange trees at Kulnura in association with black scale suggests that rough surfaces and micro-environments associated with sooty mould fungi may favour development of Tetracrium coccicolum. My observations also suggest that microclimate, not host scale species, governs the distribution of this fungus within canopies of mature trees. It was observed on white louse scale and purple scale more commonly than on red scale, presumably because white louse scale and purple scale occur more commonly on inner canopies of mature trees than does red scale, which prefers outer surfaces.
Table 10.4. Morphological descriptions of *Tetracrium novae-zealandiae* in this study and descriptions by Dingley (1954) and (Rossman 1977, 1978).

<table>
<thead>
<tr>
<th>Character</th>
<th>Dingley (1954) and Rossman (1977, 1978)</th>
<th>This study</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Teleomorph</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perithecia features</td>
<td>broadly obpyriform or globose to subglobose; covered with straight to slightly flexuous, hyaline hairs</td>
<td>covered with straight hyaline hairs</td>
</tr>
<tr>
<td>Perithecia dimensions</td>
<td>450–600 μm tall × 500–600 μm wide</td>
<td>372.7–562.5 (mean = 438.8) μm tall × 153.9–310.8 (mean = 213.4) μm wide; with hairs, 310–543 (mean = 410) μm × 375–516.4 (mean = 433.8) μm wide</td>
</tr>
<tr>
<td>Asci</td>
<td>bitunicate, long-clavate to cylindric</td>
<td>bitunicate, long-clavate to cylindric</td>
</tr>
<tr>
<td>Asci dimensions</td>
<td>225–275 × 20–24 μm (Rossman 1978); 250–360 × 20–25 μm (Dingley 1954)</td>
<td>200–305.3 (mean = 242.2) μm × 17.8–27 (mean = 22.3 μm</td>
</tr>
<tr>
<td>Ascospore feature</td>
<td>long-clavate, apically rounded, tapering to narrowly rounded base, often slightly sigmoid to vermiform, multisepitate, 13–23 septa</td>
<td>long-clavate, round at apex, tapering to narrowly rounded base, 13–23 septa</td>
</tr>
<tr>
<td>Ascospore dimension</td>
<td>110–150 × 7.5–10 μm</td>
<td>121.1–173.6 (mean = 150) μm × 8.1–13.4 (mean = 9.7) μm</td>
</tr>
<tr>
<td><strong>Anamorph</strong></td>
<td>unknown</td>
<td></td>
</tr>
<tr>
<td>Sporodochia</td>
<td></td>
<td>white on brown cushion-shape stroma</td>
</tr>
<tr>
<td>Sporodochia dimension</td>
<td></td>
<td>to be done</td>
</tr>
<tr>
<td>Conidia</td>
<td></td>
<td>hyaline, holoblastic, staurospore or not, mostly 4 arms, rarely 3 arms, mature arm acute apex, arms united at base of globose cells</td>
</tr>
<tr>
<td>Conidiophore</td>
<td></td>
<td>short</td>
</tr>
<tr>
<td>Conidia dimension</td>
<td>117.1–161.6 (mean = 143.8) μm × 7.3–11.9 (mean = 10) μm,</td>
<td></td>
</tr>
<tr>
<td>Septa</td>
<td></td>
<td>Each arm has 16–30 septa</td>
</tr>
</tbody>
</table>
10.4.2. Tubercularia coccicola

The anamorph of *Tubercularia coccicola* was first described by John Albert Stevenson in 1917 on purple scale and serene scale (*Serenaspis minima* (Maskell) (cited as *Hemichionaspis minor*)) in Porto Rico. The teleomorph of *Tubercularia coccicola* was described by Thomas Petch (1921a,b) as *Nectria tuberculariae* from specimens found on *Lepidosaphes* sp. in Sri Lanka. Petch (1921a,b) wrote ‘this fungus occurred in company with *Nectria aurantiicola* B. and Br., *Ophionectria coccicola* E. and E. and *Septobasidium rameale* (Berk.) on a tree which had been imported from West Australia five years before’. Fawcett (1948) recorded the anamorph on white louse scale and purple scale in Porto Rico, and on an unidentified scale in Brazil. He regarded the fungus as one of four teleomorphic *Nectria* species attacking scale insects of citrus and recorded the anamorph as *Tubercularia coccicola*.

Despite these records, my results suggest that *Tubercularia coccicola* belongs to the Bionectriaceae, not to the Nectriaceae. The morphology of the anamorph stage and culture characteristics are consistent with descriptions of the anamorph of *Clonostachys* by Schroers (2001), the telemorphs of which are species of *Bionectria*. If the fungus belongs to the Bionectriaceae then, on the basis of unitary names and precedence being given to the oldest generic names and species epithets, it is a species of *Clonostachys* Corda 1839 not *Bionectria* Spegazzini 1919 (see Schroers 2001). If so, the putative name for the species would be *Clonostachys coccicola*. My study represents the first occasion on which this fungus has been recorded parasitising armoured scales in Australia since the teleomorph was described as *Nectria tuberculariae* by Petch (1921a,b).

Although, I was not able to prove Koch’s postulates, my observations indicate that the fungus is a common and effective entomopathogen of armoured scale insects in citrus orchards in coastal New South Wales, particularly white louse scale, inside tree canopies where microclimates may favour development of the fungus.

10.4.3. Myriangium citri

*Myriangium citri* was first described by Paul Hennings from specimens collected in Osasco in Brazil on ‘Citrus nobilis’ (probably mandarin, *Citrus reticulata*) (Hennings 1908). Marcelino et al. (2007) reported that *Myriangium* sp. was the most
prevailing entomopathogenic fungus of *Fiorinia externa* on eastern hemlock trees in New York, Connecticut and New Jersey, United States of America. *Myriangium floridanum* Höhn. collected from *Citrus* in Florida is a synonym of *Myriangium citri* (Petch 1924, Arx 1963). My morphological descriptions of *Myriangium citri* are similar to those of Hennings (1908), Miller (1940) and von Arx (1963). Furthermore, my field observations on its occurrence are similar to those of Miller (1940), who found it on various scale insects on *Citrus*. Millar (1940) noted that it occurred in the southern United States, the West Indies, and Africa. I did not record the seasonal development of the fungus. However, I observed mature ascospores under humid conditions during winter and spring. My molecular results confirmed that my isolates of the ‘black-headed’ fungus represent a species of *Myriangium*, and that it is possibly an endophyte in citrus trees. The distribution of the fruiting body only on bark and the limited dispersal ability also suggested that the fungus might live in the plant tissues and parasitise armoured scale hosts particularly under favourable weather conditions.

I did not test the pathogenicity of *Myriangium citri* against armoured scale insects, but my observations indicated that it was abundant on these scale in the field, particularly in the orchards on Somersby Plateau. I attributed the higher incidence of *Myriangium citri* in the orchards on the Somersby Plateau compared to orchards in Hawkesbury Valley to differences in rainfall between the two areas. The annual, average, long-term rainfall at Peats Ridge is 1264 mm compared to 809 mm at Richmond (Chapter 2).

Batista & Silvia Maia (1957) described what they considered to be the anamorph of *Myriangium citri*, but they did not name it. Their research appears to have been overlooked by other workers aside from von Arx (1963), but it has not been confirmed, and anamorphs have not been described for other species of *Myriangium*. However, the structures I observed and regard as the anamorph of *Myriangium citri* appeared to be identical to those described by Batista & Silvia Maia (1957).

My *Myriangium citri* ITS region sequences showed high similarity endophytic fungus (isolate 9166 GenBank accession EF419944) that appears to belong to the Myriangiaceae. Further research is required to determine if *Myriangium citri* and other species of *Myriangium* are endophytes.
10.5. Conclusions

This study led to the discovery of four putative entomopathogens of armoured scales on citrus trees in coastal New South Wales:

- *Tetracrium coccicolum* (Höhn.) Ellis & Everh. [Pleosporaceae] n. comb., adopted as the unitary name for teleomorphic *Podonectria coccicola* (Ellis & Everh.) Petch, and anamorphic *Tetracrium coccicolum* (Höhn.) Ellis & Everh. [Tubeufiaceae];
- *Tetracrium novae-zealandiae*, the adopted unitary name for teleomorphic *Podonectria novae-zealandiae* Dingley;
- *Clonostachys coccicola* (Stevenson) [Hypocreales: Bionectriaceae] n. comb., formerly teleomorphic *Nectria tuberculariae* Petch, and anamorphic *Tubercularia coccicola* Stevenson [Hypocreales: Nectriaceae]; and
- *Myriangium citri* Henn.

The morphological identifications were confirmed by molecular studies.

These four species have not been previously reported to occur in Australia, although records suggest that references to *Podonectria* in Australian literature appear to be related to *Tetracrium coccicolum*, not *Tetracrium novae-zealandiae*. It is also of interest to note that Petch (1921a,b) mentions ‘*Ophionectria coccicola* E.’ as one of four fungal species he recorded on a tree imported into Sri Lanka from Australia, albeit five years before he collected the specimens.

All four species were grown in pure cultures for the first time. This important outcome should enhance future research on these and other putative entomopathogens. The systematic position of three of the four species needs to be resolved through additional research. Current placement of *Tetracrium coccicolum* and *Tetracrium novae-zealandiae* in the Tubeufiaceae appears to be, as noted above, incorrect, as my molecular results place the genus in the Pleosporaceae. *Tubercularia coccicola*, as noted above, appears to belong to the genus *Clonostachys* in the family Bionectriaceae, not to the genus *Tubercularia* in the Nectriaceae.
Chapter 11. General discussion, conclusions and recommendations

This study represents the most comprehensive investigation of the ecology of red scale (*Aonidiella aurantii*) undertaken in Australia since an article summarising a meeting of citrus growers that discussed ‘orange blight’ of citrus trees was published in *The Sydney Morning Herald* on 5 August 1862. In the article, a ‘red insect’ was mentioned. In a subsequent article in the same newspaper on 7 August 1862, Mr Robert L King reported that, of three insects displayed at the meeting, ‘the black, the red, and the white’, the red was more disliked: ‘the black’ was a reference to black scale, ‘the white’ a reference to white louse scale.

The field studies reported herein were undertaken in citrus orchards on the Central Coast of New South Wales where the natural enemies of red scale included five primary parasitoids, four native ladybirds and six entomopathogens. Most studies on red scale have been conducted in California, South Africa and Mediterranean countries where relatively dry climates prevail, and the biodiversity of natural enemies is less than in coastal New South Wales. In this chapter, I summarise the results and implications of my study, as presented in Chapters 4 to 10.

### 11.1 Red scale phenology on the Central Coast of New South Wales

In Chapter 4, I showed that, on the Central Coast of New South Wales:
- there are three annual generations of red scale;
- cold weather in winter, particularly ambient temperatures at sunset and sunrise, influence scale phenology and scale mortality;
- winter/spring, summer and autumn generation male flight peaks occur approximately 800, 1500 and 2200°D (90–120, 180–210, 244–282 d), respectively, after the winter solstice (21 June); and
- the abundance of scale in summer generations is related to the its abundance in the preceding winter/spring generation.
Use of pheromone traps was an effective means of determining the number of generations of the scale. Their use led me to recognise the late winter/early spring generation. Prior to my study, the number of generations of the scale on the Central Coast was uncertain, and relationships between scale densities in the first and second annual generations had not been recognised. I recommend that pheromone traps be used elsewhere in Australia to determine the phenology of red scale, and as a management tool for predicting the need for augmentative releases of natural enemies and/or application of pesticides, preferably horticultural and/or agricultural mineral oils, in order to limit the extent of infestation of the scale in the summer and autumn generations.

11.2. Seasonal incidence and impacts of parasitoids and predators

In Chapter 5, my studies indicated that:

- five primary parasitoids and a several predators, particularly *Halmus chalybeus*, suppress populations of red scale in citrus orchards on the Central Coast of New South Wales;
- *Halmus chalybeus* appeared to be the most important natural enemy of red scale;
- *Aphytis chrysomphali* and *Aphytis melinus* coexist, the former being more common than the latter;
- *Comperiella bifasciata* is an effective parasitoid of red scale and was more abundant in the relatively more humid orchards on the Somersby Plateau than in the relatively less humid orchards in the Hawkesbury Valley; and
- *Encarsia citrina* and *Encarsia perniciosi* are more effective parasitoids of red scale in Australia than has been previously recognised.

I rarely observed encapsulation of eggs and larvae of *Comperiella bifasciata* by red scale. This led me to question the strain of the parasitoid on which previous observations were made, particularly studies reported by Brewer (1971).

My study is the first to assess the effectiveness of *Halmus chalybeus* as a predator of red scale since Koebele (1892) and Crichton (1893a) recorded it as an important natural enemy of red scale in coastal New South. However, although *Halmus chalybeus* is a voracious predator, little is known about its biology and ecology.
Research is required to assess its role as a natural enemy of introduced and native prey in orchards and in natural ecosystems. Due to its cryptic behaviour, care will be required in developing accurate assessment procedures for assessing population sizes and levels of predation. Despite its effectiveness in the region, my results and the reports in the literature, suggest that it is not suitable for commercial mass-rearing and release, as it does not thrive in ‘dry climates’, such as in inland Australia. However, my results and observations, and published accounts of the biology and ecology of *Rhyzobius* species, suggest that *Rhyzobius lophanthae* should be evaluated for commercial mass-rearing and release for control of red scale in inland citrus producing regions of Australia. The possibility, given their oviposition behaviour, that species of *Rhyzobius* may be density-dependent warrants further research.

Although *Orcus australasiae* can occur in coastal and inland citrus districts of Australia, I only observed it in my study orchards on the Somersby Plateau. As for *Halmus chalybeus, Orcus australasiae* was first recorded as a predator of red scale late 1880s (Koebele 1892, Crichton 1893a), but its role as a natural enemy of native and introduced hosts has been ignored since then. It is voracious and, unlike *Halmus chalybeus*, preys on red scale in the presence of ants. Therefore, further studies should be undertaken to understand its role in the biological control of native and introduced armoured scales and soft scales, in orchards and in natural ecosystems. As noted above for species of *Rhyzobius*, the oviposition behaviour of *Orcus australasiae* suggests that studies on its biology and ecology should determine if its relationships with its hosts are density-dependent.

My study is the first in which eggs of *Orcus australasiae* have been observed, in this instance, under the integuments of dead adult black scale, mostly scales killed species of *Metaphycus*. These observations are valuable for future research on the biology and ecology of *Orcus australasiae*, as its oviposition behaviour suggests that the presence of soft scales may be important for its populations to thrive. However, my cultures of *Orcus australasiae* on butternut pumpkin infested with oleander scale showed that *Orcus australasiae* is able to complete its life cycle on armoured scales. I assume, in this instance, that the beetles laid their eggs under oleander scale covers. Thus, the beetle may also lay its eggs under armoured scales in nature.
Despite variation in levels of parasitism and predation, scale populations in my study did not exceed economic thresholds. The major factors governing abundance of red scale in the region were the severity of low temperatures in winter and natural enemy activity in summer and autumn. In the absence of its natural enemies, red scale populations would exceed economic thresholds. This conclusion is supported by reports of impacts of pesticides on natural enemies of the scale (DeBach & Bartlett 1951, DeBach et al. 1951, Furness et al. 1983, Papacek & Smith 1992).

11.3. Density and other interactions between red scale, its parasitoids, and the coccinellid predator, *Halmus chalybeus*

In this Chapter, I indicated that there was no evidence of density-dependent relationships between red scale and its parasitoids, and red scale and predation by *Halmus chalybeus*. However, as noted above, further research is required to determine if relationships between *Orcus australasiae*, *Rhyzobius* species, particularly *Rhyzobius lophanthae*, and their hosts are density-dependent. I found no evidence of intraguild predation by *Aphytis* species on *Encarsia* species, as reported in California by Borer (2002) and Borer et al. (2003).

11.4. Impacts of ant-black scale mutualism on the biological control of red scale

In Chapter 7, I showed that *Iridomyrmex rufoniger*:

- disrupted predation by *Halmus chalybeus* and *Rhyzobius* species;
- disrupted parasitism of red scale by *Aphytis* species, *Encarsia* species and *Comperiella bifasciata*;
- but did not suppress predation by *Orcus australasiae*.

I commonly observed *Orcus australasiae* feeding on red scale, including adult mated females, but rarely on black scale. Further research is required to determine its relationship with black scale and other soft scales. It may feed on the young instars, as older instars and adults are too large, and it is possible that integuments of dead, young instars may not as suitable for oviposition as integuments of older scale.
In Chapter 7, I also recorded self-asphyxiation of black scale by its honeydew in the absence of *Iridomyrmex rufoniger*. This was the first record of self-asphyxiation of black scale since Way (1954) reported self-asphyxiation of *Saissetia zanzabiensis* in the absence of ants foraging on its honeydew. The result of my experiment led me to recommend banding of trees as a simple means of minimising black scale infestations, particularly in nursery and immature orchards in which black scale can be a serious pest. At the completion of my study, I banded all trees in the three Hamlin orange tree blocks that I used for the experiment. Populations of the scale declined rapidly within eight weeks.

The results of my studies, and mutualistic relationships between native lycaenid butterflies and native ants, e.g., *Iridomyrmex rufoniger* and *Anonychomyrma itinerans* (Atsatt 1981, Eastwood & Frazer 1999, Eastwood et al. 2006) led me to hypothesise that associations between native ant species and native honey-dew producing hemipterans may influence choice of oviposition sites by the butterflies.

**11.5. Confirmation of taxonomic identities of red scale, yellow scale, their parasitoids and a honeydew-seeking ant**

In Chapter 8, I used morphological and molecular methods to confirm the presence or absence of red and yellow scale in my study orchards and the identity of their parasitoids. I:

- confirmed the identities of the scale;
- found evidence indicating that red scale has been introduced to Australia on more than one occasion;
- showed that *Aphytis melinus* and *Aphytis chrysomphali* occur in citrus orchards in the Central Coast of New South Wales;
- found no evidence of *Aphytis lingnanensis* being present in the region;
- found evidence that suggests *Aphytis melinus* may have been introduced to Australia on more than one occasion; and
- found evidence that suggests that the form of *Encarsia citrina* I recorded in my study orchards may be a specialised armoured scale strain of the species that does not appear to occur in California.
The possibility that *Aphytis melinus* may have been introduced to Australia on more than one occasion stemmed from my molecular results that showed that the 28S rDNA of an accession from Western Australia and two accessions from Kulnura differed to specimens from a commercial insectary that produces *Aphytis melinus* introduced to Australia from California in the 1960s. Additional research is required to confirm these differences. If confirmed, research will also be required to confirm the origin of the second strain, and how the two strains co-exist. A plausible explanation for the origin of a second strain may be that two species of *Aphytis*, not one, were introduced to Western Australia by George Compere in the early 1900s (see Chapter 1). Some records reviewed in Chapter 1 suggest that Compere introduced *Aphytis chrysomphali*, other records suggest that he introduced *Aphytis lingnanensis* (Wilson 1960, Furness et al. 1983, Smith et al. 1997). If two species were introduced, the evidence suggests that one was most probably *Aphytis chrysomphali*, as it was introduced to the New South Wales from Western Australia in 1925–1926 (Smith et al. 1997). The other may have been a form of *Aphytis melinus* from southern China, if populations from which Robert Luck collected specimens in Guangdong were not derived from material imported from California. However, this is highly speculative and can only be confirmed through future research.

Further research is also required to confirm the identity and natural distribution of the form of *Encarsia citrina* I recorded parasitising red scale in my study orchards. This research should focus on whether it is a native species, an Australasian species, or a species native Southeast Asia. My results also suggested it may be a suitable candidate for introduction to other countries for the biological control of armoured scales. It does not appear to be the form of *Encarsia citrina* introduced to California in the early 1900s by George Compere (Compere 1961), and it is unlikely to be the form he introduced to Western Australia (Despeissis 1903a). I found no evidence of *Encarsia citrina* parasitising red scale in Western Australia.
11.6. The two red-headed fungi (*Microcera coccophila* Desm. and *Microcera larvarum* (Fuckel) Gräfenhan, Seifert & Schroers): morphological description and identification, molecular verification and studies on pathogenicity and dispersal

In Chapter 9, I confirmed the presence of *Microcera coccophila* in citrus orchards in coastal New South Wales and recorded *Microcera larvarum*, the first record of it in Australia.

I also:

- confirmed connections, based on cultural and molecular characteristics, between the anamorph and teleomorph of *Microcera coccophila*, and the anamorph and teleomorph of *Microcera larvarum*;
- developed methods for culturing both species;
- confirmed that both fungi are entomopathogens by fulfilling Koch’s postulates in bioassays with oleander scale; and
- showed that workers of *Iridomyrmex rufoniger* can passively move conidia of *Microcera coccophila*.

My development of methods for culturing should enhance future research on the taxonomy and pathogenicity of *Microcera coccophila* and *Microcera larvarum*.

My field observations indicated that *Microcera coccophila* plays an important role in biological control of armoured scale insects in the region. Its role in the biological control of these scales has been overlooked, probably due to impacts of Bordeaux and other chemicals for control of citrus diseases, white louse scale, and other pests (Scott 1982) reducing the level of the pathogen. Its abundance in my study may stem from the reduced use of these chemicals in the orchards since the late 1990s (Lister, Hitchcock and Britten, pers. comm., 2012). Use of home-made Bordeaux mixture became illegal in 1998 (NSW Agriculture, Coastal and Hunter Valley Fruitgrowers’ Newsletter 28, Autumn 1998).
My studies on *Iridomyrmex rufoniger* in this chapter (Chapter 9) and in Chapter 7 suggested a cycle of events related to its mutualistic association with black scale. When foraging on honeydew of the scale, *Iridomyrmex rufoniger* disrupts the activities of parasitoids and predators of armoured scales, in this instance red scale. Populations of the scale species increase until *Lecanicillium lecanii* establishes on black scale and *Microcera coccophila* on red scale. *Iridomyrmex rufoniger* passively spreads conidia of the latter, and possibly the former, over wet surfaces of the scales, and surfaces of their host plants. This leads to fungal epizootics, with black scale populations declining more rapidly than those of red scale.

Further studies are required to:

- understand biology and ecology *Microcera coccophila* and *Microcera larvarum*;
- quantify impacts of Bordeaux and lime sulphur, and currently used copper sprays on the incidence of entomopathogenic fungi; and
- determine influences of climate and scale densities on the abundance of the fungi in the field.

### 11.7. Studies on four other fungi associated with armoured scales on citrus: descriptions and molecular verifications

In Chapter 10, I identified and cultured four putative entomopathogens, that, in addition to *Microcera coccophila* and *Microcera larvarum*, I recorded in association with red scale and other armoured scales in citrus orchards on the Central Coast of New South Wales. The fungi were:

- *Tetracrium coccicolum*;
- *Tetracrium novae-zealandiae*;
- *Clonostachys coccicola*; and
- *Myriangium citri*.

Thus, I recorded six entomopathogenic fungi of armoured scales in the citrus orchards. Three of these may have been, in addition to *Microcera coccophila*, among the four entomopathogens of armoured scales that Tyron (1894) observed in Queensland. Two of them, particularly one of the two species of *Tetracrium*, may
have been fungi from Queensland and Victoria reported by McAlpine (1899), in addition to *Microcera coccophila* from Sydney.

Summerville (1934) recorded an undetermined species of *Tetracrium* (cited as *Podonectria*) on purple scale in Queensland. Hely (1968) mentioned the presence of *Microcera* sp. (cited as *Sphaerostilbe* sp.) and *Tetracrium* sp. (cited as *Podonectria* sp. on scale insects in citrus trees in coastal New South Wales. Smith et al. (1997) mentioned *Microcera coccophila* as an entomopathogen of armoured scale insects in Australia. There are no reports in the literature of *Myriangium citri* being present in Australia, but there are specimens, collected by Lillian Fraser, of it in Plant Pathology Herbarium, Orange, New South Wales.

My study is the first to:

- record and described anamorph *Tetracrium novea-zealandiae* and the connection between the anamorph and the teleomorph;
- obtain pure cultures of each species, and cultures from conidia and ascospores of *Tetracrium coccicolum* and *Tetracrium novae-zealandiae*; and
- obtain conidia from cultures of the teleomorph of *Tetracrium coccicolum*.

Thus, my studies on entomopathogens provided a breadth of new knowledge about the biodiversity of entomopathogens of armoured scales on citrus in coastal New South Wales and more widely. My development of culturing techniques for the fungi should greatly assist future work on their systematics and pathogenicity bioassays.

Five of the six species can occur within close proximity on an orange branch. Five species can be seen in Figs 11.1 and 11.2.

With the exception of *Microcera coccophila*, the fungi I observed were more common inside tree canopies than in outer tree canopies. The current abundance of these fungal species in orchards on the Central Coast of New South Wales appears to be associated with reduced use of Bordeaux since the 1990s and a decline in the abundance and importance of white louse scale, once considered a damaging pest of citrus if control is neglected (Hely et al. 1982). Watson & Berger (1932) reported vast increases in populations of armoured scale insects following the use of fungicides applied for control plant pathogens, also killing entomopathogens. They
wrote ‘indeed it is possible to kill a citrus tree in the course of a year or so by repeated sprayings with bordeaux. Such experiences afford the most convincing proof of the importance of the entomogenous fungi in controlling scale-insects.’ Rossman (1978) noted that the three species of entomopathogens (Tetracrium coccicola, cited as Podonectria coccicola, Microcera coccophila cited as Sphaerostilbe coccophila, and Myriangium duriae) mentioned by Rolfs & Fawcett (1908) as often occurring together on citrus trees in Florida, had undoubtedly been usurped by chemical sprays.

Figure 11.1. Five of the six entomopathogens recorded in the study: on a Valencia branch in Lister’s orchard in September 2009
Figure 11.2. Four of the six entomopathogens recorded in the study: on a Valencia branch in Lister’s orchard in September 2009
Appendix I. Ambient twilight temperatures at Richmond and Mangrove Mountain over four years: 2008–2011

The following figures are presented:

Appendix I – Figure 1. Ambient twilight temperatures 1 h 45 min before sunset, at sunset and 45 min after sunset at Richmond RAAF base (station number 067033) during 2008.

Appendix I – Figure 2. Ambient twilight temperatures 1 h 45 min before sunset, at sunset and 45 min after sunset at Richmond RAAF base (station number 067033) during 2009.

Appendix I – Figure 3. Ambient twilight temperatures 1 h 45 min before sunset, at sunset and 45 min after sunset at Richmond RAAF base (station number 067033) during 2010.

Appendix I – Figure 4. Ambient twilight temperatures 1 h 45 min before sunset, at sunset and 45 min after sunset at Richmond RAAF base (station number 067033) during 2011.

Appendix I – Figure 5. Ambient twilight temperatures 1 h 45 min before sunset, at sunset and 45 min after sunset at Mangrove Mountain (AWS) (station number 061375) in 2008.

Appendix I – Figure 6. Ambient twilight temperatures 1 h 45 min before sunset, at sunset and 45 min after sunset at Mangrove Mountain (AWS) (station number 061375) in 2009.

Appendix I – Figure 7. Ambient twilight temperatures 1 h 45 min before sunset, at sunset and 45 min after sunset at Mangrove Mountain (AWS) (station number 061375) in 2010.

Appendix I – Figure 8. Ambient twilight temperatures 1 h 45 min before sunset, at sunset and 45 min after sunset at Mangrove Mountain (AWS) (station number 061375) in 2011.
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Appendix I – Figure 5. Ambient twilight temperatures 1 h 45 min before sunset, at sunset and 45 min after sunset at Mangrove Mountain (AWS) (station number 061375) in 2008.
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Appendix I – Figure 7. Ambient twilight temperatures 1 h 45 min before sunset, at sunset and 45 min after sunset at Mangrove Mountain (AWS) (station number 061375) in 2010.
Appendix I – Figure 8. Ambient twilight temperatures 1 h 45 min before sunset, at sunset and 45 min after sunset at Mangrove Mountain (AWS) (station number 061375) in 2011.
Appendix II. *Microcera coccophila* synonyms, host scales, host plants of scales, and locations where recorded.

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Teleomorph</th>
<th>Anamorph</th>
<th>Host scale(s)</th>
<th>Plant on which host scale and the fungus were found</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desmazières (1848)</td>
<td>Microcera coccicola Desm.</td>
<td>scale insect</td>
<td>willow (<em>Salix</em> sp. [Malpighiales: Salicaceae]) and ash (<em>Fraxinus</em> sp. [Lamiales: Oleaceae]):</td>
<td>Normandy, France</td>
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<tr>
<td>Tulasne &amp; Tulasne (1861)</td>
<td>Sphaerostilbe coccophila Tul.</td>
<td>not mentioned</td>
<td></td>
<td>Florence, Italy</td>
<td></td>
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<tr>
<td>Cooke (1871)</td>
<td>Microcera coccicola Desm.</td>
<td>‘Cocci on bark of trees’</td>
<td></td>
<td>United Kingdom</td>
<td></td>
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<tr>
<td>Tryon (1889)</td>
<td>Microcera coccicola Desm.</td>
<td>red scale</td>
<td><em>Citrus</em> sp. [Sapindales: Rutaceae]</td>
<td>Queensland, Australia</td>
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<tr>
<td>Tryon (1894)</td>
<td>Microcera coccicola Desm.</td>
<td>red scale, circular black scale, Glover’s scale</td>
<td>orange</td>
<td>Wide Bay (Maryborough) region, Queensland</td>
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<tr>
<td>Rolfs (1897)</td>
<td>Sphaerostilbe coccicola Tul.</td>
<td>San José scale</td>
<td>peach (<em>Prunus persica</em> (L.) Batsch) and plum (<em>Prunus × domestica</em> L.) [Rosales: Rosaceae])</td>
<td>Florida, United States of America</td>
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<tr>
<td>McAlpine (1899)</td>
<td>Microcera coccophila Desm.</td>
<td>red scale</td>
<td>orange (<em>Citrus × aurantium</em> L.) and pomelo (shaddock: <em>Citrus maxima</em> (Burm.) Merr.) [Sapindales: Rutaceae]</td>
<td>Sydney, Australia</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>lemon (<em>Citrus × limon</em> (L.) Osb. [Sapindales: Rutaceae]: cited as lemon</td>
<td>Queensland, Australia</td>
<td></td>
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<tr>
<td>Author (year)</td>
<td>Teleomorph</td>
<td>Anamorph</td>
<td>Host scale(s)</td>
<td>Plant on which host scale and the fungus were found</td>
<td>Location</td>
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<tr>
<td>Earle (1899)</td>
<td><em>Sphaerostilbe coccicola</em></td>
<td>obscure scale (<em>Melanaspis obscura</em> (Comstock))</td>
<td>water oak (<em>Quercus nigra</em> L. Walter [Fagales: Fagaceae]): cited as <em>Quercus aquatica</em> (Lam.)</td>
<td>Alabama, United States of America</td>
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<tr>
<td>Fuller (1901)</td>
<td><em>Sphaerostilbe coccicola</em></td>
<td>red scale</td>
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<td>Natal, South Africa</td>
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<td>Gossard (1903)</td>
<td><em>Sphaerostilbe coccicola</em></td>
<td><em>Aleyrodes citri</em></td>
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<td>not stated</td>
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<td>Fawcett (1908)</td>
<td><em>Sphaerostilbe coccicola</em> Tul.</td>
<td><em>Microcera coccophila</em> Desm.</td>
<td>San José scale, obscure scale (<em>Melanaspis obscura</em> (Comstock))</td>
<td>water oak (<em>Quercus nigra</em> L. Walter [Fagales: Fagaceae]): cited as <em>Quercus aquatica</em> (Lam.)</td>
<td>Florida, United States of America</td>
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<td></td>
<td></td>
<td></td>
<td>circular black scale, oleander scale, purple scale, Glover’s scale, chaff scale</td>
<td>not mentioned</td>
<td>Florida, United States of America</td>
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<tr>
<td>Reinking (1921)</td>
<td><em>Microcera coccophila</em> Desm.</td>
<td>red scale, purple scale, Glover’s scale, gingging scale <em>Pseudooonidia trilobitiformis</em> (Green),</td>
<td><em>Citrus sp.</em> [fruit] [Sapindales: Rutaceae]</td>
<td>Philippines</td>
<td></td>
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<tr>
<td>Author (year)</td>
<td>Teleomorph</td>
<td>Anamorph</td>
<td>Host scale(s)</td>
<td>Plant on which host scale and the fungus were found</td>
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<tr>
<td>Summerville (1934)</td>
<td><em>Sphaerostilbe coccicola</em> Tul.</td>
<td>red scale, pink wax scale</td>
<td>circular black scale, red scale, dictyospermum scale (<em>Chrysomphalus dictyospermi</em> (Morgan)), green coffee scale, purple scale, Glover’s scale, apple parlatoria (<em>Parlatoria cinerea</em> Hadden), chaff scale (<em>Parlatoria pergandii</em> Comstock), orchid parlatoria scale (<em>Parlatoria proteus</em> (Curtis)), black parlatoria (<em>Parlatoria ziziphi</em> (Lucas)), gingging scale (<em>Pseudoaonidia trilobitiformis</em> (Green))</td>
<td><em>Citrus</em> sp. (leaves, twigs)</td>
<td>Queensland, Australia</td>
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<td>scale insects</td>
<td><em>Dysoxylum spectabile</em> (A. Juss.) Hook. f. [Sapindales: Meliaceae])</td>
<td>Auckland, New Zealand</td>
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<td></td>
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<td>scale insects</td>
<td><em>Melicytus ramiflorus</em> Forst. [Malpighiales: Violaceae])</td>
<td>Auckland, New Zealand</td>
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<td></td>
<td></td>
<td></td>
<td>scale insects</td>
<td><em>Ribes rubrum</em> L. [Saxifragales: Grossulariaceae]</td>
<td>Auckland, New Zealand</td>
</tr>
<tr>
<td>Author (year)</td>
<td>Teleomorph</td>
<td>Anamorph</td>
<td>Host scale(s)</td>
<td>Plant on which host scale and the fungus were found</td>
<td>Location</td>
</tr>
<tr>
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</tr>
<tr>
<td>Booth (1971)</td>
<td><em>Nectria flammea</em></td>
<td><em>Fusarium coccophilum</em></td>
<td>scale insects</td>
<td>cited as <em>Ribes sativum</em> Syme</td>
<td>Auckland, New Zealand</td>
</tr>
<tr>
<td></td>
<td>(Tulasne) Dingley</td>
<td>(Desm.)</td>
<td>scale insects</td>
<td><em>Solanum mauritianum</em> Scop. [Solanales: Solanaceae]: cited as <em>Solanum auriculatum</em></td>
<td>Wellington, New Zealand</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>scale insects</td>
<td>unknown</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>scale insects</td>
<td>bushman’s toilet paper (<em>Brachyglottis repanda</em> Forst [Asterales: Compositae])</td>
<td>New Zealand</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>scale insects</td>
<td>pomelo (<em>Citrus maxima</em> L. [Sapindales: Rutaceae]): cited as <em>Citrus grandis</em></td>
<td>Sabah, Malaysia</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>scale insects</td>
<td>lemon (<em>Citrus × limon</em> L.[Sapindales: Rutaceae]): cited as <em>Citrus medica</em> var. <em>limonum</em></td>
<td>Zambia</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>scale insects</td>
<td><em>Citrus</em> sp. [Sapindales: Rutaceae]</td>
<td>Australia</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>scale insects</td>
<td><em>Coffea arabica</em> L. [Gentianales: Rubiaceae]</td>
<td>New Guinea &amp; Tanzania</td>
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<td>scale insects</td>
<td>oriental raisin tree (<em>Hovenia dulcis</em> Thunb. [Rosales: Rhamnaceae])</td>
<td>Uganda</td>
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<td></td>
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<td>scale insects</td>
<td>blackcurrant (<em>Ribes nigrum</em> L. [Saxifragales: Grossulariaceae])</td>
<td>New Zealand</td>
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<td></td>
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<td>scale insects</td>
<td><em>Camellia sinensis</em> (L.) Kuntze [Ericales: Theaceae]:</td>
<td>Sri Lanka</td>
</tr>
<tr>
<td>Author (year)</td>
<td>Teleomorph</td>
<td>Anamorph</td>
<td>Host scale(s)</td>
<td>Plant on which host scale and the fungus were found</td>
<td>Location</td>
</tr>
<tr>
<td>---------------</td>
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<tr>
<td>Gao &amp; Ouyang (1981)</td>
<td><em>Fusarium coccophilum</em> (Desm.) Wr. &amp; Rg.</td>
<td>scale insects</td>
<td>unidentified host</td>
<td>cited as <em>Thea sinensis</em></td>
<td>Australia</td>
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<tr>
<td>Hely et al. (1982)</td>
<td><em>Fusarium coccophilum</em></td>
<td>red scale, purple scale, white louse scale</td>
<td><em>Citrus</em> sp. [Sapindales: Rutaceae]</td>
<td>New South Wales, Australia</td>
<td></td>
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<tr>
<td>Zhou et al. (2001)</td>
<td><em>Fusarium coccophilum</em></td>
<td>red scale, purple scale,</td>
<td>orange</td>
<td>China</td>
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<td>Rossman et al. (1999)</td>
<td><em>Cosmospora flammea</em> (Tul. &amp; C. Tul.)</td>
<td>scale insects</td>
<td>not mentioned</td>
<td>warm temperate and tropical regions</td>
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<tr>
<td>Smith et al. (1997)</td>
<td><em>Fusarium coccophilum</em></td>
<td>red scale, white louse scale, purple scale, Glover’s scale, chaff scale</td>
<td><em>Citrus</em> sp. [Sapindales: Rutaceae]</td>
<td>Australia</td>
<td></td>
</tr>
<tr>
<td>Gräfenhan et al. (2011)*</td>
<td><em>Microcera coccophila</em></td>
<td>scale insect</td>
<td>bay laurel (<em>Laurus nobilis</em> L. [Laurales: Lauraceae])</td>
<td>Italy</td>
<td></td>
</tr>
</tbody>
</table>

CBS KNAW Fungal Biodiversity Centre, Utrecht Netherlands
Appendix III. *Tetracrium coccicolum* and *Podonectria coccicola* synonyms, host scales, host plants of scales, and locations where the fungi were originally recorded.

<table>
<thead>
<tr>
<th>Author</th>
<th>Teleomorph</th>
<th>Anamorph</th>
<th>Host(s)</th>
<th>Host plant of scale</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hobbard (1885)</td>
<td>‘bark fungus’</td>
<td></td>
<td></td>
<td>Citrus sp.</td>
<td>Florida</td>
</tr>
<tr>
<td>Ellis &amp; Everthart (1886)</td>
<td><em>Nectri coccicola</em> Ellis &amp; Everh.</td>
<td>scale insects</td>
<td>bark of living orange trees</td>
<td>Florida</td>
<td></td>
</tr>
<tr>
<td>Ellis &amp; Everthart (1886)</td>
<td><em>Dialonectria coccicola</em> Ellis &amp; Everh.</td>
<td>scale insects</td>
<td>orange trees</td>
<td>Florida</td>
<td></td>
</tr>
<tr>
<td>Berlese &amp; Voglino (1886)</td>
<td><em>Ophionectria coccicola</em> (Ellis &amp; Everh.)</td>
<td>scale insects</td>
<td>orange trees</td>
<td>Florida</td>
<td></td>
</tr>
<tr>
<td>Cooke (1887)</td>
<td><em>Microcera rectispora</em> Cooke &amp; Mass.</td>
<td>coccus</td>
<td>orange tree</td>
<td>Brisbane, Australia</td>
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</tr>
<tr>
<td>Cooke (1892)</td>
<td><em>Microcera rectispora</em> Cooke &amp; Mass.</td>
<td>not stated</td>
<td>not stated</td>
<td>not stated</td>
<td></td>
</tr>
<tr>
<td>Tryon (1894)</td>
<td><em>Microcera rectispora</em></td>
<td>white louse scale</td>
<td>orange trees</td>
<td>Wide Bay (Maryborough) region, Queensland</td>
<td></td>
</tr>
<tr>
<td>McAlpine (1899)</td>
<td><em>Microcera rectispora</em> (Cooke &amp; Mass.)</td>
<td>white louse scale</td>
<td>orange (<em>Citrus × aurantium</em> L.)</td>
<td>Queensland, Australia</td>
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<tr>
<td>Seaver (1903)</td>
<td><em>Scoleconectria coccicola</em> (Cooke &amp; Mass.)</td>
<td>on dead scale insect</td>
<td>bark of living orange trees</td>
<td>Florida, Cuba</td>
<td></td>
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<tr>
<td>von Höhnel f (1911)</td>
<td><em>Tetracrium coccicolum</em> Höhn.</td>
<td></td>
<td></td>
<td>Sri Lanka, Java, Taiwan, Jululand (South Africa),</td>
<td></td>
</tr>
<tr>
<td>Author</td>
<td>Teleomorph</td>
<td>Anamorph</td>
<td>Host(s)</td>
<td>Host plant of scale</td>
<td>Location</td>
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<tr>
<td>Miyabe &amp; Sawada (1913)</td>
<td><em>Ophionectria coccicola</em> (Ellis &amp; Everh.) Berl. &amp; Vogl.</td>
<td>black parlatoria scale, circular black scale, Glover’s scale, purple scale</td>
<td>‘Citrus nobilis Lour.’</td>
<td>Taiwan</td>
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<tr>
<td>Petch (1921a,b)</td>
<td><em>Podonectria coccicola</em> (Ellis &amp; Everh.) Petch</td>
<td>purple scale, black parlatoria, Glover’s scale</td>
<td><em>Citrus</em> sp., orange tree, lime tree, ‘Citrus nobilis’ orange tree</td>
<td>Florida, Florida, Florida, Taiwan, Sri Lanka</td>
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</tr>
<tr>
<td>Watson &amp; Berger (1926)</td>
<td><em>Podonectria coccicola</em> (Ellis &amp; Everh.) Petch</td>
<td>Glover’s scale, purple scale, chaff scale</td>
<td><em>Citrus</em> sp.</td>
<td>Florida, United States of America</td>
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<tr>
<td>Dingley (1954)</td>
<td><em>Podonectria coccicola</em> (Ellis &amp; Everh.)</td>
<td><em>Leucaspis</em> sp.</td>
<td>unknown host</td>
<td>Auckland, New Zealand</td>
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<tr>
<td>Rossman (1977)</td>
<td><em>Podonectria coccicola</em> (Ellis &amp; Everh.) Petch</td>
<td><em>Leucaspis</em> sp.</td>
<td>bushman’s toilet paper (<em>Brachyglottis repanda</em> Forst. [Asterales: Compositae])</td>
<td>Auckland, Auckland, New Zealand</td>
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</tr>
<tr>
<td>Rossman (1978)</td>
<td><em>Podonectria coccicola</em> (Ellis &amp; Everh.) Petch</td>
<td><em>Tetracrium coccicolum</em> Höhn.</td>
<td>scale insects</td>
<td>not stated, Puerto Rico</td>
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<tr>
<td>Rossman (1978)</td>
<td><em>Podonectria coccicola</em> (Ellis &amp; Everh.) Petch</td>
<td><em>Tetracrium coccicolum</em> Höhn.</td>
<td>scale insects</td>
<td>not stated, Florida</td>
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<td>Rossman (1978)</td>
<td><em>Podonectria coccicola</em> (Ellis &amp; Everh.) Petch</td>
<td><em>Tetracrium coccicolum</em> Höhn.</td>
<td>scale insects</td>
<td>orange leaves and twigs, Melbourne, Florida</td>
<td></td>
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<tr>
<td>Gao &amp; Ouyang (1981)</td>
<td><em>Ophionectria coccicola</em> (Ell. &amp; Ev.) Berl. et Vogl.</td>
<td>scale insects</td>
<td>orange</td>
<td>southeast China</td>
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</table>
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