Development of Biochemical and Physiological Indices of Maturity of Dessert Stone Fruit in Relation to Cool Storage

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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

The aim of this research was to develop a new harvest maturity index for highly coloured stone fruit that is not affected by seasonal or climatic factors. The judgement of commercial maturity is difficult with some cultivars of stone fruit because they develop intense skin pigmentation several days before they are ripe. Studies of the changes in the physicochemical and physiological parameters associated with ripening confirmed that no single measurement is suitable for the assessment of harvest maturity in the Japanese type plum cultivars (*Prunus salicina* Lindl). These studies identified two distinct patterns of ripening behaviour; ‘Gulfruby’ and ‘Beauty’ showed a typical climacteric pattern of development, whilst ‘Shiro’ and ‘Rubyred’ exhibited a suppressed-climacteric phenotype. Fruit of the latter cultivars ripen slowly, both on the tree and after harvest. This phenotype appears to result from an inability of the fruit to produce sufficient quantities of ethylene since ripening was accelerated by postharvest application of propylene (500 µL/L). All cultivars except Gulfruby exhibited the presence of a ‘tree factor’ in terms of ethylene production. Successive harvests of fruit showed that fruit harvested at a preclimacteric stage began to evolve ethylene earlier than fruit from the same population attached to the tree. To further examine the suppressed-climacteric phenotype, internal ethylene concentrations were assessed in attached and detached Rubyred fruit. No ethylene was detected in attached fruit at any sampling time, but maximum ethylene production in detached fruit was 0.15 µL/kg.h at 20°C. This rate of production was associated with an internal concentration of 0.4 µL/L.

The cool storage (0°C) responses of fruit harvested at three stages of maturity were examined. Symptoms of chilling injury, developed after 2, 3, 4 and 7 weeks cool storage in cultivars Gulfruby, Shiro, Radiance and Rubyred, respectively. Fruit harvested at the first maturity stage withstood cold storage better than more mature fruit and had the capacity to attain high quality when ripened at 20°C.

Total soluble proteins were extracted and separated from fruit during maturation and ripening using 2-dimensional gel electrophoresis. At least three proteins were first detected in fruit at optimum commercial harvest maturity. These polypeptides had the following molecular weights (Mr) and isoelectric point (pI): Gulfruby at 25 days after pit hardening (DAPH), 17.5, 34.5 and 35 kDa and pI 3.5, 3.6 and 3.5, respectively, Beauty at 42 DAPH, 18.3, 37 and 38 kDa and pI 3.5, 3.5 and 4.0 respectively, Shiro at 45 DAPH, 17, 41 and 41 kDa and pI 3.6, 3.7 and 3.8, respectively and Rubyred at 85 DAPH, 19, 40 and 40 kDa and 4.0, 4.0 and 4.0, respectively. Although the protein profiles were similar in both climacteric and suppressed-climacteric types, a clear association between protein changes and the respiratory climacteric was observed only in the climacteric type. The molecular weight of
protein Y approximates that of ACC oxidase from other plant species. Further work is required to determine if the proteins that have been found to increase at optimum harvest maturity are suitable for development an immunoassay of maturity.

The differences in ripening behaviour of climacteric and suppressed-climacteric plums were examined following fumigation with 13, 26 and 39 μL/L of 1-methylcyclopropene (1-MCP) and treatment with propylene (500 μL/L). Application of propylene alone advanced the onset of the respiratory and ethylene climacterics in Gulfruby and Beauty plums, whilst 1-MCP in the presence of propylene delayed these events for several days. Shiro and Rubyred plums exhibited suppressed-climacteric patterns of respiration and ethylene production, with rates of ethylene production 15 to 500-fold lower than in Gulfruby and Beauty. When treated with 1-MCP, Shiro and Rubyred did not develop an ethylene or respiratory climacteric unless propylene was applied. These results showed that the development of skin colour in all cultivars was partially ethylene-independent whilst aroma production was ethylene-dependent in Beauty and Shiro and ethylene-independent in Gulfruby and Rubyred plums. The use of 1-MCP, particularly in combination with the suppressed-climacteric phenotype, may have commercial application for delaying ripening, thus permitting sea transport of plums to distant markets.

Since Gulfruby, Beauty, Shiro and Rubyred accumulated similar concentrations of aminocyclopropane-1-carboxylic acid (ACC), it was suggested that the suppressed-climacteric phenotype is the result of an impaired ability of the fruit to convert this compound to ethylene. This phenotype also seems to have a reduced capacity to perceive ethylene and to produce new receptors following treatment with 1-MCP. Identification of genetic markers for this trait will assist introgression of this trait into new cultivars.
DECLARATION AND PUBLICATIONS

I declare solely that this work has not been submitted for a higher degree at any other university or institution.

Nasser Abdi

Parts of the work in this dissertation have been published, presented, or are being prepared for publication.

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SECTION 5


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IN PREPARATION

ACKNOWLEDGMENTS

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<tr>
<td>ACC</td>
<td>aminocyclopropane-1-carboxylic acid</td>
</tr>
<tr>
<td>ACO</td>
<td>ACC oxidase</td>
</tr>
<tr>
<td>ACS</td>
<td>ACC synthase</td>
</tr>
<tr>
<td>AOA</td>
<td>aminooxycetic acid</td>
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<tr>
<td>APS</td>
<td>ammonium persulphate</td>
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<tr>
<td>ATPase</td>
<td>adenosine triphosphatase</td>
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<tr>
<td>AVG</td>
<td>aminoethoxyvinylglycine</td>
</tr>
<tr>
<td>Bis</td>
<td>N,N-methylene bisacrylamide</td>
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<tr>
<td>cDNA</td>
<td>complementary deoxyribonucleic acid</td>
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<tr>
<td>CHAPS</td>
<td>3-3-cholamidopropyl dimethylammonio-1-propane sulfate</td>
</tr>
<tr>
<td>CI</td>
<td>chilling injury</td>
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<tr>
<td>DACP</td>
<td>diazocyclopentadine</td>
</tr>
<tr>
<td>DAFB</td>
<td>days after full bloom</td>
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<tr>
<td>DAPPH</td>
<td>days after pit hardening</td>
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<td>DMCP</td>
<td>3,3-dimethylcyclopropene</td>
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<td>DTT</td>
<td>dithiothreitol</td>
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<td>EDTA</td>
<td>ethylenediaminetetra acetic acid</td>
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<td>EFE</td>
<td>ethylene forming enzyme</td>
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<td>ELISA</td>
<td>enzyme-linked immunoabsorbant assay</td>
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<td>Fd</td>
<td>fruit diameter</td>
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<td>FDP</td>
<td>fruit development period</td>
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<td>Fw</td>
<td>fruit weight</td>
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<td>GB</td>
<td>gel breakdown</td>
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<td>IEC</td>
<td>induced ethylene climacteric</td>
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<td>IEF</td>
<td>isoelectric focusing</td>
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<tr>
<td>kD</td>
<td>kilodalton</td>
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<td>kgf</td>
<td>kilogram force</td>
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<tr>
<td>mA</td>
<td>milliampere</td>
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<td>MACC</td>
<td>1-malonyl ACC</td>
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<td>MAPK</td>
<td>mitogen-activated protein kinase</td>
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<td>MASE</td>
<td>L-methionine, s-adenosylmethionine, L-aminocyclopropane-1-carboxylic acid-</td>
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<td></td>
<td>to ethylene</td>
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<tr>
<td>1-MCP</td>
<td>1-methylcyclopropene</td>
</tr>
<tr>
<td>Mr</td>
<td>relative molecular weight</td>
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<tr>
<td>2,5- NBD</td>
<td>2,5- Norbornadiene</td>
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<td>NP-40</td>
<td>nonidet phosphor-40</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<tr>
<td>NSW</td>
<td>New South Wales</td>
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<td>P</td>
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<td>polyacrylamide gel electrophoresis</td>
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<td>PG</td>
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<td>pI</td>
<td>isoelectric point</td>
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<td>PIP</td>
<td>diacrylопiperazine</td>
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<td>PME</td>
<td>pectin methylesterase</td>
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<tr>
<td>PPO</td>
<td>polyphenol oxidase</td>
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<tr>
<td>SAM</td>
<td>s-adenosyl methionine</td>
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<tr>
<td>SDS</td>
<td>sodium dodecyl sulfate</td>
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<tr>
<td>SE</td>
<td>standard error</td>
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<td>SSC</td>
<td>soluble solids concentration</td>
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<td>STS</td>
<td>silver thiosulphate</td>
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<tr>
<td>TA</td>
<td>titratable acidity</td>
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<tr>
<td>TCA</td>
<td>trichloroacetic acid</td>
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SECTION 1
INTRODUCTION

Plums (Prunus spp.), which belong to the family Rosaceae, are one of the major groups of stone fruit. Their centre of origin, along with other stone fruit is Western Asia, including China, Iran, Afghanistan and Turkey (Bailey and Hough, 1975; Weinberger, 1975). This study concentrates on cultivars of the Japanese plum (P. salicina Lindl) one of 15 species of plums cultivated world-wide. This species is a member of the oriental plum group which was introduced to the USA from Japan in the late 19th century by Luther Burbank and others. In many parts of the world it is the most important species producing fruit for the fresh market because of its good size, attractiveness, firmness, and good keeping qualities. Although oriental plums were introduced from Japan, they are thought to be native to China. P. domestica the European plum, is a member of the Eurasian group which originated in Western Asia (Couvillon and Krewer, 1991).

Among stone fruits, plums rank next to peach in commercial importance. In Australia plums comprise 20% of an annual production of about 130,000 tonnes/year of stone fruit (Anon, 1996a). World production of plums and prunes is about 3,260,000 tonnes/year (Couvillon and Krewer, 1991). Plums are cultivated between the latitudes 30° North and 45° South (Hammerschlag and Litz, 1992). The low-chill stone fruit industry commenced in Australia in the subtropical area of northern New South Wales with the planting of Sungem and Sundowner nectarines and Gulfruby plum cultivars. High-chill stone fruit are grown in NSW, Victoria, Tasmania, South and Western Australia (Porter et al., 1992).

Major problems in the production of plums include the accurate determination of optimum harvest maturity and correct postharvest handling to maximize storage life and quality. The timing of harvest is of utmost importance if fruit, either for the fresh market or for storage, are to reach the customer in prime condition. The judgement of commercial maturity is difficult with some cultivars because they develop an overall pigmentation of the skin many days before the fruit are ripe. Harvesting plums at an early stage of maturity may result in a product that has a good appearance, transports and stores well, however, yield may be sacrificed and the flavour poor. On the other hand, fruit which are harvested late may perish before they are sold. Therefore, for these highly coloured cultivars, there is a need for physiological markers which will allow the stage of maturity to be determined with precision.

A number of parameters can be used to assist judgement of harvest maturity of fruit. These include changes in flesh texture, tissue permeability, soluble solids concentration (SSC), aroma volatiles, ethylene and CO₂ production (Kader and Mitchell, 1989a; Pratt, 1975). Many of these parameters are not reliable because they vary with cultivar, production area and season (Kader and Mitchell, 1989a). Molecular markers, developed from an
understanding of the physical and biochemical changes which occur in fruit as they mature, may help overcome these problems. It was proposed that since plums are a climacteric fruit (Romani, 1984) it is expected that new enzymes will be synthesized during the onset of ripening. Therefore, as shown in several other climacteric fruits (including apples and pears, which are also members of the Rosaceae family), if the appearance of these enzymes can be detected reliably they could provide the basis for the development of harvest indices that will be independent of environmental and cultural conditions.

Aims of this Project

In view of the desirability to develop new markers of harvest maturity the specific aims of this study were to investigate:

- Fruit development on the tree, including accompanying changes in fruit size, firmness, fruit composition and colour (Section 4).

- The cool storage responses of fruit harvested at different stages of maturity (Section 5).

- Physiological responses of fruit harvested at different stages of maturity in terms of respiration, ethylene production and responses to propylene (an active analogue of ethylene) and 1-methylcyclopropene (an ethylene antagonist) (Sections 4 & 7).

- The use of two-dimensional gel electrophoresis to detect the appearance of any proteins that correlate with ideal harvest maturity for cool storage (Section 6).
SECTION 2
REVIEW

2.1 Taxonomy and Origin of Commercial Cultivars (cvs)

Many cvs of plums have been developed from interspecific crosses between *P. salicina* and other species. Five cvs of plum were used in this study:

**Radiance (Syn. Radiant)** A high-chill variety that produces fruit of medium size, with red skin and yellow to orange flesh that ripen in early March in the Western Sydney region. This cultivar originated from *P. salicina* (Anon, 1996b) (Fig 2.1).

**Gulfruby** A low-chill cv. with fruit of medium size, red skin and flesh colour that ripen in late November. This cv. is a hybrid of the Japanese plum (*P. salicina*) and is a product of the Prunus breeding program in Florida, USA (Sherman and Lyrene, 1983) (Fig 2.2).

**Beauty** A high-chill cv. with fruit of medium size, red skin and flesh colour that ripen in late December. This variety originated from *P. salicina* (Howard, 1945) (Fig 2.3).

**Shiro** A high-chill variety with fruit of medium size, yellow skin and flesh colour that ripen in early January. This cv. is a interspecific hybrid bred by Luther Burbank. It was selected from crosses between Robinson (*P. munsoniana*), Myrobalan (*P. cerasifera*) and Wickson (cross of *P. triflora* and *P. simonii*) (Hedrick, 1919) (Fig 2.4).

**Rubyred** A high-chill cv. with fruit of large size, deep red skin and flesh colour that ripen in mid-February. This cv. originated in Japan (Hedrick, 1919) (Fig 2.5).
Fig. 2.1. Changes in skin colour from the mid to late maturity stages in the cv. Radiance (syn. Radiant).
Fig. 2.2. First colour (A) and full colour (B) development in the cv. Gulfruby.
Fig. 2.3. First colour (A) and full colour (B) development in the cv. Beauty.
Fig. 2.4. First colour (A) and full colour (B) development in the cv. Shiro.
Fig. 2.5. First colour (A) and full colour (B) development in the cv. Rubyred.
2.2 Fruit Growth and Development of Plums

2.2.1 Physicochemical Changes

2.2.1.1 Growth Patterns

Upon the completion of pollination and fertilization, the flower ovary begins to enlarge and develops into the fruit. This process is termed "fruit set". Stone fruit typically have a double sigmoidal growth curve pattern that comprises three distinct stages of growth (Fig. 2.6).

![Growth pattern graph]

Fig. 2.6. Growth pattern of a stone fruit. Pit hardening occurs during stage II and was chosen as zero-time for studying fruit growth and development. Stage III includes the final period of rapid fruit growth, maturation and ripening (after Kader and Mitchell (1989) in LaRue and Johnson (eds)).

As plum fruit develop from a single ovary they are classified as drupes. The endocarp, or innermost layer of the pericarp, becomes lignified and is called the pit or stone, while the fleshy mesocarp is covered by a thin epicarp or skin. In plums, cell division continues for about 40 days after anthesis and this period of rapid growth is referred to as Stage I (Fig. 2.6) in which cell division continues only in the epidermis and mesocarp. The endocarp begins to harden approximately 60 to 90 days after full bloom, an event referred to as pit hardening (Fig. 2.7). During this period of growth the endosperm and embryo inside the seed develop (Romani and Jennings, 1971).
Fig. 2.7. Appearance of fruit from the cv. Gulfruby (A) before and (B) after pit hardening. Pit hardening was chosen as the beginning of fruit maturation (time zero) in this study. Samples I, II and III are samples of fruit from three different trees.
The time of pit hardening also marks Stage II when fruit growth is relatively slow and fruit shedding is complete. The duration of Stage II varies considerably among varieties. Pit hardening provides a consistent starting point to study changes during maturity and ripening. Four to six weeks before harvest, cell enlargement resumes in the flesh (mesocarp) and the fruit begins its final, rapid growth phase. This is referred to as Stage III (Fig. 2.6) and the fruit continue to increase in size and weight until they reach full maturity. The various changes associated with ripening, such as softening and colour development, occur during Stage III (LaRue and Johnson, 1989).

2.2.1.2 Definition of Maturation and Ripening

Physiological maturity is defined as the stage when growth ceases and when ripening begins (Reid, 1992). Commercial maturity parameters have been based on components of the fruit which change during fruit development (Reid, 1992). To enable commercial handling, peaches, plums and nectarines are usually harvested while still firm and are allowed to ripen later before consumption (Kader and Mitchell, 1989a). Maturity indices are usually based on a mixture of physicochemical characteristics that are judged either objectively or subjectively. Experienced growers rely mainly on subjective assessments based on previous observations (Kays, 1991). The determination of the number of days between full bloom and maturity gives an approximate guide to harvest date, but the length of this period depends on seasonal climatic conditions (Gur, 1986). Several physicochemical factors can be integrated to give an assessment of harvest date as no single factor is reliable. However, the assessment of maturity still remains problematic.

Fruit ripening involves many physiological and biochemical changes in the tissues of fruit which, under ideal conditions, are integrated and lead to the production of acceptable, ripe fruit (Thompson, 1996). These changes in quality make the fruit attractive to consumers, and in nature, these changes are thought to favour seed dispersal. Ripening frequently involves changes in fruit colour (often involving the destruction of chlorophyll and the synthesis of new pigments) (Rhodes, 1980; Seymour and Tucker, 1993), the production of the volatiles responsible for aroma (Davies and Hobson, 1981), the conversion of starch into sugars (Biale and Young, 1981), an increase in carbon dioxide and ethylene production in climacteric fruits (McGlasson et al., 1978), a decline in acidity (Kader and Mitchell, 1989a) and flesh textural changes associated with fruit softening (Huber, 1983). Changes in these parameters are thought to be regulated and coordinated by the expression of specific proteins (Brady, 1992). Overall, ripening processes are probably under the control of plant growth regulators (McGlasson et al., 1978).

Changes in ripening parameters differ qualitatively and quantitatively according to fruit type. For example, mango has a large increase in CO₂ production during ripening,
whereas in apple, the increase is much smaller (Biale and Young, 1981). Similarly, most
dessert peaches and plums soften to a melting texture when ripe, while apples soften only
slightly, remaining firm and crisp. The changes in ripening parameters which occur in plums
are detailed in the following sections.

2.2.1.3 Changes in Skin Colour

Like other stone fruit, the appearance of plums attracts customers, but if eating quality
does not match appearance customers will not repeat purchases. Fruit colour or visual
appearance is determined by the various pigments present in the skin and flesh (Rood, 1957;
Senter and Forbus, 1992). As fruit mature and ripen, green colour declines and they develop
yellow, red or other colours due to pigments which are characteristic of the various cultivars
(Kader and Mitchell, 1989b; Romani and Jennings, 1971). In the yellow-fleshed plum
cultivars, carotenoids and low molecular weight proanthocyanidins are present, while
anthocyanins are lacking (Senter and Forbus, 1992). Anthocyanins and proanthocyanins are
dominant in the dark-fleshed plums (Senter and Forbus, 1992). Colour changes can be either
dependent or independent of ethylene action according to the pigments involved and the fruit
species (Lelievre et al., 1997b).

The development of skin pigmentation in peaches and nectarines depends on exposure
to light, the location of the fruit on the tree, and therefore, is not a reliable marker of harvest
maturity (Kader and Mitchell, 1989b; Mitchell et al., 1979). In contrast, changes in flesh
colour are not affected by sunlight, thus, they are a more reliable index of maturity (Crisosto,
1994).

2.2.1.4 Changes in Fruit Firmness

Fruit firmness depends on skin toughness, flesh firmness, and internal structure such as
the thickness of the flesh, stone size and separation of the stone from the flesh. Most fruit
soften during ripening and this is a major quality attribute that influences consumer
acceptance and shelf life. Fruit softening is regulated by three mechanisms; loss of turgor,
degradation of protopectin and the enzymatic breakdown of the fruit cell walls (Seymour et
al., 1993). Loss of turgor is a non-physiological process associated with the loss of water
from harvested fruit.

Firmness of stone fruit, apples and pears is objectively measured as the force required
for a defined probe to penetrate the fruit to a specific depth (Gur, 1986; Watada and Abbott,
1985). Examples of these probes are the Magness-Taylor penetrometer, the Effegi
penetrometer and the Instron Universal testing instrument. Other non-destructive methods
that have been used to assess firmness/texture are sonic transmission and impact response
(Meredith et al., 1990; Zhang et al., 1994). Fruit firmness, as measured by puncture tests
following removal of small patches of skin, is one of the most widely used indicators of fruit
quality (Bourne, 1980). For example, the recommended firmness for picking plums using an Effegi penetrometer varies between 4.1 to 9.1 kgf depending on the cultivar (Haller, 1941).

Two broad mechanisms for the softening of fruit cell walls have been proposed. One proposal involves a leakage of organic acids into the apoplast or a decrease in the pH of the apoplast due to the action of a plasma membrane bound H⁺ ATPase which results in the removal of Ca⁺⁺ bridges between pectin chains (Brady, 1992). According to the second proposal, softening is the result of enzymatic degradation of protopectin and cell wall hydrolysis during fruit ripening and senescence (Perring and Pearson, 1988; Weichmann, 1986). This mechanism will be discussed in Section 2.2.2.3. Other changes in cell wall composition that accompany fruit softening include decreases in wall bound pectic polysaccharides and corresponding increases in soluble pectic components (Pilnik and Voragen, 1970), and changes in neutral sugar components such as arabinose, galactose and xylose (Dowson et al., 1992; Gross and Sams, 1984; Gur, 1986).

2.2.1.5 Changes in Fruit Composition

To achieve acceptable fruit quality, mature peaches and nectarines should exceed 10 % SSC (Biggs, 1976). SSC consists of sugars, acids, amino acids and mineral salts and is commonly measured with a refractometer. The major acids in plums are quinic, malic, citric and succinic acids (Meredith et al., 1992). SSC generally increases during fruit maturation and ripening, while titratable acidity (TA) declines. Measured values of SSC in plums vary with the cultivar and range between 7 and 24 % (Money and Christian, 1950). It has been observed that SSC and TA vary among cultivars with production area, season and position of fruit on the tree (Dann and Jerie, 1988; Kader and Mitchell, 1989b). SSC:TA ratios have been found to give values more closely related to quality and harvest maturity than either SSC or TA alone, but the ratio still varies between years (Kader et al., 1982; Kader and Mitchell, 1989b). Thus, these attributes can only be used in association with other observations as a guide to harvest maturity.

2.2.1.6 Changes in Flavour and Aroma Production

Volatile compounds are responsible for fruit aroma. Aroma production by ripening fruit is an important quality criterion and has been found to be a ripening-related process (Visai and Vanoli, 1997). The formation of flavour and aroma compounds in fruit is a dynamic process, because volatile substances are continuously synthesized during fruit development and ripening. They change both qualitatively and quantitatively, especially during ripening (Severnants and Jennings, 1966; Visai and Vanoli, 1997).

Thirty four volatile compounds have been isolated from P. salicina plums; among them n-nonanal and binalol were present in the largest amounts (Ismail et al., 1980).
Nonanal, which is also present in the epicuticular wax of *P. domestica* plums, was found to be responsible for the strong aroma emanating from the surface of these fruits (Ismail *et al.*, 1977). In contrast, in peach and nectarine fruits, aroma is not attributable to one or a few compounds. About 100 aroma compounds have been identified and aroma is considered to be due to the integrated response of the human nose to this complex of aroma compounds (Sevenants and Jennings, 1966). Although peak evolution of volatiles roughly coincides with the climacteric peak in respiration in apple fruits (Song *et al.*, 1997), they are not a useful guide to harvest maturity, because they vary widely among cultivars and with growing conditions.

This review of published work on stone fruit shows that physicochemical changes vary among cultivars, seasons, growing area, crop load, position of fruit on the tree and fruit size. Therefore, they can only be used as a general guide to harvest maturity. Clearly, there is a need for indices that are more closely linked to the physiological changes that control ripening in stone fruits. These changes are discussed in Section 2.2.2.

2.2.2 Physiological Changes

2.2.2.1 Respiratory Climacteric

Kidd and West (1925) divided fruits into two main categories, ‘climacteric’ and ‘non-climacteric’, according to changes in respiratory behaviour. Members of the climacteric class include banana (McMurchie *et al.*, 1972), apple (Dilley *et al.*, 1993a), peach and nectarine (Gur, 1986) and plum (Sekse, 1988). The respiratory climacteric is accompanied by an increase in autocatalytic, endogenous ethylene production (Sekse, 1988). Application of exogenous ethylene or propylene to fruit of the climacteric class at a preclimacteric stage advances the onset of endogenous ethylene production and ripening (McMurchie *et al.*, 1972). In non-climacteric fruits, the respiration rate gradually decreases during maturation and senescence and ethylene production remains low (Biale and Young, 1981). Examples of such fruits are blueberries (*Vaccinium*), strawberries (*Fragaria*), citrus species, grapes and sweet cherries (*Prunus avium*) (Sekse, 1988). Exposure of non-climacteric fruit to ethylene or propylene stimulates respiration, but does not induce ethylene synthesis. However, ethylene treatment may induce chlorophyll breakdown, the synthesis of carotenoids, and in general, it hastens senescence (Knee *et al.*, 1988; McMurchie *et al.*, 1972).

The function of increased respiration during the ripening of climacteric fruit has been the subject of much debate. A popular view is that an increase in respiration is necessary to provide ATP and substrates for the many anabolic processes associated with ripening (Blanke, 1991). Respiration in fruits usually involves the oxidation of glucose to carbon dioxide and water which produces energy that is stored as adenosine triphosphate (ATP). This
is a very efficient system with about 90% of the energy generated being conserved within the fruit and the remainder being lost as heat (Blanke, 1991; Wills et al., 1998). After harvest, fruit are dependent on stored carbohydrates for respiration (Blanke, 1991; Wills et al., 1998). Some of the mechanisms proposed to explain the regulation of respiration, and the possible influence of ethylene on this process are discussed in the following section.

2.2.2.2 Ethylene and Ripening

The role of ethylene in the ripening of climacteric fruit has attracted an enormous amount of research since Gane (1934) showed that apples produce ethylene. The basic questions have been whether ethylene is a product or a cause of ripening and how it acts (Biale et al., 1954). A causative role for ethylene in the ripening process was proved in the 1960's with the advent of gas chromatography, and during the last 30 years the biochemical pathways of ethylene synthesis have been determined. The enzymes involved with ethylene biosynthesis and their corresponding genetic sequences have been isolated and identified, and progress has been made in understanding ethylene perception and signal transduction (Kieber, 1997).

Ethylene plays a critical role in ripening of climacteric fruit by coordinating the expression of genes that are responsible for a variety of processes that include autocatalytic ethylene production and increased respiration rate, chlorophyll degradation, anthocyanin and carotenoid synthesis, conversion of starch to sugars, aroma production and increased activity of cell wall degrading enzymes (Abeles et al., 1992; Gray et al., 1992). In contrast, it is generally considered that the ripening of non-climacteric fruits is ethylene-independent (Lelievre et al., 1997b). However, in these fruits, new enzymes (proteins) are synthesized during ripening, indicating that changes in gene expression are involved. Little is known of the regulatory mechanisms underlying these biochemical changes (Lelievre et al., 1997b).

Some of the most important research findings relating to fruit ripening are reviewed in the following sections. Since the enzymes responsible for ethylene synthesis have been measured in climacteric fruits including apples, tomatoes, avocados and bananas, it is highly possible that the synthesis of these enzymes may serve as a harvest maturity index for plums.

2.2.2.3 Enzymes Associated with Fruit Softening

In order to understand the changes occurring in cell walls during softening, knowledge is required of the molecular components of the primary cell wall and middle lamella, and factors, either enzymic or non-enzymic, which could influence cell wall architecture. However, there is little detailed knowledge of the cell wall structure of many mature fruit (Brady, 1987).
The change in texture of fruits during ripening has been linked with several enzymatic changes including the loss of neutral-sugar side chains, changes in the synthesis of cell wall components products and the breakdown of calcium-pectate (Hobson, 1993). Some of the products of cell wall metabolism have been shown to promote ripening (Gross, 1990).

It has been established that certain proteins, at least some of them metabolically active, are bound to fruit cell walls, while those rich in hydroxyproline residues play an intrinsic role in wall structure (Carpita and Gibeaut, 1993). There is evidence that endo-polygalacturonase (PG), one of the enzymes responsible for the degradation of pectin, is not evenly distributed in cell walls. Because it is immobile, the enzyme would require continuing accumulation if extensive softening is to occur (Brady et al., 1987). It has been demonstrated that swelling and extensive hydration of cell walls accompanies ripening, that pectin becomes solubilized and that hemicelluloses are also degraded at this time (Gross, 1990; Hobson, 1993). Therefore, cellulose microfibrils are left without support and start to breakdown and this leads to cell separation or to changes in wall elasticity. These changes are thought to be caused by a small group of hydrolytic enzymes (Huber, 1983).

Enzymes thought to be involved in fruit cell wall metabolism during ripening include PG, pectin methyl esterase (PME), β-glucanase or cellulase and β-galactosidase (Awad and Young, 1979; Seymour and Tucker, 1993). PME removes methyl groups from the pectin chains which may then be attacked by PG (Tucker and Grierson, 1987). The relative importance of these various cell wall hydrolases may vary among species; e.g., cellulase and PG activity control softening of avocado (Awad and Young, 1979), different isoenzymes of PG correlate with softening of tomato fruits (Hobson, 1965), cellulase, endo- and exo-PG activity are associated with softening in peaches and pears (Ben-Arie et al., 1979; Downs et al., 1991). Clingstone peaches have an exo-PG while freestone cvs have both endo and exo-PG activity (Dick and Labavitch, 1989; Pressey and Avants, 1977). Hinton and Pressey (1974), reported that cellulase and PME are not present in immature peach fruit and increase in the early stages of ripening. The contribution of PG, cellulase or other enzymes to the softening of plums or apricot is unknown (Brady, 1993). As plums, nectarines and peaches are climacteric members of Prunus, similar biochemical changes can be expected in these species during fruit softening.

2.2.3 Ethylene Biosynthesis and Action

2.2.3.1 Biochemical Pathway and Molecular Biology

The pathway of ethylene biosynthesis proceeds from methionine through S-adenosylmethionine (SAM) to the cyclic amino acid 1-methylcyclopropane-1-carboxylic acid (ACC), which is the immediate precursor of ethylene (Adams and Yang, 1979). Ethylene biosynthesis may be controlled by competition for one of its precursors. There are two key
enzymes required for the synthesis of ethylene; one is ACC synthase (ACS) (Kende, 1989) and the second is ACC oxidase (Yang and Hoffman, 1984). This enzyme was formerly referred to as the ethylene forming enzyme (EFE), later was renamed ACC oxidase (ACO) by Ververidis and John (1991) (Fig. 2.8).

ACS was first identified in tomato by Boller et al. (1979) and Yu et al. (1979). Several isoforms of ACS with pI values of 5.3, 7 and 9 have been found in wounded tomato fruit tissue (Mehta et al., 1988). These data are consistent with the existence of multiple ACS genes that encode proteins showing diversity in molecular mass as well as in their pI values. SDS-PAGE analysis of ACS has shown molecular weights of 48 (pI, 7.03), 55 (pI, 7.25), 56 (pI, 5.6-8.0) and 58 (pI, 8.17) Kda, respectively, for apple (Dong et al., 1991), zucchini (Sato et al., 1991), tomato (Van Der Straeten et al., 1990) and squash fruits (Nakajima et al., 1990).

To date, nine ACS genes have been found in tomato, of which, only three are expressed in ripening fruit (Zarembinski and Theologis, 1994). As well as in tomato, differential expression of ACS genes has been demonstrated in melon (Yamamoto et al., 1995) and winter squash (Nakajima et al., 1990). In tomato fruits, ACS is a soluble enzyme and there is no evidence for membrane localization of ACS as in other plant tissues (Boller et al., 1979). It has been reported that ACS activity may be regulated at the post-transcriptional level (Kende, 1993).

The final step in ethylene biosynthesis, the conversion of ACC to ethylene, is catalyzed by ACO (Yang and Hoffman, 1984). ACO was first identified by expressing the tomato cDNA, pTOM13, in an antisense orientation. This resulted in greatly reduced ethylene production in tomato fruit (Hamilton et al., 1990). The pTOM13 sequence has homologues in a wide range of plants: mRNAs with homology to pTOM13 have been demonstrated to increase during ripening in apple (Dong et al., 1991), peach (Callahan et al., 1990), avocado (Buse and Laties, 1991) and melon (Balague et al., 1993). SDS-PAGE of apple fruit tissue indicated that ACO has a molecular weight of 35.7 kD, whereas by gel filtration the Mr was estimated to be 39 kD and by size exclusion HPLC 36 kD(Dilley et al., 1993b). Isoelectric focusing gel electrophoresis showed that ACO in apple has one isoform with a pI of 5.3 (Dilley et al., 1993b). Three gene members of ACO have been identified in tomato (Bouzayen et al., 1993). All three ACO genes accumulate during fruit ripening, tissue wounding and senescence (Barry et al., 1996).
Fig. 2.8. The MSAE (L-methionine, s-adenosylmethionine, 1-aminocyclopropane-1-carboxylic acid-to-ethylene) pathway showing the conversion of L-methionine into ethylene. The conjugation of ACC into MACC is indicated on the lower right pathway. The detoxification of HCN by cysteine is shown on the lower left (after Abeles et al., 1992).
It has been demonstrated that ACO is monomeric (Dong et al., 1992) and catalyzes the oxidation of ACC and its co-substrate ascorbate as follows (Yang and Dong, 1993):

\[
\text{ACC} + \text{O}_2 + \text{ascorbate} \xrightarrow{\text{Fe}^{2+}, \text{CO}_2} \text{C}_2\text{H}_4 + \text{CO}_2 + \text{HCN} + \text{dehydroascorbate} + 2\text{H}_2\text{O}
\]

ACO expression is often constitutive, at least at a low level of activity. However, there is evidence that the activity of ACO also increases in some plants in response to internal and external factors that induce ethylene formation (Kende, 1993). All evidence indicates ACO is probably attached to cell walls (Pech et al., 1995).

ACC can be metabolized to 1-malonylaminocyclopropane-1-carboxylic acid (MACC), a process which may contribute to the regulation of ACC levels and the rate of ethylene formation (Hoffman et al., 1982). There is also evidence that autoinhibition of ethylene biosynthesis is based on enhanced malonylation of ACC (Liu et al., 1985). Although malonylation was thought to be irreversible under physiological conditions (Yang and Hoffman, 1984), high levels of MACC may result in some conversion of MACC to ACC as has been shown in avocado and apple fruits during the preclimacteric stage (Mansour et al., 1986; Sitrit et al., 1986). A new conjugate, 1-(γ-L-glutamyl) aminocyclopropane-1-carboxylic acid (GACC), of ACC has been identified in tomato (Martin et al., 1995). MACC or GACC can serve as a source of ACC (Fluhr and Mattoo, 1996; Hanley et al., 1989).

In conclusion, ethylene biosynthesis is subject to positive and negative feedback regulation. The availability of molecular probes for genes encoding the enzymes of ethylene biosynthesis and analysis of the promoter sequences of these genes will aid in determining the mechanism of such feedback regulation in fruit ripening.

2.2.3.2 Action of Ethylene and Propylene

Ethylene is produced from many sources including internal combustion engines, ripening fruits, fluorescent ballasts, fungi, smoke, plastics in UV light and virus infected plants (Abeles et al., 1992). The use of kerosene stoves for sweating or forced curing of citrus was a well-established commercial practice by the late 1900s (Sherman, 1985). Denny's work (1924) marked the beginning of the controlled use of ethylene gas in commercial degreening and fruit ripening practices.

There are three potential sources of ethylene for commercial use: liquids, compressed gas and catabolic production from ethanol. Liquid sources include 2-chloroethyl phosphonic acid known as ethephon (Dennis, 1976). Pure ethylene gas is flammable in air at concentrations 3.1 to 32 % and must be used with care. Ethylene mixed with compressed carbon dioxide is available commercially as a non-flammable source (Abeles et al., 1992). Harvey (1928) published the first comprehensive bulletin describing the commercial
application of ethylene for ripening bananas, pineapples, dates, Japanese persimmons, tomatoes, apples and muskmelons.

The threshold concentration of exogenous ethylene required to trigger ripening has been extensively studied (Biale, 1960; Burg and Burg, 1967; Wang et al., 1972). It is not clear whether this threshold concentration causes ripening or it stimulates endogenous ethylene production to physiologically active levels which in turn causes the fruit to ripen (Sfakiotakis and Dilley, 1973a). Sensitivity to ethylene is not consistent throughout the life of the fruit, and the threshold level needed to cause the ripening of climacteric fruit is dependent on the age of the tissue. Fruits attached to the tree are less sensitive to ethylene than harvested fruits (Meigh et al., 1967), suggesting the presence of a ripening inhibitor supplied by the parent plant: this is usually refereed to as the ‘tree factor’ (Abeles et al., 1973; Burg, 1964). Application of ethylene or propylene can remove, overcome or hasten the disappearance of tree factors (Abeles et al., 1992; Sfakiotakis and Dilley, 1973a).

Propylene, like ethylene, promotes fruit ripening, inhibits elongation of pea subapical sections, and causes epinasty (Crocker et al., 1935). Burg and Burg (1967) determined the molecular requirements for ethylene action and found propylene to be the next most active ethylene analogue to show ethylene-like action. The equivalent concentration of propylene to cause half-maximum ethylene response was found to be 130 times that of ethylene. Gerasopoulos and Richardson (1996) reported that application of 500 μL/L propylene is equivalent 4 μL/L ethylene for pears.

Propylene is a useful treatment in studies involving ethylene because it can be separated from ethylene by gas chromatography (McMurchie et al., 1972; Sfakiotakis and Dilley, 1973a). Sensitivity of the fruit to exogenous ethylene increases with time after full bloom (Wang et al., 1972). This feature of climacteric fruit has been shown with a range of species including apples and avocados (Sfakiotakis and Dilley, 1973a; Zauberman and Fuchs, 1973) treated either with ethylene or propylene. The differences between the response of climacteric and non-climacteric fruits to propylene led McMurchie et al. (1972) to propose that there are two systems of ethylene production in fruit. System 1 is responsible for basal ethylene production in fruit before the onset of ripening in climacteric and non-climacteric fruit, as well as in vegetative tissues and during wound-induced ethylene production (Burg and Burg, 1962). In climacteric fruit, such as apple, pear, peach, plum and nectarine, System 2 is thought to be responsible for the upsurge of ethylene production with the onset of ripening (McMurchie et al., 1972; Tonutti et al., 1991). Exogenous ethylene exerts a negative feedback regulation on ethylene production in immature climacteric fruit (System 1), such as sycamore figs (Zeroni et al., 1976) and banana (Vendrell and McGlasson, 1971). Whereas, in mature climacteric fruit, ethylene is autostimulatory (Dupille and Sisler, 1995). Measurement
of changes in internal ethylene concentrations have been used as a guide to harvest maturity in apple (Dilley, 1980).

2.2.3.3 Evaluation of Internal Ethylene Concentrations as a Guide to Maturity

The fact that the ethylene concentration in fruit increases when ripening processes have started (Sfakiotakis and Dilley, 1973b) makes the detection of this burst of ethylene a potential indicator for the measurement of physiological maturity. Dilley (1980) has indicated that analysis of internal ethylene concentrations at harvest can be used to determine the optimum harvest date and can also be used to predict the potential storage life of apples in air or controlled atmospheres. Changes in internal ethylene concentrations have also been reported to be a useful index of harvest maturity in apples (Liu et al., 1985). However, there is large variability from fruit to fruit that is influenced by orchard, latitude, cultivar, harvest time and temperature (Blanpied et al., 1985; Saltveit, 1982) and there has been limited commercial application of this technique. This idea led to development of the induced ethylene climacteric (IEC) procedure to predict the onset of the endogenous ethylene climacteric in attached fruit (Dilley and Dilley, 1985). This was based on the observation that harvested fruit ripen sooner than comparable fruit left on the tree due to tree factor (s). The IEC procedure works well for some early to mid season cvs that have high rates of ethylene production once ripening commences. However, the IEC technique is laborious and is affected by seasonal factors, location of fruit on the tree and rates of ethylene production (Blankenship and Unrath, 1988, Watkins et al., 1989).

Internal ethylene concentrations in apples remain at about 0.1 µL/L until they reach the optimal harvest date. At this time the rate of ethylene production increases 10- to 1000-fold depending on cv. (Abeles et al., 1992). The relationship between internal concentrations of ethylene and the rate of production can be used to calculate a conversion constant (Burg and Burg, 1962). This conversion constant is expressed as the ratio of the internal concentration in the tissue divided by the rate of ethylene production. The reported conversion constants for some climacteric fruits are 1 (apples), 5 (pears and peaches), 13 (nectarines) and 4 (plums) (Abeles et al., 1992).

To better understand the role of ethylene action in ripening processes, more information is needed about the mechanisms of ethylene perception and signal transduction.

2.2.3.4 Ethylene Perception and Signal Transduction

Research is now focusing on the ways that plants perceive and transduce hormonal signals and the consequences of signal recognition for gene expression. An understanding of ethylene perception and signal transduction is being gained from genetic mutants of
Arabidopsis thaliana and tomato where the genes for putative ethylene receptors have been cloned (Chang et al., 1993; Hua et al., 1995; Wilkinson et al., 1995).

Since the first ethylene insensitive mutant was isolated by Bleecker et al. (1988) a number of both ethylene-insensitive and so-called constitutive response mutants have been found (Guzman and Ecker, 1990; Kieber, 1997). When seedlings from many different dicotyledonous plant species are grown in the dark, in the presence of ethylene, they adopt a striking morphology, referred to as the ‘triple response’. In pea, the triple response consists of an inhibition of elongation, radial swelling of the epicotyl and an altered response to gravity (Knight et al., 1910; Neljubow, 1901). In Arabidopsis, the triple response consists of an inhibition of root and hypocotyl elongation, radial swelling of the hypocotyl, and an exaggeration of the curvature of the apical hook (Kieber, 1997). This response provides a way for the isolation of ethylene responsive mutants and has provided a useful tool for the studying the molecular basis of ethylene signalling (Kieber, 1997).

Hormonal signals are first perceived by receptors and all receptors are members of families of related proteins and are located in the plasma membrane, nucleus, cytoplasm, or even the cell wall (Trewavas and Malho, 1997). Trewavas (1982) summarized the relationship between a plant growth substance and its receptors with plant tissue in the equation:

Growth substance (GS) + Receptor (R) → (GS:R) → Biological response

In the concept of fruit ripening, the change in sensitivity to ethylene can be interpreted as a change in R. Thus, the nature and concentration of R, the receptor, is crucial.

Thirty years ago it was proposed that ethylene binds to its receptor via a transition metal (Burg and Burg, 1967). A direct interaction between ethylene and its receptors is favoured because of the strength of the σ bonds which could be created (Collman et al., 1987). However, the amino acid sequence of the putative ethylene receptors isolated to date do not contain a recognised metal-binding motif although, a number of amino acids which could interact with metals have been identified. It is proposed that the cofactor is chelated by amino acid residues in the three hydrophobic domains of the amino terminus of the protein (Bleecker and Schaller, 1996). The identity of the transition metal is unknown but is presumed to be Zn\(^{++}\) or Cu\(^{++}\) based on the affinity of alkenes for transition metals and from observations on the physiological activity of ethylene analogues (Burg and Burg, 1967). Proof of the involvement of a transition metal would aid our knowledge of how ethylene is perceived. There are several ethylene receptors with different functions in plants which made their isolation difficult (Goren and Sisler, 1982).
One of these receptors is ETR1 (ethylene-resistant), which is inherited as a single gene (Bleecker et al., 1988). This ethylene receptor protein, which is a homodimer, closely resembles the two-component signal transduction systems of prokaryotes (Chang et al., 1993). The ETR1 protein is a disulfide-linked dimer (Schaller et al., 1995) with an N-terminal sensor domain adjacent to the histidine kinase domain, and with a putative receiver domain in the C-terminal region (Chang, 1996). Heterologous expression of the ETR1 protein in yeast or bacteria generated saturable ethylene-binding activity which could be antagonized by competitors of ethylene action (Schaller and Bleecker, 1995). Several ETR1 homologues are known in Arabidopsis (Chang et al., 1993). In one of these, EIN2 (ethylene insensitive), ethylene production rates are raised, which may indicate that feedback inhibition of ethylene on its production necessitates ethylene perception. ERS (ethylene-response sensor) which shows high similarity to ETR1, but lacks the receiver domain. ERS or ETR1 may transfer its phosphate to the receiver domain in ETR1 or to other independent unknown receiver domains (Fluhr and Matteo, 1996). A tomato homolog of ETR1 has been cloned from N. riparia, a ripening impaired tomato mutant called Never Ripe and its function is similar to ERS-like gene product (Payton et al., 1996; Wilkinson et al., 1995).

The CTR1 (constitutive triple response) gene product acts downstream of ETR1. Once the response regulator domain has been activated the ethylene signal is transduced via CTR1, a negative regulator of the transduction pathway (Kieber, 1993). It is proposed that the amino-terminal domain of the ethylene receptor perceives ethylene to the response regulator domain. This latter protein is closely related to serine/threonine protein kinase which suggests that signal transduction is mediated by a process similar to the mitogen-activated protein kinase (MAPK) cascade that regulates cell differentiation in mammals, worms and flies (Nishihama et al., 1995). In the absence of ethylene, ETR1 could activate CTR1, which would impose quiescence by phosphorylation of a putative MAPKK (Fluhr and Matteo, 1996).

2.2.3.5 Ethylene Inhibitors and 1-methylcyclopropene (1-MCP)

There are several types of ethylene inhibitors that may be applied exogenously to plants (Burg and Burg, 1967; Yang, 1985). Aminoxyacetic acid (AOA) and aminooethoxy vinylglycine (AVG) prevent ethylene synthesis (Yang and Hoffman, 1984). AOA or AVG inhibit ACC synthase by preventing the conversion of SAM to ACC (Yang, 1980). Complete inhibition of ethylene production by AOA or AVG has not been reported in any fruit tissue (Lieberman, 1979).

Competitive inhibitors of ethylene action include 2,5-norbornadiene (2,5-NBD) and trans-cyclooctene, both of which bind transiently to the ethylene receptor (s) (Sisler and Pain, 1973). 2,5-NBD is a toxic and offensive smelling compound while trans-cyclooctene is
unstable (Sisler, 1991; Sisler and Yang, 1984). Diazocyclopentadiene (DACP) is also an inhibitor of ethylene action which has potential as a photoaffinity label for ethylene binding (Serek et al., 1994a; Sisler and Blankenship, 1993). Silver thiosulphate also inhibits ethylene action and is used by the floriculture industry to extend flower life. Its continued use is questioned as silver is a potent environmental pollutant (Sisler and Blankenship, 1993).

Recently the cyclopropenes, 1-MCP and 3,3-dimethylcyclopropene (3,3-DMCP), have been found to be effective antagonists of the ethylene response (Fig. 2.8) (Sisler et al., 1996a,b). 1-MCP is about 1000 times more active than 3,3-DMCP. These materials are non-toxic, odourless and relatively simple, organic compounds that irreversibly inhibit ethylene binding at concentrations as low as 0.5 μL/L (Serek et al., 1995). Both of these compounds are gases at room temperature and 1-MCP is the most useful compound because it is believed to bind irreversibly to the ethylene receptor (Serek et al., 1994a; Sisler and Serek, 1997). 1-MCP is a flat molecule while in 3,3-DMCP, the methyl groups stick out from the plane of the molecule. This would hinder the binding of 3,3-DMCP to the receptor (Sisler et al., 1996b). 1-MCP interacts with the ethylene production mechanism and stops autocatalytic production of ethylene (Sisler et al. 1996a).

![Molecular structures](image)

**Fig. 2.8. Molecular structures of cyclopropene, 1-MCP and 3,3-DMCP (after Sisler and Serek, 1997).**

It has been reported that 1-MCP inhibits skin colour and ethylene production in tomato and banana fruits (Sisler et al., 1996b) and prevents the climacteric rise of ethylene production, respiration and volatile production in apple (Song et al., 1997).

2.2.4 Genetic Control of Fruit Ripening

It has been demonstrated that ethylene plays an essential role in fruit ripening by stimulating the expression of ripening-related genes (Grierson et al., 1985). Thus, there is a tendency to consider the role of ethylene in gene activation as a "switching" mechanism.
implying that once a switch is thrown, climacteric ripening will follow as a matter of course (Brady, 1992).

The changes in flavour, texture and colour in fruits are considered to be genetically regulated and coordinated. There are basically three major questions to be asked about genetic control:

- How is fruit ripening initiated?
- Once initiated, how is ripening regulated?
- How are the various diverse biochemical changes coordinated during ripening?

The answers to these questions will be complex, but they must depend on the relation between the regulation of gene expression and enzyme activity and substrate concentration (Seymour et al., 1993; Speirs and Brady, 1991).

Distinguishing between ethylene dependent and independent pathways and gene expression during ripening has been facilitated by the availability of transgenic plants and the molecular analysis of naturally-occurring mutant lines such as the Nr tomato mutant (Lelievre et al., 1997a). The capacity to convert ACC to ethylene increases in apples prior to the climacteric rise in ethylene (Lau et al., 1986; Mansour et al., 1986), suggesting that ACO gene expression is developmentally controlled and that it precedes the expression of ACS. Hoffman and Yang (1980) suggested that preclimacteric fruit lack the capability to convert ACC to ethylene. Later, during the post-climacteric stage, ACC may increase when ethylene production declines. The other ripening-related genes in tomato fruits are E4, E8 and PG. E8 encodes a protein that strongly reduces ethylene production, but its function is unknown (Penarubia et al., 1992). However, application of exogenous ethylene in this fruit activated E8 gene expression during ripening, but not PG (Lincoln et al., 1987).

In peach fruits, no increase in ACS activity was observed during the ethylene climacteric and this enzyme remained undetectable during the fruit growth cycle (Tonutti et al., 1991). Tonutti et al. (1991) also found that the ACC concentrations in the epicarp were lower than in the mesocarp, even during the climacteric, while the ACO activity was consistently higher. The slow-ripening characteristic of mutant nectarines appears to be controlled by a single recessive gene (Ramming, 1991). These mutants had low levels of ACC and, at the time when ACC was applied, ethylene production increased to much higher rates than in the normal type (Brecht and Kader, 1984a). The addition of ethylene to these fruits stimulated ACS and reduced ACO activities (Brecht and Kader, 1984b). They suggested that ACS is responsible for the onset of ripening in these fruits.

In preclimacteric avocado, tomato and apple fruits, ACO activity is at a low level and increases within 24 hours of ethylene treatment, before autocatalytic ethylene production is
detected (Dilley et al., 1993a; Sistrit et al., 1986). ACO activity in vivo decreases rapidly after the climacteric peak in avocado, whereas the AVOe3 protein, with Mr 35 kDa homologous to ACO in apples, continues to accumulate. ACO activity may be limited by physiological factors such as the cellular environment in postclimacteric fruit (McGarvey et al., 1990).

2.2.5 Potential Biochemical Indices of Maturation

Older procedures for determining harvest maturity in stone fruit, as described in Section 2.2, are not satisfactory because of the variations among cultivars, climatic conditions, crop load, production area and seasons. Therefore, the development of new indices of harvest maturity based on protein changes and gene expression may be more efficacious. The principal aims of this research were to develop new harvest maturity indices of ripening in stone fruit. The above review of published work shows that there are changes in the protein composition of fruit as they mature and ripen that could provide the basis for developing new harvest maturity indices.

Analysis of proteins present in avocado mesocarp at different stages of ripening suggests that both synthesis and degradation of proteins are involved in the ripening process (Baker et al., 1985). Differences in mRNAs have been detected between maturity stages in apples that were associated with changes in the protein population (Lay-Lee et al., 1990). The protein changes observed between stages I (immature) and II (mature) and were associated with increasing internal ethylene concentrations and declining fruit firmness (Lay-Lee et al., 1990).

Changes in protein profiles during various maturity stages (preclimacteric, early, climacteric and post climacteric) in banana have been reported (Dominguez-Puigjaner et al., 1992). SDS-PAGE analyses revealed that a 23 kD polypeptide was prominent in immature fruit and decreased with ripening. Other polypeptides (28, 30 and 43 kD) increased from the pre-climacteric to the post-climacteric stage. An interesting observation was the accumulation of a 28 kD polypeptide in post-climacteric fruit (Dominguez-Puigjaner et al., 1992). Similar observations have been reported in cherimoya fruit at different maturity stages (Montero et al., 1993). In a pI range from 5.2 to 5.85, a set of polypeptides of about 50 kD was present in unripe fruit, decreased at the early ripening stage, then increased at the maximum ethylene production stage, and finally declined at the post ripening stage (Montero et al., 1993).

Large increases in specific proteins have been found in some fruits. For example, ACO in apple fruit rises from nil in immature fruit to about 5% of the soluble protein in ripe fruit in only a few days (Kuai and Dilley, 1992). If either ACS or ACO could be detected before ethylene production they might be useful as tools to determine harvest maturity. The level of ACO activity increases before ACS in apple fruits which could make ACO a good
candidate for a maturity index (Pekker et al., 1993). Pekker et al. (1993) discovered 8 specific proteins in apples that increased in quantity as the ethylene climacteric developed; some of them were not detectable in preclimacteric fruits. They also found one protein termed PAP3 that has been identified as ACO. There is high homology in amino acid sequence among the ACO-related proteins. It has been proposed that monoclonal antibodies for ACO from apples could be useful for developing a harvest maturity index (Kuai and Dilley, 1992). Pekker et al., (1993) have further suggested that application of an ELISA (enzyme-linked immunoabsorbant assay) to detect the development of the ethylene biosynthetic pathway could be a simple way to determine harvest maturity.

This review has revealed the current knowledge of the changes in proteins and genetic control of ripening in several species of fruit including, tomatoes, apples, pears, avocados, bananas and cherimoyas. There is some information for peaches (Brady, 1987), but little for plums. Since plums are members of the same plant family as apples, pears and peaches, it is reasonable to expect the biochemical changes during ripening will be similar. Thus, if changes in proteins in plums can be correlated with optimum harvest maturity it should be possible to develop an ELISA system similar to that which has been attempted for apples by Dilley et al. (1993b) and Pekker et al. (1993).

2.3 Factors Limiting Cool Storage Life

2.3.1 Cool Storage of Plums and Other Stone Fruit

At present only 4% of the Australian stone fruit production is exported, mostly by air to Asian and Middle East countries (Wade, 1993). Plums account for almost 60% of these exports (Anon, 1996b). However, the stone fruit industry wishes to expand its export trade and this will require sea freight because air freight is expensive and capacity is limited. Storage life of up to 6 weeks will be required to allow the assembly of shipments, shipping time and time for an orderly distribution in the importing countries. At present, most peaches, nectarines and plums have a maximum storage life of about 3 weeks. Improved cultivars, harvested at ideal maturity and improved storage technology are required if the industry is to achieve its goal. To define maximum storage life of stone fruit, they must be harvested at optimum maturity and stored near 0°C. However, the fruit will eventually develop chilling injury (CI) at this temperature (Taylor et al., 1995; Wade, 1993).

One of the recommended ways to extend storage life is to intermittently warm the fruit to about 18°C for one day before CI occurs. Theoretically warming can be repeated at intervals to prevent CI (Anderson, 1979, 1982). However, application of this method commercially is difficult because of, the cost of the energy required to warm the large masses of fruit. Another recommended methodology for the storage of stone fruit is controlled
atmosphere storage which is a standard method for storing product in an atmosphere with reduced oxygen and increased carbon dioxide (Smock, 1979). Plums were stored at 2% O₂ and 12% CO₂ at 1°C, but the storage period did not exceed 4 weeks (Strief, 1989). Off-flavour can occur in fruit held in high levels of carbon dioxide (Olson and Schomer, 1975). Therefore, further work is required before this technology can be used commercially.

2.3.2 Chilling Injury (CI) and Other Changes in Quality

The postharvest life of stone fruit is limited by physiological CI disorders that result in poor quality fruit (Hall et al., 1989). CI in stone fruit occurs at temperatures above freezing point and below 5-15°C depending on the cultivar. The most common symptoms of CI are internal discoloration (browning) of the flesh under the epidermis, pitting, water-soaked areas, failure to ripen, off-flavour development, and an increased incidence of surface moulds and decay (Kader, 1992). Research on CI has concentrated on the activities of enzymes that are primarily responsible for the degradation of pectic substances of the cell wall during ripening, e.g., PG and PME (Ben-Arie and Sonego, 1980; Buescher and Furmanski, 1978; King et al., 1989).

Symptoms of CI observed in plum fruit include internal breakdown (IB) and gel breakdown (GB), which occur in different parts of the flesh (Lill et al., 1989; Taylor et al., 1993a, 1995). The flesh of plum fruit can be divided into the inner and outer mesocarp. SSC, juice pH, internal ion conductivity and the juice viscosity of water-soluble pectin are higher in the inner than the outer mesocarp tissue. Cell membrane permeability, as indicated by high levels of electrolyte leakage, is also higher in the inner tissue. In contrast, titratable acid in the inner mesocarp is lower. These differences suggest that, at harvest, and throughout cool storage, the inner mesocarp of plums is ripper and more sensitive to CI than the outer mesocarp (Taylor et al., 1993b). In plums, higher levels of bound juice in the inner tissue results in less extractable juice than in the outer tissue. Usually, exposure of fruit to chilling temperatures causes a hardening of membrane lipids which increases the permeability of cell membrane. Providing that fruit are exposed to chilling temperatures for only short periods the membranes may recover when the fruit are returned to non-chilling temperatures (Lyons, 1973).

Symptoms of IB develop directly below the epidermis. The flesh becomes watery and colour changes from light to dark brown. Flesh browning is due to the activity of polyphenol oxidase (PPO), an enzyme which oxidises polyphenols to produce tannins (Dodd, 1984; Von Mollendorff et al., 1992a). Both harvest maturity and temperature regime during storage have a significant effect on the incidence of IB in plums with immature plums being more susceptible. The incidence of IB was lower in plum fruit harvested at optimum maturity, if the storage temperature was about -0.5-0°C (Kotze et al., 1988).
Another symptom of CI in stone fruit is GB which develops following the appearance of IB during cool storage. The symptoms consist of a gelatinous breakdown of the flesh between the stone and the inner mesocarp (Taylor et al., 1993b). GB in plums is associated with loss of juiciness (Taylor et al., 1993c), and is similar to woolliness in peaches (Ben-Arie and Lavee, 1971) and mealiness in nectarines (Von-Mollendorff et al., 1992a). In peaches and nectarines this occurs when low-methyloxy pectins with high molecular weights bind water in a gel complex in the middle lamella regions (Ben-Arie and Lavee, 1971; Taylor et al., 1995). Von-Mollendorff et al. (1989) suggested that because of membrane disfunction at low storage temperatures, water and solutes leak through cell membranes into the intercellular spaces where they form gel complexes with high molecular mass pectins released following transfer of fruit to a ripening temperature. As a result, free juice levels decline (Wang, 1982). It is probable that pectic substances also play a similar role in the development of GB in plums. Since, high-methyloxy pectins only bind water in the gel complexes if the pH and sugar levels are favourable (Doesburg, 1965) and gel strength is promoted by higher levels of sugar, ripe plums would be expected to be more susceptible to GB (Taylor et al., 1995). It is apparent that it is necessary to harvest stone fruits within a narrow range of maturities to minimise CI and maximum cool storage life.

Despite improvements in storage life of stone fruit with controlled atmospheres and intermittent warming techniques, storage life is often too short to enable shipping to export markets by sea. However, storage life can be maximised by harvesting the fruit at optimum maturity. This again requires the development of reliable markers of maturity.
SECTION 3

SCOPE OF THIS THESIS

The main purposes of this research was to develop a new harvest maturity index for highly coloured stone fruit that is not affected by seasonal or climatic factors. The most likely candidate was thought to be a marker protein closely correlated with maturation and ripening.

Preliminary experiments were conducted in the 1993/94 season with one nectarine (cv. Sungem) and one Japanese type plum (cv. Gulfruby) to develop techniques to be used in subsequent seasons. Subsequent studies (1994-1997) were confined to Japanese-type plums. Physicochemical changes were measured during fruit development, physiological responses of fruit harvested at defined stages of development were described (Section 4) and optimum cool storage life at 0°C was correlated to these stages of fruit development (Section 5). Procedures for the extraction and separation of total proteins by 2-D gel electrophoresis were perfected and changes were related to fruit maturation (Section 6). The final set of experiments examined in more detail the role of ethylene in ripening of plums by treating fruit with the ethylene antagonist, 1-methylcyclopropene (1-MCP), and propylene, an active analogue of ethylene (Section 7). In Section 8 the genetic basis of the major physiological differences among plum cultivars that were discovered in this research are discussed. Proposals for future research to explore these differences are presented.
SECTION 4

PHYSICOCHEMICAL AND PHYSIOLOGICAL STUDIES

4.1 Introduction

Among the 15 species of plums used for fruit production in the world, the most important fresh market species is the Japanese plum which is noted for good size, attractiveness and storage properties. Plums, including the Japanese plum, are extremely variable in skin and flesh colour (light green, yellow, red or purple), fruit size and ripening behaviour (Couvillon and Krewer, 1993). This variability makes harvest maturity difficult to judge.

Harvest date is the most important factor determining consumer acceptability as quality is reduced by either premature or late harvesting. Harvesting plums at an early stage of maturity may result in a product that has a good appearance and which transports and stores well. However, yield may be sacrificed and the flavour poor. On the other hand, fruit which are harvested late may ripen quickly and perish before they are sold. Therefore, to ensure optimal quality, there is a need for markers or indices that allow the stage of maturity to be determined with precision.

A number of parameters have been used to assist with the judgement of harvest maturity of fruit. These include changes in skin colour, flesh texture, tissue permeability, SSC, aroma volatiles, ethylene and CO₂ production and gene expression (Brady, 1992; Pratt, 1975). Many of these parameters are not reliable because they vary with cultivars, production area, location of fruit on the tree and season (Kader and Mitchell, 1989b). Plum cultivars develop their pigmentation early in growth, and therefore, this attribute may be of little value in determining harvest time. Markers of maturity, developed from an understanding of the physiological and biochemical changes which occur in fruit as they mature, may help overcome the problems associated with other indices of harvest time.

The aims of the experiments described in this section were to compare the biochemical and physiological changes which occurred during maturation and ripening of four cultivars (cvs) of plums, Gulfruby, Beauty, Shiro, and Rubyred, together with a cultivar (cv.) of nectarine called Sungem. Changes in skin colour, flesh firmness, aroma volatiles, SSC, TA, SSC:TA ratio, rates of respiration and ethylene production were examined to determine their relationship with harvest maturity and applicability as markers of this event. To clarify the role of ethylene in the ripening processes, fruit were treated with propylene, an active ethylene analogue known to promote ripening and stimulate the respiratory climacteric (McMurchie et al., 1972).
4.2 Material and Methods

4.2.1 Source of fruit

The cultivars used in this study were hybrid Japanese-type plums which have been derived from crosses with *P. salicina* (Lindl). Fruit were harvested from three matched, ten-year-old trees of the cvs Beauty, Shiro and Rubyred grown on Myrobalan rootstocks at Bilpin (about 25 km from North Richmond), NSW. Plums from the early ripening cv. Gulfruby, grown on low-chill peach rootstocks, and Sungem nectarines, were harvested from the orchards at the Centre for Horticulture and Plant Science, UWS Hawkesbury, Australia.

4.2.2 Fruit growth measurement

The diameter of the fruit midway between the stem and blossom ends, was determined on 20 fruit per tree from each of three trees during 1993/94, 1994/95 and 1995/96 seasons. Fruit weight was measured only in the 1994/95 season. Ten fruit from three replicate trees of approximately the same size were harvested from around the widest part of the tree perimeter at pit hardening and at subsequent intervals of 4-14 days for the plums or according to skin colour development (green, 1/4 red, 1/2 red, 3/4 red and full red) for the nectarine until the fruit reached a tree ripe stage (soft to touch, full colour development, juicy, sweet and aromatic). Fruit were transported on the day of harvest within 30 minutes to the Postharvest Laboratory, Centre for Horticulture and Plant Science for further analysis.

4.2.3 Physicochemical measurements

Skin colour, determined as the hue angle and ‘a’ value’ was measured on Gulfruby plums and Sungem nectarines using a Minolta CR-200 colorimeter (McGuire, 1992). Flesh firmness was determined on paired surfaces from opposite sites of the fruit using an Effegi penetrometer fitted with a 7.5 or 11 mm tip. The units used were newtons (N= kgf X 9.807). Samples of flesh taken from 10 fruit from each of three trees were combined and stored at -20 °C for chemical analyses. Separate samples of fruit from each maturity stage were pitted, sliced and stored at -80°C for protein analysis.

For chemical analysis, frozen samples were thawed and then homogenized in a Waring blender. Samples of the homogenate were centrifuged (6000 g for 6 min) and aliquots of the supernatant used to measure SSC % using a hand held refractometer (Atago, Japan) and TA by titrating 5 mL of juice diluted with 5 mL distilled water to pH 8.1 with 0.1 N NaOH (Robertson et al., 1991). Duplicate titrations were conducted and the results were expressed as mmol H⁺/ mL juice.

4.2.4 Physiological measurements

Plums were harvested at pit hardening and at subsequent intervals of 6 days during 1993/94 and 4-14 days during 1994/95 and 1995/96 seasons. At each harvest, four (1993/94
and 1994/95) or three (1995/96) fruit from each of three trees of each cv. were weighed and enclosed singly in 0.2 L chambers at 20°C. The chambers were ventilated with water-saturated air (2.5-4.1 mL/min). In 1995/96, replicate samples of fruit were similarly ventilated with water-saturated air containing 500 µL/L propylene. Samples of air (1.0 mL) exiting from the chambers were taken to determine rates of CO₂ and ethylene production on the day after harvest and at subsequent 24 h intervals. Ethylene concentrations were measured with a Gowmac Model 580 gas chromatograph. Carbon dioxide concentrations were determined by pulsed infra-red gas analysis (Horiba, Model PIR-2000) following the method of Jobling et al. (1991). Measurements were standardised using calibrated compressed gas mixtures purchased from the British Oxygen Co., Australia. The lowest measurable rate of ethylene production was about 0.01µL/kg.h. Aroma volatiles produced by fruit were assessed organoleptically by ten panellists for three replicates fruit from each maturity stage using the following scale: 0, no aroma; 1, low; 2, medium; and 3, strong aroma.

4.2.5 Internal ethylene concentrations

The open end of a plastic tube (40 x 15 mm) was attached to one cheek of a fruit using acid-free silicone (Dow-Corning 3040). A hole was drilled in the opposite end of the tube which was then sealed with a silicone rubber serum cap. Internal ethylene concentrations in fruit from the cv. Rubyred were estimated during the 1995/96 season by extracting 1 mL gas samples from the attached tubes (Fig. 4.1). Gas samples were taken from fruit attached to the tree at intervals of 2 or 3 days. Fruit with tubes attached were also harvested from 80 to 110 days after pit hardening (DAPH) and enclosed in 0.2 L plastic chambers ventilated with water-saturated air, with or without the addition of 500 µL/L propylene. Ethylene in the tubes and air exiting from the chambers was measured daily.

4.2.6 Statistical analyses

Data concerning skin colour, SSC, TA and SSC:TA ratio were subjected to analyses of variance using COSTAT version 4.02 (Weasel Software, Berkeley, CA, USA). Least significant differences (LSD) were calculated using Duncan’s multiple range test. Correlation coefficients between aroma and ethylene production were calculated according to Parsons (1974). Regression analyses of fruit size, weight and firmness were made also using COSTAT, version 4.02.
Fig. 4.1. Methodology for the measurement of internal ethylene concentrations in the cv. Rubyred. Polyethylene plastic tube was sealed on the surface of fruit with a small amount of acid-free silicone cement. The tube was closed with a silicon serum septum.
3. Results

4.3.1 Preliminary Study

A preliminarily experiment was conducted on Gulfruby plums and Sungem nectarines during the 1993/94 season to establish the patterns of fruit growth, physicochemical characteristics and rates of respiration and ethylene production during maturation and ripening. Since there were similarities in the patterns for Gulfruby plums and Sungem nectarines (Table 4.1 and Fig. 4.2). Subsequent work was concentrated only on highly coloured cultivars of plums.

Skin colour developed from green to red during maturation in Gulfruby plums and Sungem nectarines. This visual change was associated with a decrease in hue angles and an increase in a values (Table 4.1). SSC increased and TA decreased during maturation. Sungem nectarines and Gulfruby plums had maximum SSC values of 16.5 and 13.5, respectively, while the corresponding TA values were 9.5 and 38 mmol H⁺/L juice (Table 4.1).
Table 4.1  
Average skin colour, soluble solids concentration (SSC), titratable acidity (TA) and SSC:TA in Gufruby plums and Sungem nectarines during the 1993/94 season.

<table>
<thead>
<tr>
<th>Maturity Stages (% of skin colour)</th>
<th>Skin colour (a value)</th>
<th>SSC (°)</th>
<th>TA (mmols H⁺/L Juice)</th>
<th>SSC/TA ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Gufruby plums:</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>0</td>
<td>-21.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>122.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.5&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>25</td>
<td>-13.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>111.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>49.3&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>50</td>
<td>-7.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>101.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>48.5&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>75</td>
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<td>78.3&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>42.2&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>100</td>
<td>20.7&lt;sup&gt;e&lt;/sup&gt;</td>
<td>46.3&lt;sup&gt;e&lt;/sup&gt;</td>
<td>13.5&lt;sup&gt;d&lt;/sup&gt;</td>
<td>38.0&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>B. Sungem nectarines:</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>0</td>
<td>-20.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>120.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.0&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>25</td>
<td>-16.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>110.6&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>50</td>
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<td>75</td>
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<td>80.0&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>20.2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>39.5&lt;sup&gt;e&lt;/sup&gt;</td>
<td>16.5&lt;sup&gt;d&lt;/sup&gt;</td>
<td>9.3&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The value for all parameters represent the means of 10 fruit taken from three trees (total 30 fruit). Fruits of plums and nectarines were harvested at different maturity stages according to skin colour (0%, green; 25%, 1/4 red; 50%, 1/2 red; 75%, 3/4 red and 100% full red colour). Data for each parameter within each variety were compared separately using an ANOVA and within each column data followed by the same letters were not significantly different at P≤ 0.05 according to Duncan’s multiple range test.
Fig. 4.2 Mean fruit diameter (A & B) and firmness (C & D) of Gulfruby plums and Sungem nectarines during the 1993/94 season. The data represent the means of 20 fruit for diameter and 10 fruit for firmness from each of three trees (i.e., n=60 and 30 respectively). The error bars represent the SEs of the means.
4.3.2 Fruit Development

Fruit development of four cvs of plums, Gulfruby, Beauty, Shiro and Rubyred was measured in the 1993/4, 1994/5 and 1995/6 seasons. In these fruit pit hardening occurred about 60, 65, 75 and 95 days after full bloom (DAFB), respectively, during the 1993/94, 1994/95 seasons and one week earlier in 1995/96. Fruit diameter and weight of all fruit increased after pit hardening which was chosen as the beginning of the experiment (0-time). In the 1994/95 season, a significant, positive correlation $R^2 = 0.86$ to 0.96; $p \leq 0.05$ was found between fruit diameter and weight (Fig. 4.3A and Appendices 4.1 & 4.2). The highest average values of 42.3, 45.3, 45.0 and 61.0 mm for fruit diameter and 57.0, 59.8, 58.6 and 134.6 g for fruit weight, recorded at harvest during 1994/95, were found 31, 49, 49 and 95 DAPH respectively (Fig. 4.3A and Appendices 4.1 & 4.2). During 1995/96, maximum values of 43.0, 52.0, 51.0 and 52.5 mm for fruit diameter were recorded 32, 49, 49 and 95 DAPH in Gulfruby, Beauty, Shiro and Rubyred, respectively, (Fig. 4.3B and Appendix 4.4). Fruit growth ceased before the development of full colour in Shiro and Rubyred, but in Gulfruby and Beauty fruit growth continued after full colour had developed. Fruit firmness decreased from pit-hardening until the fruit ripened during 1994/95 and 1995/96 seasons (Fig. 4.3 C & 4.3 D and Appendices 4.3 & 4.5).

4.3.3 Physicochemical changes

First colour appeared 14 (red), 28 (red), 28-30 (yellow), and 56 (deep red) DAPH and full colour was observed 31-32, 49, 49 and 95 DAPH in Gulfruby, Beauty, Shiro and Rubyred plums during 1994/95 and 1995/96, respectively. As Shiro is a greengage the colour change during ripening was from green to yellow as chlorophyll was lost: in the other cvs the colour change was from green to red due to the accumulation of anthocyanins. Fruit firmness in all cvs decreased after pit hardening and softening continued until the fruit reached full colour (Figs 4.3 C & D). The lowest values for this parameter 5.8, 7.8, 5.6, and 15 N, 1994/95 season; 8.5, 8.8, 9.8, and 19.6 N, 1995/96 season were recorded at 31-32, 49, 49, and 105 DAPH for the cvs Gulfruby, Beauty, Shiro and Rubyred, respectively (Figs 4.3 C & D). SSC increased from pit hardening until the development of full colour in all cvs, whilst TA declined during maturation in both the 1994/95 and 1995/96 seasons (Tables 4.2 & 4.3). Maximum levels of SSC were recorded after full colour developed in all cvs. The SSC:TA ratio increased during ripening in all cvs (Tables 4.2 & 4.3).
Fig. 4.3. Mean fruit diameter (fd) (A & B), weight (fw) (A) and firmness (C & D) of four cultivars of plums measured at weekly intervals during the 1994/95 and 1995/96 seasons. The data represent the means of 20 fruit for diameter and 10 fruit for firmness from three different trees (i.e., n= 60 and 30 respectively). Regression analysis showed that the data could be described by a 2nd order polynomial equations (Appendices 4.1-4.5).
Table 4.2
Average skin colour, soluble solids concentration, titratable acidity and SSC:TA in Gulfruby, Beauty, Shiro and Rubyred plums during the 1994/95 season.

<table>
<thead>
<tr>
<th>Cultivar and Maturity Stage</th>
<th>Skin colour (hue angle)</th>
<th>SSC (%)</th>
<th>TA (mmol H⁺ / L Juice)</th>
<th>SSC/TA Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Gulfruby:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pit-hardening</td>
<td>126.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.15&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>First colour</td>
<td>114.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.18&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Full colour</td>
<td>48.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>39.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.33&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>B. Beauty:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pit-hardening</td>
<td>126.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.13&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>First colour</td>
<td>107.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>41.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.23&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Full colour</td>
<td>45.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>30.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.44&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>C. Shiro:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pit-hardening</td>
<td>126.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.11&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>First colour</td>
<td>118.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>39.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.20&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Full colour</td>
<td>103.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>26.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.42&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>D. Rubyred:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pit-hardening</td>
<td>129.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.18&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>First colour</td>
<td>109.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>39.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.24&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Full colour</td>
<td>46.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>29.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.46&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The values for all parameters are the means of 10 fruit taken from three trees (total 30 fruit). Data in each column for each cultivar followed by the same letter are not significantly different (P≤ 0.05) according to Duncan’s multiple range test.
Table 4.3  
Average skin colour, soluble solids concentration, titratable acidity and SSC:TA in Gulfruby, Beauty, Shiro and Rubyred plums during 1995/96 season.

<table>
<thead>
<tr>
<th>Cultivar and Maturity Stage</th>
<th>Skin colour (hue angle)</th>
<th>SSC (%)</th>
<th>TA (mmol H⁺ / L Juice)</th>
<th>SSC/TA ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Gulfruby:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pit-hardening</td>
<td>126.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>49.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.16&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>First colour</td>
<td>115.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.17&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Full colour</td>
<td>51.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.35&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>B. Beauty:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pit-hardening</td>
<td>125.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.17&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>First colour</td>
<td>108.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.23&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Full colour</td>
<td>45.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>29.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.45&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>C. Shiro:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pit-hardening</td>
<td>126.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>45.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.14&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>First colour</td>
<td>112.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>41.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.19&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Full colour</td>
<td>98.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.50&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>D. Rubyred:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pit-hardening</td>
<td>131.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.18&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>First colour</td>
<td>110.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.23&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Full colour</td>
<td>50.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>32.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.40&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The values for all parameters are the means of 10 fruit taken from three trees (total 30 fruit). Data in each column for each cultivar followed by the same letter are not significantly different (P≤ 0.05) according to Duncan’s multiple range test.
4.3.4 Physiological Changes

Rates of ethylene and CO₂ production were measured daily for 4-14 days after harvest. In Gulfruby plums, ethylene production could just be detected one day after harvest in fruit taken at the 25% red colour stage in 1993/94, 26 DAPH in 1994/95 and 21 DAPH in 1995/96 (Figs 4.4A, 4.5A & B). After 6 days at 20°C, ethylene was detected in fruit harvested 14 DAPH in the last seasons. This increase in ethylene production coincided with the appearance of first colour. The highest rates of ethylene production (80, 120 and 85 μL/kg.h) after 6 days storage at 20°C, occurred at 100% red colour in 1993/94, 26 DAPH in 1994/95 and 32 DAPH in 1995/96, respectively (Figs 4.4A and 4.5A & B). Carbon dioxide production, measured one day after harvest, remained constant at about 27-30 mL/kg.h for fruit harvested at the 75 to 100% red stages in 1993/94 (Fig. 4.4A), 18 mL/kg.h for fruit harvested 7, 14 and 21 DAPH in 1994/95 (Fig. 4.5C) and 7-9.5 mL/kg.h in fruit harvested at 14, 21 and 25 DAPH in 1995/96 (Fig. 4.5D). During storage respiration rates rose to levels in excess of 30.5 mL/kg.h in fruit harvested at the 75% red stage in 1993/94 and at 14 DAPH or later in 1994/95 and to 21.5 mL/kg.h in fruit from all harvest dates during 1995/96. With the exception of the harvest at pit hardening in 1994/95, all increases in CO₂ production coincided with increases in ethylene production.

Fruit from Beauty showed patterns of ethylene and CO₂ production similar to Gulfruby in both years (Fig. 4.6). The main difference between these cvs was the period after pit hardening at which ethylene was first detected or when CO₂ production increased. These events occurred approximately 20 days later in Beauty than in Gulfruby (Fig. 4.6). The highest rate of ethylene production (68.0 μL/kg.h) was recorded after 6 days storage and occurred 42 DAPH in 1994/95: in 1995/96 the maximum was 78 μL/kg.h at 49 DAPH (Figs 4.6 A & B). In Beauty, after 6 days at 20°C, ethylene was detected in fruit harvested 35 DAPH in 1994/95 and 28 DAPH in 1995/96 season coincident with first colour development.
Fig. 4.4. Ethylene and CO₂ production from Gulfruby plums (A) and Sungem nectarines (B) during the 1993/94 season. The data represent the means of five fruit from three different trees (n=15). The error bars represent the SEs of the means.
Fig. 4.5. Rates of ethylene (A & B) and CO₂ (C & D) evolution from Gulfruby plums harvested at different times after pit-hardening during the 1994/95 and 1995/96 seasons. The data represent the means of four fruit from three trees (i.e., n=12) during the 1994/95 season and three fruit from three trees (i.e., n=9) during the 1995/96 season. The rates of gas production were measured 1 and 6 days after harvest. The error bars represent the SEs of the relevant means.
Fig. 4.6. Rates of ethylene (A & B) and CO₂ (C & D) evolution from Beauty plums harvested at different times after pit-hardening during the 1994/95 and 1995/96 seasons. The data represent the means of four fruit from three trees (i.e., n=12) during the 1994/95 season and three fruit from three trees (i.e., n=9) during the 1995/96 season. The rates of gas production were measured 1 and 6 days after harvest. The error bars represent the SEs of the relevant means.
For both seasons, patterns of ethylene and CO₂ production were strikingly different in Shiro and Rubyred from the other two cultivars (Figs 4.5 to 4.8). In Shiro and Rubyred, ethylene was not produced until late in the fruit development period, and essentially, was only produced when full colour and size were reached. In comparison, in the cvs Gulfruby and Beauty, ethylene was readily detected in stored fruit following the development of first colour. Furthermore, the rates of ethylene production in Shiro and Rubyred were low.

In Shiro, a trace of ethylene production was detected one day after harvest in fruit taken 42 DAPH in 1994/95 and 49 DAPH in 1995/96 (Figs 4.7A & B). The highest rate of ethylene production (28.5 μL/kg.h) was recorded after 6 days storage at 20°C and occurred 42 DAPH in 1994/95 and the maximum was only 4.5 μL/kg.h at 49 DAPH in 1995/96 (Figs 4.7 A & B). During storage at 20°C, respiration rates increased to 24.3 mL/kg.h in fruit harvested 49 DAPH in 1994/95 and to 17.0 mL/kg.h in 1995/96 (Figs 4.7 C & D).

In Rubyred, ethylene production was only detected one day after harvest after full colour had developed. After 6 days storage the highest rate of ethylene production recorded was 13 μL/kg.h in fruit harvested 105 DAPH during the 1994/95 season and only 0.1 μL/kg.h from fruit harvested 110 DAPH in the 1995/96 season(Figs 4.8A & B). These values of ethylene production contrast to the levels of 85 and 78 μL/kg.h for Gulfruby and Beauty. The highest rate of CO₂ production was 27.5 mL/kg.h one day after harvest at pit hardening in 1994/95 and about 9 mL/kg.h in 1995/96 seasons (Figs 4.8 C & D).

Changes in CO₂ production were not clearly associated with changes in ethylene production in both cvs and in general, the average rates of CO₂ evolution in Shiro and Rubyred were half of those produced by the other cvs.
Fig 4.7. Rates of ethylene (A & B) and CO₂ (C & D) evolution from Shiro plums harvested at different times after pit-hardening during the 1994/95 and 1995/96 seasons. The data represent the means of four fruit from three trees (i.e., n=12) during the 1994/95 season and three fruit from three trees (i.e., n=9) during the 1995/96 season. The rates of gas production were measured 1 and 6 days after harvest. The error bars represent the SEs of the relevant means.
Fig. 4.8. Rates of ethylene (A & B) and CO₂ production (C & D) in Rubyred plums during 1994/95 and 1995/96 seasons. The data represent the means of 4 fruit for 1994/95 and 3 fruit for 1995/96 from 3 different trees (i.e., n = 12 & 9). The error bars represent the SEs of the relevant means.
The production of aroma volatiles was assessed organoleptically in all cultivars. Volatile production by fruit of Beauty and Shiro occurred at the same time as the rise of ethylene production (Table 4.4 and Figs 4.6 & 4.7). In the other cvs, aroma production was detected late in fruit development and no correlation with ethylene synthesis was observed.

**Table 4.4**

**Aroma production by Beauty and Shiro fruit harvested during the 1994/95 and 1995/96 seasons.**

<table>
<thead>
<tr>
<th>Cultivar and Maturity Stage</th>
<th>Aroma Intensity Score</th>
<th>Aroma Intensity Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1994/95</td>
<td>1995/96</td>
</tr>
<tr>
<td><strong>Beauty:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28 (DAPH)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>35</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>42</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>49</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Correlation equation:</td>
<td>Y = -10.6 X + 31.0</td>
<td>Y = -6.4 X + 22.9</td>
</tr>
<tr>
<td></td>
<td>R = 0.90 (P=0.002)</td>
<td>R = 0.92 (P=0.003)</td>
</tr>
<tr>
<td><strong>Shiro:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28 (DAPH)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>35</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>40</td>
<td>No sample</td>
<td>0.25</td>
</tr>
<tr>
<td>42</td>
<td>2.5</td>
<td>No sample</td>
</tr>
<tr>
<td>45</td>
<td>No sample</td>
<td>0.50</td>
</tr>
<tr>
<td>49</td>
<td>3</td>
<td>2.50</td>
</tr>
<tr>
<td>Correlation equation:</td>
<td>Y = -0.5 X + 5.5</td>
<td>Y = -0.32 X + 1.8</td>
</tr>
<tr>
<td></td>
<td>R = 0.98 (P=0.006)</td>
<td>R = 0.97 (P=0.07)</td>
</tr>
</tbody>
</table>

Data are the means of four fruit from three trees (i.e., n=12) during the 1994/95 season and three fruit from three trees (i.e., n=9) during the 1995/96 season. Aroma produced by fruit were assessed by ten panellist using the following scale: 0, no aroma; 1, low; 2, medium; and 3, strong aroma. The equation represents the correlation between aroma and ethylene production.
4.3.5 Propylene treatment

Propylene (500 μL/L) was applied continuously to the fruit from Gulfruby, Beauty, Shiro and Rubyred until full colour was reached. Only trace amounts of ethylene were detected in control fruit of Gulfruby for the first 72 h. After 96 h storage at 20°C, significant quantities of ethylene were produced by fruit from the 21-day harvest and after one further day in storage ethylene production from fruit harvested 14 DAPH also increased (Fig. 4.9A). Propylene induced ethylene production within 48 h of harvest by samples of fruit from both harvest dates. Respiration was stimulated to twice the rate of similar control fruit before any increase in ethylene was detected (Fig. 4.9C).

The response to propylene of fruit from Beauty (Fig. 4.9A & C) was similar to that of Gulfruby (Fig 4.9B & D). Propylene induced ethylene production in this cv. within 48 h of harvest in fruit from 28 and 35 DAPH. After 120 h storage, significant quantities of ethylene were produced by fruit from the 35 DAPH harvest (Fig. 4.9B). Respiration was stimulated to twice the rate of similar control fruit harvested 28 DAPH and coincided with increased ethylene, but in fruit harvested at 35 DAPH the response to propylene was small (Fig. 4.9D).
Fig. 4.9. Rates of ethylene (A & B) and CO₂ (C & D) evolution from Gulfruby and Beauty plums harvested at different times after harvest during the 1995/96 season. The data represent the means of three fruit from three trees (i.e., n=9). The fruit were ventilated with water saturated air, with or without the continuous application of propylene (500 μL/L). The rates of gas production were measured one days after harvest and at subsequent 24 hour intervals. The error bars represent the SEs of the relevant means.
The results for Shiro and Rubyred (Figs 4.10) were different from Gulfruby and Beauty (Fig. 4.9). In control fruit of Shiro, no ethylene could be detected for 96 h following harvest from fruit taken at all sampling times. The exception to this were fruit from the 45 DAPH harvest where trace levels of ethylene could be detected (Fig. 4.10A). In propylene-treated fruit, the time between harvest and the detection of significant levels of ethylene production was related to harvest date, with fruit from the later harvests producing measurable amounts of ethylene sooner (Fig. 4.10A). Propylene stimulated CO₂ production of fruit harvested 35, 40 and 45 DAPH within 96 h after picking with rates rising to approximately twice those of the control fruit (Fig. 4.10C). Fruit from the later harvests also showed a trend for higher levels of CO₂ production.

The response to propylene of fruit from Rubyred was similar to that of Shiro. In control, air-treated fruit of the cv. Rubyred, no ethylene could be detected for 120 h following harvest from fruit taken at all sampling times. In propylene-treated fruit the time between harvest and significant levels of ethylene production was related to harvest date: the later harvest date the shorter the period postharvest before ethylene was produced (Fig. 4.10B). The magnitude of ethylene production was also related to harvest time. Propylene stimulated CO₂ production within 24 h of picking in fruit from all harvest dates (Fig. 4.10D). Average CO₂ production rates were doubled within 24 h of the commencement of the propylene treatment. The magnitude of the stimulation of CO₂ production also increased with time postharvest.
Fig. 4.10. Rates of ethylene (A & B) and CO₂ (C & D) evolution from Shiro and Rubyred harvested at different times after harvest during the 1995/96 season. The data represent the means of three fruit from three trees (i.e., n=9). The fruit were ventilated with water-saturated air, with or without propylene (500 µL/L). The rates of gas production were measured one day after harvest and at subsequent 24 hour intervals. The error bars represent the SEs of the relevant means.
4.3.6 Evaluation of internal ethylene concentrations

To determine if harvesting stimulated ethylene production, internal ethylene concentrations were measured in three separate samples of Rubyred fruit during 1995/96. One sample remained on the tree whilst the two other samples were harvested. The harvested fruit were ventilated with water-saturated air and one sample was continuously treated with propylene. No ethylene could be detected in fruit which remained attached to the tree at any sampling time. At 110 DAPH, these fruit were ripe, soft, aromatic and sweet and after this period unharvested fruit fell from the trees.

Ethylene production from harvested fruit is presented in Fig. 4.11. Ethylene production from control fruit not treated with propylene correlated with harvest date. Ethylene was detected 24 h after picking from fruit from the last two harvests and, in these fruit, production increased with the number of days postharvest (Fig. 4.11). Fruit from the earlier harvest dates required a longer storage period before ethylene was produced and this gas could not be detected from samples taken 90 DAPH or before. A similar pattern of ethylene production occurred in propylene-treated fruit, although the magnitude of production was clearly increased. For example, a comparison of data from the 100 DAPH harvest suggests a 300-fold ethylene increase in the propylene-treated fruit compared with the appropriate control.
Fig. 4.11. Concentration of internal ethylene in Rubyred plums sampled at different times after harvest during the 1995/96 season. The fruit were ventilated with water-saturated airs with (B) or without (A) propylene (500 µL/L). The data represent the means of three fruit from three trees (i.e., n=9). The rates of gas production by detached fruit was measured one day after harvest and at subsequent 24 hour intervals. The error bars represent the SEs of the relevant means.
4.3 Discussion

The results of the preliminary study with Gulfruby plums and Sungem nectarines showed that there were no significant differences in fruit growth and development between these species (Figs 4.2 and 4.3). Therefore, the study concentrated on the ripening behaviour of plum cultivars, especially because of the difficulty in assessing harvest maturity in highly coloured varieties.

In an attempt to overcome the problems associated with maturity assessment, the physicochemical, biochemical and physiological changes that occurred during ripening were examined to determine their suitability as indices of harvest maturity. Fruit size and firmness have been proposed as indices of fruit maturity (Crisosto, 1994). Plums continue to grow after pit hardening until they are ripe, a process which involves both cell division and cell enlargement (Coombe, 1976). However, fruit size alone cannot be used as a maturity index in plums, because it may vary with cultivar, fruit load, climatic conditions and cultural practices. Fruit growth in the varieties used in this study ceased when fruit were ready for harvest. However, the point where a particular fruit or batch of fruit has ceased to grow can only be judged retrospectively and, therefore, this time point cannot be used as an indicator of harvest time. Fruit firmness in the four cvs studied decreased gradually from pit hardening and no distinct changes in softening associated with ripening could be detected. Overall, it seems that the decrease in firmness may simply reflect cell enlargement during fruit growth (Kader and Mitchell, 1989b), which also makes this attribute unsuitable as a marker of maturity.

SSC and TA have been suggested as maturity indicators for peaches (Crisosto, 1994) and apples (Dirinck and Schamp, 1989). Although there were large increases in SSC and reductions in TA during maturation in the cvs used in this study, these attributes and their ratio are not reliable indices of harvest maturity. The values at harvest vary with fruit development period with early maturity cultivars, such as Gulfruby and Beauty, having high SSC and TA values compared to mid- and late-season cvs, such as Shiro and Rubyred. Appropriate values associated with optimum harvest date would have to be determined for each cultivar. Levels of SSC and TA have also been shown to vary with season, fruit position on the tree, variety and climatic conditions (Dann and Jerie, 1988; Kader and Mitchell, 1989b). Although SSC and TA are good indices of fruit quality in plums, like size and firmness they are again unsuitable as markers of harvest maturity (Gur, 1986).

Aroma production in Beauty and Shiro fruit was associated with ethylene evolution and the intensity of aroma production increased with increasing emissions of this hormone. In contrast, aroma production from Gulfruby and Rubyred gradually increased as the fruit ripened and was ethylene-independent. The production of aroma volatiles is one of a number biochemical changes which occur in fruit as they ripen. In many fruit these changes are co-
ordinated by the production of ethylene during the climacteric (Spiers and Brady, 1991). In plums the co-ordinational role of ethylene appears to be variable among cultivars. Aroma production was correlated with ethylene in Beauty and Shiro but not in Gulfruby and Rubyred. Skin colour was ethylene-dependent in all four cvs, while aroma was dependent in Beauty and Shiro and independent in Gulfruby by and Rubyred. The enzyme systems associated with these physiological changes appear to be controlled by different mechanisms and the role of ethylene (if any) in their regulation provides a fascinating area for further study.

Since plums are classified as climacteric fruit, ethylene production was assayed 1 and 4 or 6 days after harvest at each successive sampling time. Differences in ethylene production between assay times revealed a strong suppression of the climacteric whilst the fruit were on the tree. This phenomenon termed the 'tree effect' has been observed in a number of other fruit such as apple (Sfakiotakis and Dilley, 1973a; Blanpied, 1993) and avocado (Biale and Young, 1971). In this study the 'tree factor' was most clearly seen in all cvs except Gulfruby. For example fruit from the cv. Beauty harvested 28 DAPH produced ethylene within 6 days of harvest whereas the fruit which remained on the tree did not produce ethylene for further 14 days (Fig. 4.12). Fruit from the other cvs showed this effect, but to different extents. This study is the first to show the magnitude of the tree effect in plums, although the phenomenon has previously been reported in cultivars such as Late Santa Rosa, Queen Ann and Cassleman (Gonzalez et al., 1980). The many reports concerning the tree effect suggest that it is common to climacteric fruit, although the mechanism causing this effect is still unknown. Lau et al. (1986) reported that a tree factor not only delayed the accumulation of ACC in attached Golden Delicious apples, but also inhibited the conversion of ACC to ethylene indicating that the factor interferes with ethylene production. A number of studies (Brecht and Kader, 1984a; Sfakiotakis and Dilley, 1973a), including this one, have shown that the tree effect can, to some extent, be overcome by applications of propylene or ethylene, also suggesting that the tree effect is mediated through the action of ethylene.
Fig. 4.12. Ethylene production by the cv. Beauty during the 1994/95 season showing the 'tree effect'. Data are the means of four fruit from three trees (i.e., n=12). The fruit were harvested after first colour development at 28 DAPH and at subsequent six day intervals. Rates of ethylene production were measured after 24h (A) and then daily for six days (B, C, & D). Rates of production in panel A are assumed to approximate rates produced by fruit on the tree. Rates in panels B, C & D are postharvest production rates. The error bars represent the SEs of the relevant means.
In this study two distinct types of ripening behaviour were observed. Both Gulfruby and Beauty showed ripening patterns typical of climacteric fruit with a distinct rise in CO₂ and ethylene production. In contrast, Shiro and Rubyred exhibited a suppressed-climacteric phenotype. In this phenotype, ethylene production rates increased during the latter stages of the ripening process, but were low when compared to the climacteric cultivars and ripening attributes appeared not to be correlated with ethylene production. However, this suppressed-climacteric behaviour appears not to affect the fruit development period as both Shiro and Beauty ripened 109 and 119 days after full bloom, respectively (Figs 4.7 and 4.8). Further work on these suppressed-climacteric types is discussed in Section 5.

As the role of ethylene in the ripening of these suppressed-climacteric types is very different from that in normal climacteric fruit, the question was raised as to whether these cultivars should be classed as non-climacteric. In order to clarify the role of ethylene in initiating the ripening of these suppressed-climacteric types, internal ethylene concentrations were measured. In fruit of the cv. Rubyred it was not possible to detect ethylene at any sampling time in the internal tissues of fruit attached to the tree. In detached fruit maintained in air, internal ethylene concentrations reached a maximum of 0.12 μL/L in fruit harvested 110 DAPH after 5 days at 20°C. Since the measurement of internal ethylene concentrations in fruit is difficult, estimates of this parameter were also determined using the relationship between production rates and internal concentrations proposed in plums by Burg and Burg (1962). In Rubyred the maximum production rate was 0.1 μL/kg.h which suggests an internal ethylene concentration of 0.4 μL/L. It is generally considered that the threshold ethylene concentration for biological activity is between 0.06 and 0.1 μL/L (Abeles et al., 1992). The internal ethylene concentrations in Rubyred are close to this threshold value and the suppressed-climacteric phenotype can be explained by the failure of the fruit to produce sufficient ethylene to co-ordinate ripening. Some cvs of pears (Downs et al., 1991) and apples (Sfakiotakis and Dilley, 1973b) also exhibit this suppression and this behaviour was again ascribed to an inability of the fruit to produce enough ethylene to develop a climacteric. To examine further the role of ethylene in the ripening behaviour of Rubyred, fruit were treated with the ethylene analogue, propylene. Fruit from all harvest dates responded to propylene treatment within 24 h by doubling their CO₂ production rates. The fruit also reacted by producing a marked increase in their internal ethylene concentrations. This typical response of Rubyred to propylene demonstrated that this cv. clearly belongs to the climacteric class.

A procedure using induced ethylene climacterecs (IEC) has been developed as an aid to the judgement of harvest maturity in apples (Dilley and Dilley, 1985). In this procedure a relationship between the time in hours for detached apples to show an increased ethylene production and the time in days for comparative attached fruit to increase in ethylene production is used to estimate harvest time. This technique may be applicable to Gulfruby and
Beauty where large increases in ethylene production were measured. Clearly, the use of IEC would not be applicable to suppressed-climacteric cvs such as Shiro and Rubyred, as was also found for certain varieties of apples (Jobling and McGlasson, 1995).

This section has shown that none of the physicochemical and physiological parameters commonly used by plum growers to judge harvest maturity are reliable and can readily be adapted to all cultivars. There is still a need to develop a system which is applicable to all varieties of plums for detecting or determining optimal harvest maturity. Changes in protein profiles during maturation and ripening have been proposed as a more reliable system for the determination of maturity in apples (Dilley et al., 1993b). Section 6 explores the applicability of protein changes for maturity assessment in plums.

4.4 Summary of Results
1) The patterns of fruit growth and physiological behaviour of Gulfruby plums and Sungem nectarines were similar. Therefore, further studies concentrated on highly coloured cultivars of plum only.

2) Pit-hardening occurred with one week difference between seasons.

3) Since there was a positive correlation between fruit diameter and weight during the 1994/95 season for four cvs of plums, only fruit diameter was used as an index of fruit growth during subsequent studies.

4) The development of first colour coincided with ethylene production in the cvs of Gulfruby and Beauty, but not in Shiro and Rubyred.

5) Fruit firmness decreased after pit-hardening in all cvs and softening continued until the fruit reached full colour. There was no sharp reduction in firmness which could be used as an index of maturity.

6) SSC increased and TA decreased during maturation and ripening in all four cvs. Again, there was no clear change in these parameters which could be used as a maturity index.

7) The patterns of ethylene and CO₂ production by Beauty were similar to Gulfruby in both seasons. Fruit of these two cvs exhibited typical climacteric patterns of production of these gases.

8) The patterns of ethylene and CO₂ production were different in Shiro and Rubyred from the other two cultivars. In these cvs, ethylene was not detected until late in fruit development, and
was only produced after full colour and size were reached. Furthermore, the rates of ethylene production in Shiro and Rubyred were also low in comparison with Gulfruby and Beauty.

9) Aroma production in Beauty and Shiro fruit was associated with ethylene production, and the intensity of aroma increased with increase of this hormone. In the other cvs, these correlations were not found.

10) The response to propylene of fruit from Gulfruby and Beauty was similar. Propylene induced ethylene production within 48 of harvest in these cvs. In contrast, for Shiro and Rubyred fruit, propylene stimulated the levels of ethylene and CO₂ production. The magnitude of ethylene production rates was also related to harvest time and much lower than Gulfruby and Beauty.

11) To determine the role of ethylene on ripening in plums, internal ethylene concentrations were measured in fruit of Rubyred. No ethylene could be detected in fruit which remained attached to the tree at any harvest time, but in detached fruit a small amount of ethylene was detected. In propylene treated detached fruit, the magnitude of ethylene production was a 300-fold that of control fruit.

12) Although plums are classified as climacteric fruit, two distinct types of ripening behaviour were observed. Both Gulfruby and Beauty showed a typical climacteric pattern. In contrast, Shiro and Rubyred exhibited a suppressed-climacteric phenotype where the fruit failed to produce sufficient ethylene to induce a climacteric.

13) In this study the effects of a ‘tree factor’ were observed.
SECTION 5

RELATION BETWEEN COOL STORAGE LIFE AND MATURITY

5.1 Introduction

Plums account for almost 60% of Australian’s stone fruit exports (Anon, 1996b). Because of their short storage life the fruit must be shipped by air. If sent by sea, the shipping time should not exceed 3 weeks. Expansion of the export trade will require sea transport of fruits able to be stored for up to 6 weeks to provide sufficient time for assembling consignments, sea transport and for orderly distribution in the importing countries (Wade, 1993).

The longest storage life is achieved by storage plums at 0°C (they freeze at -0.8°C) (Mitchell et al., 1974). However, the fruit at temperatures below 10°C suffer chilling injury. There are two forms of chilling injury, internal browning (IB) and gel breakdown (GB). These develop slowly in cool storage, and become most evident after the fruit are returned to a ripening temperature. Affected fruit are unable to ripen normally (Hall et al., 1989; Taylor et al., 1993a). IB is the first symptom of chilling injury that appears as a dark brown area under the epidermis, while GB develops as a gel-like texture around the stone later in storage (Lill et al., 1989). Chilling injury can be minimized by harvesting fruit within a narrow, optimum maturity range (Taylor et al., 1995).

Indices currently used to determine harvest maturity have been described previously (Section 2.2.1). However, no single index is satisfactory and judgement is usually based on several subjective indices.

The main aim of this section was to define the relationships between cool storage life and harvest maturity of four cvs for plums: Radiant (syn. Radiance), Gulfruby, Shiro and Rubyred. This information will provide base line data for later studies of protein changes in maturing fruit that will help towards developing a novel commercial maturity test (Section 6).

5.2 Materials and Methods

5.2.1 Source of fruit

The four cultivars of plums used in this study were Radiant, Gulfruby, Shiro and Rubyred. The fruit were harvested after first colour development. Fruit of Radiance were harvested in March 1994 from a commercial orchard at Young, New South Wales. Commencing in November 1995, fruit of Gulfruby were picked from trees at the Centre for Horticulture and Plant Science, UWS Hawkesbury. Fruit of Shiro and Rubyred were obtained
from a commercial orchard at Bilpin, NSW, with harvests starting from December 1995 to March 1996 respectively. Fruit from the 4 cvs were considered to be commercially mature at the mid (Radiant), 29 (Gulfruby), 45 (Shiro) and 85 DAPH (Rubyred) harvests. For all cvs samples of fruit were taken from each of 3 trees and treated separately. All fruit were hand picked and transported on the day of harvest to the Postharvest Laboratory at the UWS Hawkesbury. On arrival they were dipped in a 1 g/L solution of Rovral (Merck, Germany) then air dried. Radiant plums were graded into 3 maturity stages based on visual skin colour: early (green with a trace of red); mid (half of the skin red, the remainder green); and late (bluish-red over the whole of the fruit). Fruit of cv. Gulfruby were harvested at 25, 29 and 32 DAPH, and those of cvs Shiro at 35, 40 and 45 and Rubyred at 85 and 95 DAPH. The fruit were placed in trays lined with low density polyethylene film which was folded over them, and stored at 0°C for up to 10 weeks. Batches of 20 fruit from each sample of fruit were then removed from cool storage at intervals of 2 weeks and allowed to ripen at 20°C for either 5 (Gulfruby and Shiro) or 7 days (Radiance and Rubyred).

5.2.2 Skin colour and flesh firmness assay

After harvest and subsequent storage, 10 fruit taken from 3 trees (i.e., 30 in total) after ripening at 20°C, the skin colour and flesh firmness were measured (Section 4.2.3).

5.2.3 Assessment of internal and gel breakdown

After ripening at 20°C, the batches of 20 fruit from each sample (i.e. 60 of each cultivar) were cut in half along the equatorial axis and examined for symptoms of IB and GB according to Taylor et al. (1993a) and the percentage showing chilling injury determined.

5.2.4 Extractable juice content

The amount of extractable juice in the flesh from cv. Radiance was determined using 10 fruit from each sample (30 fruit in total) (Lill and Van Der Mespel, 1988). Segments of tissue (about 1.5 g) were taken from each fruit, the skin was removed and the flesh homogenised by forcing it through a 5 mL disposable syringe with no needle. The homogenate was collected in an Eppendorf centrifuge tube, weighed and centrifuged (10000 x g for 5 min). The supernatant juice was collected into a fresh Eppendorf tube and weighed. The weight of supernatant was recorded and expressed as a percentage of the weight of the sample. This value was considered to be the apparent juice content.

5.2.5 Chemical measurements

At each harvest time and following storage, fruit were assessed for SSC and TA. Measurements of these parameters were made on 10 fruit taken from 3 trees (i.e. 30 in total). Samples of juice were obtained by homogenising fruit tissue in a Waring blender followed by
centrifugation at 3700 x g for 5 min. The supernatant, was used for the determination of SSC (%), and TA (mmol H⁺/ L juice) (Section 4.2.3).

5.2.6 Statistical analyses

Data were subjected to completely randomized 2-way factorial analyses of variance using COSTAT version 4.02 (Weasel Software, Berkely, CA, USA, 1990). The graphs were drawn using Microsoft Excel version 5.0.

5.3 Results

5.3.1 Physicochemical measurement at harvest

The development of skin colour, SSC and the SSC:TA ratio increased over successive harvests, whilst firmness and TA decreased in all cultivars studied (Figs 5.1-4, Table 5.1).
Table 5.1
Effects of harvest maturity and cool storage at 0°C on skin colour (i.e. hue angle) of Radiant, Gulfruby and Shiro plums after ripening at 20°C.

<table>
<thead>
<tr>
<th>Harvest Maturity</th>
<th>Storage Time (weeks)</th>
<th>Average by harvest</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Early</td>
<td>90.0</td>
<td>89.0</td>
</tr>
<tr>
<td>Mid</td>
<td>84.5</td>
<td>84.0</td>
</tr>
<tr>
<td>Late</td>
<td>76.3</td>
<td>76.0</td>
</tr>
<tr>
<td>Average by storage time</td>
<td>83.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>25 DAPH</td>
<td>49.0</td>
<td>46.0</td>
</tr>
<tr>
<td>29 DAPH</td>
<td>43.0</td>
<td>43.0</td>
</tr>
<tr>
<td>32 DAPH</td>
<td>39.3</td>
<td>39.0</td>
</tr>
<tr>
<td>Average by storage time</td>
<td>43.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>35 DAPH</td>
<td>112.0</td>
<td>110.0</td>
</tr>
<tr>
<td>40 DAPH</td>
<td>107.0</td>
<td>104.6</td>
</tr>
<tr>
<td>45 DAPH</td>
<td>96.0</td>
<td>94.3</td>
</tr>
<tr>
<td>Average by storage time</td>
<td>105.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>103.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The maturity stages of Radiant plums were defined as: early (skin colour green with a trace red), mid (half of skin colour red and the reminder green) and late (skin colour bluish red). The maturity stages of Gulfruby and Shiro plums were defined by the number of days after pit hardening (DAPH) (85, 55 and 70 days after full bloom for the cvs Radiance, Gulfruby and Shiro plums, respectively. Data are the means of 10 fruit from three trees (n=30); means followed by the same letters are not significantly different at P ≤ 0.05 according to Duncan’s multiple range test; there was no significant interaction between storage period and harvest maturity.
5.3.2 Physicochemical measurements during storage

In Radiant (Radiance) plums there was a significant decrease in fruit firmness during the first 2 weeks of storage (Fig. 5.1A). After this period, fruit firmness remained constant or increased slightly until week 8 of storage and then declined. However, all fruit were softer after storage than at harvest. In cvs Gulfruby and Shiro, fruit stored for 2 weeks showed a reduction in firmness, but the firmness of fruit stored for longer periods steadily increased, eventually surpassing the level recorded at harvest (Figs 5.2 & 3A). In Rubyred plums there was a significant decrease in firmness of fruit stored for 8 weeks, and then it increased (Fig. 5.4A).

In general, SSC decreased with longer periods of storage (Figs 5.1, 2, 3D & 4C). The exceptions to this were fruit from the early and mid harvests of all cultivars which had been stored for 2 weeks. These fruit showed a slight increase in this parameter from the level measured at harvest. However, fruit from these harvests which were stored for longer periods showed a reduction in SSC. The TA of all cultivars decreased significantly during storage in fruit from all harvests (Figs 5.1, 2, 3 & 4D) and, as a consequence, the SSC:TA ratio increased with storage period. For all cultivars except Rubyred, the hue angle of the skin colour after ripening at 20°C decreased with increasing periods of cool storage (Table 5.1). The hue angle as well as other parameters also varied with the maturity of the fruit at harvest with the more mature fruit having smaller hue angle.
Fig. 5.1. Effects of harvest maturity and storage time on (A) fruit firmness (kgf), (B) percentage of fruit exhibiting chilling injury, (C) extractable juice content and (D) SSC (solid line) and TA (broken line) in Radiant plums during the 1993-94 season. Data for chilling injury represent the means of 20 fruit taken from 3 trees (i.e., n=60) and for the other parameters the means of 10 fruit from each of 3 trees (i.e., n=30). Fruit were harvested at an early (♦, open bar), mid (●, shaded bar) or late (△, solid bar) stage as assessed by skin colour. The bars represent the standard error of the mean. l.s.d. (p≤ 0.05) values were calculated from the residual mean square from a 2-way analysis of variance of all data.
5.3.3 Internal and gel breakdown

After 6, 4, 4 and 8 weeks cool storage in Radiant, Gulfruby, Shiro and Rubyred, respectively, IB was the first symptom of the chilling injury observed. It appeared as a dark browning of the under epidermis. The symptoms of GB developed as a gel-like texture around the pit after 2 weeks later in all cvs (Figs 5.1-4 & Fig. 5.5).

Fig. 5.2. Effects of harvest maturity and storage time on (A) fruit firmness (kgf), (B) percentage of fruit exhibiting chilling injury, (C) SSC and (D) TA in plums of cv. Gulfruby during the 1995/96 season. Data for chilling injury represent the means of 20 fruit taken from 3 trees (i.e., n=60) and for the other parameters the means of 10 fruit from each of 3 trees (i.e., n=30). Fruit were harvested 25 (◆, open bar), 29 (●, shaded bar) and 32 days (Δ, solid bar) after pit hardening. The bars represent the standard error of the relevant mean. l.s.d. (p ≤ 0.05) values were calculated from the residual mean square from a 2-way analysis of variance of all data.
In Radiant plums no IB or GB was observed in fruit stored for up to 4 weeks (Fig. 5.1B). Fruit stored for 6 weeks showed slight symptoms of IB. Fruit stored for longer periods showed both IB and GB. The severity of these disorders increased with the length of the storage period and to a lesser extent with the maturity of the fruit at harvest. In the late maturity stage of Radiant fruit, maximum percentage occurrences of CI at 25, 95 and 100 were obtained after 6, 8 and 10 weeks cool stored, respectively (Fig. 5.1B). In cvs Gulfruby and Shiro, IB developed after 2 weeks of cool storage. Fruit stored for longer periods exhibited both IB and GB (Figs 5.2 & 3B). Maximum percentage of 65 and 100 in Gulfruby at 32 DAPH and 75 and 100 in Shiro at 45 DAPH were observed after 4, 6 and 8 weeks cool storage (Figs 5.2 & 3B). Rubyred plums, stored for 8 weeks, showed slight symptoms of IB while fruit stored for longer periods showed both IB and GB (Fig. 5.4B). Maximum percentage of 5, 35 and 100 CI were observed in Radiant harvested at 95 DAPH after 8, 9 and 10 weeks cool storage respectively.

5.3.4 Extractable juice content

The extractable juice content of Radiant plums increased with fruit maturity (Fig. 5.1C). During storage the juice content of mid- and late-picked fruit declined. Fruit from the early harvests also showed a reduction in juice content after they had been stored for 8 weeks. The percentage reduction in this parameter was related to maturity at harvest with the more mature fruit losing the greatest amount of extractable juice. No significant change was observed in juice content when the symptoms of chilling injury became most noticeable.
Fig. 5.3. Effects of harvest maturity and storage time on (A) fruit firmness (kgf), (B) percentage of fruit exhibiting chilling injury, (C) SSC and (D) TA in plums of cv. Shiro during the 1995/96 season. Data for chilling injury represent the means of 20 fruit taken from 3 trees (i.e., n=60) and for the other parameters the means of 10 fruit from each of 3 trees (i.e., n=30). Fruit were harvested 35 (●, open bar), 40 (●, shaded bar) and 45 days (△, solid bar) after pit hardening. The bars represent the standard error of the relevant mean. l.s.d. (p≤ 0.05) values were calculated from the residual mean square from a 2-way analysis of variance of all data.
Fig. 5.4. Effects of harvest maturity and storage time on (A) fruit firmness (kgf), (B) percentage of fruit exhibiting chilling injury, (C) SSC and (D) TA in plums of cv. Rubyred during the 1996/97 season. Data for chilling injury represent the means of 20 fruit taken from 3 trees (i.e., n=60) and for the other parameters the means of 10 fruit from each of 3 trees (i.e., n=30). Fruit were harvested 85 (Δ, open bar) and 95 (●, solid bar) after pit hardening. The bars represent the standard error of the relevant mean. l.s.d. (p≤ 0.05) values were calculated from the residual mean square from 2-way analysis of variance of all data.
Fig 5.5. Comparison between internal browning (IB) and gel breakdown (GB) development after cool storage and subsequent ripening at 20°C in the cv. Gulfruby plum. (B) IB and GB are compared to 'mealiness' or 'woolliness' in nectarines.
5.4 Discussion

Fruit of Gulfruby and Shiro could be stored for at least 2 and 3 weeks at 0\(^\circ\)C whilst those of Radiant and Rubyred could be stored for at least 5 and 7 weeks, respectively, before chilling injury was evident. This study suggests that the development of chilling injury is influenced by the genetic background of the cultivars. Gulfruby and Shiro have short or medium fruit development periods (FDP) and exhibited a typical ripening pattern climacteric, whilst Radiant and Rubyred have a long FDP and exhibit a suppressed-climacteric phenotype (Section 4.3). The possibility that storage life is related to the length of the FDP and a suppressed-climacteric suggest that these are valuable genetic traits that should be incorporated in a plum breeding program.

Changes in fruit quality were noted during the storage period. Long storage at 0\(^\circ\)C resulted in a reduced hue angle of the ripened fruit (Table 5.1). The hue angle was also lower in the batches of fruit which were maturer at harvest. This reduction in hue angle is associated with a change in skin colour from green to red in cvs Gulfruby, Radiant and Rubyred and from green to yellow in Shiro. A comparison of fruit firmness after ripening for cvs Radiant and Rubyred showed that there was a decrease in this parameter which correlated with storage time. This trend was reversed for fruit of the other 2 cultivars where an increase in firmness occurred during storage. Decreases in fruit firmness during ripening have been shown to relate more to cell wall enlargement than to a biochemical softening of the cell walls (Lill and Van Der Mespel, 1988). On the other hand, other researchers have observed that the conversion of insoluble pectic substances to soluble forms is an important factor in the mechanism of fruit softening associated with ripening (Ben-Arie and Lavee, 1971; Pressey et al., 1971). In this study, increases in fruit firmness of Gulfruby and Shiro correlated with the development of chilling injury. The chilling injury syndrome includes the conversion of soluble pectins to insoluble forms (Taylor et al., 1993b, 1995) and may be the cause of the observed increases in firmness. In cvs. Radiant and Rubyred there was an increase in firmness of fruit stored for 6 or 8 and 9 weeks, respectively. This increase again correlated with the development of chilling injury. Reduction of firmness after this period may be due to other components of the chilling injury syndrome.

In addition to changes in firmness, a slight decrease in SSC with storage time was observed for each of the four cultivars of plums. Vangdal (1982) also noted a decrease in the soluble solids concentration of Norway plums during cool storage. This reduction contrasts to the situation in peaches and nectarines (Aly et al., 1981; Taylor et al., 1993b) and apricot (Salunkhe et al., 1968) where levels increase or remain constant over increasing periods of storage. In our study TA also decreased but at a faster rate than SSC. Therefore, the SSC:TA ratio increased with increasing periods of storage. If the relationship between SSC:TA ratio and flavour in plums is similar to apricots then a higher ratio should be indicative of better
flavour (Taylor et al., 1993b). Storage, therefore, appears to have had a beneficial as well as a negative effect on quality parameters. Further work on the interactions between plum cultivars and holding temperatures may enable a high quality stored product to be placed on the market.

The increase in SSC, decrease in TA, and improvement in skin colour with successive harvests are consistent with the expected improvement in quality with increasing maturation. This study has demonstrated the effect of fruit maturity at harvest on the development of chilling injury. However, although the changes during maturation in this study were clear, it has been shown (Taylor et al., 1993b) that these parameters vary between years and locations which renders them unsuitable for establishing harvest maturity. More mature fruit are more likely to suffer from IB or GB and also reduction of extractable juice content during storage. Therefore, the incidence of chilling injury can be minimized by harvesting early, an effect which has also been seen in Songold plums (Taylor et al., 1993a). In Radiant plums, less extractable juice content may result from high levels of bound juice in inner tissue. Similarly, it has been observed in Songold plums (Taylor et al., 1993b) and in peaches a reduction in juiciness occurs when the cell fluids which leak through membranes form gel complexes with pectins (Ben-Arie and Lavee, 1971). In plums it has also been suggested that during the early stage of ripening there is less soluble pectin in the intercellular spaces of the fruit (Pressey et al., 1971) and this may be involved in the lower incidence of chilling injury. A similar proposal was offered in regard to cold-stored peaches (Von Mollendorff et al., 1992).

Although fruit harvested at an earlier maturity tend to withstand the cool storage better than more mature fruit, this study has shown that less mature fruit have a lower quality when ripened than mature fruit. If storage is inevitable, growers need to be able to determine the precise stage of development of their fruit in order to maximise the effects of maturity on quality whilst minimising its effect on chilling injury. Markers need to be developed which will allow harvest date to be determined and which are independent of fluctuations caused by variations in the growth season. The changes in protein expression which occur during fruit development may provide suitable targets for marker production.

5.5 Summary of Results

1) The maturity indices including skin colour, SSC and SSC:TA increased, while fruit firmness and TA decreased during ripening in all four cvs studied.

2) Fruit firmness decreased during the first 2 weeks storage for the cvs Radiant, Gulfruby and Shiro, but not until the 8th week for Rubyred. After this period, fruit firmness remained
constant or increased slightly. This increase in firmness was associated with chilling injury syndrome.

3) SSC increased during the first 2 weeks storage and then decreased with prolonged storage in all four cvs. The TA decreased during storage in all four cvs.

4) Skin colour development was affected by the storage time in the cvs studied.

5) Symptoms of chilling injury, such as IB and GB, developed after 5, 3 and 7 weeks in the cvs Radiant, Gulfruby, Shiro and Rubyred, respectively. In general, early or mid maturity stages showed a better tolerance of cool storage than did the late maturity stage.

6) It has been found that the suppressed-climacteric phenotype had a longer cool storage than climacteric type, and it is valuable for a plum breeding program.
SECTION 6

EVALUATION OF CHANGES IN TOTAL PROTEINS DURING MATURATION AND RIPENING

6.1 Introduction

Harvest maturity is the most important factor determining consumer acceptability and storage life of stone fruit (Kader and Mitchell, 1989a). A number of indices that can be used to assist with the judgement of harvest maturity of fruit were discussed previously in Section 4. Many of these indices are not reliable because of the variation among cvs, seasons, production areas, climatic conditions and crop loading (Kader and Mitchell, 1989b). The application of IECs in apples has been used by Dilley and Dilley (1985) to determine harvest date, but again is not reliable. However, this technique may be applicable to cultivars where large increases in ethylene are produced (Jobling and McGlasson, 1995). Therefore, there is a need to develop a molecular system for determining optimal harvest time which is applicable to all varieties of stone fruit.

It has been demonstrated that fruit ripening is a process during which biochemical differentiation occurs and which involves de novo protein synthesis (Brady and O’Connell, 1976; Dilley et al., 1993b). During the onset of ripening of several climacteric fruit, the concentration of protein increases e.g., apple (Hulme, 1972), avocado (Christoffersen et al., 1982), banana (Brady et al., 1970), tomato (DeSwardt et al., 1973), and pear (Frenkel et al., 1968). Ripening-related mRNAs and proteins, which are absent or at low levels in immature fruit, increase dramatically during ripening and decline post-ripening (Grierson, 1984).

In plant cells, the vacuole contains many components such as phenolic compounds, polysaccharides, organic acids, proteases, pigments and inhibitory ions that may interfere with protein extraction, separation and purification (Granier, 1988; Meyer et al., 1988). Several protein extraction protocols have been described and their use depends on the plant species, the organ or tissue, and the nature of proteins (soluble and insoluble) (Faurobert, 1997). These methods include the use of sodium dodecyl sulphate (SDS)/Tris lysis, urea/Nonidet-40 (NP-40) or phenol extraction buffers (Granier, 1988; Meyer et al., 1988; Schuster and Davies, 1983).

The choice of procedure for the solubilization of proteins depends on the physicochemical nature of the protein to be analysed. There is not a universal method that will result in optimal solubilization or resolution of all protein samples to be analysed by electrophoretic methods (Dunbar, 1988). However, the ideal sample solubilization method for 2-D PAGE should result in the disruption of all protein complexes and aggregates into a
solution of single polypeptides. If polypeptide interactions are incompletely disrupted, a protein may present simultaneously in an aggregated state and as a single polypeptide (Dunn and Burghes, 1983).

For protein analysis by 1- and 2-D PAGE, it is required that proteins are well resolved, and that gels are substantially free of smearing, and streaking and lack artefacts due to proteolysis or other reasons (Grainer, 1988; Hurkm and Tanaka, 1986). Among the protein extraction methods, the use of phenol extraction protocols has resulted in the best resolution of plant membrane proteins because of low background staining and lack of streaking, and is most often suitable for 2-D PAGE (Hurkm and Tanaka, 1986).

The separation of proteins by one-dimensional gel electrophoresis using optimal conditions will only resolve about 100 proteins from any sample (Dunn and Burghes, 1983). For example, the application of one-dimensional gel electrophoresis to the separation of proteins from apples and peaches only resolved 30 to 40 individual polypeptides, despite the fact that plants contain thousands of proteins (Mahhou and Dennis, 1994). This technique only separates proteins according to their relative molecular weight (Mr) and is unable to resolve proteins having similar Mr but different net charges (Dunbar, 1988; Laemmli, 1970). 1-D PAGE is also unsuitable for analysis of complex mixtures of proteins extracted when studying gene expression (Dunbar, 1988; Dunn and Burghes, 1983).

2-D PAGE has been used widely in plant biology to study changes in gene expression during development. This analytical technique for the separation and quantification of protein species from complex mixtures was pioneered by O'Farrell (1975). In this technique, the proteins separate according to net charge in the first dimension and size or molecular weight in the second dimension (Dunn, 1993). In plants, the aim of protein studies by 2-D PAGE has often been to identify gene products of interest, as related to morphological or physiological changes (Dunn and Burghes, 1983). The main problem associated with 2-D PAGE is the standardization of conditions used in different laboratories which cause variation in protein profiles (Dunbar, 1988).

The proteins of stone fruit have been studied by different analytical methods including starch gel electrophoresis and SDS-PAGE (Faurobert, 1997). Proteins have been extracted from the leaves and stems of apricots by Tris/urea, SDS and TCA extraction buffers and separated by 2-D PAGE (Faurobert, 1997). However, to date there is a lack information about protein extraction and separation methods for use with plums.

The purpose of this section of work was to find a procedure giving the largest number of clearly resolved proteins after separation by one-and two-dimensional gel electrophoresis.
Once developed, the optimal methodology for 2-D PAGE was used to follow changes in protein profiles during maturation and ripening which correlate with optimal harvest maturity time in plums.

6.2 Materials and Methods

6.2.1 Source of fruit

Fruit of Gulfruby, Beauty, Shiro and Rubyred plums were selected from commercial orchards (Section 4.2.1). Five fruit per tree from each of three trees were harvested every 4-6 days from pit-hardening until the fruit reached a tree ripe stage. The fruit were transported on the day of harvest to the Postharvest Laboratory, Centre for Horticulture and Plant Science, for further analysis.

6.2.2 Buffers and Solutions

Coomassie brilliant blue stain

- Coomassie blue R-250 0.1 % (w/v)
- Methanol 30.0 % (v/v)
- Acetic acid 10.0 % (v/v)

IEF gel solution (after Hochstrasser et al., 1988)

- Urea 17.0 M
- Stock solution P* 2.5 mL
- Ampholine (Biolyte) 3/10* 0.8 % (v/v)
- Ampholine (Biolyte) 5/7* 0.2 % (v/v)
- CHAPS 0.3 % (w/v)
- NP-40 1.0 % (v/v)

* Modified during the optimization of the separation procedure. The solution was stored at -80°C in a 1.5 mL Eppendorf tube.

IEF equilibration buffer (after Bjellqvist et al., 1993)

- Urea 6.0 M
- Glycerol 20.0 % (v/v)
- SDS 2.0 % (w/v)
- 0.5 M Tris buffer, pH=6.8 10.0 mL

Lower tank electrolyte buffer (1X) (1-and 2-D PAGE)

- Tris base pH = 8.3 0.025 M
- Glycine 0.192 M
- SDS 0.1 % (w/v)

This buffer was retained for more than 3 months at 5°C without affecting the quality of the protein separations.

O'Farrell lysis buffer (after O'Farrell, 1975)
- Urea 9.5 M
- Tris base, pH = 7.5 35.0 mM
- DTT 1.0 % (w/v)
- Ampholine (Biolyte) 3/10 0.7 % (v/v)
- Ampholine (Biolyte) 5/7 1.3 % (v/v)
- PMSF 2.0 mM (originally omitted, see Section 6.2.4)

The solution was stored at -20°C in a 1.5 mL Eppendorf tube.

Overlay loading buffer
- Glycerol 20 % (v/v)
- Bromophenol blue trace

The solution was stored at room temperature.

Phenol-Protein extraction buffer (after Barent and Elthon, 1992)
- Sucrose 700.0 mM
- Tris base, pH = 7.6 50.0 mM
- HCl 30.0 mM
- KCl 100.0 mM
- Na₂ EDTA 5.0 mM
- DTT 2.0 mM (originally omitted, see Section 6.2.4)
- PMSF 2.0 mM

The solution was stored at -20°C in a dark bottle.

Resolving or separating gel solution (12 %, 0.375 M Tris, pH 8.8) (1 & 2-D PAGE)
- Stock solution A 20.0 mL
- 1.5 M Tris base, pH 8.8 12.5 mL
- 10 % (w/v) SDS 0.5 mL
- ddH₂O 16.7 mL

This solution is enough for one 2-D slab gel (180 x 180 x 3 mm). The solution was stored at 5°C in a dark bottle.

Silver staining solutions (after Rabilloud, 1992)

A. Fixation solution
- Ethanol 38 % (v/v)
- Acetic acid 10 % (v/v)
- ddH2O 52 % (v/v)

B. Incubation solution
- 95 % Ethanol 30.0 % (v/v)
- Sodium acetate 6.8 % (w/v)
- Sodium thiosulphate 0.2 % (w/v)
- 25 % Glutaraldehyde 0.5 % (v/v)

C. Developing solution
- Sodium carbonate 3.6 % (w/v)
- 25 % formaldehyde 0.05 % (v/v)

Stacking or concentrating gel solution (4 %, 0.125 M Tris, pH 6.8) (1 & 2-D PAGE)
- Stock solution A 1.3 mL
- 0.5 M Tris base, pH 6.8 2.5 mL
- 10 % (w/v) SDS 0.1 mL
- dd H2O 6.1 mL

This solution is enough for one 2-D slab gel (180 x 180 x 3 mm). The solution was stored at 5 °C in a dark bottle.

Stock solution A
- Acrylamide 29.2 % (w/v)
- Bisacrylamide 0.8 % (w/v)

Made to 100 mL with deionized water. Filtered and stored at 5°C in a dark bottle (30 days).

Stock solution P
- Acrylamide 29.2 % (w/v)
- PIP 0.8 % (w/v)

Made to 10 mL with deionized water. Filtered and stored at 5°C in a dark bottle (30 days).

Upper tank electrolyte buffer (2X)
- Tris base pH = 8.3 0.05 M
- Glycine 0.382 M
- SDS 0.2 % (w/v)

Discarded after each run.
Tris/ Urea extraction buffer (after Orr and Brady, 1993)

- Tris- HCl, pH = 7.6  
  68 mM
- Urea  
  6 M
- DTT  
  100 mM
- SDS  
  5 % (w/v)

The solution was stored at -20°C in a 1.5 mL Eppendorf tube.

6.2.3 Extraction of soluble proteins

Proteins were extracted from the tissue of plum fruit using the protocols detailed below.

6.2.3.1 Use of Tris/urea extraction buffer

Soluble proteins from frozen or lyophilized fruit tissues of Gulfruby and Shiro were extracted with Tris/urea extraction buffer according to the method of Orr and Brady, 1993. About 100 to 500 mg fruit tissue was ground in a 2 mL Eppendorf tube in the presence of 300 mL Tris/urea extraction buffer. The samples were centrifuged at 7000 x g for 10 min in an JA-20 rotor (LKB). Then 50 mL of supernatant was taken, 10 mL of overlay loading buffer added and subsequently heated at 100°C for 3-5 min. The aliquots were either immediately subjected to electrophoresis or stored in 500 mL Eppendorf tubes at -80°C.

6.2.3.2 Use of Phenol-Protein extraction buffer

Individual fruit were pitted, sliced and stored at -80°C. Samples of fruit from each maturity stage were extracted with phenol-protein extraction buffer according to Barent and Elthon (1992) with the following modifications:

Two to four g of plum fruit tissue were frozen in liquid N₂ and ground in a mortar to generate a powder. The frozen powder was rapidly suspended in 10 mL of phenol-protein extraction buffer in a 45 mL centrifuge tube. An equal volume of water-saturated phenol (Sigma-Aldrich, Australia) was then added. The tube was sealed and shaken vigorously for 5 min at room temperature after which the phases were separated by centrifugation at 7000 x g for 10 min. The phenol phase was recovered and re-extracted with an equal volume of protein extraction buffer and 1 mL of water-saturated phenol. After three or four re-extractions, the proteins were precipitated from the phenol phase by the addition of five volumes of 0.1 M ammonium acetate in methanol (previously cooled at -20°C): Precipitation occurred overnight at -20°C. The following day, proteins were collected by centrifugation at 7000 x g for 10 min following which the protein pellets were washed three times with ammonium acetate in methanol and once with acetone (precooled at -20°C). The pellet was dried under vacuum for 5 min and solubilized in a 350 mL of O’Farrell lysis buffer. The protein solution was clarified by centrifugation for 5 min at 7000 x g following which aliquots were either
immediately subjected to electrophoresis or stored in 1.5 mL Eppendorf tubes at -80°C. Each extraction procedure was repeated twice for each sample of fruit. Three to four gels were obtained from one extract of fruit from each maturity stages of each cultivar. All chemicals were purchased either from Bio-Rad or Sigma-Aldrich.

6.2.4 Use of Tris/urea and phenol-protein extraction buffers

(1) Proteins were extracted from 0.5 g of fresh plum tissue using the Tris/urea extraction protocol (Section 6.2.3.1). Proteins were also extracted from 2 to 4 g tissue using the phenol-protein extraction buffer which did not contain DTT and PMSF (Section 6.2.3.2). Equal amounts of these proteins were separated by 1 & 2-D PAGE. In 1-D PAGE, the protein was separated according to molecular weight (Mr) as bands. In 2-D PAGE, the protein was separated according to net charge in the first dimensional IEF gel and then separated according to Mr in the second dimension as spots.

(2) 2 mM DTT and 2 mM PMSF were added to the phenol-protein extraction buffer of Barent and Elthon (1992). 2 to 4 g of fruit tissue were extracted using either the original phenol-protein extraction buffer or with the addition of the above compounds.

(3) 2 mM PMSF was added to the O’Farrell (1975) lysis buffer. Twenty to 40 μg of proteins, extracted using this procedure, were separated by 1 & 2-D PAGE.

6.2.5 Assessment of protein content

The protein concentration of each sample was determined using a modified Bradford protein assay (Ranagli and Rodriguez, 1985).

6.2.6 Protein separation

The extracted proteins were separated using one and two dimensional gel electrophoresis as below.

6.2.6.1 1-D PAGE (after Laemmli, 1970)

Gels for one-dimensional electrophoresis (3.0 x 200 x 200 mm) were cast in a Multicell-II-xi-Protean chamber (Bio-Rad). The 12 % resolving gel was degassed and polymerization initiated with 1.6 mL of 10 % ammonium persulfate (APS) and 0.25 mL of TEMED. Fifty mL aliquots of this solution was poured to give gels 180 mm in depth. The resolving gels were then overlaid immediately with water-saturated sec-butanol for dissolving oxygen and to level the surface of the gels. The polymerization was generally complete after about 1 h, however, the gels were stored overnight to assure complete polymerization. The gels were washed and then overlaid with 4 % stacking gel about 15 mm in depth.
Electrophoresis was conducted using tank and chamber buffer solution at a constant current of 40 mA/gel until the bromophenol blue tracking dye was 10 mm from the bottom of the gel. After fixing in 12.5 % (w/v) TCA for 1 h, protein bands were visualised by staining the gels overnight with Coomassie brilliant blue solution followed by destaining in 30 % methanol and 10 % acetic acid for 24 h. Gels were photographed and stored at 5°C in plastic bags containing a small amount of water.

6.2.6.2 2-D PAGE

6.2.6.2.1 First-Dimension; Isoelectric Focusing (IEF)

First dimensional gel electrophoresis, or IEF, was performed according to the method of Hochstrasser et al. (1988). After degassing 4 mL IEF gel solution for 2 min, 6.5 mL of 10 % (w/v) APS and 3.2 μL of TEMED were added to accelerate the polymerization. The glass tubes in which the gels were formed were sealed at the bottom with parafilm and then filled with about 350 μL of gel solution per tube using a syringe fitted with a 180 mm long-needle (Bio-Rad). The gels were then overlaid with 10 μL of overlay loading buffer. For the separation, an upper catholyte buffer (20 mM NaOH) and lower anolyte buffer (10 mM H₃PO₄) were placed in the appropriate sections of the electrophoresis chambers (175-IEF, Bio-Rad). About 25- 40 μg protein sample was loaded at the acidic end of the IEF gel, with or without 10 μL of 2-D SDS PAGE standard proteins (Bio-Rad). IEF was conducted for 3 h at 300 V, 15 h at 700 V and then at 1000 V to give 35000 V-hour using a 1000/500 Bio-Rad power supply.

After the separation was complete, the IEF gels were extruded using a water-filled syringe with an attached Eppendorf pipette tip. Once extruded, gels were either equilibrated for 2-D PAGE or stored in a plastic tube at -80°C.

6.2.6.2.2 Second-Dimension; Separation by Molecular Weight

IEF gels from 6.2.6.2.1 were equilibrated prior to SDS-PAGE according to the method of Bjellqvist et al. (1993) by soaking in IEF equilibration buffer for 2 x 5 min. The 100 mL equilibration solution was divided into 2 x 50 mL. To solution A 2 % (w/v) DTT was added for the first 5 min and to solution B for a further 5 min 2.5 % (w/v) iodoacetamide plus 1 % (w/v) bromophenol blue was added.

For the second separation, six slab gels (3.0 x 200 x 200 mm) were cast in a Multicell-II- xi-Protean chamber (Bio-Rad). The 12 % resolving gel was degassed and polymerization initiated with 1.6 mL of 10 % APS and 0.25 mL of TEMED. Three hundred mL aliquots of this solution were poured simultaneously in six gels to give a 180 mm in depth. The resolving gels were then overlayed immediately with water-saturated sec-butanol for dissolving oxygen
and levelling the surface of the gels. The polymerization was generally complete after about 1 h again, however, the gels were stored overnight to assure complete polymerization. The gel were then overlaid with 15 mm of 4 % stacking gel. Each IEF gel was then placed on a piece of parafilm, straightened and placed on the slab gel. Any bubbles were removed using a spatula by pressing the IEF gel gently.

Electrophoresis was performed using lower and upper tank electrolyte buffer solution at a constant current of 40 mA/gel until the dye front was approximately 10 mm from the bottom of the gel.

6.2.7 Optimization procedure for the first and second separations

The composition of IEF gel solution was modified during the optimization of the 2-D PAGE procedure as detailed below:
(1) Originally a 29.2 % : 0.8 % mixture of acrylamide/bisacrylamide was used to make the IEF gel solution. This was later changed to a 29.2 % : 0.8 % mixture of acrylamide: PIP.

(2) Different proportions of ampholines were used: (a) 0.2 % 3/10, 0.8 % 5/7; (b) 0.4 % 3/10, 0.6 % 5/7; and (c) 0.8 % 3/10 and 0.2 % 5/7.
The development of the IEF gel was changed from 400 v/13 h and 800 v/5 h to 300 v/3 h, 700 v/15 h and 1000 v/24 to 30 h.

6.2.8 Silver-nitrate staining procedure

The 2-D gels were fixed and stained using a modification of the method of Rabilloud (1992). Gels were fixed in fixation solution A for 60 to 90 min and were gently agitated during this period. Individual gels were then soaked overnight in incubation solution B. The incubation solution was then removed and the gel washed for 3 x 15 min in distilled water (250 mL/gel). The gels were then stained with 0.2 % (w/v) silver nitrate dissolved in deionized water for 60 min. After staining, the gels were washed with distilled water for 5 min and the stained proteins visualized by soaking the gels in a plastic box containing 250 mL of developing solution C. After 15-20 min development with gentle shaking, the visualization reaction was stopped by the addition of 250 mL of 1.5 % acetic acid. After visualization, the gels were washed with distilled water and the stained gels were sealed in a plastic bags and with a small amount of distilled water and stored at 5°C.

6.2.9 Analysis of polypeptide patterns

The absolute position of a polypeptide on a 2-D gel varies from one gel to the next. The relative position of a protein, i.e. position relative to neighbouring proteins, however, is constant. For comparative analysis, the distance travelled by each standard with respect to the edge of the gel was correlated with the Mr and pI of the protein using linear regression. From
the regression equations the Mr and pI of individual polypeptides were determined. Estimates of Mr and pI were made on all proteins of interest from each cv. at each maturity stage. Differences in these attributes of each polypeptide were determined between cvs using analysis of variance and Duncan’s multiple range test. The relative expression of proteins of interest was assessed visually using the following scale: 0 = no expression; + = polypeptide just visible; ++ = low expression; +++ = moderate expression; ++++ = high expression.

6.2.10 Gel Scanning

The stained gels from each maturity stage from each cultivar were scanned and analysed using Molecular Analyst 2-D PAGE software at the Department of Microbiology, University of Sydney. Every polypeptide in every gel was given an identification number unique for that gel. After detection and editing of a gel series, the protein spots were measured recording intensity, quantities and qualities. Each edited gel was matched with a special reference or master gel.

6.3 Results

6.3.1 Proteins extraction

To determine the changes in protein profiles during ripening, soluble proteins were extracted at different maturity stages in the cvs studied. Two protein extraction buffers were used, a tris/urea and a phenol-protein buffer (Fig 6.1 A & B). The number and quality of polypeptides obtained revealed the different efficiencies of the two extraction methods for the solubilization of proteins from plum fruit. The tris/urea protocol (Fig 6.1A) recovered few polypeptides which separated poorly with smearing and streaking occurring. The phenol-protein protocol (Fig 6.1B) extracted a greater number of polypeptides which resolved more clearly, despite the absence of DTT and PMSF in the extraction buffer and also PMSF in the O’Farrell lysis buffer (these compounds were added during later extractions). According to the modified Bradford assay (Ranagli and Rodriguez, 1985), 25-40 μg/g fresh weight of protein were extracted with phenol-protein buffer from each cultivar at each maturity stage.

6.3.2 Protein separation

As a preliminary experiment to determine if plum proteins could be extracted and separated by SDS-PAGE, samples were subjected to one-dimensional electrophoresis using a Multicell-II-xi-Protean chamber (Bio-Rad). Clearly separated bands were obtained after this procedure and changes in protein expression could be detected during ripening (Fig. 6.2).

A number of modifications to the original 2-D protein separation protocols of Hochstrasser et al. (1988) and O’Farrell (1975) were made during this study which allowed a good resolution of plum polypeptides:
(1) The amount of bisacrylamide in the IEF tube gel was reduced from 0.8 % to 0.67 %. This reduced the level of streaking and background staining (Fig 6.1 B & 6.3A). The replacement of bisacrylamide by PIP at 0.67 % in the IEF gels further improved polypeptide resolution reducing the streaking and tailing of polypeptide spots even further (Fig 6.3B).

2) Different mixtures of biolytes in the IEF gel solution were trialed. When a 0.2 % 3/10 and 0.8 % 5/7 mixture was used a poor separation of polypeptides was obtained with most remaining at the basic side of the gel (Fig 6.4A). In comparison, when the IEF gel contained 0.4 % 3/10 and 0.6 % 5/7, a better separation was obtained, however, there was still a considerable amount of streaking (Fig 6.4B). The best separation was obtained with a 0.8 % 3/10 and 0.2 % 5/7 mixture of ampholytes which gave a good separation with minimal streaking and allowed proteins of interest to be identified (Figs 6.4C).

(3) It was determined that similar 2-D polypeptide profiles could be obtained when samples were stored at -80°C or were separated immediately after preparation.

After optimization of the protein extraction and separation procedures, some polypeptides with high molecular weights were still diffuse and poorly resolved on the top of the gel. However, most polypeptides were less diffuse and were clearly resolved. Computer analysis of scanned-gels revealed that more than 900 polypeptides could be detected by 2-D PAGE (Fig. 6. 14).

Similar patterns of protein expression were found in fruit of the four cvs studied. Differences were observed in the levels of certain proteins during maturation. At least three proteins (X, Y and Z) of interest were observed to change in expression when the fruit ripened. These are indicated by letters on Figs 6.6 to 6.13. The molecular weights and pI's of these polypeptides are given in Table 6.1.
Table 6.1.
Molecular weights (Mr) and isoelectric points (pI) of three proteins of interest (X, Y and Z) in four cvs studied.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>X Mr</th>
<th>X pI</th>
<th>Y Mr</th>
<th>Y pI</th>
<th>Z Mr</th>
<th>Z pI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gulfruby</td>
<td>17.3 ± 0.43a</td>
<td>3.4 ± 0.10a</td>
<td>35.0 ± 0.57a</td>
<td>3.6 ± 0.12a</td>
<td>35.7 ± 0.66a</td>
<td>3.6 ± 0.16a</td>
</tr>
<tr>
<td>Beauty</td>
<td>20.1 ± 0.90b</td>
<td>4.0 ± 0.57a</td>
<td>38.3 ± 0.70b</td>
<td>3.9 ± 0.55a</td>
<td>39.3 ± 0.66b</td>
<td>4.1 ± 0.55a</td>
</tr>
<tr>
<td>Shiro</td>
<td>17.0 ± 0.28a</td>
<td>3.7 ± 0.21a</td>
<td>40.3 ± 0.32c</td>
<td>3.8 ± 0.25a</td>
<td>40.1 ± 0.10b</td>
<td>3.9 ± 0.20a</td>
</tr>
<tr>
<td>Rubyred</td>
<td>20.0 ± 0.57b</td>
<td>3.5 ± 0.25a</td>
<td>39.2 ± 0.58bc</td>
<td>3.6 ± 0.17a</td>
<td>39.4 ± 0.40b</td>
<td>3.7 ± 0.28a</td>
</tr>
</tbody>
</table>

The data are the means and standard errors of three gels from three harvest maturities of each cultivar (i.e., n=9). Means followed by the same letter are not significantly different according to Duncan’s multiple range test at p≤ 0.05.

As Table 6.1 shows, significant differences could be detected in the molecular weights of each of these proteins from the four cvs. Estimates of the size of protein X gave two values for the Mr of this protein, approximately 17 kDa from Gulfruby and Shiro and 20 kDa from Beauty and Rubyred. Estimates of the size of protein Z also suggests that there may be two forms of this protein as Beauty, Shiro and Rubyred all had sizes of approximately 40 kDa whilst the size for Gulfruby was 35.7 kDa. Estimates of the size of protein Y exhibited the greatest variability among the cvs with the possibility of three isoforms existing. The pIs of the three proteins did not show any variation among cvs (Table 6.1).

The expression of these polypeptides in relation to optimal harvest time, the ethylene and respiratory climacterics and softening are shown in Table 6.2. The proteins of interest were expressed 4 to 7 days before optimum harvest time (as defined in Section 4) in all four cvs studied (Table 6.2). Their expression also occurred before the onset of the climacteric. The level of expression of all of these proteins increased as the fruit ripened. In the climacteric cvs, harvested fruit also entered their climacteric 4-7 days after these proteins started to be expressed. However, any association between the expression of these proteins and the climacterics in the suppressed-climacteric cvs was unclear. In Shiro, the climacteric commenced nine days after these proteins were expressed. However, high levels of these proteins were found in fruit from Rubyred without a respiratory or ethylene climacteric occurring. Associations can also be found between the expression of these proteins and other parameters of ripening. Table 6.2 shows a positive relationship between softening and expression of X, Y and Z. A positive relationship also exits between expression and SSC, and a negative relationship with TA (Table 4.3).
Fig. 6.1. Separation of proteins from plum fruit extracted by tris/urea extraction buffer (A) and phenol-protein extraction buffer (B). These two extraction procedures were conducted according to Orr and Brady (1993) and Hochsterasser et al. (1988), respectively, without any modifications.
Fig. 6.2. 1-D PAGE separation of proteins from plums. Protein were revealed by staining with Coomassie brilliant blue.

<table>
<thead>
<tr>
<th>Lane</th>
<th>Description</th>
<th>Size (DAPH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>cv. Shiro, proteins extracted</td>
<td>35</td>
</tr>
<tr>
<td>2</td>
<td>&quot;</td>
<td>28</td>
</tr>
<tr>
<td>3</td>
<td>cv. Gulfruby, proteins extracted</td>
<td>21</td>
</tr>
<tr>
<td>4</td>
<td>&quot;</td>
<td>14</td>
</tr>
<tr>
<td>5</td>
<td>&quot;</td>
<td>21</td>
</tr>
<tr>
<td>6</td>
<td>&quot;</td>
<td>14</td>
</tr>
<tr>
<td>7</td>
<td>&quot;</td>
<td>26</td>
</tr>
<tr>
<td>8</td>
<td>&quot;</td>
<td>31</td>
</tr>
<tr>
<td>9</td>
<td>Protein standard (Bio-Rad)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>cv. Shiro, &quot; &quot;</td>
<td>35</td>
</tr>
<tr>
<td>11</td>
<td>&quot;</td>
<td>28</td>
</tr>
<tr>
<td>12</td>
<td>&quot;</td>
<td>42</td>
</tr>
<tr>
<td>13</td>
<td>&quot;</td>
<td>49</td>
</tr>
</tbody>
</table>
Fig. 6.3. Separation by 2-D PAGE of proteins extracted from the cv. Shiro. Proteins were extracted with phenol-protein extraction buffer. In (A) the IEF contained 3 mL bisacrylamide and in (B) the bisacrylamide was replaced by 2.5 mL PIP.
Fig. 6.4. Separation of proteins by 2-D PAGE from the cv. Shiro harvested 49 DAPH. In (A) the IEF gel solution contained 0.2 % 3/10 & 0.8 % 5/7, in (B) 0.4 % 3/10 & 0.6 % 5/7 and in (C) 0.8 % 3/10 & 0.2 % 5/7 biolytes.
Fig. 6.5. (A) IEF tube gel showing proteins separated according to net charge (pH 3 to 10). (B) is 2-D PAGE separation of protein molecular weight (Mr) and isoelectric point (pI) standards (Bio-Rad).
Table 6.2
Correlations between protein profiles with ethylene, carbon dioxide production and flesh firmness in the four cvs of plums after 24 h at 20°C.

<table>
<thead>
<tr>
<th>Maturity stages (DAPH)</th>
<th>Ripening-related proteins</th>
<th>C$_2$H$_4$ (µL/kg.h)</th>
<th>CO$_2$ (mL/kg.h)</th>
<th>Flesh firmness (Newton)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gulfruby:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7.0</td>
</tr>
<tr>
<td>21</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>25*</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>2.0</td>
</tr>
<tr>
<td>32</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>18.0</td>
</tr>
<tr>
<td>Beauty:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>35</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>0</td>
</tr>
<tr>
<td>42*</td>
<td>++++</td>
<td>+++</td>
<td>++++</td>
<td>2.5</td>
</tr>
<tr>
<td>49</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>5.2</td>
</tr>
<tr>
<td>Shiro:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>40</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>45*</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>0</td>
</tr>
<tr>
<td>49</td>
<td>++++</td>
<td>+++</td>
<td>++++</td>
<td>0.5</td>
</tr>
<tr>
<td>Rubyred:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>56</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>80</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>85*</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>0</td>
</tr>
<tr>
<td>95</td>
<td>++++</td>
<td>++</td>
<td>++++</td>
<td>0</td>
</tr>
</tbody>
</table>

The data for protein expression are the means of three replicate gels with 0 representing no expression, + low expression and ++++ the highest level of expression. Data for ethylene and CO$_2$ were taken from three fruit per three trees (i.e., n=9) and for flesh firmness from 10 fruit from three trees (i.e., n=30). The rates of ethylene and CO$_2$ production were measured 24 h after harvest. * optimum harvest time.
Fig. 6.6. Separation of proteins by 2-D PAGE from Gulpurby plum harvested 14 DAPH (A) or 21 DAPH (B). Proteins X, Y and Z with potential for the production of maturity markers are shown (B). In (A) the numbers show 2-D PAGE protein standards (Bio-Rad). The main protein spots have similar patterns in fruit from both harvesting times.
Fig. 6.7. Separation of proteins by 2-D PAGE from Gelfruby plum harvested 25 DAPH (C) or 32 DAPH (D). Proteins X, Y and Z with potential for the production of maturity markers are shown. The main protein spots have a similar patterns in both harvesting times.
Fig. 6.8. Separation of proteins by 2-D PAGE from Beauty plum harvested 28 DAPH (A) or 35 DAPH (B). Proteins X, Y and Z with potential for the production of maturity markers are shown (B). The main protein spots have similar patterns in fruit from both harvesting times.
Fig. 6.9. Separation of proteins by 2-D PAGE from Beauty plum harvested 42 DAPH (C) or 49 DAPH (D). Proteins X, Y and Z with potential for the production of maturity markers are shown. The main protein spots have similar patterns in fruit from both harvesting times.
Fig. 6.10. Separation of proteins by 2-D PAGE from Shiro plum harvested 28 DAPH (A) or 40 DAPH (B). Proteins X, Y and Z with potential for the production of maturity markers are shown (B), but only protein Z in (A). The main protein spots have similar patterns in fruit from both harvesting times.
Fig. 6.11. Separation of proteins by 2-D PAGE from Shiro plum harvested 45 DAPH (C) or 49 DAPH (D). Proteins X, Y and Z with potential for the production of maturity markers are shown. The main protein spots have similar patterns in fruit from both harvesting times.
Fig. 6.12. Separation of proteins by 2-D PAGE from Rubyred plum harvested 56 DAPH (A) or 80 DAPH (B). Proteins X and Z with potential for the production of maturity markers are shown (B). The main protein spots have similar patterns in fruit from both harvesting times.
Fig. 6.13. Separation of proteins by 2-D PAGE from Rubyred plum harvested 85 DAPH (C) or 95 DAPH (D). Proteins X, Y and Z with potential for the production of maturity markers are shown. The main protein spots have similar patterns in fruit from both harvesting times.
Fig. 6.14. Separation of proteins by 2-D PAGE from Gulfruby plum harvested 25 DAPH. About 700 proteins were detected after scanning the gel by computer.
6.4 Discussion

In an attempt to overcome the difficulty in assessing harvest maturity in highly
coloured cultivars of plums using physiological parameters, protein profiles were analysed by
2-D PAGE to determine if changes in protein expression could be used to identify ideal
harvest date. The 2-D PAGE technique described here for plums has shown that the
polypeptides produced by ripening plum fruit can be adequately separated.

It was found that the proteins extracted with the protein extraction buffer and water-
saturated phenol were better resolved with lower smearing and streaking in comparison to
those extracted using the tris/urea extraction buffer. The use of water-saturated phenol and
urea in the protein lysis buffer allowed denatured and dissociated plum proteins to be
obtained for separation by breaking non-covalent hydrogen bonds and hydrophobic
interactions. Protein profiles were further improved by the addition of the zwitterionic
detergents, CHAPS and NP-40, to the IEF gel solution. These detergents are commonly used
for solubilizing and disaggregating protein complexes and their inclusion in the protocol
described here reduced the amount of protein tailing seen on the gels. The separation of plant
protein samples by electrophoresis can also be complicated by interactions between proteins
and other compounds, mostly carbohydrates. The phenol extraction protocol also appears to
be helpful in the removal of these compounds from extracts of plum proteins. The presence of
proteolytic enzymes can degrade proteins during extraction. PMSF, an inhibitor of
proteinases, added to the O’Farrell lysis buffer in which the proteins were dissolved for
separations also significantly improved the quality of the protein profiles obtained by 2-D
PAGE as was reported by Damerval et al. (1987), Hurkman and Tanaka (1986) and Meyer et
al. (1988).

Visualisation of proteins with silver staining revealed more protein spots with a low
level of background staining in comparison to Coomassie blue staining. These differences
between silver and Coomassie blue staining have been reported in a wide range of plant
studies (Hurkman and Tanaka, 1986; Monroy and Schwartzbach, 1983). 2-D PAGE resolved
of approximately 900 individual polypeptides after silver staining, whereas the 1-D PAGE
resolved about 32 bands at most in plum fruits. Polypeptides separated by 2-D PAGE with
high molecular weights were diffuse and poorly resolved on the top of gel as has been found
in separations of protein from maize (Granier, 1988), cherimoya (Montero et al., 1993) and
banana (Dominguez-Puigjaner et al., 1992). However, medium and low molecular weight on
the middle and bottom of the gels were less diffuse and better resolved and allowed proteins
to be identified whose expression changed during ripening. Comparison of the various stages
of ripening by 2-D PAGE showed that three proteins (X, Y and Z) increased as ripening
proceeded. Variation was observed in the molecular weights, but not the pI’s, of proteins X,
Y and Z, again suggesting that isoforms of these proteins exist. Isoforms of ripening-related
genes have been previously demonstrated. For example, different forms of ACC oxidase have been found in apples (Poneleit and Dilley, 1993), pears (Lelievre et al., 1997a), tomatoes and melons (Pech et al., 1995). Further work is required to be performed to confirm whether the differences in Mr found in proteins X, Y and Z are due to different amino acid compositions or other subsituations such as carbohydrates.

These same three proteins always increased in each individual fruit of each cultivar examined, in the same pattern in relation to optimal harvest time. This was in spite of the significant differences in the ripening behaviour of the climacteric and suppressed-climacteric plum cvs (Section 4). Changes in protein expression are to be expected as ripening is a distinct developmental stage during which new proteins are synthesised as has been shown in apples (Lay-Lee et al., 1990), banana (Dominguez-Puigjaner et al., 1992), cherimoya (Montero et al., 1993) and avocado (McGarvey et al., 1992). This study is the first to show such changes in plums.

A number of ripening-related proteins have been identified from a range of species and their molecular weights and pI’s are presented in Table 6.3. The molecular weight of protein X most closely resembles that of E4 (Lincoln et al., 1987). However, the largest estimate of the Mr of protein X from the four cvs used in this study is still 5 kDa smaller that E4 and, therefore, it is probably unrelated. Proteins Y and Z have molecular weights similar to ACC oxidase as the estimates of its Mr (35.0-40.3 kDa) are within the range reported for other species (35.7 - 41). However, the pI of proteins Y and Z are different from the values obtained for ACC oxidase from other species, therefore, these proteins may not be related to this enzyme. The molecular weights of proteins Y and Z are also close to the protein E8 which is also expressed during ripening (Lincoln et al., 1987). Putative identification of protein identities based on Mr and pI alone can never be very accurate and the identity of proteins X, Y and Z must be determined using antibodies or through amino acid sequencing.

This study has shown that changes in protein expression can be correlated with the ripening process. If these proteins can be purified, they may be used as antigens to prepare monoclonal antibodies for use as analytical reagent. These antibodies may then be used to develop an ELISA system for predicting optimum harvest maturity for use by growers as proposed in apple by Pekker et al. (1993).
Table 6.3
Comparison of Mr and pIs of three proteins of interest in plum with other proteins (enzymes) which have been identified in the other fruits.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Species</th>
<th>Mr (kDa)</th>
<th>pI</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>plums</td>
<td>17.3-20.1(^a)</td>
<td>3.82(^a)</td>
<td>This study</td>
</tr>
<tr>
<td>Y</td>
<td>&quot;</td>
<td>35.0-40.3(^b)</td>
<td>4.05(^a)</td>
<td>&quot;</td>
</tr>
<tr>
<td>Z</td>
<td>&quot;</td>
<td>35.7-40.1(^b)</td>
<td>4.10(^a)</td>
<td>&quot;</td>
</tr>
<tr>
<td>ACO</td>
<td>apples, pears, banana</td>
<td>35.7-41.0</td>
<td>4.9 - 5.1</td>
<td>(Ponelit and Dilley, 1993; Lelievre et al., 1997a; Lopez-Gomez et al., 1997)</td>
</tr>
<tr>
<td>ACS</td>
<td>tomatoes, apples, zucchini</td>
<td>48 - 58</td>
<td>5.6 - 7.0</td>
<td>(Van der Straeten et al., 1990; Dong et al., 1991; Sato et al., 1991)</td>
</tr>
<tr>
<td>E4</td>
<td>tomatoes</td>
<td>25</td>
<td></td>
<td>(Lincoln et al., 1987)</td>
</tr>
<tr>
<td>E8</td>
<td>tomatoes</td>
<td>42</td>
<td></td>
<td>(&quot; &quot; &quot; )</td>
</tr>
<tr>
<td>PG</td>
<td>tomatoes, peaches</td>
<td>54-43</td>
<td></td>
<td>(Orr and Brady, 1993; Speirs and Brady, 1991)</td>
</tr>
</tbody>
</table>

The data are the means of three gels from four cvs for X, Y and Z (i.e., n=12). Means followed by the same letter are not significantly different according to Duncan’s multiple range test at p ≤ 0.05.

6.5 Summary of Results

1) One dimensional gel electrophoresis detected protein changes during maturation and ripening in the cvs Gulfruby and Shiro.

2) The phenol extraction protocol was found to be the best procedure for protein extraction from plum fruit.

3) There was no difference in protein patterns between IEF gel used immediately for separation by Mr or kept at -80°C.

4) 2-D PAGE resolved about 900 polypeptides from plum fruit.
5) The visualisation of proteins using silver staining had a higher sensitivity with a reduced background staining compared to the use of Coomassie blue.

6) In spite of differences in physiological patterns between the climacteric and suppressed-climacteric types, but there were similarities in the pattern of three proteins of interest and the rest of protein spots.

7) In the climacteric cvs harvested fruit entered their climacteric 4-7 days after the expression of the three proteins of interest, but the association between climacteric and protein expression was unclear in the suppressed-climacteric types.

8) There was positive correlation between softening and other ripening parameters and the expression of these three proteins.

9) Three proteins whose time of appearance correlated with optimal harvesting time were detected in all cvs. The average Mr are 18.6, 38.2 and 38.6 with pIs of 3.82, 4.05 and 4.1.

10) It is possible that the proteins Y and Z are similar to ACO detected in apple and pear fruits or may be E8 as detected in tomato fruits.
SECTION 7
APPLICATION OF 1-MCP TO EVALUATE THE ROLE OF ETHYLENE IN RIPENING

7.1 Introduction

Traditionally, the ripening behaviour of fruits has been categorised as being either climacteric or non-climacteric (Biale, 1964) with the classification depending on whether or not they produce a peak in respiration (McMurchie et al., 1972). The Rosaceae family contains a number of species of commercial significance which have been placed in the climacteric category. However, our work with plums has identified a suppressed-climacteric phenotype as described previously in Section 4. Fruit exhibiting this phenotype produce ethylene during the latter parts of the ripening process. However, the levels of this hormone are low when compared to normal, climacteric types and they exhibit a reduced respiratory climacteric. Some cultivars of pears (Downs et al., 1991) and apple (Jobling and McGlasson, 1995; Sfakiotakis and Dilley, 1973b) also exhibit this type of ripening behaviour. Further, in other species such as kiwifruit (Sfakiotakis et al., 1997), the ability to develop a climacteric depends on environmental conditions. Therefore, it has become evident that placing a species into either a climacteric or non-climacteric category is an oversimplification.

Given the importance of the climacteric in determining ripening time and fruit quality, it is important that the physiological factors associated with this event are fully understood and can be controlled. This is especially important with stone fruit as once the climacteric has commenced they ripen quickly and may spoil before they reach their market. The ripening of climacteric fruit can be delayed by a number of inhibitors of ethylene production and action. Amongst these inhibitors, only silver thiosulphate (STS) has commercial applications but only on ornamental crops. However, its continued use is questioned as silver is a potent environmental pollutant, and many countries propose to prohibit its use. Recently, 1-methylcyclopropene (1-MCP), a new inhibitor of ethylene perception, has been synthesized (Magid et al., 1971; Serek et al., 1994a, b). This compound is non-toxic and odourless and may be a good replacement for STS, used on ornamentals and may be approved for use on fruits and vegetables.

The objectives of this section were to characterise further the differences in ripening behaviour between climacteric (Gulfruby and Beauty) and suppressed-climacteric (Shiro and Rubyred) plums and to determine if 1-MCP is able to delay their ripening. To this end, preclimacteric fruit from four cultivars (Gulfruby, Beauty, Shiro and Rubyred) were fumigated with 1-MCP and then treated continuously with propylene, an active ethylene analogue. Respiration and ethylene production were then monitored in treated and control fruit, and an assessment of aroma and colour made as the fruit ripened.
7.2 Materials and Methods

7.2.1 Source of Fruit

Fruit were harvested from three matched, eleven-year-old trees of the cvs Beauty, Shiro and Rubyred and also five-year-old trees of Gulfruby during the 1996/97 season (Section 4.2.1). In attached fruit, pit-hardening occurred about 55, 60, 70 and 90 days after full bloom, first colour was attained 14 (red), 28 (red), 30 (yellow) and 56 (deep red) days after pit hardening (DAPH) and full colour 32, 49, 49 and 95 DAPH for the cvs Gulfruby, Beauty, Shiro and Rubyred, respectively. Preclimacteric fruit were harvested 15, 28, 30 and 80 DAPH in the cvs Gulfruby, Beauty, Shiro and Rubyred, respectively.

7.2.2 1-MCP Synthesis and Application

1-MCP was synthesised by Dr M. Williams at UWS (Nepean) following the protocol of Magid et al. (1971) and was stored under nitrogen in a solution of methylallylchloride. On the day of harvest, samples of fruit were placed in sealed 6 L plastic containers, following which quantities of methylallylchloride solution (1-MCP precursor) were injected onto a glass filter paper disc within the container via a rubber port and allowed to volatilise. Application rates were designed to give 13, 26 or 39 μL 1-MCP/L container volume. Fruit were exposed to these concentrations for six h at 20°C. A second set of fruit were enclosed and exposed to a mixture of volatiles, mainly ethylether, that are the by-products of the synthesis of 1-MCP.

7.2.3 Gas Analysis

After treatment with 1-MCP, three fruit of each cultivar were weighed and enclosed singly in 0.2 L chambers at 20°C ventilated with water-saturated air at a rate of 2.0-2.6 mL/min. Three treated fruit were similarly ventilated, however, using water-saturated air containing 500 μL/L propylene. The untreated, control fruit were ventilated with water-saturated air either with or without the addition of propylene. 1-MCP-treated fruit were also treated with propylene.

On the day after harvest and at subsequent 24 h intervals 1.0 mL samples of air exiting from the chambers were taken to determine rates of CO₂ and ethylene production. Ethylene and carbon dioxide concentrations were measured as detailed in Section 4.2.4.

7.2.4 Assessment of Aroma Volatiles and Skin Colour

Aroma volatile production and skin colour were assessed as described in Section 4.2.3.
7.2.5 ACC Measurement

The fruit of Gulfruby, Beauty, Shiro and Rubyred were harvested during the 1996 and 1997 seasons from first colour development until fruit were tree ripe. Harvested were stored at -80°C for subsequent ACC assay. For each of two seasons (1996 and 1997) two fruit from each cultivar and at each maturity stage (i.e., n=4) were assayed for ACC concentrations according to the method of Lizada and Yang (1979). The assay was performed at 20°C. About one gram fresh weight tissue was selected from the equatorial region of fruit using a No. 8 (10 mm) cork borer and a razor blade. The fruit tissues were placed in 25 x 180 mm test tubes and heated for 30 seconds at full power in a Panasonic Model NN-6558 (100 Watt) microwave oven. Five mL of 0.1 N HCl in 80 % methanol was then added and the tubes were held at 20°C for 4 hours. ACC was determined in 0.5 mL of aliquot of unfiltered extracts in 13 x 180 mm test tubes.

To determine recovery rates, 0.5 mL of aliquots of unfiltered extract was added to two tubes (labeled +ACC and -ACC). 100 μL of 1 nm ACC standard was added to the +ACC tube and both tubes adjusted to a volume of 850 μL with distilled water. The tubes were neutralized by the drop-wise addition of 10 % KOH, using phenolphthalein to determine the end point. Mercuric chloride (200 μL of 0.1 M) was added and the tubes were then sealed with serum caps. Ten drops of a mixture of 5 % NaOCl and 50 % (w/v) NaOH (2:1) were injected into the tubes using a 25-gauge needle and the solutions mixed for 20 seconds to convert ACC to ethylene. Gas samples were taken for ethylene analysis after 15 minutes. The tubes were weighed with their contents and then filled with water and weighed again to determine their volume. ACC concentration was calculated using the percentage recovery of ACC added to the paired samples.

Percent recovery of ACC = A/(B+C)

where  A= total recovery = nL in void space (ppm • void volume) of spiked sample
B = 22.4 nL = 1 nmol gas
C = nL from sample = nL in void space (ppm sample • void volume)

ACC production (nmol) = ppm sample • 100%/ recovery

ACC concentration (nmol/g) = nmole produced ÷ 1/sample weight (g)

7.2.6 Statistical Analyses

Differences between ethylene and carbon dioxide production rates were determined by analysis of variance. LSDs were determined using Duncan’s multiple range test and
standard errors of the means were calculated with Microsoft Excel version 5.0. Correlation coefficients between aroma and ethylene production were calculated with COSTAT version 4.02.

7.3 Results

7.3.1 1-MCP and propylene treatments

The two climacteric cvs of plums, Gulfruby and Beauty, reacted in similar ways to treatment with either 1-MCP and/or propylene, with only the magnitude of the changes in CO₂ and ethylene production differing (Figs 7.1A & D). Fruit treated with 1-MCP for 6 h, and then allowed to ripen in air, showed a delay in the onset of the respiratory and ethylene climacterics of three (Gulfruby; Figs 7.1A & C) or 4-7 days (Beauty; Figs 7.2A & C). Fruit of the cv. Gulfruby, treated with either 13 or 26 μL/L 1-MCP, had similar delays in their climacterics as comparison to air control fruit, whilst with Beauty, the higher levels of 1-MCP (26 and 39 μL/L) delayed the climacteric by a further three days as compared to those exposed to the lowest concentration (13 μL/L). Treatment with 1-MCP also reduced the rate of increase in CO₂ and ethylene production, as well as the maximal levels of these two gasses, especially at the highest levels of 1-MCP treatment.

Fruit of both climacteric cvs, ripened without prior exposure to 1-MCP, responded to propylene within 24 h of treatment (Figs 7.1D & 7.2D). Propylene increased the rate of CO₂ production and initiated the respiratory climacteric one day earlier than in the controls, however, maximal rates of respiration were similar. Treatment with propylene also initiated an earlier onset of the ethylene climacteric by one day in both cvs and stimulated an increase in the maximal rates of ethylene production. Exposure of 1-MCP-treated fruit to propylene failed to influence the onset of the climacteric, however, once the climacteric had commenced, rates of CO₂ and ethylene production were higher than in control fruit (Figs 7.2A & 7.2B).

Fruit of the climacteric cvs entered their respiratory climacterics before any detectable levels of ethylene production were observed. In the cv. Beauty, changes in skin colour and aroma production were also assessed in relation to ethylene production. In both the control and 1-MCP-treated fruit, pigment production commenced before the production of ethylene (compare Figs 7.2A & E). However, ethylene production stimulated the rate of pigment production and the greatest change in colour occurred during the ethylene climacteric. In all fruit of this cv., aroma volatiles were also produced (Fig. 7.5) before the ethylene climacteric and their production was more closely associated with changes in CO₂ production.
Fig. 7.1. Comparison of rates of ethylene (A & B) and CO₂ production (C & D) by control and 1-MCP-treated Gulfruby plums. After treatment the fruit were ventilated with water-saturated air with or without the continuous application of propylene at 500 μL/L. The data represent the means of three fruit from three different trees (i.e., n=9). The bars indicate LSD at p≤ 0.05 by Duncan’s multiple range test.
Fig. 7.2. Comparison of rates of ethylene (A & B), CO₂ production (C & D) and skin colour development (E & F) by control and 1-MCP-treated Beauty plums. After treatment the fruit were ventilated with water-saturated air with or without the continuous application of propylene at 500 μL/L. The data represent the means of three fruit from three different trees (i.e., n=9). The bars indicate LSD at P ≤ 0.05 by Duncan’s multiple range test.
The response of the two suppressed-climacteric cvs (Shiro and Rubyred) to propylene and 1-MCP were also similar to each other but differed markedly from the climacteric types. Firstly, the magnitude of both the CO₂ and ethylene climacterics of fruit ripened without exposure to either compound were markedly lower in the suppressed-climacteric types than in the climacteric ones (Table 7.1 and Figs 7.1-7.4). Treatment of the suppressed-climacteric types with 1-MCP completely abolished both the ethylene and CO₂ climacterics when the fruit were ripened in air (Figs 7.3C & 7.4C). Secondly, the response of the suppressed-climacteric types to propylene was less clear than in the climacteric types. With the cv. Shiro, there was no immediate respiratory response to propylene. However, propylene advanced the occurrence, but not the magnitude, of the respiratory climacteric in fruit which had not been treated with 1-MCP.

Propylene also accelerated both the production rate and magnitude of the ethylene climacteric in Shiro and Rubyred. With 1-MCP-treated fruit, propylene restored their ability to produce both an ethylene and respiratory climacteric, although the magnitude of these events was lower than in non-1-MCP-treated fruit (Table 7.1 and Figs 7.3B & D).

Fruit of the cv. Rubyred, ripened without prior 1-MCP treatment, showed an increased respiration rate after about four days of propylene exposure, and then quickly entered their respiratory climacterics, the magnitude of which was about two-fold higher than in the appropriate control. Peak ethylene production was reached nine days after the commencement of propylene treatment, an advance of fourteen days over the controls, whilst the maximal rate of ethylene production was about 160-fold higher (Table 7.1 and Figs 7.4A & B). As with Shiro, fumigation with propylene restored the ability of 1-MCP-treated fruit to produce a climacteric. The climacteric in these fruit occurred 10-14 days after the climacteric in the non-1-MCP-treated controls. Also, the peak levels of CO₂ and ethylene production were less than those of the controls as well as being substantially lower than the climacteric cvs.
Fig. 7.3. Comparison of rates of ethylene (A & B), CO₂ production (C & D) and skin colour development (E & F) by control and 1-MCP-treated Shiro plums. After treatment the fruit were ventilated with water-saturated air with or without the continuous application of propylene at 500 μL/L. The data represent the means of three fruit from three different trees (i.e., n=9). The bars indicate LSD at P≤ 0.05 by Duncan’s multiple range test.
Fig. 7.4. Comparison of rates of ethylene (A & B), CO$_2$ production (C & D) and skin colour development (E & F) by control and 1-MCP-treated Rubyred plums. After treatment the fruit were ventilated with water-saturated air with or without the continuous application of propylene at 500 $\mu$L/L. The data represent the means of three fruit from three different trees (i.e., n=9). The bars indicate LSD at $P \leq 0.05$ by Duncan’s multiple range test.
Table 7.1

The peak rates of ethylene and CO₂ production, mean number days after harvest to the onset of the ethylene and respiratory climacterics and 50% colour changes during the climacterics after treatment with propylene and 1-MCP in plum fruit.

<table>
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<th>Variety</th>
<th>Treatment</th>
<th>Ethylene Climacteric</th>
<th>Respiratory Climacteric</th>
<th>50% colour prodn</th>
<th>Propylene (µL/L)</th>
<th>1-MCP (µL/L)</th>
<th>Onset (days)</th>
<th>Peak Rate (µL/kg.h)</th>
<th>Onset (days)</th>
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</table>

The fruit were ripened with (+) or without (-) a continuous application of propylene (500 µL/L) and were given a 24 h treatment with different concentrations of 1-MCP immediately after harvest. Means labelled with different letters for each parameter in each cv. are significantly different at P ≤ 0.05 according to Duncan’s multiple range test. nc = no climacteric; nd = not determined.
7.3.2 Skin colour and aroma assessment

Significant changes in skin colour could be detected in the two suppressed-climacteric types ripened in air before the commencement of either the respiratory or ethylene climacterics (Figs 7.3E & 7.4E and Table 7.1). As Shiro is a greengage-type plum the colour change during ripening was from green to yellow as chlorophyll was lost. In the other cvs the colour change was from green to red due to the accumulation of anthocyanins. Where the fruit had been treated with 1-MCP, full colour was obtained despite the absence of either a clear respiratory climacteric or the production of detectable levels of ethylene. Fumigation of fruit with propylene hastened full colour production (Figs 7.3F, 7.4F & Fig. 7.7).

Aroma production by fruit of the cv. Shiro was also assessed (Figs 7.5C & D). Treatment with 1-MCP prevented the fruit from producing aroma volatiles when they were ripened in air. Fumigation of 1-MCP-treated fruit with propylene restored aroma production, with both the level and timing of production being associated more closely with respiration than with ethylene production.
Fig. 7.5. Aroma production by control and 1-MCP- treated fruit of the cvs Beauty (A & B) and Shiro (C & D). After treatment the fruit were ventilated with water-saturated air with or without the continuous application of propylene at 500 μL/L. The data represent the means of three fruit from three different trees (i.e., n=9). The correlation coefficients between aroma and ethylene production were R= 0.86 for Beauty and R= 0.97 for Shiro calculated according to Parson (1974).
7.3.3 ACC concentrations

ACC concentrations at harvest were measured in all cvs from samples taken in both the 1996 and 1997 seasons. Data for the two seasons were similar, and averaged concentrations for the two seasons are presented in Figure 7.6. Concentrations of ACC were similar during the corresponding maturation and ripening periods among the cvs studied but were slightly lower in the suppressed-climacteric phenotypes (Shiro and Rubyred).

Fig. 7.6. ACC concentrations in Gulfruby, Beauty, Shiro and Rubyred plums at harvest. Optimum harvest maturity for these cultivars are shown with arrows. The data represent the means of two samples from each cultivar for 1996 and 1997 (i.e., n=4). The error bars represent SEs of the relevant means.
Fig. 7.7. Ripening responses of Gulfruby plums ripened either with a continuous propylene treatment (500 μL/L) (P), after six hours exposure to 1-MCP (13 μL/L) (M) or without treatment with these compounds (control) (C).
7.4 Discussion

In this section the contrasting ripening behaviour shown by climacteric and suppressed-climacteric cultivars of Japanese-type plums has been studied. This latter phenotype, described previously in Section 4, results from the fruit’s inability to produce sufficient amounts of ethylene to co-ordinate ripening efficiently. The suppressed-climacteric phenotype was highly expressed, as the cvs Shiro and Rubyred produced maximal levels of CO₂ during their climacteric which were approximately half of those of the climacteric types. Also, maximal ethylene production was 15 to 500-fold less. Propylene increased respiration in the climacteric cvs within 48 h of application, as would be expected after treatment with this ethylene analogue. This fast response to propylene was abolished in fruit treated with 1-MCP. This result is believed to be the first demonstration that an ethylene-specific receptor, is involved in the early respiratory response shown by preclimacteric fruit to added propylene. In comparison to the climacteric cv., the enhancement of respiration rates of the suppressed-climacteric types by propylene was delayed with increases in respiration occurring four days after the commencement of propylene treatment for Rubyred and Shiro (Table 7.1). The reason for this lag is unclear but may also be a result of impaired ethylene/propylene perception. Despite this lag, propylene advanced the onset of the respiratory and ethylene climacteric in both the climacteric and suppressed-climacteric cvs. However, the maximum rates of ethylene production from fruit of Shiro and Rubyred remained at a fraction of those produced by Gulfruby and Beauty. The reactions of Shiro and Rubyred to propylene confirmed that they should be classified as climacteric despite their low levels of CO₂ and ethylene production.

Similar (though not identical) concentrations of ACC accumulated in both types of fruit during maturation and ripening. This result suggests that Shiro and Rubyred have a reduced capacity to convert ACC to ethylene, a lesion which would give rise to the suppressed-climacteric phenotype. This limitation was partially alleviated by the application of propylene, particularly in the cv. Rubyred. Non-1-MCP-treated fruit of this cv. ripened in propylene showed a 160-fold increase in ethylene production over the level of production by the appropriate controls. This increase suggests that the gene(s) for ACC oxidase encode a fully functional protein(s), and propylene may have induced changes in transcript levels. In preclimacteric apple (Bufler, 1986) and melon and tomato fruits (Liu et al., 1985) a short treatment with ethylene was shown to increase ACC oxidase activity and propylene may also induce a similar effect. However, if there is a lesion preventing the conversion of ACC to ethylene then it would be expected that ACC should accumulate, as was observed in the non-ripening (nor) mutant of tomato (Terai, 1993). Although ACC concentrations were similar during fruit development between the two classes, levels in the suppressed-climacteric cvs were, in fact, lower. These small differences in ACC content between the climacteric and
suppressed-climacteric types may be a consequence of the lower levels of ethylene production in these cvs as the genes for ACC synthase can also be upregulated by this hormone (Olson et al., 1991; Van Der Straeten et al., 1990). Alternatively, ACC may be more efficiently converted to the malonyl or glutamyamino derivatives (Fluhr and Mattoo, 1996).

This study has revealed some other differences in the physiology of the two classes of plums. Application of 1-MCP to Gulfruby and Beauty, the climacteric cvs, merely delayed the onset of the ethylene climacteric whilst 1-MCP treated fruit of Shiro and Rubyred became overripe (soft, juicy and aromatic) and rotted without the production of an ethylene climacteric or a clear respiratory climacteric. The application of propylene to 1-MCP-treated fruit has a marginal effect on the time to the onset of the ethylene climacteric. However, in 1-MCP-treated fruit of the suppressed-climacteric cvs, propylene was able to restore an ethylene climacteric. It has been proposed by Sisler and Serek (1997) that plants overcome inhibition by 1-MCP by synthesising new receptors. The suppressed-climacteric varieties appear to be unable to do this without stimulation by an ethylene analogue.

Two models can be proposed to explain the suppressed-climacteric phenotype and the reaction of fruit expressing it to treatment with 1-MCP and propylene. The first model assumes that in normal climacteric fruit there is a receptor that triggers autocatalytic ethylene production, and the expression of the receptor is, itself, enhanced by the production of ethylene. Hence, when the system is activated there is a cycle of events which increases ethylene production and leads to the ethylene climacteric. In suppressed-climacteric cvs, there could be a lesion in the production or function of this receptor which prevents the upward spiral of ethylene production. When such plants are treated with 1-MCP receptor levels are reduced below a level where this spiral of events can happen. The application of exogenous propylene, however, is able to prime and maintain the system.

A second model to explain the suppressed-climacteric phenotype assumes that there are two receptors involved with the production of the climacteric, and in normal plants the function of the second receptor is dependent on the function of the first. This model is similar to Systems 1 and 2 proposed by McMurchie et al. (1972). Again there may be a lesion in the expression or function of the second receptor which results in the suppressed-climacteric phenotype. When the action of the first receptor is blocked, and perhaps a developmental period is bypassed (as would occur with 1-MCP treatment), there is insufficient signal to stimulate the production of the second receptor, hence, the loss of the climacteric. In this second model, propylene would again act to prime and maintain the production of the second receptor.

Elements of these models have been proposed by Payton et al. (1996) for the action of the Nr gene in tomato. This gene is proposed to encode an ethylene receptor which is both
developmentally regulated as well as being upregulated by ethylene (Payton et al., 1996; Wilkinson et al., 1995). It may well be a lesion in a similar gene in plums which results in the suppressed-climacteric phenotype. Also, the level of expression of the Nr gene differs in different genetic backgrounds, as the Nr gene is more able to block ripening in fruit derived from the variety Ailsa Craig than from Pearson (Lanahan et al., 1994) and Rutgers cultivar (McGlasson and Adato, 1977). This feature of Nr may explain different expressivity of the suppressed-climacteric phenotype in the cvs used in this study. It should also be stated that other possibilities for the suppressed-climacteric phenotype also exist. Signal transduction is a complex network of events involving the interaction of many genes and their products (Trewavas and Malho, 1997) only a few of which have been identified in the pathway for ethylene signal transduction (Ecker, 1995). Lesions in these genes could also result in the suppressed-climacteric phenotype, the precise nature of which will, therefore, have to await further characterisation at the molecular level.

Skin colour changes in both the climacteric and suppressed-climacteric cvs started before ethylene production could be detected, and in the latter types treated with 1-MCP, full colour was attained without the development of an ethylene climacteric. The association between colour changes and ethylene varies with the type of pigment, the species, and the tissue in which the pigment is being produced. Colour production, therefore, can be either ethylene-dependent or independent (Lelievre et al., 1997b). In plums, the role of ethylene seems to be that of a catalyst hastening and coordinating pigment production.

In contrast to colour changes, aroma production was more closely linked to respiration rate than to ethylene levels in both Beauty (typical climacteric) and Shiro (suppressed-climacteric). This relationship was exemplified by the elimination of aroma production in 1-MCP-treated Shiro fruit, and the restoration of aroma production when these fruit were treated with propylene. The highest intensity of aroma production was achieved at peak respiration rate at which time ethylene also peaked. Our results differ somewhat from those found with Golden Delicious apples. In this cultivar, ethylene production and aroma synthesis were again abolished in fruit treated with 1-MCP, however, an application of ethylene for 24 h to 1-MCP-treated fruit could not restore volatile production (Song et al., 1997) as precursor compounds were limiting. These differences between apple and plum may simply be the result of these species having characteristic aroma compounds which come from different biochemical pathways that are affected to a greater or lesser extent by ethylene.

Lastly, we have determined that application of 1-MCP has the potential to control the ripening of plum fruit. Single applications of this compound are able to give significant delays in the ripening of suppressed-climacteric types, whilst either pulses or a continuous low dose would be required for climacteric cultivars. Further work on the effects of 1-MCP
will allow its future commercial use: this would be facilitated by the development of further
cultivars expressing the suppressed-climacteric phenotype. Delays in the ripening of plums or
other fruits, in conjunction with appropriate harvesting and environmental conditions, will
allow them to be stored for several weeks and will permit sea freight to distant markets.

7.5 Summary of Results

1) The climacteric types of plum fruit, Gulfruby and Beauty, responded in similar ways to
treatment with either 1-MCP or propylene, differing only in the magnitude of changes in CO₂
and ethylene production rates.

2) Fruit treated with 1-MCP for 6 h, and then ripened in air, showed delays the onset of the
respiratory and ethylene climacteric for several days in Gulfruby and Beauty, and for several
weeks in Shiro and Rubyred cvs.

3) Different concentrations of 1-MCP produced similar delays in inhibition of the climacteric
in Gulfruby, but in Beauty higher levels of 1-MCP delayed the climacteric by a further 3
days as compared to lowest concentrations.

4) Propylene increased the rate of CO₂ production and initiated the climacteric one day earlier
than in the controls. It also stimulated increases in the maximal rates of ethylene production
in both Gulfruby and Beauty.

5) Application of propylene to 1-MCP-treated fruit of climacteric types failed to influence the
onset of the climacteric, but after the commencement of the climacteric, rates of CO₂ and
ethylene production were higher than in control fruit.

6) In Beauty, skin colour changed before the production of ethylene in both the control and 1-
MCP-treated fruit, however, ethylene production stimulated the rate of pigment production.

7) The reaction of two suppressed-climacteric cvs Shiro and Rubyred to either propylene or
1-MCP were markedly different from the climacteric cvs. The magnitude of both the CO₂ and
ethylene climacterics of fruit ripened without exposure to these compounds were markedly
lower than in the climacteric types.

8) Treatment of the suppressed-climacteric phenotypes with 1-MCP completely abolished
both the ethylene and CO₂ climacterics. The response of these cvs to propylene was less clear
than in the climacteric types.
9) Propylene accelerated both the production and magnitude of the ethylene climacteric in Shiro and Rubyred. In 1-MCP-treated fruit, propylene restored their ability to produce both an ethylene and respiratory climacteric, although the magnitude of these events was lower than in untreated fruit. The maximal ethylene production in propylene treated fruit was about two-fold and 160-fold higher in Shiro and Rubyred respectively.

10) Significant changes in skin colour could be detected in the suppressed-climacteric types ripened in air or treated with 1-MCP before the commencement of either the respiratory or ethylene climacterics. Fumigation of fruit with propylene hastened full colour production in these cvs.

11) Aroma production by fruit of the cvs Beauty and Shiro was assessed. Fruit treated with 1-MCP delayed aroma production for several days in Beauty, but completely prevented it in Shiro. Application of propylene to 1-MCP-treated and untreated fruit stimulated aroma production in Beauty and restored with both the level and timing of production being more closely associated with respiration rate than with ethylene production in Shiro.

12) Concentrations of ACC were similar during the maturation and ripening periods in both the two climacteric and suppressed-climacteric types.
SECTION 8

GENERAL DISCUSSION AND CONCLUSION

After many decades of research it is somewhat disconcerting that the determination of harvest maturity in many tree fruits is still dependent on subjective judgement and some empirical measurements. This is despite our ever increasing knowledge of the physiology and biochemistry of fruit ripening as evidenced by the following quotation:

"Pome fruit ripening is dependent upon ethylene and de novo protein synthesis; enzyme activities of ripening derive from a process of biochemical differentiation involving directed protein synthesis; methionine is the precursor for ethylene biosynthesis; ethylene action in ripening involves binding to a receptor, requires O₂ and is competitively inhibited by CO₂; mitochondrial oxidative phosphorylation remains fully competent over the course of ripening (Clijsters, 1969)."

The need for improved ways of determining harvest maturity is now more urgent as consumers demand fruit of guaranteed quality. This has led to the preceding study of more highly coloured cultivars of stone fruit where the use of traditional methods for determining maturity, based on skin colour changes, are impractical.

Clearly, there is a need to develop a maturity index that is closely linked to the genetically controlled ripening process and that is independent of environmental influences on qualities changes. Dilley and his coworkers demonstrated that measurements of ethylene production could provide a reliable maturity index for some cultivars of apples, but the procedure requires an expensive gas chromatograph and is time consuming (Dilley and Dilley, 1985). Dilley et al. (1993b) and Pekker et al. (1993) then examined the feasibility of using SDS-PAGE to detect newly synthesized proteins during ripening in apples. They detected the expression of at least two new proteins, including ACC oxidase, in apples as they matured. The approach used by Dilley et al. (1993b) has been applied successfully in the present study to Japanese plums. At least three proteins that appear at optimum commercial harvest maturity have been detected. It is possible that ACO is among these proteins as estimates of its Mr are similar to those from other plant species.

An exciting discovery arising from the current study was the recognition that some cvs of Japanese type plums have a suppressed-climacteric phenotype. In the typical climacteric types, some of the ripening parameters were found to be ethylene-dependent, whilst in suppressed-climacteric phenotype, ethylene production is at least an order of magnitude lower than in the climacteric type and some ripening attributes appear not to be
correlated with ethylene production. The physiological behaviour of this phenotype appears to result from an inability of the fruit to produce sufficient quantities of ethylene since ripening can be accelerated by treatment with propylene, an active analogue of ethylene.

This research also revealed a strong suppression of the climacteric in attached fruit. This phenomenon, termed the ‘tree factor’, has been recognised for many years in other Rosaceae fruit including apples (Blanpied, 1993; Sfakiotakis and Dilley, 1973a) and also in avocados (Biale and Young, 1971). Whether this ‘tree factor’ is the same factor which prevents the ripening in early mature fruit after application of ethylene immediately after harvest or there are separate factors for inhibiting the ripening of attached fruit remains an open question.

The differing responses to 1-MCP and propylene of the climacteric and suppressed-climacteric phenotypes provided valuable research material for the study of ethylene perception. This study has suggested that there may be different receptors for ethylene production and the respiratory climacteric. The concentrations of ACC were examined in all four cvs studied. Since ACC concentrations were similar in these cvs, it suggests that the suppressed-climacteric phenotype is the result of an impaired ability of the fruit to convert this compound to ethylene. Thus, it seems that ethylene production by ACC synthase is not limited, but it could be by activity of ACC oxidase or other regulators of the conversion of ACC to ethylene.

Future prospects and recommendations

This thesis has confirmed that traditional physicochemical measurements (skin colour, flesh firmness, SSC and TA) are not reliable indices of commercial harvest maturity in Japanese plums. However, this study has shown that molecular markers, such as proteins, that change during maturation are a more accurate index for determining optimum harvest maturity. If sufficient quantities of these ripening-related proteins identified in this study can be purified, an assay system for use by growers could be developed. The first step in this procedure would be to raise monoclonal antibodies against these proteins. After evaluation of the monoclonal antibodies to determine if they can be used as a probe for assessing ideal harvest time of Japanese plums, it should be practicable to develop an ELISA system for commercial use. It is envisaged that growers would directly test samples of fruit in the orchard to assist them to judge the best time to harvest the crop.

The studies of ripening in this thesis have shown the presence of the suppressed-climacteric phenotype in Japanese plums improves shelf life and may be useful for delaying ripening when fruit are sent to distant markets. Applications of 1-MCP delay ripening even further and treatment with ethylene may be required to overcome the effects of 1-MCP so that
they are suitable for consumption. Continuation of physiological and genetical studies of cvs with the suppressed-climacteric phenotype should enable the detection of molecular markers that will assist the introgression of this trait into new commercial cultivars.
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PLEASE NOTE

The greatest amount of care has been taken while scanning the following pages. The best possible results have been obtained.
APPENDIX I

RELATED PUBLICATIONS


Effects of harvest maturity on the storage life of Japanese type plums

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\textbf{Summary.} Plums are highly perishable and once harvested have a short commercial life. Hence, this crop would benefit from the determination of conditions which permit extended storage. This study investigates the effects of fruit maturity at harvest on the physical and chemical characteristics of the plum cultivars, Radiant, Gulfruby and Shiro, after periods of cool storage. Fruit from these cultivars were separated into 3 maturity categories according to either skin colour at harvest or time after pit hardening. The storage disorders, internal breakdown and gel breakdown, were measured after periods of storage at 0°C and subsequent ripening at 20°C. Skin colour, flesh firmness, concentration of soluble solids and titratable acidity were measured at harvest and after ripening. Internal breakdown and gel breakdown, symptoms of chilling injury, developed after 2 weeks cool storage in Gulfruby and Shiro and after 4 weeks in Radiant. These symptoms were most severe in the oldest fruit. Significant differences were also found in skin colour, firmness, soluble solids and acidity during ripening and after cool storage. Fruit harvested at an earlier maturity withstand cold storage better than more mature fruit. However, these less mature fruit initially have a poorer quality than those which are more mature. Therefore, it is important for the grower to be able to determine the precise stage of crop development in order to allow harvesting at a time which is optimal for the storage process.

\textbf{Introduction.} Plums are an important fruit crop which, in Australia, account for almost 60% of stonefruit exports (Anon. 1993). Transport to distant markets may involve prolonged storage periods during which time the fruit may ripen and decay. As a result of periods of hot weather, or due to other seasonal factors, fruit for domestic markets may be sent in excess quantities, resulting in low prices and a loss of profitability. Thus, there is a need for extended periods of fruit storage to reduce fluctuations in supply and to facilitate exports. Unfortunately, however, plums are highly perishable and, depending on the cultivar, may only have a commercial life of 2–6 weeks, even when stored at 0°C.

Postharvest storage of plums is limited by 2 main types of physiological injury, internal breakdown (IB) and gel breakdown (GB), which occur in different parts of the flesh. The flesh of plum fruit can be divided into the inner and outer mesocarp and there are physiological differences between these parts in properties such as their concentrations of soluble solids and electrolytes, viscosity of water-soluble pectins and acidity. Internal breakdown develops directly below the epidermis and manifests itself as a browning of the flesh which is due to the enzymic oxidation of polyphenols and tannins (Dodd 1964). Gel breakdown arises after the appearance of IB in the inner mesocarp around the pit and results in a gelatinous appearance of the flesh. Gel breakdown is thought to be caused by changes in the permeability of cell membranes and the presence of water-soluble pectins in the intercellular spaces (Taylor et al. 1993a, 1993b). Gel complexes are formed which bind water resulting in low levels of extractable juice (Dodd 1984; Kotze et al. 1987; Lill and Van Der Mepel 1988). These disorders, therefore, reduce quality and limit the possibilities for storage.

Temperature can be another limiting factor in the storage of plums since below 10°C fruit may suffer from chilling injury which is exhibited as IB and/or GB; below -0.8°C the flesh freezes. Symptoms of chilling injury can develop after storage between these temperatures when the fruit are subsequently ripened at 20°C (Hall et al. 1989; Mitchell and Kader 1989; Taylor et al. 1993a, 1995). Gel breakdown, however, develops more rapidly in fruit stored between 2 and 5°C than at lower temperatures (Mitchell et al. 1992). Therefore, the most appropriate temperature for the storage of the plums appears to be near to 0°C (Mitchell et al. 1974).

Gel breakdown can be reduced by harvesting within a narrow maturity range. However, this is not always possible in practice as the developmental stage of the fruit is difficult to determine (Taylor et al. 1995). Indices used to judge harvest maturity include fruit size, shape, skin colour, firmness, concentration of soluble solids
(SSC) and titratable acidity (TA) (Robertson et al. 1991). Unfortunately, these indices are influenced by crop load, cultivar and seasonal factors, and cannot be used with a high degree of confidence. This study was undertaken to establish the influence of harvest maturity on cool storage life and quality of fruit of plum cultivars Radiant, Gulfruby and Shiro, and forms part of a research program which aims to develop new ways of judging optimum, commercial harvest maturity.

Materials and methods

Source of fruit

The 3 cultivars of plums used in this study were Radiant, Shiro and Gulfruby. Scions of Radiant and Shiro were grafted onto Myrobalan root stocks and of Gulfruby onto a low chill Prunus persica stock. Fruit of Radiant were harvested in March 1994 from a commercial orchard at Young, New South Wales. Commencing in November 1995, fruit of Gulfruby were picked from trees at the School of Horticulture, University of Western Sydney, Hawkesbury. Fruit of Shiro were obtained from a commercial orchard at Bilpin, New South Wales, with harvests starting from December 1995. Fruit from the 3 cultivars were considered to be commercially mature at the mid (Radiant), 29-day (Gulfruby) and 45-day (Shiro) harvests. For all cultivars, samples of fruit were taken from each of 3 trees and treated separately. All fruit were hand picked and transported on the day of harvest to the Postharvest Laboratory at the University of Western Sydney, Hawkesbury. On arrival they were dipped in a 1 g/L solution of Rovral (Merck, Germany) then air dried. Radiant plums were graded into 3 maturity stages based on skin colour: early (green with a trace of red); mid (half of the skin red, the remainder green); and late (bluish red over the whole of the fruit). Fruit of cv. Gulfruby were harvested 25, 29 and 32 days after pit hardening (DAPH), and those of cv. Shiro at 35, 40 and 45 DAPH. The fruit were placed in trays lined with low density polyethylene film which was folded over them and stored at 0°C for up to 10 weeks. Aliquots of 20 fruit from each sample of fruit were then removed from cool storage at intervals of 2 weeks and allowed to ripen at 20°C for either 5 (Gulfruby and Shiro) or 7 days (Radiant).

Assessment of internal and gel breakdown

Aliquots of 20 fruit from each sample (i.e. 60 of each cultivar) were cut in half along the equatorial axis and the occurrence of IB and GB assessed at weekly intervals according to Taylor et al. (1993a). The percentage area of these injuries was summed and expressed as percentage chilling injury.

Extractable juice content

The amount of extractable juice in the flesh from cv. Radiant was determined using 10 fruit from each sample (30 fruit in total) according to the method of Lill and Van Der Mespel (1988). Segments of tissue (about 1.5 g) were taken from the fruit, the skin was removed and the flesh homogenised by forcing it through a 5 mL disposable syringe with no needle. The homogenate was collected in an Eppendorf centrifuge tube, weighed and centrifuged (10,000 g for 5 min). The weight of the supernatant was recorded and expressed as a percentage of the weight of the sample. This value is considered to be the apparent juice content.

Physical and chemical measurements

At each harvest time and following storage, fruit were assessed for skin colour, firmness, SSC and TA. Measurements of these parameters were made on 10 fruit taken from 3 trees (i.e. 30 in total). Skin colour, measured as the hue angle (McGuire 1992), was assayed using a Minolta CR-200 colorimeter. After the removal of the skin, flesh firmness was determined using an Effegi penetrometer mounted in a drill press and fitted with an 11 mm tip. Two measurements were made on each fruit using opposite cheeks of the fruit. Extracts of juice were made by homogenising fruit flesh in a Waring blender. The homogenate was centrifuged at 3700 g for 5 min and the supernatant used for the determination of SSC and TA. Concentration of soluble solids was determined using a hand-held refractometer (Atago Company, Japan) and TA by titration to an end point of pH 8.1 using 0.1 mol NaOH/L (Robertson et al. 1991).

Statistical analyses

Data were subjected to completely randomised 2-way factorial analyses of variance using COSTAT version 4.02 (Wesel Software, Berkeley, CA, USA, 1990).

Results

Physical and chemical measurements at harvest

The development of skin colour, SSC and the SSC:TA ratio measured at harvest increased with maturity, whilst firmness and TA declined in all cultivars (Figs 1–3, Table 1).

Physical and chemical measurements during storage

In Radiant plums there was a significant decrease in fruit firmness during the first 2 weeks of storage (Fig. 1a). After this period, fruit firmness remained constant or increased slightly until week 8 of the storage period and then declined again. However, all fruit were softer after storage than at harvest. In cvv. Gulfruby and Shiro, fruit stored for 2 weeks showed a reduction in firmness. However, the firmness of fruit stored for longer periods steadily increased eventually surpassing the level recorded at harvest (Figs 2a and 3a).

In general, SSC decreased with increasing periods of storage (Figs 1d, 2c and 3c). The exceptions to this were fruit from the early and mid harvests of all 3 cultivars which had been stored for 2 weeks. These fruit showed a slight increase in this parameter from the level measured
at harvest. However, fruit from these harvests which were stored for longer periods showed a reduction in SSC. The TA of all cultivars decreased significantly during storage in fruit from all harvests (Figs 1a, 2a, and 3d) and, as a consequence, the SSC:TA ratio increased with storage period. For all cultivars the hue angle of the skin colour after ripening at 20°C decreased with increasing periods of cool storage (Table 1). The hue angle also varied with the maturity of the fruit at harvest with the more mature fruit having smaller hue angles.

**Internal and gel breakdown**

Internal breakdown was the first symptom of the chilling injury syndrome and exhibited itself as a change in the flesh colour under the epidermis which became dark brown. Following the development of IB, GB occurred producing a gel-like texture around the stone. In Radiant plums no IB or GB was observed in those fruit stored for up to 4 weeks (Fig. 1b). Fruit stored for 6 weeks showed the symptoms of IB. Fruit stored for longer periods showed both IB and GB. The severity of this syndrome increased with the length of the storage period and to a lesser extent with the age of the fruit at harvest. In cvv. Gulfruby and Shiro, IB developed after 2 weeks of cool storage. Fruit stored for longer periods exhibited both IB and GB (Figs 2b and 3b). In all cultivars, GB was most severe when fruit were harvested after they were considered to be commercially mature.

**Extractable juice content**

The extractable juice content of Radiant plums increased with fruit maturity (Fig. 1c). During storage
the juice content of mid- and late-picked fruit declined. Fruit from the early harvest also showed a reduction in juice content after they had been stored for 8 weeks. The percentage reduction in this parameter was associated with the fruits' maturity at harvest with the more mature fruit losing the greatest amount of water. No significant change was noted in juice content when the symptoms of chilling injury became most noticeable.

Discussion
This study has looked at the storage properties of 3 commonly grown cultivars of plums. Gulfruby and Shiro plums could be stored for at least 2 weeks at 0°C whilst those of Radiant could be stored for at least 4 weeks before chilling injury was evident. This study suggests that the development of chilling injury is influenced by the genetic background of the cultivars. Gulfruby and Shiro have short or medium fruit development periods (FDP) whilst Radiant has a long FDP. Rubyred, which also has a long FDP, also appears to be less prone to chilling injury (N. Abdi unpublished data). The possibility that storage duration is related to the FDP would make this a useful feature for influencing the choice of cultivar if storage is desired. Further study, however, would be needed to confirm this association.

Changes in fruit quality were noted during the storage period. Storage for increasing periods at 0°C reduced the hue angle of the ripened fruit. The hue angle was also lower in the batches of fruit which were more mature at harvest. This reduction in hue angle is associated with an
increase in quality as it represents the change from green to red in cv. Gulfruby and Radiant and from green to yellow in Shiro. A comparison of fruit firmness after ripening for cv. Radiant showed that there was a decrease in this parameter which correlated with storage period. This trend was reversed for fruit of the other 2 cultivars where an increase in firmness occurred with storage period. Decreases in fruit firmness during ripening have been shown to relate more to cell enlargement than to a biochemical softening of the cell walls (Lill and Van Der Mespel 1988). On the other hand, other researchers have observed that the conversion of insoluble pectic substances to soluble forms is an important factor in the mechanism of fruit softening associated with ripening (Ben-Arie and Lavee 1971; Pressey et al. 1971). In our study, increases in fruit firmness of Shiro and Gulfruby correlated with the development of chilling injury. Part of this syndrome includes the conversion of soluble pectins to insoluble forms (Taylor et al. 1993b, 1995) and may be the cause of the observed increases in firmness. With cv. Radiant there was an increase in firmness in fruit stored for 6 or 8 weeks; this increase again correlated with the development of chilling injury. Reduction of firmness after this period may be due to other components of the chilling injury syndrome.
Table 1. Effects of harvest maturity and cool storage at 0°C on skin colour (i.e. hue angle) of Radiant, Gulfruby and Shiro plums after ripening at 20°C

The maturity stages of Radiant plums were defined as: early (skin colour green with a trace red), mid (half of skin colour red and the remainder green) and late (skin colour bluish red)

The maturity stages of Gulfruby and Shiro plums were defined by the number of days after pit hardening (DAPH) (85, 55 and 70 days after full bloom for the cvv. Radiant, Gulfruby and Shiro plums respectively)

Data are the mean of 10 fruit from three trees (n = 30); means followed by the same letter are not significantly different at P = 0.05 according to Duncan's multiple range test; there was no significant interaction between storage period and harvest maturity

<table>
<thead>
<tr>
<th>Harvest maturity</th>
<th>0</th>
<th>2</th>
<th>Storage time (weeks)</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>Average by harvest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early</td>
<td>90.0</td>
<td>89.0</td>
<td>88.6</td>
<td>88.0</td>
<td>86.6</td>
<td>86.0</td>
<td>88.5</td>
<td>83.6y</td>
</tr>
<tr>
<td>Mid</td>
<td>84.5</td>
<td>84.0</td>
<td>84.0</td>
<td>82.0</td>
<td>81.6</td>
<td>81.0</td>
<td>83.0y</td>
<td>74.1z</td>
</tr>
<tr>
<td>Late</td>
<td>76.3</td>
<td>76.0</td>
<td>75.0</td>
<td>74.0</td>
<td>73.0</td>
<td>70.0</td>
<td>74.1z</td>
<td>79.1c</td>
</tr>
<tr>
<td><strong>Average by storage time</strong></td>
<td>83.5a</td>
<td>82.9a</td>
<td>82.6a</td>
<td>81.3b</td>
<td>80.5b</td>
<td>79.1c</td>
<td><strong>85.5</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Gulfruby</strong></td>
<td>25 DAPH</td>
<td>49.0</td>
<td>46.0</td>
<td>44.6</td>
<td>43.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>29 DAPH</td>
<td>43.0</td>
<td>43.0</td>
<td>42.0</td>
<td>40.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>41.8y</td>
</tr>
<tr>
<td>32 DAPH</td>
<td>39.3</td>
<td>39.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>38.6z</td>
</tr>
<tr>
<td><strong>Average by storage time</strong></td>
<td>43.5a</td>
<td>43.2a</td>
<td>42.9a</td>
<td>39.5b</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td><strong>41.9</strong></td>
</tr>
<tr>
<td><strong>Shiro</strong></td>
<td>35 DAPH</td>
<td>112.0</td>
<td>110.0</td>
<td>109.6</td>
<td>108.6</td>
<td>108.0</td>
<td>-</td>
<td>109.8x</td>
</tr>
<tr>
<td>40 DAPH</td>
<td>107.0</td>
<td>106.0</td>
<td>103.3</td>
<td>102.3</td>
<td>100.0</td>
<td>-</td>
<td>-</td>
<td>101.0y</td>
</tr>
<tr>
<td>45 DAPH</td>
<td>96.0</td>
<td>94.3</td>
<td>95.4</td>
<td>93.3</td>
<td>93.3</td>
<td>-</td>
<td>-</td>
<td>94.5z</td>
</tr>
<tr>
<td><strong>Average by storage time</strong></td>
<td>105.0a</td>
<td>103.0b</td>
<td>102.4b</td>
<td>101.4c</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td><strong>104.4</strong></td>
</tr>
</tbody>
</table>

In addition to changes in firmness, a slight decrease in SSC with storage time was observed for each of the 3 cultivars of plums used in this study. Vandal (1982) also noted a decrease in the soluble solids concentration of Norway plums during cool storage. This reduction is in contrast to the situation in peaches and nectarines (Aly et al. 1981) and apricots (Salunkhe et al. 1968) where levels increase or remain constant after increasing periods of storage. In our study TA also decreased but at a faster rate than SSC. Therefore, the SSC:TA ratio increased with increasing periods of storage. If the relationship between SSC:TA ratio and flavour in plums is similar to apricots then a higher ratio would be indicative of better flavour (Taylor et al. 1993b). Storage, therefore, appears to have had a beneficial as well as a negative effect on quality parameters. Further work on the interactions between plum cultivars and holding temperatures may enable a high quality stored product to be placed on the market.

The increases in SSC, decreases in TA and improvement in skin colour with successive harvests are consistent with the expected improvement in quality with maturation. This study has demonstrated the effect of fruit maturity at harvest on the development of chilling injury. However, although the changes during maturation in this study were clear, it has been shown (Taylor et al. 1993b) that these parameters vary between years and locations which renders them unsuitable for establishing harvest maturity. More mature fruit are more likely to suffer IB or GB during storage. Therefore, the incidence of chilling injury can be minimised by harvesting early, an effect which has also been seen in Sungold plums (Taylor et al. 1993a). In plums it has been suggested that during the early stage of ripening there is less soluble pectin in the intercellular spaces of the fruit (Pressley et al. 1971) and this may be involved in the lower incidence of chilling injury. A similar proposal was offered in regard to cold-stored peaches (Von Mollendorff et al. 1992).

Although fruit harvested at an earlier maturity tend to withstand the storage process better than more mature fruit, this study has shown that less mature fruit have a lower quality when ripened than mature fruit. If storage is inevitable, growers need to be able to determine the precise stage of development of their fruit in order to maximise the effects of maturity on quality whilst minimising its effect on chilling injury. Markers need to be developed which will allow harvest date to be determined and which are independent of fluctuations caused by variations in the growth season. The changes in protein expression which occur during fruit development may provide suitable targets for marker production.

Acknowledgments

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Storage life of Japanese type plums

References


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Ripening behaviour and responses to propylene in four cultivars of Japanese type plums

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Abstract

The aim of this study was to determine the physicochemical changes in highly coloured cultivars of plums that could be used as a guide to assessing optimum harvest maturity. The patterns of fruit growth and maturation were investigated in the cultivars: Gulfruby, Beauty, Shiro and Rubyred. Changes in the rates of respiration and ethylene production, skin colour, firmness, soluble solids concentration and titratable acidity were recorded at intervals from pit-hardening until the fruit were tree ripe. In order to evaluate the role of ethylene in the ripening process, propylene was applied to harvested fruit. Internal ethylene concentrations in the cv. Rubyred were also measured at intervals after pit-hardening either in harvested fruit or fruit attached to the tree. Studies of the changes in the physiological parameters associated with ripening showed that none were suitable for the assessment of harvest maturity in all cultivars of plums. However, this analysis revealed two distinct patterns of ripening behaviour in the cultivars studied. Gulfruby and Beauty showed a typical climacteric pattern of development, whilst Shiro and Rubyred exhibited a suppressed-climacteric phenotype. This phenotype appears to result from an inability of the fruit to produce sufficient quantities of ethylene to co-ordinate ripening. However, treatment with propylene showed that fruit displaying the suppressed-climacteric phenotype should still be placed in the climacteric class. This suppressed-climacteric character could be incorporated into plum breeding programs to produce new varieties with improved storage properties.

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Keywords: Prunus spp.; Ethylene; Propylene; Tree effect; Suppressed-climacteric

1. Introduction

Plums are the largest group of stonefruit grown commercially in Australia and there is potential for an expansion in production (Anon., 1996).

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This potential will only be achieved if fruit, either for the fresh market or for storage, reach the customer in prime condition. Harvest date is the most important factor determining consumer acceptability, as quality is reduced by either premature or late harvesting. Harvesting plums at an early stage of maturity may result in a product that has a good appearance, and transports and stores well. However, yield may be sacrificed and the flavour poor. On the other hand, fruit which are harvested late ripen quickly and may perish before they are sold. Therefore, to ensure optimal quality, there is a need for markers which will allow the stage of maturity to be determined with precision.

A number of parameters have been used to assist with the judgment of harvest maturity of fruit. These include changes in skin colour, flesh texture, tissue permeability, soluble solids concentration (SSC), aroma volatiles, ethylene and CO₂ production and protein expression (Pratt, 1975). Many of these parameters are not reliable because they vary with cultivar, production area and season (Kader and Mitchell, 1989). Studies on fruit firmness, for example, have shown that large fruit soften faster than small fruit of the same variety (Kader and Mitchell, 1989). In some varieties of stonefruit, changes in skin colour may be useful indicators of ripening (Rhodes, 1980; Kader and Mitchell, 1989). However, many plum cultivars develop their pigmentation early in growth and therefore, this attribute may be of little value in determining harvest time. Markers of maturity developed from an understanding of the physiological and biochemical changes which occur in fruit as they mature may help overcome the problems associated with other indices of harvest date.

In this paper we report the results of a comparative study of the biochemical and physiological changes which occurred during the ripening of four cultivars of plums. Changes in skin colour, flesh texture, aroma volatiles, rates of respiration and ethylene production were monitored to determine their relationship with harvest maturity and applicability as markers of this event. To clarify the role of ethylene during ripening, fruit were treated with propylene, an ethylene analogue known to promote ripening and stimulate the respiratory climacteric (McMurchie et al., 1972).

2. Methods

2.1. Source of fruit

The cultivars used in this study were all Japanese type plums which have been derived from crosses with P. salicina. Fruit from Beauty, Shiro and Rubyred were selected from 10-year-old trees grown on Myrobolan rootstocks at Bilpin, NSW. Plums from the early ripening cultivar Gulfruby, grown on low-chill peach rootstocks, were harvested from orchards at the School of Horticulture, University of Western Sydney Hawkesbury, NSW, Australia.

2.2. Fruit growth measurement

Fruit growth was measured by determining the diameter and weight of 20 fruit per tree from each of three trees in 1994/95, and fruit diameter only in 1995/96. Ten fruit from three replicate trees of approximately the same size were harvested from around the widest part of the tree perimeter at pit-hardening and at subsequent intervals of 4–14 days until the fruit reached a tree ripe stage (soft to the touch, full colour development, juicy, sweet and aromatic). Fruit were transported on the day of harvest to the Postharvest Laboratory, School of Horticulture, for further analysis.

2.3. Physical and biochemical measurements

Skin colour, determined as the hue angle, was measured using a Minolta CR-200 colorimeter. Flesh firmness was assayed on pared surfaces from opposite sides of the fruit using an Effegi penetrometer fitted with a 7.5 or 11 mm tip. At each harvest, samples of flesh of approximately equal weight (approx. 25 g) were taken from 10 fruit from each of three trees of each cultivar. Samples were combined and stored at −20°C. For chemical analysis, samples were thawed and then homogenised in a Waring blender. Samples of the homogenate were centrifuged (6000 g for 8 min) and aliquots of the supernatant used for the measurement of soluble solids concentration (SSC) using a hand refractometer (Atago, Japan) and titratable acidity (TA) according to the method of Robertson et al. (1991).
3.4. Physiological measurements

Fruit were harvested at pit-hardening and at subsequent intervals of 4–6 days during the 1994/5 and 1995/6 seasons. At each harvest, four (1994/5) or three (1995/6) fruit from each of three trees of each cultivar were weighed and enclosed singly in 0.2 l chambers at 20°C. The chambers were ventilated with water-saturated air (2.5–4.1 ml/min). In 1995/6, replicate samples of fruit were similarly ventilated with water-saturated air containing 500 μl/l propylene. Samples of air (1.0 ml) exiting from the chambers were taken to determine rates of CO₂ and ethylene production on the day after harvest and at subsequent 24 h intervals. Ethylene concentrations were measured with a Gowmac Model 580 gas chromatograph. Carbon dioxide concentrations were determined by pulse infra-red gas analysis (Horiba, Model PIR-2000) (Dobling et al., 1991). Measurements were standardised using calibrated compressed gas mixtures purchased from the British Oxygen Co., Australia. The lowest measurable rate of ethylene production was about 0.01 μl kg⁻¹ h⁻¹. Aroma volatiles produced by fruit were assessed organoleptically by ten panellists using the following scale: 0, no aroma; 1, low; 2, medium; and 3, strong aroma.

2.5. Internal ethylene concentrations

The open end of a plastic tube (40 x 15 mm) was attached to one cheek of a fruit using acid-free silicone (Dow-Corning 3040). A hole was drilled in the opposite end of the tube which was then sealed with a silicone rubber serum cap. Internal ethylene concentrations in fruit from the cv. Rubyred were estimated during the 1995/6 season by extracting 1 ml gas samples from the attached tubes. Gas samples were taken at intervals of 2 or 3 days from fruit attached to the tree. Fruit with tubes attached were also harvested from 80 to 110 days after pit-hardening (DAPH) and enclosed in 0.2 l plastic chambers ventilated with water-saturated air, with or without the addition of 500 μl l⁻¹ propylene. Ethylene in the tubes and air exiting from the chambers was measured daily.

2.6. Statistical analyses

Data concerning fruit size, firmness, skin colour, SSC and TA were subjected to analyses of variance using COSTAT version 4.02 (Weasel Software, Berkeley, CA, USA). Least significant differences (LSD) were calculated using Duncan’s multiple range test. Correlation coefficients between aroma and ethylene production were calculated according to Parsons (1974). Regression analyses of fruit size and firmness were made also using COSTAT, version 4.02.

3. Results

3.1. General

In general, all parameters of fruit development were similar between the two seasons, and therefore, data from Beauty, Shiro and Rubyred are given for the 1995/6 season only. To allow a comparison between years, data from Gulfruby from both seasons are presented (Fig. 1). An exception to the similarities was observed in the peak concentrations of ethylene production and this is detailed below.

3.2. Fruit development

Pit hardening occurred about 55, 60, 70 and 90 days after full bloom during the 1995/6 season in the cvs. Gulfruby, Beauty, Shiro and Rubyred respectively and about 1 week later in 1994/5. Fruit diameter and weight of all cultivars increased after pit-hardening which was chosen as the beginning of the experiment (0-time). During 1994/5 there was a positive correlation between fruit diameter and weight ($R^2 = 0.96–0.98$ depending on cv.; $P < 0.05$ in all cases). The highest average values of 43.0, 52.0, 51.0, and 52.5 mm for fruit diameter and 47.6, 59.8, 56.5, and 127.5 g for fruit weight, recorded at harvest, occurred 31, 49, 49, and 95 DAPH in Gulfruby, Beauty, Shiro and Rubyred, respectively (Fig. 2). Fruit growth ceased before the development of full colour in Shiro and Rubyred, but in Gulfruby and Beauty fruit growth continued after full colour had developed.
3.3. Physicochemical changes

First colour appeared 14 (red), 28 (red), 28 (yellow), and 56 (deep red) DAPH and full colour was obtained 32, 49, 49, and 95 DAPH in Gulfruby, Beauty, Shiro, and Rubyred, respectively. Fruit firmness in all cultivars decreased after pit-hardening and softening continued until the fruit reached full colour (Fig. 1, Fig. 2). Soluble solids concentration (SSC) increased from pit hardening until the development of full colour in all cultivars, but titratable acidity (TA) declined during maturation (Table 1). Maximum levels of SSC were recorded after full colour developed in all cultivars.

Fig. 1. Fruit growth (A), flesh firmness (B) and skin colour development of Gulfruby plums during the 1994/5 and 1995/6 seasons. Data are the means of 60 fruit for diameter (Df), 12 fruit for fruit weight (fw) and 30 fruit for flesh firmness (ff). Second-order polynomial regression lines were fitted to each data set with $R^2$ values of 0.87 (Df 94/5), 0.83 (Df 95/6), 0.96 (fw), 0.93 (ff 94/4) and 0.92 (ff 95/6).

Fig. 2. Fruit growth (A) and flesh firmness (B) of Gulfruby, Beauty, Shiro and Rubyred plums during the 1995/6 season. Data are the means of 60 fruit for diameter and 30 fruit for flesh firmness. Second-order polynomial regression lines were fitted to each data set. The $R^2$ values for diameter were 0.83, 0.95, 0.91 and 0.96 and 0.92, 0.95, 0.94 and 0.93 for firmness for the four cvs. respectively.

Rates of ethylene and CO$_2$ production were measured daily for 6 days after harvest. In Gulfruby, ethylene evolution could just be detected 1 day after harvest in fruit taken 26 DAPH in 1994/5 and after 21 DAPH in 1995/6 (Fig. 3). After 6 days at 20°C, ethylene was detected in fruit harvested 14 DAPH in both seasons. This increase in ethylene production coincided with the appearance of first colour. The highest rate of ethylene production (120 µl kg$^{-1}$ h$^{-1}$) was recorded after 6 days storage and occurred 26 DAPH in 1994/5; in 1995/6 the maximum was only 85 µl kg$^{-1}$ h$^{-1}$ which was reached 32
Table 1
Average measurements of skin colour, soluble solids concentration, titratable acidity and SSC/TA ratio in Gulfruby, Shiro, Beauty and Rubyred plums in the 1995/6 season and for Gulfruby during 1994/5

<table>
<thead>
<tr>
<th>Maturity stages (skin colour)</th>
<th>Skin colour (hue angle)</th>
<th>SSC (%)</th>
<th>TA (mmol H+ / juice)</th>
<th>SSC/TA</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Gulfruby (1994/95):</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Re-hardening</td>
<td>126.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.15&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>First colour</td>
<td>114.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.18&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Full colour</td>
<td>48.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>39.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.33&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Re-hardening</td>
<td>126.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>49.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.16&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>First colour</td>
<td>115.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>51.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.17&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fall colour</td>
<td>51.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.35&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>C. Beauty:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Re-hardening</td>
<td>125.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.17&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>First colour</td>
<td>108.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.22&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fall colour</td>
<td>45.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.45&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>D. Shiro:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Re-hardening</td>
<td>126.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>45.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.14&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>First colour</td>
<td>112.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>41.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.19&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fall colour</td>
<td>98.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.50&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>E. Rubyred:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Re-hardening</td>
<td>131.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.18&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>First colour</td>
<td>110.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.23&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fall colour</td>
<td>50.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>32.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.40&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The values represent the means of 10 fruit taken from three trees (total 30 fruit).

Data with the same letters in each cultivar for each composition are not significantly different at P<0.05 according to Duncan’s multiple range test.

DAPH. Carbon dioxide, measured 1 day after harvest, remained constant at about 18 ml kg<sup>-1</sup> h<sup>-1</sup> for fruit harvested 7, 14, and 21 DAPH in 1994/5 (Fig. 3C) and at 7–9.5 ml kg<sup>-1</sup> h<sup>-1</sup> in fruit taken 14, 21, and 25 DAPH in 1995/6 (Fig. 1D). During storage respiration rates rose to levels in excess of 30.5 ml CO<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup> in fruit harvested 14 DAPH or later in 1994/5 and to 21.5 ml kg<sup>-1</sup> h<sup>-1</sup> in fruit from all harvest dates during 1995/6. With the exception of the harvest at pit-hardening in 1994/5, all increases in CO<sub>2</sub> production coincided with increases in ethylene production. Fruit from Beauty showed patterns of ethylene and CO<sub>2</sub> production similar to Gulfruby (Fig. 4). The main difference between these cultivars was the period after pit-hardening at which ethylene was first detected or where CO<sub>2</sub> production increased. These events occurred approximately 20 days later in Beauty than in Gulfruby (Fig. 4).

For both seasons, patterns of ethylene and CO<sub>2</sub> production were strikingly different in Shiro and Rubyred from the other two cultivars and the pattern for the 1995/6 season is presented in Fig. 5. In Shiro and Rubyred, ethylene was not produced until late in fruit development, and essentially, was only produced after full colour and size were reached. In comparison, in the cvs. Gulfruby and Beauty, ethylene was readily detected in stored fruit following the development of first colour. Furthermore, the rates of ethylene production in Shiro and Rubyred were low. Maximum rates of 4.5 and 0.1 µl kg<sup>-1</sup> h<sup>-1</sup> were recorded for these cvs. respectively (Fig. 5A, Fig. 5B) which contrasts with the values of 85 and 87 µl kg<sup>-1</sup> h<sup>-1</sup> for Gulfruby and Beauty (Fig. 4). Changes in CO<sub>2</sub> production were not clearly associated with changes in ethylene production in both cultivars and, in general, the average rates of CO<sub>2</sub> evolution in Shiro and Rubyred were half of
those produced by other two cultivars (Fig. 5C, Fig. 5D).

The production of aroma volatiles was assessed organoleptically in all cultivars. Volatile production by fruit of Beauty and Shiro occurred at the same time as the rise in ethylene production (Fig. 4, Fig. 5). In the other cultivars, aroma production was detected late in fruit development and no correlation with ethylene synthesis was found (data not shown).

3.4. Propylene treatment

Propylene (500 µl l⁻¹) was applied continuously after harvest to fruit from Gulfruby, taken 14 and 21 DAPH, and Rubyred, taken 80–100 DAPH (Fig. 6, Fig. 7). Only trace amounts of ethylene were detected in control, air-treated fruit of Gulfruby for the first 72 h. After 96 h storage, significant quantities of ethylene were produced by fruit from the 21-day harvest and after a further day in storage ethylene production in fruit harvested 14 DAPH also increased. Propylene induced ethylene within 48 h of harvest in fruit from both harvest dates. Respiration was stimulated to twice the rate of similar control fruit before any increase in ethylene was detected (Fig. 6B). The response to propylene of fruit from Beauty was similar to that of Gulfruby (data not shown).

In control, air-treated fruit of the cv. Rubyred, no ethylene could be detected for 120 h following
Fig. 4. Rates of ethylene (A) and CO₂ (B) evolution from plums of the cv. Beauty harvested at different times after pit-hardening during the 1995/6 season. Data are the means of three fruit from three trees (i.e. n = 9). The rates of gas production were measured 1 and 6 days after harvest. The numbers on graph A refer to aroma production and the equation represents the correlation between aroma and ethylene production. The error bars represent the S.E.s of the relevant means.

Harvest from fruit taken at all sampling times. In propylene-treated fruit the time between harvest and the occurrence of significant levels of ethylene production was related to harvest date: the later the harvest date the shorter the period postharvest before ethylene was produced (Fig. 7A). The magnitude of ethylene production was also related with harvest date. Propylene stimulated CO₂ production within 24 h of picking in fruit from all harvest dates (Fig. 7B). Average CO₂ production rates were doubled by propylene 24 h after the commencement of the treatment and the magnitude of the stimulation increased with time postharvest. Fruit from the later harvests also showed a trend for higher levels of CO₂ production.
3.5. Internal ethylene concentrations

To determine if harvesting stimulated ethylene production, internal ethylene concentrations were measured in three separate samples of Rubyred fruit. One sample remained on the tree whilst the two other samples were harvested. The harvested fruit were ventilated with water-saturated air and one sample was continuously treated with propylene. No ethylene could be detected in fruit which remained attached to the tree at any sampling time. At 110 DAPH these fruit were ripe, soft, aromatic and sweet and after this harvest fell from the trees.

Ethylene production from harvested fruit is presented in Fig. 8. Ethylene production from control fruit not treated with propylene correlated with harvest date. Ethylene was detected 24 h after picking from fruit from the last two harvests and, in these fruits, production increased with the number of days postharvest (Fig. 8). Fruit from the earlier harvest dates required a longer storage period before ethylene was produced and this gas could not be detected from samples taken 90 DAPH or before.
A similar pattern of ethylene production occurred in propylene-treated fruit, although the magnitude of production was clearly increased. For example, a comparison of data from the 100% harvest suggests a 300-fold ethylene increase in the propylene-treated fruit compared to control fruit.

4. Discussion

This study has concentrated on the ripening of plum cultivars due to their commercial significance and the difficulty in assessing harvest maturity in highly coloured varieties. In an attempt to overcome this difficulty, the physical and bio-
Fig. 7. Rates of ethylene (A) and CO₂ (B) production from Rubyred plums harvested 80–100 DAPH during the 1995/6 season. Data are the means of three fruit from three trees (i.e., n = 9) and represent measurements made at 24 h intervals postharvest. The fruit were enclosed singly in 0.2 L chambers at 20°C which were ventilated with water-saturated air with or without the addition of 500 µL L⁻¹ propylene. The error bars represent the S.E.s of the relevant means.

chemical changes that occurred during ripening were assessed for their suitability as indices of maturity.

Fruit size and firmness have been proposed as indices of fruit maturity (Crisosto, 1994). Plums continue to grow from pit-hardenening until they are tree ripe, a process which involves both cell division and cell enlargement (Coombe, 1976). However, fruit size alone cannot be used as a maturity index in plums, because it may vary with cultivar, fruit load, climatic conditions and cultural practices. Fruit growth in the varieties used in this study ceased when fruit were ready for harvest. However, the point where a particular
fruit or batch of fruit has ceased to grow can only
be judged retrospectively and, therefore, this time
point cannot be used as an indicator of harvest
time. Fruit firmness in the four cultivars studied
decreased gradually from pit-hardening and no
distinct changes in softening associated with
opening could be detected. Overall, it seems that
fruit enlargement during fruit growth (Kader and
Mitchell, 1989), which also makes this attribute
unsuitable as a marker of maturity.

SSC and TA have been suggested as maturity
indicators for peaches (Crisosto, 1994) and apples
(Dirinck and Schamp, 1989). Although there were
large increases in SSC and reductions in TA dur-
ing maturation in the cultivars used in this study,
these attributes and their ratio are not reliable indices of harvest maturity. Absolute values at harvest vary with fruit development period with early maturing cultivars such as Gulfruby and Beauty having high SSC and TA values compared to mid- and late-season cultivars, such as Shiro and Rubyruby. Appropriate values associated with optimum harvest date would have to be determined for each cultivar. Levels of SSC and TA have also been shown to vary with fruit position on the tree, variety, season and climatic conditions (Donn and Jerie, 1988; Kader and Mitchell, 1989), although in this study little difference between years was observed for each of the cultivars. Although SSC and TA are good indices of fruit quality in plums, like size and firmness they are again unsuitable as markers of harvest maturity.

Aroma production in fruit of Beauty and Shiro was associated with ethylene evolution and the intensity of aroma production increased with increasing emissions of this hormone. In contrast, aroma production from Gulfruby and Rubyruby gradually increased as the fruit ripened and was not correlated with ethylene production. The production of aroma volatiles is one of a number of biochemical changes which occur in fruit as they ripen. In many fruit these changes are co-ordinated by the production of ethylene during the climacteric (Spiers and Brady, 1991). In plums the co-ordinational role of ethylene appears to be variable between cultivars. Aroma production was correlated with ethylene in Beauty and Shiro but not in Gulfruby and Rubyruby. Colour production was associated with ethylene production in Gulfruby and Beauty but not in Shiro and Rubyruby. The enzyme systems associated with these physiological changes appear to be controlled by different mechanisms and the role of ethylene (if any) in their regulation provides a fascinating area for further study.

Since plums are classified as climacteric fruit, ethylene production was assayed 1 and 6 days after harvest at each successive sampling time. Differences in ethylene production between assay times revealed a strong suppression of the climacteric whilst the fruit were on the tree. This 'tree effect' has been observed in a number of other fruit such as apple (Sfakiotakis and Dilley, 1973a) and avocado (Biale and Young, 1971; Seymour and Tucker, 1993). In this study the 'tree effect' was most clearly seen in the cv. Beauty. Fruit from this cultivar harvested 28 DAPH produced ethylene within 6 days of harvest whereas the fruit which remained on the tree did not produce ethylene for a further 14 days. Fruit from the other cultivars showed this effect but to different extents. This study is the first to show the magnitude of the tree effect in plums, although the phenomenon has previously been reported in cultivars such as Late Santa Rosa, Queen Ann and Cassleman (Gonzalez et al., 1980). The many reports concerning the tree effect suggest that it is common to climacteric fruit, although the mechanism causing this effect is still unknown. Lau et al. (1986) reported that a tree factor not only delayed the accumulation of 1-aminoacyclopropane-1-carboxylic acid (ACC) in attached Golden Delicious apples, but also inhibited the conversion of ACC to ethylene indicating that the factor interferes with ethylene production. A number of studies (Sfakiotakis and Dilley, 1973a; Brecht and Kader, 1984), including this one, have shown that the tree effect can, to some extent, be overcome by applications of propylene or ethylene, also suggesting that the tree effect is mediated through the action of ethylene.

In this study two distinct types of ripening behaviour were observed. Both Beauty and Gulfruby showed ripening patterns typical of climacteric fruit with a distinct rise in CO₂ and ethylene production. In contrast, Shiro and Rubyruby exhibited what we have termed a suppressed-climacteric phenotype. In this phenotype, ethylene production rates increased during the latter stages of the ripening process but were low when compared to the climacteric cultivars and ripening attributes appeared not to be correlated with ethylene production. However, this suppressed-climacteric behaviour appears not to affect the fruit development period, as both Shiro and Beauty ripened 109 and 119 days after full bloom, respectively. Further work in our laboratory on these suppressed-climacteric types (Abdi et al. in preparation) suggests that this trait could be incorporated into plum breeding programs to produce
new varieties with improved storage lives which would facilitate shipping to distant markets.

As the role of ethylene in the ripening of these suppressed-climacteric types is very different from that in normal climacteric fruit, the question was raised as to whether these cultivars should be classed as non-climacteric. In order to clarify ethylene's role in initiating the ripening of these suppressed-climacteric types internal ethylene concentrations were measured. In fruit of the cv. Rubyred it was not possible to detect ethylene at any sampling time in the internal issues of fruit attached to the tree. In detached fruit maintained in air, internal ethylene concentrations reached a maximum of 0.12 μl l⁻¹ in fruit harvested 110 DAP after 5 days at 20°C. Since the measurement of internal ethylene concentration in fruit is difficult, estimates of this parameter were also determined using the relationship between production rates and internal concentrations proposed in plums by Burg and Urg (1962). In Rubyred the maximum production rate was 0.1 μl kg⁻¹ h⁻¹ which suggests an internal ethylene concentration of 0.4 μl l⁻¹. It generally considered that the threshold ethylene concentration for biological activity is between 0.06 and 0.1 μl l⁻¹ (Abeles et al., 1992). The internal ethylene concentrations in Rubyred are close to this threshold value and suppressed-climacteric phenotype can be explained by the failure of the fruit to produce sufficient ethylene to co-ordinate ripening. Some cultivars of pears (Dowse et al., 1991) and apples (Sakaiotakis and Dilley, 1973b) also exhibit a suppressed-climacteric phenotype and this behavior was again ascribed to an inability of fruit to produce enough ethylene to develop climacteric.

A procedure using induced ethylene climacterics has been developed as an aid to the judgment of harvest maturity in apples (Dilley and Dilley, 1985). In this procedure fruit are harvested prematurely and the time between harvest and the development of the climacteric is used to estimate harvest time. This technique may be applicable to Gehruby and Beauty where large increases in ethylene are produced. Clearly, the use of induced ethylene climacterics would not be applicable to suppressed-climacteric cultivars such as Rubyred and Shiro, as was also found for certain varieties of apples (Jobling and McGlasson, 1993).

This study has shown that none of the physiological parameters commonly used by plum growers to judge harvest maturity are reliable and can readily be adapted to all cultivars. There is still a need to develop a system which is applicable to all varieties of plums for detecting or determining ideal harvest maturity. Preliminary analyses by 2-dimensional gel electrophoresis in our laboratory has shown changes in protein profiles during maturation. If these patterns can be correlated with optimum harvest maturity it may be possible to develop a maturity test system based on the detection of changes as proposed for apple by Dilley et al. (1993). This analysis will determine if a single system can be developed for all cultivars irrespective of whether they exhibit a climacteric or suppressed-climacteric phenotype.

Acknowledgements

The authors would like to thank to Sean Lonergan and family for access to their orchard and for providing some of the fruit used in this study. We would also like to thank Gary Morgan for technical support throughout the project, Tony Haigh for his help in the statistical analysis and preparation of the graphs and Heather Nonhebel for her criticism of this manuscript. This study was partially funded by a grant (SF602) from the Horticultural Research and Development Corporation. Nasser Abdi was supported by a scholarship from the Iranian government.
References


Responses of climacteric and suppressed-climacteric plums to treatment with propylene and 1-methylcyclopropane

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Abstract

The aim of this study was to characterise further the ripening behaviour of climacteric ('Gulfruby' and 'Beauty') and suppressed-climacteric ('Shiro' and 'Rubyred') plums by treating preclimacteric fruit with 1-methylcyclopropane (1-MCP) followed by continuous treatment with propylene. Analyses showed that the development of skin colour was an ethylene-independent phenomenon, whilst aroma production was either ethylene-dependent or ethylene-independent, depending on the cultivar. Typical climacteric patterns of ripening were shown by 'Gulfruby' and 'Beauty' fruit, as the application of propylene alone advanced the onset of the respiratory and ethylene climacterics whilst 1-MCP delayed these events. 'Shiro' and 'Rubyred' fruit exhibited suppressed-climacteric patterns or ripening associated with 15–50-fold less ethylene production than the climacteric cultivars. Since 1-aminocyclopropane-1-carboxylic acid (ACC) concentrations were similar in all four cultivars, it is suggested that the suppressed-climacteric phenotype is the result of an impaired ability of the fruit to convert ACC to ethylene. Fruit of these cultivars treated with 1-MCP did not develop an ethylene or respiratory climacteric unless exogenous propylene was applied. We suggest that the inability of 1-MCP treated, suppressed-climacteric fruit to develop a climacteric results from an impaired ability of the fruit to perceive ethylene and to produce new receptors. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: ACC concentrations; Ethylene perception; 1-MCP; Propylene; Prunus salicina Lindl; Receptor(s); Suppressed climacteric

1. Introduction

Traditionally, the ripening behaviour of fruits has been categorised as being either climacteric or
non-climacteric (Biale, 1964), with the classification depending on whether or not they produce a peak in respiration (McMurchie et al., 1972). The Rosaceae family contains a number of species of commercial significance which have been placed in the climacteric category. However, our work with plums has identified what we have termed a suppressed-climacteric phenotype (Abdi et al., 1997). Fruit exhibiting this phenotype produce ethylene during the latter parts of the ripening process. However, levels of this hormone are low when compared to normal, climacteric types and they exhibit a reduced respiratory climacteric. Some cultivars of pears (Downs et al., 1991) and apple (Sfakiotakis and Dilley, 1973) also exhibit this type of ripening behaviour. In other species, such as kiwifruit (Sfakiotakis et al., 1997) and some cultivars of pears (Leijèvre et al., 1997a), the ability to develop a climacteric depends on environmental conditions. It has become evident, therefore, that placing a species into either the climacteric or non-climacteric category is an oversimplification.

Given the importance of the climacteric in determining ripening time and fruit quality, it is important that the events associated with this developmental phase are fully understood. This is especially important with stonefruit as once the climacteric has commenced they ripen quickly and may spoil before they reach their market. The ripening of climacteric fruit can be delayed by a number of inhibitors of ethylene production and action. Amongst these inhibitors, only silver thiosulphate (STS) has commercial applications. However, its continued use is questioned as silver is a potent environmental pollutant, and many countries propose to prohibit its use. Recently, 1-methylcyclopropene (1-MCP), a new inhibitor of ethylene perception, has been synthesised (Magid et al., 1971, Serek et al., 1994). This compound is non-toxic and odourless and may be a good replacement for STS (Serek et al., 1994).

The objectives of this study were to characterise further the differences in ripening behaviour between climacteric (‘Gulfruby’ and ‘Beauty’) and suppressed-climacteric (‘Shiro’ and ‘Rubyred’) plums and to determine if 1-MCP is able to delay ripening. To this end, preclimacteric fruit from the four cultivars were treated with 1-MCP and then treated continuously with propylene, an active ethylene analogue. Respiration and ethylene production were then monitored in treated and control fruit, and assessments of aroma and colour made as the fruit ripened.

2. Materials and methods

2.1. Source of fruit

All cultivars used in this study were hybrid, Japanese-type plums involving crosses to Prunus salicina Lindl. Fruit were harvested from three matched, 11-year-old trees of ‘Beauty’, ‘Shiro’ and ‘Rubyred’ cultivars grown on Myrobalan rootstocks at Bilpin, NSW, or from the early ripening cultivar ‘Gulfruby’, grown on a low chill Prunus persica stock at the Centre for Horticulture and Plant Sciences, UWS Hawkesbury. In attached fruit, pit-hardening occurred about 55, 60, 70 and 90 days after full bloom and first colour was attained 14 (red), 28 (red), 30 (yellow) and 56 (deep red) days after pit-hardening (DAPH) and full colour 32, 49, 49 and 95 DAPH for ‘Gulfruby’, ‘Beauty’, ‘Shiro’ and ‘Rubyred’ fruit, respectively. Preclimacteric fruit were harvested 15, 28, 30 and 80 DAPH.

2.2. 1-MCP synthesis and application

On the day of harvest, samples of fruit were placed in sealed 6.4 plastic containers, following which quantities of 1-MCP dissolved in methylallyl chloride were injected onto a glass filter disc within the container via a rubber port and allowed to volatilise. Application rates were designed to give 1-MCP concentrations of 13, 26 or 39 μl l⁻¹ container volume. Fruit were exposed to these concentrations for 6 or 24 h at 20°C. An additional set of fruit was enclosed and exposed to a mixture of volatile (mainly ethyl ether) that are the by-products of the synthesis of 1-MCP. 1-MCP was synthesised at UWS Nepean following the protocol of Magid et al. (1971), and was stored under nitrogen in a solution of methylallyl chloride.
2.3. Gas analysis

After treatment with 1-MCP, three fruit of each cultivar were weighed and enclosed singly in 0.2 l chambers at 20°C, then ventilated with water-saturated air at a rate of 2.0-2.6 ml min⁻¹. Three treated fruit were similarly ventilated using water-saturated air with the addition of 500 μl l⁻¹ propylene. Untreated fruit were ventilated with water-saturated air either with or without the addition of propylene to act as controls.

On the day after harvest, and at subsequent 24-h intervals, 1.0 μl samples of air exiting from the chambers were taken to determine the rates of CO₂ and ethylene production. Ethylene concentrations were measured using a Gowmae Model 580 gas chromatograph. CO₂ concentrations were determined by pulse infrared gas analysis (Horiba Model PIR-2000, Japan; Jobling et al., 1991). Measurements were standardised using calibrated compressed gas mixtures purchased from British Oxygen Co., Australia. The lowest measurable rate of ethylene production was about 0.01 μl kg⁻¹ h⁻¹.

1.24. Assessment of aroma volatiles and skin colour

Aroma volatiles produced by fruit were assessed organoleptically by ten panellists who assessed three fruit from each cultivar at each harvest date. The assessments of aroma were made using the following scale: 0, no aroma; 1, low aroma; 2, medium aroma; 3, strong aroma.

Skin colour, measured as the hue angle, was assessed daily using a Minolta CR-200 colorimeter (Japan).

2.5. ACC measurement

Samples of fruit tissue stored at −80°C were used for 1-aminocyclopropane-1-carboxylic acid (ACC) measurements. For each of two seasons (1996 and 1997), two fruit from each cultivar at each maturity stage (i.e. n = 4) were assayed for ACC concentrations according to the method of Lizada and Yang (1979).

2.6. Statistical analyses

Least significant differences were determined using Duncan’s multiple range test, and standard errors of the means were calculated with Microsoft Excel version 5.0. Correlation coefficients between aroma and ethylene production were calculated according to Parsons (1974).

3. Results and discussion

In this study we have examined the contrasting ripening behaviour shown by climacteric and suppressed-climacteric cultivars of Japanese-type plums. The two climacteric cultivars ('Gulfruby' and 'Beauty') reacted in similar ways to treatment with either 1-MCP or propylene, with only the magnitude of the changes in CO₂ and ethylene production differing. The responses of the two suppressed-climacteric cultivars ('Shiro' and 'Rubyred') to propylene and 1-MCP were also similar to each other but differed markedly from the climacteric types. Therefore, only the patterns of ethylene and CO₂ for 'Beauty' and 'Rubyred' are presented, with a summary of the data from 'Gulfruby' and 'Shiro' being given in Table 1. The suppressed-climacteric phenotype, described by Abdi et al. (1997), results from the fruit’s inability to produce sufficient amounts of ethylene to coordinate ripening efficiently. The suppressed-climacteric phenotype was again expressed in this study as the cultivars ‘Shiro’ and ‘Rubyred’ produced maximal levels of CO₂ during their climacteric, which were approximately half of those in the climacteric types; in addition, maximal ethylene production was 15-500-fold less (Figs. 1 and 2 and Table 1). Propylene increased respiration in the climacteric cultivars within 48 h of application, as would be expected after treatment with this ethylene analogue (Fig. 1D). This fast response to propylene was abolished in fruit treated with 1-MCP. This result, to our knowledge, is the first demonstration that an ethylene-specific receptor is involved in the early respiratory response shown by preclimacteric fruit to added propylene.

In comparison to the climacteric cultivars, the enhancement of respiration rates of the sup-
<table>
<thead>
<tr>
<th>Treatment</th>
<th>1-MCP (μM)</th>
<th>1-MCP (μM)</th>
<th>1-MCP (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.001</td>
<td>0.01</td>
<td>1.0</td>
</tr>
<tr>
<td>1-MCP</td>
<td>0.001</td>
<td>0.01</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Legend: ND = not determined, NC = no effect

The data were analyzed using a non-parametric test (Kruskal-Wallis) to determine the significance of the differences among treatments. The null hypothesis was rejected at a significance level of p < 0.05.
Fig. 1. Comparison of rates of ethylene (A, B), CO₂ production (C, D) and skin colour development (E, F) by control and 1-MCP treated 'Beauty' plums. After treatment, the fruit were ventilated with water-saturated air, with (B, D, F) or without (A, C, E) the continuous application of propylene at 500 μL l⁻¹. The data are the means of three fruit from three different trees (i.e., n = 9). The error bars represent S.E. of the relevant means. Filled symbols represent fruit ripened in air, open symbols represent fruit ripened in the presence of propylene: • and ○, controls; ♦ and △, 1-MCP at 13 μL l⁻¹; ■ and ▽, 1-MCP at 26 μL l⁻¹; ▲ and ▼, 1-MCP at 39 μL l⁻¹.
Fig. 2. Comparison of rates of ethylene (A, B), CO₂ production (C, D) and skin colour development (E, F) by control and 1-MCP treated 'Rubyred' plums. After treatment the fruit were ventilated with water-saturated air with (B, D, F) or without (A, C, E) the continuous application of propylene at 500 µL l⁻¹. The data are the means of three fruit from three different trees (i.e. n = 9). The bars indicate least significant difference at ≤0.05 by Duncan’s multiple range test. Filled symbols represent fruit ripened in air, open symbols represent fruit ripened in the presence of propylene: ● and ○, controls; ◇ and ▲, 1-MCP at 13 µL l⁻¹; ▲ and ▲, 1-MCP at 26 µL l⁻¹; ▲ and ▲, 1-MCP at 39 µL l⁻¹.
pressed-climacteric types by propylene was delayed, with increases in respiration occurring 4 days after the commencement of propylene treatment (Fig. 2 and Table 1). The reason for this lag is unclear, but may be a result of impaired ethylene/propylene perception. Despite this lag, propylene advanced the onset of the respiratory and ethylene climacteric in both the climacteric and suppressed-climacteric cultivars. However, the maximum rates of ethylene production from fruit of 'Shiro' and 'Rubyred' remained at a fraction of those produced by 'Gulfruby' and 'Beauty' (Table 1). The reactions of 'Shiro' and 'Rubyred' to propylene confirmed that they should be classified as climacteric, despite their low levels of CO2 and ethylene production.

Similar (though not identical) concentrations of ACC accumulated in both types of fruit during maturation and ripening (Fig. 3). This suggests that 'Shiro' and 'Rubyred' have a reduced capacity to convert ACC to ethylene, a lesion that would give rise to the suppressed-climacteric phenotype. This limitation was partially alleviated by the application of propylene, particularly in 'Rubyred' fruit. Non-1-MCP treated fruit of this cultivar, ripened in propylene, showed a 150-fold increase in ethylene production over the level of production by the appropriate controls. This suggests that the gene(s) for ACC oxidase encode a fully functional protein(s), and that propylene may have induced changes in transcript levels. In preclimacteric apple (Bufler, 1986), melon and tomato (Liu et al., 1985) fruits, a short treatment with ethylene was shown to increase ACC oxidase activity; propylene may therefore also induce a similar effect. However, if the suppressed-climacteric fruit contain a lesion preventing the conversion of ACC to ethylene, then ACC should accumulate, as was observed in the non-ripening (nor) mutant of tomato (Terai, 1993); this did not occur in 'Shiro' or 'Rubyred' fruit. Although ACC concentrations were similar during fruit development between the two classes, levels in the suppressed-climacteric cultivars were, in fact, lower. These small differences in ACC content between the climacteric and suppressed-climacteric types may be a consequence of the higher levels of ethylene production in the climacteric cultivars as the genes for ACC synthase can be upregulated by this hormone (Van Der Straeten et al., 1990, Olson et al., 1991). Alternatively, ACC may be more efficiently converted to malonyl or glutamylamino derivatives in the suppressed-climacteric cultivars.

This study has revealed other differences in the physiology of the two classes of plums. Application of 1-MCP to the climacteric cultivars, 'Gulfruby' and 'Beauty', merely delayed the onset of the ethylene climacteric. In contrast, suppressed-climacteric fruit treated with 1-MCP became overripe (soft, juicy and aromatic as described in Abdi et al., 1997) and rotted without the production of an ethylene climacteric or a clear respiratory climacteric (Fig. 2). The application of propylene to 1-MCP treated fruit had a marginal effect on the time to the onset of the ethylene climacteric. However, in 1-MCP treated fruit of the suppressed-climacteric cultivars, propylene was able to restore an ethylene climacteric. It has been proposed by Sisler and Serek (1997) that plants overcome the inhibition caused by 1-MCP through the synthesis of new receptors. The suppressed-climacteric varieties appear to be unable to do this without stimulation by an ethylene analogue.

Two models can be proposed to explain the suppressed-climacteric phenotype and the reaction of fruit expressing it to treatment with 1-MCP and propylene. The first model assumes that in normal, climacteric fruit there is a receptor that triggers autocatalytic ethylene production and that the expression of the receptor is itself enhanced by the production of ethylene. Hence, when the system is activated, there is a cycle of events that increases ethylene production and leads to the ethylene climacteric. In suppressed-climacteric cultivars there could be a lesion in the production or function of this receptor which prevents the upward spiral of ethylene production. When such plants are treated with 1-MCP, receptor levels are reduced below a level where this spiral of events cannot happen. The application of exogenous propylene, however, is able to prime and maintain the system.

A second model to explain the suppressed-climacteric phenotype assumes that there are two
receptors involved with the production of the climacteric, and in normal plants the function of the second receptor is dependent on the function of the first. This model is similar to systems 1 and 2 proposed by McMurchie et al. (1972). A lesion in the expression or function of the second receptor may result in the suppressed-climacteric phenotype. When the action of the first receptor is blocked, and perhaps a developmental period is bypassed (as would occur with 1-MCP treatment), there is insufficient signal to stimulate the production of the second receptor, hence the loss

Fig. 3. ACC concentrations in ‘Gulf Ruby’ (A), ‘Beauty’ (B), ‘Shiro’ (C) and ‘Ruby Red’ (D) plums at harvest. Optimum harvest maturity for these cultivars occurred at 25, 42, 45 and 85 days after pit hardening respectively (Abdi et al., 1997). The data are the means of two samples from each cultivar for 1996 and 1997 (n = 4). The error bars represent S.E. of the relevant means. Arrows indicate optimal harvest time for cool storage.
of the climacteric. In this second model, propylene would again act to prime and maintain the production of the second receptor.

Elements of these models have been proposed by Payton et al. (1996) for the action of the Nr gene in tomato. This gene is proposed to encode an ethylene receptor that is both developmentally regulated as well as not being regulated by ethylene (Wilkinson et al., 1995, Payton et al., 1996). It may well be a lesion in a similar gene in plums that results in the suppressed-climacteric phenotype. Also, the level of expression of the Nr gene differs in different genetic backgrounds, as the this gene is more able to block ripening in
fruit derived from the variety ‘Ailsa Craig’ than from ‘Pearson’ (Lanañan et al., 1994) and ‘Rutgers’ cultivars (McGlasson and Adato, 1976). This feature of Nr may explain the different expression of the suppressed-climacteric phenotype in the cultivars used in this study. It should also be stated that other possibilities for the suppressed-climacteric phenotype exist. Signal transduction is a complex network of events involving the interaction of many genes and their products (Trevawas and Malho, 1997), only a few of which have been identified in the pathway for ethylene signal transduction (Ecker, 1995). Lesions in these genes could also result in the suppressed-climacteric phenotype, the precise nature of which will, therefore, have to await further characterisation at the molecular level.

Skin colour changes in both the climacteric and suppressed-climacteric cultivars started before ethylene production could be detected (Fig. 1E, F; Fig. 2E, F). As ‘Shiro’ is a greenengage plum, the colour change in this cultivar during ripening was from green to yellow as chlorophyll was lost; in the other cultivars the colour change was from green to red due to the accumulation of anthocyanins. Fruit of the suppressed-climacteric types treated with 1-MCP attained full colour without the development of an ethylene climacteric (Fig. 2). The association between colour changes and ethylene varies with the type of pigment, the species and the tissue in which the pigment is being produced. Colour production, therefore, can be either ethylene-dependent or ethylene-independent (Lelièvre et al., 1997b). In plums, the role of ethylene seems to be that of a catalyst hastening and coordinating pigment production and chlorophyll loss.

In contrast to colour changes, aroma production was closely linked to ethylene levels in both ‘Beauty’ (typical climacteric) and ‘Shiro’ (suppressed-climacteric) fruit (Fig. 4). This relationship is exemplified by the elimination of aroma production in 1-MCP treated ‘Shiro’ fruit, and the restoration of aroma production when these fruit were treated with propylene. Our results differ somewhat from those found with ‘Golden Delicious’ apples. In this cultivar, ethylene production and aroma synthesis were abolished in fruit treated with 1-MCP. However, an application of ethylene for 24 h to treated fruit could not restore volatile production (Song et al., 1997) as precursor compounds were limiting. These differences between apple and plum may simply be the result of these species having characteristic aroma compounds which come from different biochemical pathways that are affected to a greater or lesser extent by ethylene.

This study has shown that 1-MCP has the potential to control the ripening of plum fruit. Single applications of this compound were able to give significant delays in the ripening of suppressed-climacteric types, whilst either pulses or a continuous low dose would be required for climacteric cultivars to achieve the same effect. Further work on the effects of 1-MCP will allow its future commercial use and, in conjunction with appropriate harvesting and environmental conditions, will extend the storage period of plums and other fruit, allowing sea freight to distant markets. This would be facilitated by the development of further cultivars expressing the suppressed-climacteric phenotype.

Acknowledgements

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References


APPENDIX II

REGRESSION ANALYSIS FOR PLUM CULTIVARS DURING 1994/95 & 1995/96 SEASONS.

SECTION 4

Appendix 4.1
Y = Fruit diameter (mm)
X = Time (days from pit hardening)

<table>
<thead>
<tr>
<th>Fruit diameter - 1994/95</th>
<th>Regression Equation</th>
<th>$R^2$</th>
<th>Prob.</th>
<th>Sig.</th>
</tr>
</thead>
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<tr>
<td>Cultivars</td>
<td></td>
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<tr>
<td>Gulfruby</td>
<td>$Y = 3.46 X - 0.071 X^2$</td>
<td>0.86</td>
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<tr>
<td>Beauty</td>
<td>$Y = 2.18 X - 0.027 X^2$</td>
<td>0.91</td>
<td>0.0000</td>
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<tr>
<td>Shiro</td>
<td>$Y = 2.40 X - 0.032 X^2$</td>
<td>0.90</td>
<td>0.0000</td>
<td>***</td>
</tr>
<tr>
<td>Rubyred</td>
<td>$Y = 1.42 X + 1.050 X^2$</td>
<td>0.96</td>
<td>0.0000</td>
<td>***</td>
</tr>
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</table>

Appendix 4.2
Y = Fruit weight (g)
X = Time (days from pit hardening)

<table>
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<th>Fruit weight - 1994/95</th>
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<th>$R^2$</th>
<th>Prob.</th>
<th>Sig.</th>
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<tr>
<td>Cultivars</td>
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<td></td>
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</tr>
<tr>
<td>Gulfruby</td>
<td>$Y = 2.07 X - 0.016 X^2$</td>
<td>0.97</td>
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<tr>
<td>Beauty</td>
<td>$Y = 0.96 X + 0.004 X^2$</td>
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<td>0.0000</td>
<td>***</td>
</tr>
<tr>
<td>Shiro</td>
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<td>0.99</td>
<td>0.0000</td>
<td>***</td>
</tr>
<tr>
<td>Rubyred</td>
<td>$Y = 1.47 X - 0.009 X^2$</td>
<td>0.95</td>
<td>0.0000</td>
<td>***</td>
</tr>
</tbody>
</table>

Appendix 4.3
Y = Flesh firmness (N)
X = Time (days from pit hardening)
### Flesh firmness - 1994/95

<table>
<thead>
<tr>
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<th>Regression Equation</th>
<th>R²</th>
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<th>Sig.</th>
</tr>
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<tbody>
<tr>
<td>Gulfruby</td>
<td>Y = 10.32 X - 0.331X²</td>
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<td>***</td>
</tr>
<tr>
<td>Beauty</td>
<td>Y = 7.00 X - 0.150 X²</td>
<td>0.51</td>
<td>0.0001</td>
<td>***</td>
</tr>
<tr>
<td>Shiro</td>
<td>Y = 6.64 X - 0.141 X²</td>
<td>0.52</td>
<td>0.0000</td>
<td>***</td>
</tr>
<tr>
<td>Rubyred</td>
<td>Y = 3.92 X - 0.037 X²</td>
<td>0.66</td>
<td>0.0002</td>
<td>***</td>
</tr>
</tbody>
</table>

Appendix 4.4
Y = Fruit diameter (mm)
X = Time (days from pit hardening)

### Fruit diameter - 1995/96

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Regression Equation</th>
<th>R²</th>
<th>Prob.</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gulfruby</td>
<td>Y = 15.70 X - 1.350 X²</td>
<td>0.96</td>
<td>0.0002</td>
<td>***</td>
</tr>
<tr>
<td>Beauty</td>
<td>Y = 10.50 X - 0.525 X²</td>
<td>0.96</td>
<td>0.0015</td>
<td>**</td>
</tr>
<tr>
<td>Shiro</td>
<td>Y = 10.96 X - 0.570 X²</td>
<td>0.95</td>
<td>0.0003</td>
<td>***</td>
</tr>
<tr>
<td>Rubyred</td>
<td>Y = 5.89 X - 0.165 X²</td>
<td>0.97</td>
<td>0.0000</td>
<td>***</td>
</tr>
</tbody>
</table>

Appendix 4.5
Y = Flesh firmness (N)
X = Time (days from pit hardening)

### Flesh firmness - 1995/96

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Regression Equation</th>
<th>R²</th>
<th>Prob.</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gulfruby</td>
<td>Y = 7.90 X - 0.249X²</td>
<td>0.45</td>
<td>0.0001</td>
<td>***</td>
</tr>
<tr>
<td>Beauty</td>
<td>Y = 5.94 X - 0.125X²</td>
<td>0.45</td>
<td>0.0002</td>
<td>***</td>
</tr>
<tr>
<td>Shiro</td>
<td>Y = 5.66 X - 0.116X²</td>
<td>0.48</td>
<td>0.0000</td>
<td>***</td>
</tr>
<tr>
<td>Rubyred</td>
<td>Y = 3.10 X - 0.029X²</td>
<td>0.60</td>
<td>0.0000</td>
<td>***</td>
</tr>
</tbody>
</table>