CHAPTER 1

LITERATURE REVIEW

This thesis has been compiled as a series of stand-alone but related chapters reporting on various ecophysiological and applied aspects of the avocado. As each chapter carries its own literature review both in the introductory remarks and the discussion of results it is not this authors' intention to provide a detailed literature review at this point which would duplicate information provided in later chapters. Instead a brief review covering some of the broader aspects which relate to this thesis is offered. For comprehensive reviews on the environmental physiology of avocado and on trunk-injected phosphonates for Phytophthora root rot control of avocado see Whiley and Schaffer (1994)[‡] and Guest *et al.* (1994)[‡‡] .

1. 1 ENVIRONMENTAL PHYSIOLOGY

Since 90 to 95% of dry weight of plants is derived from atmospherically fixed carbon the importance of photosynthesis to crop productivity is self-evident. Nevertheless, CO_2 assimilation (A) seldom limits crop productivity which is usually more a function of dry matter partitioning to the harvested organs (Evans 1975). However, there is increasing evidence of endogenous and exogenous factors which periodically limit A and may potentially effect crop yield (Flore and Lakso 1989; Arp 1991; Thomas and Strain 1991; Schaffer *et al*. 1994). Light, temperature and water are amongst the most important environmental parameters influencing crop photosynthesis. In well managed horticultural systems water is usually not a limiting factor and this review focuses on the response of avocado to light and temperature.

[‡] **Whiley, A.W. and Schafffer, B., 1994.** Avocado. In: *Handbook of Environmental Physiology of Fruit Crops, II: Sub-tropical and Tropical Crops.* B. Schaffer and P.C. Anderson (Eds.). CRC Press Inc., Boca Raton, Florida. pp. 3-35.

[‡‡] **Guest, D.I., Pegg, K.G. and Whiley, A.W., 1994.** Control of *Phytophthora* diseases of tree crops using trunk-injected phosphonates. *Hort. Rev.* 17, 299-330.

1. 1. 1 Irradiance

Growth

In a six-week study under glasshouse conditions, Chirachint and Turner (1988) were unable to demonstrate any effect of reduced light on growth of cv. Fuerte trees. Plants were grown at maximum PPFs of ≈ 1350 μmol quanta m^{-2} s^{-1} (full sunlight in the glasshouse at noon on a clear day) and under shade which reduced PPFs to ≈ 725 μmol quanta m^{-2} s^{-1}. New shoot growth was produced during the experiment, but there were no differences in dry mass accumulation, top:root ratios and stomatal conductance (g_s) between treatments. Since shading did not reduce g_s or biomass accumulation, it was assumed that photosynthesis was not significantly reduced in plants grown under 54% of incident light. Scholefield *et al*. (1980) observed that for container-grown avocado trees, the light saturation for *A* was at a PPF of < 600 μmol quanta m^{-2} s^{-1}. In Chirachint and Turner's (1988) experiment, trees growing in 54% full sunlight for the major portion of the day would receive above the PPF for maximum *A*, accounting for the lack of growth differences. Incident light below 600 μmol quanta m^{-2} s^{-1} would only occur early in the morning, late in the afternoon, or on overcast days. Hence, with respect to this data it could be argued that for most of the day both treatments gave light saturating levels of PPF and the small differences that occurred with shading may have only been expressed by a longer time frame for the experiment.

Light interception and orchard design

Dry matter accumulation in many crops is directly proportional to the interception of radiant energy by the canopy (Duncan *et al.* 1973; Monteith 1977; Jackson 1978). At full canopy development, annual crops usually intercept most of the available light (Sceicz 1974). However, full canopies of orchard crops only intercept 65 to 70% of the available radiant energy, thereby limiting potential production (Jackson 1980). In most orchard crops advanced technology is available to control and limit tree size, e.g. dwarfing rootstocks, pruning and root restriction (Heinicke 1964; Jackson and Blanco 1973; Williamson *et al*. 1992). These techniques allow the development of a relatively stable planting density with tree size contained once filling their allocated space. This is not the case for avocados as the polyaxial, terminally flowering architecture of the avocado dictates that to remain productive the tree must continue to increase in size. This presents some unique problems which are yet to be satisfactorily resolved.

High-density orchards initially offer the most effective means of maximising light interception and yield during the first few years following planting due to a greater number of finite canopies in a given area. In the absence of dwarfing rootstocks, management strategies must be designed to maintain effective lighting of the side canopies of individual trees as the orchard matures and trees begin to crowd. In California, high-density planting and progressive tree removal is advocated (Lee 1974). This provides for precocity in the early years of the orchard with sustained production at maturity. Trees are planted 6 m apart (280 trees ha^{-1}) and are removed in two stages as they become crowded. A 12-year plan results in mature orchards with trees 12 m apart (70 trees ha^{-1}). Failure to thin high-density orchards results in a decline in yield once tree crowding begins (Platt *et al*. 1970).

Variations of this strategy have been adopted in subtropical regions with initial planting densities ranging from 200 trees ha^{-1} to 400 trees ha^{-1} while experimental blocks at 800 trees ha^{-1} have been established using paclobutrazol (Cultar®; a plant growth retardant) to reduce tree growth (Toerien and Basson 1979; Köhne and Kremer-Köhne 1991; Crane *et al*. 1992). At 800 trees ha^{-1}, cumulative yield was doubled over the first five years compared with the standard planting density of 400 trees ha^{-1}, and girdling of trees in the year of thinning also substantially increased yield (Köhne and Kremer-Köhne 1991; Köhne 1992).

Timely management of the orchard canopy is critical to maintain fruit production. In South Africa, tree thinning has been recommended when 90% of the orchard floor becomes shaded (Toerien and Basson 1979). When alternate diagonal rows were removed in a 6 m x 6 m spaced planting, thereby reducing tree numbers to half, yields increased by 50% the following season compared with trees remaining at the original planting density. In Florida, Crane *et al*. (1992) rejuvenated crowded orchards which had lost one third to two thirds of their lower canopy, by topping and tree removal. Trees had been planted at 276 ha^{-1} and before treatment were yielding 6.9 t ha^{-1}. By topping trees at 4.8 m and reducing numbers to 130 trees ha^{-1}, fruit yield increased to 19.8 t ha^{-1} three years after treatments began. Although results clearly demonstrate the benefits from well-lit orchard canopies, there is a lack of information on light interception *per se*, and a study of the dynamics of light interception in a growing orchard and its impact on yield, would significantly contribute to better informed orchard management. This is particularly important in the humid subtropics, where growth is far more vigorous than in semi-arid production areas such as California, Chile and Israel.

Leaf Gas Exchange

(i) Effect on stomatal response

There is considerable evidence that stomates in avocado leaves respond to changes in light. Scholefield *et al*. (1980) showed that at 0600 and 1800 hrs when PPF was near zero, g_s was < 10 mmol m^{-2} s^{-1} compared to a day peak of 170 mmol m^{-2} s^{-1}, indicating that stomates close during the night. This diurnal response to PPF was also reported by Bower (1978), who measured pre-dawn g_s at < 10 mmol m^{-2} s^{-1}, increasing to ≈ 200 mmol m^{-2} s^{-1} by 0800 hrs. Similar responses have been reported for citrus (Hall *et al.* 1975), apples (Warrit *et al*. 1980) and macadamia (Lloyd 1991). Stomatal response to PPF has also been shown to occur in avocados independent of atmospheric and internal water deficit. When PPF was reduced from > 1700 μmol quanta m^{-2} s^{-1} to < 130 μmol quanta m^{-2} s^{-1}, g_s in both well-watered and stressed trees fell by $\approx 40\%$ (Sterne *et al*. 1977).

(ii) Effect on CO_2 assimilation

The relatively few studies reported in the literature on the effects of light on net CO_2 assimilation (*A*) in mature leaves of container-grown avocados are in general agreement that light saturation for *A* occurs between PPFs of 400 to 660 μmol quanta m^{-2} s^{-1}, or between 25 to 33% of full sunlight (Bower *et al*. 1978; Kimelmann 1979; Scholefield *et al*. 1980). Mature leaves of container-grown plants (cv. Fuerte) had a light saturation point for *A* at PPFs between 400 to 500 μmol quanta m^{-2} s^{-1}, i.e. 20 to 25% of full sunlight (Scholefield *et al*. 1980). For whole-tree canopies of container-grown trees (cv. Edranol) light saturation was reached at about 33% of full sunlight (PPF ≈ 660 μmol quanta m^{-2} s^{-1}, estimated from radiation units) (Bower *et al*. 1978). The higher light saturation point for a population of leaves measured by Bower *et al*. (1978) is consistent with results reported for apple (Lakso and Seeley 1978). It is attributed to a portion of canopy leaves being shaded, thereby requiring a greater PPF to saturate all leaves in the canopy compared to the PPF required to saturate individual leaves (Lakso and Seeley 1978).

1. 1. 2 Temperature

Limitations to Crop Production

The three ecological races of avocado provide a wide range of temperature adaptation. The Mexican race is considered the most cold-tolerant, the Guatemalan race is intermediate in cold-tolerance and the West Indian race the most tropically adapted (Bergh 1975; Sampson 1986). Their respective ability to withstand freezing temperatures reflects the different cold tolerances of Mexican and West Indian races (Scorza and Wiltbank 1976). Containerised plants pre-hardened to cool temperatures, and then exposed for 1 hr to freezing conditions, showed that lethal damage to Mexican race seedlings occurred at approximately -7.0^{o}C, while West Indian seedlings suffered lethal damage at -5.6^{o}C. These results are further supported by the field observations of Campbell *et al*. (1977) following a severe freeze in southern Florida in 1977. Temperatures fell to ≈ -4^{o}C for 12 hrs, causing varying degrees of damage to mature avocado trees. West Indian race cultivars were the most severely damaged, with large branches killed. Damage was less to Guatemalan race cultivars, which suffered minimal structural damage to twigs and small branches, while Mexican race cultivars only lost leaves, sustaining no damage to wood.

The record freeze in California during 1990 provided additional information on critical temperature limits for tree survival under field conditions. The damage sustained by trees was confounded by the length of exposure to sub-zero temperatures, soil moisture and tree health factors (Witney and Arpaia 1991). Mature trees of ‘Zutano’ (Mexican race) were severely damaged or killed following 14 consecutive nights where temperatures were at or below -2.8^{o}C with eight of these nights below -6.7^{o}C. ‘Hass’ trees suffered minimal damage to shoot terminals when exposed to ≈ -4^{o}C for 12 hours on two consecutive nights, but trees were defoliated and fruit damaged when temperatures were between -4^{o}C to -8^{o}C for 10 nights. Fruit are generally more sensitive to chill and may drop without significant visible damage to the foliage, 8 to 10 days after temperatures of -4^{o}C to -5^{o}C (Witney and Arpaia 1990).

Growth

In controlled temperature studies with grafted ‘Fuerte’ and ‘Hass’ trees, growth and dry matter accumulation were maximised when temperatures were held between the range of $21/14^{o}$C to $33/26^{o}$C (day/night) (Lahav and Trochoulias 1982). Root and shoot dry matter partitioning had a similar pattern to that described by Whiley *et al.* (1989) for mango with reduced allocation to roots as temperature increased. Growth and dry matter accumulation in ‘Hass’ was less affected by the low and high temperature extremes than ‘Fuerte’, which had reduced growth at temperatures below or above $25/18^{o}$C and above $29/22^{o}$C. The greater environmental adaptability of ‘Hass’ is no doubt an important reason for its commercial viability under diverse climatic conditions.

The effect of root temperature on growth has been reported from several studies (Haas 1939; Yusof *et al*. 1969; Whiley *et al*. 1990). Although experimental conditions were not identical, root dry matter production was generally highest with soil temperatures were between 18 to 28^{o}C. Dry matter accumulation in roots was reduced at soil temperatures of 32^{o}C and at 13^{o}C (Yusof *et al*. 1969; Whiley *et al*. 1990).

Leaf Gas Exchange

(i) Net CO_2 assimilation

The response of photosynthesis to temperature is complex due to the multitude of changes in physical and biological processes which occur as a function of temperature. Vapour pressure deficit (temperature responsive) has a strong effect on the stomatal component of gas exchange of several tree fruit species (Jones *et al*. 1985) including avocado (Sterne *et al*. 1977). When defining short-term effects as measured in temperature response curves, factors such as leaf age (Schaffer *et al*. 1991), and prior conditioning of leaves (Flore and Lakso 1989) will increase the complexity of interpreting the response. In container-grown ‘Edranol’ trees, $A_{\max}$ was observed at air temperatures of 20 to 24^{o}C (Bower *et al*. 1978). Within $\pm$ 5^{o}C of this temperature range, A declined by $\approx$ 20%. While relative humidity was measured during the determinations, there is no indication that changes in VPD were monitored when establishing the response of A to temperature. The photosynthetic characteristics of container-grown ‘Fuerte’ trees indicated that the optimum temperature for A was between 28 to 31^{o}C; A was maintained at approximately 33%

of maximum at temperatures below 15^{o}C and above 40^{o}C (Scholefield *et al*. 1980).. However, it is again unclear if VPD was considered in establishing the response of *A* to temperature.

(ii) Photosystems I and II

Chlorophyll fluorescence research has indicated the presence of a heat acclimation mechanism in avocado leaves which prevents damage to the Photosystem II (PS II) over a moderate increase in temperature (from 21 to 35^{o}C) (Havaux and Lannoye 1987). The two photosystems of photosynthesis preferentially absorb light of different wavelengths, Photosystem I (PS I) from the far-red edge of the spectrum (>690 nm) and PS II below 690 nm. Normally the light energy absorbed by the chlorophyll *a/b* light-harvesting complex is equally distributed between PS I and PS II; however, the regulation of light distribution can be disrupted when plants are exposed to environmental conditions which induce stress (Baker *et al*. 1983; Havaux and Lannoye 1985; Havaux *et al.* 1987). Avocado leaves were exposed to saturating blue-green (≈ 450-550 nm) or far-red light (≈ 728 nm), respectively, and the light distribution ratio of PS II:PS I (β) calculated for each wavelength from chlorophyll fluorescence measurements (Havaux *et al.* 1987). At 20^{o}C there was an adjustment in β from 49% with blue-green light to 58% when leaves were irradiated with far-red light. However, at 35^{o}C there was a relatively equal distribution of light energy between the two photosystems independent of spectral quality. The adjustment of β was reversible and occurred when temperatures exceeded 25^{o}C, increasing almost linearly to 37^{o}C. Irreversible heat damage to PS II, measured by the inhibition of O_2 evolution, was detected when temperatures exceeded 37^{o}C (Havaux *et al.* 1987). As PS II is much more heat sensitive than PS I, it was concluded that the adjusted light distribution in favour of PS II as temperatures increase provides a protective mechanism limiting over-excitation and subsequent photo-oxidative damage to PS II (Havaux and Lannoye 1987). The significance of the heat acclimation mechanism in avocado leaves to prevent photo-oxidative damage to PS II under field conditions is uncertain, since in full sun (full spectral range of light) light distribution between photosystems may differ to that where specific wavelengths were used to target either PS I or PS II.

1. 2 CARBOHYDRATE CYCLING IN TREE FRUIT CROPS

Trees accumulate carbohydrate reserves during periods of excess production and deplete these when use exceeds the rate of photoassimilation. They are highly integrated systems of competing carbohydrate sinks with large spatial and temporal variations occurring in the use of reserve and currently produced carbohydrates, for metabolism and growth of shoots, stems, roots and reproductive structures (Kozlowski 1992). Patterns of carbohydrate allocation within trees also vary seasonally and between deciduous and evergreen trees. Deciduous trees produce new leaves in the spring at low carbon cost per unit of leaf area but at high cost to reserve carbohydrates (Dickson 1989). By comparison the carbon costs of evergreen leaves are relatively high (Pearcy *et al*. 1987). Only a small percentage of the foliage of most evergreens is renewed annually. The older leaves remain photosynthetically active and carbon gain may be similar to that in fast growing deciduous trees (Dickson 1989; Matyssek 1986).

Spatial and temporal variations have been shown in the allocation of carbohydrates for growth of different organs and tissues (Cannell 1985, 1989). The variations in partitioning are often strongly influenced by edaphic and climatic factors. For instance, some evergreen shrubs and trees of semi-arid environments partition proportionally more carbohydrates to the foliage and roots (and less to stems and branches), than do species of more mesic habitats (Miller *et al.* 1990). Similarly, soil water or nutrient depletion often results in preferential allocation of carbon to roots rather than shoots (Kozlowski *et al.* 1991) while reduced PPF maybe followed by greater allocation to shoots than roots (Ledig 1983). Where stress is alleviated by irrigation or fertilisation there is greater carbohydrate allocation to shoots, thus decreasing the root-shoot ratio (Ledig 1983; Axelsson and Axelsson 1986). Cannell (1989) reported that improved mineral nutrition decreased the rate of turnover of fine roots and stimulated shoot growth.

Reproductive structures are strong sinks for carbohydrate (Cannell 1971, 1985; Kozlowski *et al.* 1991). This is shown by lower amounts of nonstructural carbohydrates in branches, stems and roots of bearing trees than in those on non-bearing trees. Examples include *Carya illinoensis* (Worley 1979; Wood and McLeans 1981), *Citrus spp.* (Jones *et al*. 1975; Smith 1976; Goldschmidt and Golomb 1982), *Coffea arabica* (Wormer and Ebagole 1965), *Malus* (Mochizuki 1962; Hansen 1967; Grochowska 1973), *Mangifera indica* (Suryanarayana and Rao 1976), *Persea americana* (Cameron and Borst 1938; Scholefield *et al*. 1985), *Phoenix dactylifera* (Aldrich and Young 1941), *Pistacia vera* (Crane *et al*. 1976; Takeda *et al.* 1980), P*runus avium*

(Roper *et al*. 1988), *Prunus domestica* (Ryugo *et al.* 1977; Hansen and Ryugo 1979; Hansen *et al.* 1982) and *Prunus persica* (Ryugo and Davis 1959). Considerable success has been achieved in increasing the partitioning of dry matter to fruit over vegetative tissue (Jackson 1985; Wolstenholme *et al.* 1990; Whiley *et al.* 1991). Heavy fruit loads lead to starch depletion and death of feeder roots of citrus trees (Smith 1976) which has been suggested as a factor contributing to alternate bearing patterns (Monselise and Goldschmidt 1982). In small roots of ‘Kinnow’ mandarin trees starch content was reduced by half in “on” (cropping) years compared with “off” years (Jones *et al*. 1975) while in ‘Wilking’ mandarin trees starch concentrations in roots were 17.4 times higher in non-bearing trees compared with bearing trees (Goldschmidt and Golomb 1982).

Scholefield *et al.* (1985) reported on seasonal carbohydrate concentrations, shoot growth, floral initiation and yield of cv. Fuerte avocado growing in a semi-arid Mediterranean climate in southern Australia. Trees showed a marked alternate bearing cycle which was directly related to starch content in large branches (Fig. 1). Heavy yield (“on” year) followed a high accumulation of starch in large branches (ca. 18%) during the preceding winter which declined with flowering, shoot growth and fruit development. The “on” year was characteristically followed by low winter starch levels (ca. 3%) in branches and subsequently low yield. The concentration flux of soluble sugars was also determined but showed little seasonal variation and was not considered to be a major source of storage carbohydrate but rather a “pool” for immediate use within the tree.

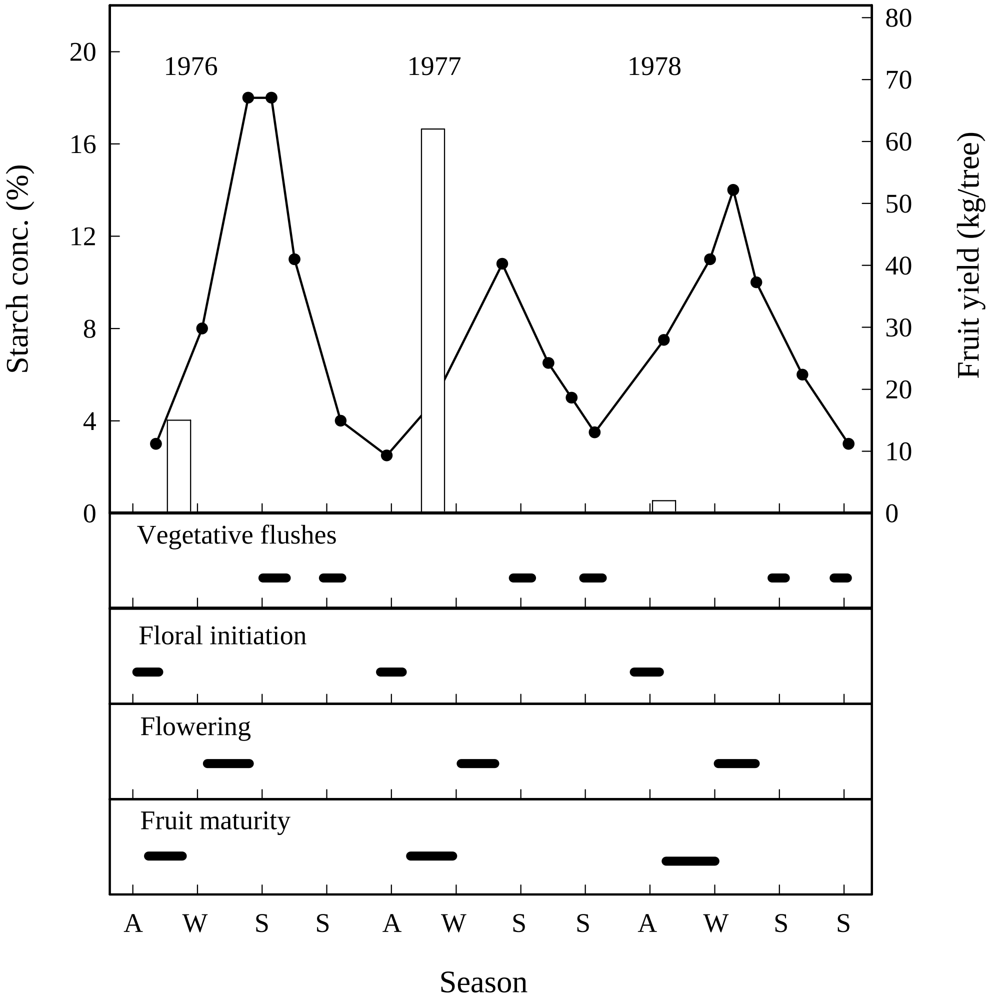

Fig. 1 Diagrammatic representation of phenological events and variations in starch concentrations (large branches) of cv. Fuerte avocado trunk wood from trees grown at Coomealla, Murray Valley Irrigation Area, southern Australia. Histograms show the yield of fruit recorded each year. Redrawn from Scholefield *et al.* (1985).

1. 3 AVOCADO AND TRUNK-INJECTED PHOSPHONATES

Trunk injection is a technique that delivers a low volume of fungicide with minimum wastage and environmental contamination, and with maximum persistence. If the fungicide is phloem-translocated, the fungicide will move according to the source-sink balance within the plant.

Xylem-translocated fungicides will move with the transpiration stream to the leaves. Both modes of translocation may be exploited to target the infection site of the pathogen.

The only commercially available phloem-translocated fungicides are the phosphonates. These are active against oomycetes such as *Phytophthora* and *Pythium* species and the downy mildews, all serious pathogens of horticultural species. A comprehensive record of the diseases controlled in horticultural crops by phosphonates is published in *Australasian Plant Pathology* (1990), 19 (4).

The earliest published agricultural research with phosphonates was in the 1930's, when salts of phosphonic acid were tested as substitutes for phosphate fertilisers in Germany (Mengdehl 1933). Later studies in the USA (McIntyre *et al*. 1950, Lucas *et al*. 1979) confirmed Mengdehl’s conclusion that phosphonates were poor phosphorus fertilisers. In 1977 Rhone-Poulenc released Aliette, a fungicide containing fosetyl-Al (aluminium tris-O-ethyl phosphonate). This remarkable chemical has activity against not only stem and foliar, but also soil-borne diseases caused by oomycete pathogens such as *Phytophthora* and *Pythium*. These diseases had always been considered recalcitrant because conventional fungicides with protectant or eradicant activity, had no therapeutic activity against existing root infections. Aliette was quickly adopted in the fight against oomycete pathogens. As a wettable power (Aliette WP) it was used as a conventional soil drench and foliar spray.

The precise biochemical mode of action of phosphonate against oomycetes is yet to be elucidated, but it is almost certain that phosphonate disrupts phosphorus metabolism leading to a fungistatic state of induced phosphate starvation (Barchietto *et al*. 1990). Why this effect is specific for oomycetes is a matter of fundamental as well as practical interest.

What is of immediate interest to horticulturalists is that the state of phosphate starvation leads to a serious disruption of virulence mechanisms of the pathogen. As a result, phosphonate-treated plants respond to inoculation as if they were resistant, and their dynamic defence mechanisms are significantly enhanced (for a review see Guest and Grant 1991). Thus the effectiveness of phosphonates against plant diseases caused by oomycetes depends not only on the sensitivity of the pathogen to phosphonate, but also the latent resistance of the host and environmental factors, combining to form a "complex mode of action" (Guest and Bompeix 1990, Guest and Grant 1991).

One apparent advantage of this complex mode of action is that failure of disease control due to resistance has not been confirmed after almost 20 years of use. A disadvantage is that disease control in one host cultivar-pathogen isolate combination cannot always be extrapolated from results from analogous combinations, because the sensitivity of different pathogen isolates varies *in vitro*, and because of the different latent defence capacity of different host cultivars (Guest *et al*. 1994).

Root rot of avocado caused by *Phytophthora cinnamomi* is the limiting factor for avocado production in most areas of the world (Zentmyer 1980). The fungus attacks the fine white feeder roots of the avocado tree producing a brownish-black firm rot, and only occasionally affects the suberised woody tissue of the major roots or collar. Trees affected by root rot usually wilt, defoliate and eventually die. Although rootstocks such as ‘Duke 7’, ‘Barr Duke’, ‘Thomas’, ‘G755’, ‘G6’ and ‘Toro Canyon’, which provide limited resistance to root rot (Coffey *et al.* 1988), are now available, control in the past has been largely based on careful site selection to avoid areas of impeded soil drainage. Chemicals such as metalaxyl (Ridomil) and fosetyl-Al (Aliette) became available for testing in the mid 1970's but failed to live up to expectations. Soil applications of a granular formulation of Ridomil initially provided good recovery of root rot affected trees but soon problems developed due to the rapid biodegradation of the chemical in the soil (Pegg *et al.* 1987). Foliar applications of Aliette WP six times a year have rarely been used commercially due to the slow response of affected trees.

The development of the trunk injection technique for root rot control in South Africa (Darvas *et al.* 1983) and the successful field testing and registration of trunk-injected potassium phosphonate in Australia (Pegg *et al.* 1985) added a new dimension to root rot control. Trunk injection is now used in all parts of the world where root rot is a problem. Phosphonates are also effective for root rot control when applied as a foliar spray, through chemigation or as a trunk paint (Snyman and Kotzé 1983, 1984) but injection is the preferred application technique because of quicker tree recovery and lower cost. Initial concerns about the detrimental effects on tree health were soon allayed. Extensive white exudation of the unique seven-carbon sugar mannoheptulose, weeps from the injection wound. This is a normal injury reaction from the tree and as the compound is highly water soluble, it quickly disappears during wet weather. Infection holes callus rapidly without secondary infections and no long term adverse effects on tree health, even though a dark-brown wood discolouration is present in the xylem tissue at the injection site (Guest *et al*. 1994).

The initial use of trunk-injected phosphonates to control root rot of avocado relied on several injections of the formulation over the duration of the high risk period for infection (spring through to autumn) (Pegg *et al*. 1987). However, due to increasing labour costs for trunk injection more efficient application methods were sought and research leading to the more precise timing of application is detailed in Chapters 5 and 7 of this thesis.