Aspects of the Biology of the Ladybird Beetle *Stethorus vagans* (Blackburn) (Coleoptera: Coccinellidae)

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Doctor of Philosophy

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PLEASE NOTE

The greatest amount of care has been taken while scanning this thesis,

and the best possible result has been obtained.
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Thesis Summary

This thesis reports laboratory and field investigations on the aspects of biology of the *Stethorus vagans* predator of two-spotted mite *Tetranychus urticae* (Acarina: Tetranychidae).

*Stethorus vagans* (Coleoptera: Coccinellidae) is an indigenous Australian ladybird, which mostly occurs in the coastal regions and to a lesser extent inland. Adults and larvae are both voracious predators, feeding on all stages of two-spotted mite, *T. urticae*. Aspects of the biology of *S. vagans* were studied in the laboratory at constant and fluctuating temperatures, and regularly fed on all stages of *T. urticae*. For field studies potted French bean plants infested *T. urticae* were exposed in the field.

The lower developmental threshold temperature for egg, 1\textsuperscript{st}, 2\textsuperscript{nd}, 3\textsuperscript{rd}, 4\textsuperscript{th} larval instars, and pupal stage was estimated to be 10.1, 9.5, 9.5, 9.1, 8.2, and 8.0°C respectively, and for all stages combined it was 9.1°C. The degree-days (DD) required for development were calculated for each stage of *S. vagans* from the developmental data and the mean threshold temperature. Total mean DD for the development of all stages combined was from 189.9 to 207.8 (calculated at constant temperatures of between 12–30°C respectively), and 189.1 at fluctuating temperatures (12.7–32.1°C).

There was no significant difference in male and female longevity with mean survival of 33.21 days at 25°C. The mean number of eggs laid per female was 190 in a mean reproductive period of 28 days at the same temperature. The mean generation time (including pre-oviposition
period) was 15.5 days, while the total life cycle from egg to adult death was 41.9 days. No diapause nor any overwintering stage was recorded in either laboratory or field investigations.

Adult *S. vagans* was found to consume a range of alternative prey for survival if the primary host was not available. However, none of the alternative hosts tested had the potential to support reproduction, except for broad mite *Polyphagotarsonemus latus* although this treatment resulted in significantly lower fecundity and egg hatchability then their primary host *T. urticae*. Adults survived for 4-5 days without food and water, while their longevity increased up to 6, 7, 8, 18, 21, 23, and 26 days on whitefly *Trialeurodes vaporariorum* eggs, predatory mite *Phytoseiulus persimilis* eggs, water, honey & water, pollen & water, rust mite *Auculops lycopersi* and broad mite *Polyphagotarsonemus latus* respectively. There was no significant difference in male and female longevity of *S. vagans* with any of the alternative hosts tested.

Time partitioning behaviour was assessed for newly emerged, satiated and 24 & 48 hour starved adults *S. vagans* as well as 4th larval instars. Adults & 4th instar larvae spent most of their time searching and feeding if they were starved, but satiated and newly emerged predators spent a greater proportion of time resting and walking.

Prey consumption rates were assessed for both immature and adult *S. vagans*. For functional and numerical responses, all motile stages of *S. vagans* were exposed to prey densities varying between 0, and 200 *T. urticae* eggs per day per individual. All stages of *S. vagans* were voracious feeders and fed on all stages of two-spotted mite, but preference was for eggs. The mean rate of consumption increased from 1st instar to 4th instar larvae (i.e. 27.9, 50.1, 71.6, and 152.4 mite eggs per day respectively), while adult males, pre-ovipositing, ovipositing, and post-ovipositing females consumed 63.5, 94.3, 57.1 and 142.7 mite eggs per day respectively.
All stages of *S. vagans* showed strong positive functional responses to *T. urticae*. None of the larval instars completed development of the stadium at the lowest prey density (2 mite eggs/day), except for a few 1st instar larvae. Prey consumption increased linearly from low prey density to high prey density until it reached a plateau for each stage. Adults and larvae also showed a significant numerical response to prey density. Starved adults survived up to 5 days, and no mortality was recorded for up to 7 days at very low prey density (i.e. 2 mite eggs/day). However high mortality occurred with prey densities less than 2, 8, 15, and 31 mite eggs for 1st, 2nd, 3rd and 4th instar larvae respectively. Fecundity (both mean daily and total) increased from lower to higher prey densities. Cannibalism was observed with all stages of *S. vagans* at low prey density and adult females fed on their own eggs. Cannibalism ceased when prey density exceeded 42 mite eggs per female /day.

In investigations to determine how *S. vagans* located their prey, they were unable to locate prey in a Y-tube olfactometer but were readily able to reach prey on potted bean plants in a room. Satiated adults took longer to locate their prey than did starved ones, and found high prey populations in a shorter time medium or low prey populations. This latter result was also observed in field investigations, when significantly more predators were collected from the high prey density treatment, while none were found on mite free plants

*S. vagans* exhibited many of the attributes of an effective biological control agent such as high reproductive potential, location of prey at low levels, reproduction at low densities, and ability to feed on alternative hosts if the primary host was not available.

It is concluded from this study that *S. vagans* has a number of characteristics which are likely to be much useful natural enemy of two-spotted mite *T. urticae*. 

DECLARATION

I declare that this work has not been submitted for a higher degree at any other University or Institute

Inam ullah
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CHAPTER 1

INTRODUCTION

Ladybirds, members of the family Coccinellidae are among the most familiar of the beetles and have common names around the world, such as lady cows, God’s cow and the Virgin’s insect (Moreton 1969). Ladybirds are one of the first insects that most children learn to recognise. They are unique among the insects in being almost universally regarded as benign. This is surprising because many people show an instinctive dislike of other members of the order Coleoptera (Majerus 1994). The wide popularity of the ladybird is manifest in the number of commercial and charitable organisations that use them as motif. For instance, in Britain, Woolworth uses the ladybird as a brand name for its range of children’s clothes. Ladybird books have helped many youngsters in their early reading. In Germany, “Coccinelle” became a nickname for an early model of Volkswagen, which in Britain was nicknamed “the beetle” (Majerus 1994).

The family Coccinellidae comprises 5,200 described species worldwide (Hawkeswood 1987). These are medium size beetles with an oval, oblong or hemispherical body shape (Majerus 1994). Most of them are of brightly shining colours with a pattern of spots or patches against a contrasting background. Many appear to be distasteful to birds, and their conspicuous appearance is likely to be an example of warning coloration (Moreton 1969).

Numerous species of ladybirds are major biological control agents of pests such as aphids, mealybugs, scale insects, thrip, and mites in all parts of the globe (Alagen 1962; Moreton 1969). Some are specific in their choice of food, while many are polyphagous. The introduction of the vedalia ladybird, Rodolia cardinalis Mulsant, from Australia into
California in 1888 to control cottony cushion scale, *Icerya purchasi* Maskell, which threatened the citrus industry, is widely regarded as the first and still to this day, one of the most spectacularly successful instances of biological pest control (Majerus 1994).

A less familiar group of ladybirds is the very small black genus, *Stethorus* Weise, which are primary predators of spider mites (Acarina: Tetranychidae). They can also utilise other food such as flower nectar, pollen, honeydew and plant resins for survival (Moreton 1969; Helle & Sabelis 1985b). Most of the species are relatively small, 0.8-1.5 mm in length. They are remarkably well adapted to live and search for prey in the habitats of plant feeding mites and have been reported in many countries where spider mites have been studied (Jeppson *et al.* 1975). *Stethorus* spp. lay high numbers of eggs per female and have potentially high daily oviposition rates when food is abundant (McMurtry *et al.* 1970).

Most spider mite species (Tetranychidae) are polyphagous and occur on a wide variety of plants. Among the tetranychids, however, some are quite host specific. *Schizotetranychus* spp. for example mostly occur on monocotyledonous plants except for *S. baltazari* Rimando which is an injurious pest of citrus. The genus *Platytetranynchus* Oudemans occurs on conifers. The genera *Oligonychus* Berlese, *Eotetranychus* Oudemans, and *Tetranychus* Dufour, however, occur on a range of hosts. Within a host these mites may have microhabitat preferences. For example, *Oligonychus mangiferus* (Rahman and Sapra) occurs only on the upper leaf surfaces of grapevines, while the lower surfaces of the same leaf may be infested by *Eotetranynchus truncatus* Estebanes (Gupta 1985).

Two-spotted mite, *Tetranychus urticae* Koch is the most polyphagous species of the tetranychids (Readshaw 1975). More than 200 economic plants are recorded as hosts of *T. urticae* (Hill 1987). It feeds on a large number of greenhouse crops, a wide range of field
crops, vegetables, pot plants, strawberry, deciduous fruit trees, walnuts, almond, berries, hops, cucurbits, cutflowers, ornamental shrubs and vines (Pritchard & Baker 1952; Huffaker et al. 1969; Helle & Sabelis 1985a; Costello et al. 1992). *T. urticae* is a cosmopolitan species which occurs in Australia (Helle & Sabelis 1985a). It is a key pest in glasshouse crops (Gough 1992) but also attacks annual flowers, cutflowers, roses, azalea, dahlias, polyanthus, violets, strawberry, beans, cotton, deciduous fruit trees, citrus, vegetables, ornamental plants, and shrubs including many weeds in orchards (Bailey & Caon 1986; Spooner-Hart 1990; Hutchison 1992; Thwaite 1993; Bower & Thwaite 1995; McMaugh 1998).

In Australia *T. urticae* is costly to control in deciduous fruit orchards. It mainly feeds on leaves, causing bronzing and premature leaf fall that reduce the yield and quality of fruit. It may have to be disinfested from export fruit and can cause irritating skin rashes on orchard personnel. It is a serious pest in 40,000 ha. of apples and pear and 16,000 ha. of peaches, incurring an annual expenditure on acaricides of about $A 3 million (Readshaw 1975).

The effective control of spider mites has become increasingly difficult due to withdrawal of some effective miticides and the development of resistance against a wide range of miticides and insecticides (Readshaw 1975). This suggests that the problem of controlling *T. urticae* deteriorates if the cost of developing and marketing of new compounds becomes prohibitive (Readshaw 1975). Therefore interest has increased in biological control of this pest. Many insects as well as several families of mites are recorded as predators of spider mites (Hussey & Huffaker 1976). One of these are ladybirds in the genus *Stethorus*, a cosmopolitan group found throughout all continents (Gordon & Anderson 1979; Houston 1980; Helle & Sabelis 1985b).
LITERATURE REVIEW

1.1 Morphology and Taxonomy of the Genus Stethorus

Ladybirds (Coleoptera: Coccinellidae) are such important predators that considerable attention has been given to many aspects of their biology and ecology. Ladybirds are small to medium sized often-brightly coloured beetles. They are oval or convex in shape with their head partly concealed by the pronotum, and they have three distinct tarsal segments, with the third tarsal segment deeply bilobed (Majerus 1994).

The tiny black coccinellids that belong to the genus Stethorus are important predators of spider mites (Acarina: Tetranychidae) (Moreton 1969; Britton & Lee 1972; Helle & Sabelis 1985b). Chapin revised the genus Stethorus in 1969, while historic descriptions of its taxonomy and morphology were published in 1979 (Gordon & Anderson 1979) and 1983 (Gordon et al. 1983). Dobzhansky (1924) placed it in the tribe Stethorini. Korchefsky (1931) synonymised Stethorini with Scymnini, but Kapur (1948), Sasaji (1968) and Gordon et al. (1983) consider Stethorini a valid tribe. Stethorus is easily separated from all other genera of Scymnini because the clypeus is not emarginate around the antennal base, and the pro- sternum is acutely produced in front, partly concealing the mouthparts (Gordon & Anderson 1979; Gordon et al. 1983).

Stethorus spp. are minute shiny black beetles, with spherical or oval bodies, covered with fine yellow hairs. They all look very similar, but can be differentiated by their morphological features, including body colour and size, dorsal structure, surface, leg and mouthpart colouration and abundance and appearance of setae (Moreton 1969; Gordon et al. 1983). The major morphological characters of species of Stethorus are given in Table 1.1.
Table 1.1 The major morphological characters of some adult *Stethorus* species.

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<td>Width (mm)</td>
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<td><em>S. keralicus</em>, Kapur</td>
<td>0.92-1.07</td>
<td>0.52-0.67</td>
<td>pale yellow</td>
</tr>
<tr>
<td><em>S. vagans</em> (Blackburn)</td>
<td>1.12</td>
<td>-</td>
<td>pale yellowish</td>
</tr>
<tr>
<td><em>S. nigripes</em>, Kapur</td>
<td>1.15</td>
<td>-</td>
<td>black</td>
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<tr>
<td><em>S. histrio</em>, Chazeau</td>
<td>1.00-1.20</td>
<td>0.80-0.86</td>
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<tr>
<td><em>S. fenestralis</em>, Houston</td>
<td>0.90-1.10</td>
<td>0.65-0.80</td>
<td>yellow-reddish yellow</td>
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<td><em>S. obscuripennis</em>, (Lea)</td>
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<td>0.78-0.88</td>
<td>yellow</td>
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<td><em>S. punctum punctum</em></td>
<td>1.35-1.55</td>
<td>0.95-1.15</td>
<td>yellow</td>
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<td><em>S. punctillum</em>, Weise</td>
<td>1.35-1.57</td>
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<td><em>S. darwini</em>, (Brethes)</td>
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</tbody>
</table>
Houston provided descriptions of the Australian species including *S. vagans* in 1980. "The femoral line of *S. vagans* reaches about three-quarters the distance between the hind coxa and the posterior margin of abdominal sternite one; the lateral part of the metasternum and area within the femoral line is without reticulation and the male abdominal sternite 6 has a deep triangular emarcation. In *S. nigripes* the metasternum is convex posteriorly with oblique grooves anteriorly on either side of the midline, the male abdominal sternite 6 is evenly rounded. In *S. histrio* the metasternum is flattened posteriorly, and without anterior grooves; while the male abdominal sternite 6 is truncate posteriorly. In *S. fenestralis*, the femoral line reaches about half the distance between the hind coxa and the posterior margin of the abdominal sternite; the lateral part of the metasternum and the area within the femoral line is partly reticulate and the male abdominal sternite 6 is evenly rounded".

Ladybirds lay their eggs in clusters, rows or singly on leaves and stems of plants and sometimes on stones or other non-living objects (Moreton 1969; Majerus 1994) *S. vagans* eggs are elongate, shiny white to pale yellow, and usually deposited horizontally on the substrate. *S. nigripes* eggs are pink and laid vertically (Britton & Lee 1972), in *S. punctillum* eggs are oval, pale yellow and laid flat on the underside of leaves (Moreton 1969), *S. keralicus* eggs are creamy, oval, and laid singly on the lower surface of the leaves (Daniel 1976) and eggs of *S. pauperculus* are smooth, elongated and bluntly round at both ends the newly deposited eggs pink and laid either singly or occasionally in groups of 2-5 on the lower surfaces of leaves (Puttaswamy & ChannaBasavanna 1977).

Larvae of *Stethorus* moult three times during their larval period before pupating in the last larval skin (Moreton 1969; Richardson 1977). The last instar larvae are elongate-oval, wide across the thorax and from 1.60 to 2.50 mm long (Moreton 1969; Gordon & Anderson 1979). The measurement recorded for different *Stethorus* spp. were for *S. vagans*, 1.82-2.34 mm; *S.
nigripes, 1.56-1.95 mm; S. histrio, 1.43-2.21 mm and S. fenestralis 1.69-2.34 mm (Houston 1980), while S. puntillum were, 2.5 mm (Moreton 1969), S. keralicus 1.53-2.04 mm (Daniel 1976) and S. pauperculus, 1.80 mm long (Puttaswamy & ChannaBasavanna 1977). Houston (1990) has given a brief description of the last instar of New Zealand species of Stethorus. Almost all Stethorus larvae have numerous setae with brown-black sclerotised areas at their base. Larva colouration ranges from white-pale white in S. keralicus, (Daniel 1976), pale cream white in S. vagans (Britton & Lee 1972), yellowish grey in S. nigripes (Britton & Lee 1972), blackish-grey in S. punctum (Colburn & Asquith 1971) and dark-brown in S. pauperculus (Puttaswamy & ChannaBasavanna 1977).


1.2 Distribution of Stethorus

The genus Stethorus has a worldwide distribution, and is present throughout the tropical and temperate regions of the world (Gordon & Anderson 1979; Houston 1980; Guoyue 1996). It is found in areas with very different climates, e.g. from Canada to New Guinea; and in many ecosystems, including tropical rain forests, dry savannas, orchards and various crops (Helle & Sabelis 1985b).

Approximately 90 species are known around the world of which 24 have been recorded from China, including a new species S. convexus (Guoyue 1996). Kapur described 20 species from North America, while 5 members were recorded from Central and South America, including S. tridens, S. ogloblini and S. darwini (Gordon & Anderson 1979; Gordon 1982). Fatemi (1983) listed 19 species of coccinellids from Esfahan province, Iran of which S. punctillum,
was the only one preying on *Tetranychus turkestani*. Gordon & et al. (1983) reported 21 species of *Stethorus* from the Western Hemisphere, of which 11 were described as new species. A number of *Stethorus* species have been recorded from Australia, New Zealand, the Pacific and South East Asia. *S. bifidus*, *S. histrio* and *S. griseus* commonly occur in New Zealand (Houston 1990). *S. vagans*, *S. nigripes*, *S. histrio*, *S. fenestralis* and *S. obscuripennis* have been recorded from Australia. *S. vagans* and *S. nigripes* were previously described by Blackburn (1892) and Kapur (1948). However Britton & Lee (1972) redescribed these species and in doing so described a new species, *S. loxtoni* Britton and Lee. Houston (1980) revised the taxonomy and morphology of *S. vagans*, *S. nigripes* and *S. histrio* and concluded that *S. loxtoni* was synonymous with *S. nigripes*. He also described a new species, *S. fenestralis*, and redescribed *S. obscuripennis* from Norfolk Island in Australia (Houston 1980, 1983). *S. vagans* and *S. histrio* are widespread and common species in Australia, but are also found in New Caledonia. The latter species is also found on Reunion Island (Indian Ocean) (Houston 1980). *S. nigripes* is restricted to hotter inland areas, while *S. fenestralis* has been commonly recorded on coastal and sub-coastal areas of hot inland Australia (Readshaw 1975; Houston 1980).

1.3 Rate of Development of *Stethorus* spp.

Few studies have been undertaken to establish rates of development and degree-day models for coccinellids, especially for the genus *Stethorus*.

Developmental studies have been conducted on *S. bifidus* on a range of temperatures from 8.5 to 27.5°C with *Tetranychus lineatus* used as prey. The relationship between temperature and developmental rate was linear between 12.5 and 27.5°C for eggs, 4th instar larvae and pupae. Development threshold temperatures ranged from 9.4°C for third-instar larvae to 11.9°C for eggs, while development from egg to adult took 217 degree days (Petersons et al.
1994). Richardson (1977) also investigated minimum threshold temperature for development of *S. loxtoni* (=*S. nigripes*). This was 12°C for the pupal stage, followed by the egg and 1st instar larva at 11.5°C, and 6.0, 6.0, and 6.5°C for 2nd, 3rd, and 4th instar larvae respectively.

### 1.4 Biology of *Stethorus*

Biological studies have been conducted on several species of *Stethorus*. Most of the species complete their life cycle (egg-egg) in two weeks and have five generations a year under optimal temperature conditions, which is slightly longer than that required for the development of most mite species (Jeppson *et al.* 1975; Pavlova 1975; Singh & Ray 1977). The number of generations recorded per year for *S. punctum* was 3 (Colburn & Asquith 1971), 2 for *S. punctillum* (Moreton 1969), 14-16 for *S. siphonulus* (Puttaswamy & Rangaswamy 1976) and 15-18 for *S. pauperculus* (Puttaswamy & ChannaBasavanna 1977), per year. Two Chinese species, *S. guangxiensis* and *S. aptus* predators of the citrus mite *Panonychus citri* undergo 9-10 generations per year (Lie *et al.* 1990).

The sex ratio in all *Stethorus* species seems to be 1: 1 male: female, as has been demonstrated in *S. nigripes* (Richardson 1977) and *S. loi* (Shih *et al.* 1991). Mating can occur within 24 hours of adult emergence at high temperatures and multiple matings have been observed in many species (Helle & Sabelis 1985b). Females can lay up to several hundred eggs in clusters rows, or singly on leaves, stems, and other objects as reported previously (Section 1.1). The mean number of eggs recorded for various species of *Stethorus* in their life span are: *S. siphonulus*, 170 (Raros & Haramoto 1974), *S. keralicus*, 295 (Puttaswamy & Rangaswamy 1976), *S. nigripes* 281 (Richardson 1977) and *S. punctillum* 100 (Jiang *et al.* 1982). The developmental time of some *Stethorus* species is summarised in Table 1.2.

*Throughout this thesis the name of *S. nigripes* will be used for this thesis.*
Table 1.2 Developmental time (days) of *Stethorus* spp. at different temperatures.

<table>
<thead>
<tr>
<th></th>
<th>Egg</th>
<th>Larva</th>
<th>Pupa</th>
<th>Adult</th>
<th>Total</th>
<th>Temperature</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Stethorus punctum</em></td>
<td>5.0</td>
<td>12.0</td>
<td>5.5</td>
<td>-</td>
<td>47</td>
<td>-</td>
<td>Colburn &amp; Asquith 1971</td>
</tr>
<tr>
<td><em>S. siphonulus</em></td>
<td>2.8</td>
<td>6.8</td>
<td>3.0</td>
<td>29.8-32.4</td>
<td>43</td>
<td>27-30°C</td>
<td>Raros &amp; Haramoto 1974</td>
</tr>
<tr>
<td><em>S. keralicus</em></td>
<td>3-4</td>
<td>5-6</td>
<td>3.5-4.0</td>
<td>38</td>
<td></td>
<td>26-34°C</td>
<td>Daniel 1976</td>
</tr>
<tr>
<td><em>S. pauperculus</em></td>
<td>4.36</td>
<td>7.63</td>
<td>3.8-4.0</td>
<td>29.8-32.4</td>
<td>43</td>
<td>24-26.2°C</td>
<td>Puttaswamy ChannaBasavanna 1977</td>
</tr>
<tr>
<td><em>S. nigripes</em></td>
<td>3.5</td>
<td>8.4</td>
<td>3.4</td>
<td>45.1</td>
<td>16.0</td>
<td>25°C</td>
<td>Richardson 1977</td>
</tr>
<tr>
<td><em>S. punctillum</em></td>
<td>3-4</td>
<td>8-9</td>
<td>6-8</td>
<td>32-53</td>
<td></td>
<td>24-28°C</td>
<td>Jingo <em>et al.</em> 1982</td>
</tr>
<tr>
<td><em>S. gilvifrons</em></td>
<td>2.9-3.5</td>
<td>5.3-12.1</td>
<td>2.0-5.6</td>
<td>44.8-145.9</td>
<td>20-35°C</td>
<td>Ahmed &amp; Ahmed 1989</td>
<td></td>
</tr>
<tr>
<td><em>S. loi</em></td>
<td>1.79</td>
<td>5.53</td>
<td>3.3</td>
<td>48.4-56.6</td>
<td>17</td>
<td>24°C</td>
<td>Shih <em>et al.</em> 1991</td>
</tr>
</tbody>
</table>
Coccinellids commencing overwinter as adults. The hibernation site selected by ladybirds varies greatly. They may shelter among conifer foliage, in curled dead leaves, hollow plant stems, grass tussocks, under loose bark, in crevices in trees or walls or at the base of dense plant growth including grass. In the shelter of deciduous woodlands, they may overwinter on exposed trunks, branches, or low herbage (Moreton 1969; Majerus 1994). During hibernation, they may congregate in groups to pass the winter. Some may form aggregations of several thousand, while others are usually found in small groups of up to several dozen (Majerus 1994). It has been suggested that they are attracted to one another in the autumn by aggregation pheromones, possible due to dead individuals previously left at the site (Moreton 1969; Majerus 1994).

Some ladybirds hibernate in the temperate climates as adults, while other just become inactive at lower temperatures and resume activities on warm days. A number of *Stethorus* species do not overwinter, e.g., *S. nigripes, S. pauperculus, S. gilvifrons S. picipes* and *S. punctillum* (Richardson 1977; Puttaswamy & ChannaBasavanna 1977; Helle & Sabelis 1985b; Lyoussoufi et.al. 1992). Their activity is continuous in tropical areas such as with *S. madecassus* and *S. punctum punctum* (Helle & Sabelis 1985b; Felland et al. 1995). Some species with large geographical ranges may or may not hibernate. *S. punctillum* has been recorded throughout the year in pear orchards in France, although in low numbers in winter, whereas in China it has been reported to hibernate as adults in winter under the bark of peach trees and in the soil around apple and pear trees, at distances 0-264 cm from trees and at a depth of 0-16.5 cm (Moreton 1969; Anonymous 1984). Adults of *S. punctum* commence hibernation in mid autumn in the leaf and grass debris beneath apple trees. Approximately 75% of overwintering beetles can be found in debris within a 60 cm radius of trees (Colburn
1971). Felland et al. (1995) collected more than 70% of hibernating S. punctum punctum adults from tree trunks, fallen leaves and root suckers of apple trees. However McMurtry et al. (1974) suggested that there appears to be no true diapause observed in any Stethorus species except for a facultative reproductive diapause induced by short day-length in S. picipes.

1.5. Primary and Alternative Hosts

The relationship between ladybirds and their hosts have attracted considerable attention, largely because of the economic importance of some of their prey. Ladybirds are regarded as beneficial because most eat plant pests such as aphids, scale insects and mites. They also feed on a variety of plant materials including honeydew, pollen, nectar, sap & resin and will even scavenge on dead organisms if their principal food is not available (Majerus 1994).

The feeding habits of Stethorus differ from other families of coccinellids. Most coccinellids are predaceous on insects in the sub-order Homoptera, but species in the genus Stethorus feed almost exclusively on spider mites (Tetranychidae: Acarina) (Gordon & Anderson 1979). Forty percent of known species of Stethorus have been reported to attack spider mites of economic importance and, to lesser extent, tenuipalpids (Tenuipalpidae: Acarina). Both adults and larvae are highly specialised predators of tetranychid mites (Houston 1980; Helle & Sabelis 1985b). When primary prey is scarce Stethorus are reported to eat a variety of other insects and mites such as aphids, scales, white flies and phytoseiid mites (Helle & Sabelis 1985b; Majerus 1994). They are also feed on honeydew, pollen, nectar, mildew, sweet sap, and plant resins or may even become cannibalistic (Helle & Sabelis 1985b; Majerus 1994).

Putman (1955a) observed feeding of starved adults and larvae of S. punctillum on aphids. He concluded that this diet was inadequate to complete development or induce oviposition,
although it increased adult longevity. He also observed predation on phytoseiid mites (Acarina: Phytoseidae). Kamiya (1966) listed several aphids and diaspidids as prey for _Stethorus japonicus_, but did not state the importance of these prey, and also reported that feeding on plant resins and sweet foliar secretions extended adult longevity. Kehat (1967) reported predation of adult _S. punctillum_ on the scale _Parlatoria blanchardi_.

While some species of _Stethorus_ feed on a range of tetranychid species, others are more selective in their choice of host. For example _S. punctillum_, _S. punctum_ and _S. gilvifrons_ do not readily feed or oviposit if they are reared on some mite species, such as _Bryobia_ spp. (Putman 1955a; McMurtry et al. 1970). _S. punctillum_ and _S. faerschi_ seem to restrict their feeding to palm mite species only (Helle & Sabelis 1985b), and _S. keraticus_ appears to be a specific predator of mites in the genus Raoiella (Nageshchandra & ChannaBasavanna 1983). Adults and larvae of _S. nigripes_ failed to feed on eggs of _Panonychus ulmi_ or _P. citri_ and avoided all stages of _Bryobia rubrioculus_ in the laboratory; honey and water alone were also not adequate for their survival (Hoy & Smith 1982). _S. gilvifrons_ was identified as a specialist predator of _T. urticae_ on beans in Turkey (Aydemir & Toros 1990).
Fig 1.1 Two-spotted mite adult females *T. urticae* the primary host of *S. vagans*.

1.6. Functional and Numerical Response to Prey Density by *Stethorus* spp.

The importance of predacious organisms in the control of pests depends on many factors, including their functional and numerical response to prey, their reproductive rate, prey selection and searching capacity. Functional response is one of the most important aspects in the dynamics of predator-prey interactions (Holling 1959).

The extent to which a pest population suffers as a result of predation by natural enemies depends on the number of predators present and their ability to find and consume hosts. Solomon (1949) identified prey density as the crucial factor determining response by predators. In an attempt to distinguish between its effect on predator abundance and its effect on predation efficiency, he defined the terms "functional" and "numerical" response. The
functional response is the change in number of prey consumed per unit time by each predator in relation to changes in prey density. The numerical response describes the growth and death rates of the predator population as a function of prey density.

Several field investigations have confirmed that Stethorus spp. regulate T. urticae populations and appear to be active at low prey densities. Adults are capable of locating small isolated patches of prey even at very low levels (less than one mite per leaf) (Hull et al. 1977b; Helle & Sabelis 1985b; Congdon et al. 1993). The natural levels of mites in unsprayed apple orchards have been reported to be brought down to 1 mite per leaf or less where Stethorus spp were present (Readshaw 1973). S. nigripes was first recognised in Australian commercial fruit growing areas where it was the only predator of T. urticae. It maintained mite populations at 0.1 per leaf, well below the level at which economic loss is incurred (Richardson 1977).

Raworth (1990) conducted a major investigation of predator prey-relationships in a strawberry field in British Colombia over a three-year period. He found that S. punctum picipes responded numerically to introductions of T. urticae. A similar study in red raspberries showed that S. punctum picipes was capable of detecting and attacking populations of T. urticae at very low density, distributed in small and widely scattered patches. In addition to conventional leaf sampling methods, the interaction of S. punctum picipes with its prey was examined by observing its response to prey patches introduced into the field from laboratory cultures. S. punctum picipes appeared to be active at low prey densities (Congdon 1993).

It has also been suggested that dispersal and searching ability, rather than numerical response, are key components of the prey-predator association. Zadeh et al. (1995) studied the functional response of adult S. gilvifrons in an apple orchard in Iran against P. ulmi. There
were correlated population fluctuations of both species beginning in early spring and continuing throughout summer. Populations of the pest decreased due to the wide distribution of the predator throughout the orchard. He concluded that *S. gilvifrons* was an effective natural control agent of *P. ulmi*.

In a laboratory study the effectiveness of *S. gilvifrons* was investigated against *T. turkestani*. The predators were observed at prey densities of 5, 10, 20, 40, and 80 mites/unit area at 30°C and 70 ± 5% RH. The development of *S. gilvifrons* was greatly affected by prey density. Predation and reproduction rates were highest at a prey density of 80 mites/unit area and lowest at a prey density of 5 mites/unit area. From the data it was concluded that *S. gilvifrons* was an efficient predator of *T. turkestani* (Ahmed & Ahmed, 1988). Chen et al. (1993) observed that *S. chengi* exhibited a clear aggregate response to *P. citri* prey and spent more total time on high-density prey patches. There were negative correlations between the predator density, average individual searching efficiency, and average individual predation. Density-dependent, density-independent, and inversely density-dependent predation was mainly caused by mutual interference and aggregation among the predators. *S. gilvifrons* adults and larvae showed Type III and Type II responses respectively, when fed *Panonychus ulmi* in the laboratory (Zadeh et al. 1995).

In general, *Stethorus* species do not remain in a locality when mites are scarce (McMurtry et al. 1970). However, in specific cases they appear to be capable of maintaining mite populations below that at which economic crop losses occur (McMurtry & Johnson 1966; Colburn & Asquith 1971; Readshaw 1971 1973; Richardson 1972; Hull et al. 1976). In many cases, however, *Stethorus* species do not exert a suppressive effect on mite populations before the economic injury level is exceeded. This has been largely attributed to the fact that high
prey densities are generally required before the predators begin to increase substantially in numbers (Clancy & Pollard 1952; Putman 1955a).

1.7. Host Finding and Host feeding by *Stethorus*

The way that ladybirds find food is the subject of considerable controversy. A number of authors have reviewed feeding behaviour and rate of consumption in coccinellids. Hodek (1973) conducted a number of experiments and concluded that coccinellids find their prey by direct contact. Banks (1957) supported this idea and found that prey could be missed if they were only a few millimetres away, even if the coccinellids were down-wind of the prey. Allen *et al.* (1970) reported that hungry adults stopped momentarily and took quick snatches at a distance of 1.3-1.9 cm from the prey, and apparently did not require previous physical contact or visual stimuli.

Stubbs (1980) came to a different conclusion. He used adults and final instar larvae of seven-spot ladybird (*Coccinella septempunctata*) and found that the adults detected their prey by sight and larvae detected prey by smell. Nakamuta (1984) using video film of the seven-spot ladybird and concluded that it attacked the prey from a distance of 7 mm, but not at all in the dark. He emphasised that sight was important in prey detection. The views of both Stubbs and Nakamuta were also supported by Ciuffardi (cited in Majerus 1994) who reported that adults of two-spotted ladybird (*Coccinella bipunctata*) detect prey by smell at least from a short distance (Majerus 1994). It has been demonstrated that *Stethorus* may also detect their prey by chemicals such as kairomones (Sabelis & van de Baan 1983).
The genus *Stethorus* are also believed to find their prey by contact (Houston 1983). They search in random patterns for prey but after finding and consuming a few mites, searching intensity increases in the vicinity similar to that occurs in a number of other coccinellids. *Stethorus* spp. exhibit positive phototropism similar to their mite prey, so they tend to deposit their eggs among mite populations even in situations not favourable for their larvae. For example, oviposition on plants with hooked trichomes will impede larval movement and feeding activity, and may even kill larvae (Moreton 1969).

Alternative hosts and numerical response to prey in *Stethorus* have previously been discussed in sections 1.5 and 1.6. Other aspects of feeding behaviour and rate of consumption by several *Stethorus* species have been studied by a number of authors indicating Fleschner (1950), Robinson (1953), Collyer (1953), Putman (1955a), Kaylani (1967) and Hodek (1973). Both adults and larvae appear to feed on all stages of spider mites, but prefer immature stages. The first instar larvae feed predominantly on eggs, while later instars and adults feed on all stages of *T. urticae* (Dhoooria 1981; Richardson 1977). Adult *Stethorus* actively fly and aggregate on mite colonies and eat mite eggs, while larvae consume motile stages (Helle & Sabelis 1985b). A preference for large mobile prey has been observed in *S. madecassus* (Chazeau 1974) and *S. nigripes* (Richardson 1977). They suck, chew, damage or eat whole adult mites. In temperate conditions, *S. punctillum* mostly devoured overwintering mite eggs in autumn (Collyer 1953). The daily rate of prey consumption by ovipositing females may exceed 40 adult mites or a greater number of immature stages per day. Consumption by fourth instar larvae may be even higher during later larval development (in excess of 200 mites) than the total consumption for the rest of the larval development (McMurtry et al. 1970). Zhou et al. (1991) recorded that last larval instars of *S. punctillum* consumed a mean of 84.6 mites/day.
Laboratory and field studies have been conducted with different species of *Stethorus* against different species of tetranychid mites to assess their rate of consumption. For example in a greenhouse environment, adult *S. punctum* consumed an average of 20 adult female mites per day (Bravenboer 1959). Moreton (1969), recorded that adult *S. punctum* required 20-40 mites per day but could consume as many as 140 mites per day at high temperatures. Larvae of the same species accounted for up to 250 mites a day, if they did not chew, but sucked the body haemolymph of their prey. He suggested that if starting with one female *S. punctum* and 100 female red spider mites, the latter would be eliminated after three generations. Colburn & Asquith (1970) studied the average feeding rate of *S. punctum* on *P. ulmi* and found that adult beetles consumed an average of 8.75 motile stages of mites and larvae consumed 9.67 per hour. *S. nigripes* is capable of consuming all stages of *T. urticae* but mite eggs are preferred over other stages (Richardson, 1977).

The potential of *S. punctum* as a biological control agent against the apple pest, *P. ulmi*, was evaluated in the laboratory. It killed significantly more *P. ulmi* at a mite density of 45/ arena than any other predator tested (Parrella *et al.* 1980). A laboratory study carried out in India used the leaf-disc technique to determine the duration of feeding by various predators on different stages of the citrus pest *Eutetranychus orientalis* (Klein) (Dhoooria 1981). He found that 1st instar larva of *S. pauperculus* fed only on eggs and larvae of the mite, but later instars fed on deutonymphs and adult females. A 4th instar larva of this species required an average of 56, 44, 58.12, 95.71, 233.75, and 117.5 eggs, larvae, protonymphs, adult male and female mites respectively. Eleven predators of *Tetranychus* mites were noted in India with the most effective being *Stethorus* sp., the adults of which destroyed 40 mites and the final instar larvae 104 mites per day respectively (Rustamova 1981).
In a number of *Stethorus* spp., starved individuals have been reported to consume more mites than satiated ones (Helle & Sabelis 1985b). For example, satiated larvae of *S. punctum* spent most of their time searching (74.4%) but when starved, time was equally shared between feeding and searching (Houck 1980). Feeding time increased significantly with the number of motile stages eaten, but not for mite eggs consumed. Starved adults concentrated their searching efforts within leaf searching patterns, while satiated adults travelled greater distances. *S. punctum* larvae, however, concentrated their efforts on prey once it was located and travelled further when starved (Houck 1980). Gikorashvili (1983) compared feeding by adult phytoseiids with *Stethorus* species in the USSR and concluded that adult phytoseiids destroyed an average of 3.6 *Panonychus ulmi* in a 24 hours, while larvae and adults of *Stethorus* destroyed 95 and 41 mites respectively. Gravid females consumed twice as many prey as did males (Helle & Sabelis 1985b).

Houck (1991) in laboratory studies examined the proportion of time *S. punctum* spent on searching, feeding and resting with *T. urticae* as host. Satiated female beetles spent 45.1% of their time searching, 14.4% feeding and 40.5% resting, while starved females spent a greater proportion of their time on feeding. Satiated larvae spent 78.4% of their time searching and 21.6% resting. The percentage of time spent by starved larvae on feeding and handling increased with increasing prey density.

1.8 Integrated Mite Control

Integrated pest management (IPM) can be defined as a strategy for managing pest populations by taking advantage of all available control measures. It emerged as a result of significant changes in attitude prompted by the excessive use of chemical pesticides during 1940-1960 period. The trend away from total reliance on chemicals for insect and mite control is
continuing throughout the world. Considerable research is now focused on non-chemical methods (including natural enemies) to control pests (DeBach & Rosen 1991).

The control of two-spotted mites using IPM strategies is probably a very good example of the use of biological agents. Spider mites (especially two-spotted mite, *T. urticae*) are key pests of a wide range of crops and ornamental plants throughout the world. They are notorious pests for their capacity to develop resistance against most of the registered insecticides and miticides (Penrose *et al.* 1999). Therefore chemical control has to be integrated with use of natural enemies, pathogens or plant resistant varieties (Penrose *et al.* 1999).

Markov & Isakulova (1982) reported that *T. urticae* was a serious pest on sugar beet which was mainly controlled by predators, and of them, *S. punctillum* was the most important. Yigit & Uygun (1982) studied the population dynamics of *Tetranychus viennensis* Zacher and its predators *Stethorus* spp., in 5 apple orchards of Adana province, Turkey. It was shown that the predators were able to control *T. viennensis* when broad-spectrum insecticides were not applied repeatedly. Populations of *T. urticae* built up after applications of pesticides on lucerne seed crops in South Australia to control *Theroaphis trifolii*, because of the susceptibility of the predator *S. nigripes* to these chemicals. When pesticide applications ceased *S. nigripes* was able to successfully control *T. urticae* in these crops (Bailey *et al.* 1982).

Atanasov (1983) reported that the spider mites *T. urticae* and *T. turkestanii* infesting strawberries in greenhouses could be controlled by releasing *Phytoseiulus persimilis* at a rate of 5 individuals /m²; these releases could be supplemented by the application of dicofol, dinobuton or diphenyldiazene 1-oxide, if necessary. Spooner-Hart (1991) reported that *P.*
persimilis has been successfully used in Australia against T. urticae in commercial horticultural crops, such as strawberries, vegetables, berries, hedges, nursery and a variety of foliage plants and deciduous fruits.

Studies carried out on four species of spider mites, viz. T. urticae, T. turkestani, T. atlanticus and T. cinnabarinus in maize crops showed S. punctillum to be the most abundant natural enemy. The appearance of this predator was fairly well synchronised with changes in the abundance of spider mites and the ratio between adults and larvae of the predator and motile mites was 1:15 (Nikolov et al. 1983). Both Stethorus spp. and Amblyseius spp. play a very important role in suppressing populations of mites Eriophyes zeasinis and T. cinnabarinus in Indian crops. Of several insecticides endosulfan, chinomethionat and carbaryl (Sevin) afforded the best control of mites with the least adverse effects on these predators (Rather 1983).

Sacco & Girolami (1988) evaluated four different control programs (including IPM) against apple mite, P. ulmi in an apple orchard. P. ulmi populations reached very high levels towards the end of the season, but declined due to the presence of Stethorus spp. The only phytoseiid mite observed was Typhlodromus pyri. Lorenzato (1988) reported a similar observation in apple orchards in which P. ulmi was the most common pest with its abundance inversely proportional to populations of its predators S. darwinii, phytoseiid mites and chrysopids. Orman & Bakanli (1989) studied prey consumption capacity, functional and numerical responses to prey density and the effect of starvation of S. punctillum on its apple pest host Tetranychus viennensis. The population dynamics of both species were also observed in apple orchards, and it was concluded S. punctillum was an effective predator of T. viennensis. Deng et al. (1990) reported that in integrated control of P. citri in China, Stethorus spp. were one of
the predators that could be integrated with cultural, chemical or other methods for the control of *P. citri*. Field (1979) commented on the role *Stethorus* spp in Victorian (Australia) peach orchards.

Injac & Dulej (1992) reported that *S. punctillum* together with *Orius minutus* and *Chrysopa carnea* reduced populations of *P. ulmi*, *T. urticae* and the rust mite *Aculus schlechtendali* in Serbian orchards if acaricides were not applied. Similar results were reported from red raspberry crops in Quebec Canada (Roy *et al*. 1999), where the spider mites *Tetranychus mcdanieli* and *T. urticae* were most abundant in pesticide treated commercial crops, followed by untreated crops and wild berries. *S. punctillum* was abundant in the unsprayed crops but rare in the pesticide treated and wild systems. *Amblyseius fallacis* (Garman) was the most abundant predator in the sprayed crops, whereas a complex of other predatory mite species dominated the wild system. A complex of naturally occurring beneficials, including *S. punctillum* controlled *T. urticae* in strawberry fields in Spain (Garcia & Zamora 1999).

However, the predators colonized the crop when only when the mites reached medium to high levels. In Israel a netted peach orchard in which populations of *T. urticae* and *T. cinnabarinus* increased were suppressed by *Typhlodromus athiasae* and *S. gilvifrons* (Erez *et al*. 1993).

Espinha & Torres (1995) observed that the population density of *S. punctillum* was closely associated with that of *P. ulmi* and concluded that *S. punctillum* controlled this species of mite effectively.

The nature of the host plant species can also influence effectiveness of control of *T. urticae* by *Stethorus* and other predatory species. Rott and Ponsonby (2000a; 2000b) reported that performance of both *S. punctillum* and the phytoseiid mite *Amblyseius californicus*, was superior on pepper and tomato and least on aubergine. This is likely to be a function of the leaf surface characteristics, especially pubescence, which may impede predator movement.
1.9 Rationale and Aims of the Thesis

The selection, rearing and manipulation of natural enemies as biological control agents against crop pests have been successful in agricultural and horticultural production systems worldwide. A number of agents have been used to reduce dependency on pesticides, which have become a major problem due to environmental contamination, pest resistance, and animal and human safety. The increasing number of natural enemies are being evaluated for their potential as a biological agents against their pests. The vedalia ladybird *Rodolia cardinalis* was highly successful against cottony cushion scale, *Icerya purchasi* (Majerus 1994).

Two-spotted mite, *T.urticae*, is distributed worldwide and is a major pest of a wide range of crops such as fruits, vegetables and ornamental plants (Helle & Sabelis 1985a; Hills 1987). In Australia it also attacks a number of field crops, nursery crops, flowers, fruits, vegetables and ornamental plants. The Queensland flower industry lost more than A$ 30 million per annum due to the damage of *T.urticae* (Parker 1991). A number of miticides have been introduced for control of *T.urticae*, but they have developed resistance against these miticides and some populations are now impossible to control with registered miticides (Parker 1991; Penrose et al 1999). However the predatory mites *P. persimilis* and *T. occidentalis* have been successfully used to control *T.urticae* in various crops. After the success of these introduced predatory mites, interest developed in other overseas predators, especially predatory mites. However, little attention has been paid to local predators such as *Stethorus* spp., which may be better adapted to our climate than those imported from overseas. In Europe it has been the practice that many biological control agents are selected by discovery in local environments. The recognition of such species may also be advantageous to building the world pool of natural enemies.
Ladybirds of the genus *Stethorus* are effective predators of two-spotted mite. Several species of *Stethorus* are known in Australia in the Pacific and South East Asia. However, except for *S. nigripes* (Richardson 1977), no detailed investigations have been conducted on any aspect of their biology, ecology, rearing, or their potential as biocontrol agents in Australia. The importance of *Stethorus* spp. in biological control has been more extensively investigated overseas (although much than less for many other species of predators) with a view to the introduction of more ecological sound, effective, and sustainable methods for spider mite management (Moreton 1969; Colburn & Asquith 1971; Gutierrez & Chazeau 1972; Raros & Haramoto 1974; Daniel 1976; Puttaswamy & ChannaBasavanna 1977; Puttaswamy & Rangaswamy 1977; Richardson 1977; Jiang *et al.* 1982; Ahmed & Ahmed 1989; Lie *et al.* 1990; and Shih *et al.* 1991).

This thesis reports investigation on biological aspects of *S. vagans*, one of the most common Australian species of *Stethorus*. Prior to this study there had been no published report on any aspect of *S. vagans* except its description, taxonomy and occasional references to its presence in crops infested with *T. urticae* (Readshaw 1975), although Lamacraft (1972) undertook a brief study on prey preference of two mite species, *T. urticae* and *Bryobia arbores* by *Stethorus* spp. The following parameters were investigated, based on laboratory and field studies:

(i) Influence of temperature, humidity and day-length on the developmental longevity and mortality of *S. vagans* (Chapter 3).

(ii) General biology, reproductive behaviour and diapause (Chapter 4).
(iii) Host location and feeding behaviour (Chapter 5).

(iv) Host preference for primary host stages and alternative host (Chapter 5).

(v) Time partitioning, functional and numerical responses to prey density (Chapter 5).

(vi) Effect of different natural and artificial hosts as well as starvation on mating, fecundity and longevity (Chapter 5).

(vi) Searching behaviour and location of prey populations (Chapter 6).
CHAPTER 2

GENERAL MATERIALS AND METHODS

2.1 INTRODUCTION

Development of effective and economical technologies for mass rearing of prey and predators are key issues for successful biological control programs. Nutritional quality for prey and predatory arthropod rearing is a fundamental unit in biological control. Inadequate nutrition can cause comprehensive changes in the metabolism, behaviour and other characteristics of arthropods. Rearing entomophages is generally more complex than rearing phytophagous species, especially if the species is an obligate predator or parasite. In such cases prey must also be reared, which may in turn require the rearing of host plants for the prey. All these activities must be synchronised.

A number of different systems for rearing predatory coccinellids have been developed. The ladybird *Hippodamia sinuata* Mulsant, was reared in plastic petri dishes and supplied either greenbugs or corn leaf aphids (Michels & Behle 1991). *Stethorus* spp. were reared on orange fruits in 15.46 x 28 hard ware-cloth trays, which held 138 orange fruit of 55 size. These trays were maintained in a linting box (Scriven & Fleschner 1960). Daniel (1976) reared *S. keralicus* in microcages made of cork and glass sheets (5 x 5 cm) into which a portion of a mite infested leaflet could be inserted. Adults and larvae of *S. pauperculus*, were reared in the laboratory in petri dishes (10 cm diameter) inverted one above the other and held in place by a paper band along the rim. Fresh leaves bearing heavy populations of the mite *Tetranychus cucurbitae* were provided daily in these petri dishes (Puttaswamy & ChannaBasavanna
1977). Richardson (1977) used two methods for culturing *S. nigripes* rearing. In the first method he maintained mite on orange fruit in a close container; in a second he brushed mites into 250 ml waxed paper cups in which predators were located. Methods used for mass rearing are not those commonly used for detailed biological studies. Richardson (1977) for example used 2 cm diameter Munger cells for individual stage rearing or biological studies.

### 2.2. Culture

#### 2.2.1. *Stethorus*

The stock culture of the ladybird *Stethorus vagans* was started with adults caught from the field. Thirty potted (15 cm diameter pots) French bean plants infested heavily with *T. urticae* > 100 /leaf, were placed at 10 sites around the Centre for Horticulture and Plant Sciences, University of Western Sydney, Hawkesbury, Richmond (33° 36'S, 150° 44 E), New South Wales, Australia. Beetles collected weekly from these sites were examined under a binocular microscope to identify them to species. In general two species of *Stethorus* were caught from the field, namely *S. vagans* and *S. nigripes*. The number of *S. vagans* caught each week was consistently three times more than *S. nigripes*. After identification *S. nigripes* were released in the field, while *S. vagans* were retained and cultured on detached French bean leaves infested with *T. urticae*, that were placed over wet sponge in ventilated plastic boxes with (dimension 30 x 12 x 6 cm). The plastic boxes were covered with nylon cloth (mesh 70 μ) and their upper lids had a rectangle hole of dimensions 22 x 7 cm cut out (Fig 2.1). The culture was maintained as a stock colony in an illuminated incubator (Thermoline Industries, Thermoline Scientific Equipment Pty Ltd., 40 Blackstone Street, Wetherill Park, NSW 2164, Australia) with internal dimensions 153 x 60 x 61 cm at 25 ± 0.10°C with a photoperiod of L: D 16: 8 hour and relative humidity 44-66 % ± 10%.
Fig 2.1 Rearing of *S. vagans*.

2.2.2 *Two spotted mites*

*T. urticae* were used as prey throughout the three year study period and were obtained from a culture maintained in a heated glasshouse with temperature ranged 21-35°C and daily mean temperature 27 ± 5°C. The French bean *Vicia faba* (cv Redland Pioneer) host plants were grown in plastic trays 60 x 30 x 6 cm in a composted seed potting mix with a basal fertiliser added, and were infested with *T. urticae* one week after emergence. These plants were transferred to 15 cm pots with the same potting mixture and kept in the insectary to use either as food for the culture or for field collecting predators.
Infested potted bean plants were changed every second week in the field, while infested leaves were supplied every day to the *Stethorus* rearing culture.

![Image of two people in a greenhouse with bean plants]

**Fig.2.2.** Rearing of two-spotted mite cultures.

### 2.3 Identification of the Species

The identification of both predators and prey species were confirmed by the Entomology Section of the Biological and Chemical Research Institute Rydalmere; NSW Agriculture and later by the Australian National Insect Collection (ANIC), Canberra. ANIC also provided named specimens of *Stethorus* spp to assist in later identification.

### 2.4 Methodologies for Biological Studies
A range of experimental systems were evaluated prior to biological studies. These were initially selected on the basis of previously reported successful methodologies.

2.4.1 Munger cell

For life history, rate of development, fecundity, mortality and adult longevity modified Munger cells (2 cm diameter) were trialed. Munger (1942), Huffaker (1948) and Laing (1968) have described the construction of and use of Munger cells. There were 10 cells in each set, which were modified by drilling 10 holes in each cell (10 x 1 mm holes) in the upper lid to reduced relative humidity in the cell. A small moist cotton ball was placed in each cell to maintain leaf disc freshness. A pair of S. vagans was released in each cell to study their biology. This method proved unsuccessful, because the leaf discs commonly had free water on their surface probable from the humidity associated the moist cotton balls and the prey become wet and some time died. Both the prey and leaf surface were unacceptable for the Stethorus, resulted in high mortality.

Fig.2.3. Munger cell trialed for S. vagans rearing.
2.4.2 Small cages

A second method evaluated was a cage with a frame made of 5-6 cm diameter aluminium rods and covered with nylon mesh (70μ), except for the front sleeve (12 cm long) which was tied by a rubber band. The volume of each cage was 30 x 30 x 15 cm and a total of 10 cages were used. The base of each cage had a shallow metal tray 25 x 12 x 4 cm, which was regularly filled with water to maintain nutrient levels in potted plants. In each cage a potted strawberry (Fragaria ananassa) plant heavily infested with T. urticae was placed. A pair of S. vagans was released on each plant to study their biology. This method was also not successful because observation of the immature stages under a stereo microscope was very difficult and disturbed them.

2.4.3 Small petri dishes

Sealable petri dishes (5.0 x 0.9 cm) Falcon® (Becton and Dickinson Labware, Becton Dickinon & Co. Franklin Lake, New Jersey, USA) were also evaluated. Leaves were detached from mite infested French bean plants (cv Redland Pioneer) and punched into 2.5cm diameter discs with a metal cork-borer. These discs were placed upside down on 4cm diameter wet sponge on the base of each petri dish. A pair of S. vagans was placed on each leaf disc to assess this method for reproduction. However vapour condensation on the upper lid of the dishes resulted in high mortality of adult S. vagans due to drowning. In an alternative method infested leaf discs were placed on dry sponge, but these discs dried rapidly, again resulting in high mortality of S. vagans.

2.4.4 Modified petri dishes

A number of modifications were made to the sealable petri dishes (Section 2.4.3) to reduce problems with condensation. Initially 30 x 1 mm diameter holes were drilled in the upper lid,
but this did not sufficiently reduce relative humidity. A second modification involved drilling a 3 cm diameter hole in the upper lid and covering it with fine nylon mesh (70μ). This method was successful in reducing the humidity, but the problem was now to maintain sufficient humidity to prevent desiccation of prey and predators in the forced air temperature chambers. The dishes were placed in 35 x 30 x 6 cm plastic containers with the upper lid drilled with 60 x 1 mm diameter holes. This method resulted in low mortality and satisfactory completion of life cycles. The dishes also allowed for adequate observation of *S. vagans* with minimal disruption. This methodology was therefore used to study rates of development, mating behaviour, fecundity, longevity, diapause, time partitioning, and rate of prey consumption.

Leaf discs (2.5 cm diameter) infested with *T. urticae* were placed on wet sponge (4 cm diameter) in these modified petri dishes for experiments with adult *S. vagans*, while dry filter papers (4.7 cm diameter) in the same dishes were used for immature stages. Immature stages were reared on dry filter paper for two reasons: first, dry filter paper provided a large surface area with similar characteristics to leaves; second the last instar larvae become impaled on trichomes on the surfaces of French bean leaves. All stages of *T. urticae* were supplied daily to *S. vagans* larvae by brushing infested French bean leaves with a mite brushing machine (Moreton 1969; Helle & Sabelis 1985b) (Fig 2.5).
Fig 2.4 Modified sealable petri dishes used for biological studies of *S. vagans*.

Fig 2.5 Mite brushing machine.
Assessment of alternative hosts sources was undertaken in the same petri dishes, but with several differences. Wet cotton balls (concentration of distilled water and honey of 50:50 v/v) were placed on the mesh screen; while fresh pollen from pecan (*Carya illinoensis*) were supplied in a small plastic lid (3 mm diameter) placed on the filter paper in the base of petri dish. All other potential prey were either provided on 2.5 cm leaf discs of their respective host plants or removed from the leaf with a fine camel hair brush and placed directly on the filter paper. The moist cotton balls were replaced daily, while pollen was replaced every second day to prevent fungal contamination. For living hosts, their number was counted at the time of introduction and removal, and they were changed daily.

### 2.5 Constant Temperature Incubator

Thermoline® incubators (internal dimension 122 x 52.5 x 43 cm) (Catalogue number RI 250, Thermoline Scientific Equipment Pty Ltd., 40 Blackstone Street, Wetherill Park, NSW 2164, Australia) were used to investigate effects of temperature, relative humidity and photoperiod on the development of *S. vagans*. These were operated at constant temperatures of 10, 15, 20, 25, 30, and 35°C ± 0.1°C with 16 hour photoperiod.

The temperatures in the incubators were automatically controlled. Engineering parameters were configured to provide stable temperature control with minimum overshoot and quick recovery times. However, temperature and RH were regularly confirmed with a digital thermometer and RH probes placing inside in the plastic container beside the petri dishes.

Light intensity was provided by 3 x 30 watt white fluorescent tubes in the door of incubators, which were controlled by a 24 hour time clock. There were air vents located in the top and bottom of the door enclosure to minimise the effect of heat generated by the fluorescent tubes, while most internal surfaces were white to maximise lighting.
2.6. Climate Controlled Growing Room

Host detection behaviour was investigated using infested French beans plants in a temperature control room 2 x 3 x 2.4 m (Bondor® James Hardie Building Systems, Sydney, Australia). The room contained low intensity of light 3 x 30 fluorescent tubes, a refrigerator unit and a humidifying unit (Defensor 505, Axair AG 88108 Pfaffiker, Switzerland). The temperature and relative humidity were controlled automatically. The temperature was set at 25 ± 5°C with relative humidity 44-66 % ± 10% and confirmed with a digital thermometer and RH probes.
CHAPTER 3

RATE OF DEVELOPMENT OF S. VAGANS AT CONSTANT AND FLUCTUATING TEMPERATURES

3.1 ABSTRACT

The development of the ladybird S. vagans was assessed at seven constant (from 10 to 35°C) and variable (12.7-32.1°C) temperatures. Immature stages were reared in modified sealable petri dishes and were supplied with excess prey of two-spotted mite, T. urticae. Observations were made 12 hourly. Larvae developed through 4 larval instars and a pre-pupal stage. The mean development times from egg to adult were 65.2 ± 0.8, 33.2 ± 0.6, 18.2 ± 0.5, 13.1 ± 0.4, and 9.2 ± 0.3 days at constant temperatures of 12, 15, 20, 25, and 30°C respectively, while 15.4 ± 0.3 days was calculated for fluctuating temperatures (varying from 12.7 to 32.1°C). Developmental rate was calculated at each temperature for each stage. There was a strong positive correlation (r = 0.99) between temperature and rate of development. The lower development threshold temperature for egg, 1st, 2nd, 3rd, 4th larval instars, pupa and all immature stages combined was 10.1, 9.5, 9.5, 9.1, 8.2, 8.0, and 9.1°C respectively. Degree day (DD) accumulation was also calculated for each stage as well as for all stages combined. It was estimated to be between 189.2 ± 4.8 - 207.8 ± 6.9 from 12 - 30°C constant and 189.1 ± 5.0 at fluctuating temperatures (12.7-32.1°C) respectively for immature stages to complete their development.

Average egg incubation period decreased from 16.5 ± 0.84 to 2.18 ± 0.24 days with increasing temperatures from 12 to 30°C respectively and was 4.1 ± 0.5 at fluctuating
temperatures (12.7-32.1°C). No egg hatch was observed at 10°C and 35°C. Eggs appeared to develop normally at 35°C, but larvae were unable to emerge. While eggs did not develop at 10°C, they were able to survive a long period of exposure to this temperature. Of approximately 200 eggs that were placed at a constant temperature of 10°C for 60 days then exposed to ≥ 15°C, more than 120 eggs hatched.

Total mortality of immature stages was 28.2, 13.9 and 21.9 % at 25 and 30°C respectively. Mortality was highest in the egg stage and 1st larval instar, and lowest in the 4th larval instar at a constant temperature of 12°C. No mortality was observed in 4th instar, pre-pupal and pupal stages at any other temperatures. Diapause was not observed in any stage in any treatment.
3.2 INTRODUCTION

Temperature and relative humidity are probably the most important physical environmental factors influencing rate of development and survival of living organisms. Degree day (DD) modelling is used to predict developmental events for plants, insects and other poikilothermic organisms (Arnold 1959; Higley et al. 1986). A degree day thermal scale is more accurate in predicting events than a chronological scale.

Rate of development and DD models have been developed for a number of insects: e.g., corn leaf aphid, *Rhopalosiphum maidis* (Elliott et al. 1988), navel orange worm *Amyelois transitella* (Sanderson et al. 1989), Mexican bean beetle *Epilachna varivestis* (Fan et al. 1992), and the coccinellids *Hippodamia sinuata* (Michels & Behle 1991), and *Stethorus bifidus* (Peterson et al. 1994). No data have been published on the degree day requirement development in the genus *Stethorus*, except for *S. bifidus*.

To understand the influence of biotic factors on development of *S. vagans* experiments were conducted at constant as well as fluctuating temperatures, to assess its effect on the development of immature stages. The goal was to obtain developmental data over a wide range of constant and fluctuating temperatures, which could be used to calculate the lower developmental threshold temperatures and to construct a degree day model for all life stages of *S. vagans*. This study was undertaken to also assist interpretation and prediction of seasonal development of *S. vagans* in the field.
3.3 MATERIALS AND METHODS

3.3.1 Field Collection and Stock Colony

Adult *S. vagans* were collected from the field on potted French bean plants (*P. vulgaris cv* Redland Pioneer) infested with two-spotted mites *T. urticae*. Infested bean plants were transferred to 15cm diameter pots from the glasshouse (Chapter 2) and all potted plants in the field were replaced after two weeks. These plants were examined every two days for beetle collection and were watered every day. Field collected beetles were brought to the laboratory and identified to species, because *Stethorus* species other than *S. vagans* were also present in the field. A colony was established from field collected *S. vagans* which was maintained at a constant temperature 25°C with a photoperiod of 16:8 (L: D) and relative humidity 46-75 % in a controlled temperature cabinet in the laboratory. All collections and investigations were carried out at the Centre for Horticulture and Plant Sciences, University of Western Sydney, Hawkesbury, Richmond (33° 36'S, 150° 44'E), New South Wales.

3.3.2 Development at Constant Temperatures

Beetles were randomly selected from the mass colony and paired on 2.5 cm leaf discs infested with *T. urticae* over moist sponge in 5cm diameter modified sealable petri dishes (Chapter 2). These dishes were maintained in modified large plastic containers with dimensions of 35 x 30 x 6 cm. These were placed in different illuminated incubators (122.0 x 52.5 x 43.0 cm) at seven constant temperatures (i.e. 10, 12, 15, 20, 25, 30, and 35°C) in the laboratory (Chapter 2). Eggs of *S. vagans* were collected from the above constant temperatures every 12 hours from the paired beetles and placed singly on a 4 cm diameter dry filter paper in modified petri dishes. Every egg was placed at the same temperature from which it was collected. A cohort of 50 eggs (replicates) per temperature treatment was
allocated to each constant temperature. The photoperiod for all temperatures was 16: 8 (L: D) hours and relative humidity ranged from 44 to 66 %. Separate experiments for higher temperatures (30 and 35°C) were conducted at higher relative humidity (70-85%), because no egg hatching was observed at this lower relative humidity. All dishes were monitored at 12 hourly intervals to assess egg hatching, larval molting and survival. The presence of exuviae in conjunction with head capsule measurements (Chapter 4) was used to determine development to various larval stages. Developmental changes were recorded, and unhatched eggs or dead individuals were removed. These immature stages were supplied daily with excess prey mites obtained by brushing infested bean leaves with a mite brushing machine (Chapter 2).

3.3.3 Development at Fluctuating Temperatures

A separate experiment was also conducted using fluctuating temperatures, to compare rate of S. vagans development with that recorded at constant temperatures.

All adults were randomly selected from the laboratory-established colony and paired over infested bean leaf discs in the modified petri dishes. As oviposition was observed, leaf discs containing an egg were carefully cut out and placed in similar petri dishes as previously described for other studies. These dishes were maintained in two uncovered plastic containers on a table at ambient temperature and relative humidity in a non-climate controlled laboratory. Larval instars were supplied daily with all stages of T. urticae mite as described in section 3.3.2. Temperature and humidity was constantly logged hourly using data loggers (Tini tags®, Hastings Data Loggers, Kempsey, NSW Australia), which were placed beside the petri dishes in the containers. The insect developmental data were recorded at ambient temperatures at 12 hourly intervals as occurred for constant temperatures.
3.3.4 Mortality

During the life cycle study any dead individuals observed were recorded and immediately removed while any immature stage killed accidentally during observation of feeding was removed and excluded from the data.

3.3.5 Data Analysis

The duration of development for each individual was recorded in hours and converted into days for analysis. Standard errors were calculated for all life stages using Excel 5.0 (Microsoft Office, SYBEX Inc., 2021 Challenger Drive, Alameda, CA 94501, USA). The effect of temperatures on the developmental rate of stage specific and combine stages were calculated by analysis of variance (ANOVA) using CoStat (CoHort Software P.O.Box 19272 Minneapolis, MN 55419, USA).

Developmental time of each life stage and all stages combined was expressed as the reciprocal of their duration (as proportion /day). The relationships between temperature and developmental rate were estimated by linear regression analysis using Origin 4.1 (Software for Technical Graphics and Data Analysis for Windows. Microcal Software. Inc., Northampton, MA 01063, USA). The lower developmental threshold temperatures for each specific stage as well as for all stages combined were derived from the regression equation, \( y = a + bx \), where as “y” is the developmental rate (expressed as 1/days) at temperature “x”; and “a” and “b” are estimates of the “y” intercept and slope respectively (Sokal & Rohalf 1995).

Degree-days (DD) were computed for development of each life stage and total stages using the method outlined by Price (1997):

\[ DD = D (T-t), \]
DD = Degree-days needed for development at a specific temperature.

D = Mean numbers of days required for development at a certain temperature.

T = Temperature at which the development was observed.

t = Minimum threshold temperature for development.

The mean DD required for development of each life stage was obtained by averaging its DD associated with all temperature regimes separately for eggs, larval instars and pupae. The mean DD for total development (egg-adult) used data obtained for all stages combined.
3.4 RESULTS

3.4.1 Developmental Time

The rate of development of all immature stages and total stages of *S. vagans* was inversely related to temperature, within the range tested. The mean total development times from egg to adult emergence were 65.2 ± 0.8, 33.2 ± 0.6, 18.2 ± 0.5, 13.1 ± 0.3, and 9.2 ± 0.3 days at constant temperatures of 12, 15, 20, 25, and 30°C respectively (Table 3.1).

The mean egg developmental period for eggs varied from 16.5 ± 0.9 days at 12°C to 2.2 ± 0.3 days at 30°C, although in the latter case eggs only hatched at the higher relative humidity tested (70-85%). No eggs hatched at either humidity level at 10°C and 35°C. Eggs appeared to develop normally at 35°C at the higher relative humidity until just prior to hatching, but the larvae failed to emerge. At 10°C, eggs survived for a long period without developing. Of 200 eggs that were initially exposed to 10°C for 60 days, none showed signs of development. However more than 120 of these eggs hatched within the normal development period when they were subsequently exposed to temperatures of 15 and 20°C.

The duration of the four larval instars were 7.8 ± 0.6, 7.7 ± 0.7, 8.0 ± 0.9 and 9.13 ± 0.6 days at 12°C, and 0.97 ± 0.3, 0.96 ± 0.3, 1.04 ± 0.3, 1.2 ± 0.3 days at 30°C, respectively. The developmental time for the pupa was 16.3 ± 0.5, 9.5 ± 0.4, 4.4 ± 0.5, 3.4 ± 0.4, 2.8 ± 0.3 days at 12, 15, 20, 25, and 30°C, respectively (Table 3.1). There were significant differences in all immature stages combined and for stage specific development times at different temperatures (0.0001<p<0.0008) (Table 3.3).
The mean developmental time of all stages combined was 15.4 ± 0.2 days at fluctuating temperatures (from 12.7 to 32.1°C, and computed to be an average of 21.4°C). Mean development times for eggs, 1st, 2nd, 3rd, 4th larval instars and pupae were 4.1 ± 0.2, 1.6 ± 0.3, 1.7 ± 0.4, 1.6 ± 0.3, 1.7 ± 0.4 and 4.7 ± 0.4 days respectively at the above temperature.

3.4.2 Developmental Rate

The reciprocals of mean development time in days at both constant and fluctuating temperatures were calculated as a percentage of developmental per day (Table 3.2). The development rate for each stage and for all stages combined increased as the temperature increased. The development rate of the egg stage increased from 6.1% per day at 12°C to 46% per day at 30°C. The daily rate of development recorded for all four larval instars combined was 18% per day at 12°C and 96% per day at 30°C, and was 6.2% and 36% per day for the pupal stage at the same temperatures respectively. The daily rate of development for eggs, larval and pupal stages was 24.6, 60.1, 21.5% respectively at fluctuating temperatures. The mean daily development rates of all immature stages at constant temperatures of 12 and 30°C and for fluctuating temperatures were 6%, 36% and 21% respectively.

The figures for mean development rate were plotted against temperature for each stage and for all stages combined to show the effect of temperature (Figs 3.1-3.4). Based on the linear regression equations the lower developmental threshold temperatures were estimated to be 10.2, 9.5, 9.5, 9.1, 8.2, and 8.0°C for egg, 1st, 2nd, 3rd, 4th instars, and pupal stages respectively. The mean lower developmental threshold temperatures for all stages combined was 9.1°C (Table 3.3).
The linear regression statistic (intercept and slope) described the relationship between the developmental rate (y) and temperature (x) for each stage and for all stages combined in *S. vagans*. The correlation coefficient (r) for each stage and all stages combined was very high (from 0.98 to 0.99) indicating a good fit of data to the linear degree-day model within the temperature range of 12 to 30°C (Table 3.4 and Fig. 3.1-3.4). There were no significant differences (α = 0.05) among immature stages at the lower threshold as the 95% confidence intervals overlapped broadly. Therefore the lower threshold temperature for all stages combined was used to determine the number of degree-days required to complete development for each stage and for all stages combined.

### 3.4.3 Degree Day (DD)

Degree-day (DD) requirements (Table 3.4) were calculated for each stage of *S. vagans* and for all immature stages combined from the developmental data and the mean threshold temperature (9.1°C) (Section 3.4.2) (Table 3.3). Total mean DD estimated for development from egg to adult were 189.2 ± 4.8, 195.8 ± 7.4, 198.4 ± 5.6, 207.8 ± 6.9 and 191.7 ± 5.9 at constant temperatures of 12, 15, 20, 25 and 30°C respectively, while it was calculated to be 189.1 ± 5.0 DD under fluctuating temperatures. The DD computed for eggs was 47.9, 58.5, 56.4, 61.1, and 45.6 at 12, 15, 20, 25, and 30°C, and 49.9 at fluctuating temperatures respectively. The DD calculated for the four larval instars ranged between 19.5 to 26.5 per instar over the range of constant and fluctuating temperatures, while for the pupal stage it varied from 47.2 to 58.1 DD at the same temperatures.
3.4.4 Mortality

Mortality throughout the developmental period for all the immature stages combined was highest (34.9%) at 12°C, followed by 15°C (22.2%) and 30°C (21.9%), while the lowest mortality (13.9%) occurred at optimum temperatures (20-25°C). Egg mortality was 9.3, 6.7, 5.6, 5.6, and 8.6% at 12, 15, 20, 25, and 30°C respectively. Larval mortality was highest during the 1st instar, with 12.8% and 12.5% at 12°C and 30°C respectively, but lower at other temperatures, i.e. 9.5, 5.9, and 5.9% at 15, 20, and 25°C respectively. Mortality for the 2nd larval instars ranged from 3.1% at 25°C to 8.8% at 12°C and for 3rd instars from 6.5% at 12°C to 3.8% at 30°C. In the 4th larval instar, mortality was only observed in the 12°C treatment (3.5%). No mortality was observed for pre-pupal or pupal stages in any temperature treatment (Table 4.6).

For fluctuating temperature (12.7-32.1°C) total mortality (ie. all immature stages combined) was 5.9%, which was significant lower that for the same parameter at constant temperatures. Mortality was highest (5.6%) at the egg stage, followed by the 1st and 2nd instars (3.0% each). No mortality was observed in any other stage.
Table 3.1 Development time (days) of immature stages of *S. vagans* at constant and fluctuating temperatures.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Constant Temperature</th>
<th>Fluctuating Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12°C</td>
<td>15°C</td>
</tr>
<tr>
<td>Egg</td>
<td>16.5 ± 0.8</td>
<td>9.9 ± 0.7</td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; instar larva</td>
<td>7.8 ± 0.6</td>
<td>3.4 ± 0.7</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt; instar larva</td>
<td>7.6 ± 0.7</td>
<td>3.3 ± 0.5</td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt; instar larva</td>
<td>8.0 ± 0.9</td>
<td>3.5 ± 0.6</td>
</tr>
<tr>
<td>4&lt;sup&gt;th&lt;/sup&gt; instar larva</td>
<td>9.1 ± 0.6</td>
<td>3.6 ± 0.5</td>
</tr>
<tr>
<td>Pupa</td>
<td>16.26 ± 0.5</td>
<td>9.5 ± 0.5</td>
</tr>
<tr>
<td>All stages combined</td>
<td>65.24 ± 2.3</td>
<td>33.18 ± 0.6</td>
</tr>
</tbody>
</table>
Table 3.2 Rate of development of immature stages of S. vagans (proportion /day).

<table>
<thead>
<tr>
<th>Stage</th>
<th>Constant Temperature</th>
<th>Fluctuating Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12°C</td>
<td>15°C</td>
</tr>
<tr>
<td>Egg</td>
<td>0.061</td>
<td>0.100</td>
</tr>
<tr>
<td>1st instar</td>
<td>0.129</td>
<td>0.298</td>
</tr>
<tr>
<td>2nd instar</td>
<td>0.132</td>
<td>0.303</td>
</tr>
<tr>
<td>3rd instar</td>
<td>0.125</td>
<td>0.286</td>
</tr>
<tr>
<td>4th instar</td>
<td>0.110</td>
<td>0.277</td>
</tr>
<tr>
<td>Pupa</td>
<td>0.062</td>
<td>0.105</td>
</tr>
<tr>
<td>All stages</td>
<td>0.015</td>
<td>0.030</td>
</tr>
</tbody>
</table>

Table 3.3 Regression of rate of development (1/y) with calculated values of correlation coefficient (r), probability (p) and minimum threshold development temperature (t) at constant temperatures.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Regression equations</th>
<th>r</th>
<th>P</th>
<th>t (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg</td>
<td>$Y = -0.21223 + 0.02092x$</td>
<td>0.98</td>
<td>0.0006**</td>
<td>10.14</td>
</tr>
<tr>
<td>1st Instar</td>
<td>$Y = -0.46514 + 0.04898x$</td>
<td>0.99</td>
<td>0.0003***</td>
<td>9.50</td>
</tr>
<tr>
<td>2nd Instar</td>
<td>$Y = -0.46034 + 0.04855x$</td>
<td>0.98</td>
<td>0.0006**</td>
<td>9.48</td>
</tr>
<tr>
<td>3rd Instar</td>
<td>$Y = -0.4076 + 0.04478x$</td>
<td>0.99</td>
<td>0.0001***</td>
<td>9.10</td>
</tr>
<tr>
<td>4th Instar</td>
<td>$Y = -0.30784 + 0.03735x$</td>
<td>0.99</td>
<td>0.0003***</td>
<td>8.24</td>
</tr>
<tr>
<td>Pupa</td>
<td>$Y = -0.13447 + 0.01689x$</td>
<td>0.98</td>
<td>0.0008**</td>
<td>7.96</td>
</tr>
<tr>
<td>All stages</td>
<td>$Y = -0.04655 + 0.00509x$</td>
<td>0.99</td>
<td>0.0001***</td>
<td>9.07</td>
</tr>
</tbody>
</table>

** Significant at p ≤ 0.05

*** Significant at p ≤ 0.01
Table 3.4 Degree-days (DD) needed for development of immature stages of *S. vagans* at constant and variable temperatures.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Constant Temperature</th>
<th>Fluctuating Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12°C</td>
<td>15°C</td>
</tr>
<tr>
<td>Egg</td>
<td>47.91</td>
<td>58.47</td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; instar larva</td>
<td>22.53</td>
<td>19.82</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt; instar larva</td>
<td>21.92</td>
<td>19.47</td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt; instar larva</td>
<td>23.20</td>
<td>20.65</td>
</tr>
<tr>
<td>4&lt;sup&gt;th&lt;/sup&gt; instar larva</td>
<td>26.48</td>
<td>21.30</td>
</tr>
<tr>
<td>Pupa</td>
<td>47.15</td>
<td>56.05</td>
</tr>
<tr>
<td>All stages</td>
<td>189.94</td>
<td>195.76</td>
</tr>
</tbody>
</table>
### Table 3.5 Mortality (%) of life stages of *S. vagans* at different temperatures.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Mortality</th>
<th>Survive</th>
<th>Mortality</th>
<th>Survive</th>
<th>Mortality</th>
<th>Survive</th>
<th>Mortality</th>
<th>Survive</th>
<th>Mortality</th>
<th>Survive</th>
<th>Mortality</th>
<th>Survive</th>
<th>Fluctuating Temp.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12°C</td>
<td>15°C</td>
<td>20°C</td>
<td>25°C</td>
<td>30°C</td>
<td>12.7-32.1°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egg</td>
<td>9.30</td>
<td>6.67</td>
<td>5.56</td>
<td>5.56</td>
<td>8.57</td>
<td>5.56</td>
<td>34</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st instar</td>
<td>12.82</td>
<td>9.52</td>
<td>5.88</td>
<td>5.88</td>
<td>12.5</td>
<td>3.03</td>
<td>33</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2nd instar</td>
<td>8.82</td>
<td>5.26</td>
<td>4.17</td>
<td>3.13</td>
<td>7.14</td>
<td>3.13</td>
<td>32</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3rd instar</td>
<td>6.45</td>
<td>2.78</td>
<td>2.17</td>
<td>0.00</td>
<td>3.8</td>
<td>0.00</td>
<td>32</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4th instar</td>
<td>3.45</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>32</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Larva (1-4)</td>
<td>28.20</td>
<td>16.67</td>
<td>11.76</td>
<td>8.82</td>
<td>21.88</td>
<td>5.88</td>
<td>32</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-pupa</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>32</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pupa</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>32</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>32</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (egg-adult)</td>
<td>34.88</td>
<td>22.22</td>
<td>16.67</td>
<td>13.89</td>
<td>21.88</td>
<td>5.88</td>
<td>32</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fig. 3.1 Effect of various constant temperatures on the rate of development of immature stages of *S. vagans*: (a) Egg, (b) 1\textsuperscript{st} larval instar.
Fig. 3.1 Effect of various constant temperatures on the rate of development of immature stages of *S. vagans*: (c) 2nd larval instar, (d) 3rd larval instar.
Fig. 3.1 Effect of various constant temperatures on the rate of development of immature stages of *S. vagans*: (e) 4th larval instar, (f) Pupa
Fig. 3.1 Effect of various constant temperatures on the rate of development of immature stages and all immature stages of *S. vagans*: (g) All stages combined.
3.5 DISCUSSION

3.5.1 Effect of Temperature on the Development of S. vagans

The developmental time decreased linearly with increasing temperature in the range 10-35°C for all immature stages of S. vagans (Table 3.1). Total developmental time of preimaginal stages of S. vagans (33.2 ± 0.6 at 15°C, and 9.17 ± 0.3 days at 30°C) was different to that reported by Richardson (1977) for S. nigripes (52.3 ± 3.4 and 8.3 ± 0.4 respectively). This slight difference in development rates between these two Australian species may be because S. vagans commonly occurs in coastal and sub-coastal climates, while S. nigripes is largely restricted to hotter inland areas (Britton & Lee 1972; Readshaw 1975; Houston 1980). The total developmental time for S. vagans was also shorter than that reported for S. punctillum (Berker 1958; Jiang et al. 1982), S. gilvifrons (Kaylan 1967; Ahmed & Ahmed 1989) and S. loi (Shih et al. 1991). The reason may be that S. vagans is a smaller species and is also adapted to different climatic conditions. Another reason may be that some of these species have different primary hosts.

Developmental time for individual life stages also reflected the same trend. S. vagans eggs took 9.9 ± 0.7 days at 15°C and 2.2 ± 0.3 days at 30°C to complete development whereas S. nigripes took 14.3 ± 0.9 and 1.9 ± 0.3 days respectively (Richardson 1977). The developmental times for 1st, 2nd, 3rd, and 4th, larval instars and pupal stage of S. vagans (3.4 ± 0.7, 3.3 ± 0.5, 3.5 ± 0.6, 3.6 ± 0.5 and 9.5 ± 0.5 days at 15°C and 0.97 ± 0.3, 0.96 ± 0.3, 1.04 ± 0.3, 1.24 ± 0.3, and 2.78 ± 0.3 days at 30°C respectively) were generally shorter than for S. nigripes (10.54 ± 1.8, 3.95 ± 1.56, 4.19 ± 0.91, 7.67 ± 0.47 and 9.81 ± 0.59 days at 15°C and 1.26 ± 0.28, 0.92 ± 0.19, 0.85 ± 0.23, 1.23 ± 0.28 and 2.03 ± 0.12 days at 30°C respectively) (Richardson 1977).
The lower developmental threshold temperature for preimaginal stages of *S. vagans* was 10.1, 9.5, 9.1, 8.2, 8.0, and 9.1°C for egg, 1st, 2nd, 3rd, 4th larval instars, pupa and all stages combined respectively. Methodology may have had some influence on the higher threshold recorded for eggs. Because the eggs were kept with the portion of the leaf on which they were laid, and other immature stages were reared directly on dry filter paper, there may have been resultant higher humidity in those petri dishes. However differences in threshold temperatures for different immature stages such as those reported here are not uncommon in insect species.

For example, Richardson (1977) recorded different lower threshold temperatures for different immature stages of *S. nigripes*, with the highest for the egg stage (11.5°C) and lowest for 2nd and 3rd instars (6.5 and 6.0°C respectively), and Nordin & O’Canna (1985) recorded lower thresholds for fall webworm, *Hyphantria cunea* of 14, 11, and 12°C for egg, larva, and pupa, respectively. Our results are also consistent with those recorded for *S. bifidus*, (11.9°C for egg stages and 9.4°C for 3rd instars) (Peterson *et. al.* 1994).

The lower developmental threshold temperature for eggs appears to be valid based on the data showing that eggs did not develop at 10°C (Section 3.4.1), although they remained viable when exposed to this temperature for a long time (61 days) and subsequently hatched in the normal time period when exposed to temperatures of ≥ 15°C. This characteristic may enable this species to survive winter in the egg stage and hatch when temperature and other climatic conditions become favourable. However, in the field very few eggs appear to hatch in winter even when the temperature rises above from 10°C, because field counts over a two year period indicated the abundance of motile *S. vagans* declined (Chapter 4).

Van de Vrie (1972) calculated a lower threshold temperature of 10°C for *T. urticae*, while Readshaw (1975) reported a lower threshold of around 9 to 10°C for eggs and immature
stages of this mite species. Therefore the lower threshold temperatures of prey and predator appear to be in harmony. The period of development reported for *T. urticae* is, however substantially shorter than that for *S. vagans* (i.e. from egg-to-egg 36.4, 16.6, and 7.3 days at 15, 20 and 30°C) (Bodman *et al.* 1993). The development period of *S. vagans* recorded was 43.8, 23.6, and 10.3 days at the same temperatures. The oviposition rate reported for *T. urticae* is also higher (i.e 200 eggs at a rate of 3-14 eggs /female /day in their life span of 3-4 weeks) (Bodman *et al.* 1993). However the rate of predation by *S. vagans* larvae and adults we recorded is much higher than the intrinsic rate of *T. urticae* increase (i.e. larval instars of *S. vagans* consume 27.9-152 eggs /day, and adult males and ovipositing females 63.5 – 142.7 eggs /day). This high predation rate appears to explain reported field observations of rapid declines in spider mite populations in the presence of *Stethorus* spp.

Eggs of *S. vagans* developed into larvae at a constant temperature of 35°C, but they could not survive for a long time. The temperature during summers in the Hawkesbury valley (the region in which the investigations were conducted) does not often rise above 35°C, although temperatures >40°C are occasionally recorded (Pat Hanson, climatic data recorder, UWS, personal communication). On the other hand temperatures in the microclimate within trees, shrubs and ground cover plants are frequently lower than the normal maximum ambient temperature, and humidity at the leaf surface is also higher than ambient conditions. This is likely to provide suitable environments for all *S. vagans* stages to survive and reproduce under otherwise inhospitable environments.

### 3.5.2 Degree Days (DD)

The degree-day (DD) model is the most widely used approach for describing insect development rate and in predicting insect developmental times as a function of temperature. There were slight differences between the DD calculation for preimaginal stages and all
stages combined depending on whether data was generated from different constant and/or fluctuating temperatures. These slight differences may be due to temperature fluctuations occurring when petri dishes were removed from the temperature cabinets for observation, although every attempt was made to minimise this influence. However, differences in DD resulting from constant and fluctuating temperatures has been reported by a number of authors. For example, Hanula et al. (1987) recorded different DD for immature stages of pine the coneworm, Dioryctri amatella, at different constant and fluctuating temperatures, and Tolley & Neimczyk (1988) also reported considerable variation in DD for the fruit fly, Oscinella frit calculated from eight constant temperatures. Our results (207.8 DD) recorded at a constant 25°C for all stages combined for S. vagans is quite close to that recorded for all stages combined of S. bifidus (217 DD) estimated at a constant 27.5°C (Peterson et al. 1994).

3.5.3 Mortality:
The total mortality observed for S. vagans (13.9%) at a constant 25°C was much less than that recorded by Richardson (1977) for S. nigripes (40%). Mortality in the egg stage and 1st larval instar was also comparatively lower than for S. nigripes (viz. egg mortality 9.2% and 1st larval instar 19.5%). However while mortality in the 2nd larval instar was not very different, no mortality was observed for 3rd instar, 4th instar, prepupal and pupal stages of S. vagans compared with 4.2, 3.3, 4.5, and 1.2% for S. nigripes receptively. The reason may be that Richardson (1977) used 2.5 cm diameter Munger cells with bean leaf discs over moist cotton balls, while we used 5 cm diameter petri dishes and brushed mite stages on dry filter paper. Richardson (1977) also reported that 3rd and 4th instar often become impaled at leaf disc trichomes and died. In the preliminary investigations for rearing S. vagans reported in Chapter 2, high mortality was also recorded. However our rearing techniques were modified to reduce mortality to a more acceptable level.
CHAPTER 4

BIOLOGY OF *STETHORUS VAGANS*

4.1 ABSTRACT

Aspects of the biology of *Stethorus vagans* were studied at constant and fluctuating temperatures. The constant temperatures used were 12, 15, 20, 25 and 30°C and temperature varied from 12.7-32.1°C, with a photoperiod L:D 16:8 hours and at varying relative humidity (45-80 %). Larvae and adults were regularly fed with all stages of two-spotted mite, *T. urticae*, in modified petri dishes.

Newly laid eggs were translucent white, turning pale yellow after 4-5 hours. The mean egg dimensions were 0.36 x 0.19 mm. Eggs laid by unmated females did not hatch or show any signs of development. After egg hatching the newly emerged larva was white in colour, but soon become pale creamy-white. There were four larval instars, which were differentiated from each other by the presence of shed exuviae and differences in head capsule size. The pre-pupa, not a distinct stage in the life cycle but a quiescent period at the end of the 4th larval instar, lasting for a several hours. Pupae were oval, flattened and black-brown with fine hair-like setae on their dorsal side and a mean length and width of 1.06 x 0.74 mm. The adults were oval, convex and black with small yellow setae on their dorsal side.

The sex ratio was consistently 1:1. Mating was observed at all experimental temperatures (ie. 12-30°C). The mean duration of copulation decreased from 157.2 to 51.0 minutes as temperatures increased from 12°C to 30°C. There were also large variations in the duration of pre-oviposition, oviposition and post-oviposition periods over these temperatures. The pre-
oviposition period ranged from 12.5 ± 0.5 days at 12°C to 1.1 ± 0.4 days at 30°C and the oviposition period varied from 100.2 ± 7.5 to 18.3 ± 2.5 days at 12 and 30°C respectively. Mean fecundity was highest at 25°C with a lifespan oviposition of 189.7 ± 20.6 eggs/female and a mean oviposition rate during the reproductive period of 6.6 ± 0.3 eggs/female/day. Egg hatchability was also higher at 25°C compared with other constant and fluctuating temperatures. The post-oviposition period was 13.1 ± 0.6 and 1.3 ± 0.3 days at 12 and 30°C respectively. There was no significant difference between the longevity of males and females, with means of 125.6 ± 11.0 and 125.7 ± 7.6 days respectively at 12°C and 16.3 ± 4.1 and 17.9 ± 2.2 days at 30°C. The mean duration from commencement of egg laying to death of adults was 190.9 ± 10.0 days at 12°C and 27.1 ± 2.0 days at 30°C, while the mean generation time (egg to egg) was 77.8 ± 1.7, 15.5 ± 0.8 and 10.3 ± 0.4 days at 12, 25 and 30°C respectively.
4.2 INTRODUCTION

Biological control requires choice of appropriate natural enemies, which in turn requires knowledge of rearing, release, specific life history and behavioural characteristics of the biological control agent concerned (Albuquerque et. al. 1994).

Biological studies have been conducted overseas on several Stethorus species, including one Australian species, S. nigripes (Richardson 1977). In general the mean generation time for Stethorus spp. may be two weeks under favourable conditions, which is slightly longer than the development time required for most plant feeding mites (Moreton et. al. 1969; Helle & Sabelis 1985b). However their oviposition rate is higher and their oviposition period is longer than their prey if food is abundant (Moreton et. al. 1969; Jeppson 1975; Pavlova 1975; Singh & Ray 1977). Houston (1980), Gordon & Andreson (1979) and Britton & Lee (1972) described the holotypes of adults, pupae, and last instar larvae of the Australian species of Stethorus, viz S. vagans, S. nigripes, S. fenestralis, S. obscuripensis and S. histrio. However they did not attempt to describe their life histories. A review of the literature reveals no information on the biology and ecology of S. vagans, except for some scattered references to its presence in Australia (Bower & Thwaite 1995). Therefore the present studies were undertaken to elucidate information on aspects of its biology. This was considered essential in assessing its potential rate of population increase and for predicting of the number of generations that may occur within a year. The monitoring, collection and biological studies were all carried out at the Centre for Horticulture & Plant Sciences, University of Western Sydney, Hawkesbury, Richmond, NSW.
4.3 MATERIALS AND METHODS

4.3.1 Cultural Colony

Cultures of *S. vagans* were established in the laboratory from field collected adults on potted French bean plants at the Centre for Horticulture and Plant Sciences, University of Western Sydney Hawkesbury, Richmond, NSW (Chapter 2). Cultures were maintained under controlled conditions of 25 ± 2°C, RH 46-75% and a photoperiod L:D 16:8 hours in a constant temperature cabinet. Beetles were fed all stages of two-spotted mite, *T. urticae*, on French bean leaves (Chapter 2).

4.3.2 Biological Studies

4.3.2.1 Life cycle

All life cycle observations were made on first generation offspring from *S. vagans* adults collected in the field. The parental pairs were randomly selected from the stock colony. Each pair (male & female) was placed on a 2.5-cm diameter leaf disc infested with all stages of *T. urticae*. These leaf discs were maintained on water-saturated foam in modified sealable petri dishes (Chapter 2). Five pairs were randomly allocated to various constant (12, 15, 20, 25, and 30°C) and fluctuating (12.7-32.1°C) temperatures in the laboratory. Eggs deposited by pairs at each temperature were collected 12 hourly. Each egg was carefully isolated by cutting the section of leaf containing the egg and placing it individually on a 4.7-cm diameter filter paper (Whatman: Catalogue number 1820 047), in a new 5-cm diameter petri dish. Eggs collected from each temperature were exposed to the same temperature as previously, and replicated 50 times. After egg hatching, the larval instars were supplied daily with all stages of *T. urticae* and were retained in the same petri dish until adult emergence. The presence of shed exuviae as well as changes in head capsule size were used to differentiate between larval instars. Measurements of the head capsule as well as dimensions of life stages of *S. vagans*
were made using a stereo-zoom microscope (compound microscope, BM series, Olympus Optical Co. Japan) fitted with an ocular micrometer (at 400 times magnification). The duration of immature stages was assessed at 12 hourly intervals as described in Chapter 2.

After adult emergence, sexes were identified and paired on the day of emergence. All pairs were placed on mite infested bean leaf discs (2.5 cm diameter), and kept at the same temperature at which they had been previously raised to study their reproductive biology. Reproduction was evaluated by observing each pair 12 hourly until the commencement of oviposition. The number of eggs laid by each female was counted daily until its death. Eggs were removed and placed at the same temperature in separate petri dishes, where the number of newly hatched larvae was recorded daily until no eggs hatched for several days. Egg hatchability (%) was calculated as well as pre-oviposition, oviposition and post-oviposition periods and adult longevity.

4.3.2.2 Sex ratio and mating behaviour

The sex ratio of S. vagans was investigated because of its importance in population dynamics. Sex ratio was determined for each constant and fluctuating temperature. Twelve hourly observations were made of the petri dishes containing pupae. Any newly emerged adults observed were transferred singly into empty scalable petri dishes to identify their sex by observing them with a binocular microscope. Identification was based on size (males are slightly smaller than females) as well as the presence of a cleavage in the 10th abdominal segment of males (Britton & Lee 1972).

Mating behaviour during pre-oviposition, oviposition and post-oviposition periods was observed for each temperature regime in which the corresponding immature stages had been
previously raised. Adults were paired after emergence on two-spotted mite infested leaf discs and the frequency and duration of copulation was noted at frequent (3 hourly) intervals. Once copulation was noted commencing pairs were observed constantly to determine the duration of copulation.

4.3.2.3 Effect of male and female ratio on oviposition and fertility

A separate experiment was also conducted to assess the effect of mating and the male: female ratio on oviposition and fertility, as well as on frequency and duration of copulation. Virgin male and female cultures were produced by isolating newly emerged adults which were raised singly from eggs maintained at a constant temperature of 25 ± 2°C. The following treatments were established using the same modified petri dishes used for assessment of immature stage development.

(i) T1: 10 newly emerged unmated females confined singly.

(ii) T2: 10 newly emerged females mated once and then confined singly.

(iii) T3: 10 newly emerged females and males confined in pairs (control).

(iv) T4: 10 newly emerged females each confined with 2 males.

All adults were paired on mite infested leaf discs (Chapter 2). Each day the number of eggs laid were recorded and the leaf discs were transferred to new dry filter papers in petri dishes. The number of eggs laid per day and their viability were compared between treatments. Conditions during the experimental period were 25°C ± 2°C, photoperiod 16L: 8D hours and relative humidity 46-80%. Observations were continued until adult death.

Data were collected for the following parameters where appropriate:

- Duration of copulation
-Frequency of copulation
-Behaviour of the two males during copulation
-Oviposition and fecundity
-Egg hatching

4.3.3 Diapause Studies

4.3.3.1 Laboratory experiment

To investigate the possibility of diapause in any stage of *S. vagans* a separate experiment was conducted at a range of temperatures and daylengths in the laboratory as described below.

<table>
<thead>
<tr>
<th>Light: Dark</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>6: 18</td>
<td>11 ± 0.5°C</td>
</tr>
<tr>
<td>6: 18</td>
<td>32 ± 0.5°C</td>
</tr>
<tr>
<td>18: 6</td>
<td>11 ± 0.5°C</td>
</tr>
<tr>
<td>18: 6</td>
<td>32 ± 0.5°C</td>
</tr>
</tbody>
</table>

To commence this study virgin males and females were raised at a constant temperature of 25 ± 2°C as previously described. Newly emerged adults were randomly selected and confined in pairs on 2.5 cm leaf discs infested with all stages of *T. urticae*. Twenty replicates were randomly allocated to each of the four treatments. These pairs of adults were placed in their allocated treatment for mating and oviposition. Eggs deposited in each treatment were placed singly on dry filter paper in separate modified petri dishes (Chapter 2) and placed back in the same treatment. Development was followed by examining each petri dish every 12 hours from egg incubation until adult death.
4.3.3.2 Field collection

Adult *Stethorus* were field collected over a three-year period from potted bean plants for a stock colony (Chapter 2). However from March 1996 to February 1998 all leaves collected from the field were thoroughly examined under a stereo microscope for different stages of *S. vagans*. The numbers of each stage were recorded, to assess whether diapause occurred at any stage in the field.

4.3.4 Statistical Analysis

4.3.4.1 Life cycle

The variability of duration of all life cycle stages at all temperatures was assumed to be normally distributed; thus an analysis of variance (ANOVA) of the data was undertaken using the statistical software package CoStat (CoHort Software P.O.Box 19272, Minneapolis, MN 55419, USA).

4.3.4.2 Sex ratio

Sex ratios were analysed to test the null hypothesis that female: male = 1:1. For this purpose, the $\chi^2$ goodness of fit test was applied using the statistical function of Excel 5 (Microsoft Office).

4.3.4.3 Effects of male: female ratio on oviposition and fertility

The effect of male to female ratios on fecundity and egg viability (ie. female fertility) was also analysed with ANOVA from CoStat CoStat (CoHort Software P.O.Box 19272, Minneapolis, MN 55419, USA).
4.3.4.4 Diapause

The oviposition and fertility rates of all S. vagans females were calculated by analysis of variance (ANOVA) using CoStat.
4.4 RESULTS

4.4.1 Description of Life Cycle

4.4.1.1 Egg

Newly laid eggs were translucent white, turning pale yellow after 4-5 hours. They were smooth, elongate and rounded at both ends. The mean size of the eggs was 0.36 x 0.19 mm (Table 4.1). They were laid horizontally on the lower surface of the leaves along the midrib and lateral veins in an exposed position. They were commonly deposited singly, but occasionally in pairs among large mite colonies. Fertilised eggs appeared granular in the first two days, while two red eyespots developed one-day before hatching. The eggs became transparent on the day of hatching and the developing embryo could clearly be seen through the chorion of the egg. Unfertilised eggs were slightly smaller and did not show any colour changes as they gradually shrivelled and died.

Fig. 4.1 Egg of *S. vagans*
4.4.1.2 Larva

Larvae moulled three times with instars closely resembling each other: the presence of a shed exoskeleton and head capsule size was used to differentiate between instars (Table 4.1). Prior to moulting, larvae stopped moving and fixed themselves by the 10th abdominal segment to a surface and remained inactive for 3-5 hours. Ecdysis began at the head and continued along the back throughout the abdomen. The shed exoskeleton remained fixed the substrate and the larva emerged from the exoskeleton of the former instar.

Four larval instars were recorded. The newly emerged larvae were white, but soon become pale creamy-white. The pink coloured contents of the alimentary canal (turning dark after feeding) were visible through the larval body. All instars possessed numerous dark brown setae over the tergites and pleurites with dark brown pigmentation at the bases of the dorsal setae. The measurements of body length, width and head capsule are presented in Table 4.1. The analysis of variance shows that the mean body length, width and head width of each instar different significantly (p ≤ 0.05).

![Fig. 4.2 Larva of S. vagans](image)
4.4.1.3 Pre-pupa

When the final instar stopped feeding, it attached itself by the anal cremaster to the substrate. The larva shrank and gradually hunched up its back. This marked the prepupal stage, which was cream in colour. This stage was not a distinct stage in the life cycle, but a quiescent period at the end of 4th instar, lasting for only a few hours.

Fig. 4.3 Pre-pupal stage of *S. vagans*

4.4.1.4 Pupa

The pupa was oval, flattened, and subtruncate anteriorly and tapered posteriorly. It was creamy in colour for the first few hours then became uniform black-brown. The body was covered with fine hair-like setae and the abdominal segments, wing pads and legs of the adult were very prominent. In the field, pupae were found in different locations but most frequently
on the underside of the leaves. In the laboratory they were found on leaf discs as well as on
the inner surface of petri dishes. The mean length and width at widest point of the pupa was
1.06 x 0.74 mm respectively (Table 4.1).

Fig. 4.4 Pupal stage of S. vagans

4.4.1.5 Adult

Adults emerged from the pupal skin, by splitting it transversely and longitudinally on the
ecdysial sutures in the thoracic region. The beetles took several minutes to emerge
completely. Once free they sat beside their pupal cases, where they remained for
approximately an hour. The newly emerged beetles were light yellow in colour for first the
few minutes then slowly changed through orange to black. The pronotum, however was
completely black at eclosion. Adults were convex and oval, being wide in the middle and narrow at both ends. Males were smaller than females and were differentiated from the females by a cleavage of their 10th abdominal sternite. The mean length and width of males and females were 1.04 x 0.75 mm and 1.15 x 0.79 mm respectively. The mean adult longevity was 125.7 ± 7.6, 72.6 ± 3.3, 41.9 ± 3.3, 26.6 ± 2.3, and 17.9 ± 2.2 days at 12, 15, 20, 25, and 30°C respectively, and 30.0 ± 1.6 days at the fluctuating temperatures (12.7-32.1°C). The total life span from commencement of oviposition to adult death ranged from 190.9 ± 10.0 to 27.1 ± 2.0 days at constant temperatures of 12 and 30°C respectively, and 27.1 ± 2.0 days at fluctuating temperatures (Table 4.2).
Table 4.1 Measurements (mm) of all stages of *S. vagans*

<table>
<thead>
<tr>
<th>Stages</th>
<th>n</th>
<th>Length</th>
<th></th>
<th></th>
<th>Width</th>
<th></th>
<th></th>
<th>Head capsule</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Range</td>
<td>Mean</td>
<td>Range</td>
<td>Mean</td>
<td>Range</td>
<td>Mean</td>
<td></td>
</tr>
<tr>
<td>Egg</td>
<td>10</td>
<td>0.33-0.40</td>
<td>0.36</td>
<td>0.18-0.20</td>
<td>0.19</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>1(^{st}) instar</td>
<td>10</td>
<td>0.50-0.75</td>
<td>0.64</td>
<td>0.10-0.13</td>
<td>0.12</td>
<td>0.10-0.11</td>
<td>0.106(^{a})</td>
<td></td>
</tr>
<tr>
<td>2(^{nd}) instar</td>
<td>10</td>
<td>0.85-0.98</td>
<td>0.91</td>
<td>0.15-0.18</td>
<td>0.16</td>
<td>0.13-0.14</td>
<td>0.131(^{b})</td>
<td></td>
</tr>
<tr>
<td>3(^{rd}) instar</td>
<td>10</td>
<td>1.08-1.25</td>
<td>1.14</td>
<td>0.20-0.28</td>
<td>0.22</td>
<td>0.15-0.16</td>
<td>0.156(^{c})</td>
<td></td>
</tr>
<tr>
<td>4(^{th}) instar</td>
<td>10</td>
<td>1.5-2.08</td>
<td>1.84</td>
<td>0.38-0.45</td>
<td>0.41</td>
<td>0.18-0.19</td>
<td>0.181(^{d})</td>
<td></td>
</tr>
<tr>
<td>Pupa</td>
<td>10</td>
<td>1.0-1.25</td>
<td>1.06</td>
<td>0.63-0.80</td>
<td>0.74</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Adult</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>10</td>
<td>0.98-1.08</td>
<td>1.04</td>
<td>0.70-0.80</td>
<td>0.75</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>10</td>
<td>1.10-1.20</td>
<td>1.15</td>
<td>0.78-0.80</td>
<td>0.79</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\)Numbers in same columns followed by different letters are significantly different at \(p \leq 0.05\%\)
Table 4.2 Mean duration ± SE (days) of *S. vagans* life stages at different constant and fluctuating temperatures.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Pre-oviposition</th>
<th>Oviposition</th>
<th>Post-oviposition</th>
<th>Female longevity</th>
<th>Male longevity</th>
<th>Adult longevity</th>
<th>Total life cycle</th>
<th>Mean generation time (egg-egg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Constant Temperature</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12°C</td>
<td>12.5 ± 0.5</td>
<td>9.0 ± 0.5</td>
<td>5.4 ± 0.6</td>
<td>2.4 ± 0.2</td>
<td>1.1 ± 0.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15°C</td>
<td>100.0 ± 7.5</td>
<td>60.9 ± 3.9</td>
<td>34.5 ± 3.8</td>
<td>28.2 ± 2.7</td>
<td>18.3 ± 2.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20°C</td>
<td>13.1 ± 0.6</td>
<td>9.7 ± 0.4</td>
<td>8.2 ± 0.85</td>
<td>2.6 ± 0.2</td>
<td>1.3 ± 0.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25°C</td>
<td>125.7 ± 11</td>
<td>79.6 ± 5.7</td>
<td>48.1 ± 1.8</td>
<td>33.3 ± 3.7</td>
<td>20.7 ± 3.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30°C</td>
<td>125.6 ± 11</td>
<td>72.6 ± 6.7</td>
<td>39.4 ± 5.1</td>
<td>26.6 ± 2.3</td>
<td>16.3 ± 4.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Fluctuating Temperature</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21.4°C (12.7-32.1°C)</td>
<td>125.7?? ±</td>
<td>76.2 ± 4.4</td>
<td>42.5 ± 4.3</td>
<td>29.8 ± 2.1</td>
<td>17.9 ± 2.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>7.6</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45.4 ± 4.5</td>
<td>77.8 ± 1.7</td>
<td>43.8 ± 1.5</td>
<td>23.6 ± 1.12</td>
<td>15.5 ± 0.8</td>
<td>10.3 ± 0.6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.4.2. Reproductive Biology

4.4.2.1 Sex ratio and mating behaviour

The number of larvae successfully reaching adulthood in all constant temperatures was 164; these comprised 82 males and 82 females, showing a sex ratio of 1:1. At fluctuating temperatures 32 beetles were raised of which 16 were males and 16 were females; again giving a 1:1 sex ratio (Table 4.3).

Mating was observed at all times of the day and throughout adult life. The mean lifetime mating frequency generally increased with increasing temperature (except for 30°C), ie. 5.2 ± 0.4, 9.7 ± 0.6, 11.1 ± 0.5, 14.6 ± 0.7, 10.3 ± 0.6 respectively at 12-30°C constant, and was 15.5 ± 0.7 at fluctuating temperatures (12.7-32.1). However the mean copulation period decreased with increasing temperature, ie. from 157.2 minutes at 12°C to 51.0 minutes at 30°C and 120.2 minutes at fluctuating temperatures (Table 4.3).

Fig 4.5 A mating pair of S. vagans.
Table 4.3 Sex ratio, mating frequency and duration of copulation of adult *S. vagans* at constant and fluctuating temperatures.

<table>
<thead>
<tr>
<th></th>
<th>Constant Temperature</th>
<th>Fluctuating Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12°C</td>
<td>15°C</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>14.0</td>
<td>17.0</td>
</tr>
<tr>
<td>Female</td>
<td>14.0</td>
<td>18.0</td>
</tr>
<tr>
<td>Male: Female</td>
<td>1: 1</td>
<td>1:1.06</td>
</tr>
<tr>
<td>Mating Frequency</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Duration of copulation (min)</td>
<td>157.2</td>
<td>136.5</td>
</tr>
</tbody>
</table>

4.4.2.2 Reproductive period

There were significant differences in the length of pre-oviposition, oviposition and post-oviposition periods between the different temperatures (Table 4.2). The mean duration of these periods decreased as temperature increased.

The mean pre-oviposition period was 12.5 ± 0.5 days at 12°C and 1.1 ± 0.1 days at 30°C. The mean oviposition period ranged from 18.3 ± 2.5 days at 30°C to 100.2 ± 7.5 days at 12°C. The longest oviposition period maintained by an individual female was 175 days at 12°C and the shortest recorded was 5 days at 30°C. Oviposition ceased 1.3 ± 0.4 days before death at 30°C and 13.1 ± 0.6 days at 12°C. There were no significant differences between female and male longevity. Mean adult longevity was 125.7 ± 11.0 days at 12°C and 27.1 ± 2.0 days at 30°C (Table 4.2). The longest adult longevity recorded for an individual *S. vagans* was 197 days at 12°C.
4.4.2.3 Fecundity and egg hatchability

More eggs were produced at 20 and 25°C than at the other three constant temperatures (Table 4.4). The highest mean total eggs laid per female throughout adulthood was 189.7 ± 20.6 at 25°C and the lowest were 96.6± 9.0 at 12°C and 96.3 at 30°C. The highest mean daily fecundity per female was 6.6 ± 0.5 eggs per day at 25°C, while on several occasions individual females laid, more than 10 eggs per day at this temperature. The lowest number of eggs oviposited was 0.95 egg/female/day at 12°C. The oldest reproductive age was 175 days at 12°C. The greatest number of eggs laid by an individual at 25°C was 327 (33 days) and the lowest was 52 eggs (10 days). The highest percentage of egg hatch was 84.1 ± 1.7% at 25°C and the lowest 46.6 ± 2.3% at 12°C (Table 4.4).
Table 4.4 Reproduction of *S. vagans* reared on *T. urticae* at different constant temperatures

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Number of females (n)</th>
<th>Duration of preoviposition (days)</th>
<th>Duration of oviposition (days)</th>
<th>Total eggs laid Mean ± SE</th>
<th>Eggs /day Mean ± SE</th>
<th>Total number eggs hatched Mean ± SE</th>
<th>Percent egg hatchability Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>12°C</td>
<td>14</td>
<td>12.5 ± 0.5</td>
<td>100.2 ± 7.5</td>
<td>96.6 ± 9.0</td>
<td>1.0 ± 0.3</td>
<td>57.3 ± 6.0</td>
<td>58.7 ± 2.3</td>
</tr>
<tr>
<td>15°C</td>
<td>17</td>
<td>9.0 ± 0.4</td>
<td>60.9 ± 3.9</td>
<td>127.0 ± 10.5</td>
<td>2.1 ± 0.2</td>
<td>86.5 ± 7.0</td>
<td>68.9 ± 2.0</td>
</tr>
<tr>
<td>20°C</td>
<td>22</td>
<td>5.4 ± 0.2</td>
<td>34.5 ± 3.4</td>
<td>148.3 ± 16.0</td>
<td>4.3 ± 0.2</td>
<td>114.2 ± 12.9</td>
<td>75.8 ± 1.5</td>
</tr>
<tr>
<td>25°C</td>
<td>15</td>
<td>2.4 ± 0.1</td>
<td>28.2 ± 2.7</td>
<td>189.7 ± 20.6</td>
<td>6.6 ± 0.3</td>
<td>161.9 ± 18.5</td>
<td>84.1 ± 1.7</td>
</tr>
<tr>
<td>30°C</td>
<td>12</td>
<td>1.1 ± 0.1</td>
<td>18.4 ± 2.5</td>
<td>96.3 ± 14.0</td>
<td>5.5 ± 0.4</td>
<td>72.7 ± 10.8</td>
<td>75.1 ± 2.1</td>
</tr>
</tbody>
</table>
4.4.2.4 Effect of male: female ratio on oviposition and egg hatchability

The mean oviposition rate of females allowed to mate continuously with only one male was significantly higher (p ≤ 0.0001) than for unmated females, those mated only once and those confined continuously with two males (Table 4.5). There was no significant difference in the fecundity of females mated only once or those confined with two males permanently, although both were significantly higher than in unmated females. All females were able to lay eggs throughout their life span, except for unmated females, which laid very few eggs only in the first week after emergence.

Viability was significantly higher (p ≤ 0.0005) in eggs laid by females confined with one male than for females that were unmated, those mated only once and those that were confined with two males. Egg hatchability from females mated once and females confined with two males did not differ significantly from each other, but both were significantly greater than for unmated females (Table 4.5). The egg viability in females that had mated at least once was >76% compared with 0% in unmated females.
Table 4.5 Mean fecundity and egg hatchability of unmated females, females mated once, and females confined with one or two males.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>n</th>
<th>Eggs laid/• /day</th>
<th>Eggs hatched</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
</tr>
<tr>
<td>1• (Unmated)</td>
<td>20</td>
<td>1.9 ±0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.0 ±0.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1• (Mated once)</td>
<td>20</td>
<td>5.3 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.1 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>1• +1•</td>
<td>20</td>
<td>6.3 ± 0.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.1 ± 0.6&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>1• + 2•</td>
<td>20</td>
<td>5.6 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.3 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Numbers in same columns followed similar letters are not significantly different at p ≤ 0.05%*

4.4.6 Diapause

No diapause was observed in any stage of *S. vagans* at any of the treatments assessed, as determined by developmental times and reproductive rates at different constant temperatures and photoperiods (Table 4.7). All females oviposited when exposed to either short (6L: 18D) or long (18L: 8D) days with low (11 ± 0.5°C) or high (32 ± 0.5°C) temperatures. There was no significant difference in oviposition rates in females reared at the same temperature but different photoperiods.

The mean total number of eggs laid per female under the treatments ranged from 37.9 ± 1.2 to 39.2 ± 1.3 and egg viability ranged from 60.1 to 62.9%. Analysis of the data showed that neither temperature nor daylength had a significant influence (p ≥ 0.87) on the mean fecundity and egg hatchability. A minimum of 65% *S. vagans* preimaginal stages completed their development at all treatments tested.
No diapause in any stage of *S. vagans* was observed in the field, as indicated by the continuous collection of all stages of *S. vagans* over a two-year period (Table 4.8). Populations were highest in autumn, spring and summer and lowest in winter. However throughout the year, the relative proportion of different life stages remained fairly constant, which further indicated that diapause in the field was unlikely.
Table 4.6 Effect of temperature and day length on oviposition and egg hatchability of *S. vagans*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Males and Females</th>
<th>Oviposition</th>
<th>Egg hatching</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Total eggs</td>
<td>Mean ± SE</td>
</tr>
<tr>
<td>Short day and Low temperature</td>
<td>20</td>
<td>758</td>
<td>37.9 ± 1.2 <em>a</em></td>
</tr>
<tr>
<td>Short day and High temperature</td>
<td>20</td>
<td>783</td>
<td>39.2 ± 1.3 a</td>
</tr>
<tr>
<td>Long day and Low temperature</td>
<td>20</td>
<td>761</td>
<td>38.1 ± 1.3 a</td>
</tr>
<tr>
<td>Long day and High temperature</td>
<td>20</td>
<td>777</td>
<td>38.9 ± 1.1 a</td>
</tr>
</tbody>
</table>

*a*Numbers in same columns followed by similar letters are not significantly different at *p* ≤ 0.05%
Table 4.7 *Stethorus* species collected from the field during a two year period (March 1996-March 1998)

<table>
<thead>
<tr>
<th>Season</th>
<th>Stethorus vagans</th>
<th></th>
<th></th>
<th></th>
<th>Stethorus nigripes</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adult</td>
<td>Egg</td>
<td>Larva</td>
<td>Pupa</td>
<td>Total</td>
<td>Adult</td>
<td>Egg</td>
<td>Larva</td>
</tr>
<tr>
<td>Autumn 96</td>
<td>301</td>
<td>70</td>
<td>27</td>
<td>14</td>
<td>412</td>
<td>98</td>
<td>38</td>
<td>14</td>
</tr>
<tr>
<td>Winter 96</td>
<td>101</td>
<td>13</td>
<td>8</td>
<td>9</td>
<td>131</td>
<td>38</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>Spring 96</td>
<td>272</td>
<td>76</td>
<td>32</td>
<td>19</td>
<td>399</td>
<td>96</td>
<td>40</td>
<td>20</td>
</tr>
<tr>
<td>Summer 96-97</td>
<td>137</td>
<td>35</td>
<td>20</td>
<td>12</td>
<td>204</td>
<td>37</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>Autumn 97</td>
<td>304</td>
<td>82</td>
<td>34</td>
<td>14</td>
<td>461</td>
<td>105</td>
<td>50</td>
<td>17</td>
</tr>
<tr>
<td>Winter 97</td>
<td>83</td>
<td>15</td>
<td>4</td>
<td>8</td>
<td>110</td>
<td>27</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>Spring 97</td>
<td>247</td>
<td>108</td>
<td>49</td>
<td>18</td>
<td>422</td>
<td>73</td>
<td>32</td>
<td>13</td>
</tr>
<tr>
<td>Summer 97-98</td>
<td>187</td>
<td>29</td>
<td>8</td>
<td>6</td>
<td>230</td>
<td>58</td>
<td>21</td>
<td>7</td>
</tr>
</tbody>
</table>
Fig. 4.7 Field collected stages of *S. vagans* over two years (March 1996-March 1998).

Fig. 4.8 Maximum and minimum temperatures during field collection of *S. vagans* (March 1996 - Feb. 1998).
4.5 DISCUSSION

4.5.1 Life Cycle

4.5.1.1 Egg

*S. vagans* appears to have a similar egg morphology and development as that reported for a number of other *Stethorus* species, such as *S. punctillum* (Putman 1955b, *S. gilvifrons* (Mathur 1969; Ahmed & Ahmed 1989) *S. keralicus* (Daniel 1976) *S. pauperculus* (Puttaswamy & ChannaBasavanna 1977) and *S. nigripes* (Richardson 1977). However the mean egg size of *S. vagans* (0.36 x 0.19 mm) is relatively small compared with *S. punctum* (0.43 x 0.33 mm) (Colburn & Asquith 1971), *S. keralicus* (0.37 x 0.32 mm) (Daniel 1976) but similar to *S. pauperculus* (0.38 x 0.20 mm) (Puttaswamy & ChannaBasavanna 1977). No such measurements have been reported for *S. nigripes*.

More than 60% of eggs hatched within the normal time period at ≥ 15°C, after a long exposure (61 days) to 10°C (Chapter 3). This confirms that *S. vagans* eggs are tolerant of low temperature (10°C) and can survive during winter without loss of viability. The data obtained from field collected eggs over a two year period also confirmed this. At higher temperatures (30 and 35°C) and lower relative humidity, eggs of *S. vagans* lost viability; however with higher humidity (70-85%) many remained viable at 30°C. At the higher relative humidity at 35°C, however, eggs appeared to develop completely but the larvae were unable to emerge. Similar results were reported for *S. nigripes*, which retained egg viability up to 35°C (Richardson 1977), but which decreased greatly above that temperature. Based on the data obtained for both fluctuating (12.7-32.1°C) and at high constant temperatures (30 and 35°C), its appears that normal field development of *S. vagans* is likely to occur under the climatic conditions experienced in the Hawkesbury district (see Fig 4.7), especially given that in crop
plants, microclimates are commonly created where temperatures are lower and relative humidity is higher than ambient conditions.

4.5.1.2 Larva

We recorded four larval instars for *S. vagans*, the same as that recorded for most other coccinellids including *Stethorus* spp., although five instars have been recorded for the *Chilocorus bipustulatus* (Yinon 1969). The larvae were similar to that described by Britton & Lee (1972) and Houston (1980). The larval instars were differentiated by body length and width as well as head capsule width (Table 4.1). *S. vagans* (i.e. 1st, 2nd, 3rd and 4th instar larvae had dimensions of 0.64 x 0.12, 0.91 x 0.16, 1.14 x 0.22 and 1.84 x 0.41 mm respectively) which is smaller than *S. punctum*, {ie. 1st larval instar 1.03 x 1.57 mm and 4th larval instar 2.5 x 2.2 mm} (Colburn & Asquith 1971), but similar to *S. pauperculus* (0.61 x 0.20, 1.0 x 0.32, 1.2 x 0.46 and 1.8 x 0.68 mm, for 1st, 2nd, 3rd, and 4th larval instars, respectively) (Puttaswamy & ChannaBasavanna 1977).

We differentiated larval instars from one another by the presence of shed exoskeletons as well as by head capsule size. Head capsule measurements are the most reliable method of differentiating between larval instars (Dyar 1890). However, its appears that apart from Daniel (1976) who reported head capsule measurements for 1st instar *S. keralicus*, no such measurements have been taken for other *Stethorus*.

4.5.1.3 Pre-pupa

The duration of the pre-pupal stage for *S. vagans* we recorded at 25°C varied from 8 to 13 hours, which is similar to that reported for most other species of *Stethorus*. For example Daniel (1976) recorded 10-15 hours for *S. keralicus* at 26-34°C, while Puttaswamy &
ChannaBassavanna (1977) reported 8 hours for *S. pauperculus* at 24-26°C. However it is much shorter than that reported for *S. nigripes* (24 hours) at 25-35°C (Richardson 1977).

**4.5.1.4 Pupa**

The pupal appearance was similar to that reported by Britton & Lee (1972) and Houston (1980) for the species. Its length and width was 1.06 x 0.74 mm, which is larger than *S. keraticus* (1.03 x 0.7 mm) (Daniel 1977), but smaller than *S. punctum* (1.36 x 0.97 mm) (Colburn & Asquith 1971) and *S. pauperculus* (1.8 x 1.1 mm) (Puttaswamy & ChannaBassavanna 1977).

The duration of the pupal stage was shorter than for *S. keraticus* (3.5-4.0 days at 26-30°C) (Daniel 1976) and *S. pauperculus* (3.8-4.0 days at 25.4-26°C) (Puttaswamy & ChannaBassavanna 1977), but longer than *S. gilvifron* (2.5 days at 35°C) (Ahmed & Ahmed 1989), *S. loi* (3.3 days at 23.8°C) (Shih et al. 1991) and *S. nigripes* (3.16 days at 25°C) (Richardson 1977). It possible that for the latter species, Richardson (1977) recorded an extended pre-pupal stage and a shorter pupal stage (see 4.5.1.3).

**4.5.1.5 Adult**

The colour of newly emerged adult *S. vagans* was light yellow, which turned to a uniform black-brown. Similar results have been described for *S. pauperculus* (Puttaswamy & ChannaBassavanna 1977) and for *S. nigripes* (Richardson 1977) as well as other ladybird genera (Majerus 1994). The males and females were easily distinguished on the basis of characteristics described by Britton & Lee (1972) and Houston (1980). Britton & Lee (1972)
also reported a mean length only for "adult" *S. vagans*, of 1.12mm, while our dimensions were 1.04 and 0.75mm for males and 1.15 and 0.79 mm for females, respectively. As with the immature stages, the adults are smaller than most other *Stethorus* spp., such as *S. pauperculus* (1.47mm length and 1.0 mm width) (Puttaswamy & ChannaBasavanna 1977) and *S. punctillum* (1.5 and 1.01 mm, respectively) (Gordon & Chapin 1983).

Adult longevity of *S. vagans* was less than that recorded for most other species of *Stethorus*, including *S. pauperculus* (30-61 days) at 24-26°C (Puttaswamy & ChannaBassvanna 1977), *S. punctillum* (32-53 days) at 24-28°C (Jiang et al. 1982) and *S. loi* (48-57 days) at 24°C (Shih et al. 1991). This may be because *S. vagans* has a smaller body size than other *Stethorus* spp. and has a larger surface area to volume ratio, therefore requiring more energy to maintain normal physiological and behavioural functions such as location of prey.

**4.5.2 Reproductive Biology**

**4.5.2.1 Sex ratio and mating behaviour**

The sex ratio of *S. vagans* was 1:1, which is similar to observations made for most ladybirds and for many other insect species. For example Richardson (1977) reported a similar sex ratio in *S. nigripes*, as did Shih et al. (1991) and Kumar & Chakraborty (1997) for *Scymnus nubilus* (Coleoptera: Coccinellidae).

Frequent mating was observed in *S. vagans* throughout their life. This is similar to that recorded for other *Stethorus* spp. such as *S. gilvifrons* (Mathur 1969), *S. pauperculus* (Puttaswamy & ChannaBasavanna 1977) and *S. nigripes* (Richardson 1977). The duration of copulation in *S. vagans* was similar to that recorded for *S. pauperculus* (30 minutes) (Puttaswamy & ChannaBasavanna 1977) and *S. gilvifrons* (50 minutes to 6 hours) (Mathur
1969). However it was much shorter than that noted for *S. nigripes* (2-5 minutes) (Richardson 1977). It appears that frequent mating is not necessary for *S. vagans* to maintain fecundity up to 18 days. However based on the data presented in this thesis, some females needed to mate again to maintain high fecundity after this period. This phenomenon occurs in *S. nigripes*, where fecundity was reduced after 20 days and regained when males were placed with females (Richardson 1977). The duration of copulation appears to have no effect on oviposition and fertility in *Stethorus* species. For example the duration of copulation in *S. vagans* is substantially longer than for *S. nigripes*, but in both species, fecundity declined approximately 20 days after the initial mating.

4.5.2.2 Reproductive period

The pre-oviposition period of *S. vagans* was almost double that recorded by Richardson (1977) for *S. nigripes* for all temperatures tested (15, 20, 25 and 30°C), while the mean oviposition period was only half that recorded for *S. nigripes* at 25°C. The post-oviposition period has not been recorded for any other species of *Stethorus* other than *S. vagans*. As the mean daily oviposition rate for *S. vagans* and *S. nigripes* are similar, it appears that the critical factor influencing fecundity is adult longevity.

The mean adult female longevity of *S. vagans* was 33.3 ± 3.7 days at 25°C, much shorter than that recorded for *S. nigripes* (50.5 ± 5.3 days). One possible reason for the differences may be a result of the different methodologies used in recording their life cycle studies. We used (5 cm diameter) petri dishes, while Richardson (1977) used 2 cm diameter munger cells. As explained in section 4.5.1.5, *S. vagans* is smaller than *S. nigripes*, but the searching area in the comparative investigations for *S. vagans* was much greater than that for *S. nigripes*. Therefore more energy may have been expended searching by *S. vagans*, contributing to.
reduced longevity. Our methodology is likely to be more closely correlated with field conditions and may more accurately represent likely field activity. In addition, the same methodologies were used to rear immature stages of the respective species, and we recorded substantially lower mortality rates (Chapter 2). It therefore appears likely that our experimental conditions were better suited to maximising longevity of *S. vagans*.

4.5 2.3 Fecundity and egg hatchability

The mean total fecundity of *S. vagans* (189.7 ± 20.6 eggs) at 25°C was much less than that recorded by Putman (1955a) for *S. punctillum* (1290 eggs) and *S. pauperculus* (339 eggs) (Puttaswamy & ChannaBasavanna 1977) and slightly less than *S. nigripes* (281 eggs) (Richardson 1977). The most likely reasons are that the oviposition periods and adult longevity of the above species are almost double that recorded for *S. vagans*. A second reason for the lower fecundity may be the smaller relative size of *S. vagans* females 1.15 x 0.78 mm compared with *S. pauperculus* (1.47 x 1.0 mm) and *S. punctillum* (1.46 x 1.01 mm) and therefore inherently lower oviposition capacity. The mean daily fecundity of *S. vagans*, however was similar to that published for *S. punctillum* (Robinson 1953), *S. bifidus* (Collyer 1964b), *S. gilvifrons*, (Kaylani 1967), *S. picipes* (Sandness & McMurtry 1970) and *S. nigripes* (Richardson 1977).

The number of eggs produced by isolated female *S. vagans*, those mated only once and those paired continuously are similar to results obtained by Richardson (1977) for *S. nigripes*. However he did not assess fecundity of females maintained with multiple males. The egg rate decline in females confined with two males may be due to disturbance during copulation, because during mating the second male either attempted to ride on the first male or followed
the united pair. However, if mating interference occurred this was not reflected by reduced egg viability over the oviposition of life of females.

The egg viability in *S. vagans* at 25°C was slighter higher (84.1%) than that recorded for other ladybird species. For example Richardson (1977) recorded 80% in *S. nigripes* at 25°C, while Elhag & Zaitoon (1996) recorded egg hatchability of 81.8, 74.5, 72.8, and 68.5% for *Adonia variegata*, *Coccinella undecimpunctata*, *C. novemnotata*, and *C. septempunctata* at 25°C respectively. On the other hand, the females mated only once and confined singly or confined with two males had significantly reduced fertility. These results are supported by Richardson (1977) who observed isolated and paired *S. nigripes* at 25°C and Majerus (1994) who studied a number of other ladybird species.

4.5.2.4 Effect of male: female ratio on the oviposition and egg hatchability

We observed multiple mating in *S. vagans*. This is similar to that reported for *S. punctillum* (Putman 1955a), *S. gilvifrons* (Mathur 1969), *S. pauperculus* (Puttaswamy & ChannaBasavanna 1977) and *S. nigripes* (Richardson 1977). However a single mating was sufficient to produce fertile eggs for 18 days, presumably because viable spermatozoa could be maintained in the spermatheca for that period. *S. nigripes* can produced fertile eggs for 20 days (Richardson 1977) and *Adalia bipunctata* for three months after a single mating, while *Chilocorus renipustulatus* store sperm in their spermatheca throughout winter (Majerus 1994). There was a slightly reduction in the fertility of *S. vagans* as determined by egg viability after 18 days. Richardson (1977) obtained similar results for *S. nigripes* in which fertility was maintained by the introduction of males 20 days after the initial mating. All eggs produced by unmated females were not viable and failed to hatch, as was also reported by
Richardson (1977) for *S. nigripes*. Clearly, mating is essential both for egg production and egg development in these species.

4.5.4 Diapause

Diapause may occur in any insect life stage as a result of one or more factor(s) such as day-length, temperature and food availability (Waage *et al.* 1985). However in most insects it is induced by a specific day: night ratio with low or high temperatures (Daly *et al.* 1998). We conducted our investigations at selected photoperiods and temperatures. These combinations of parameters chosen were not exhaustive; however were considered to be practical and consistent with those reported by other authors. For example Swift (1987) studied diapause in the predatory mite *Amblyseius fallaci*, Canard (1990) in the lacewing *Nineta pallida* and Okuda & Hodek (1994) in *Coccinella septempunctata* all at 16L: 8D and 12L: 12D at 20, 25 and 30 C.

No diapause was recorded in any stage in any temperature /day-length treatment, either in the laboratory or in the field. Rate of development was related to temperature alone. The laboratory data are supported by the results of the *Stethorus* collections from the field over a two-year period (Table 4.7 and 4.8). These results are also supported by Richardson (1972, 1977), who reported no diapause in the Australian species *S. nigripes* in Adelaide (South Australia) and California, and Readshaw (1975) who observed *Stethorus* spp. (including *S. vagans*) in Canberra, Australia, which experiences cooler winters than those recorded at either Richmond or Adelaide. While it is unclear whether *S. vagans* or other Australian *Stethorus* spp. diapause in the coolest regions in Australia (eg. Tasmania) in the field it appears unlikely, based on our results.
While the field observations confirmed the laboratory results that *S. vagans* do not diapause in winter (Table 4.7), their major prey *T. urticae* spider mites do undergo diapause (Helle & Sabelis 1985a). Although some *T. urticae* activity may continue in protected areas (e.g., near walls, rocks or under trees and bushes) or in subtropical regions, further south or at elevation in central NSW or in unprotected areas, spider mite activity ceases and they diapause. Readshaw (1975) found adults and larvae of *Stethorus* spp (including *S. vagans*) in tree bands throughout the winter feeding on diapausing *T. urticae*. Active mite populations in protected areas and diapausing mites in crevices or under tree bark or in leaf litter, as well as availability of alternative hosts (Chapter 5) (Helle & Sabelis 1985a) may be sufficient to support populations of *S. vagans* and other *Stethorus* spp. to continue limited activity during winter.
CHAPTER 5

FEEDING BEHAVIOUR OF THE LADYBIRD S. VAGANS

5.1 ABSTRACT

Adult longevity and fertility of S. vagans were recorded on a range of alternative hosts in the laboratory, via a series of no choice tests. None of the alternative hosts were able to maintain fertility in female S. vagans, except broad mites, Polyphagotarsonemus latus. Although some eggs were laid when S. vagans were supplied with pollen & water, they did not hatch. The most effective food source for maintaining adult longevity was twospotted mite, T. urticae (38 ± 6.0 days), followed by broad mite, Polyphagotarsonemus latus (26.1 ± 0.9), rust mite, Auclops lycopersi (23.47 ± 1.3), pollen & water (21.1 ± 2.7), and honey & water (18.94 ± 2.3). There was no significant difference between male and female longevity on the same host.

Time partitioning and prey preference of both adults and 4\textsuperscript{th} instar larvae of S. vagans were studied in the laboratory. The behaviour of the predators was examined under different feeding regimes i.e. newly emerged, satiated, and starved for 24 & 48 hours. The parameters assessed were the proportion of time spent by the predators searching, feeding, resting, walking and drinking water. All stages of the preferred host T. urticae were freely available during the experiment. The influence of starvation on predation and preference for prey stages were assessed. Newly emerged and fully fed predators spent most of their time resting and walking, while those starved for 24 & 48 hours spend significantly more time searching (28.7 & 27.9%) and feeding (46.7 & 54.9%).
All stages of S. vagans preferred eggs of T. urticae to nymphal and adult stages, irrespective of their previous feeding regimes. More than 80% of the prey of newly emerged adults, satiated adults and newly emerged 4th instar larvae consisted of mite eggs, while they comprised more than 70% of the diet of 24 hour starved adults and 4th instar larvae. Adults and 4th instar larvae starved for 48 hours consumed less than 70% of their prey as eggs.

The rate of consumption by both adults and immature stages of S. vagans was observed at various densities of prey eggs and adult female mites. The total number of mite eggs consumed by 1st, 2nd, 3rd, and 4th larval instars were 27.93 ± 1.1, 50.12 ±1.0, 71.64 ± 1.5, and 152.36 ± 1.6 respectively. This compared with the numbers consumed by adult males, pre-ovipositing females, ovipositing females and post-ovipositing females, of 63.53 ± 0.4, 94.25 ± 0.5, 142.69 ± 0.53 and 57.1 ± 0.6 eggs per day respectively.

All motile stages of S. vagans responded positively to prey density and showed a type-II functional response. Their numerical response was measured by the successful completion of immature stages as well as the reproductive response of resultant adult females. To assess reproductive response, both gross and net oviposition rates were recorded at all prey densities. The gross fecundity was higher than net fecundity at low prey densities, because the adult predators were cannibalistic, eating their eggs because of lack of suitable prey. However both gross and net fecundity increased linearly until they reached a plateau at higher densities.
5.2 INTRODUCTION

Predation is an important component of ecological aspects because through predators the flow of energy continues throughout a community. It also regulates the populations on which they feed and maintains the fitness of these prey populations (Price 1997). Predation is common among ladybirds, and this aspect of their behaviour has received considerable attention, because of the economic importance of many of their prey (Majerus 1994; Price 1997).

Ladybirds are usually highly voracious and often have access to a wide range of prey. Records of prey taken in nature or accepted in the laboratory are numerous, and indicate a broad range of possible animal and plant sources eaten by adults, and to lesser extent larvae. Although the utilisation of alternative food may not be important for the reproductive potential of coccinellids, it appears to be essential for their survival during periods when their natural food sources are unavailable, scarce or building up (Helle & Sabelis 1985b; Majerus 1994).

An effective biological control agent is mainly selected on the basis of its functional and numerical responses to its prey. Functional response is the change in the number of prey eaten per unit time by each predator in relation to changes in prey density (Solomon 1949), while the numerical response is defined as the change in the predator numbers due to prey populations (Crawley 1975). Functional response curves were first derived by Solomon (1949) and then modified by Holling (1959, 1965 and 1966). He described four curves for different predator responses to their prey:
i) A linear rise to a plateau for crustacean predators (Type I).

ii) A curvilinear rise to a plateau for predatory insects (Type II).

iii) A s-shape curve rising to a plateau for vertebrate predators (Type III).

iv) A dome-shape response created by the disturbance of predators by prey activity at high prey density (Type IV).

The manner by which predators search, select, handle and consume prey, as well as their functional and numerical responses to prey densities are major parameters determining the success of biological control agents. These aspects have been reported in ladybirds by several authors, eg. the numerical response of *S. punctum picipes* to *T. urticae* populations in strawberries in British Colombia, Canada (Raworth 1990) and the functional response of *Chilocorus kuwanae* to the scale insect *Unaspis yanonensis* (Yang et al. 1997).

The studies described in this Chapter were undertaken to better understand the relationship of the coccinellid *S. vagans* to its prey in terms of its host location, alternative hosts, time and resource partitioning, rate of prey consumption and functional & numerical responses.
5.3 MATERIALS AND METHODS

5.3.1 Culture of *S. vagans*

Large numbers of *S. vagans* pupae were collected from the field on gerbera (*Gerbera jamesonii*) leaves which were naturally infested with *T. urticae*. These field collected pupae were cultured in plastic boxes 30 x 12 x 6 cm in which a hole 22 x 7 cm was cut in the lid and covered with 70 μ nylon mesh. All adults were identified to species after emergence and *S. vagans* were cultured in separate boxes. These culture were maintained in a controlled temperature cabinet at 25°C, under photoperiod L:D 16: 8. All collections and experimental work were conducted at the Centre for Horticulture and Plant Sciences, University of Western Sydney, Richmond campus (Chapter 2).

5.3.2 Alternative hosts

Two hundred newly emerged beetles were randomly selected from the stock colony and paired in 20 replicates on each of the potential food sources in modified petri dishes (Chapter 2). Rate of predation by paired adult *S. vagans* was measured in the laboratory at 25 ± 2°C on the different food hosts. Other parameters assessed were mating, fecundity, egg hatchability and adult longevity.

Treatments were:

1. Starved, no food
2. Water only
3. Honey and water
4. Pollen and water
5. Citrus aphids, *Toxoptera citricidus* (Hemiptera: Aphididae) (all stages)
6. White fly, *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae) (eggs only)
10. Twospotted mite *T. urticae* (Acarina: Tetranychidae) (all stages) (Control)

The above diets were exposed to adult *S. vagans* either inside or on the nylon screen of the modified petri dishes. The base was covered with dry filter paper to absorb any excrement of hosts or predators, thereby preventing contamination. The number and quantity of each food type was consistent in each treatment, except for Treatment 1 (starved). In each dish a fresh bean leaf disc not infested with *T. urticae* was supplied daily to the pair for oviposition.

In Treatment 2, water was supplied constantly through a cotton roll placed on the mesh screen, and was renewed daily. In Treatment 3, honey diluted with water in a ratio of 3:1 (v/v) was provided in the same manner as water in Treatment 2. These cotton rolls were renewed every second day; to prevent rolls from becoming dry and also to avoid any risk of contamination. In Treatment 3, mixed pollen obtained from honeybees, *Apis mellifera*, was supplied in a small plastic lid (3mm diam.) placed on the dry filter paper, while water-soaked cotton rolls were placed over the nylon screen covering the dishes as in Treatment 2. Cotton rolls were renewed daily, while pollen was replaced every two days, because it developed fungal growth if kept longer. The number of aphids, white fly eggs, predatory mite eggs, rust mites, broad mites and twospotted mites were counted carefully on the host leaf arenas before the predators were confined. After a 24 hour feeding period the pairs of *S. vagans* were transferred to new containers and the remaining number of each host counted and recorded. Mating, fecundity and longevity of *S. vagans* were determined by observing all dishes 12
hourly. The number of eggs laid was counted and the period between adult emergence and death was calculated as longevity.

5.3.3 Time partitioning behaviour

We examined time partitioning behaviour in both adults and 4th instar larvae. Newly emerged beetles were randomly selected from the foundation colony to observe their time partitioning behaviour. Their behaviour was examined individually and compared between satiated and starved for (24 & 48 hours) as well as newly emerged (less than 24 hours) adults. Satiated and newly emerged individuals were tested immediately after selection, while the remainder were maintained in a culture fed with excess *T. urticae* for a week. Each adult treatment was replicated 30 times (15 males and 15 females). Each trial comprised of one individual adult on a 2.5 cm diameter leaf disc infested with all stages of *T. urticae* placed on moist foam in a 5 cm diameter modified petri dish.

To observe time partitioning behaviour of newly emerged, satiated, and starved (24 & 48 hours) 4th instar larvae, 150 newly laid eggs of *S. vagans* were exposed at 25°C ± 2 in a controlled temperature cabinet. The newly emerged larvae were fed excess all stages of *T. urticae* until the 4th instar. Newly emerged 4th instar larvae were immediately exposed to the prey arena as they shed their 3rd larval instar exoskeleton. For satiated 4th instars they were fully fed for one day before assessing their time partitioning behaviour, while other larvae were starved for 24 & 48 hours; however they were fully fed for one day prior to starvation. Each treatment was replicated 30 times. In each trial prey were supplied with all stages of *T. urticae* on 4.7 cm diameter dry filter papers by brushing infested French bean leaves (Chapter 2). Both adults and larvae of *S. vagans* were observed individually for a two hour period.
under a binocular microscope (20 x magnification) and tested only once. Four stopwatches were used (one for each behaviour category) to record the following behaviours:

(i) Searching: defined as slow forward movement
(ii) Feeding: defined as a successful capturing, manipulating and consuming prey. It was considered to be terminated when the predator discarded the exoskeleton.
(iii) Resting: the residual time during which the beetle was not actively searching or feeding, but located in one place.
(iv) Other activities eg. walking (rapid movement, not searching & drinking water): In this category beetles were also observed with other activities such as rubbing their elytra on the edge of leaves or foam.

In these experiments the time spent in each behaviour was recorded as well as the number and stage of each mite prey consumed.

5.3.4 Prey consumption

Newly emerged *S. vagans* adults were randomly selected soon after emergence from the stock colony to investigate the rate of prey consumption for adult males and for pre-ovipositing, ovipositing and post-ovipositing females. Twenty individuals from each of the above groups were exposed to a *T. urticae* density of 200 eggs/arena. For the pre-oviposition category females were selected prior to mating, while for oviposition and post-oviposition they were chosen at the peak of the oviposition period and several days after ceasing egg laying respectively.
The prey consumption rate of immature stages of *S. vagans* was also observed. Approximately 45 newly laid eggs of *S. vagans* were incubated at $25 \pm 2^\circ$C in a controlled temperature cabinet (as previously described in Chapter 2). The newly emerged 1st instar larvae were confined with the same mite egg density used for adult stages. All larval instars were observed at the same prey density and followed until they moulted to the subsequent instar or died.

The prey consumption experiments were conducted in modified sealable petri dishes (Chapter 2) with a 4.7 diameter dry filter paper (Whatman Catalogue Number 1870 047) on their base onto which the mite eggs were placed. The total internal surface area was 32.48 cm$^2$ (diameter = 8 cm, height = 0.3). Mite eggs were obtained by brushing infested French bean leaves onto sheets of paper using a mite-brushing machine (as described in Chapter 2). The sheets were retained for approximately 20 minutes to enable the motile mite stages to move off; any remaining motile stage were removed by an aspirator. Each predatory stage of *S. vagans* was provided with 200 mite eggs, which were transferred to the filter paper with a fine camel hair brush. The predators were transferred to a new container at the end of each 24 hour period and the number of mite eggs remaining in each dish was recorded.

5.3.5 Functional and Numerical Response

The functional and numerical responses of *S. vagans* to various densities of *T. urticae* were conducted in the laboratory at $25 \pm 2^\circ$C and photoperiod L:D 16: 8. Initially adult predators were randomly selected from the culture colony and paired on infested bean leaf discs in modified petri dishes. *S. vagans* eggs obtained from these leaf discs were kept at the same temperature for incubation. Fifteen newly hatched larvae were confined singly at nine prey density levels until they died or pupated. Mite eggs were supplied at eight levels of
abundance from 2 to 200, while no mite eggs were supplied for the starvation control (Chapter 2). The level of prey densities selected for these experiments were based on the number of *T. urticae* observed in field and greenhouse populations. The number of mite eggs at different levels of mite infestation on young leaves of French bean plants (i.e., developing populations) was estimated by randomly selecting approximately 20 leaves infested with different levels of twospotted mite from the glasshouse culture and cutting 2.5 cm diameter discs from them. Mite egg numbers on these discs were counted using a stereomicroscope. It was noted that young leaves showing marked symptoms of mite feeding (i.e., very heavy populations) contained > 40-50 mite eggs/disc, while heavy, moderate, light and very light infestations had approximately 30-40, 20-30, 10-20, and < 10 mite eggs/disc respectively. The number of mite eggs consumed and the development of each *S. vagans* individual were recorded 12 hourly. The predators were transferred to new dishes containing the appropriate number of mite eggs every 24 hours. Any larval instar that survived for some time but was unable to moult or pupate and eventually died was excluded from the data.

A separate series of investigations were run for ovipositing female *S. vagans* at eight levels of *T. urticae* eggs (from 0 to 200) similarly to that described in Section 5.3.4. Ten predators were confined individually at each density in modified petri dishes and observed 8 hourly for five consecutive days. Every 24 hours the predators were transferred to new dishes containing the same commencing number of prey. To establish adult *S. vagans* functional response to adult *T. urticae*, 20 ovipositing *S. vagans* females were confined individually in modified petri dishes at 5, 10, 20 and 30 adult female mites on 2.5-cm leaf discs. Leaf discs were cut from mite-free French bean plants, which were infested with the appropriate number of adult *T. urticae* prior to exposure to *S. vagans* females. These discs were observed 12 hourly, and
the predators were transferred to a new arena after 24 hours, with the number of prey remaining counted and recorded. This experiment was run for 5 consecutive days.

The numerical response of adult females $S. \, vagans$ was also studied at various prey densities with $T. \, urticae$ eggs. Newly emerged females were kept with males and fed for one week. The female predators were then exposed individually in modified petri dishes to 0, 10, 25, 50, 75, 100, 125, 150 and 200 mite eggs per day. Predators in the control treatment were provided with all stages of twospotted mite. All predators were observed at 12 hourly intervals and any prey eggs consumed were recorded as well as predators eggs oviposited. The beetles were examined twice daily, because at low prey levels they were observed to consume their own eggs. They were transferred to new level of prey with relevant number of eggs every 24 hours. Eggs oviposited were recorded at 12 hourly intervals and the investigations were conducted over a five day period.

5.4.4 Statistical Analysis

Analysis of variance (ANOVA) was used to determine whether there was any significance difference in $S. \, vagans$ longevity and fecundity associated with different alternative hosts. Where significant differences occurred means were separated by using Least Significant Difference (LSD). ANOVA was also used to identify significant differences between mean predation rates and handling time as well as to determine significance differences in the rate of prey consumption between the motile stages of $S. \, vagans$. In all cases the statistical package CoStat (CoHort Software P.O.19272, Minneapolis, MN 55419, USA) was used. Graphs were drawn using Origin 4.1 (Software for Technical Graphics and Data Analysis for Windows).
5.5 RESULTS

5.5.1 Alternative Hosts

The effect of alternative hosts on adult *S. vagans* survival, mating frequency, fecundity, and egg hatchability is summarised in Table 5.1. Mating was not recorded in any host treatment except for broad mites and twospotted mites. No oviposition was therefore recorded on any alternative hosts. The mean mating frequency recorded with twospotted mite was 16 times more than for broad mite, while fecundity was 9 times higher with the same host. Egg hatchability and daily egg production was also greater on the primary host, *T. urticae*. Broad mite was the only alternative prey treatment in which viable eggs were laid. A few eggs were laid in the pollen & water treatment, but they did not hatch and were considered to be non-viable. There were also significant differences in adult *S. vagans* longevity associated with different alternative hosts. It was highest in the broad mite treatment and lowest in the starved and rust mite treatments, while it significantly increased in pollen & water, honey & water, predatory mite, water, white-fly and aphid treatments, respectively (Fig 5.1).
Table 5.1 Mean mating, fecundity, egg hatchability and adult longevity in starved adult *S. vagans* with different food sources.

|                | n  | Mating Mean ± S.E. | Fecundity Mean ± S.E. | %Hatch Mean ± S.E. | Egg/day Mean ± S.E. | Adult Longevity
<table>
<thead>
<tr>
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<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Female Mean ± S.E.</td>
</tr>
<tr>
<td>Starved</td>
<td>20</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>4.9 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Water</td>
<td>20</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>9.0 ± 0.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Honey &amp; Water</td>
<td>20</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>18.9 ± 2.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pollen &amp; water</td>
<td>20</td>
<td>0.0</td>
<td>4</td>
<td>0.0</td>
<td>0.4</td>
<td>21.1 ± 2.7&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>Aphid</td>
<td>20</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>5.0 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Whitefly (egg)</td>
<td>20</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>5.9 ± 0.6&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Predatory Mite</td>
<td>20</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>7.2 ± 0.3&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Rust Mite</td>
<td>20</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>23.5 ± 1.3&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td>Broad Mite</td>
<td>20</td>
<td>1 ± 0.0</td>
<td>10.1 ± 1.0</td>
<td>67.0 ± 2.6</td>
<td>2.98 ± 0.7</td>
<td>26.1 ± 0.9&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Twospotted</td>
<td>20</td>
<td>16</td>
<td>90.7 ± 4.3</td>
<td>81.6 ± 8.1</td>
<td>6.23 ± 6.4</td>
<td>38.0 ± 6.0&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* Numbers followed by similar letters are not significantly different at p ≤ 0.05%
5.5.2 Time Partitioning

The results for time partitioning behaviour are presented in Table 5.2. Satiated adult *S. vagans* spend most of their time resting (60.3 ± 5.3%), followed by walking (30.5 ± 5.6%), feeding (4.97 ± 0.7%) and searching (4.23 ± 0.8%). Adults starved for 24 & 48 hours spent a higher proportion of their time feeding (i.e. 46.7 ± 8.6% & 54.9 ± 6.5% respectively). This was followed by searching, resting, and walking with proportions of 28.9 ± 7.7 & 27.9 ± 6.4, 13.4 ± 1.7 & 9.5 ± 1.5, and 10.4 ± 1.7 & 3.9 ± 0.6% respectively. They were also recorded drinking water, although for short periods (0.6 ± 0.4% & 3.9 ± 1.2 % at 24 & 48 hours starved respectively) (Fig 5.2).
Adults tested within 24 hours of emergence spent most of their time either resting (44.4 ± 5.2%) or walking (40.7 ± 5.3%), and very little time searching (7.6 ± 0.6%) or feeding (7.3 ± 0.6%). There was no significant difference between behaviour of male and female adults in any category, whether satiated, starved or newly emerged.

The mean time spent by satiated 4th instar larvae in searching was 2.9 ± 0.8 %, feeding 2.8 ± 0.6 %, resting 65.4 ± 6.5 % and walking 28.9 ± 5.8 %. This pattern differed greatly in larvae starved for 24 hours, which spent significantly more time searching (30.5 ± 6.3) and feeding (47.7 ± 5.8) than resting (18.9 ± 2.2) and walking (2.9 ± 1.3 %). This pattern was even more pronounced in the 48 hour starved larvae with results recorded in the above categories recorded as 33.5 ± 4.2, 56.5 ± 3.8, 10.0 ± 1.7 % and 0.0% respectively. Newly emerged 4th instars spent more time resting (41.1 ± 5.9 %) with only 8.7 ± 0.9 % spent on searching, 9.3 ± 0.6 % for feeding, 40.9 ± 6.0% and 0.0% for walking (Table 5.2).

Both adults and 4th instar larvae consumed more eggs > nymphs > adults of T. urticae irrespective of whether they were fully fed, starved or newly emerged (Table 5.3). Satiated adult S. vagans ate more eggs (82.0 ± 5.1%) than nymphs (11.0 ± 4.3%) and adult (6.8 ± 2.7%) prey. Predation on eggs was lower in adult predators starved for 24 and 48 hours, however numerical consumption of eggs was still higher than of nymphal or adult mites. Newly emerged 4th instar larvae also preferred eggs to all other mite stages and consumed the highest percentage of eggs (83.0 ± 4.8%) compared with fully fed (70.0 ± 1.2%) and starved larvae (73.3 ± 4.4 & 69.6 ± 4.3% for 24 & 48 hours starved respectively). However the total consumption of nymphs and adult T. urticae decreased in satiated larvae compared with newly emerged larvae.
Table 5.2 Percentage time spent by *S. vagans* in various activities when provided *T. urticae* as prey.

<table>
<thead>
<tr>
<th>Stages</th>
<th>n</th>
<th>Searching Mean ± SE</th>
<th>Feeding Mean ± SE</th>
<th>Resting Mean ± SE</th>
<th>Walking Mean ± SE</th>
<th>Drinking Water Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Satiated adult</td>
<td>30</td>
<td>4.2 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.0 ± 0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>60.3 ± 5.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>30.5 ± 5.6&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Starved adult (24hour)</td>
<td>30</td>
<td>28.9 ± 7.7&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>46.7 ± 8.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.4 ± 1.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.4 ± 1.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.6 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Starved adult (48hour)</td>
<td>30</td>
<td>27.9 ± 6.4&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>54.9 ± 6.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.5 ± 1.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.9 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.9 ± 1.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Newly emerged adult</td>
<td>30</td>
<td>7.6 ± 0.6&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.3 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>44.4 ± 5.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40.7 ± 5.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.0</td>
</tr>
<tr>
<td>Satiated 4&lt;sup&gt;th&lt;/sup&gt; instar</td>
<td>30</td>
<td>2.9 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.8 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>65.4 ± 6.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>28.9 ± 5.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Starved 4&lt;sup&gt;th&lt;/sup&gt; instar (24h)</td>
<td>30</td>
<td>30.5 ± 6.3&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>47.7 ± 5.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.9 ± 2.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.9 ± 1.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Starved 4&lt;sup&gt;th&lt;/sup&gt; instar (48h)</td>
<td>30</td>
<td>33.5 ± 4.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>56.5 ± 3.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.0 ± 1.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Newly emerged 4&lt;sup&gt;th&lt;/sup&gt; instar</td>
<td>30</td>
<td>8.7 ± 0.9&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>9.26 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40.94 ± 6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>41.1 ± 5.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* Numbers in same columns followed similar letters are not significantly different at *p* ≤ 0.05%
Table 5.3 Mean number and percentage of stages of *T. urticae* consumed by *S. vagans* adults and 4th instar larvae.

<table>
<thead>
<tr>
<th>Stages</th>
<th>Feeding</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. eggs</td>
<td>% eggs</td>
<td>No. nymph</td>
<td>% nymph</td>
<td>No. adults</td>
<td>% adults</td>
<td>Mean No</td>
<td>% Total</td>
<td></td>
</tr>
<tr>
<td>Satiated adults</td>
<td></td>
<td>5.4 ± 0.7</td>
<td>82.0 ± 5.1</td>
<td>1.2 ± 0.1</td>
<td>11.3 ± 4.3</td>
<td>1.0 ± 0.0</td>
<td>6.8 ± 2.7</td>
<td>6.4 ± 0.6</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Starved adults (24hour)</td>
<td></td>
<td>12.5 ± 1.5</td>
<td>77.6 ± 5.3</td>
<td>2.6 ± 0.4</td>
<td>17.8 ± 3.1</td>
<td>1.4 ± 0.2</td>
<td>4.6 ± 1.8</td>
<td>15.8 ± 1.3</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Starved adults (48hour)</td>
<td></td>
<td>7.4 ± 5.8</td>
<td>54.7 ± 2.6</td>
<td>3.0 ± 2.0</td>
<td>25.5 ± 14.6</td>
<td>1.9 ± 1.2</td>
<td>19.8 ± 17.8</td>
<td>12.1 ± 6.5</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Newly emerged adults</td>
<td></td>
<td>6.4 ± 0.6</td>
<td>89.4 ± 3.2</td>
<td>0.6 ± 0.2</td>
<td>8.6 ± 3.2</td>
<td>0.2 ± 0.1</td>
<td>3.7 ± 2.5</td>
<td>7.1 ± 0.5</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Satiated 4th instars</td>
<td></td>
<td>3.8 ± 0.7</td>
<td>70.1 ± 1.2</td>
<td>1 ± 0.0</td>
<td>6.9 ± 2.9</td>
<td>1.0 ± 0.0</td>
<td>3.1 ± 2.1</td>
<td>4.4 ± 0.9</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Starved 4th instars (24h)</td>
<td></td>
<td>25.8 ± 2.6</td>
<td>73.3 ± 4.4</td>
<td>6.5 ± 1.0</td>
<td>19.4 ± 3.2</td>
<td>2.5 ± 0.5</td>
<td>7.3 ± 1.4</td>
<td>34.8 ± 2.0</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Starved 4th instars (48h)</td>
<td></td>
<td>31.4 ± 3.0</td>
<td>69.6 ± 4.3</td>
<td>10.8 ± 1.7</td>
<td>24.9 ± 4.1</td>
<td>2.1 ± 0.3</td>
<td>4.8 ± 0.7</td>
<td>44.6 ± 2.4</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Newly emerged 4th instar</td>
<td></td>
<td>6.7 ± 0.6</td>
<td>83.6 ± 4.8</td>
<td>0.8 ± 0.2</td>
<td>12.4 ± 3.4</td>
<td>0.2 ± 0.1</td>
<td>4.0 ± 2.7</td>
<td>6.9 ± 0.9</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>
Fig 5.2 Proportion of time spent by adult *S. vagans* in different activities when provided with *T. urticae* as prey.
Fig 5.3 Proportion of time spent by fourth instar larvae of *S. vagans* in different activities when provided with *T. urticae* as prey.
5.5.3 Rate of prey consumption

The rates of consumption by immature and mature stages of *S. vagans* when provided surplus *T. urticae* eggs are given in Table 5.4. The 4th instar larva was the most voracious stage followed by 3rd, 2nd and 1st larval instars. The mean number of mite eggs consumed by 1st, 2nd, 3rd, and 4th instar larvae was 27.0 ± 1.1, 50.0 ± 1.0, and 71.0 ± 1.5 and 152.4 ± 1.7 per larva in a 24 hour period, respectively. Ovipositing adult females consumed more than did pre-ovipositing and post-ovipositing females (viz. mean 142.7 ± 0.5, 94.3 ± 0.5 and 57.1 ± 0.6 eggs per day respectively), while males consumed 63.5 ± 0.4 eggs per day (Table 5.4).

**Table 5.4 Mean number of *T. urticae* eggs consumed by various stages of *S. vagans*.

<table>
<thead>
<tr>
<th>Predator Stages</th>
<th>Number observed</th>
<th>Mite eggs consumed /day /individual Mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larva</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st instar larva</td>
<td>30</td>
<td>27.9 ± 1.1a</td>
</tr>
<tr>
<td>2nd instar larva</td>
<td>25</td>
<td>50.1 ± 1.0b</td>
</tr>
<tr>
<td>3rd instar larva</td>
<td>25</td>
<td>71.6 ± 1.5c</td>
</tr>
<tr>
<td>4th instar larva</td>
<td>25</td>
<td>152.4 ± 1.7h</td>
</tr>
<tr>
<td>Adult</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>20</td>
<td>63.5 ± 0.4d</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-oviposition</td>
<td>20</td>
<td>94.3 ± 0.5f</td>
</tr>
<tr>
<td>Oviposition</td>
<td>20</td>
<td>142.7 ± 0.5g</td>
</tr>
<tr>
<td>Post-oviposition</td>
<td>20</td>
<td>57.1 ± 0.6c</td>
</tr>
</tbody>
</table>

*Numbers in same columns followed similar letters are not significantly different at p ≤ 0.05%*
5.5.4 Functional Response

The functional response and development of immature stages of *S. vagans* were studied at nine prey density levels. Newly emerged larvae failed to survive more than one day without food; therefore “zero prey density” was not included in the data analysis.

The results of the functional response of all larval instars of *S. vagans* are presented in Table 5.5. The response of the 1st, 2nd and 3rd instars increased linearly from low to medium prey densities and then plateaued for higher densities (i.e 150 & 200 mite egg/density), while for 4th instars it increased linearly until it reached a plateau at the highest density (i.e 200 mite eggs/density) (Fig. 5.3). There was no significant difference in the response of adult *S. vagans* to mite eggs at low prey densities, because in the 10 and 25 mite egg treatments (per petri dish for 24 hours), they were able to be consumed within the first 8 hours of the feeding period. In the 50 mite egg treatment adult *S. vagans* were able to completely consume all prey within 16 hours and in the 100 egg treatment most eggs were consumed within 24 hours. The consumption response increased linearly at low prey densities until it plateaued at higher prey densities (Table 5.6, Fig 5.4). A similar response was observed when adult *S. vagans* were fed on adult mite prey. The number of adult *T. urticae* consumed by adult female *S. vagans* increased linearly from low (5 mites/day) prey density until it reached a plateau at higher prey densities (10-30 mites/day) (Fig.5.5). The mean number of mites consumed by a *S. vagans* female at the various prey densities tested was 4.5 ± 0.1, 7.9 ± 0.3, 8.6 ± 0.3 and 8.2 ± 0.3 per day respectively (Table 5.7).

Development of immature stages of *S. vagans* was also observed at all eight prey densities. The larval instars consumed all mite eggs at lower prey densities within the 24 hour
investigation period. The minimum number of prey eggs required for normal development of each instar is shown in Table 5.8. The greatest number of prey was required for completion of the 4th larval instar followed by the 3rd instar. There were differences in the relative response of 2nd instar larvae, depending on prey density. In general, more prey were consumed at high prey densities to complete each S. vagans instar. For 1st instar larvae this difference was almost 5 fold (ie. from prey density of 2 to 200 mite eggs), for 2nd instars 4 fold (from 2 to 200 mite eggs) and for 3rd and 4th instars 2 fold (from 10 to 200 mite eggs).

No 2nd instar larva completed development at prey levels < 5 eggs/day and no 3rd or 4th instar larvae completed their development at < 10 mite eggs/day.
### Table 5.5 Functional response of immature stages of *S. vagans* to prey density (number of *T. urticae* eggs consumed per day).

<table>
<thead>
<tr>
<th>Stages</th>
<th>Number of predatory days</th>
<th>Prey density (mite eggs /day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; instar</td>
<td>2.0 ±0.0</td>
<td>4.8 ± 0.1</td>
</tr>
<tr>
<td>N*</td>
<td>93</td>
<td>49</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt; instar</td>
<td>2.0 ±0.0</td>
<td>5.0 ± 0.1</td>
</tr>
<tr>
<td>N*</td>
<td>3</td>
<td>51</td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt; instar</td>
<td>-</td>
<td>5.0 ± 0.0</td>
</tr>
<tr>
<td>N*</td>
<td>-</td>
<td>16</td>
</tr>
<tr>
<td>4&lt;sup&gt;th&lt;/sup&gt; instar*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>N*</td>
<td>125</td>
<td>64</td>
</tr>
</tbody>
</table>

N* = Number of predator-days (replicates 15 X days) for each density level.
Table 5.6 Functional response of adult *S. vagans* to *T. urticae* egg density at 25 ± 2°C.

<table>
<thead>
<tr>
<th>Prey density (Mite egg/day)</th>
<th>Number of prey (mite eggs) consumed</th>
<th>Proportion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8 hour*</td>
<td>16 hour*</td>
</tr>
<tr>
<td>10</td>
<td>9.8 ± 0.0</td>
<td>9.9 ± 0.1</td>
</tr>
<tr>
<td>25</td>
<td>24.9 ± 0.1</td>
<td>25.0 ± 0.0</td>
</tr>
<tr>
<td>50</td>
<td>41.0 ± 1.0</td>
<td>50.0 ± 0.0</td>
</tr>
<tr>
<td>75</td>
<td>43.0 ± 0.9</td>
<td>72.3 ± 0.6</td>
</tr>
<tr>
<td>100</td>
<td>43.98 ± 4.6</td>
<td>83.7 ± 0.6</td>
</tr>
<tr>
<td>125</td>
<td>53.5 ± 0.7</td>
<td>97.2 ± 0.7</td>
</tr>
<tr>
<td>150</td>
<td>41.0 ± 0.8</td>
<td>102.0 ± 0.9</td>
</tr>
<tr>
<td>200</td>
<td>52.2 ± 1.3</td>
<td>112.8 ± 1.2</td>
</tr>
</tbody>
</table>

*Each reading is the mean of 50 predatory days (i.e. 10 replicates x 5 days)*

Table 5.7 Functional response of adult *S. vagans* to adult *T. urticae* density at 25 ± 2°C.

<table>
<thead>
<tr>
<th>Prey density (adult mite /day)</th>
<th>Number of observations</th>
<th>Total (mites eaten /day)</th>
<th>Mean (mites eaten /day/S. vagans)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>20</td>
<td>90</td>
<td>4.5 ± 0.1</td>
</tr>
<tr>
<td>10</td>
<td>20</td>
<td>158</td>
<td>7.9 ± 0.3</td>
</tr>
<tr>
<td>20</td>
<td>20</td>
<td>171</td>
<td>8.6 ± 0.3</td>
</tr>
<tr>
<td>30</td>
<td>20</td>
<td>164</td>
<td>8.2 ± 0.3</td>
</tr>
</tbody>
</table>
Table 5.8 Total number of eggs consumed by each immature stage of *S. vagans* at various prey densities and total number of eggs required to complete development at 25°C.

<table>
<thead>
<tr>
<th>Stages</th>
<th>Number of observations</th>
<th>Prey density (mite eggs/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; instar</td>
<td></td>
<td>12.7 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>15</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt; instar</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>10</td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt; instar</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>11</td>
</tr>
<tr>
<td>4&lt;sup&gt;th&lt;/sup&gt; instar</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>11</td>
</tr>
</tbody>
</table>
Fig. 5.4 Functional response of immature stages of *S. vagans* to *T. urticae* egg density.

Fig. 5.5 Functional response of adult *S. vagans* to *T. urticae* egg density.
Fig 5.6 Functional response of adult *S. vagans* to adult *T. urticae*.

Fig. 5.7 Number of prey required by immature stages of *S. vagans* for development.
5.5.5 Numerical response

The developmental time and survival of preimaginal stages of *S. vagans* were both strongly influenced by prey density. The relative development time for all immature stages combined (i.e. 1st instar emergence to pupation) was 17.9 days at low prey density (25 mite eggs/day). There was no significant difference in rate of development at higher prey densities. However survival rate was significantly higher at prey densities of 100 eggs/day or above (Table 5.9, Fig 5.7).

The minimum number of prey required for survival of 1st, 2nd, 3rd and 4th larval instar survival were 10, 25, 100 and 100 eggs/day respectively. Without prey no development occurred and the 1st instar larvae could not survive more than one day, while only 15% of 1st instar larvae completed this stage at the lowest prey density (2 mite eggs/day), but died soon after molting. At low prey levels (i.e. 5 mite-eggs per day) 75% of the 2nd instar larvae completed this stage but could not survive to the 3rd instar. Only 3 out of 16 larvae completed the 4th instar at 10 mite eggs/day, with a mean development period of 18 days. The minimum number of prey needed for 90% survival of 1st, 2nd, 3rd and 4th instars were 2-5, 7.5, 17.5 and 37.5 mites per day respectively (Table 5.10). Maximum survival (100%) of immature stages was recorded only at prey densities of ≥ 100 eggs/day, while none reaching the pupal stage that had consumed less then 25 mite eggs/day.

The numerical response of adult female *S. vagans* was also examined using the parameters of survival and reproduction in response to changes in prey density (Table 5.11). Adults could not survive after the 5th day of starvation, but they could survive for short periods (11 days) at low prey density (i.e. 10 mite eggs/day). Adult *S. vagans* responded to prey density. Both mean gross and mean net fecundity for adult females was low at low prey densities, but
increased linearly with increasing prey density, plateauing at high densities (i.e. 200 mite eggs/day) (Table 5.11). Gross fecundity was higher than net fecundity at lower prey densities, because the predators ate their own eggs. However the net fecundity increased consistently with prey number until it equalled gross fecundity at the highest prey density. It required 100 ± 0.0 mite eggs at low prey density (10 mite eggs/day) and 18.8 ± 0.3 mite eggs at high prey density (200 mite eggs/day) to produce one *S. vagans* egg (Table 5.12).
Table 5.9 Number of days required for immature stages of *S. vagans* to complete development at various *T. urticae* densities at $25 \pm 2^\circ$C.

<table>
<thead>
<tr>
<th>Stages</th>
<th>Number of individuals commencing each stage</th>
<th>Prey density (mite eggs /day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; instar</td>
<td>N</td>
<td>6.2 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt; instar</td>
<td>N</td>
<td>3.4 ± 0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt; instar</td>
<td>N</td>
<td>4.9 ± 0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>4&lt;sup&gt;th&lt;/sup&gt; instar</td>
<td>N</td>
<td>15.0 ± 0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13</td>
</tr>
<tr>
<td>Pupa</td>
<td>N</td>
<td>4.3 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>N</td>
<td></td>
</tr>
</tbody>
</table>
Table 5.10 Numerical response of immature stages of *S. vagans* to *T. urticae* densities and their survival to the next stage at 25 ± 2°C.

<table>
<thead>
<tr>
<th>Predator stages</th>
<th>Prey density (mite eggs/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; instar larva</td>
<td>15</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt; instar larva</td>
<td>0</td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt; instar larva</td>
<td>0</td>
</tr>
<tr>
<td>4&lt;sup&gt;th&lt;/sup&gt; instar larva</td>
<td>0</td>
</tr>
<tr>
<td>Pupa</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 5.11 Numerical response and conversion of prey into progeny by female *S. vagans* at different prey (*T. urticae*) densities.

<table>
<thead>
<tr>
<th>Prey density (mite eggs/day)</th>
<th>Mean mite eggs consumed/• /day</th>
<th>Eggs laid/∗ /day (gross fecundity)</th>
<th>Eggs surviving/∗ /day (net fecundity)</th>
<th>/S. vagans egg laid</th>
<th>/S. vagans surviving egg</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>10.0 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>50.0 ± 0.0</td>
<td>100.0 ± 0.0</td>
</tr>
<tr>
<td>25</td>
<td>25.0 ± 0.0</td>
<td>0.6 ± 0.1</td>
<td>0.2 ± 0.0</td>
<td>43.0 ± 3.7</td>
<td>125.0 ± 0.0</td>
</tr>
<tr>
<td>50</td>
<td>50.0 ± 0.0</td>
<td>2.2 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>23.3 ± 1.0</td>
<td>67.4 ± 6.1</td>
</tr>
<tr>
<td>75</td>
<td>75.0 ± 0.0</td>
<td>3.3 ± 0.1</td>
<td>2.1 ± 0.2</td>
<td>23.3 ± 0.8</td>
<td>40.1 ± 3.8</td>
</tr>
<tr>
<td>100</td>
<td>93.9 ± 0.7</td>
<td>5.8 ± 0.1</td>
<td>4.8 ± 0.1</td>
<td>16.1 ± 0.3</td>
<td>19.7 ± 0.4</td>
</tr>
<tr>
<td>125</td>
<td>121.8 ± 0.4</td>
<td>6.7 ± 0.2</td>
<td>5.7 ± 0.1</td>
<td>18.3 ± 0.5</td>
<td>21.5 ± 0.6</td>
</tr>
<tr>
<td>150</td>
<td>127.7 ± 0.9</td>
<td>7.0 ± 0.1</td>
<td>6.8 ± 0.1</td>
<td>18.3 ± 0.2</td>
<td>18.8 ± 0.3</td>
</tr>
<tr>
<td>200</td>
<td>139.0 ± 0.5</td>
<td>7.5 ± 0.1</td>
<td>7.5 ± 0.1</td>
<td>18.8 ± 0.3</td>
<td>18.8 ± 0.3</td>
</tr>
</tbody>
</table>
Fig 5.8 Number of days required for immature stages of *S. vagans* to complete their development at various prey densities.

Fig 5.9 Numerical response of adult female *S. vagans* to prey densities.
5.6 DISCUSSION

5.6.1 Alternative prey to T. urticae

No mating or oviposition was recorded for S. vagans on any host, except for pollen & water, broad mite and twospotted mite (control). The mean number of eggs laid per female when reared on pollen & water and broad mites was very low (4.0 ± 0.4 and 10.1 ± 1.0, respectively) when compared with T. urticae (90.7 ± 4.3). Eggs laid in the pollen & water treatment did not hatch, whereas egg hatchability in the broad mite treatment was 67%. This was, however, significantly less than that recorded in the T. urticae treatment (81.6%) (Table 5.1). Adult longevity increased compared with the starved control, with the provision of alternative hosts such as water, honey & water, aphids and white fly eggs but no reproduction occurred on these hosts. Adult longevity in relation to host was T. urticae > broad mites > rust mites > pollen & waters > honey & water > water > P. persimilis eggs > white flies > aphids > starved (control). However no significant difference in longevity was recorded between adults fed on aphids or white flies. There was no significant difference recorded between male and female longevity on any of the above diets.

Our results with S. vagans are similar to reports of authors working with other Stethorus spp. such as Putman (1955a) and Kehat (1967) who concluded that S. punctillum fed on aphids, phytoseiid mites and scale insects but these hosts were not sufficient for development or oviposition, and Kamiya (1966) and Hoy et al. (1979) who reported that S. japonus fed on plant resins, sweet foliar secretions, and honey & water, which increased longevity but did not result in copulation or reproduction.
In this study broad mite was found to be the only effective alternative host for *S. vagans*, not only increasing adult longevity, but also producing fertile eggs. While the other alternative hosts did not support reproduction, all significantly increased adult longevity. Therefore these alternative prey may assist in sustaining *S. vagans* in localities where its primary host is in low in numbers or diapausing.

It appears from the results that *T. urticae* is an important food source for *S. vagans*, not only for adult survival but also for successful reproduction. A puzzling aspect is that *S. vagans* is native to Australia, but *T. urticae* and broad mites are introduced species; therefore it remains unclear that what was the original host(s) of *S. vagans* before the introduction of these species. Other mite species native to Australia such as tydeids, oribatids, stigmatideids and tetranychids, such as *T. ludeni* may have been primary hosts of *S. vagans* before the introduction of *T. urticae* to Australia. None of these species were included in these investigations.

### 5.6.2 Time partitioning and stage preference

The results in Table 5.2 show that adult *S. vagans* spent more time searching and feeding when starved for 24 or 48 hours than when satiated or newly emerged. Adults starved for 48 hours spent more time drinking water than when starved for only 24 hours. Less drinking also occurred in 48 hour starved adults once they had a successfully prey capture and ingested haemolymph. However they also attacked a greater proportion of adult mites under these conditions, in which case each individual provided more food than did individual prey eggs or nymphs. Beetles starved for 48 hours occasionally regurgitated the mite haemolymph back into the prey’s body for a considerable time after commencing feeding.
Larvae of *S. vagans* responded similarly to adults with respect to time partitioning. Fourth instar larvae starved for 24 & 48 hours spent more time searching and feeding than did fully fed and newly emerged larvae. The proportion of time spent searching and feeding by larvae starved for 24 hours was greater than for the larvae starved for 48 hours (Table 5.2). The reason may be that 48 hour starved larvae attacked more motile stages than those starved for 24 hours, which may have provided more haemolymph for nourishment than did eggs.

All stages of *S. vagans* had a strong preference for mite eggs when given a choice of all mite stages (Table 5.3). Starved predators consumed more motile stages of *T. urticae* than did satiated ones, but this was still a lower proportion than eggs. Satiated and newly emerged predators were more selective and preferred eggs to other mite stages which they encountered during searching. Houck (1991) recorded similar results for satiated adult *S. punctum*, which consumed 92.7% eggs, 3.6% nymphs and 3% adult *T. urticae* respectively, while for 24 hour starved adults these figures were 88.4, 3.6, 7.5% respectively. Therefore it appears that *Stethorus* spp. have a strong preference for eggs when satiated, but this preference changes (ie. they become less selective) when they are starved.

The process of feeding consists of piercing prey, sucking haemolymph and its regurgitation back into the body of the captured prey. It also includes the predator chewing and consuming body. In a number of encounters motile mites were obsessive being attacked and even damaged, but still were able to escape from their predators. This was more common when the predators were satiated or newly emerged.

5.6.3 Rate of consumption
The rate of prey consumption for all motile stages of *S. vagans* was similar to those recorded for relative stages of *S. nigripes* (Richardson 1977). However the consumption rate was less than that reported for some other species of *Stethorus*, namely *S. punctillum* and *S. punctum* (Putman 1955a; Hull *et al.* 1977b). This is possibly because *S. vagans* is smaller than these other species.

5.6.4 Functional response

The functional response of all motile stages of *S. vagans* to *T. urticae* increased curvilinearly to a plateau at high prey densities. This type of curve is characteristic of a type-II functional response (Holling 1965), which has been demonstrated in a number of insect and other invertebrate predators to their prey. Richardson (1977) recorded a similar curve for *S. nigripes* with the same host, *T. urticae*. Munyaneza & Obrycki (1997) also reported a type-II functional response for the coccinellid *Coleoegilla maculata* feeding on Colorado potato beetle *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae).

An interesting functional response has been reported in *S. bifidus* and its prey, *T. linterarius*, in which an increasing proportion of mites were killed as mite density increased, because of decreased mean feeding time and reduced extraction of the body contents. This behaviour was not observed in *S. vagans*.

5.6.5 Numerical response

The mean developmental time for *S. vagans* immature stages was significantly shorter at higher prey densities i.e. 14.7 days at a prey density of 25 mite eggs per day, but only 11.1 days at 200 mite eggs /day. The developmental rate recorded in the control treatment was
13.7 ± 0.2 days at 25 ± 2°C, which is longer than that recorded at higher densities of mite eggs. This may be because more motile stages of prey were present in the control treatment and therefore may be less nutritious than mite eggs or it may be because it is more difficult to locate and capturing motile stages. Another reason is that motile mites may interfere with *Stethorus* activities such as searching or eating, although this was not observed. Our results are very similar to those reported by Richardson (1977) for *S. nigripes*, another Australian species.

The numerical response of larval instars of *S. vagans* was also strongly correlated with mite egg densities (Table 5.8, Fig. 5.6). For 95% survival immature *S. vagans* required 75 mite eggs per day compared with 70 mite eggs/day for *S. nigripes* (Richardson 1977). Both larvae and adults of *S. vagans* had a type-II response at higher prey densities, which has been recorded for a number of insect predators including *S. nigripes* (Richardson 1977).

The reproductive response of adult *S. vagans* females was positively correlated with mite egg densities. The gross and net fecundity increased linearly with mite density and plateaued at high prey density. A similar response was reported for *S. nigripes* (Richardson 1977). The net fecundity rate was lower than gross fecundity because adults ate their own eggs at lower prey densities. Cannibalism ceased at higher prey densities. Cannibalism is a common phenomenon in a number of insect predators especially ladybirds, at low prey densities. Richardson (1977) for example observed the same type of behaviour in *S. nigripes* and Majerus (1994) reported that ladybirds *Adalia bipunctata* and *F. hebraea* fed on their own eggs or early instar larvae if prey was scarce. The cannibalism of *S. vagans* at lower prey densities has important ecological significance. First it provides enough energy and water to
enable predators to survive when prey is scarce. Second it likely to reduce inter-specific competition in the population and hence prevents population collapse.

We was calculated that at lower prey densities S. vagans required 25-35 mite eggs to produced an egg, which declined to 16 to 20 mite eggs at higher prey densities. The efficiency in converting prey to predator progeny was correlated with prey density as sigmoid curve (Fig.5.9). These results were supported by Richardson (1977) who reported that S. nigripes required 30-40 T. urticae eggs to produced one egg.

This is likely to make S. vagans an effective predator at all prey densities, as while the number of mite eggs consumed at high prey density is lower and their rate of oviposited under these condition is higher.
CHAPTER 6

HOST LOCATION BY S. VAGANS AT DIFFERENT PREY DENSITIES

6.1 ABSTRACT

Host location by S. vagans was observed at four densities of its prey T. urticae, commencing at a nominal 10, 20, and 50 mites per plant in the field. Leaves from potted French bean plants located in the field and infested with the respective number of mites were examined daily for presence of S. vagans. The mean number of adult S. vagans recorded was 0.54, 2.5 and 5.18 at low, medium and high mite densities respectively, while none were found on mite free plants. Immature stages of S. vagans were only found on plants with high mite density. The mean number of all stages of T. urticae counted on leaves at the time of S. vagans collection was 4.27, 19.5 and 490.4 mites at low, medium and high densities respectively.

Both sexes of adult S. vagans were assessed in the laboratory in relation to their prey searching ability using a Y-tube olfactometer. Both satiated and starved adults were tested with and without an air current flowing from the prey chamber. No adults in any treatment were able to locate their prey in this device.

Host detection by S. vagans was also observed in a controlled temperature room at 25°C. For each observation, 4 adults (2 males and 2 females) were released in the middle of potted French bean plants, one infested with T. urticae and the other untreated as a control. All released adults, both satiated and starved, were able to located their prey. Adults located their prey at higher densities significantly faster than at medium or low densities. The mean time taken by satiated adults was longer than by starved adults, viz. 259.0 ± 5.3, 144.1 ± 2.5, 50.2
± 1.3 minutes and 199.5 ± 3.4, 93.3 ± 2.0 and 20.2 ± 1.1 minutes at low, medium and high prey densities respectively.
6.2 INTRODUCTION

How adult coccinellids find their prey was been the subject of some controversy. Most authors are not in agreement even on their method of prey detection. For example, Thompson (1951) reported that coccinellids detect their prey by sight, whereas Dixon (1959) using Adalia bipunctata concluded that both sight and scent are involved. Stubbs (1980) and Nakamuta (1984) supported this conclusion while working with Coccinella septempunctata. Sabelis & van de Baan (1983) reported that Stethorus larvae might use kairomones to detect phytoseiid hosts. Still other authors have confirmed the importance of physical searching in locating prey, including Colburn & Asquith (1970) who worked with adult S. punctillum. Hodek (1973) reported that adult coccinellids found their prey by actual contact, while Dixon (1959) and Kesten (1969) indicated that ladybirds walk upwards when searching for prey, because they are both positively phototaxic and negatively geotaxic. This chapter reports laboratory and field studies to investigate detection of their primary prey, T. urticae by adult S. vagans, because in the field the adult is the only stage capable of locating prey over any distance.
6.3 MATERIAL AND METHODS

6.3.1 Host Finding

To determine whether S. vagans could find their prey, T. urticae, over a range of densities, investigations were conducted in the field, in the laboratory and in a constant temperature room.

6.3.1.1 Field experiment

Initially, French bean plants (cv. Redland Pioneer) were germinated in trays then transferred to 15 cm diameter plastic pots in potting mix after one week. The potted plants were infested with the required number of gravid adult female T. urticae, which had been cultured in a greenhouse at a mean temperature of 27 ± 2°C (Chapter 2). The mites were transferred and evenly distributed on each plant with a fine camel hair brush. Four treatments, three with mite densities: low (10 mites/plant), moderate (20 mites/plant), high (50 mites/plant) and a control (plants without mite) were set up at the same time with each density replicated on four potted bean plants. Thus, a total of 4 treatments x 4 replicates were exposed in the field at four different sites, a total of 64 plants. The sites were in four representative habitats: under pine (Pinus radiata) trees, in an ornamental area comprising shrubs and various flowers, in a peach (Prunus persica) orchard and in an orange (Citrus sinensis) orchard at the Centre of Horticulture and Plant Sciences, University of Western Sydney, Hawkesbury, Richmond (33° 36’S, 150° 44 E) in the central coast of NSW, Australia. The site for each density (treatment) was randomly changed weekly and the old plants were replaced with new ones. The distance between potted plants was 5 metres within the same density (replicates) and more than 150 metres between the different densities (treatments). These field-exposed plants were monitored daily and each plant was carefully inspected for at least five minutes. Leaves on
which adult *S. vagans* were observed were detached, then sealed separately in bottles and
brought back to the laboratory. Each leaf was examined under a binocular microscope (at 20
X magnification) (Carl Zeiss, Germany) and all stages of *S. vagans, S. nigripes* and *T. urticae*
were counted. All *S. vagans* were subsequently transferred to the laboratory culture. As the
mite density was reduced as result of this leaf detachment, the plants were replaced weekly
with new plants infested with the appropriate number of adult mites. The meteorological data
during the collection period was obtained from a weather station located less than 1km from
the experimental site on the campus of University of Western Sydney, Hawkesbury,
Richmond. These investigations were conducted from March 1997 to March 1998.

### 6.3.1.2 Y-tube olfactometer

A Y-tube olfactometer was used to test the ability of adult *S. vagans* to locate the prey in the
laboratory. The technique was similar to that used to assess olfactory responses of other
predacious species to tetranychid mites (Dicke & Groeneveld 1986; Dong & Chant 1986).
The apparatus consisted of a Y-tube with arms 30 cm long and square in section (30 mm x 30
mm). Each arm was lined with absorbent paper to provide a non-slippery surface for the
beetles. The absorbent paper was replaced after each trial. Each arm terminated in a 10 x 11 x
5.5 cm chamber with an open lid at the top.

Adult *S. vagans* of both sexes were randomly selected from the founder colony, maintained at
25 ± 2°C in the laboratory. Some of the adults were starved for 24 hours, while others
(satiated) were used in the Y-tube olfactometer immediately after selection. There were six
treatments each replicated three times. A replicate was comprised of 10 adults (5 males and 5
females). The beetles were tested with and without airflow in the Y-tube. The air current was
blown into each of the target chamber by a double outlet aquarium pump, exiting via the
release chamber. The temperature and relative humidity during the investigations was 21.5-27°C and 45-70% respectively, while light intensity during the investigation was supplied by two 40 Watt fluorescent tubes.

The treatments were:

1. Bean leaves with all stages of *T. urticae* vs bean leaves without *T. urticae*

2. Bean leaves with all stages of *T. urticae* vs damaged (by hand) bean leaves without *T. urticae*

3. Bean leaves with all stages of *T. urticae* vs empty cage

4. Bean leaves without *T. urticae* vs damaged bean leaves without *T. urticae*

5. Bean leaves without *T. urticae* vs empty cage

6. Damaged bean leaves without *T. urticae* vs empty cage

On each occasion 10 adult *S. vagans* (5 males and 5 females) were released in one chamber. The beetles were observed at hourly intervals for 8 hours then left over night (16 hours) and re-examined the next morning to determined their ability to locate their host.

### 6.3.1.3 Controlled temperature room

The host finding behaviour of adult *S. vagans* was also studied in a constant temperature room (Defensor® Axir Ltd. WMH Walter Meier Holding Co. Switzerland) with dimensions 2 x 3 x 2.4 m. The walls and roof of the control room were white and the room was equipped with a refrigerator unit, a humidifier (Atomizer 505 S) and two white fluorescent tubes at a height of 1.2 m from the floor. The experimental conditions for these investigations were 25 ± 2°C with RH 50 ± 10% and 80 W/m² light intensity without any air movement.
French bean plants (cv. Redland Pioneer) were grown in trays and later transferred to pots as described in Section in 6.3.2. Adult female *T. urticae* were released uniformly on these plants at rates of 20, 50, and >100 mites /plant, one hour before the investigation commenced. Each experiment was conducted by simultaneously exposing two plants (treatments) at a distance of 3 m from each other in the room from the following treatment combination, providing a choice for the released *Stethorus* adults:

1. Bean plant without mites vs Bean plant with damaged (damaged by hand) leaves
2. Bean plant without mites vs Bean plant with 20 mites /plant.
3. Bean plant without mites vs Bean plant with 50 mites /plant.
4. Bean plant without mites vs Bean plant with >100 mites /plant.

For each trial 4 *S. vagans* adults (2 males and 2 females) were randomly selected from the mass culture and simultaneously released mid way between the two plants (i.e. 1.5 m from each plant). This was repeated 6 times for both satiated and 24 hour-starved beetles for each treatment. The time between each treatment was 16 hours, while the position of the plants was changed after each test (replicate). Beetles were marked with different florescent powder (Radiant® Colour Division, Imperial Colour and Chemical Department, Hercules Inc., 2800 Radiant Ave., CA. 94804) before their release to assist in their recognition when recording their movements. They were observed continuously until they located their host (in the case of highest densities), or for a maximum five hours. The beetles were used only once to prevent previous experience influencing results.
6.3.4 Data Analysis:

The hypothesis was that *S. vagans* is able to locate populations of two-spotted mite, *T. urticae* at very low densities.

Costat Statistical Package (CoHort Software, Minneapolis, MN 55419, USA) was used to analyse the data. Treatment effects were compared by using Analysis of Variances (ANOVA). Least significant differences and standard errors were calculated by Duncan's multiple range test. Graphical representations were made using Origin 4.1 (Software for Technical Graphics and Data Analysis for Windows).
6.4 RESULTS
6.4.1 Host Finding:

6.4.1.1 Field Experiment:
Adult *S. vagans* were first observed on the field exposed plants on the 6th, 4th, and 2nd day after exposure at light, medium, and heavy mite treatments respectively. No predators were found on any mite-free (control) plant throughout the investigations. Only those leaves on which adult *S. vagans* were observed were collected from the infested plants. After 8 weeks of collection, the mean number of *Stethorus* recorded on high mite density plants was significantly greater than on medium and low density plants. The mean number of *Stethorus* was twice as high as that which occurred on plants with medium mite density and almost three times more than on plants with low mite density (Table 6.1). The mean number of all mite stages recorded from these treatment plants was 490.4 ±20.5, 19.5 ± 2.0, and 4.27 ± 0.5 respectively. No immature stages of *S. vagans* were found on plants with light or medium mite infestation. However in the high mite infestation treatment both eggs and larvae of *S. vagans* were found. Analysis of the data confirmed that mite density on plants significantly influenced the number of *S. vagans* found on them.

The number of all stages of *T. urticae* was also counted on each leaf on which adult *S. vagans* were observed. The mean number of eggs, nymphs, and adults were 49.5 ± 8.0, 18 ± 3.5 and 14.3 ± 2.1 respectively in the light mite density, 188 ± 13.2, 90.25 ± 7.4 and 94.5 ± 2.5 for medium and 757.25 ± 32.1, 673.25 ± 26.4 and 699.5 ± 18.3 for high density treatment.
Table 6.1 Mean number of leaves containing mites and *S. vagans* on plants exposed in the field.

<table>
<thead>
<tr>
<th>Treatments (Nominal mite numbers)</th>
<th>Number of leaves with <em>S. vagans</em></th>
<th>Number of TSM / leaf</th>
<th>Number of <em>S. vagans</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mite/plant (control)</td>
<td>16</td>
<td>0.0 ± 0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.0 ± 0.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>10 mites/plant</td>
<td>16</td>
<td>0.4 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.3 ± 2.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>20 mites/plant</td>
<td>16</td>
<td>1.7 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.5 ± 5.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>50 mites/plant</td>
<td>16</td>
<td>3.7 ± 0.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>490.4 ± 21.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* n = Number of plants in each treatment

* Numbers followed by different letters are significantly different (p ≤ 0.05) using Duncan's multiple range test.

Fig. 6.1 Maximum and minimum temperatures recorded in the field during *S. vagans* host detection studies.
6.4.1.2 Y-Tube Olfactometer:

No *S. vagans*, whether starved or satiated were able to locate their host in the Y-tube olfactometer within an initial period of 8 hours, nor when left overnight (an additional 16 hours). The adults were observed walking from the release chamber up in the tube and trying to fly in the chamber, but were still not successful in locating their prey in the opposite chamber over the 24 hour investigation period.

6.4.1.3 Controlled temperature room:

Both starved and satiated adult *S. vagans* were able to locate their prey, *T. urticae*, at all three prey densities in the control temperature room. No beetles moved onto any mite-free bean plant (whether or not they had damaged leaves). They generally flew directly upwards and sat on the roof of the room immediately after their release in the middle of the two plants (ie. infested or damaged and untreated control). However after several minutes, they commenced searching by walking in the vicinity. This walking movement continued for approximately three to four minutes, after which the adult made a short flight and landed in an area approximate 5-10cm from the area already searched, and recommenced searching. With each flight and search they moved closer to the infested plant until the final short flight when they alighted on the plant. None moved in the direction of the identical mite-free plant. The mean searching time to locate their host plants decreased significantly (*p* ≤ 0.05) as mite populations increased, for both satiated and starved beetles (Table 6.2). However beetles starved for 24 hours located their prey significantly faster (*p* ≤ 0.003) than did satiated beetles. There was no significant difference between male and female searching times, whether satiated or starved, in any treatment.
Table 6.2 Mean searching time for satiated and starved adult *S. vagans* at different mite densities.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>S. vagans adults (Satiated)</th>
<th>S. vagans adults (Starved)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control undamaged plants</td>
<td>Mean time to locate host</td>
<td>Mean time to locate host</td>
</tr>
<tr>
<td>(No mites /plant)</td>
<td>(n) (min.)</td>
<td>(min.)</td>
</tr>
<tr>
<td>Plants with damaged leaves</td>
<td>12  &gt; 300^a</td>
<td>&gt;300^a</td>
</tr>
<tr>
<td>(No mites /plant)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low (20 mites /plant)</td>
<td>259.0 ± 5.3^b</td>
<td>199.55 ± 3.4^b</td>
</tr>
<tr>
<td>Medium (50 mites /plant)</td>
<td>144.08 ± 2.5^c</td>
<td>93.24 ± 2.0^c</td>
</tr>
<tr>
<td>High (100 mites /plant)</td>
<td>50.16 ± 1.3^d</td>
<td>20.22 ± 1.1^d</td>
</tr>
</tbody>
</table>

* n = number of plants used for each treatment.

*Numbers followed by different letters are significantly different (p ≤ 0.05) based on Duncan’s multiple range test*
6.5 DISCUSSION

6.5.1 Host finding

6.5.1.1 Field experiment

The results clearly demonstrate that the adult *S. vagans* are capable of detecting and locating their prey from short and medium distances (Section 6.4.1.3) at densities at least as low as 10 mites per potted French bean plant. The number of *S. vagans* found on infested plants increased as mite densities increased. It therefore seems reasonable to assume that *S. vagans* are able to regularly establish on field grown plants infested with low populations (minimum 10) adult *T. urticae* per plant. Our results are strongly supported by Raworth (1990) and Zadeh et al. (1995), who reported that *S. punctum* and *S. gilvifrons* were active at a very low prey densities (1 mite/leaf) in the field. Congdon et al. (1993), Helle & Sabelis (1985b), Hull et al. (1977b), Richardson (1977) and Readshaw (1973) all reported that *Stethorus* spp. were able to find *T. urticae* in small isolated patches with one mite or less/leaf. Our investigations did not assess mite densities at this level. The view that *Stethorus* spp. are "high prey density dependent predators" has relied mostly on the study of random leaf samples (Congdon et al. 1993), and is not supported by our results, if this view is based at least in part, on their ability to locate prey populations population at low density.

Female *S. vagans* only oviposited in high-density mite populations, and no eggs were found at medium or low mite densities. However the leaves containing adult *S. vagans* were detached from plants at one-day intervals. This may not have been allowed females sufficient time to adjust to the new plant conditions and respond via oviposition. Hull et al. (1977) reported that *S. punctum* laid its eggs on mite infested leaves which had 0.3-1.9 mites/leaf,
while a similar investigation by Congdon et al. (1993) reported that *S. punctum picipes* deposited a few eggs at very low prey density (1 mite/leaf).

### 6.5.1.2 Y-tube olfactometer

The investigations in the laboratory using the Y-tube olfactometer were not successful. None of the predators whether satiated or starved were able to find their host through the tube. However they were observed walking from the release chamber up in the tube, but invariably returned to same chamber. Most attempted to fly in the release chamber as well as in the tube, but there was insufficient room for successful flight. We also conducted some trials using fans to blow air down the tubes from the prey, but this was also not successful. Even when the olfactometer was placed perpendicular (90°) to the bench top, none of the beetles were able to locate their prey. This was initially surprising, given their reported negative geotaxic behaviour (Dixon 1959; Kesten 1969). Based on the information discussed in the Section 6.5.1.3, it is hypothesised that for successful host location adult *S. vagans* must make short flights, then search by walking. Such behaviour was not possible in the closed Y-tube olfactometer.

### 6.5.1.3 Controlled temperature room

In the enclosed room, all satiated and starved male and female *S. vagans* were able to find their prey at all mite densities, while none were attracted to mite-free plants even when they had been mechanically damaged. Starved beetles took significant less time to locate their prey at all mite densities than did satiated beetles. The time taken to locate their prey increased with reduced mite density for both satiated and starved predators. When the beetles were first released, they flew and sat on the roof of the room for few minutes and then commenced searching. Each beetle (whether satiated or starved) made a low short flight and
sat to search their vicinity by walking. This process continued until they located their host, with each flight bringing them closer. A possible reason is that *T. urticae* emit some chemical, e.g., kairomone, which attracts predators, and this attraction is not merely associated with plant feeding damage. Sabelis & van de Baan (1983) reported that larval stages of *Stethorus* were attracted to *T. urticae* by kairomones, as has also been reported for predatory mites (Dong & Chant 1986). It is likely that adult *Stethorus* are also attracted by kairomones. This may explain reports that adult *Stethorus* can fly actively to locate even isolated mite colonies (Helle & Sabelis 1985b).

Based on the results presented above, it is likely that adult *Stethorus* have two modes of searching to locate their prey, i.e., from a distance they locate by smell, and initially fly in the direction of their prey. To actively locate prey, they search an area by walking which may rely on sight, direct encounters and/or perhaps smell. After a period of unsuccessful searching, they fly again in the direction of their prey, landing and searching in a new area.
CHAPTER 7

DISCUSSION AND CONCLUSION

This thesis is the first to report details of the biology of the Australian ladybird *Stethorus vagans*. Prior to this, *S. vagans* was only known in the literature from its taxonomy, description and occasional references to its presence in the field. While *Stethorus* spp. have been studied in Europe, Asia and USA, only one other Australian species *S. nigripes* (=*S. loxtoni*) has investigated in depth (Richardson 1977). Where possible, this thesis has compared data from *S. vagans* with that reported for *S. nigripes*.

Because of the lack of basic biological data on *S. vagans* the studies primary focussed on the laboratory and semi-field investigations. These concentrated on elucidating information on its development, host location, feeding and reproductive behaviour.

A summary of the major specific thesis outcomes is given in Table 7.1.
<table>
<thead>
<tr>
<th>Major finding of the thesis</th>
<th>Chapter/s</th>
<th>New</th>
<th>Previously reported in S. vagans.</th>
<th>Previously reported in other Stethorus spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Development of improved rearing method.</td>
<td>2</td>
<td>yes</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>2. Calculation of minimal developmental threshold temperature for all stages of S. vagans.</td>
<td>3</td>
<td>no</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>3. Calculation of DD for all stages.</td>
<td>3</td>
<td>no</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>4. Confirmation of sex ratio 1:1.</td>
<td>4</td>
<td>no</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>5. Determination of no diapause in S. vagans.</td>
<td>4</td>
<td>no</td>
<td>no</td>
<td>variable</td>
</tr>
<tr>
<td>6. Confirmation of description of some stages of S. vagans and description of previously undescribed stages, including measurements.</td>
<td>4</td>
<td>no</td>
<td>yes</td>
<td>N/A</td>
</tr>
<tr>
<td>7. Mating requirement for oviposition. One mating sufficient for maximum production of viable eggs (for 20 days).</td>
<td>4</td>
<td>no</td>
<td>no</td>
<td>yes</td>
</tr>
</tbody>
</table>
8. Most alternative hosts (live arthropods and substrates) increased longevity, but did not support reproduction.

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<tbody>
<tr>
<td>5</td>
<td>no</td>
<td>no</td>
<td>yes</td>
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</table>

9. All stages of *S. vagans* have prey preference for eggs of *T. urticae*, but this is influenced by level of starvation.

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<tbody>
<tr>
<td>5</td>
<td>no</td>
<td>no</td>
<td>yes</td>
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</table>

10. *S. vagans* has type-II numerical response to prey density.

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<tbody>
<tr>
<td>5</td>
<td>no</td>
<td>no</td>
<td>no</td>
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11. *S. vagans* show response in time partitioning behaviour between different stages and level of starvation. New activities were identified (ie. walking and drinking water).

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<tbody>
<tr>
<td>5</td>
<td>no</td>
<td>no</td>
<td>yes</td>
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12. *S. vagans* adults were shown to be able to locate their prey at low prey densities, provided their normal searching behaviour was not inhibited.

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<tbody>
<tr>
<td>6</td>
<td>no</td>
<td>no</td>
<td>yes</td>
</tr>
</tbody>
</table>
7.1 *S. vagans* and its similarity to other *Stethorus* species

*S. vagans* is similar to other *Stethorus* species in its morphology and life cycle (Moreton 1969; Daniel 1976; Richardson 1977), its reliance on spider mites as its primary prey and its ability to feed on alternative food sources to maximise its longevity (Putman 1955a; Helle and Sabelis 1985; Majerus 1994). On the other hand, it differs from a number of other species in that is smaller, has a shorter body and has a lower total fecundity (although the rate of oviposition /female /day is approximately the same).

One of the most important characteristics is the conversion of prey (*T. urticae*) eggs into eggs of *S. vagans*. At lower prey densities, this conversion factor was 25-30, but declined to 16-20 at higher densities. The only comparable data has been provided by Richardson (1977) for *S. nigripes* with a conversion factor of 30-40 (although the prey density from which this was determined was not given).

7.2 Evaluation of *S. vagans* as an effective natural enemy

Huffaker *et al* (1969) identified the most important attributes of a natural enemy to be high searching ability, sufficient power of increase to overtake and suppress the prey population, synchrony with the prey and its habitat and a density-dependent response to its prey. *S. vagans* possesses many of these attributes, based on the laboratory and limited field investigations reported in this thesis. It is able to maintain itself at relatively low prey densities, and demonstrates good host location and searching ability. While it has a high preference for one prey species, *T. urticae*, it is also able to survive on alternative food sources likely to be present in the field, including other arthropod species and other organic substrates. Reproduction, however, is severely limited with alternative food. The native
spider mite *T. ludeni* may be a possible source of prey for *S. vagans*, although this species was not investigated in the work reported here. However, it is predominantly a tropical species and except for mid-late summer in coastal NSW is unlikely to inhabit much of the known distribution of the predator. *S. vagans* generally appears to be behaviourally and reproductively synchronised with the habitat of its primary prey, except that it does not diapause in conditions where its preferred prey does, including ecosystems primarily composed of deciduous flora. *S. vagans* has many of the qualities expected in a biological control agent. It is able to locate its prey at low prey density and has high powers of increase. It behaviour and reproduction appear to be synchronised with its prey and its habitat (van de Vrie 1972; Readshaw 1975; Bodman 1993; Chapters 4 and 5). It also exhibits a density-dependent response to prey by possessing strongly developed functional and numerical responses to prey density, including intra-specific competition in the form of cannibalism.

### 7.3 The strategy of *S. vagans*

Natural enemies of invertebrate pests have generally been classified as *r* or *K* strategists (MacArthur and Wilson 1967, Pianka, 1972, Matthews 1976) although Greenslade (1972) has also introduced the concept of beyond *K* selection in specific environments. *R* strategists have a rapid intrinsic rate of increase to exploit the situation of high food density with few other constraints. They are usually tolerant of a broad range of physiological conditions, but are poor competitors, avoiding competition by emigration or other behavioural mechanisms when the prey density is low. They are typically opportunists that arrive early and quickly become numerous in disturbed or unstable ecosystems such as agro-ecosystems. In contrast *K* strategists are generally less voracious and less responsive to changes in prey density and have a narrow tolerance of environmental conditions, including food source. They are, however, good competitors. In general, the relative influence of *r* or *K* selection depends on
the degree of stability of the environment (Matthews 1976). It has been argued that the most suitable biological control agents are r strategists. In biological control programs, agents are typically imported, mass reared and released into the target area, aiming at long-term establishment in the environment and exertion of some level of suppression on the target species (pest) over time. This has been referred to as “Classical Biological Control”. In more recent years, there has been the development of “Inundative Biological Control” in which agents are mass-reared and released in higher numbers in specific target areas, frequently on a regular schedule or in response to pest levels, to achieve rapid reduction of the target species (Broadley & Thomas 1995). In this case, the agent is akin to a biological pesticide. In classical biological control, long-term survival of the agent in the field is essential to its success although this is less important with inundative strategies.

Thus, a natural enemy should exhibit attributes of a r strategist when initially introduced, or at times when the prey population may rapidly increase due to disruption of the ecosystem. Yet, for its long-term survival in the field, it must also exhibit attributes of a K strategist when pest populations are low. This thesis reports that S. vagans exhibits both of these attributes. It behaves as a r strategist when populations of T. urticae are high, but as a K strategist when prey density is low.

7.4 Potential for mass rearing Stethorus and field augmentation

A number of insect species as well as several families of mites have been recorded as predators of spider mites (especially T. urticae) in the field, and several have been mass reared and used in augmentive biological control programs. There has been only one report of successful mass rearing of Stethorus spp. for biological control of spider mites (Scriven &
Fleschner 1960), although Richardson (1977) mass reared almost 20,000 S. nigripes which were released in Californian strawberry fields, but apparently failed to establish. This contrasts with the common use of predatory mites in the family Phytoseiidae (e.g. *Phytoseiulus persimilis* (Helle and Sabelis, 1985b; Spooner-Hart 1990), *Typhlodromus = Metaseiulus = Galandromus occidentalis* (Readshaw 1975; Hoy et al 1979; Thwaite 1993) and *T. pyri* (Hardman et al. 1997; Croft et al. 1998; Courtieux & Pierre 1999).

A number of authors have reported that *Stethorus* spp. are active fliers and are capable of locating and controlling *T. urticae* populations at low prey densities and in isolated patches (Hull et al. 1977b; Helle & Sabelis 1985b). We recorded similar results for *S. vagans* (Chapter 6). The ability to of phytoseiid mites to locate isolated prey is at least partially restricted by their relatively limited mobility.

Another of the major differences between phytoseiid mites and *Stethorus* spp is their prey consumption rate. *P. persimilis* consumes 10-12 mites /day /female, while we recorded *S. vagans* consuming 94-143 mite eggs /day /female, and Richardson (1977) reported similar levels of consumption for *S. nigripes*.

Based on our studies, in which a *S. vagans* culture was maintained for a 3 year period, mass rearing appears feasible. However, preliminary investigations to scale up the colony using the culture method described earlier in this thesis (with minor modifications from Scriven & Fleschner (1960)) proved difficult. There seems to be no immediate alternative to the use of *T. urticae* as prey as evidenced by the results of the investigations reported in Chapter 5, in which alternative food sources were able to significantly increase longevity (although this was still much shorter than with *T. urticae* prey), but were unable to support reproduction.
The lack of suitable artificial diets for rearing arthropods is a limit to production of a number of natural enemies. While Singh (1977) and Singh & Moore (1985) reported more than 750 species that had been successfully reared on artificial diets few have been developed for natural enemies (Anderson & Leppla 1992). The likely reasons given were:

i. Many predators eat only living moving prey.

ii. Predators use chemical cues for prey searching.

iii. Nutrients in the diets must be qualitatively and quantitatively appropriate to support normal growth, fecundity, fertility and behaviour.

If large scale culturing of *S. vagans* using its natural prey was developed, a suitable alternative plant host to French bean for rearing *T. urticae* would be required, because of the presence of leaf trichomes on which larvae become impaled. This contrasts with the rearing of some phytoseiid mites (but not *P. persimilis*) where pollen can be an alternative or supplementary food source, and the presence of pollen and melliferous flowers can prolong their longevity and fecundity in the field (James & Whitney 1993).

Given the absence of diapause in *S. vagans*, this predator provides not only opportunities for continuous rearing, but also for use in field and greenhouse production in winter, where temperatures are able to be maintained above the minimum development threshold of approximately 9.1°C (Chapter 4). In cool areas in Australia and overseas where greenhouse crop production is conducted in winter, houses are frequently heated above this threshold temperature. Rott and Ponsonby (2000a) reported activity of *S. punctillum* against *T. urticae* occurred in heated greenhouse grown crops above 20°C with increased activity to 30 °C.

7.6 The use of *S vagans* in Integrated Pest Management (IPM) Programs
S. vagans is a highly host specific predator. While its target prey T. urticae is a major pest in both field and protected cropping (Helle and Sabelis 1985a), it is frequently not the only pest species in the production system, and control measures need to be undertaken for arthropods, pathogens and weeds. The use of a specific biological control agent in such complex ecosystems may therefore, be problematic. The use of pesticides against non-target pests has frequently been associated with resurgence of secondary pests, as a result of their effect on natural enemies.

There appears that Stethorus spp may be highly susceptible to a range of pesticides commonly used in agriculture (Charles et al. 1985; Hull et al 1985; Gurr et al 1999; Roy et al. 1999). It is possible that there are also sublethal effects of these and other pesticides, such as reduced fecundity or longevity, as this has been reported for other predacious species (Wang & Guo 1995; Wright & Verkerk 1995; Smith & Krischik 1999). The selection of compatible pesticides is therefore a key component of any IPM program involving S. vagans.

An alternative option to use of pesticides against other pests is the use of augmentative or inundative releases of biological control agents. In Australia, a number of biological control agents are produced commercially (Broadley and Thomas 1995), and for some crops such as citrus, the naturally occurring biocontrol agents are well documented (Smith et al.) The range of agents available for release is very limited in comparison with Europe (Malais and Ravensberg 1992) and USA (Darr et al. 1996). However, there may be sufficient for their incorporation in some IPM programs where they can replace insecticides and acaricides (but not fungicides). In general, it appears that herbicides have less impact on natural enemies than do the other pesticide groups (Nicholas, 2000).
Using a complex of predators against spider mites may also have some validity. Rott and Ponsonby (2000a) demonstrated that in a heated greenhouse, a combination of *S. punctillum* and *Amblyseius californicus* were superior to the commercially available *Phytoseiulus persimilis* in controlling *T. urticae*.

Based on the above, the most likely crop ecosystems where *S. vagans* could be incorporated into IPM programs are protected cropping (vegetables, nursery, floriculture, indoor landscapes), pome fruit, berries and hops.

### 7.5 Future prospects and recommendations

This thesis raises a number of issues that require further investigation. Some key aspects are:

More detailed field studies are required to confirm the data generated from the studies discussed in this thesis, in particular to determine predator-prey interactions under more natural conditions.

Prior to any assessment of augmentative field releases, a reliable production system for mass *S. vagans* needs to be developed. Mass rearing on its primary host in the laboratory proved to be difficult, due to production of webbing by heavy infestations of *T. urticae* which impeded movement of all stages of *Stethorus*, and the presence of trichomes on the French bean leaves on which the prey were reared which impaled young *S vagans* larvae. French beans were used because they had previously been identified as the most suitable for mass rearing *T. urticae* for production of the phytoseiid predator *P. persimilis*. 
Additional investigations on alternative food sources for *S. vagans*, both in the laboratory but particularly in the field may elucidate the full effect of these on survival and reproduction. The investigations may also provide some suggestions for development of artificial diets.

Further assessment of the host location behaviour is required to clarify the means by which *S. vagans* detects its host, especially at a distance, but also at close quarters. Such studies may identify kairomones or other chemical cues that would assist in understanding this still vexed issue, and provide opportunities to enhance host location or predator aggregation in the field.

7. 6 Conclusion

This thesis concludes that *S. vagans* is likely to be an effective biological control agent of two-spotted mite *T. urticae* based on its attributes elucidated in this thesis. These are its ability to locate hosts in the field at low prey densities, its continuous activity throughout the year, its strong positive functional and numerical response to its primary prey density yet its ability to utilise alternative food and hosts for survival.

However, before this potential can be fully realised, more information is required on its performance in the field, development of reliable methods for mass rearing, and its integration into crop IPM or IPDM programs.


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