ECOPHYSIOLOGICAL STUDIES AND TREE MANIPULATION FOR MAXIMISATION OF YIELD POTENTIAL IN AVOCADO (*Persea americana* Mill.)

by

Anthony W. Whiley

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ABSTRACT

Tree fruit crops generally consist of scion and rootstock components, which through interactive synergism affect tree performance. Coupled with tree architecture, sink/source relationships (both spatial and temporal), genotypic responses to environments, and carry-over seasonal effects present a high level of complexity which often confounds research results. The development, description and use of pheno/physiological models as research and crop management tools is a new holistic approach to reduce complexity and improve understanding of the critical factors which influence crop productivity.

A pheno/physiological model is described for cv. Hass avocado growing in a cool, mesic subtropical environment in S.E. Queensland, Australia. Seasonal shoot and root growth had bimodal periodicity with root growth offset and delayed with respect to shoot growth. The priority sink strength of developing shoots compared with roots was confirmed with ^{14}C studies. Root growth in summer extended through until late winter when there was a substantial decline following anthesis - a critical time in fruit development with competition between reproductive and vegetative sinks for limited resources. Delayed harvesting of fruit over several seasons resulted in alternate bearing patterns, while removal of fruit at the minimum legal maturity of 21 to 24% dry matter sustained successive high yields. With cv. Hass, production was directly related to starch concentrations in trunks or shoots in July (mid-winter) immediately prior to anthesis. However, seasonal starch concentration fluxes in trunks were much lower in coastal subtropical Australia compared with those previously reported from interior areas in more southerly latitudes (7.5% vs. 18% maximum). Current assimilate from over-wintered leaves was necessary to bridge the gap in early spring between the depletion of starch reserves by new reproductive and vegetative shoot growth, and the sink/source transition of the spring shoot growth.

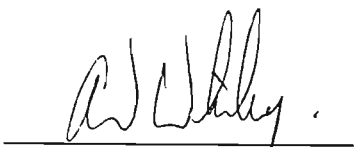
Net CO_2 assimilation of summer grown leaves reached ca. 17 $\mu mol\ CO_2\ m^{-2}\ s^{-1}$, approximately twice as high as previously reported rates on container-grown plants or trees in

minimum temperatures were < 10°C for 50 days, this being the first report of this phenomenon in field-grown avocado trees. Partial recovery occurred prior to senescence of previous season's leaves in spring after minimum temperatures increased above 10°C. The plasticity of the light response was high with the compensation point for net CO_2 assimilation at 30 μmol quanta m^{-2} s^{-1} and the light saturation point at 1270 μmol quanta m^{-2} s^{-1}. Net CO_2 fixation from fruit photosynthesis was always less than losses through respiration but was highest during the first few weeks of ontogeny, perhaps contributing to the fruit's own carbon economy at a time when competition for assimilates was greatest. In general, CO_2 assimilation studies with current technology applied to orchard trees in non-restrictive soils have elucidated efficiencies more akin to deciduous than evergreen trees - thereby compensating for short-lived leaves and energy expensive fruits.

Pheno/physiology models were used to substantiate the most effective timing for trunk injection of ambimobile phosphonate fungicides for the control of Phytophthora root rot, a serious disease of avocados, viz. at the completion of the leaf expansion phases when leaves were strong net exporters. Preliminary studies demonstrated potential yield increases when the assimilation efficiency of photoinhibited over-wintered leaves was improved through increased nitrogen concentration, and spring shoot growth was partially suppressed with foliar sprays of the growth retardant paclobutrazol.

DECLARATION

I hereby declare that the research reported in this thesis is the result of my own investigations, except where acknowledged, and has not, in its entirety or in part, been previously submitted to any University for degree purposes.

Signed 

(A W Whiley)

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TABLE OF CONTENTS

INTRODUCTION

The avocado is a fruit of subtropical and tropical central America and Mexico though its precise origin is obscure due to its long history of cultivation. The name for this fruit is derived from the corruption of the Spanish *ahuacate* or *aguacate*, which in turn are adaptations of the Aztec *ahuacatl* (Popenoe 1920). Its centre of diversification is thought to encompass the subtropical and highland tropical areas of Mexico, Guatemala and Honduras (Kopp 1966) where it grows as a rainforest species. When the Spanish arrived in central America the avocado was cultivated from Mexico to Peru (Hodgson 1950) where its high food value was recognised by the indigenous people. Activities associated with the European colonisation of this region soon carried the avocado to Venezuela (Serpa 1968), the West Indies (Hume 1951), Chile (Schmidt 1965), the Madeira and Canary Islands (Cabezon 1965) and other areas of the Spanish Empire suiting its cultivation. Today it is grown in many areas of the world encompassing diverse environments ranging from semi-arid/Mediterranean to humid/tropical thus demonstrating the adaptability of this fruit crop. Total world production of avocado in 1991 was estimated at 2,036,000 tonnes (Anon. 1991).

Avocado (*Persea americana* Mill.) (syn. *Persea gratissima* Gaertn. f., 1807; *Persea drymifolia* Schlecht. & Cham. 1831; *Persea nubigena* Williams 1950), belongs to the aromatic laurel family (Lauraceae) of which only one other genus, *Cinnamomum*, is appreciably cultivated yielding cinnamon and camphor (Bergh 1975). All *Persea* species studied have a chromosome number of 2n = 24 (Bergh 1975). Three ecological races are identified within *P. americana* and are given varietal status within the species (Bergh *et al.* 1973; Bergh and Ellstrand 1986; Scora and Bergh 1990); viz. *P. americana* var. *drymifolia* (Mexican race), *P. americana* var. *guatemalensis* (Guatemalan race) and *P. americana* var. *americana* (West Indian or Lowland race). Trees of the West Indian race are the most tolerant to alkaline and saline soil conditions while those of Mexican race origin are the most tolerant of low temperatures. The races hybridise freely, giving rise to genotypes with adaptation from cool semi-arid to hot humid 'tropical lowland' climates. Fruit of Mexican and Guatemalan races have more oil in their

mesocarp at maturity (10 to 30%) than fruit of the West Indian race (3 to 10%). Fruit of the Mexican race have the thinnest skins while fruit belonging to the Guatemalan race generally are the latest maturing of the three races. Production in the lowland tropics is centred around West Indian and West Indian x Guatemalan cultivars. Guatemalan and Mexican race cultivars and their hybrids dominate the technologically more advanced industries of the subtropics.

Commercial avocado production in Australia occurs in all mainland States which cover a diversity of environments, ranging from the semi-arid/Mediterranean in Western Australia to warm subtropical with predominantly summer rainfall in Queensland and northern New South Wales. Approximately 80% of production is from the subtropical areas of the country. By world standards the Australian avocado industry is small producing 20 000 tonnes in 1993, of which 95% was consumed on domestic markets. Currently the industry is in a rapid growth phase repeating the expansion cycles of the mid 1970's and the early 1980's. Interest is being shown in the development of new markets for both fresh and processed fruit. Export is a consideration, however due to geographical isolation and higher labour costs compared with other major producing countries, the Australian industry will require a technological edge to compete effectively in international markets. In particular, the assurance of well-above average yields of quality fruit will be necessary.

Major constraints identified by industry in relation to export include low yields, unreliable fruit quality and high production costs, the latter being due to expenses associated with managing large trees. Low yield with respect to other fruit crops has to some extent been explained by Wolstenholme (1986, 1987), however there remains a large gap between the $\approx$ 32 t ha^{-1} target potential (Wolstenholme 1986) and the industry average for bearing trees of ca. 12 t ha^{-1} (Whiley and Winston 1987). In some situations production of > 20 t ha^{-1} has been sustained for a number of years, but inevitably tree size becomes excessive and management strategies which result in major yield loss for a number of years are necessary, e.g. staghorning, heavy mechanical pruning.

Research reported in this thesis deals with Mexican and Guatemalan race cultivars or their hybrids growing in humid, moderate to high rainfall subtropical climates in S.E. Queensland.

The author has used an holistic approach in studying the growth and development of avocado which has been documented in pheno/physiological models. Earlier reports on some aspects of avocado physiology covered in this thesis were confined to container-grown trees (Bower *et al.* 1978; Scholefield *et al.* 1980), or field-grown trees in soil conditions in Florida, USA which mimicked container-grown responses (Schaffer *et al.* 1987). However, recent changes in technology have allowed detailed measurements reported in this thesis to be made on field-grown trees. The prime objective of the research was therefore to apply modern technology to a range of potentially yield-limiting factors, identified from the construction of a pheno-physiological seasonal growth model, with a view to a more sophisticated understanding of how to manipulate the tree during critical periods. The approach has been holistic, at the whole-tree level, with a strong eco-physiological bias, and ultimately directed at solving grower problems in the orchard situation. The result is substantially changed views on some aspects of physiological limitations on the yield performance of avocado trees. Improved understanding of tree phenology and physiology has assisted in the development of more sophisticated management strategies which potentially will reduce production costs and improve avocado yield and quality.

UNIT ABBREVIATIONS

Term	Abbreviation	Units
Net carbon dioxide assimilation (i.e. photosynthesis or assimilation rate)	A	$\mu mol\ CO_2\ m^{-2}\ s^{-1}$
Maximum net carbon dioxide assimilation	A_{max}	$\mu mol\ CO_2\ m^{-2}\ s^{-1}$
Light saturation point for A	Q_A	$\mu mol\ quanta\ m^{-2}\ s^{-1}$
Light compensation point for A	Q_O	$\mu mol\ quanta\ m^{-2}\ s^{-1}$
Dark respiration	R_d	$\mu mol\ CO_2\ m^{-2}\ s^{-1}$
Photorespiration	R_l	$\mu mol\ CO_2\ m^{-2}\ s^{-1}$
Intercellular carbon dioxide (leaf)	Ci	$\mu mol\ mol^{-1}$
Photosynthetic photon flux	PPF	$\mu mol\ quanta\ m^{-2}\ s^{-1}$
Quantum yield	Ø	$\mu mol\ CO_2\ \mu mol\ quanta^{-1}$
Stomatal conductance	g_s	$mmol\ H_2O\ m^{-2}\ s^{-1}$
Vapour pressure deficit (leaf to air gradient)	VPD	kPa
Leaf water potential	ψ_l	MPa
Soil matric potential	ψ_s	kPa

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., Bocca 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. In Press.

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 ≈ 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°C, while West Indian seedlings suffered lethal damage at -5.6°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°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°C with eight of these nights below -6.7°C. 'Hass' trees suffered minimal damage to shoot terminals when exposed to ≈ -4°C for 12 hours on two consecutive nights, but trees were defoliated and fruit damaged when temperatures were between -4°C to -8°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°C to -5°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°C to 33/26°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°C and above 29/22°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°C. Dry matter accumulation in roots was reduced at soil temperatures of 32°C and at 13°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

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), *Prunus 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.

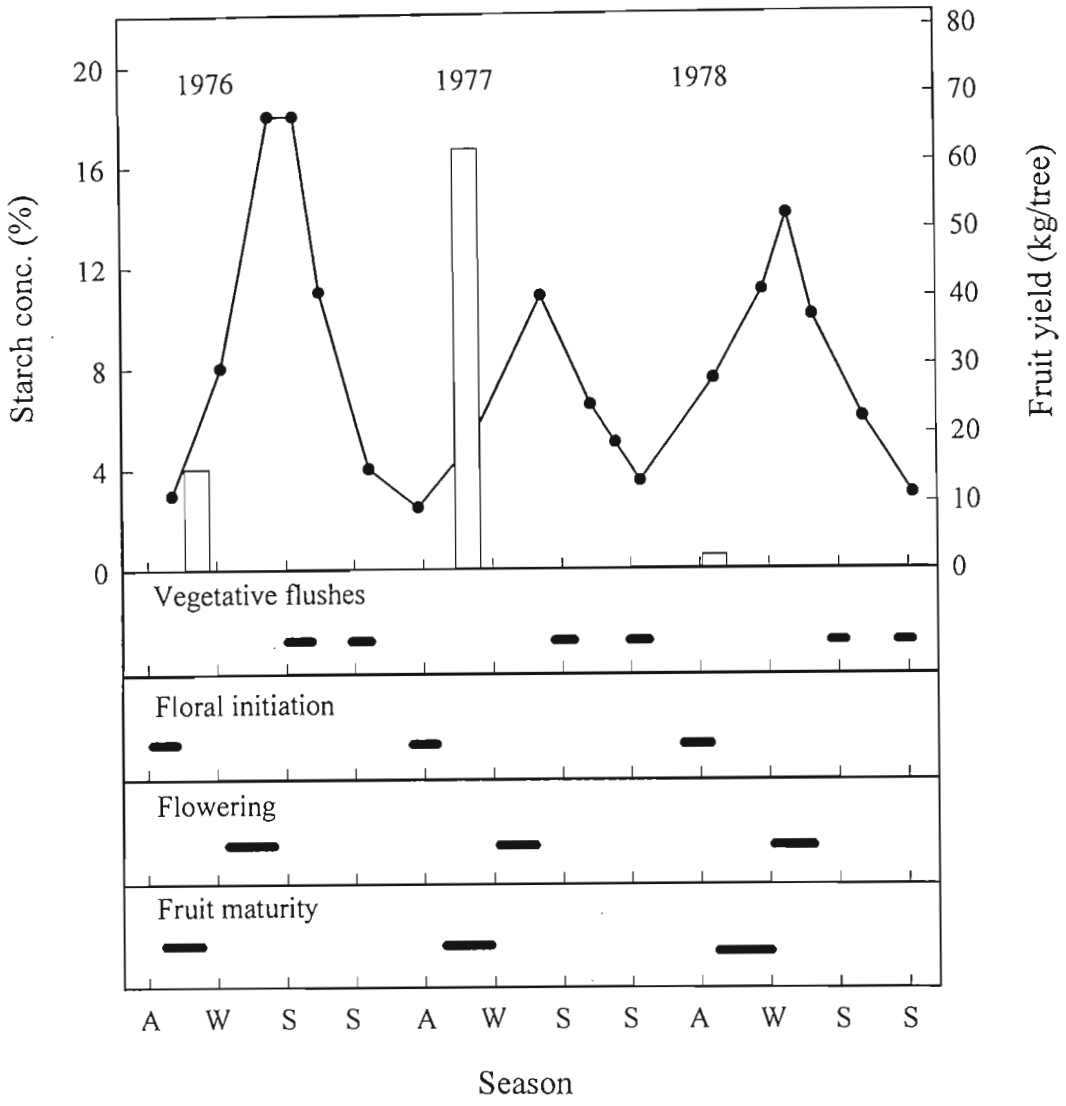

Fig. 1 Diagrammatic representation of phenological events and variations in starch concentrations (large branches) of cv. Fuerte avocado trunk wood from trees grown at Coomealla, Murray Valley Irrigation Area, southern Australia. Histograms show the yield of fruit recorded each year. Redrawn from Scholefield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

CHAPTER 2

PHENO/PHYSIOLOGICAL MODELS

To enhance competitive fitness, perennial plant species typically display seasonal variation in the production of new leaves, flowers and fruit due to genetically imprinted growth patterns (van Schaik *et al.* 1993). In individual species, biotic influences have generally selected for temporally staggered, or clumped phenological activities. For instance, some species vulnerable to seed predation have developed strongly clumped fruiting sequences by storing carbohydrate reserves to produce larger crops at longer intervals (Janzen 1971); and leaves produced in synchronised flushes sustain less insect damage than those grown asynchronously (Lieberman and Lieberman 1984).

Environmental factors have a major impact on plant growth and dictate seasonal change, within genetically determined limits. For instance, many deciduous species of seasonally dry tropical forests drop all their leaves before the dry season and re-leaf about one month before the onset of rain, thereby minimising the impact of water stress (Frankie *et al* 1974; Wright and Cornejo, 1990). Irradiance and water stress have been identified as the most important environmental factors shaping the phenology of tropical woody plants in natural communities (van Schaik *et al.* 1993). However, with the domestication of fruiting species temperature has become equally important as production has been extended into more hostile environments (Sedgley and Grant 1983; Whiley and Winston 1987; Issarakraisila and Considine 1994).

These introductory remarks are perhaps an over-simplification of the complexity of plant responses and interactions in mixed communities, and ecologists are still grappling to gain a meaningful understanding of the phenological processes and the agents which control them. As horticulturists dealing with monoculture systems we are not concerned with the complexities of mixed communities. However, by recognising and appreciating the evolutionary factors which dictate the organisation of growth and seasonal change in natural

shoots could be advantageous through increasing shoot complexity. However, pruning of mature trees was discouraged due to the unpredictable nature of the growth response.

Kotzé (1979) compiled a simple model for the annual growth of avocado (cv. Fuerte) to promote discussion and encourage research initiatives. Tentative suggestions were made with respect to irrigation and the timing of fertiliser applications. However, his model focused on the reproductive cycle from floral initiation through to fruit maturity, and failed to recognise the dynamics of seasonal contributions from competitive and complementary shoot and root growth. A more holistic approach to the dynamics of tree growth was presented by Wolstenholme (1981) who speculated on the interactions between root, shoot and fruit growth. The implications of physiological changes and their effect on the "rhythmic" growth patterns of trees were discussed and conclusions drawn on potential management strategies to improve and sustain avocado yields.

A further significant development in avocado phenology was a detailed account of above-ground growth sequences for 21 avocado cultivars growing in southern Florida (Davenport 1982). His observations on floral development, flowering and fruit set, and vegetative flushes were mostly reported in a single time dimension (seasonal change). Of particular interest were the reported relationships between tree phenology and some associated physiological changes, e.g. observations that leaf senescence was precipitated by flowering, which exposed new opportunities in relating tree physiology and phenology as a research tool. However, the dynamics of root growth were not studied.

The author began whole-tree phenology studies with avocados in the late 1970's and despite prototype models being used to assist with research approaches and as an extension tool, details were not published until 1988 (Fig. 2) (Whiley *et al.* 1988a). By this time the concept had wide recognition as an extension aid by the Queensland avocado industry, and interest in the application of the models was being shown by other avocado producing states in Australia and later internationally. The published models were based on data sets collected in a time sequence from orchard trees, superimposed with the interpretation of research results and drawn as a conceptualised framework to provide management strategies at farm level. The

incorporation of root growth patterns, even though these were deduced from simple non-destructive "surface root mat" techniques, was of particular significance to the holistic understanding of rhythmic growth patterns.

The basic phenology model (Fig. 2) is two dimensional, integrating a time scale (*x*-axis) with the magnitude of response (y-axis). It illustrates the sequence of growth events over a full fruiting cycle and the relationship between the reproductive effort and root and shoot growth. Due to the plasticity of phenological responses across environments, the model is a useful tool for providing management strategies to growers in diverse regions. The timing of the key

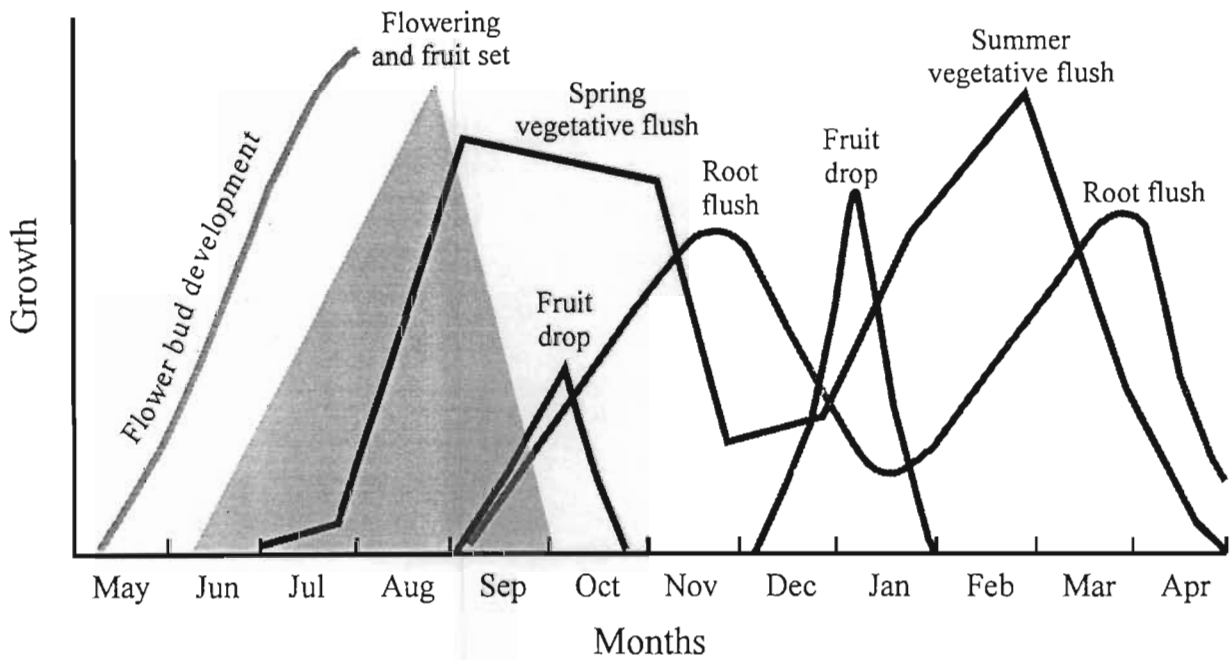

Fig. 2 Phenology model developed for cv. Fuerte avocado growing in a warm, sub-tropical climate at Nambour, S.E. Queensland. Redrawn from Whiley *et al.* (1988a).

phenological events moves left or right along the *x*-axis in response to warmer and cooler climates dictated by changing latitude or altitude. As orchard management should be timed by growth event rather than by calendar month, strategies which target major phenological changes can be recommended irrespective of where the tree is growing. However, as a cautionary note, experience has shown that at outer environmental extremes (latitudes 12° & 33° S in Australia) plasticity may fail wherein there is a requirement to redefine the model. As

researchers or managers, we are limited in the control we can exert on displacement along the *x*-axis once a cultivar has been selected for a given environment.

While there is undoubtedly an interaction with prevailing environmental conditions, it is the magnitude of each growth event which can be manipulated through management strategies to have the greatest impact on tree performance. It is largely in this area that horticultural research has been focused, defining water, nutritional and crop protection requirements and the time sequence in which they should be applied in relation to phenological events.

2. 2 INTEGRATION OF PHYSIOLOGY WITH PHENOLOGY MODELS

2. 2. 1 Introduction

The previous section has reviewed the development of phenological modelling for avocado and the implications for its use in research and extension programs. However, the model of Whiley *et al.* (1988a) was qualitative and based solely on growth measurements, and as a research tool there were limitations in the interpretation of relationships between phenological events which could only be improved by more detailed physiological knowledge. My objectives with the research reported herein were (i) to re-examine root growth with respect to whole tree phenology; and (ii) to study seasonal changes in net CO_2 assimilation (A), leaf nitrogen and chlorophyll concentrations and starch levels, with a view to providing further information on physiological limitations during critical phenological events.

2. 2. 2 Materials and Methods

A commercial 'Hass' avocado orchard at Maleny in S.E. Queensland (latitude 26.5°S, altitude 520 m) was chosen for the phenological/physiological study. The climate is cool, mesic subtropical with a high mean annual rainfall of 2000 mm in a summer/wet: winter/dry pattern. The soils of the area are of basaltic origin and are described as krasnozems. Physically they are

well drained clay loams (ca. 60% clay fraction) to between 10 to 16 m deep and show no obvious physical limitations to root growth.

Roots were observed from a rhizotron facility especially constructed for the study. It consisted of 10 wooden boxes 1.0 x 1.2 x 2.1 m, each with a 10 mm thick 690 x 845 mm clear plexiglass (poymethyl methacrylate) panel built into the two opposite 1.2 m sides: 20 panels in total (Fig. 3). Disadvantages of this approach to root study have been enumerated by Rogers (1934) and were ameliorated as far as practicable by replication of the viewing panels, planting trees after the facility had been installed and excluding light from the viewing panels. Root measurements obtained with this technique on fruit trees have compared favourably with those obtained by other methods, e.g. tracer uptake (Atkinson 1974) and excavation or water depletion (Atkinson 1978). This is believed to be the first detailed study of this nature and scope and duration (4½ yr) on avocado trees anywhere in the world, incorporating non-fruiting and fruiting trees, and light and heavy cropping.

Fig. 3 Installation of rhizotron boxes constructed for root studies on avocado trees, Maleny, S.E. Queensland, Australia.

Holes slightly larger than the boxes were excavated and the rhizotrons installed in October 1988. They were aligned in one row, 4 m apart with the plexiglass panels in each box facing outwards and the top of each window at soil level. To ensure good contact with the plexiglass, the top-soil was carefully packed against each of the viewing panels to the same dry bulk density as the surrounding soil (1.1 t m^{-3}). Once in position the rhizotrons were fitted with solid lids with an upper silver surface to block light and reflect heat. Apart from removal for access to the panels each month (ca. 30-45 mins. to collect data) the covers remained closed for the duration of the study. After installation, the soil 3 m either side of the rhizotrons was deep-ripped to 1 m, and planting sites adjacent to each window prepared by incorporating 9 l of chicken manure, 250 g of superphosphate (9% P) and 500 g of dolomite m^{-2} surface area.

During 1988 'Velvick', a Guatemalan race seedling selected in Queensland; 'G755A', a *Persea schiedeana x P. americana* hybrid rootstock, and 'Duke 7', a Mexican race selection both from California (Coffey *et* al. 1988) were clonally propagated using the nurse-seedling technique (Frolich and Platt 1971-72, modified by Brokaw), and were grafted to 'Hass' scions. A number of seedling 'Velvick' rootstocks were also grafted to 'Hass' scions. All propagation material used in this study had been previously tested and certified free of Sunblotch viroid, a potentially serious disease of avocado (da Graca 1985). In March 1989, five 'Hass' trees on each of the four rootstocks were randomly planted in central positions 1 m from the windows. To control weed growth and assist in reducing fluctuations in soil matric potential (ψ_s), trees were mulched with barley straw spread 1 m from the trunk and to a depth of 100 mm, within a week of planting. This mulch was maintained for three years after planting by which time tree canopies provided sufficient ground shade and became self-mulching through the accumulation of leaf litter.

At the time of planting, under-tree mini-sprinklers (10 l hr^{-1}) were installed at each site to supplement natural rainfall. Soil matric potential was monitored 0.5 m from rhizotron walls using permanently installed tensiometers at 30 and 75 cm depth. During dry periods tensiometers were checked at weekly intervals and irrigation given to maintain $\psi_s \leq 40$ kPa at 30 cm and ≤ 50 kPa at 75 cm depth. Trees were fertilised according to the schedule for tree age developed for

avocados growing in S.E. Queensland (Banks 1992). The exception to this program was the additional 4 g m^{-2} of canopy of Solubor (22% elemental B) soil-applied in spring and summer each year due to known boron deficiencies and the high buffering capacity of the soil at this site (A.W. Whiley, unpublished data).

Root measurements were made only of the white, unsuberised "feeder" roots. Data were collected at monthly intervals by tracing the outline of roots visible at the soil-panel interface onto transparent sheets of acetate with a black indelible pen. Only those portions of roots that were visible at the interface were recorded. No distinction was made between roots of different diameters. Only some of the white "feeder" roots became suberised (browning) after which they were no longer measured. The information on the acetate sheets was digitised by scanning to an electronic file using a Hewlett Packard ScanJet IIc. Root lengths were determined by computerised image analysis (Sci-Scan Image Analysis System, Delta T, UK). This method gave a total length (m) of visible white root at the soil-panel interface (0.58 m^2 vertical window area) each month.

Beginning in 1991 trunk girth measurements were taken during July of each year in positions demarcated by white acrylic paint marks, above and below the graft union. In 1992, the first year of heavy cropping, the diameter and height of the trees were measured and the canopy volume calculated using the models for a half sphere and a cylinder. This was the only year that canopy measurements were taken as trees began to crowd in 1993 and side canopies had to be pruned.

Reproductive and vegetative phenology data were collected for four seasons at monthly intervals, or more frequently when necessary, using the system of Whiley *et al.* (1988a). Floral bud development was ranked on a 0 to 10 scale where 0 = no visible development and 10 = opening of the first flower. Flowering was judged by recording the first and last dates of anthesis and estimating the time that 50% of the flowers had opened. All qualitative estimates were made independently by two people and the mean of the scores used for fitting the data. Fruit drop in spring was estimated by counting fruit on three tagged shoots on each of five trees at weekly intervals from the end of anthesis until numbers were relatively stable. The weekly

data were calculated as a percentage of the total fruit that dropped from the tagged shoots. In summer, fallen fruit under each tree were counted and removed at weekly intervals. These data were calculated as a percentage of the total number of fruit on the tree at harvest, reflecting the crop loss after a substantial growth investment (30-40% of full size). At the completion of anthesis three indeterminate and three determinate inflorescences on each of five trees were tagged and individual fruit lengths on each shoot measured with electronic callipers at weekly intervals for the first five months after fruit set and then monthly until harvest. When harvesting the two "on" crops (1992 & 1994), fruit which developed on either indeterminate or determinate shoots were recorded separately.

For nitrogen and starch analysis, the most recently matured summer-flush leaves were selected at monthly intervals from May until December 1992. Thirty leaves were randomly collected from each tree and equally divided into sub-samples; one for nitrogen and the other for starch analysis. Leaves for nitrogen analysis were washed in a solution of mild detergent (1 ml l^{-1}) and acetic acid (0.6 ml l^{-1}), rinsed in distilled water, dried at 52°C for three days, milled and re-dried at 105°C immediately prior to determinations. Nitrogen was measured using a Kjeldahl digest of sulphuric acid, sodium sulphate and selenium catalyst (M^{c}Kenzie and Wallace 1954). The digestate was diluted prior to automatic colorimetric analysis using the indophenol reaction with salicylate and sodium dichloroisocyanurate (Berthelot 1959).

Wood samples were collected for starch analysis from the large roots radiating from the crown of the rootstock and from the scion region of the trunk after June 1992, when it was judged that trees were large enough to support a monthly program of this nature. For each organ, samples were obtained from five sites around the tree by first removing a plug of bark and then drilling 40 mm into the wood with a 9 mm diameter bit. The drilled shavings from each hole were bulked for analysis.

Starch samples were placed in a cool, insulated box for transport back to the laboratory and within 3 hrs of collection, were transferred to a convection oven at 60°C and dried to constant mass. Dried samples were ground at 100 mesh in a Udy Mill (Udy Corporation, USA) and stored in an airtight container. Starch was determined by a two stage enzymatic hydrolysis of the starch

to glucose and the concentration measured colorimetrically using a coupled glucose oxidase/peroxidase/chromogen system as described by Rasmussen and Henry (1990).

Photo-assimilation studies were carried out from March to December 1992 on 'Hass' trees growing in the rhizotron and grafted to cloned 'Velvick' rootstocks. When summer-flush shoots were nearing maturity, five fully expanded sun-exposed leaves were tagged on the northern sides of trees. At the time of selection the leaves were ca. 40 days old and fully expanded, (Chapter 3) and to lessen "sink" effects were at least 0.5 m from the nearest fruit. Net CO_2 assimilation (A) was measured at monthly intervals with a LICOR LI-6200 portable photosynthesis meter configured as a closed system (LICOR, Nebraska, USA) and using a LI-6000-11, 1 l chamber. All measurements of A were made at or above PPFs of 1200 μmol quanta m^{-2} s^{-1} and between 0830 to 1030 h, a low stress time of day (Whiley *et al.* 1988b). Photosynthetic rates were derived from LI-6200 Software Version 2.00.

Chlorophyll concentrations were determined from five leaves on the northern side of the same trees used for photo-assimilation studies. Two discs totaling 1.0 cm^2 were sampled from either side of the midrib of each leaf and the discs pooled for each tree. Chlorophyll was extracted with 85% acetone from the discs kept in darkness at 25°C for 48 h. Measurements were made spectrophotometrically as described by Proctor (1981).

An automatic weather station (Monitor Sensors, Caboolture, AUST.) was positioned at the site to record rainfall and air and soil temperatures. The soil temperature sensor was installed at 450 mm below the surface in a position equi-distant between the top and bottom of the recording field. Data presented in the figures are the means ± SEs of pooled values from five trees.

2. 2. 3 Results and Discussion

Some trees on 'Duke 7' and 'G755A' rootstocks died in the second and third years of the project (Verticillium wilt). Therefore only data for trees propagated on cloned and seedling 'Velvick' rootstocks are presented.

Rootstocks and Root Phenology

Twenty-seven months after planting an over-growth of the scion, expressed by a scion/rootstock girth ratio of > 1.0, was detected in trees grafted to cloned 'Velvick' rootstocks (Table 1). The over-growth in this scion/rootstock combination persisted for the duration of the study indicating mild incompatibility. In contrast, trees grafted to seedling 'Velvick' were near normal (slightly favouring the rootstock) with respect to scion/rootstock relationships. The compatibility of graft unions between the same species is ultimately a function of biochemical events (Leakey 1985). In *Pinus contorta*, scion overgrowth has been attributed to translocation incompatibility wherein a degree of phloem degeneration and necrosis is evident (Copes 1975). Compatibility of this type does not necessarily indicate that the combination is without merit and indeed it may be exploited for horticultural gain.

Table 1 Scion/rootstock girth ratios of cv. Hass trees grafted to cloned and seedling 'Velvick' rootstocks. The ratios were calculated from girth measurements taken above and below the graft union. Data are mean values of five trees ± standard errors.

Rootstock	Scion/rootstock ratio			
	1991	1992	1993	1994
Cloned 'Velvick'	1.15 ±0.04	1.21 ± 0.04	1.20 ± 0.05	1.17 ± 0.01
Seedling 'Velvick'	0.94 ± 0.02	0.97 ± 0.01	0.98 ± 0.01	1.00 ± 0.01

The first roots were visible at the soil-panel interface in some boxes by March 1990, but due to insufficient numbers recording did not begin until July 1990 when all windows were colonised. Note that where time is plotted as the abscissa in Figs. 4 & 5 it refers to time elapsed since 1 June 1990 (June is early winter in the southern hemisphere); thus each calendar season refers to a three month period, e.g. spring is from September through November.

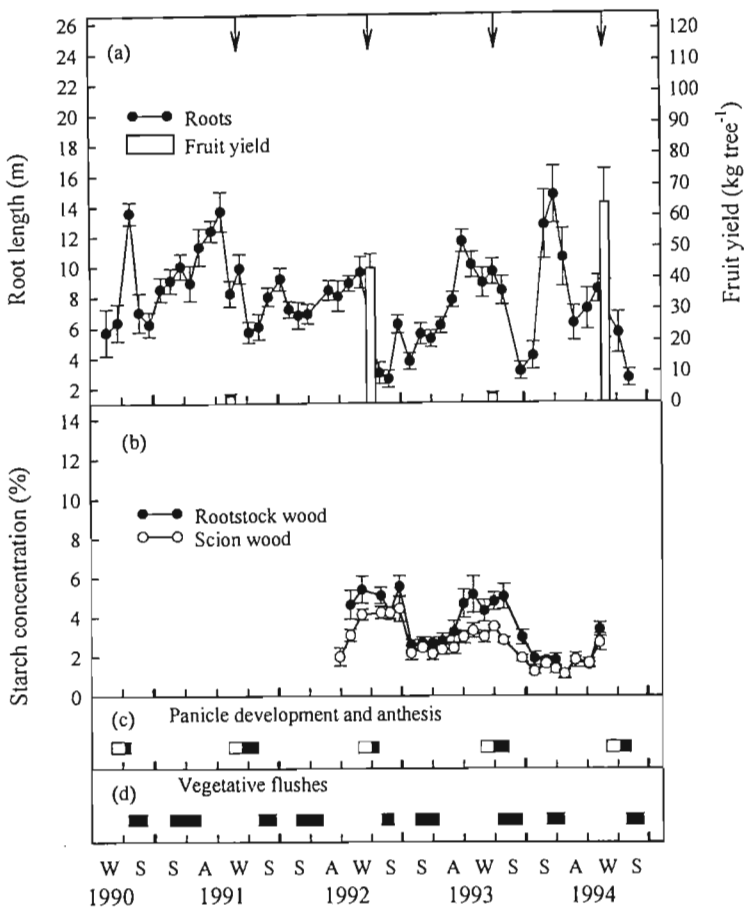

Fig. 4 Root growth and starch cycling over a 4½ yr period in cv. Hass grafted to cloned 'Velvick' rootstock as they relate to above ground tree phenology and yield where: (a) is seasonal changes in root length per 0.58 m^2 vertical window area, and annual fruit yield (arrows indicate harvest times); (b) is seasonal changes in starch concentration of the rootstock and scion portions of the tree; (c) are periods of inflorescence development represented by the open bars, and anthesis represented by the closed bars; and (d) are periods of shoot growth. Root length, yield and starch data are mean values of five trees ± vertical SE bars.

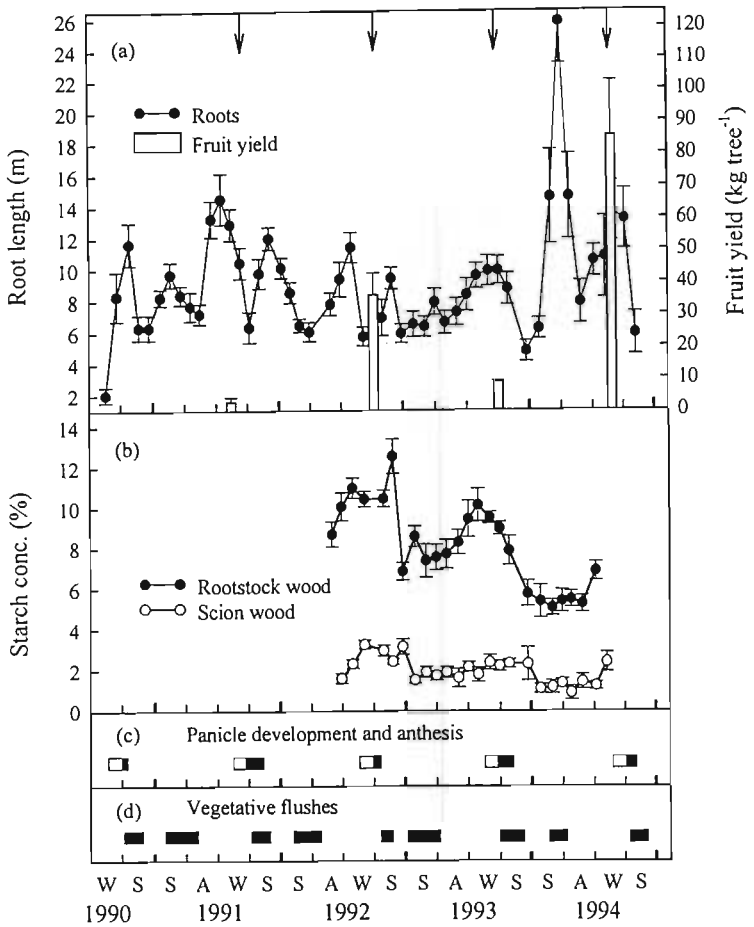

Fig. 5 Root growth and starch cycling over a 4½ period in cv. Hass grafted to seedling 'Velvick' rootstock as they relate to above ground tree phenology and yield where: (a) is seasonal changes in root length per 0.58 m^2 vertical window area, and annual fruit yield (arrows indicate harvest time); (b) is seasonal changes in starch concentration of the rootstock and scion portions of the tree; (c) are periods of inflorescence development represented by the open bars, and anthesis represented by the closed bars; and (d) are periods of shoot growth. Root length, yield and starch data are mean values of five trees ± vertical SE bars.

There were strong seasonal variations in total measured root length of both rootstocks (Figs. 4 & 5). These appeared independent of soil temperatures at 450 mm depth which varied by 7.5°C between summer (23.5°C) and winter (16.0°C). Typically, two peaks of root growth occurred over a one year period; the first of the new season in spring and generally concomitant

with spring flush, and the second beginning in late summer and peaking in winter immediately prior to anthesis (Figs. 4 & 5). The pattern was more pronounced in trees grafted to seedling 'Velvick' rootstocks and was not always apparent in trees growing on the cloned material. Similar bimodal periodicity for avocado root growth has been previously described by Whiley *et al.* (1988a) and Ploetz *et al.* (1992), although in both cases the extension of the second growth phase through winter to anthesis was not indicated. It is likely that roots observed in the surface mat technique used by Whiley *et al.* (1988a) suffered desiccation during the dry winter months giving the impression of growth cessation, while the young trees used by Ploetz *et al.* (1992) may not have developed root cycles typical of mature fruiting trees. The two-peak annual cycle has similarities with deciduous fruit crops (Head 1967; Rogers and Head 1969), however the major difference is extension of the second growth period of avocado roots into winter in this cool, mesic subtropical environment. With avocado major points of interest are alternation between shoot and root flush peaks; the prolonged winter root growth in deeper, warmer and moister soil zones; and the pronounced reduction in feeder root growth (die-off of feeder roots) during flowering and fruit set.

The bimodal periodicity observed in this study is likely due to competition between shoots and roots for photo-assimilates in this complex, much branched tree. In photosynthate translocation studies with avocado (Chapter 5) it was found that when leaves were exposed to ^{14}C during active shoot growth 43% was recovered in the new shoots while only 5% was recovered from roots. In contrast, when leaves were exposed during a period of shoot quiescence, 32% of the ^{14}C was recovered from the roots and only 5% from the most recent shoot flush (Whiley and Schaffer 1993).

The greatest root lengths were recorded during the first two years of the study when they reached ca. 14 m per 0.58 m^2 of window observation area for a month, and again in the fifth year when root length of the seedling 'Velvick' rootstock reached 25.8 m per 0.58 m^2 of window observation area. At other times root length was lower with maximum recordings from ca. 9 to 11 m for any one observation time. Root lengths during the first two years of the study may be due to either greater root activity at the peripheral zone of the tree's canopy, which was approximately aligned with the side of the rhizotrons 18 months after planting; or

proportionally greater root growth in non-fruiting trees. The latter is supported by the root patterns determined for 'Hass' trees grafted to cloned 'Velvick' where during the 1991/92 season, trees carried ca. 44 kg of fruit and maximum root lengths measured were ca. 9 m (Fig. 4). In contrast, the 1992/93 crop was small (ca. 3 kg $tree^{-1}$) and the maximum root length at the soil-panel interface reached 11.6 m. Differences were not as clear with 'Hass' trees grafted to seedling 'Velvick' rootstocks though root growth on average during the fruiting years was greater than those trees on cloned rootstocks; 8.0 ± 0.3 m compared with 7.1 ± 0.4 m respectively (Figs. 4 & 5). Trees on both rootstocks showed strong root growth following spring shoot maturation in 1993 despite trees carrying a heavy crop. This is likely due to the vigorous shoot growth which was observed following anthesis. However, root growth during the late summer and winter was markedly suppressed, which is probably related to fruit lipid accumulation (Kaiser and Wolstenholme 1994) and growth of fruit which occurs during this period.

The lower impact of fruiting on seedling rootstock may be due to the greater vigour of these trees. The effect of cropping on root growth of trees has been reported on many occasions and the outcomes have generally been consistent. Head (1969) found reduced periodicity and magnitude of new root growth in fruiting apple trees with the major summer growth peak eliminated in some years. Similarly, Ryhakov and Dzavakjanc (1967) and Dzhavakyants (1971) reported that cropping in apples reduced the number of root growth peaks from two in vegetative trees to one per season. Others finding adverse effects of cropping on root growth include Maggs (1963), Avery (1970), Cannell (1971) and Atkinson (1977). This is not surprising when considered in the context of the "priority sinks" philosophy. Here developing seeds (fruit) have a large "sink strength" or "mobilising ability" to attract photo-assimilates and are usually more competitive than other plant organs, of which roots are generally acknowledged as among the weakest sinks of the plant (Cannell 1971; Chalmers and van den Ende 1975; Lenz 1979). For example, the proportion of the annual increment of dry mass allocated to the root system decreased from 20% in young peaches, to 1% in fruiting peach trees (Chalmers and van den Ende 1975).

There were seasonal changes in the starch content of major roots and the scion trunks of both scion/rootstock combinations (Figs 4 & 5). Accumulation occurred during the autumn and winter when shoots were quiescent with concentrations falling rapidly during or just after anthesis (see Chapter 6 for more information). However, it is very noticeable that the starch concentration in the large woody roots of the seedling 'Velvick' was always about double that in the cloned 'Velvick' rootstock. There was also a direct relationship between the root starch concentration of seedling 'Velvick' roots and root length (Fig. 6). This relationship would be expected as more photo-assimilates are translocated to the roots during relatively quiescent periods in the aerial portions of tree, thereby increasing the growth of roots, and remobilised to stronger aerial sinks at critical stages of phenological development, e.g. flowering, fruit set and seed growth.

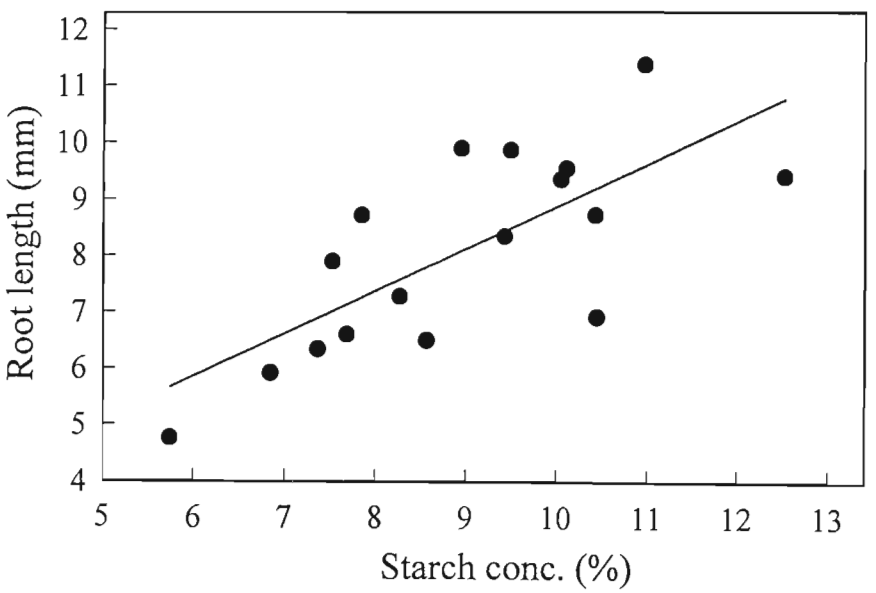

Fig. 6 Relationship between starch concentration in the rootstock and root length measured at the soil-panel interface. The regression is represented by the equation $y = 1.37 + 0.75x$, $r^2 = 0.55$*.

Differences in starch concentrations between the two rootstocks may be explained by mild incompatibility between the cloned 'Velvick' rootstock and the 'Hass' scion (Table 1). It is possible that the movement of translocate to roots was impeded due to phloem degeneration

(Copes 1975) resulting in less root growth, particularly when trees had a substantial crop load, e.g. 1992 (Figs. 4 & 5). The outcome of less starch moving to the roots was a higher concentration maintained in the scion which was ca. 30% more than the concentrations measured in the 'Hass' scions on the seedling rootstocks.

The mild incompatibility and reduced vigour of the cloned 'Velvick' root system is most likely responsible for the smaller stature of these trees when compared with those on seedling 'Velvick' rootstock. When canopy measurements were taken in September 1992, ca. 30 months after planting, 'Hass' trees grafted to clonal or seedling 'Velvick' rootstocks had canopy volumes of 12.44 ± 1.38 m^3 and 16.98 ± 1.22 m^3, respectively. When the 1992 yield performance of trees on the two rootstocks was compared on a canopy volume basis, 'Hass' grafted to clonal 'Velvick' rootstocks produced 3.57 ± 0.31 kg m^{-3} of fruit which was significantly greater than the 2.12 ± 0.42 kg m^{-3} produced by trees grafted to seedling 'Velvick' rootstocks. These data are supported by a long-term rootstock experiment on the same site where 'Hass' grafted to clonal 'Velvick' rootstocks have continued to have the highest production efficiency on a canopy volume basis when compared with clonal 'Duke 7' and seedling 'Velvick' rootstocks (A.W. Whiley, unpublished data). In this case, mild incompatibility at the scion/rootstock interface and the resultant reduced tree vigour has been an effective horticultural tool in the repartitioning of assimilates to give higher fruit yields.

The central axis or primary inflorescence of avocado is usually terminated by a vegetative bud which at the finish of anthesis grows out into a new shoot (Chandler 1958). However, in some cases the inflorescence is terminated by a panicle sub-unit. The two types are known as either indeterminate or determinate compound inflorescences, respectively (Thorp *et al.* 1994). Compared to other major Mexican/Guatemalan race cultivars, 'Hass' produces a greater proportion of determinate inflorescences which in years of heavy cropping can become a problem due to exposed fruit becoming sunburnt. In their study Thorp *et al.* (1994) reported that in 37% of the floral shoots of 'Hass' the terminal meristem remained reproductive and gave rise to determinate compound inflorescences.

In this study fruit on both indeterminate and determinate inflorescences grew rapidly in length for the first 150 days after fruit set and thereafter the growth rate substantially diminished (Fig. 7). This latter stage of diminished growth coincides with rapid lipid accumulation in the fruit (Kaiser and Wolstenholme 1994). At 40 days after set, determinate fruit were already larger than those set on indeterminate inflorescences and during the first 150 days they maintained a higher growth rate (slope of curves). This growth advantage was still apparent at maturity where determinate fruit were ca. 18% longer than those set on indeterminate inflorescences.

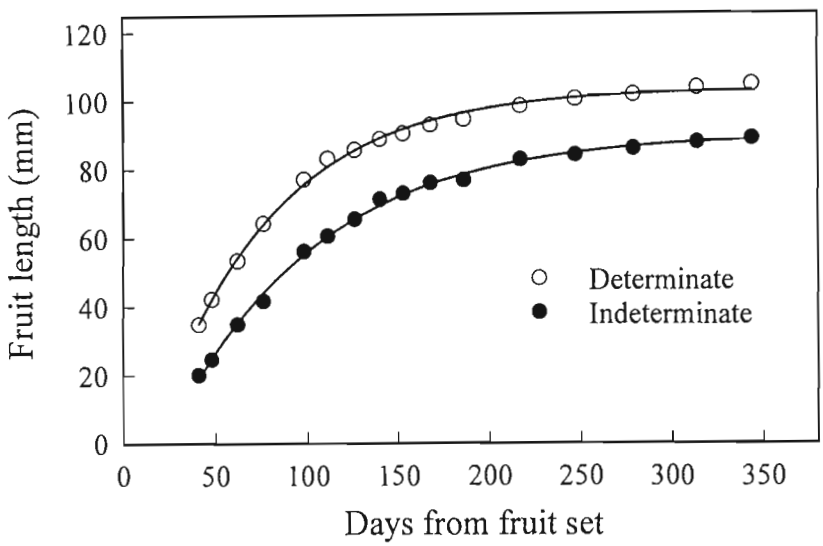

Fig. 7 The growth of cv. Hass fruit on indeterminate and determinate terminals from 40 days after fruit set through until maturity. Growth was determined by measuring the increase in fruit length. The regression for determinate fruit is represented by the equation $y = 103.05 - 130.23(0.984^x)$, $r^2 = 0.99$; and the regression for indeterminate fruit is represented by the equation $y = 89.67 - 118.62\ (0.988^x)$, $r^2 = 0.99$. The curves are significantly different in placement and shape as judged by *t* tests ($P < 0.001$). Data points are mean values of fruit from three terminals on each of five trees.

The growth advantage of fruit on determinate shoots is thought to be due to spatial separation from shoots which develop concurrently with fruit set on indeterminate inflorescences. Using

mid-bloom foliar sprays of paclobutrazol to suppress 'Hass' spring shoot growth from indeterminate inflorescences, Wolstenholme *et al.* (1990) reported increased fruit size when shoots matured. This resulted in larger fruit at maturity compared with fruit from the unsprayed control trees.

The yield data for 1992 and 1994 showed that the greatest percentage of fruit harvested from trees on either rootstock was from determinate shoots, > 80% for trees grafted to cloned 'Velvick' and > 60% for trees grafted to seedling 'Velvick' (Table 2). There were significant differences in the indeterminate/determinate yield ratios between rootstocks which was lowest for cloned 'Velvick' for the two years that data were collected.

Table 2 Effect of rootstock on the ratio of indeterminate/determinate fruit on cv. Hass. The ratios were calculated from the 1992 and 1994 crops which were "on" years for these trees. Data are mean values from five trees ± standard errors; percentage determinate fruit are given in parenthesis.

Rootstocks	Indeterminate/determinate fruit ratio	
	1992	1994
Cloned 'Velvick'	0.22 ± 0.06 (83.4)	0.17 ± 0.01 (86.0)
Seedling 'Velvick'	0.91 ± 0.33 (60.8)	0.48 ± 0.05 (67.8)

Mechanisms for the production of determinate shoots have not been elucidated but the author has observed that trees infected with *Phytophthora cinnamomi* Rands, which causes Phytophthora root rot, usually have a higher percentage of determinate inflorescences, suggesting that root damage or stress (lack of vigour) is a primary cause. In this study trees grafted to clonal 'Velvick' rootstocks carried ca. 20% more of their crop on determinate inflorescences than trees on seedling rootstocks (Table 2). Of the physiological variables measured, the major difference between trees was in root starch concentrations. There is little

doubt that roots play an important role in the regulation of flowering and promotion of bud development through the timely supply of growth regulators (Jackson 1993). It is suggested that the lower assimilate concentration in cloned compared with seedling 'Velvick' roots may have resulted in a reduced capacity of cloned roots to stimulate terminal vegetative buds in the inflorescences thereby leading to a greater proportion of determinate inflorescences. This mechanism would have ecological significance, in that trees in a cropping cycle with depleted reserves would enhance their ability to set and carry more fruit by reducing competition with indeterminate flowering shoots.

Pheno/physiological Models

Leaf starch concentrations of mature summer leaves increased rapidly from March reaching peak concentration in June/July (winter), when there was a sharp decline which coincided with inflorescence development and anthesis (Fig. 8a). The level declined again in November during the onset of leaf senescence. The leaf nitrogen concentration remained relatively stable from April until July, a period of extended quiescence in the canopy of the tree (Fig. 8a). However, there was a sharp decline during the growth of inflorescences. Leaf N concentrations showed recovery during anthesis but declined once more during fruit set and spring shoot growth. The leaf concentration flux of starch and nitrogen showed significant changes which could be related to critical stages of tree phenology. The fall in concentration of both products coincided with inflorescence development and it is likely that remobilisation occurred to support the proximal reproductive sink. The decline in leaf N concomitant with fruit set and shoot growth has similarly been reported in citrus where it was concluded that young vegetative flushes draw nitrogen from reserves in old leaves (Erner 1988).

Net CO_2 assimilation (A) of sunlit summer-flush leaves reached its highest rate in April (18.3 μmol CO_2 m^{-2} s^{-1}) and then slowly declined through to May (Fig. 8b). By June there was a rapid decline in A which remained at ca. 10.2 μmol CO_2 m^{-2} s^{-1} through to October. There was a small recovery in A by November followed by a rapid decline as leaves senesced. Except for a lag phase going into winter the pattern of chlorophyll concentrations in leaves substantially mirrored A. Levels increased from March to April but then remained stable through to July.

There was a sharp decrease in August and concentrations remained low until October when there was a rapid increase through to November, then subsequently a fall as leaves senesced (Fig. 8b).

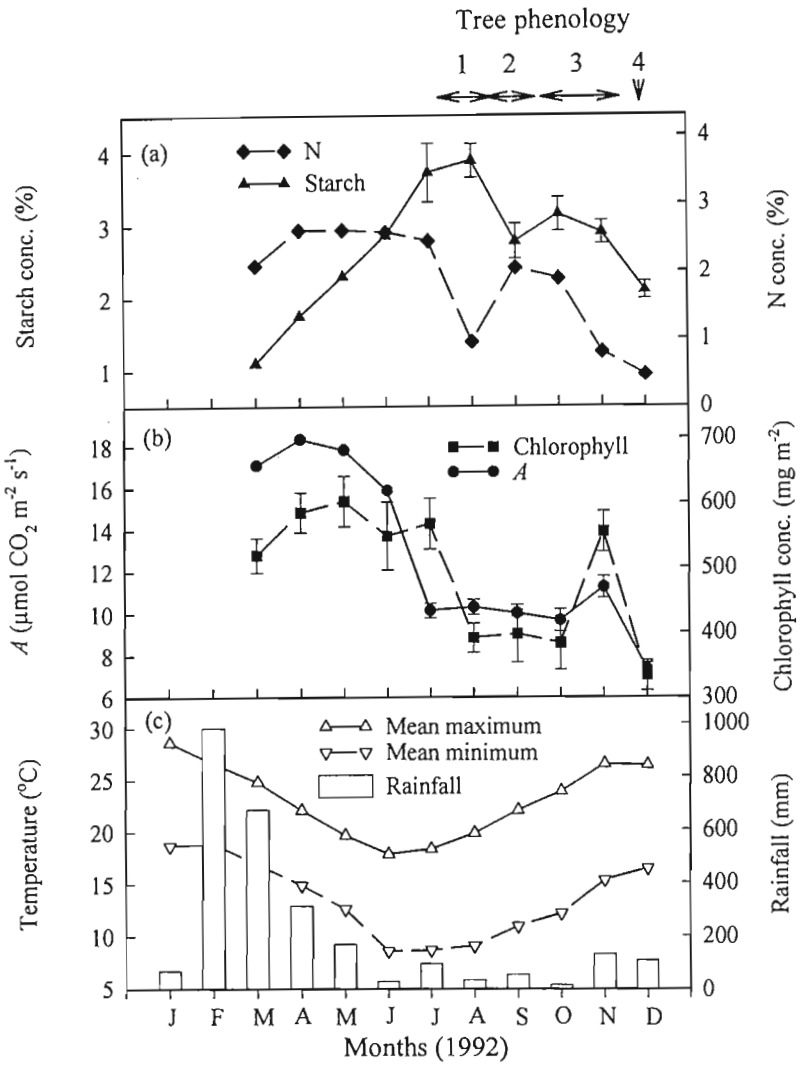

Fig. 8 Seasonal changes in nitrogen, starch and chlorophyll concentrations and net CO_2 assimilation (*A*) of summer grown leaves of cv. Hass in relation to phenology (1 = inflorescence development; 2 = anthesis; 3 = fruit set and shoot growth; 4 = leaf senescence) and temperature where: (a) are mean leaf nitrogen and starch concentrations (n = 5); (b) is the total chlorophyll concentration and *A* (n = 3); and (c) are the mean monthly temperatures and the exceptionally high rainfall recorded at the experimental site. Data are mean values of five trees ± vertical SE bars which are obscured by symbols at some points.

The autumn decline in *A* can be attributed to at least three factors. There was an increase in vapour pressure deficits (VPD) over the months that measurements were taken rising from < 1.0 kPa to ≈ 2.4 kPa. VPDs are known to affect *A* in most crops due to reduced stomatal conductance (g_s) (Schultze 1986). Bower *et al.* (1978) indirectly related lower *A* in avocado to an increase in VPD, i.e. they showed an inverse relationship between g_s and VPD. Over the duration of this study taking measurements before 1030 h reduced variation in VPD but nevertheless some effect may have occurred.

An increase in leaf starch levels was concomitant with the initial decline in *A* which may be the effect of end product feedback-inhibition. Schaffer *et al.* (1987) concluded that accumulation of leaf starch in avocado resulted in the inhibition of *A*. However, the sharp drop in July is more likely due to an inhibition of photosystem II activity brought on by exposure to low temperature stress (Chapter 3) (Smillie and Hetherington 1983). Smillie *et al.* (1988) reported that many tropical species develop photo-inhibition damage once temperatures fall below 12°C. In this case leaves had been exposed to mean minimum temperatures of < 10°C for one month prior to measuring the low *A* values (Fig. 8c). The winter fall in chlorophyll concentration is also more likely to be linked to temperature than to declining N concentrations. The one month lag in relation to the decline in *A* is consistent with photo-oxidation of chlorophyll, which develops after longer exposure to cold temperatures and an excess of absorbed light beyond that utilised in photosynthesis (van Hasselt 1974; Demmig-Adams and Adams III 1992) (see Chapter 3). The partial recovery in both *A* and chlorophyll concentrations is consistent with the release from photo-inhibition conditions (Smillie *et al.* 1988) and did not occur until October when mean minimum temperatures rose above 12°C. However, this was at a time when leaf N concentrations were rapidly declining thereby restricting the full potential of *A* recovery (DeJong 1982; Syvertsen 1984). Furthermore, it is believed that there is strong competition between reproductive and vegetative sinks at this time for available photo-assimilates, either current or from stored sources (Biran 1979; Wolstenholme *et al.* 1990). The horticultural implications of these results are further developed in Chapter 7 (section 7. 2) of this thesis.

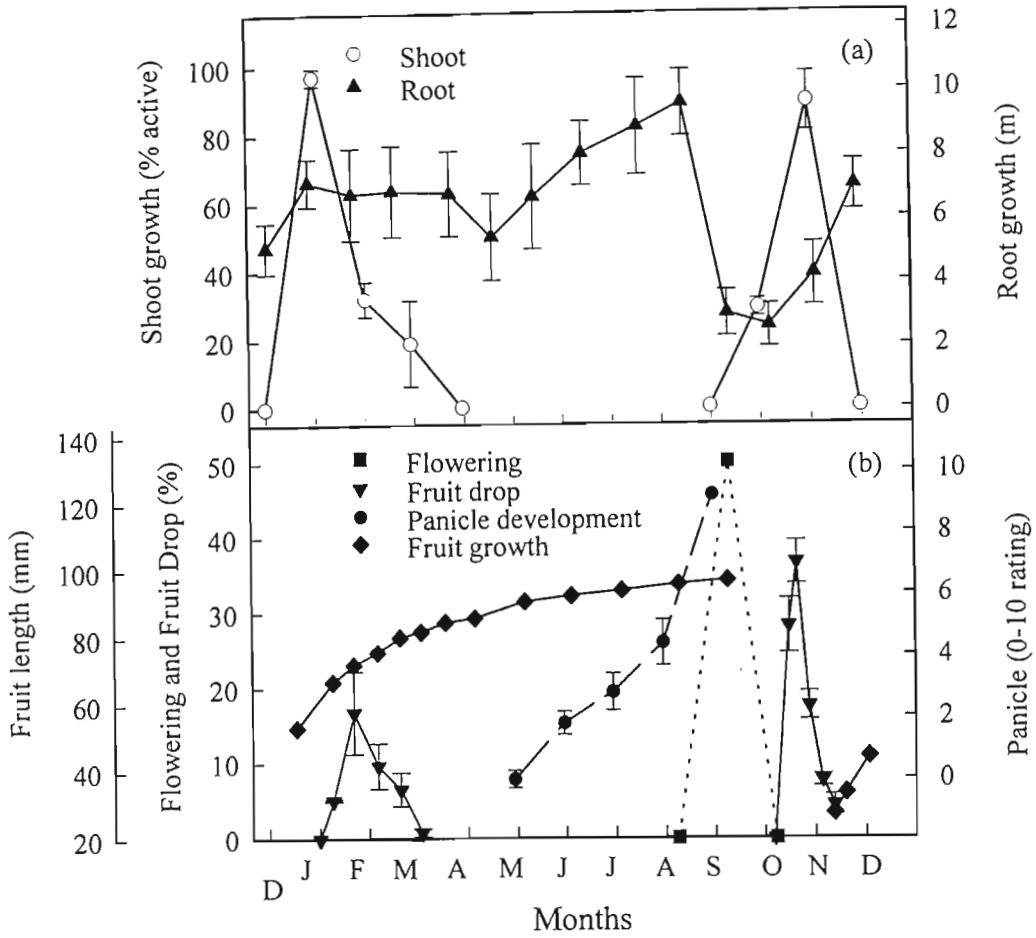

Fig. 9 Phenology of cv. Hass on cloned 'Velvick' rootstock growing at Maleny, S.E. Queensland where: (a) is the seasonal relationship between shoot and root growth; and (b) is the relationship between floral development and fruiting. Data points are mean values from five trees ± vertical SE bars which are obscured by symbols at some points.

The phenology model developed for 'Hass' at Maleny (Fig. 9) has similarities to the earlier schematic model proposed by Whiley *et al.* (1988a) for 'Fuerte' (Fig. 2). The main differences between the two models are firstly the delay of phenological events in relation to the time dimension, e.g. anthesis for 'Fuerte' was early June to late September; for 'Hass' from early August until early October; and secondly the extended root growth through winter culminating in a sharp decline concomitant with inflorescence development and anthesis. The shift in the time frame of growth events illustrates the plasticity of the phenological response which is

driven by genotypic/environmental interactions. For instance, 'Fuerte' is an early maturing cultivar and when studied was growing in a warm coastal environment compared with the later maturing 'Hass' growing in a cool, subtropical highland region. Modification by environmental factors may implement more significant changes to the model as shown by Kaiser and Wolstenholme (1994). In their studies with 'Hass' growing in the cool, mesic subtropical Natal midlands, one extended period of shoot growth was recorded over the spring and summer months in contrast to the bimodal periodicity reported for 'Fuerte' (Whiley *et al.* 1988a) and 'Hass' in this study (Fig. 9). Such changes require careful consideration of likely implications when research hypotheses or management strategies are being tested.

Differences in root growth patterns between the 'Fuerte' model (Whiley *et al.* 1988a) and 'Hass' (Fig. 9) can be explained by the different techniques used to collect the information. For 'Hass', studies were more quantitative and carried out from the surface to a depth of 820 mm thereby integrating results from a more representative zone of root activity than that used for 'Fuerte'. The extended period of root growth during summer through to mid-winter and the starch dynamics of scion/rootstock interactions have tree performance and management implications worthy of further research. Some pertinent issues will be discussed in subsequent chapters of this thesis

CHAPTER 3

LEAF AND SHOOT PHYSIOLOGY IN RELATION TO CO_2 ASSIMILATION AND FRUIT RETENTION

The harvest of solar energy by fixation of atmospheric carbon dioxide is one of the most important attributes of plants. Their success as crop plants largely depends on their ability to maintain a photosynthetically efficient canopy, which meets the demands of growth over changing environmental conditions and may shift substantially at both the daily and seasonal level (Long *et al.* 1994). A highland tropical to subtropical, evergreen species from Central America, the avocado is now cultivated over a wide range of environments from cool, semi-arid to humid, tropical climates (Whiley and Schaffer 1994) where it experiences adverse climatic conditions which induce physiological stresses. Photosynthetic activity is influenced by biotic (Wareing *et al.* 1968; Sams and Flore 1982; DeJong 1982; Schaffer *et al.* 1987) and abiotic factors (Ludlow 1983; Bongi *et al.* 1987; Jones, 1992a). This chapter examines some aspects of photo-assimilation of avocado in relation to shoot ontogeny, irradiance, and CO_2 partial pressures. The importance of current assimilate on fruit retention during spring shoot growth was also investigated.

3. 1 DYNAMICS OF GROWTH AND CO_2 ASSIMILATION OF THE FRUITING SPRING SHOOT

3. 1. 1 Introduction

Plant leaves have many functions that include recycling of a portion of the total nutrient stock, storage of carbohydrates, modification for defence purposes (spines) and a direct contribution to the carbon economy (Chabot and Hicks 1982). The product of assimilation rate and longevity ultimately determines the value of the leaf with respect to its carbon contribution, and in general the longer its life the lower its potential A_{max}, i.e. short-lived leaves are more likely to have higher A_{max} than long-lived leaves (Chabot and Hicks 1982). However, it is the CO_2

assimilation dynamics of the whole canopy, and subsequent partitioning of photoassimilate to economic end-product which are ultimately related to crop productivity. In this study, aspects of leaf age and the ontogeny of spring-grown, fruiting shoots of cv. Hass in relation to CO_2 assimilation were investigated.

3. 1. 2 Materials and Methods

Net CO_2 assimilation (A) measurements were carried out on seven-year-old cv. Hass trees (grafted to seedling 'Edranol' rootstocks), growing in a commercial orchard at Nelspruit, South Africa (latitude 25°S, altitude 660 m). The area has a warm subtropical climate with mean rainfall of 900 mm per annum and a mean min/max temperature of 18.6/29.1°C in January and 6.5/23.3°C in July. The trees were growing in a sandy loam soil with flood irrigation scheduled with a Class 'A' Evaporation Pan to ameliorate the development of water stress.

For this study, three uniform indeterminate flowering terminals on the sun-exposed northern side of each of five trees were selected near the completion of anthesis, and each leaf of the new shoot was tagged and dated as it emerged from the vegetative bud. The first leaf position on the new shoot generally produces a residual leaf and was ignored, with the second leaf designated the first leaf for the purposes of the study. Approximately 10 days after each leaf emerged, its area was measured with a portable leaf area meter (LICOR Model LI-3000) and A determined with a LICOR LI-6200 portable photosynthetic meter (see Materials and Methods, Chapter 2). Following the initial measurements, leaf areas were determined every 4 to 8 days until leaves stopped expanding (about 32 days after emergence). Photosynthesis measurements followed a similar schedule until A of the youngest leaf was constant (76 days after bud-break). Net A of the developing shoot ($\sum A$ per area of each leaf) was calculated at each point of measurement.

Ten days after bud-break, fruits set on the tagged indeterminate flowering shoots were counted. Counting was repeated at 7 to 14 day intervals for a period of 76 days after bud-break. Abscised fruit were recovered and microscopically examined for the presence of embryos.

Non-linear regression analyses using TableCurve™ (Jandel Scientific, Calif., USA) were used to correlate *A* to leaf expansion, as well as shoot ontogeny and fruit drop to the time elapsed after bud-break.

3. 1. 3 Results

The mean number of leaves and leaf area per fruiting shoot were 6.9 ± 1.1 and 496.4 ± 141.0 cm^2, respectively. Leaf growth followed a sigmoidal curve (Fig. 10) reaching 50% of full expansion after 18 days with maximum size 31 days after bud-break. There was ca. 10 days difference between full expansion of the first and last leaves on shoots which was related to the time that they developed. Hence leaf ontogeny was approximately the same duration for each position on the shoot.

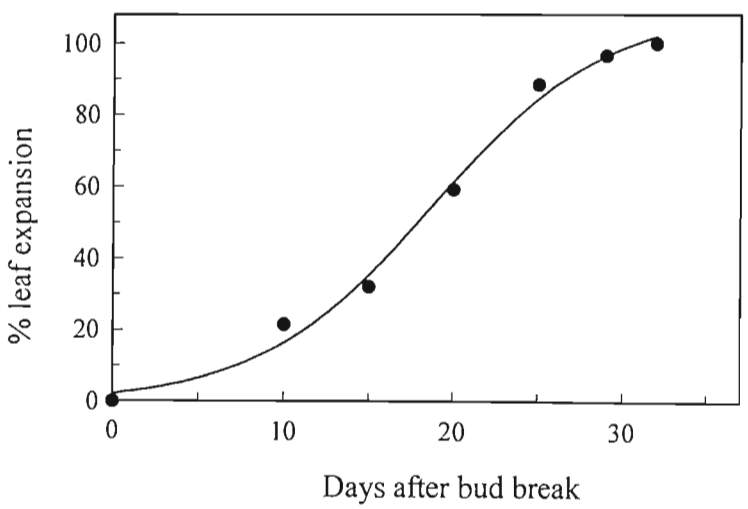

Fig. 10 Expansion of the first leaf on spring-grown fruiting shoots of cv. Hass in relation to bud-break where the regression curve is represented by: $y = - 0.776 + 110.475/(1 + \exp(-(x - 18.715)/5.168))$, $r^2 = 0.99^{**}$. Data points are mean values from three shoots on each of five trees (n = 5).

Leaves had reached ≈ 20% of their full expansion before they were large enough to fit the LI-6000-11 leaf chamber for the measurement of CO_2 exchange. At this stage of development there was a net CO_2 loss from leaves due to respiration being greater than their capacity for

assimilation (Fig. 11). Net CO_2 assimilation increased exponentially as leaves expanded, however the leaf-age compensation point at saturating PPF was not reached until individual leaves had reached 80% of their final size, i.e. individual leaves underwent the sink/source transition when ≈ 24 days old (Figs. 10 & 11). While leaves reached full expansion ≈ 31 days after bud-break (Fig. 10) they did not attain A_{max} until they were ≈ 50 days old (Fig. 12a). This time frame was approximately the same for each leaf irrespective of when it emerged.

With respect to the gas exchange characteristics of the whole shoot (excluding fruit), during early ontogeny there was a net CO_2 loss as expanding leaves had higher respiratory losses than

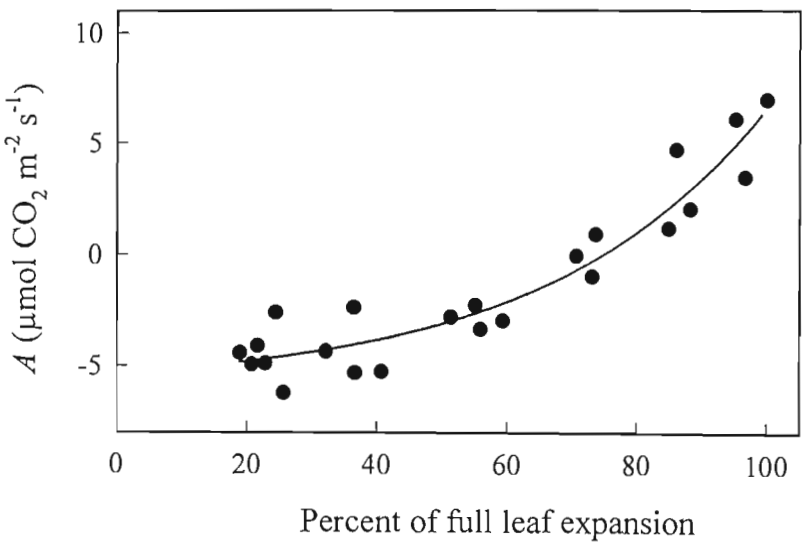

Fig. 11 Effect of leaf age on net CO_2 assimilation (A) of avocado leaves, cv. Hass, represented by the regression y = - 5.967 + 0.652 exp(-x/-33.824), $r^2 = 0.88^{**}$. Data are means from the first leaf on three fruiting spring-grown shoots on each of five trees (n = 15).

CO_2 assimilation gains (Fig. 12b). This net CO_2 loss (sink phase) from the shoot continued for the first 27 days after bud-break after which there was a net gain in CO_2 assimilated, i.e. the shoot as a whole became a source of carbon, with A_{max} for the shoot occurring ≈ 70 days after bud-break.

The mean number of fruits set on each shoot was greatest (29.1 ± 6.8) when first recorded ten days after bud-break (Fig. 12b). Fruit numbers declined rapidly as shoot ontogeny advanced with a mean of 1.5 ± 0.6 fruit per shoot remaining 76 days after bud-break. At that time, the number of fruit retained was 5% of the original number set. Of the abscised fruit recovered, 97% had formed normal embryos. From the correlations between fruit number and shoot A (Fig. 12b) it was estimated that by the time the shoot compensation point for A ($\sum R_l$ and A = 0) was reached, 25 fruit per shoot (86%) had fallen. A further 6% of the initial fruit set dropped during the period between the shoot compensation point and reaching shoot $A_{max,}$ and for the remainder of the study period fruit loss was minimal.

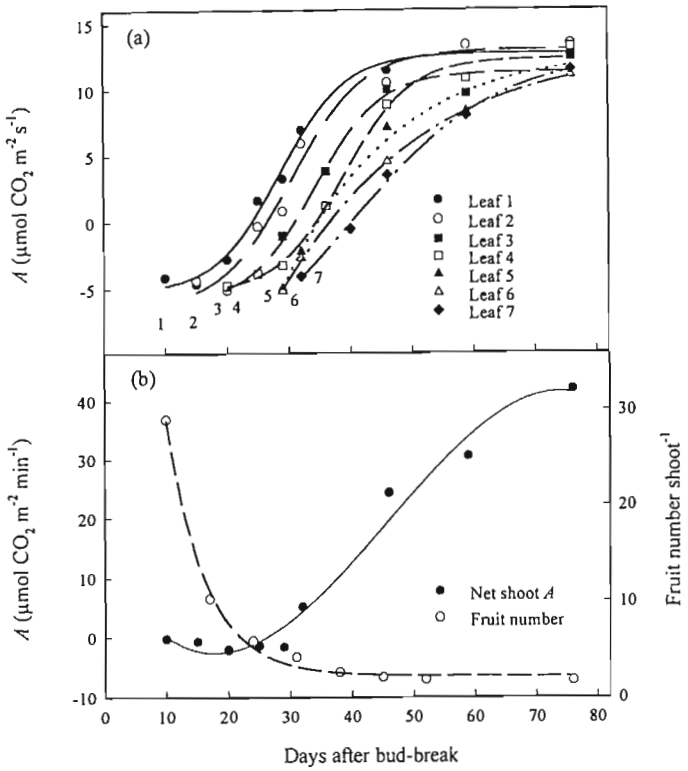

Fig. 12 CO_2 assimilation (A) and fruit loss from developing spring shoots where (a) is the A of maturing leaves from different positions along the shoot. Equations for each regression are presented below; and (b) the relationship between total A of the shoot represented by the regression $y = 13.65 - 1.988x + 0.069x^2 + 0.0005x^3$, $r^2 = 0.98$; and fruit drop represented by the regression $y = 2.213 + 130.39 \exp(-x/6.31)$, $r^2 = 0.99^{**}$. Data points are mean values from five trees (n = 15).

Leaf position	Regression	r^2
1	y = - 6.458 + 19.438/(1 + exp(-(x - 30.47)/5.77))	0.96**
2	y = 6096 + 17.361/(1 + exp(-(x - 34.20)/5.321))	0.96**
3	y = - 5.33 + 17.63/(1 + exp(-(x - 38.91)/5.437))	0.95**
4	y = - 5.264 + 17.96/(1 + exp(x - 28.635)/5.284))	0.96**
5	y = - 209.40 + 222.29/(1 + exp(-(x -+ 11.647)/16.62))	0.97*
6	y = - 119.41 + 132.34/(1 + exp(-(x + 8.697)/20.34))	0.99**
7	y = - 13.158 + 25.764/(1 + exp(-(x - 39.566)/12.23))	0.99*

3. 2 CO_2 ASSIMILATION OF CV. HASS LEAVES IN RELATION TO IRRADIANCE, CO_2 PARTIAL PRESSURES AND TEMPERATURE

3. 2. 1 Introduction

General features of subtropical and tropical fruiting trees include large leaves producing high ratios of leaf area to canopy surface; leaves can be up to 25% of the fresh mass of the tree and can contain a substantial reserve of nutrients and carbohydrate; some species have highly efficient light-harvesting chloroplasts for growth under low photosynthetic photon fluxes (PPF); leaves have a limited vascular network with high stomatal density; *A* commonly saturates at about 20 to 25% of full sunlight, and light compensation points are low (Possingham 1986).

There is still a paucity of published information on leaf gas exchange characteristics of avocado, although some results are available which have similar findings. In separate studies, Bower *et al.* (1978), Kimelmann (1979), Scholefield *et al.* (1980) and Schaffer *et* al. (1987) agreed that A_{max} of leaves was between 6 to 9 μmol CO_2 m^{-2} s^{-1}. There was also general agreement between Bower *et al.* (1978) and Scholefield *et al.* (1980) that light saturation for CO_2 assimilation (Q_A) of avocado leaves was at PPFs between 400 to 500 μmol quanta m^{-2} s^{-1}, i.e. 20-25% of full sunlight. Hence, these results added authenticity to the generally held belief that evergreen species have lower photo-assimilation capacity than deciduous crops (Larcher 1969; Chabot and Hicks 1982; Mooney and Gulmon 1982).

Rate-limitation of photosynthesis is attributed to restricted CO_2 diffusion, either through stomatal resistance or physical resistance through the mesophyll (Wareing *et al.* 1968); leaf age and longevity (Chabot and Hicks 1982; Sams and Flore 1982); sink-limited feedback inhibition (Arp 1991; Thomas and Strain 1991), nutrient deficiencies (Gulmon and Chu 1981; DeJong 1982; Schaffer and Gaye 1989) and water, temperature and light factors (Taylor and Rowley 1971; Kaiser 1987; Anderson and Brodbeck 1988a). Exposure of tropical and subtropical species to temperatures below ≈ 10°C is reported to greatly retard or stop growth causing

dysfunction of cellular processes. The effects of these temperatures on cellular processes are reversible with limited exposure to temperatures below 10°C, but ultimately irreversible leading to cell death (Taylor and Rowley 1971; Powles 1984; Smillie *et al.* 1988). Chill injury results in the inhibition of photosynthetic (Taylor and Rowley 1971; Öquist and Martin 1986) and other metabolic processes (Graham and Patterson 1982), with leaf yellowing (photo-oxidation of chlorophyll) developing after extended exposure to low temperatures (Taylor and Rowley 1971; Taylor *et al.* 1974; Powles 1984; Robinson 1993). Functionally, the consequences of photoinhibition of photosynthesis are a reduction in the maximum quantum yields for CO_2 uptake (Ø) (Powles 1984), and with prolonged exposure to excessive light, a decreased rate of light saturated photosynthesis (A_Q) (Long *et al.* 1983; Powles *et al.* 1983). Damage to leaves from chill stress may be quantified by measuring the decrease in the variable fluorescence (F_v) of photosystem II (PS II) in relation to the maximum fluorescence (F_m), usually parameterised as F_v/F_m, which implies decreased photochemical conversion efficiency of PS II (Krause 1988; Smillie *et al.* 1988). Where persistent low temperatures limit carbon assimilation, decreases in photosynthetic efficiency which may persist for months have been observed in evergreen leaves. The obvious cost is the reduction in efficiency of conversion of intercepted light into plant dry matter, which may affect avocado which is often still committed to fruit development during winter - a time of prolonged low temperature stress.

Documented research on A of avocado was carried out on container-grown trees (Bower *et al.* 1978, Kimelmann 1979, Scholefield *et al.* 1980) or field-grown trees (Schaffer *et* al. 1987) where soil conditions imposed severe root restriction (Crane *et al.* 1994). Arp (1991) and Thomas and Strain (1991) reported reduced photosynthetic capacity in a number of species grown in pots where roots had become pot-bound. They attributed this effect to end product-inhibition caused by the sink-limitation of restricted root systems. In view of these recent findings it is pertinent to re-examine the relationship between PPF and the CO_2 assimilation responses of avocado under field grown conditions.

The availability of reliable portable photosynthetic meters in the 1980s has advanced the study of CO_2 assimilation of plants in natural environments. This study investigates the response of

leaves on mature, field-grown avocado trees to light and CO_2 partial pressures before and during extended cold temperature stress over winter.

3. 2. 2 Materials and Methods

Five year old cv. Hass trees grafted to seedling 'Velvick' rootstocks (see Chapter 2), growing in a commercial orchard with a cool, mesic subtropical climate at Maleny were used in this study. Irrigation was provided by two under-tree sprinklers each delivering 10 l hr^{-1}, and watering was scheduled with permanently installed tensiometers at frequencies to ameliorate development of water stress (Banks 1992). An automatic weather station (Monitor Sensors, Caboolture, AUS.) in the orchard provided minimum and maximum temperature data for the duration of the study. For each set of gas exchange measurements, mature summer-grown leaves from at least three non-fruiting shoots on three trees were selected at a time when shoot growth and floral development were quiescent. Measurements of *A* and intercellular partial pressures of CO_2 (Ci) under variable PPFs and ambient CO_2 partial pressures were made in May 1994 on non-cold stressed (NCS) leaves, and repeated at the end of July 1994 on cold stressed (CS) leaves after prolonged exposure to orchard night temperatures < 10°C (Table 3). In each case measurements were made between 0800 and 1030 h to reduce the effect of diurnal variation on *A* and Ci. Leaf cuvette temperatures and VPD's during pre- and post cold stress measurements were 25 to 27°C and <1.0 kPa and 21 to 24°C and < 1.2 kPa, respectively. To characterise potential chill damage to PSII, chlorophyll fluorescence of leaves was measured using a BioMonitor Stress Meter (BioMonitor SCI, Umeå, Sweden). Measurements were taken monthly starting in April when summer growth had matured and concluded prior to anthesis in August. A further set of measurements were taken at the end of October during the rapid growth stage of fruitlets and spring shoots. Measurements were taken on 4 to 5 sunlit leaves proximate to where gas exchange determinations were carried out on the same three trees. Cuvettes were attached to leaves on each side of the midrib between 0900 and 1000 h and chlorophyll fluorescence measurements taken after 30 mins of dark adaptation followed by two secs irradiation with 400 μmol quanta m^{-2} s^{-1} of blue light (320-550 nm).

Fluorescence was quantified as the F_v/F_m ratio where:

F_0 = initial constant yield fluorescence

F_m = maximum fluorescence recorded

Variable fluorescence (F_v) was calculated as described by Öquist and Wass (1988); where:

$$F_v = F_m - F_0$$

For the light studies, PPF was manipulated by the use of 1 m^2 frames with a polythene mesh (Sarlon Industries, Sydney, AUS.) of different light transmission properties, i.e. by varying mesh size and density. Measurements were made on cloud-free days to ensure acclimation of leaves to a range of PPFs under stable irradiance conditions. Frames were erected 0.5 m from the tree canopy and the leaves allowed to acclimate to the new PPF conditions for at least 30 mins prior to taking measurements. Determinations of *A* and Ci were made with a LICOR LI-6200 portable photosynthetic meter (see Materials and Methods, Chapter 2). Readings of *A* taken at zero PPF were obtained by wrapping the leaf cuvette in a black cloth and waiting until CO_2 evolution stabilised.

Measurements of the responses of *A* to CO_2 partial pressures were made at saturating PPFs (Whiley and Schaffer 1994) using a CIRAS-1 portable photosynthesis meter (PP Systems, UK) configured as an open system. This equipment facilitates delivery of variable ambient CO_2 partial pressures to the leaf cuvette via compressed CO_2 cartridges. Determinations were made approximately 3 to 5 min after placing the leaf in the cuvette when *A* had stabilised.

Data were fitted to non-linear regression models (TableCurve™, Jandel Scientific, Calif., USA) and the light compensation (Q_O), and light (Q_A) and CO_2 (C_A) saturation points for *A* were predicted (Q_A and C_A = 0.9 of the maximum *A* [A_{max}]; Osman and Milthorpe 1971). Quantum yield (Ø) was estimated from the slope of the linear portion (at low PPFs) of the *A* vs. PPF curve.

3. 2. 3 Results

Mean monthly minimum and maximum air temperatures for the experimental site are presented in Table 3. During April and May when the first leaf measurements were made the mean minimum air temperatures were > 12°C. There were 22 and 21 nights in June and July respectively, when minimum temperatures were < 10°C. The coldest temperatures registered were 4.7 and 4.6°C for each of the months, respectively. Mean minimum temperatures remained below 10°C until September when they increased to 11°C.

Table 3 Mean monthly temperatures in the orchard at Maleny for 1994.

Months‡	Mean temperature (°C)	
	min.	max.
Jan	19.2	28.1
Feb	17.6	24.5
Mar	16.1	23.3
Apr	14.0	22.0
May	12.9	21.1
Jun	9.2	19.1
Jul	8.0	18.0
Aug	8.3	19.1
Sep	10.0	23.6
Oct	12.5	24.2

‡ F_v/F_m ratios were determined in May and July 1994.

Chlorophyll fluorescence

Mean min/max temperatures for the five days preceding *A* measurements were 12.7/20.2°C and 5.5/18.8°C in May and July, respectively. The F_v/F_m ratios in April and May were 0.81 ± 0.02 and 0.79 ± 0.01, respectively (Table 4) indicating that leaves had not been exposed to damaging low temperatures and photosynthetic processes were functioning normally (Öquist and Wass 1988). In June, minimum temperatures dropped below the critical threshold where chill injury may occur (Table 3) and this was reflected in a substantial decrease in the F_v/F_m ratio (Table 4). By late July, following a period of low temperatures, a few north-facing leaves had visible signs of chlorophyll photo-oxidation (Fig. 13), a condition caused by low temperatures and high PPFs (van Hasselt 1974). At this time the F_v/F_m ratio was 0.41 ± 0.03 indicating cold-induced stress. Increased mean minimum temperatures in August were concomitant with a higher F_v/F_m ratio, and substantial improvement was measured ca. eight weeks later (31 October) when the F_v/F_m ratio was 0.67 ± 0.01. The October measurement of the F_v/F_m ratio suggests that the photoinhibition that developed during the winter months in these leaves was reversible and is further supported by the seasonal changes in *A* reported on the same trees in 1992 (see Fig. 8, Chapter 2).

Table 4 Seasonal variation in chlorophyll fluorescence (F_v/F_m) measured on field-grown cv. Hass trees growing at Maleny. Data are mean values (± SEs) of 4 to 5 leaves from each of three trees.

F_v/F_m ratio					
April	May	June	July	August	October
0.81 ± 0.02	0.79 ± 0.01	0.51 ± 0.05	0.41 ± 0.03	0.48 ± 0.03	0.67 ± 0.01

Fig. 13 Photo-oxidation of the chlorophyll of a cv. Hass avocado leaf following extended exposure to night temperatures < 8°C and PPFs > 1600 μmol quanta m^{-2} s^{-1}. The decline in total chlorophyll concentration in 'Hass' leaves during winter is shown in Fig. 8b (Chapter 2).

Response of A and Ci to PPF

The photosynthetic rate increased asymptotically in response to increasing PPF, irrespective of the pre-conditioning temperatures to which the leaves had been exposed (Fig. 14a). Exposure to temperatures < 10°C reduced A_Q from the NCS leaf value of 16.12 ± 0.26 μmol CO_2 m^{-2} s^{-1} to 11.1 ± 0.29 μmol CO_2 m^{-2} s^{-1}, and reduced Q_A from 1270 μmol quanta m^{-2} s^{-1} to 1040 μmol quanta m^{-2} s^{-1}. The Q_0, estimated from regression curves, was ≈ 30 μmol quanta m^{-2} s^{-1} for NCS leaves and increased to ≈ 50 μmol quanta m^{-2} s^{-1} after leaves had been exposed to cold temperatures.

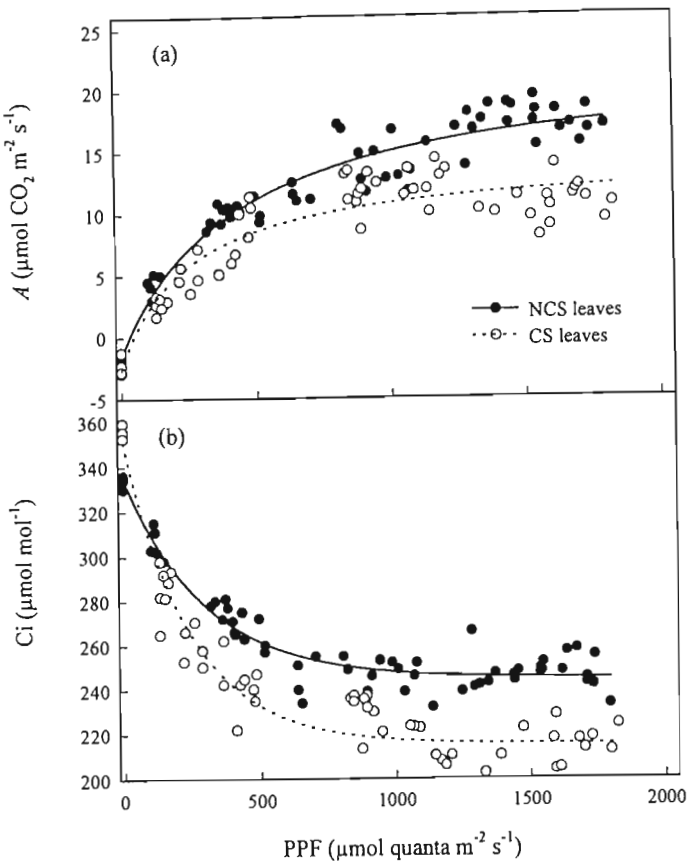

Fig. 14 Responses of CO_2 assimilation (A) and intercellular partial pressures of CO_2 (Ci) in leaves of field-grown avocado trees cv. Hass, to varying photosynthetic photon flux (PPF). Data points are for single leaves. The regression line for A of non-cold stressed (NCS) leaves is represented by (a) $y = 22.08\ ((-30.67 + x)/(427.43 + x))$, $r^2 = 0.94^{**}$; and cold stressed (CS) leaves by $y = 14.17((-46.72 + x)/(250.05 + x))$, $r^2 = 0.86^{**}$; the regression line for Ci of NCS leaves is represented by (b) $y = 244.65 + 90.2\ \exp(-0.0034x)$, $r^2 = 0.94^{**}$, and CS leaves by $y = 215.28 + 135.52\ \exp(-0.0041x)$, $r^2 = 0.92^{**}$.

For NCS and CS leaves Ci initially declined rapidly and then levelled as leaves were removed from darkness and exposed to increasing PPFs (Fig. 14b).

Q½, representing the PPF when Ci is reduced by 50% was calculated from :

$Q½ = \ln(0.5)/-k$ where k is the third constant in the standard decay curve fitted to the data(adapted from Jones 1992b)

Calculated values of Q½ indicate that NCS leaves had a higher light requirement (207 µmol quanta m^{-2} s^{-1}) compared with CS leaves (167 µmol quanta m^{-2} s^{-1}) to reduce Ci by one-half. Thus the Q_A of NCS leaves will be higher than that for CS leaves so long as stomatal conductance is not a limiting factor, supporting the information presented in Fig. 14a.

Cold temperatures significantly reduced Ø from 0.0545 µmol CO_2. µmol quanta^{-1} to 0.0336 µmol CO_2. µmol quanta^{-1} (Fig. 15).

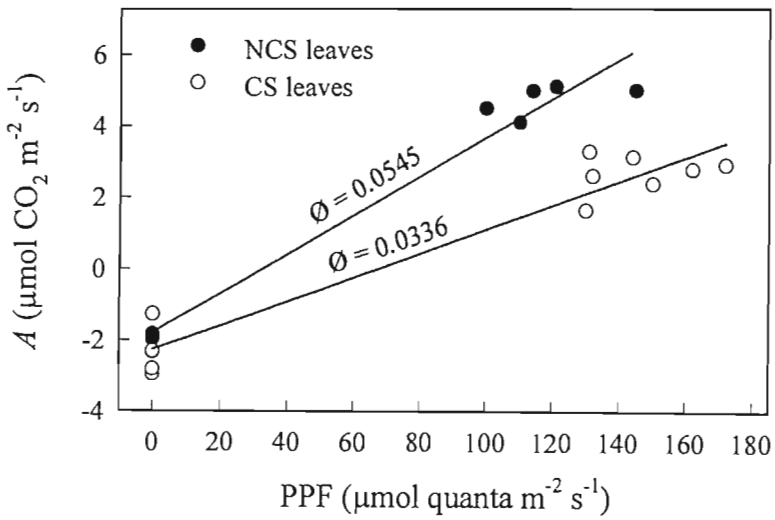

Fig. 15 Quantum yield (Ø) of non-cold stressed (NCS) and cold-stressed (CS) leaves on field-grown cv. Hass trees. Data points represent values of single leaves and CO_2 partial pressure was 330 µmol mol^{-1} at the time of measurement. Ø differ significantly ($P < 0.01$) as judged by a *t* test comparing the slopes.

Response of A and Ci to increasing partial pressures of CO_2

Net CO_2 assimilation showed a typical asymptotic response to increasing partial pressures of CO_2 for both NCS and CS leaves (Fig. 16a). After short term exposure to increasing partial pressures of CO_2, the A_{max} estimated at 2100 µmol mol^{-1} CO_2 for NCS and CS leaves, was 54.1 ± 3.7 and 42.1 ± µmol CO_2 m^{-2} s^{-1}, respectively. Exposure to temperatures < 10°C decreased the CO_2 saturation point (C_A,) from ≈ 1320 µmol mol^{-1} CO_2 for NCS leaves to ≈ 1260 µmol

mol^{-1} CO_2, and reduced the saturated CO_2 assimilation rate (A_C) of 48.7 ± 2.7 μmol CO_2 m^{-2} s^{-1} for NCS leaves to 37.9 ± 2.4 μmol CO_2 m^{-2} s^{-1}. Ci in leaves from both treatments increased in a linear relationship as the CO_2 partial pressure increased and was non-limiting for A (Fig. 16b).

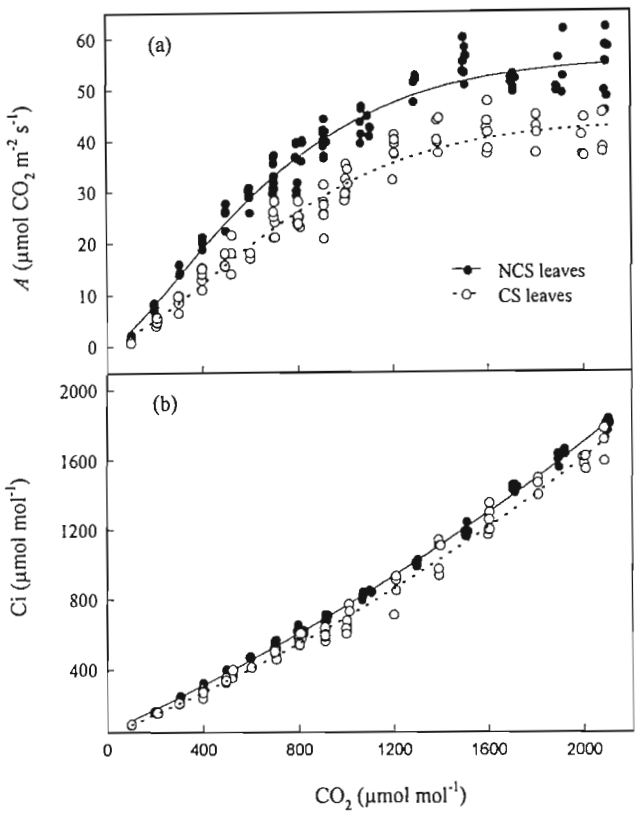

Fig. 16 Responses of CO_2 assimilation (A) and intercellular partial pressures of CO_2 (Ci) in leaves of field-grown avocado trees, cv. Hass, to increasing partial pressures of CO_2. Data points are for single leaves. The regression line for A of non-cold stressed (NCS) leaves is represented by (a) $y = 55.61(1-\exp(-((x + 745.02\ \ln(2)^{1/1.354} - 578.39)/745.02)^{1.354}))$, $r^2 = 0.96^{**}$; and for cold stressed (CS) leaves is represented by $y = 43.2-\exp(-((x + 880.14\ \ln(2)^{1/1.583} - 659.07/880.14)^{1.58}))$, $r^2 = 0.96^{**}$; and the regression line for Ci of NCS leaves is represented by (b) $y = -2372.91 + 2421.79 \exp(-x/-3845.81)$, $r^2 = 0.99^{**}$, and for CS leaves is represented by $y = -1818.11 + 1850.45 \exp(-x/-3235.892)$, $r^2 = 0.99^{**}$.

3. 3 EFFECT OF PRE-ANTHESIS DEFOLIATION OF TREES AND THE INFLUENCE OF INDETERMINATE AND DETERMINATE INFLORESCENCES ON FRUIT RETENTION

3. 3. 1 Introduction

Pre-flowering concentrations of stored carbohydrates have been positively related to yield in several fruit crop species (Hilgeman *et* al. 1967; Goldschmidt and Golomb 1982; Scholefield *et al.* 1985; Worley 1979). Based on a three-year study, Scholefield *et al.* (1985) reported that pre-flowering concentrations of trunk starch in 'Fuerte' avocado trees were directly related to subsequent yield. During flowering and fruit set these trees lost most of their leaves with no effective source of current photo-assimilates available until the maturation of the new growth in spring. Possibly as a consequence, strong biennial cropping patterns developed because fruit set and retention were dependant on a 'pool' of stored carbohydrate which did not accumulate in sufficient quantity during seasons of heavy cropping. Maximum levels of starch accumulation in 'Hass' avocado trees in subtropical production areas of Queensland, Australia and Natal, South Africa do not reach the same magnitude as those reported by Scholefield *et al.* (1985) and trees in Queensland and Natal retain their over-wintered leaves during anthesis and fruit set (Kaiser and Wolstenholme 1994; Chapter 3).

Avocado conforms architecturally to Rauh's model wherein the majority of inflorescences are compound indeterminate (Reece 1942; Hallé *et al.* 1978). However, departures from this model can occur with the production of determinate inflorescences at varying frequencies (Thorp *et al.* 1994; Whiley and Schaffer 1994). Close coupling between fruit and shoot growth from indeterminate inflorescences is thought to be partially responsible for the low fruit retention characteristic of avocado. Removal of the terminal shoot during early development has been shown to increase fruit retention and yield (Biran 1979; Cutting and Bower 1990). A similar result has been achieved with the use of strategically timed foliar applications of the growth inhibitor paclobutrazol to 'Fuerte' (Adato 1990) and 'Hass' trees (Wolstenholme *et al.* 1990; Whiley *et al.* 1991). The dynamics of fruit set and retention on naturally occurring

determinate inflorescences of avocados has not previously been reported in detail and may provide additional information on the mechanisms of fruiting in avocados.

The objective of this study was to investigate the role of over-wintered leaves produced mainly during the summer (current photoassimilate supply) on fruit set and retention during spring shoot ontogeny of cv. Hass, and to compare the dynamics of fruit set and retention of indeterminate and determinate inflorescences.

3. 3. 2 Materials and Methods

Four-year-old cv. Hass trees grafted to 'Velvick' seedling rootstock and growing in a commercial orchard at Maleny, S.E. Queensland, were selected for the defoliation study which was carried out on the same trees over two cropping cycles. The block was not irrigated but annual rainfall is very high and trees were mulched from their trunks to the drip line with barley straw (100 mm deep) and fertilised and sprayed for pest and disease control according to standard commercial recommendations (Banks 1992). Initial tree selection was made at the completion of summer growth with care being taken to select trees of similar size and appearance in relation to tree vigour. Six uniform trees were chosen and immediately prior to flower development, all leaves were hand-stripped from three of the trees which then had their trunks and limbs coated with a white acrylic-based paint on the upper and north-western surfaces to protect against sunburn. Ten shoots, between 1 and 2.5 m above soil level, were labelled on the northern side of each of the six trees. As soon as indeterminate shoots could be identified, five uniform shoots were selected from the original group chosen on each tree. The start (first flower open) and termination (last flower open) of anthesis were recorded on the selected shoots on each tree.

The number of fruits set on each of the selected shoots was counted at the end of anthesis. Nine and 18 days after bud-break of the terminal vegetative bud, fruit on each shoot were counted again and to judge shoot development, the length and breadth of youngest leaves

measured. Thereafter fruit counts and leaf measurements on each of the shoots were repeated at 3 to 4 day intervals until shortly after the youngest leaf reached full expansion.

'Hass' trees grafted to cloned 'Velvick' rootstocks were chosen at the same location for the studies on fruiting characteristics of determinate and indeterminate inflorescences. These trees were growing in the rhizotron facility under conditions already described (Chapter 2). Three terminals of each inflorescence type were selected for uniformity on each of five trees near the completion of anthesis. The mean date of bud-break of the terminal vegetative bud on indeterminate inflorescences was used as the reference point for fruit numbers on each terminal. From 12 days after bud-break fruit were counted on each terminal at weekly intervals for eight weeks with a final count being made two weeks later.

Climatic data for the duration of the experiment were recorded on an automatic weather station (Monitor Sensors, Caboolture, AUS.) near the experimental site.

TableCurve™ (Jandel Scientific, Calif., USA) was used for non-linear regression analyses to model the growth of the youngest leaf and the loss of fruit in relation to shoot ontogeny.

3. 3. 3 Results

Defoliation studies

There was no difference in the time of the anthesis period among the selected shoots (data not presented) which occurred from 27 August to 9 October 1991 and 2 September to 6 October 1992. In both years the main period of flowering occurred during September when mean min/max temperatures were >10 and 20°C, respectively (Table 5), which are non-restrictive for floral dichogamy, pollination and fertilisation of 'Hass' trees (Sedgley and Annells 1981).

Patterns of fruit set and drop and leaf expansion were similar for both years of the study so only data for 1992 are presented. Also, as there was no temporal separation in leaf development on shoots from either treatment, the 1992 data have been pooled for regression analysis. There

Table 5 Mean monthly maximum/minimum temperatures recorded in the orchard at Maleny during anthesis in 1991 and 1992.

Months	Rainfall (mm)		Temperature (°C)			
	1991	1992	1991		1992	
			min.	max.	min.	max.
Aug	0.3	34.6	10.0	21.2	9.1	19.9
Sep	4.8	54.8	11.5	24.7	11.0	22.1
Oct	82.6	15.2	13.7	24.9	12.2	23.9

was no significant difference in the initial number of fruits set on shoots of defoliated trees compared with control trees; these being 20.88 ± 2.04 and 22.50 ± 1.96, respectively. However, subsequent fruit loss from shoots on all trees was high, although it became greater on defoliated trees as shoots approached maturity (Fig. 17).

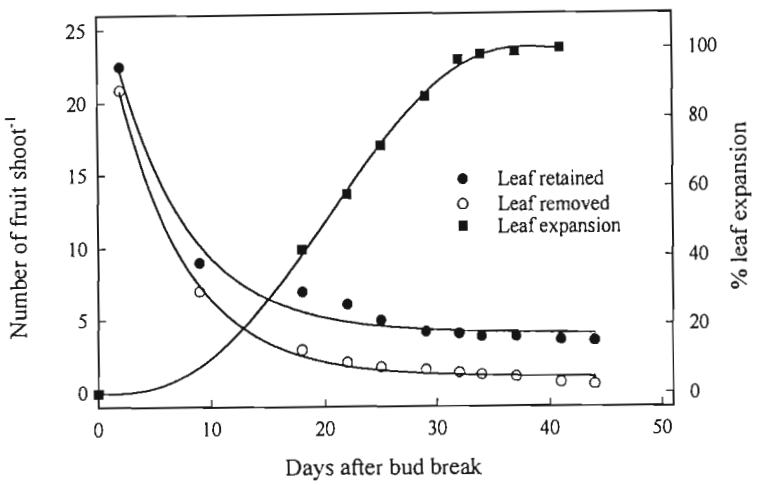

Fig. 17 Spring leaf expansion and fruit loss from shoots on trees where the mature, over-wintered leaves were either retained or removed prior to anthesis. The model for expansion of the youngest leaf is represented by $y = -2.132 + 106.44/(1 + \exp(-(x-20.274)/5.397))$, $r^2 = 0.99^{**}$ (data are mean values from six trees, n = 30); the model for fruit loss from shoots on trees where leaves were retained is represented by $y = 4.02 + 24.83 \exp(-0.154x)$, $r^2 = 0.98^{**}$, and for fruit loss from shoots on trees where leaves were removed by $y = 0.79 + 26.36 \exp(-0.136x)$, $r^2 = 0.99^{**}$. Fruit drop curves are significantly different in placement as judged by *t* test ($P < 0.01$). Fruit data are mean values for from three trees (n = 3).

Shoots were judged mature when the last-formed leaf reached full expansion, which was ≈ 37 days after bud-break (Fig. 17). With respect to the time for full expansion of leaves, the results from this study were in agreement with those reported with 'Hass' in South Africa (Chapter 3. 1. 3). At this stage of shoot development it was estimated from the regression models that defoliated trees had retained 1.1 ± 0.16 fruits shoot^{-1} while the control trees had retained 4.1 ± 0.38 fruits shoot^{-1}.

Dry matter accumulation was significantly different between shoots from the two treatments when measured at shoot maturity (60 days after bud-break). Shoots on defoliated trees produced ca. 37% more dry matter than shoots on trees where over-wintered leaves were retained (Table 6). In shoots from the control trees, 93.5% of the dry matter was partitioned to the vegetative component of the shoot with the remaining 6.5% partitioned to fruit. However, in shoots from defoliated trees 98.4% of the dry matter was partitioned to the vegetative component of the shoot with only 1.6% distributed to fruit.

Table 6 Effect of pre-anthesis defoliation on the distribution of dry matter in mature spring-grown fruiting shoots of cv. Hass trees. Data are mean values ± SEs of five shoots from each of three trees. Percentage allocation of dry matter within shoots are indicated in parenthesis.

Shoot component	Dry weight (g shoot^{-1})	
	Untreated	Pre-anthesis defoliation
Leaf	8.84 ± 0.22 (82.8)	11.57 ± 0.08 (79.3)
Stem	1.14 ± 0.35 (10.7)	2.79 ± 0.35 (19.1)
Fruit	0.70 ± 0.18 (6.5)	0.23 ± 0.20 (1.6)
Total shoot	10.68 ± 0.25	14.59 ± 0.21

With respect to final yield there was a significant reduction in both fruit number and total weight on trees defoliated prior to inflorescence development (Table 7). Based on the

cumulative yield for the two years of the experiment, pre-flowering defoliation reduced fruit numbers and yield by ca. 77%.

Table 7 Effect of pre-anthesis defoliation on the cumulative fruit numbers and yield (1992 & 1993) of cv. Hass trees. Data are mean values ± SEs of three trees.

Treatment	Fruit number ($tree^{-1}$)	Fruit weight ($kg\ tree^{-1}$)
Pre-anthesis defoliation	24.67 ± 3.93	6.73 ± 0.74
Control (retention of leaves)	111.00 ± 2.08	30.63 ± 0.94

Fruit retention on indeterminate and determinate inflorescences

There was an exponential loss of fruit over time from set to spring shoot maturity from both types of terminals (Fig. 18). Initial fruit set was considerably higher on the determinate terminals (62.5) compared with indeterminate terminals (46.2). At the completion of spring shoot growth (ca. 40 days after bud-break) an average of only 1.7 and 0.4 fruit remained on determinate or indeterminate terminals, respectively. This was equivalent to 2.7% of the initial set on determinate, and 0.9% for indeterminate terminals.

3. 3. 4 Discussion

Leaf ontogeny and A characteristics

Young leaves are heterotrophic, i.e. sinks, depending to a greater or lesser extent on photoassimilates imported from other regions of the plant for growth. However, by full expansion they are autotrophic (or sources), exporting products of photosynthesis to support growth and development in the total plant (Dale 1985; Turgeon 1989; Kozlowski 1992). The sink-source transition in leaves has been the subject of many studies, with leaves of

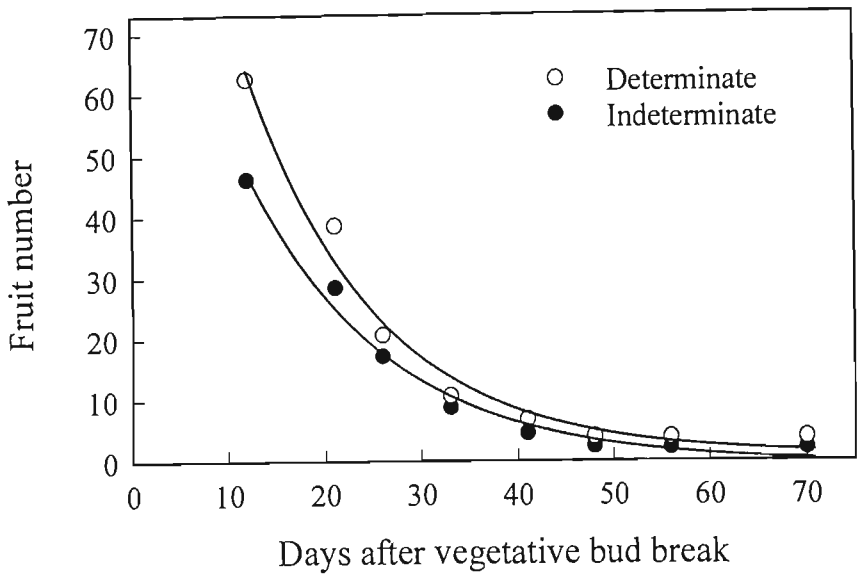

Fig. 18 Fruit loss from indeterminate and determinate flowering terminals from fruit set to maturity of the spring shoot. The regression model for fruit loss from indeterminate terminals is represented by the equation $y = -0.509 + 109.664(0.933^x)$, $r^2 = 0.99$; and for determinate terminals is represented by the equation $y = 0.907 + 156.42(0.927^x)$, $r^2 = 0.98$. The curves are significantly different in placement as judged by *t* test ($P < 0.01$). Data points are mean values of fruit numbers from three terminals on each of five trees.

expansion (Turgeon 1989). However, they continue to import photoassimilates from nearby source leaves for a period after beginning the export of their own carbon-based products (Fellows and Geiger 1974; Ho and Shaw 1977; Anderson and Dale 1983). It is likely that the longevity of leaves influences the stage of development at which they undergo the sink-source transition. For instance, leaves with a life span of less than eight months reach the sink-source transition at 25 to 50% of full expansion (*Cucumis sativis*, Hopkinson 1964 and Ho *et al.* 1984; *Vitis vinifera*, Bernard 1985; *Actinidia chinensis*, Lai *et al.* 1988), while in evergreen species it is delayed until leaves are near full size (*Citrus spp.*, Kriedemann 1969a; *Persea americana*, Buchholz 1986).

In this study, 'Hass' avocado leaves reached full expansion 31 days after bud-break which is similar to the 27-28 days reported by Schaffer *et al.* (1991) for leaves of the West Indian cvs. Booth-8 and Peterson in a warmer climate. Although a net gain in A of 'Hass' leaves in this study was recorded at 80% of full leaf expansion (24 days old), ^{14}C studies by Buchholz (1986) suggested that avocado leaves do not become an effective source until fully expanded. i.e. when about 28-30 days old. This transition period from photosynthetic competency to net exporter of assimilates supports results reported for other species (Fellows and Geiger 1974; Ho and Shaw 1977; Anderson and Dale 1983) and may be due to the requirements of growth and R_d exceeding the initial supply of photosynthetic products.

Twenty days after full leaf expansion there was a two-fold increase in A with A_{max} occurring 50 days after bud-break. This result contrasts with those reported by Kozlowski (1992) in his review where the attainment of A_{max} in leaves occurred between 35 to 90% of full expansion. However, these values refer to herbaceous dicotyledonous species with a leaf age span of less than five months (*Phaseolus vulgaris* L., Fraser and Bidwell 1974; *Fragaria virginiana,* Jurkin *et al.* 1979). Schaffer *et al.* (1991) found that A_{max} for the West Indian cvs. Peterson and Booth-8 was not reached until ca. 60 and 80 days after bud-break, respectively. The longer time taken to reach A_{max} in avocado leaves is probably a function of increasing chlorophyll and nitrogen concentrations which continue to rise until some time after full leaf expansion (Fig. 8) (Schaffer *et al.* 1991). Furthermore, avocado leaves are relatively large and more sclerophyllous than those of most other evergreen fruit trees.

The A_{max} measured on spring grown leaves reported herein (13.1 μmol CO_2 m^{-2} s^{-1}), is considerably higher than previously published values for the Guatemalan race cv. Edranol (9.1 μmol CO_2 m^{-2} s^{-1}, Bower *et al.* 1978), the Mexican cv. Fuerte (6.2 μmol CO_2 m^{-2} s^{-1}, Scholefield *et al.* 1980) and the West Indian cvs. Peterson and Booth-8 (5.5 and 8.0 μmol CO_2 m^{-2} s^{-1}, Schaffer *et al.* 1991). The higher A in this study may be due to more favourable environmental conditions during measurements; the effect of enhanced sink strength from the developing fruit on these shoots (Hansen 1970; Ghosh 1973; Fujii and Kennedy 1985); and/or most likely freedom from sink-limited feedback inhibition. The lower values reported from previous studies were measured on container-grown and on field-grown trees where root

growth was probably restricted by the container or soil factors (Arp 1991, Crane *et al.* 1994). This can suppress *A* due to limited root sinks and an accumulation of photoassimilates in leaves (Schaffer *et al.* 1987; Arp 1991). Schaffer *et al.* (1994) provide a more detailed discussion on the effect of sink-limitation on *A* with respect to container-grown vs. field grown trees.

In the second study defining the response of single 'Hass' leaves to increasing PPF (3. 2), the A_{max} of non-stressed leaves in full sunlight (1800 μmol quanta m^{-2} s^{-1}) was measured at 17.54 ± 0.39 μmol CO_2 m^{-2} s^{-1}. This was determined on mature summer-grown leaves, following a period of quiescence in the tree when leaves attain their maximum nitrogen content for the year (Fig. 8). Net CO_2 assimilation rates of this magnitude for single 'Hass' leaves have been measured on field-grown trees by the author for a number of years during the late summer/autumn period, using both LI-COR 6200 and CIRAS-1 photosynthetic meters (unpublished data). These data are contrary to the long-accepted contention that evergreen trees have lower *A*'s than deciduous trees (Larcher 1969; Chabot and Hicks 1982). Indeed, the A_{max} measured for 'Hass' avocado in this study is similar to that reported for almond (18.0), apple (15.7 ± 5.6), pecan (14.5 ± 2.1) and sweet cherry (17.9 ± 5.3) (Flore and Lakso 1989).

The quantum yield (Ø) of non-stressed 'Hass' leaves was determined at 0.0545 μmol CO_2 μmol $quanta^{-1}$ and approximates the range defined for C_3 plants (0.0524 ± 0.0014; Ehleringer and Björkman 1977). The 30 μmol quanta m^{-2} s^{-1} value determined as the light compensation point for *A* falls within the upper end of the range defined for shade tolerant species (Thompson *et al.* 1992) indicating the ability of avocado leaves to exploit low levels of incident light. Together with the high A_Q (16.12 μmol CO_2 m^{-2} s^{-1}), it demonstrates the plasticity of this species in its response to the light environment. Net CO_2 assimilation at light saturation has been reported for a diversity of rainforest tree species which range from ca. 2.4 to 12.1 μmol CO_2 m^{-2} s^{-1}, depending on their ecological niche in the forest canopy (Langenheim *et al.* 1984; Oberbauer and Strain 1984; Chazdon and Pearcy 1986; Doley *et al.* 1988;Thompson *et al.* 1992). The A_Q for avocado is considerably higher than the aforementioned range and the ecological significance for this *A* capacity is addressed under the general discussion and conclusion section of this thesis.

The *A* rates resulting from short-term exposure of single avocado leaves to enriched partial pressures of CO_2 are unlikely to be sustainable. Research with other species has shown a downward rate adjustment after long-term exposure to elevated CO_2. Although reasons for this decline have not been fully elucidated it has been suggested that it may be due to a decline in carboxylation efficiency due to reduced Rubisco activity; suppressed sucrose synthesis caused by an accumulation of starch; inhibition of the triose-P carrier; limitation of daytime photosynthate export from sources to sinks; or insufficient sinks in the plants (Guinn and Mauney 1980; DeLucia *et al.* 1985; Koch *et al.* 1986; Fetcher *et al.* 1988; Sage *et al.* 1989). Short-term CO_2 enrichment of 'Hass' leaves at saturating PPFs increased *A* by ca. 300% compared with ambient CO_2 partial pressures. An increase of similar magnitude was reported by Garcia *et al.* (1994) for *Pinus eldarica* in response to atmospheric CO_2 enrichment. They were able to continuously measure whole tree *A* at enriched CO_2 partial pressures for 4 to 5 days and found that C_A increased from 25.1 to 77.5 μmol CO_2 $tree^{-1}$ s^{-1} when CO_2 partial pressures were raised by 450 μmol mol^{-1}. These preliminary results with 'Hass' however, indicate high carboxylation efficiency, particularly when compared with the response of mangosteen (a shade-tolerant tropical rainforest species) to CO_2 enrichment (Wiebel *et al.* 1993).

Leaves of evergreen plants grown in the subtropics are often exposed to conditions during winter that favour photoinhibition due to low temperatures interacting with excess incident light. Chill injury in plants which develop photoinhibition, characteristically results in a reduction of Q_A and Ø (Powles *et al.* 1983). The reduced carboxylation efficiency of cold stressed leaves in this study was most likely due to photoinhibition following exposure to temperatures < 10°C. Indeed, this is indicated by the reduced F_v/F_m ratio of 'Hass' leaves, suggesting a lower capacity of PS II for electron transport resulting in a significant decline in Ø. However, rate limitation of photosynthesis may also be attributed to lowered stomatal conductance (Flore and Lakso 1989) which can occur following chilling temperatures (Wilson 1979). In this study it is unlikely that this factor was a major contributor to reduced *A* under light saturating conditions. When leaves were exposed to non-limiting partial pressures of ambient CO_2 and saturating PPFs, Ci increased in a linear relationship indicating unrestricted diffusion of CO_2 through the mesophyll tissues. The photoinhibition that occurred was

reversible with leaves recovering substantially (as determined by the F_v/F_m ratio) by the end of October.

Carbon balance of the fruiting shoot

Crop yields for many subtropical and tropical evergreen tree fruit species, e.g. avocado, litchi, macadamia, and mango, are low compared with "similar" temperate species. In some cases environmental conditions at critical stages of floral biology limit fruiting. However, for the most part fruit set is prolific but is followed by a heavy drop during the first few weeks of ontogeny. This normal process of yield adjustment establishes a sink/source balance, i.e. fruit/leaf ratio. In many instances this adjustment favours the vegetative bias of these trees which have not undergone the intensive selection and development programs of temperate fruit crop species. For instance, mango has had a long history of cultivation in India where it was grown for the Rajahs but selected on quality rather than yield criteria, and the large scale production of grafted avocado and macadamia as orchard trees has only occurred from the 1920s in spite of a long period of utilisation of the former. Thus, improvement of the harvest index of these crops through manipulation of resource allocation is of primary concern to the horticulturist.

With avocado the study of spring shoot ontogeny indicated that the greatest loss of fruit coincided with the period during which the shoot was a strong sink (i.e. net *A* loss) with 60% of the initial fruit set abscising during the first 27 days after bud-break. The youngest leaves of the shoots were sinks for another 15 days (42 days after bud-break) during which time a further 22% of the fruits were lost. Fruit retention stabilised at the time that spring shoots reached maximum source strength. It has also been shown that dry matter gain of individual fruit is minimal during the period of net leaf carbon loss but becomes substantial as the spring shoot approaches maximum source strength (Whiley 1990).

Previous research with avocado has suggested that the fruit and shoot components of spring growth are competitive sinks for available assimilate. Tipping during the early stages of shoot growth or chemically retarding spring shoot growth (especially indeterminate fruiting shoots)

has been effective in retaining more fruit on shoots and increasing final yield (Biran 1979; Blumenfeld *et al.* 1983; Köhne and Kremer-Köhne 1987; Adato 1990; Cutting and Bower 1990; Wolstenholme *et al.* 1990). In recent studies with partitioning of ^{14}C-photosynthates during flowering and fruit set of avocado, Finazzo *et al.* (1994) reported that the sink strength of floral, fruitlet and vegetative organs was similar on indeterminate inflorescences. They concluded that neither developing fruitlets or shoots were limited for assimilates during the critical period of fruitlet abscission as assimilate was "available" for distribution from other areas such as branch tissues. These conclusions are against the weight of evidence in the literature and it may be argued that branches themselves are strong sinks for photoassimilates. Shoots of avocado are succulent and somewhat brittle and are thicker than young shoots of most other evergreen fruit tree species (Chandler 1958). Their strength is due to a rapid increase in thickness rather than the nature of the wood suggesting that they are important sinks for assimilates (Chandler 1958). In ^{14}C-photosynthate studies with non-fruiting container-grown avocados Whiley and Schaffer (1993) found that when leaves were exposed to $^{14}CO_2$ when either shoots or roots were sinks, > 40% of the recovered ^{14}C was from trunk/shoot organs. Studies in other crops with ^{14}C-photosynthate report a high percentage (up to 76%) of the recovered ^{14}C from shoots at times of sink activity in other portions of the plant, suggesting that the "pool" of photosynthates in shoots may not be available for re-translocation (Dickson and Larson 1981; Dickson *et al.* 1990). However, the Finazzo *et al.* (1994) experiments were carried out on trees growing in soils which restrict root growth (Crane *et al.* 1994) and these conditions are known to influence some physiological responses of trees (Schaffer *et al.* 1994) and may explain their different results.

The study on the dynamics of fruit abscission from indeterminate and determinate shoots also supports the hypothesis that fruit loss is at least to some extent influenced by the closely coupled spring shoot. Not only were more fruit lost from indeterminate terminals during the first 70 days of spring growth (Fig. 17) but fruit grew faster on determinate shoots (Fig. 7) and were larger at maturity. Previous studies with 'Hass' have also shown that increased fruit size at spring shoot maturity is expressed in larger fruit at harvest (Wolstenholme *et al.* 1990; Whiley *et al.* 1991). Recently completed ^{14}C studies with developing avocado shoots (Whiley and Schaffer 1993) confirmed that expanding leaves are strong sinks. This experiment further

supports the concept of competitive vegetative sinks during the first 42 days after bud-break, i.e. incomplete temporal and spatial separation of vegetative and reproductive growth, especially in indeterminate fruiting shoots. It is suggested that the size of the assimilate pool (stored and current from existing mature leaves) and the strength of the shoot sink (i.e. vigour of growth) largely determines the success of fruit retention during the first 60 days after bud-break.

While initially competitive, renewal shoot growth during spring is necessary for the secondary development of avocado fruit. Wolstenholme *et al.* (1990) demonstrated that with severe retardation of spring shoot growth of cv. Hass with paclobutrazol sprays, fruit dry mass at flush maturity was significantly reduced compared to other treatments resulting in less growth suppression. This was reflected in lower yield at fruit maturity. Similar results were reported by Quinlan and Preston (1971) from shoot tipping and removal studies with apples. Despite the necessity of spring shoot growth, the opportunity remains to manipulate the vegetative-reproductive balance to give a more favourable economic yield. Whiley *et al.* (1991) have shown that a low concentration foliar spray of paclobutrazol (Cultar®) at full bloom, which slightly suppresses shoot growth of cv. Hass, significantly increased fruit yield. Correct timing of fertilisation with nitrogen can also assist in controlling spring flush vigour thus favouring greater fruit retention and yield (Whiley *et al.* 1988a). "Fine-tuning" of competitive vegetative: reproductive growth interactions at this critical juncture has major horticultural implications, which are becoming increasingly recognised by progressive growers.

These studies have also highlighted the importance in the summer rainfall subtropics of the over-wintered leaf canopy, a variable mixture of spring and summer leaf flush cohorts (presumably of varying overall *A*), to fruit retention during the development of the renewal spring shoot. As fruit loss was higher from shoots on defoliated trees (Fig. 17), it is not surprising that more dry matter was allocated to the combined leaf and stem components of the shoot. However, with the loss of a net source of current assimilate during the first 25 days of shoot development (Chapter 3.1) it is surprising that these shoots accumulated more dry matter than those shoots on trees where a full canopy was retained. This may be attributed to the additional respiratory loss of carbon from fruiting shoots during early ontogeny. Blanke and

Whiley (1995) report R_l and R_d of young 'Hass' fruit at ca. 12 and 14.5 μmol CO_2 m^{-2} s^{-1}, respectively. This is approximately three times greater than the maximum R_d of 4.6 μmol CO_2 m^{-2} s^{-1} reported for 'Peterson' avocado leaves (Schaffer *et al.* 1991).

CHAPTER 4

GAS EXCHANGE OF DEVELOPING FRUIT

4. 1 CARBON DIOXIDE EXCHANGE OF DEVELOPING FRUIT[‡]

4. 1. 1 Introduction

Crop yield increases have been achieved largely by increasing the proportion of assimilates partitioned to the harvested plant organs (Evans 1976). For example, in avocado (*Persea americana* Mill.), a substantial yield increase was obtained in response to a reduction in vegetative growth as a result of treatment with paclobutrazol foliar sprays (Wolstenholme *et al.* 1990; Whiley *et al.* 1991). Other key factors determining fruit yield are the respiratory cost of growth and ripening and the seasonal photosynthetic efficiency of the crop (Amthor 1984; Cannell 1985).

Respiratory losses from fleshy fruit, during growth and ripening, have been documented for several crop species (Kidd and West 1925; Jones *et al.* 1964a; Blanke *et al.* 1985). However, the contribution of fruit to their own carbon economy should not be ignored. Previous studies with young green fruit have established their photosynthetic contribution to the carbon requirement for growth and maintenance (Bean and Todd 1960; Todd *et al.* 1961; Kriedemann 1968; Bazzaz *et al.* 1979; Flinn *et al.* 1977; Jones 1981). For example, pods of pea (*Pisum sativum* L.) exhibited a net photosynthetic gain during the first 30 days after anthesis but thereafter respiration losses exceeded CO_2 assimilation (Flinn *et al.* 1977). For oranges and lemons (*Citrus sinensis* and *C. limon*), grape (*Vitis vinifera* cv. Sultana), and apple (*Malus pumila* cvs. Jonathan & Golden Delicious), fruit respiratory losses of CO_2 during a diurnal cycle exceeded photosynthetic gains throughout fruit ontogeny (Bean *et al.* 1963; Kriedemann

[‡] **Whiley, A. W., Schaffer, B. and Lara, S.P., 1992.** Carbon dioxide exchange of developing avocado (*Persea americana* Mill.) fruit. *Tree Physiol.* 11, 85-94. **APPENDIX 2**

1968; Clijsters 1969; Jones 1981). However, Clijsters (1969) demonstrated a 36% reduction in the growth of apples when photosynthetic activity was inhibited by excluding light from developing fruit.

Wolstenholme (1986, 1987) calculated that the oil-accumulating avocado fruit has a high energy requirement for growth (energy value at maturity of 807.2 kJ 100 g^{-1} for cv. Fuerte at 17% oil compared with 262.8 and 292.5 kJ 100 g^{-1} for apples and citrus, respectively). Avocado fruit are climacteric (Eaks 1980), and the respiratory sequence is initiated by detachment from the tree. Previous studies on avocado fruit respiration have been conducted exclusively with detached fruit at various stages of development and to the author's knowledge, there are no reports on net CO_2 exchange of this fruit attached to the tree throughout ontogeny. The fruits remain green from setting until maturity and have a high stomatal density (50 to 75 stomata mm^{-2} shortly after fruit set), with active stomata similar to those of leaves, facilitating gas exchange (Blanke and Bower 1990). Total chlorophyll concentration in the mesocarp is only 12 to 30% that of the peel concentration (Cran and Possingham 1973). Thus, a fruit has the potential for photosynthetic activity, thereby contributing to its own carbon requirements during growth. Refixation of respiratory CO_2 within fruit by phosphoenolpyruvate carboxylase (PEPC) may be a significant contributor to fruit photosynthesis (Blanke and Lenz 1989). This mode of CO_2 refixation in fruit may also be present in avocado, because PEPC has been found in avocado fruit (Blanke and Notton 1991).

The purpose of this study was to determine the dynamics of CO_2 efflux from avocado fruit from post anthesis to fruit maturity and to assess the contribution of fruit to their own carbon economy from fixation of atmospheric CO_2.

4. 1. 2 Materials and Methods

Avocado trees (*Persea americana* var. *americana* x *P. americana* var. *guatemalensis*, cv. Booth-7) growing at the University of Florida, Tropical Research and Education Center, Homestead, Florida (25°N latitude) were used in this study. Trees were on 'Waldin' or 'Lula' seedling rootstocks and were 35 years old at the beginning of the experiment. Trees were

maintained with standard fertilisation, irrigation and pest control practices recommended for avocado in Florida (Malo and Campbell 1983).

From three weeks after anthesis (early April, 1989) to fruit maturity (mid-September, 1989), CO_2 efflux from attached fruit was determined in the field at 14-day intervals for three fruit on each of five trees. Since fruit were harvested at the end of each measurement period, different fruit were used for each measurement date. However, fruit growth rates within and among trees were fairly uniform, and fruit were tagged at set to ensure similar aged material was measured. Net CO_2 exchange was determined from CO_2 fluxes by enclosing individual small fruit in a Parkinson's leaf chamber (Analytical Development Co., Hoddesdon-Herts, England), or larger fruit in a 14 x 14 x 13 cm Plexiglass chamber containing a battery powered fan and a thermocouple. Net CO_2 exchange was determined with an LCA-2 portable open gas exchange system (Analytical Development Co., Hoddesdon-Herts, England) as describe by Schaffer and O'Hair (1987). Flow rate of ambient air into the chamber was maintained at 400 ml min^{-1} for the first five measurement dates and 600 ml min^{-1} for the later dates. Net CO_2 exchange was calculated using equations described by Jarvis (1971) and von Caemmerer and Farquhar (1981). Light respiration (R_l) of fruit was determined by measuring CO_2 efflux throughout the day at a minimum photosynthetic photon flux (PPF) of 600 μmol quanta m^{-2} s^{-1}, which exceeds the light saturation for CO_2 assimilation of avocado leaves (Scholefield *et al.* 1980). Immediately following measurements made in the light, the chamber was covered with two layers of black polyethylene. Dark CO_2 efflux, i.e. dark respiration (R_d), was then determined. Chamber air temperature was monitored, but not controlled, during CO_2 exchange determinations, and ranged from 31°C to 45°C during the course of the study. Respiration data were standardised to 30°C by using temperature response data from each sampling date. This method has been used to standardise temperatures for peach respiration data (DeJong *et al.* 1987). Data were not collected until the CO_2 flux in the chamber had stabilised (about five minutes for small fruit during the first measurement date, and up to two hours for large, mature fruit). Immediately after each CO_2 exchange determination, the fruit measured were harvested from the tree and the dry weight of each fruit determined after slicing and drying at 60°C.

Fruit R_d and R_l were expressed on a g_{dw}^{-1} and a $fruit^{-1}$ basis. Statistical models determining fruit growth over time and comparing fruit dry weight to fruit R_d and R_l were constructed by linear and nonlinear regression analysis. Fruit photosynthetic activity was calculated from the difference between fruit R_l and fruit R_d at each point on the regression lines (Bean and Todd 1960; Clijsters 1969; Jones 1981).

Light interception by the avocado tree canopy was defined in a separate study carried out on a 5 m canopy diameter tree (cv. Hass) in subtropical south-east Queensland (lat. 27°S). During flowering, which on avocado is mostly terminal to the last vegetative flush (Whiley *et al.* 1988a), and early fruit set, spot measurements of PPF (LICOR LI-190SA quantum sensor) were made within the fruiting zone and compared to the full sunlight position. At the completion of spring shoot growth, 1 m line quantum sensors (LICOR LI-191SA) were positioned in the fruiting zone of the tree, and at 0.5 and 1.0 m inside the canopy from the fruiting zone. The sensors were aligned as closely as possible at 90° to the midday sun on the northern side of the tree. A fourth quantum sensor (LICOR LI-190SA), was positioned outside the tree canopy in full sunlight. The PPF was integrated hourly during the light period of each day using a LICOR-1000 datalogger and the accumulated quanta at each line sensor expressed as a percentage of full sunlight. Mean values of the percentage of light intercepted at each point in the canopy, were calculated for a one week period. The quantum sensors were left positioned in the tree and the same PPF measurements were again collected approximately eight weeks later, after summer shoot growth had occurred.

4. 1. 3 Results

Fruit dry weight increased exponentially over time (Fig. 19). The increase was relatively slow during the first 10 weeks after anthesis and then increased rapidly from week 10 to fruit maturity (20 weeks after anthesis).

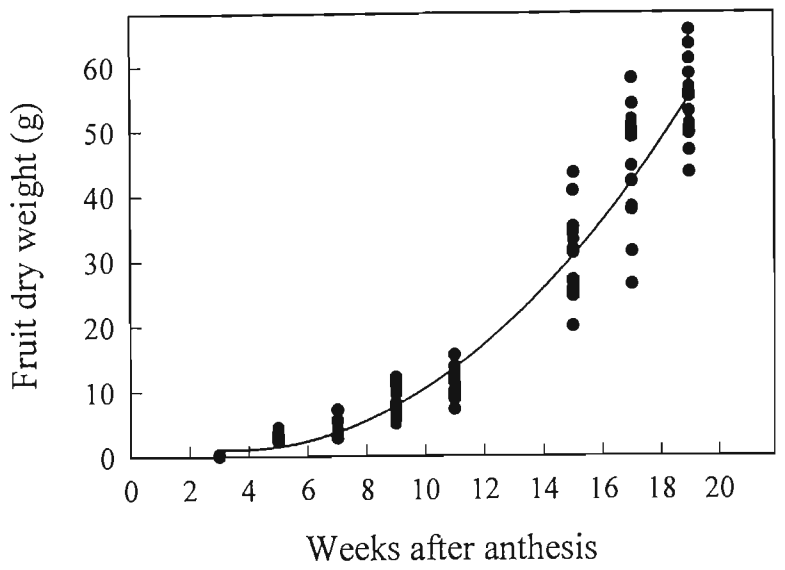

Fig. 19 Fruit dry weight of 'Booth-7' avocados during fruit development. The regression line is defined by the equation: $y = 3.94 - 1.618x + 0.227x^2$, $r^2 = 0.99$.

As fruit dry weight increased over time, R_d and R_l showed a similar CO_2 efflux pattern, on a dry weight basis (Fig. 20a). Dark respiration and R_l were highest three weeks after anthesis, 25 and 22 nmol CO_2 g_{dw}^{-1} s^{-1}, respectively. As fruit ontogeny progressed, R_d and R_l decreased and were lowest at fruit maturity, about 1.0 and 0.5 nmol CO_2 g_{dw}^{-1} s^{-1}, respectively. The difference between R_d and R_l decreased as fruit weight increased (Fig. 20a). This was concomitant with a reduction in the calculated fruit photosynthetic rate, from about 3.1 nmol CO_2 g_{dw}^{-1} s^{-1} during early fruit growth to about 0.5 nmol CO_2 g_{dw}^{-1} s^{-1} at fruit maturity (Fig. 20b).

The pattern of fruit respiration expressed on a $fruit^{-1}$ basis was similar in the dark and light (Fig. 21a). Until fruit dry weight reached 10 g, R_d and R_l (on a $fruit^{-1}$ basis) increased linearly as fruit developed. When fruit were about one-third of their maturation weight (20 g dry weight), respiration per fruit approached an asymptote and increased little until fruit were harvested (Fig. 21a). Dark respiration was always greater than R_l, and these differences were greatest when fruit dry weight was between 20 and 55 g (Fig. 21a). Respiration rates were

highest at fruit maturity and were about 208 and 152 nmol CO_2 $fruit^{-1}$ s^{-1} or 34 and 25 mg CO_2 h^{-1} for R_d and R_l, respectively. Calculated fruit photosynthesis, expressed on a $fruit^{-1}$ basis, increased linearly as fruit dry weight increased from 0 to 20 g, then levelled off when fruit reached approximately half their maturation weight (Fig. 21b). There was little increase in calculated fruit photosynthesis as fruit dry weight increased from 30 to 60 g.

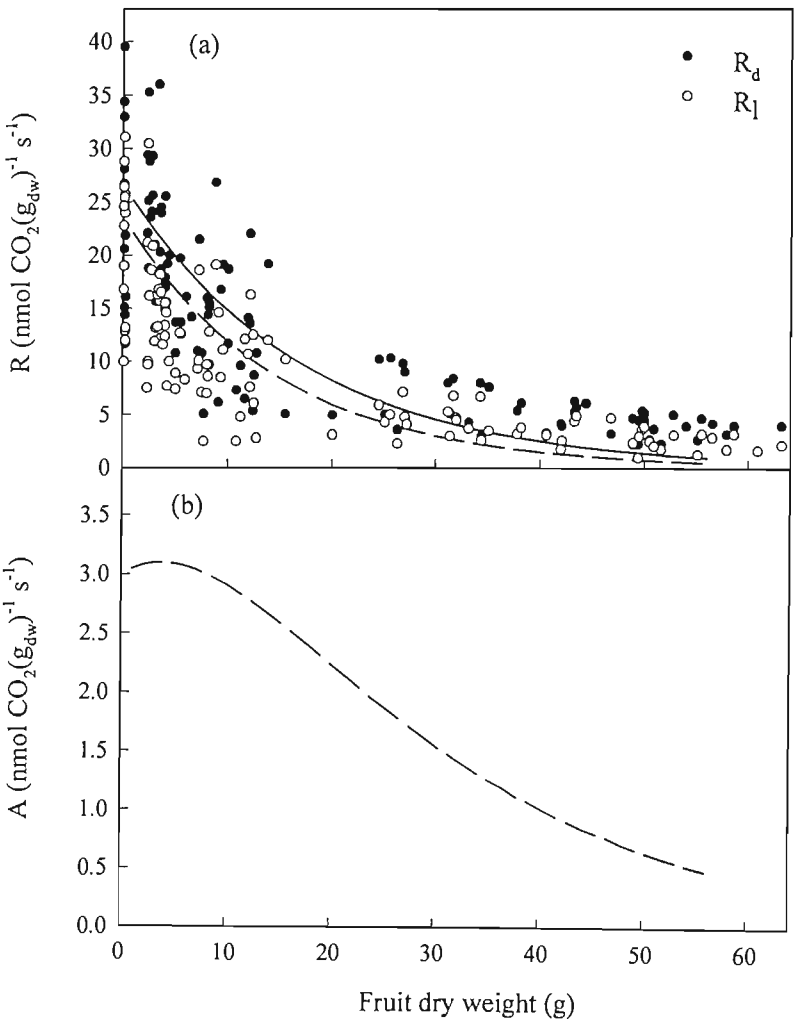

Fig. 20 (a) Fruit respiration of developing 'Booth-7' avocados in the dark (R_d) and in the light (R_l) expressed on a g_{dw}^{-1} basis where the regression line for R_d is defined by the equation: $y = 26.55e^{-0.057x}$, $r^2 = 0.60$ and the regression line for R_l is defined by the equation: $y = 19.98e^{-0.067x}$, $r^2 = 0.63$; (b) Net CO_2 assimilation (A), determined from R_d - R_l, of developing 'Booth-7' avocado fruit expressed on a g_{dw}^{-1} basis.

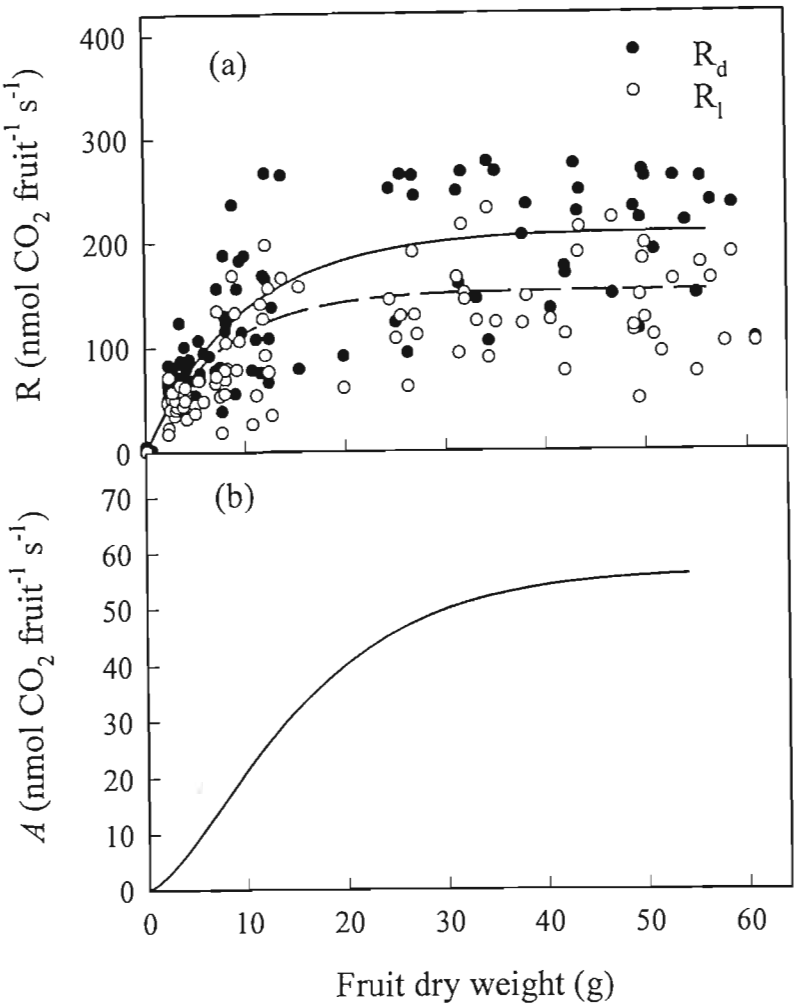

Fig. 21 (a) Fruit respiration of developing 'Booth-7' fruit in the dark (R_d) and in the light (R_l) expressed on a fruit^{-1} basis where the regression line for R_d is defined by the equation: $y = 209.01(1 - e^{-0.107x})$, $r^2 = 0.71$ and the regression line for R_l is defined by the equation: $y = 140.60(1 - e^{-0.117x})$, $r^2 = 0.66$; (b) Net CO_2 assimilation (*A*), determined from $R_d - R_l$ of developing 'Booth-7' avocado fruit expressed on a fruit^{-1} basis.

PPF measurements taken during flowering and early fruit set indicated that most young fruit were exposed to full sunlight for the first four weeks of their development (data not presented). During the two periods when light interception data were integrated, daily PPF ranged from 15.5 to 59.5 mol quanta m^{-2} resulting from overcast and cloud-free days. By the end of spring

shoot growth, light transmission to the fruiting zone had been reduced to 35.9% of full sunlight with a further reduction to 13.7 and 9.7% at 0.5 and 1.0 m respectively, inside the canopy from the fruiting zone (Fig. 22). By the end of the summer shoot growth, light transmission to the fruiting zone had further declined to 13.1% of full sunlight and 9.7 and 6.3% at the related internal monitoring positions.

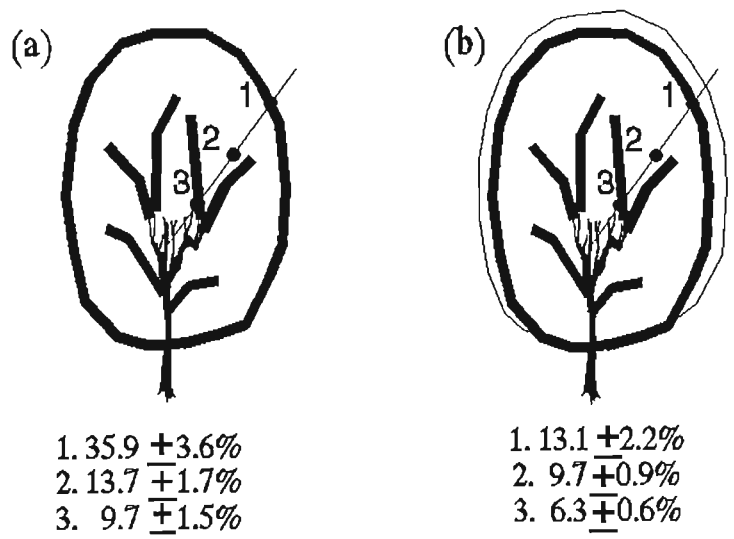

Fig. 22 Light transmission in cv. Hass avocado tree canopy at; (a) when spring shoot growth had stopped and (b) at the finish of summer shoot growth. PPF was measured in full sunlight, 1 - in the fruiting zone, 2 - 0.5 m inside the canopy from the fruiting zone and 3 - 1.0 m inside the canopy from the fruiting zone. Data are mean values ± SEs (n = 7) of the percentage of full sunlight measured at each point. PPF in full sunlight ranged between 15.5 to 56.6 μmol quanta m^{-2} (a) and 19.8 to 59.5 μmol quanta m^{-2} (b) over each seven day period.

4. 1. 4 Discussion

The dynamics of R_d and R_l observed for attached, developing avocado fruit were similar to those observed for other crops (Jones *et al.* 1964a; Clijsters 1969; Jones 1981; DeJong *et al.*

1987; DeJong and Walton 1989). The highest respiration rates were observed during the early stage of fruit growth, from the first measurement date to about 12 weeks after anthesis, decreasing to the lowest rates at fruit maturity. The period of highest respiration rates corresponds to the time that cell division is greatest in avocado fruit (Valmayor 1967). This was similar to the patterns previously reported for avocado (Todd *et al.* 1961), apple (Clijsters 1969; Jones 1981), and peach (DeJong *et al.* 1987; DeJong and Walton 1989). The maximum R_d value measured at 30°C for avocado fruit, about 25 nmol CO_2 g_{dw}^{-1} s^{-1}, was similar to R_d measured at 20°C for apple fruit, about 26 nmol CO_2 g_{dw}^{-1} s^{-1} (recalculated from Jones 1981) and peach fruit at 20°C, about 28 nmol CO_2 g_{dw}^{-1} s^{-1} (DeJong *et al.* 1987). Jones (1981) reported the greatest difference between R_d and R_l for apple during the early phase of fruit growth and the least differences at fruit maturation. A similar response was observed for avocado fruit. When the R_d and R_l of avocado were expressed on a $fruit^{-1}$ basis, respiration at 30°C was highest at fruit maturity, about 34 and 25 mg CO_2 h^{-1} $fruit^{-1}$ for R_d and R_l, respectively. These values were somewhat lower than 50 mg CO_2 h^{-1} $fruit^{-1}$ measured at 23°C reported for avocado by Blanke (1991). The discrepancy between values may be due to experimental or genotypic differences. The cultivar used in Blanke's (1991) study was *P. americana* var. *drymifolia* cv. Fuerte whereas here the hybrid *P. americana* var. *americana* x *P. americana* var. *guatemalensis* cv. Booth-7 was used. The oil concentration in 'Booth-7' fruit (about 8%) is lower than that of 'Fuerte' (about 12 to 14%) at maturity (C.W. Campbell, pers. comm.[‡]). Presumably this leads to lesser energy demands for growth and development for 'Booth-7' than for 'Fuerte' (Wolstenholme 1986), resulting in lower respiratory activity in 'Booth 7' fruit.

At all stages of fruit development, fruit photosynthesis was substantially less than dark respiration. However, the calculated photosynthetic rate of developing avocado fruit (i.e. the difference between R_d and R_l; (Todd *et al.* 1961; Jones 1981), was highest during early fruit growth, about 3.0 nmol CO_2 g_{dw}^{-1} s^{-1}. The photosynthetic rates for the developing fruit were 42 times less than rates for mature leaves, about 126.0 nmol g_{dw}^{-1} s^{-1} (B. Schaffer and A.W. Whiley, unpublished data).

[‡] Emeritus Professor C.W. Campbell, University of Florida, Homestead, Florida, USA.

Although chlorophyll concentrations in the peel of avocado fruit are similar to concentrations in the leaves (Cran and Possingham 1973; Schaffer *et al.* 1991) the differences in the maximum CO_2 assimilation rates between the two organs may be attributed to the difference in the chlorophyll a:b ratio, which is 1 to 2:1 in fruit (Cran and Possingham 1973) relative to 2 to 3.3:1 in leaves (Schaffer *et al.* 1991). However, the difference in the amount of CO_2 assimilated between the organs is more likely due to the greater surface area to volume ratio in leaves than in fruit, which results in a severe decline of light penetration into fruit tissue (only 0.02% of incident light penetrates more than 2 mm into the avocado mesocarp; Cran and Possingham 1973) and a change in the spectrum of photosynthetically active radiation (Blanke 1990). This relationship is further expressed by the declining net CO_2 assimilation (expressed as nmol CO_2 g_{dw}^{-1} s^{-1}) as fruit increase in size.

Vu *et al.* (1985) suggested that reproductive organs fix little atmospheric CO_2 via ribulose-bis-phosphate carboxylase (RuBPC) in the reductive pentose phosphate pathway. They reported the CO_2 assimilation PEPC:RuBPC ratio was 4 to 5:1 and 0.1:1 for citrus flowers and leaves, respectively. Furthermore, Blanke and Lenz (1989), Blanke (1990), and Blanke and Notton (1991) concluded that the refixation of CO_2 via the PEPC pathway, provides a significant contribution of carbon by the fruit to its own growth requirements.

4. 1. 5 Conclusion

The data from the present study indicate that avocado fruit contribute to their own carbon requirement by means of CO_2 fixation in the light, and that the relative contribution of fruit photosynthesis to the total energy requirement is greatest during the stages of early fruit development. This may be a significant factor influencing fruit retention as it coincides with the period of photoassimilate competition between reproductive and vegetative sinks (Biran 1979; Blumenfeld *et al.* 1983; Wolstenholme *et al.* 1990; Whiley *et al.* 1991), which extends for about 42 days after spring shoot growth commences (Whiley 1990). In addition the over-wintered leaf canopy has lost photosynthetic efficiency (A.W. Whiley, unpublished data) at a

time of critical assimilate demand. During this period young developing fruit are in full sunlight with the opportunity to maximise their photoassimilate contributions to growth. These data show that up to the end of spring shoot growth, when fruit have attained a size between 12 to 15 g dry weight, there is sufficient light during cloud-free conditions, to support fruit photosynthesis within the fruiting zone of the canopy. However, by the time the summer growth flush is complete (Whiley *et al.* 1988a), the light environment in the fruiting zone is unlikely to support photosynthetic activity in the fruit. At this stage of fruit ontogeny the renewed and photosynthetically efficient leaf canopy would meet all photoassimilate requirements of fruit growth.

CHAPTER 5

CARBON PARTITIONING IN CONTAINER-GROWN AVOCADO TREES

5. 1 ^{14}C-PHOTOSYNTHATE PARTITIONING IN AVOCADO TREES AS INFLUENCED BY SHOOT DEVELOPMENT[‡]

5. 1. 1 Introduction

A major consideration in the management of avocado orchards in most avocado-producing countries is Phytophthora root rot, caused by *Phytophthora cinnamomi* (Zentmyer 1971; Darvas and Bezuidenhout 1987). This disease is controlled effectively by foliar sprays or trunk injections of systemic phosphonate fungicides (Darvas *et al.* 1984; Pegg *et al.* 1985) which are transported acropetally in the xylem and basipetally along with photoassimilates in the phloem (Guest and Grant 1991). To be effective, these fungicides must be moved basipetally from the leaves to the roots in sufficient concentration to suppress disease development.

Architecturally, the avocado is defined as a polyaxial species with a usually synchronous growth pattern characterised by alternating shoot and root growth (Verheij 1986; Whiley *et al.* 1988a). Movement of systemic fungicides in the tree is related to the dynamics of photoassimilate partitioning, which varies with the activity of competing sinks, often temporally separated. The relationship between vegetative (shoot and root) flushing and photoassimilate partitioning in the tree indicates a stage of vegetative growth at which systemic fungicides are likely to be most effectively translocated to the roots. The objective of this study was to determine the influence of stage of shoot development on photoassimilate partitioning in container-grown avocado trees.

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‡ **Whiley, A.W. and Schaffer, B., 1993.** ^{14}C-Photosynthate partitioning in avocado trees as influenced by shoot development. *HortScience* 28, 850-2. **APPENDIX 3**

5. 1. 2 Materials and Methods

Two-year-old 'Simmonds' avocado trees (a West Indian race cultivar) grafted on 'Waldin' seedling rootstocks, were planted in a peat-perlite potting medium (Promix, Premier Brands, Conn., USA) in 12-l plastic pots. Plants were fertilised at 14-day intervals with a 8N-3P-9K granular fertiliser (Atlantic-Florida East Coast Fertiliser and Chemical Co., Homestead, USA) and a 7N-56P-14K soluble fertiliser with minor elements ((SOL-U-GRO; Miller Chemical and Fertiliser Corp., Hanover, USA) in the irrigation water. Trees were trained to a single leader and, to synchronise growth, were topped at ≈15 to 20 cm above the graft union, leaving 10 to 15 mature leaves per tree, and placed in an air-conditioned glasshouse in May, 1989. The glasshouse was maintained at 30 ± 2°C (12 hr day), and 20 ± 2°C (12 hr night). The axillary bud in the terminal position on each tree was allowed to develop into a new shoot; all other axillary buds were removed (Fig. 23).

Eighteen days after bud-break (DABB) of the new shoot, the oldest leaf on this shoot and the youngest leaf of the previously matured shoot were exposed to $^{14}CO_2$ (Fig. 23). Sixteen days later (34 DABB), when all leaves of the actively growing shoot were fully expanded, the oldest leaf on this shoot and the youngest leaf of the previously matured shoot on a different set of trees were exposed to $^{14}CO_2$. Thus, there were two treatments based on the position of the leaf exposed to $^{14}CO_2$: the oldest leaf of the new shoot (T-1), and the youngest leaf of the previously matured shoot (T-2). Each treatment consisted of six single-plant replicates at each exposure time in a completely randomised design.

T-1 leaf areas were measured *in situ* with a leaf area meter (model LI -3000: LI - COR, Nebraska, USA.) at the time of exposure and at shoot maturity to ascertain their stage of physiological maturity. In addition, net CO_2 assimilation was determined for T-1 and T-2 leaves immediately before treatment to ensure that leaves to be exposed were primarily net C exporters. Net CO_2 assimilation was determined with a portable infrared gas analyser (Analytical Development Corp., Haddesdon-Herts, UK) at a photosynthetic photon flux (PPF) >600 μmol quanta m^{-2} s^{-1}, which is above the light saturation level for avocado (Scholefield *et al.* 1980).

Trees were labelled with ^{14}C by exposing leaves to $^{14}CO_2$ in a sealed transparent plastic chamber attached to a CO_2 generator, as described by Schaffer *et al.* (1985). The $^{14}CO_2$ was

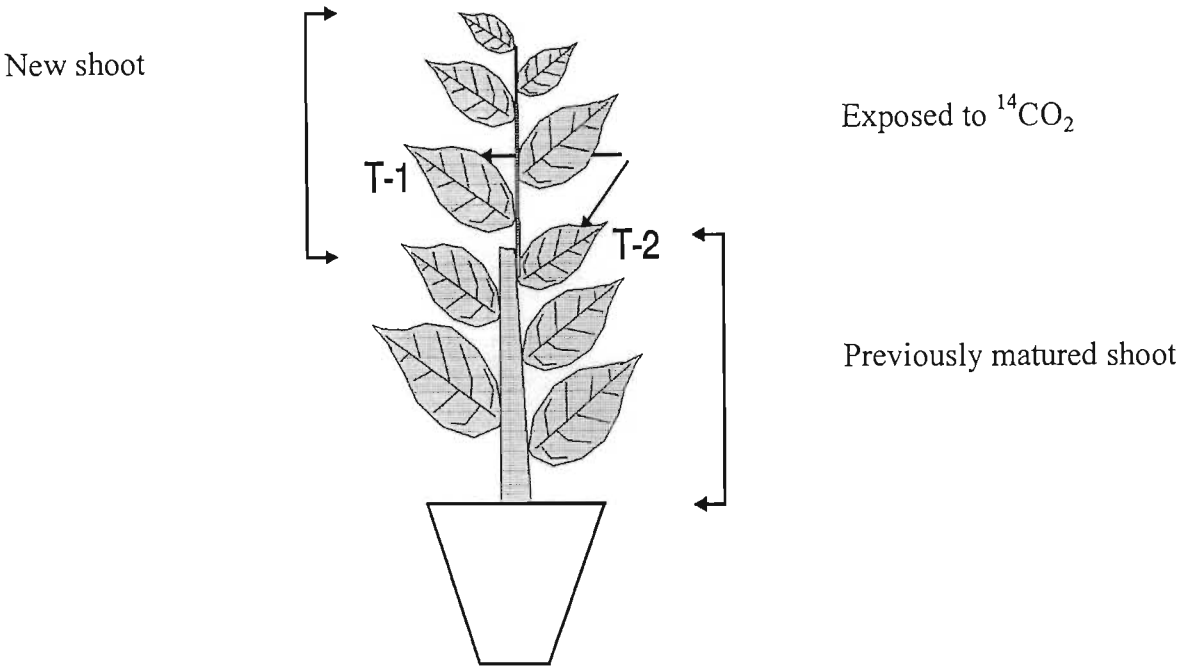

Fig. 23 Schematic diagram of a containerised avocado tree illustrating the different shoots and relative position of leaves exposed to $^{14}CO_2$.

produced by adding 1 N HCL to 1 ml of $NaH^{14}CO_3$ (18.5×10^{10} Bq ml^{-1}) in an Erlenmeyer flask. The gas was circulated continuously through the leaf chamber for 10 min at a flow rate of 2 l min^{-1} by a pump attached to the flask and chamber with plastic tubing. Excess $^{14}CO_2$ was absorbed by bubbling the gas through 1 l of saturated $Ba(OH)_2$ solution for 3 min to avoid contaminating the environment with $^{14}CO_2$, and to prevent non-treated leaves from exposure to residual $^{14}CO_2$ on removal from the leaf chamber. PPF in the glasshouse was greater than 600 µmol quanta m^{-2} s^{-1} at the time of $^{14}CO_2$ exposure.

Sixteen days after leaves were exposed to $^{14}CO_2$, trees were harvested, organs separated and oven-dried at 65°C and dry mass determined. Material from each organ was finely ground in a spice mill (Black and Decker, Shelton, Conn., USA), a measured amount of tissue was oxidised

in a sample oxidiser (model 306; Packard Instruments, Downersville, USA), and ^{14}C from each sample placed in 20 ml of 1 Carbosorb II: 2 Permaflor 5 (v:v) (Packard Instruments). Scintillation fluid (10 ml) was added to the samples for counting. The radioactivity of each sample was determined by radioassay with a liquid scintillation spectrometer (model 580l; Beckman Instrument Co., Fullerton, Calif., USA). Five nonradio-labelled samples of each tissue were prepared and assayed for use as standards. The percentage of ^{14}C in each organ was calculated from the disintegrations per min multiplied by organ dry weight and is reported as the percentage of total recovered ^{14}C in the plant.

5. 1. 3 Results and Discussion

At 18 DABB, the $^{14}CO_2$-exposed T-2 leaf was fully expanded, whereas the $^{14}CO_2$-exposed T-1 leaf was 88% expanded. Leaf area measurement of T-1 at 34 DABB indicated that all leaves of the new shoot were fully expanded, thus the new shoot was then mature.

The mean net CO_2 assimilation rates of T-1 and T-2 leaves were 6.1 and 9.2 μmol CO_2 m^{-2} s^{-1}, respectively at 18 DABB. The highest rate is lower than those for field-grown trees (Chapter 3), but typical of container-grown trees (Bower *et al.* 1978; Scholefield *et al.* 1980). The lower assimilation rate for T-1 was related to the fact that these leaves had not attained maximum photosynthetic capacity, which is reached after full expansion (Schaffer *et al.* 1991). More ^{14}C remained in the exposed T-1 than T-2 leaves at 18 and 34 DABB (Fig. 24). This most likely was due to photoassimilate requirement for leaf expansion and dry matter accumulation in the younger leaf. Although Schaffer *et al.* (1991) observed that avocado leaves reach full expansion in ≈ 28 days, dry matter accumulation continues to increase beyond this point. There was no difference between treatments in ^{14}C partitioning to the stem of the new and mature shoots and the leaves of the mature shoots at either treatment time. At 18 DABB, T-1 accumulated a higher proportion of absorbed ^{14}C in the leaves of the new shoot than T-2 (Fig. 24a). This indicates that more of the assimilates for current shoot growth were provided by the oldest leaf of the same shoot than leaves of the previously matured shoot. At 18 and 34 DABB, more ^{14}C photoassimilates were partitioned to the roots from the T-2 than T-1 treatment (Fig. 24), a result that is consistent with ^{14}C translocation patterns in orange [*Citrus*

sinensis (L.) Osb.] (Kriedemann 1969b). However, the difference in assimilate partitioning to the roots between the T-2 and T-1 was greater at 18 DABB.

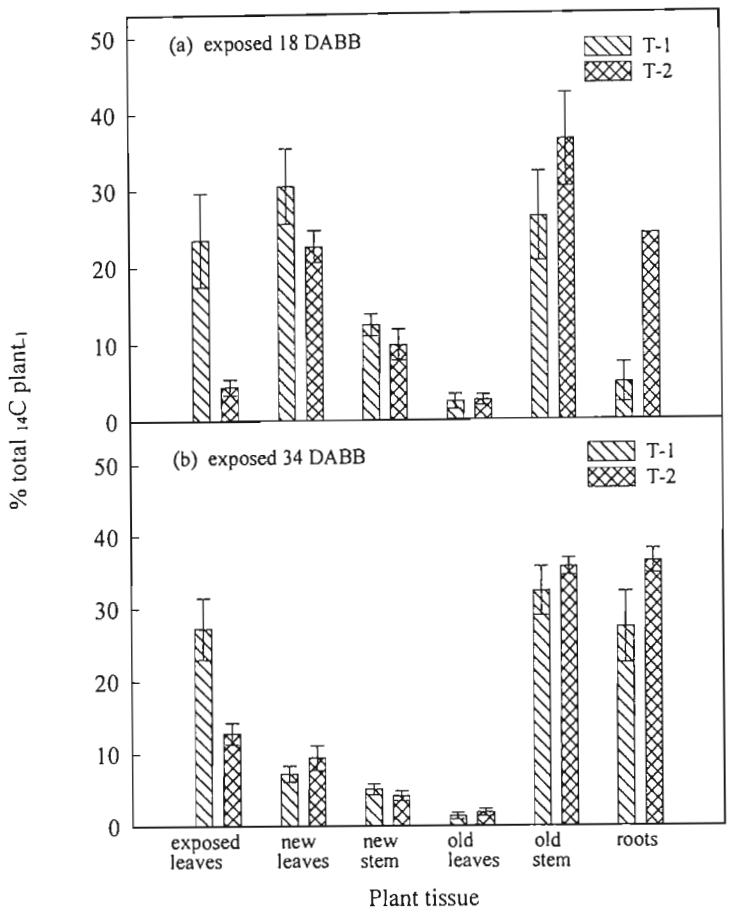

Fig. 24 Partitioning of ^{14}C in avocado trees (a) 18 days and (b) 34 days after bud-break of the newest shoot. T-1 = the oldest leaf of the new shoot, and T-2 = the youngest leaf of the previously matured shoot exposed to $^{14}CO_2$. Exposed leaves = leaves exposed to $^{14}CO_2$, new leaves = all leaves of the new shoot, new stem = stem of the new shoot, old leaves = all leaves of the previously matured shoot, old stem = stem of the previously matured shoot. Vertical lines represent ± SE where n = 6.

When ^{14}C-assimilate transport from T-1 and T-2 leaves was averaged, the developing leaves of the new shoot were a stronger photoassimilate sink than the roots 18 DABB (Fig. 25). However, by 34 DABB the roots had become a stronger sink. These results agree with those reported for grape (*Vitis vinifera* L.) (Hale and Weaver 1962), citrus (Kriedemann 1969a,

1969b), and pecan [*Carya illinoinensis* (Wangenh.) K. Koch] (Davis and Sparks 1974) where new shoots in non-fruiting trees were the strongest photoassimilate sink during their growth and development.

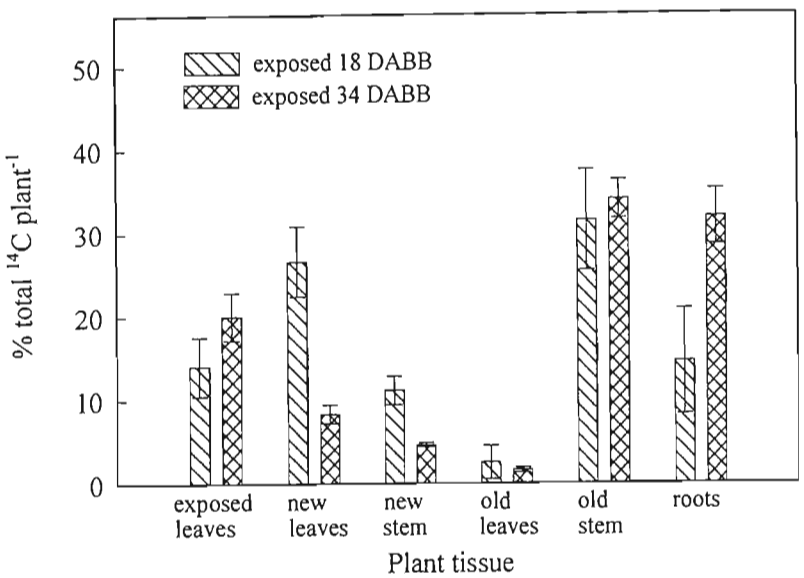

Fig. 25 Partitioning of ^{14}C in avocado trees. Leaves were exposed to $^{14}CO_2$ at 18 or 34 days after bud-break of the new shoot. The percentage of ^{14}C in each tissue was calculated by averaging the percentage translocated from the oldest leaf of the new shoot and youngest leaf of the previously matured shoot at each time. Vertical lines represent ± SE where n = 6 .

5. 1. 4 Conclusions

Spring shoot growth in avocado trees is predominantly from terminal vegetative buds of indeterminate inflorescences and is synchronised strongly by low winter temperatures, which induce flowering followed or accompanied by vegetative flushing (Venning and Lincoln 1958; Davenport 1982; Whiley *et al.* 1988a). This shoot growth occurs at a time when the over-wintered canopy is losing its photosynthetic efficiency and is approaching senescence (Chapters 2 and 3) and rising soil temperatures promote the activity of *Phytophthora cinnamomi* (Pegg *et al.* 1982). Summer growth flushes typically involve fewer branches;

portions of the canopy remain quiescent, while other branches grow actively (Whiley *et al.* 1988a). The results from this research suggest that spring phosphonate treatment will be most effective after new shoots mature, and will maximise fungicide translocation to the roots which are the target organ for protection. Timing of phosphonate application in summer is not likely to be as critical, since at any one time large portions of the canopy are quiescent and leaves on mature shoots permit more photoassimilate translocation to the roots. This hypothesis requires substantiation using phosphonate treatments at different stages of canopy development, and results from research addressing these areas is reported in Chapter 7.

CHAPTER 6

FRUIT SIZE, YIELD AND STARCH CYCLING IN RELATION TO DELAYED HARVEST OF EARLY AND LATE MATURING AVOCADO CULTIVARS

6. 1 EFFECT OF DELAYED HARVEST ON SUBSEQUENT YIELD AND FRUIT SIZE OF EARLY ('Fuerte') AND LATE ('Hass') MATURING AVOCADO CULTIVARS

6. 1. 1 Introduction

Competition with other trees in forest stands during evolution has increased the complexity of woody perennials to the extent that they have developed life-cycle strategies which optimise competitive fitness within the plant community (Dickson 1991). The storage of minerals and carbohydrates surplus to current requirements and their remobilisation during periods of critical demand, enhance the tree's competitiveness in terms of growth and reproduction. Avocados have the capacity to store significant quantities of minerals and carbohydrates which are largely re-cycled during flowering, fruit set and spring growth (Cameron and Borst 1938; Cameron *et al.* 1952; Scholefield *et al.* 1985). While mineral nutrients are essential for vegetative growth, the requirements for avocado fruit development are comparatively small (Wolstenholme 1991). However, there is large investment of "energy" to produce oil-rich fruit with large carbohydrate-rich seeds, compared with sugar-storing species, e.g.. apples, citrus, peaches, mangoes (Wolstenholme 1986; 1987). Avocado fruiting therefore places high demand on the carbon-based products of photo-assimilation.

During a current cropping cycle, biotic and abiotic factors can have a substantial impact on tree performance. Biotic factors such as shoot vigour, leaf to fruit ratios and the incidence of pests and diseases (Quinlan and Preston 1971; Chacko *et al.* 1982; Whiley *et al.* 1986), and the environmental variables such as temperature (particularly in relation to critical phenological events), humidity, light, wind and storms (Proctor and Creasey 1971; Sedgley and Annells 1981;

Whiley and Winston 1987; Issarakraisila and Considine 1994; Crane *et al.* 1994) all contribute to the quality and quantity of the harvest.

The inability of mature avocado fruit to ripen while attached to the tree (Schroeder 1952) has been widely utilised as a "tree storage" strategy to take advantage of marketing opportunities. As lipids continue to accumulate in fruit well after horticultural maturity (Eaks 1990; Kaiser and Wolstenholme 1994), this practice undoubtedly will have some impact on the total carbon economy of the tree. In some circumstances, delayed harvest of late maturing cultivars such as 'Hass', may result in the tree simultaneously carrying mature fruit while flowering and setting the following season's crop. In cooler climates where 'Hass' is grown, e.g. California, Israel and New Zealand, simultaneously carrying two seasons crops on trees is a "normal" event and may be the main cause of the pronounced alternate bearing experienced in these countries.

While currently there are no published reports on the long-term effect of delayed harvest of avocado on subsequent productivity, the consequences of this practice have been reported for 'Valencia' orange. This fruit may also be "stored" on trees in cool areas for up to eight months after legal maturity has been reached, with several months overlap of successive crops. The consequences are reduced yields of smaller fruit in subsequent crops, with the likelihood of the onset of alternate bearing (Hilgeman *et al.* 1967; Monselise and Goldschmidt 1982).

Fruit size of avocado is also an important component of yield as premium prices are often paid for larger fruit, and small size can be problematic with some cultivars, e.g. 'Hass' (Lahav and Adato 1990; Köhne 1991; Cutting 1993). This study investigates the impact of delayed harvesting on starch cycling within the tree, and the subsequent effect on fruit size and yield of early and late maturing cultivars growing in Australia.

6. 1. 2 Materials and Methods

Avocados growing in commercial orchards at Childers (latitude 25°S, altitude 40 m) and Maleny (latitude 26.5°S, altitude 520 m) in S.E. Queensland were used in this study over six seasons from 1988 until 1994. The research was carried out with the two most important avocado cultivars

grown in Australia - the early maturing 'Fuerte' and the late maturing 'Hass'. The 'Fuerte' experiment was located at Childers which has a warm, subtropical climate best suited to this cultivar. There were two 'Hass' experiments, one located at Childers and the other at Maleny. The climate at Childers is considered marginally too warm for 'Hass', with small fruit at maturity consequently a serious commercial problem. This cultivar is much more suited for production in the cool, mesic subtropical climate of Maleny. At Childers, seven-year-old 'Fuerte' and 'Hass' trees grafted to seedling Guatemalan rootstock spaced at 9 x 7 and 8 x 6 m respectively, were chosen while at Maleny 10-year-old 'Hass' trees grafted to seedling 'Velvick' spaced at 12 x 8 m were used. The 'Hass' at Maleny had previously been affected by hail and had developed a strong alternate bearing pattern prior to starting the experiment. At each location, fertilisation and pest and disease control were according to recommendations of Whiley *et al.* (1988a) and Banks (1992). Irrigation at Childers was by under-tree sprinklers (two per tree each delivering 14 l hr^{-1}) and was scheduled with tensiometers to supplement rainfall (annual average of 900 mm) while at Maleny trees relied solely on rainfall (annual average of 2000 mm).

Percentage dry mass of fruit flesh was selected as the maturity index for harvest. As maturing avocado fruit maintain a constant relationship between the percentage oil and water in the flesh (Swarts 1976; Lee 1981a; Lee *et al.* 1982), the determination of flesh dry matter is a reliable method of judging maturity with respect to the previously defined oil content standard (Lee 1981b). In Australia and South Africa this (or the reciprocal, flesh moisture percentage) has been commercially utilised for some time for determining minimum fruit maturity standards (Swarts 1978; Brown 1984). In Australia the minimum maturity standard for avocados is 21% dry matter (Brown 1984) though commercially 'Hass' is generally harvested when it reaches 23 to 25% dry matter. In many instances fruit is stored on trees for market opportunities, so that pulp dry matter may exceed 30 to 35% when harvested. Thus treatments selected spanned those of normal commercial practice and were:

for 'Fuerte'

1. All fruit harvested at 21% dry matter (21%);
2. All fruit harvested at 24% dry matter (24%);
3. Half of the fruit harvested at 21% and half at 30% dry matter (21/30%);
4. Half of the fruit harvested at 24% and half at 30% dry matter (24/30%);

5. All fruit harvested at 30% dry matter (30%); and

for 'Hass'

1. All fruit harvested at 25% dry matter (25%);
2. All fruit harvested at 30% dry matter (30%);
3. Half of the fruit harvested at 25% and half at 35% dry matter (25/35%);
4. Half of the fruit harvested at 30% and half at 35% dry matter (30/35%);
5. All fruit harvested at 35% dry matter (35%).

To determine the correct stage of maturity for harvesting, random fruit samples were periodically collected for pulp dry matter determination. In addition, at harvest pulp dry matter of five fruit from each tree was measured to establish the actual maturity of fruit from each treatment when picked.

Tree phenology was detailed by recording the date of floral bud-break; the duration of anthesis and the periods of active shoot growth. Wood samples were collected from trunks and the most recently produced shoots at regular intervals for starch analyses. Samples of wood were taken from five sites on each tree by first removing a plug of bark and then drilling 40 mm into the trunk with a 9 mm bit. The shavings were collected for analysis. Approximately 6 cm samples were removed from mature summer grown shoots and the leaves discarded. Ten non-fruiting shoots were randomly collected from each tree and sampling continued until the completion of anthesis. The procedures for processing and analysing the samples for starch content were as described in Chapter 2.

Data from each experiment were analysed by ANOVA, and covariance analysis was used to separate the effect of yield on fruit size. Where applicable, the relationship between trunk starch concentration in July and subsequent yield was established using linear regression analysis (TableCurve™, Jandel Scientific, Calif., USA).

6. 1. 3 Results

'Fuerte' at Childers

Depending on the year, fruit reached 21% dry matter from late March until mid-April; 24% dry matter from late April until mid-May and 30% dry matter from late May until mid-June (Table 8). All fruit over the duration of the study were harvested within ± 1% of the target dry matter for the respective treatments.

Table 8 Maturity of cv. Fuerte fruit at Childers indicated by dry matter at the different times of harvest in 1988 to 1993. Data are the means ± SE (n = 5) from each treatment when harvested at their respective maturity times.

1st Harvest		2nd Harvest		3rd Harvest	
Date	Dry matter (%)	Date	Dry matter (%)	Date	Dry matter (%)
14.04.88	21.5 ± 0.3	06.05.88	24.6 ± 0.4	17.06.88	31.1 ± 0.3
28.03.89	21.1 ± 0.2	26.04.89	24.9 ± 0.5	08.06.89	29.8 ± 0.4
24.04.90	21.4 ± 0.3	15.05.90	25.4 ± 0.4	19.06.90	29.7 ± 0.3
11.04.91	21.7 ± 0.1	09.05.91	24.8 ± 0.2	21.05.91	30.4 ± 0.4
09.04.92	21.2 ± 0.2	29.04.92	24.6 ± 0.2	18.06.92	30.6 ±0.3
14.04.93	21.7 ± 0.2	04.05.93	25.7 ± 0.6	24.05.93	30.0 ± 0.5

On an annual basis, there was no significant treatment effect on yield except in 1991 where trees that were strip-harvested at 21% and 24% or picked at 24/30%, had significantly higher yield than trees where fruit was harvested at 21/30% or 30% (Fig. 26a). Due to carry-over effects and natural tree to tree variation, it is unusual to demonstrate yield responses from agronomic treatments in tree crops over an annual cycle (Schaffer and Baranowski 1986). In this case

significant differences were likely due to pre-conditioning of the trees following the application of treatments over a number of years.

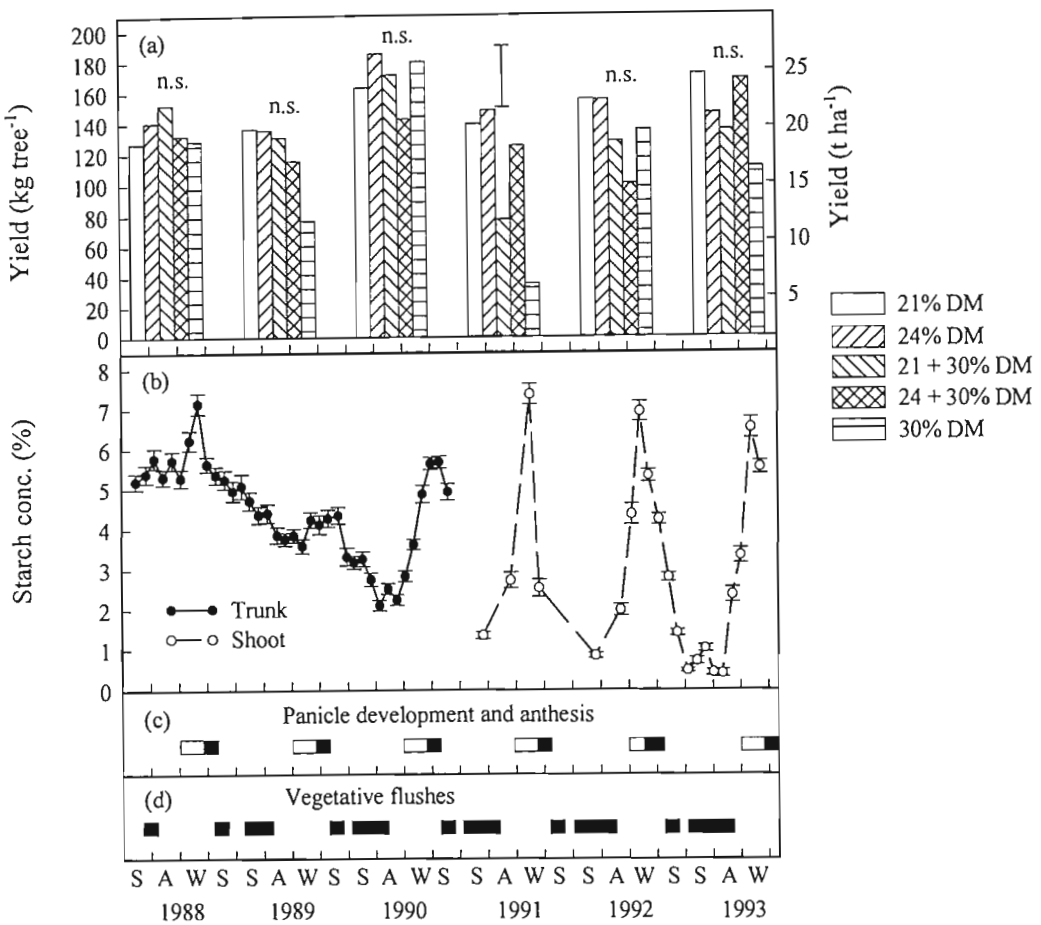

Fig. 26 Relationship between yield, seasonal starch concentration flux and tree phenology of cv. Fuerte at Childers where: (a) is yield of fruit which were harvested at different stages of maturity as judged by dry matter (DM), vertical bar indicates LSD ($P \leq 0.05$); (b) is the mean starch concentration of all treatments (n = 30), SEs are represented by vertical bars; (c) is periods of panicle growth represented by open horizontal bars, and periods of anthesis represented by closed horizontal bars; (d) is periods of vegetative growth represented by closed horizontal bars.

Trunk and shoot starch concentration data have been pooled as there were no significant differences between treatments. The concentration flux of trunk starch over three years was in the order of 5% (from ca. 2 to 7%) (Fig. 26b). Starch levels peaked during each winter and declined

after flowering. The lowest trunk starch concentrations were during the summer and autumn of 1990 when trees were carrying their heaviest crop (Figs. 26a & 26b). The seasonal concentration flux of starch in summer grown shoots was higher than in the trunks of trees (ca. 8% varying from < 1% to > 7%) and followed a defined seasonal pattern (Fig. 26b). Peak concentrations of starch accumulated during the autumn/winter period when shoot growth had ceased and the tree was in a relatively quiescent phase (Figs. 26b, 26c & 26d). During the flowering and spring flush periods shoot starch levels dropped rapidly to < 1% by the end of spring.

There were two periods of shoot growth activity in trees during a cropping cycle, viz. in spring and summer (Fig. 26d). Spring shoot growth was synchronised by flowering with most terminals flushing simultaneously in early September. Shoot growth was relatively quiescent after 60 days, followed by more sporadic summer and autumn flush growth from late December through to late April-May when all shoot growth activity ceased.

Mean fruit size was dependent on the time of harvest although significant differences were not apparent for every year of the study (Table 9). In general fruit size increased in those treatments where harvest was delayed, either by removing part of the crop early and the balance later or leaving the fruit until they had reached 30% DM. In three years out of six for example, fruit size of the 21/30% and 30% DM treatments were significantly larger than the 21% DM treatment.

From the second year of the study treatments began to affect the cumulative yield of trees (Fig. 27). Treatments with the earliest harvest times, i.e. 21% and 24% DM, had significantly higher yields than where fruit was allowed to hang until 30% DM. The yield increment increased with time, though the earliest harvested treatments never significantly out-yielded those where fruit were harvested at 21/30% or 24/30% DM. Expressed differently, split harvests did not prejudice cumulative yields over the six seasons.

Table 9 Effect of time of harvest on fruit size of cv Fuerte at Childers. Data are mean values (n = 6) of treatments for each year of the study and have been subjected to covariance analysis adjusting for yield. Figures in parenthesis are the unadjusted fruit size means. Values in columns not sharing a common letter are significantly different ($P \leq 0.05$) (ANOVA).

Treatments	Fruit mass (g)					
	1988	1989	1990	1991	1992	1993
1. 21% DM	269.3 (276.4)c	306.6 (308.5)c	318.9 (320.5)a	339.4 (325.9)a	309.3 (318.4)b	290.9 (294.3)a
2. 24% DM	309.5 (309.5)b	313.6 (315.2)abc	312.3 (307.2)a	338.2 (321.2)a	313.0 (302.8)b	321.8 (320.1)a
3. 21/30% DM	343.9 (343.9)a	331.2 (332.4)ab	329.3 (328.3)a	330.6 (341.6)a	343.6 (347.1)a	334.4 (328.3)a
4. 24/30% DM	340.2 (340.2)a	357.9 (357.5)a	340.6 (348.5)a	351.8 (343.9)a	320.2 (337.6)ab	314.3 (315.8)a
5. 30% DM	339.8 (339.8)a	343.0 (338.7)ab	327.2 (323.7)a	344.8 (367.3)a	345.5 (345.2)a	335.8 (338.7)a
Regression coefficient	-0.784**	0.102	-0.305	-0.394**	-0.505	-0.372

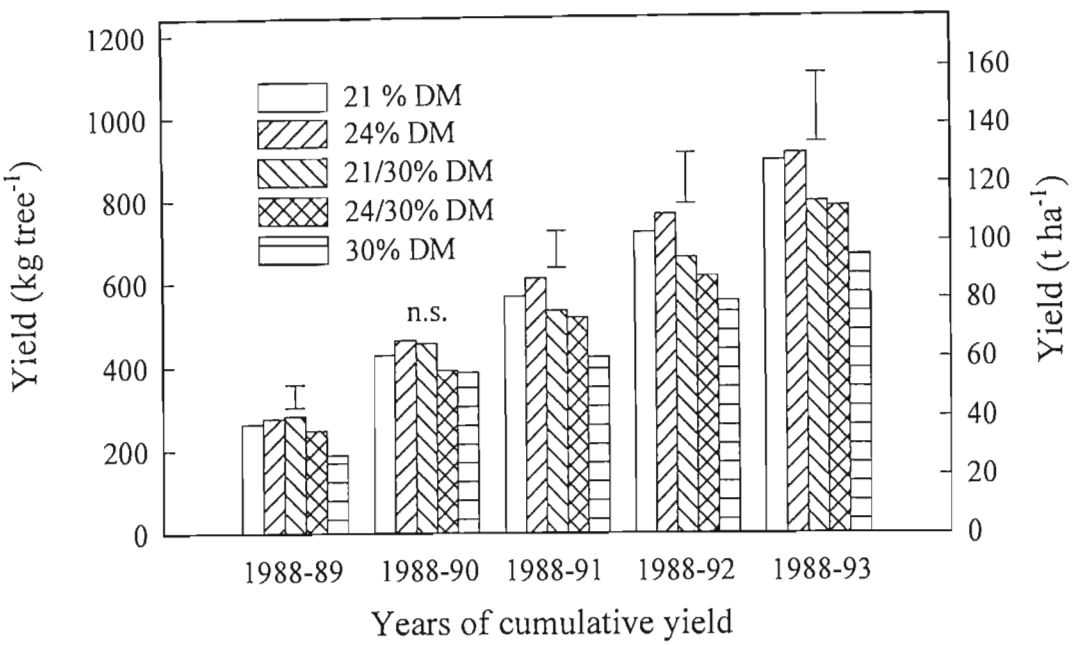

Fig. 27 Effect of time of harvest, based on fruit dry matter (DM), on the cumulative yield of cv. Fuerte avocado trees at Childers over six consecutive years. Columns are treatment means (n = 6) and vertical bars indicate LSDs ($P \leq 0.05$).

The effect of the time of harvest on yield becomes more apparent when the annual patterns for each treatment are examined over the duration of the study (Fig. 28). In the first year of this experiment treatments had no significant effect on yield, indicating absence of bias in the experimental population (Fig. 26a). The continued early harvesting of fruit at 21 and 24% for six years resulted in cropping patterns wherein annual variation was insignificant, and limited to fluxes which most likely expressed environmental conditions at critical periods of development (Fig. 28). In contrast, delayed harvesting of fruit, either 50% or all of the crop, resulted in the development of an alternate bearing cycle where the amplitude increased with time. Examination of the data show that the cycle was atypical from 1992 to 1993 where yields for the 21/30% and 30% treatments were almost identical (Fig. 28). This may be explained by the severe tropical storm experienced in the orchard in February 1992 when a significant portion of the crop was lost when fruit was about 60% grown. This effectively amounted to an unscheduled early harvest across all treatments allowing a similar sized crop to be carried the following year.

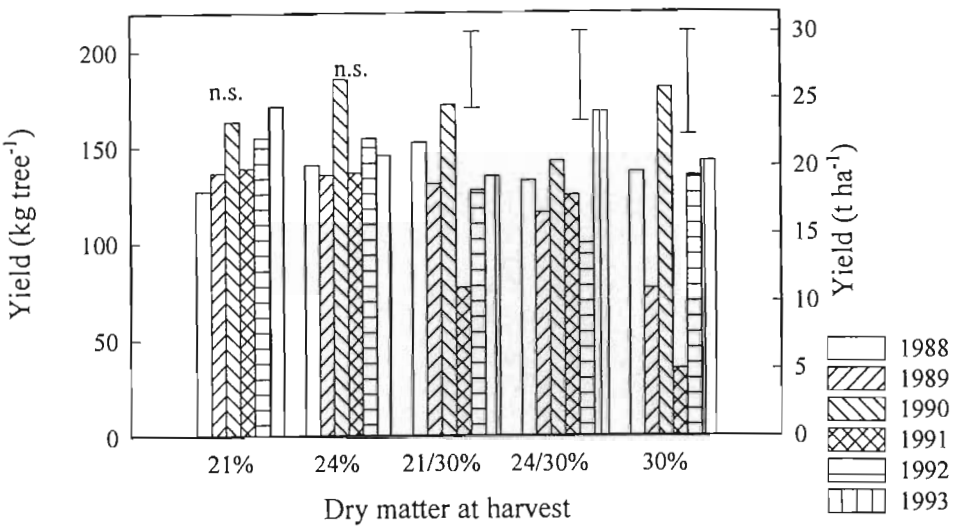

Fig. 28 Effect of time of harvest on the sustainability of yield of cv. Fuerte avocado trees at Childers over six consecutive years. Columns are mean values (n = 6) and vertical bars indicate LSDs ($P \leq 0.05$).

'Hass' at Childers

'Hass' fruit at Childers reached 24% dry matter from mid to late May over the duration of the study (Table 10). From mid- to late July, fruit had accumulated 30% dry matter with 35% being reached from late August to mid-September. All fruit were harvested within ± 1% of the target dry matter for the respective treatments.

Table 10 Maturity of cv. Hass fruit at Childers indicated by dry matter at the different times of harvest in 1991 to 1994. Data are the means ± SE (n = 5) each of treatment when harvested at their respective maturity times.

1st Harvest		2nd Harvest		3rd Harvest	
Date	Dry matter (%)	Date	Dry matter (%)	Date	Dry matter (%)
12.05.91	24.8 ± 0.2	03.07.91	31.6 ± 0.4	22.08.91	35.1 ± 0.4
18.05.92	24.7 ± 0 3	06.07.92	30.6 ± 0.3	07.09.92	34.8 ± 0.3
25.05.93	24.9 ± 0.4	27.07.93	29.4 ± 0.3	17.09.93	35.2 ± 0.4
30.05.94	24.7 ± 0.4	04.07.94	30.7 ± 0.4	28.08.94	34.8 ± 0.4

There was an increase in crop load from the start to the finish of the study, particularly in the last two years of the experiment (Fig. 29a). However, fruit yield was lower than expected in 1992 due to the effects of a tropical cyclone. There were no significant differences between treatments in annual yield except for 1993 when trees harvested at 25%, 30% and 25/35% DM out-yielded those in which harvest was delayed until 35% DM. In this case, carry-over effects from the storm may have enhanced the magnitude of treatment effects thereby precipitating a conclusive result.

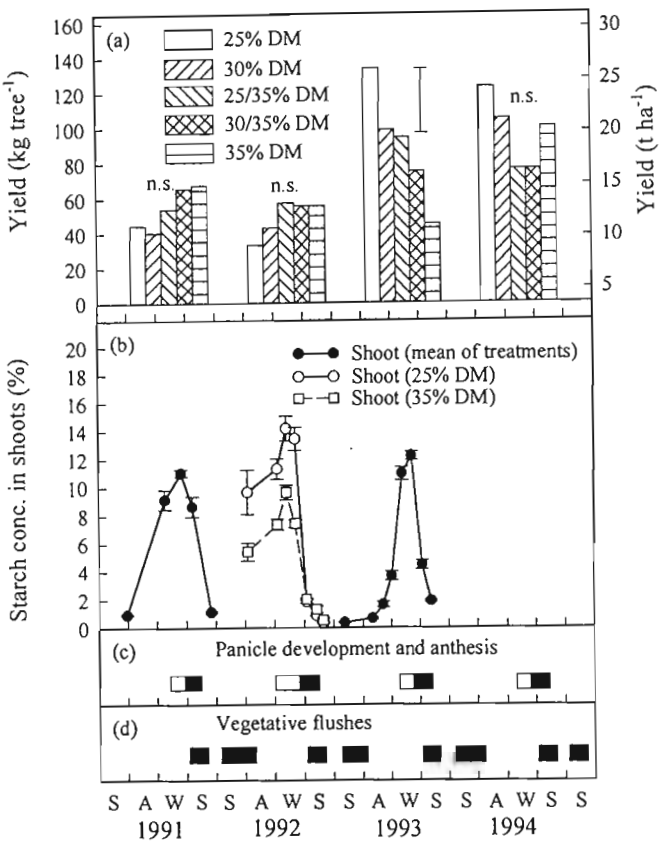

Fig. 29 Relationship between yield, seasonal starch concentration flux and tree phenology of cv. Hass at Childers where: (a) is yield of fruit which were harvested at different stages of maturity as judged by dry matter (DM), the vertical bar indicates LSD ($P \leq 0.05$); (b) is starch concentration of shoots of all treatments (●, n = 15), or 25% DM (○, n = 3), or 35% DM (□, n = 3), SEs are represented by vertical bars; (c) is periods of panicle growth represented by open horizontal bars, and periods of anthesis represented by closed horizontal bars; and (d) is periods of vegetative growth represented by horizontal closed bars.

There were no significant differences in fruit size between any of the treatments in each of the four years of the study (Table 11). However, there were trends present, stronger in some years than others, which indicated the potential for fruit size to increase the longer it is left on the tree.

Table 11 Effect of time of harvest on fruit size of cv. Hass growing at Childers. Data are mean of 6 trees for each treatment for each year of the study and have been subjected to covariance analysis adjusting for yield. Figures in parenthesis are the unadjusted fruit size means. There were no significant differences between values in columns as tested by ANOVA.

Treatment	Fruit size (g)			
	1991	1992	1993	1994
25% DM	172.9 (179.8)	169.1 (164.8)	230.2 (203.8)	188.9 (183.8)
30% DM	178.6 (188.3)	146.5 (144.7)	239.6 (241.5)	194.0 (195.2)
25/35% DM	186.2 (186.4)	160.3 (163.0)	244.8 (240.4)	192.5 (195.5)
30/35% DM	185.4 (177.6)	162.7 (164.6)	236.4 (242.5)	192.8 (195.8)
35% DM	202.1 (193.0)	176.3 (178.2)	238.5 (253.2)	206.9 (205.7)
Regression coefficient	-0.692**	0.253	-0.553**	-0.174

With respect to seasonal starch concentrations in shoots, there were no significant differences during 1991 and 1993 so data for all treatments were pooled. However, in 1992 shoot starch concentrations were significantly different and data have been presented separately for the 25% and 30% DM treatments (Fig. 29b). Starch levels for the 30%, 25/35% and 30/35% DM treatments in 1992 fell between concentrations for the 25% and 35% DM treatments and were not significantly different (data not presented). Starch concentrations in the shoots followed similar seasonal patterns for the first three years of the study when they were measured. Levels were initially low at the completion of summer shoot growth but increased rapidly as shoots entered a

period of quiescence and peaked prior to flowering (Figs. 29b, 29c & 29d). Concentrations declined rapidly during flowering and spring shoot growth.

Shoot growth occurred during two periods over each cropping cycle; a relatively short term of activity in the spring concomitant with the termination of flowering followed by intermittent growth during the summer. The spring flush was generally concluded by mid- to late November while shoot growth during summer began in early January and had ceased by mid-March.

The light crop due to storm damage and the different harvesting times of fruit are the factors most likely contributing to higher accumulation of starch in the 25% DM treatment in 1992. While all treatments lost a considerable percentage of their crop in February, the earlier harvesting of the 25% treatment (18.05.92) provided these trees with a greater period without strong sinks to accumulate starch in shoot tissues. The 1992 starch concentration in shoots was positively related to fruit yield in 1993 (Fig. 30).

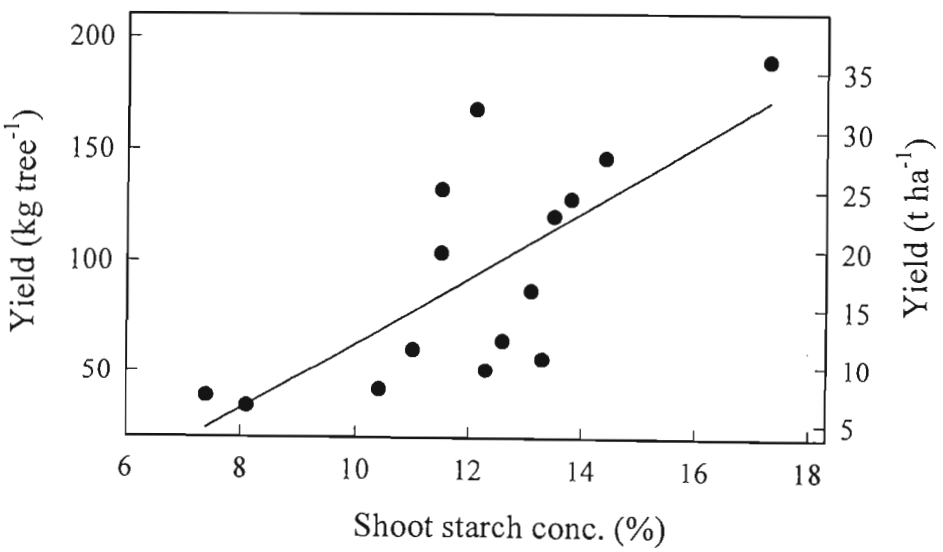

Fig. 30 Relationship between yield (1993) and July shoot starch concentration (1992) of cv. Hass growing at Childers. The regression is represented by the equation $y = 14.84x - 85.63$, $r^2 = 0.52$*.

While there were no significant differences in cumulative fruit yield until the fourth year of the study, the combined yield of the first and second years was higher for treatments which were harvested late (Fig. 31). This was undoubtedly due to the non-significant trend for an increase in size of late harvested fruit (data not presented) and before any impact was made by these fruit on the following year's crop. By the end of 1993 (three years) this trend was changing slightly favouring the earlier harvested fruit and after four years, those trees harvested at 25% dry matter produced more fruit than all the other treatments (Fig. 31).

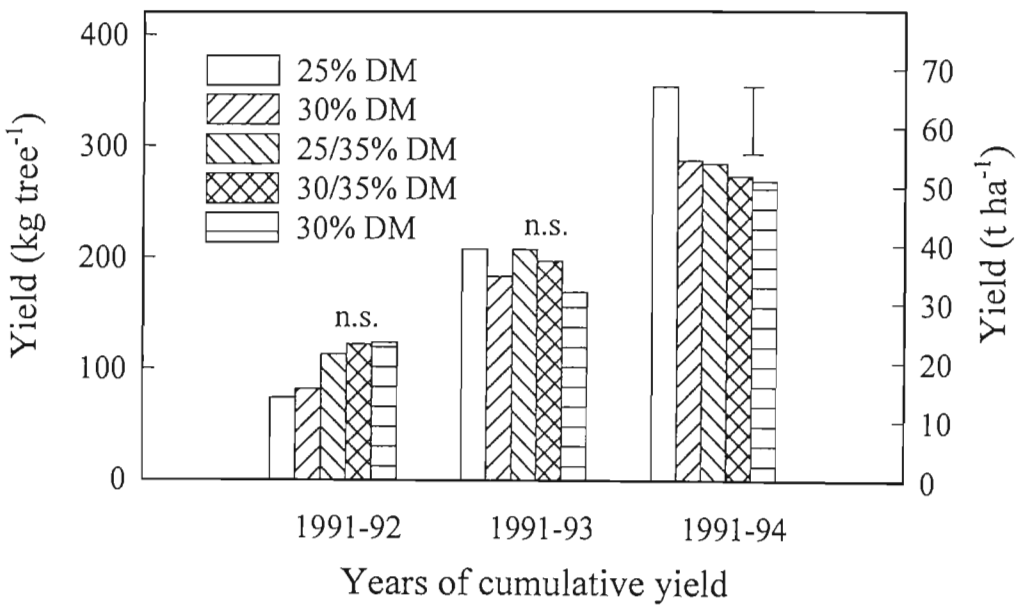

Fig. 31 Effect of time of harvest, based on fruit dry matter (DM), on the cumulative yield of cv. Hass avocado trees at Childers over four consecutive years. Columns are mean values of 6 trees for each treatment and vertical bar represents LSD ($P \leq 0.05$) determined by ANOVA..

With respect to the sustainability of production there was little impact made on yield by any of the treatments during the first two years of the study (Fig. 32). This in part may be due to the storm damage to the trees during the 1992 season. However, in the third and fourth years there is an indication that treatments were beginning to affect production sustainability. Where fruit were harvested at 25% and 30% DM, yield increased substantially in 1993 and 1994 and was similar

for both of these years. There were no significant differences in yield from year to year in those treatments harvested at 25/35% and 30/35%. However, in those trees where all fruit were harvested at 35% there was a reduction in the 1993 yield compared with production in 1994. It is suggested that this may be the beginning of a biennial fruiting pattern similar to that induced with 'Fuerte' growing in the same orchard.

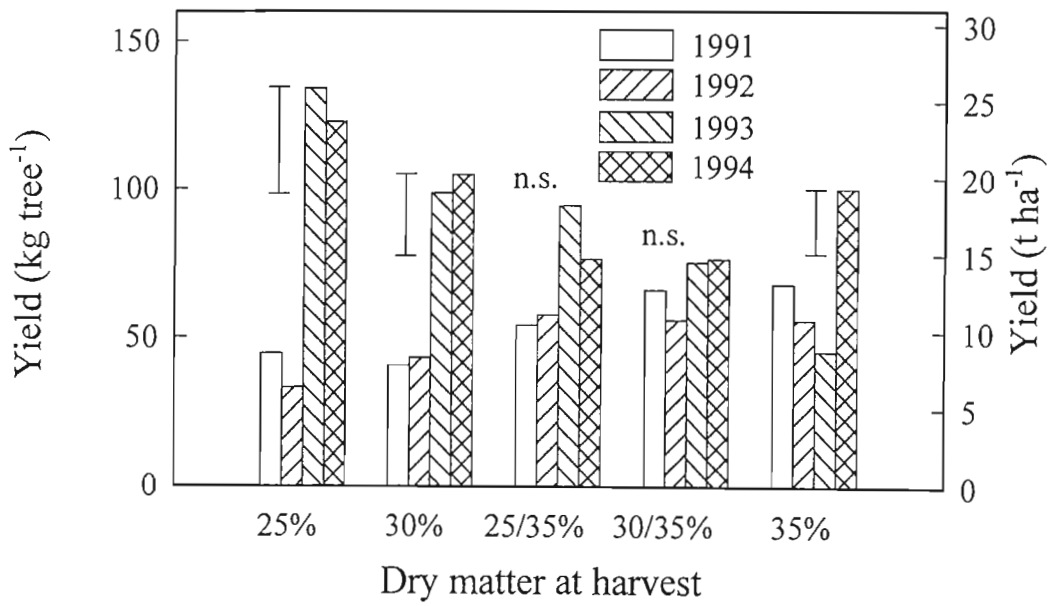

Fig. 32 Effect of time of harvest, as judged by fruit dry matter (DM), on the sustainability of yield of cv. Hass at Childers. Columns represent mean values of 6 trees for each treatment and vertical bars LSDs ($P \leq 0.05$) determined by ANOVA.

'Hass' at Maleny

At Maleny fruit reached 25% dry matter from late June to mid-July; 30% from early August to late September; and 35% in early November (Table 12). For the most part treatments were harvested within 1.5% of their defined maturity, the exceptions being the November 1989 and September 1990 harvests at which fruit were slightly more mature than planned. Some fruit were tree-stored for over 4 months, well into a second growing season.

Table 12 Maturity of cv. Hass fruit at Maleny indicated by dry matter at the different times of harvest in 1988 - 1990. Data are the means ± SE of five fruit from each of the trees when harvested at their respective maturity times.

1st Harvest		2nd Harvest		3rd Harvest	
Date	Dry matter (%)	Date	Dry matter (%)	Date	Dry matter (%)
14.07.88	24.5 ± 0.3	28.09.88	31.6 ± 0.4	09.11.88	35.5 ± 0.3
28.06.89	24.2 ± 0.4	02.08.89	31.1 ± 0.5	01.11.89	37.4 ± 0.3
18.07.90	25.0 ± 0.2	19.09.90	32.6 ± 0.5	07.11.90	35.4 ± 0.5

There were no significant differences among treatment yields in any of the three years of the study (Fig. 33a). However, there was a very strong biennial effect across all treatments with high yields in the first and third years of the experiment (equivalent to 39.2 and 37.0 t. ha^{-1}, respectively) and low yield (equivalent to 9.6 t. ha^{-1}) in the second year.

As there were no significant differences in trunk starch concentrations between treatments throughout the three years of the study data have been pooled. Seasonal fluctuations in trunk starch levels ranged from ca. 3.5 to 7.4% and maximum concentrations occurred just prior to (1988 & 1990) or during flowering (1989) (Figs. 33b & 33c). Starch concentrations tended to be relatively stable during shoot growth and were in the vicinity of 5 to 6% (Figs. 33b & 33d). The low starch concentrations in 1988 and 1990 were likely due to very heavy crop loads which were not harvested until June/July or later for some of the treatments. The highest concentration of starch occurred during winter 1989 after shoot growth had ceased and when the crop load was light (Fig. 33a & 33b).

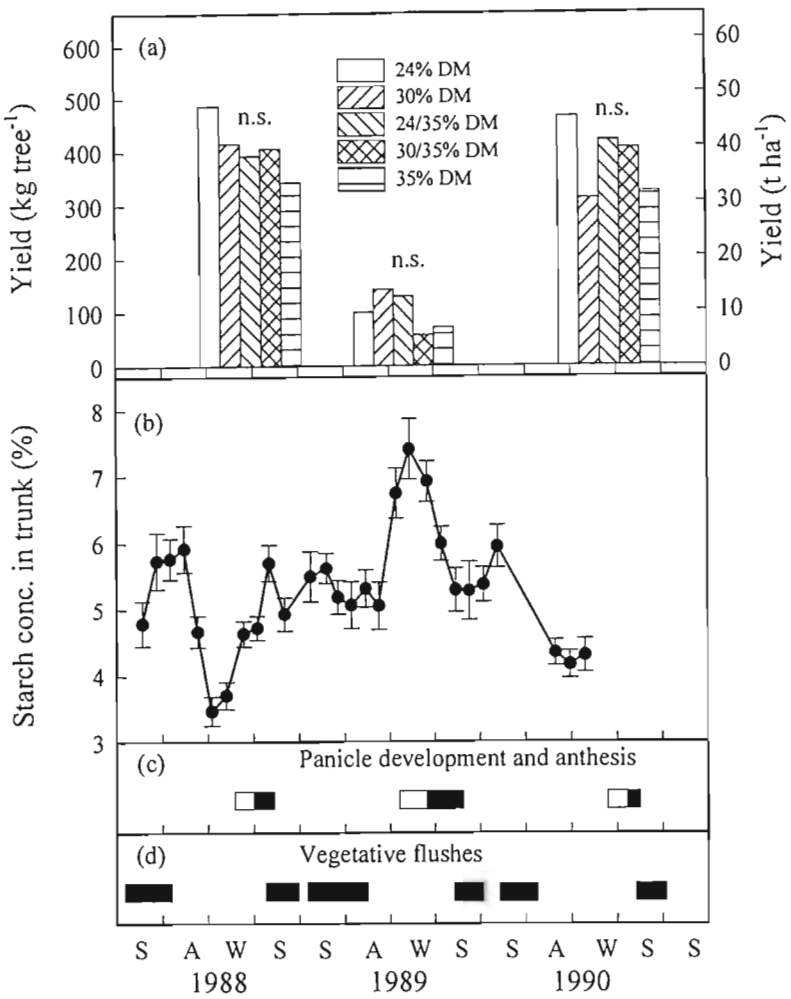

Fig. 33 Relationship between yield, seasonal starch concentration flux and tree phenology at Maleny where: (a) yield of fruit which was harvested at different stages of maturity as judged by dry matter (DM); (b) is starch concentration (n = 30), of all treatments, SEs are represented by vertical bars; (c) is periods of panicle growth represented by open horizontal bars, and periods of anthesis represented by closed horizontal bars; and (d) is periods of vegetative growth represented by horizontal closed bars.

Panicle emergence began in mid- to late July each year with anthesis mainly during September and extending into October (Fig. 33c). The flowering intensity was noticeably greater and extended for longer in the spring of the off-year preceding heavy cropping. Shoot growth occurred in two major periods over each crop cycle (Fig. 33d). In spring growth was synchronised by flowering and began near the conclusion of anthesis in early October. By late November of each year shoots had matured and following a short rest, sporadic flushing occurred

over the summer months. In 1989, summer growth began in mid-December and extended through until mid-April while in 1990 summer growth had ceased by the middle of March.

Trunk starch concentration in July of 1988 and 1989 were directly related to the yields in 1989 and 1990, respectively (Fig. 34). These data show "low" yields of ca. 100 kg tree^{-1} (10 t ha^{-1}) after July trunk starch concentrations of ca. 3 to 4%, rising to ca. 500 kg tree^{-1} (50 t ha^{-1}) at ca. 9 to 10% trunk starch the previous July.

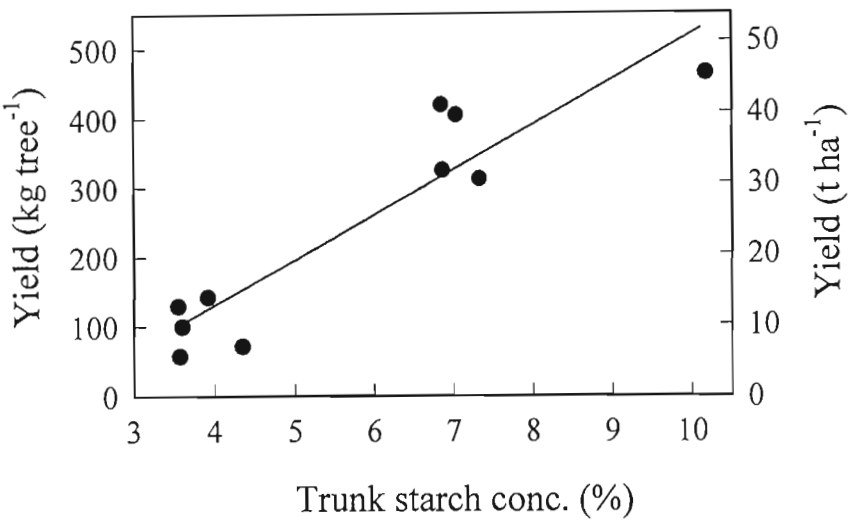

Fig. 34 Relationship between July trunk starch concentration and next season's yield of cv. Hass growing at Maleny. The regression is represented by the equation $y = 64.90x - 129.04$, $r^2 = 0.86^{**}$.

The effect of harvest time on fruit size is detailed in Table 13. General trends for each year indicate that delayed harvest increased fruit size, however a near significant ($P \leq 0.06$) increase was only recorded in the low crop year of 1989. In this season fruit which was allowed to hang late on the tree were 23% heavier than the earliest fruit harvest. This increase in fruit size was achieved without any significant reduction in fruit yield, either in the 1989 or 1990.

Table 13 Effect of time of harvest on fruit size of cv. Hass growing at Maleny. Data are means of 6 trees for each treatment for each year of the study and have been subjected to covariance analysis adjusting for yield. Values in parenthesis are the unadjusted fruit size means. Means in columns not sharing a common letter were significantly different at $P \leq 0.06$ as tested by ANOVA.

Treatment	Fruit mass (g)		
	1988	1989	1990
25% DM	219.8 a (214.0)	259.3 a (255.2)	219.2 a (214.3)
30% DM	233.5 a (233.0)	227.2 ab (214.5)	217.9 a (222.5)
25/35% DM	222.5 a (223.7)	251.2 a (243.4)	230.3 a (228.1)
30/35% DM	225.3 a (225.5)	210.3 b (214.7)	210.8 a (209.6)
35% DM	217.3 a (222.2)	236.7 ab (240.5	237.7 a (241.4)
Regression coefficient	-0.075	-0.167*	-0.062

6. 1. 4 Discussion

Yield and fruit size

Results presented in this Chapter have shown that avocado yields are strongly influenced by the synergism of fruit load and duration of the crop on the tree. With early and late maturing cultivars growing at Childers, removal of fruit within reasonable time of reaching maturity maintained yield performance over a number of seasons. However, prolonged delayed harvesting of fruit caused strong alternate bearing cycles to develop. This was particularly evident with 'Fuerte' which began this cycle the first year after starting treatments, i.e. shown in the 1989 yields. The development of the alternate bearing pattern at Childers was delayed with 'Hass', as yields were relatively low across all treatments over the first two years of the study. This was most likely due to the previous history of the trees which had suffered from Phytophthora root rot and boron deficiency prior to starting the treatments, and damage caused by the tropical storm in

1992. Nevertheless, in the final two years of the study there were signs that a biennial cycle had developed in trees where delayed harvesting was practiced. This result contrasts with that reported by Kaiser and Wolstenholme (1994) who found that late harvesting of 'Hass' in the cool, mesic, subtropical Natal midlands did not depress yields the following year. There are three possible reasons why results differ: the length (number of years) of the Kaiser and Wolstenholme experiment was insufficient to induce biennial patterns; theft of fruit from trees distorting yield data which was suggested by the authors to explain unexpected results; or environmental differences between the sites significantly changing comparative tree and fruit performance. With respect to the latter, the Childers site in Queensland is significantly warmer than Everdon in Natal. Higher mean temperatures would increase the 'cost of fruiting' at Childers through increased respiratory losses (Blanke and Whiley 1995) thereby limiting photo-assimilates for fruit growth and subsequent crops.

Picking date is also reported to have affected yield in other tree crops. In a study with apples, fruit were picked over an eight week period from when they were judged to attain maturity until they had begun to fall naturally from the trees (Williams *et al.* 1980). It was shown that fruit set the following spring was highly correlated to the harvest dates of the previous year. Early picking promoted more flower clusters which consequently set more fruit. Similarly, harvest time has been shown to affect cropping patterns of 'Valencia' oranges. After a 14-year study, Jones and Cree (1954) concluded that late picking decreased the following year's yield and increased the severity of alternate bearing. Later studies showed a curvilinear relationship between harvest date and the size of the next year's crop and suggested that 'Valencia' oranges crop to the limit of their available carbohydrates (Jones *et al.* 1964b).

'Hass' trees at Maleny were in a hail-induced biennial bearing cycle when treatments were started and this cycle, represented by "on", "off", "on" years continued for the duration of the study. Climatic stresses have been reported responsible for biennial bearing in other fruit species. For instance, spring frosts which destroy bloom have caused synchronised alternate cropping over large areas in apples, pecans and mangoes (Singh *et al.* 1974; Williams and Edgerton 1974; Sparks 1975). Unseasonably cool temperatures during flowering have also led to large scale

failure of fruit set of 'Valencia' oranges in Australia thereby initiating an alternate cropping cycle (Gallasch *et al.* 1978).

The crop at Maleny in the first year (ca. 39 t. ha^{-1} averaged across treatments) was extremely high by physiological standards for avocados (Wolstenholme 1986; 1987). It was associated with depletion of trunk starch content as the crop matured during winter, which contrasts with previously described patterns (Cameron and Borst 1938; Rodrigues and Ryan 1960; Scholefield *et al.* 1985; Kaiser and Wolstenholme 1994). Flowering, which led to a small crop in 1989, was of low intensity and short duration compared with the same event in 1989 which subsequently produced a 37 t. ha^{-1} crop (averaged across treatments). In this case the effect of harvest time on yield had no apparent impact on the pre-determined cycle, and it appears that more rigorous procedures earlier in the crop cycle of an "on" year will be necessary to break strong alternate bearing patterns. For instance, Jones *et al.* (1974) were able to influence the cropping cycle of alternate bearing 'Valencia' oranges by thinning fruit at different stages of development in an "on" year. The subsequent crop was directly related to the amount and time after set that fruit was removed, e.g. removal of 66% of the current crop three months after anthesis produced more fruit the following year than the removal of 33% of the current crop seven months after anthesis. Similarly, El-Zeftawi and Thornton (1975) showed that stripping fruit from 'Valencia' trees within four months of setting their first commercial crop, moderated alternate bearing for six years without decreasing total yield over that period. Strategic pruning of trees may also be an alternative practice to modulate biennial cropping patterns in avocado. There is currently no information available on this topic but severe pruning (topping along with tree removal) of large trees in Florida improved yield compared with non-pruned crowded trees (Crane *et al.* 1992).

There was a trend of increased fruit size with delayed harvest though results were not significant in all years or at all sites. For 'Fuerte', fruit size increased in the order of 8 to 18% with later harvested treatments. However, these gains must be balanced against lower yields as small fruit are generally not an issue in well managed 'Fuerte' orchards. There was no significant difference in fruit size with 'Hass' grown at Childers once data had been adjusted for yield, though there was a consistent trend each year suggesting larger fruit with delayed harvest. Although delayed harvest at Maleny increased fruit size by ca. 23% in the "off" year ($P \leq 0.06$), no differences were

recorded when heavy crops were carried on the trees. This may be due to assimilate limitation due to over-loading trees with fruit. Other studies have reported increased fruit size when harvesting was delayed. For instance, the size of 'Bramley's Seedling' apple improved appreciably with late harvesting (Williams *et al.* 1980), and Kaiser and Wolstenholme (1994) found larger 'Hass' fruit when harvesting was delayed. This is consistent with the well-known fact that cell division continues as long as avocado fruits are firmly attached to the tree, with effects likely to be greater in fruit in which seed coats do not abort prematurely (Schroeder 1952; Valmayor 1967).

It is worth noting the difference in 'Hass' fruit size between Childers and Maleny after adjustment for yield at both sites (Tables 11 & 13). At Childers the mean fruit size over the four years of the study was 195.0 ± 6.5 g while for the three years at Maleny mean fruit size was 227.9 ± 3.6. This represents an increase of ca. 17% in the size of the fruit at Maleny. Due to different time frames for the two experiments statistical analysis cannot be applied to the data, however these results support industry perceptions that in warmer climates the 'Hass' small fruit problem is more severe. During the first 12 weeks of fruit ontogeny (October/December) the mean min/max temperature at Childers was 3.5°C higher than at Maleny. This is the period of most rapid cell division and growth (Valmayor 1967) when respiration rates of fruit are highest (Whiley *et al.* 1992; Blanke and Whiley 1995). Blanke and Whiley (1995) have suggested that the high rates of R_d measured for 'Hass' fruit may be a contributing factor to their smaller size in warmer climates.

Seasonal starch cycling

Starch is the most common and ubiquitous reserve carbohydrate in plants and there are numerous reports on its role in alternate bearing in fruit crops (Grochowska 1973; Davis and Sparks 1974; Jones *et al.* 1975; Goldschmidt and Golomb 1982; Scholefield *et al.* 1985). It has been repeatedly confirmed that starch levels are higher during the winter of the "off" year compared to levels when the tree has cropped heavily. The three year study of Scholefield *et al.* (1985) clearly indicated a direct relationship between winter starch levels and subsequent yield of 'Fuerte' avocado trees growing in a cool, dry Mediterranean climate in southern Australia. In this six year study with 'Fuerte' there was no clear correlation between starch concentrations (measured in the

trunk or shoots) and yield, although in all years trunk or shoot levels declined rapidly during flowering and early fruit development. The small seasonal change in starch concentration relative to yield compared with that reported by Scholefield *et al.* (1985), suggests a low dependence on reserve carbohydrate of 'Fuerte' trees to maintain yield in subtropical climates. It is suggested that carbohydrates from current photo-assimilates play a proportionally more important role in cropping than is the case in cooler regions where avocados are grown. Observations at Childers indicated the trees retained most summer grown leaves through until spring shoot growth was fully developed, thereby ensuring continuity of photo-assimilate supply during the flowering and fruit set period. However, Scholefield *et al.* (1985) reported that summer grown leaves were shed from March onwards at a rate faster than new ones were produced, probably as a consequence of salinity and environmental stress. Thus the assimilation surface was substantially reduced at flowering (a period of critical demand) and setting fruit were largely dependent on storage carbohydrate until the sink/source transition of the spring growth occurred.

Starch concentrations in either the trunk or shoots of trees were a better indicator of crop performance for 'Hass'. At Childers, a direct relationship between shoot starch concentrations in July and the subsequent crop was established for the 1992/93 cycle when large differences in treatment yields were recorded. Similarly, at Maleny trunk starch and subsequent yield were directly related across two seasons when strong biennial bearing was present. Shoot starch concentrations at Childers reached higher levels in 'Hass' (ca. 14%) than 'Fuerte' (ca. 7%). The reasons for this are not clear but may be associated with magnitude and temporal differences in phenology. 'Fuerte' showed more vegetative vigour than 'Hass' and flushed longer during summer (Figs. 26 & 29). Although 'Fuerte' fruit were harvested earlier in the year, floral bud development and anthesis were also advanced compared with 'Hass'. Strong root growth could also be expected from the cessation of summer flushing through to anthesis (see Chapter 2) and this combination of sinks together with decreased CO_2 assimilation efficiency during the winter, may have produced the lower peak concentrations.

The reduced yields detailed in these experiments cannot be solely attributed to threshold concentrations of starch at critical phenological stages. Crop failure was most often related to poor flowering with either a reduced number of floral sites or expression of flowering intensity

(observed but no data recorded). The reasons for flowering failure are beyond the scope of data presented in this chapter, but can possibly be explained by the theory of "multifactorial control" which postulates that several compounds - assimilates and known phytohormones - participate in floral induction (Bernier *et al.* 1981; Bernier 1988). Bernier *et al.* (1993) suggest a complex series of physiological signals between shoots and roots which precipitate floral induction. Roots are the primary source of cytokinins which participate in the floral stimulus at apical buds. Root studies presented in this thesis (Chapter 2), clearly show suppression of root growth during the autumn/winter period, a time of floral induction in avocado (Davenport 1982; Whiley *et al.* 1988a), when trees carry heavy crop loads. Poor root growth resulting in reduced cytokinin supply may well be a factor contributing to the diminished flowering observed in trees where delayed harvesting reduced the subsequent yield.

6. 1. 5 Conclusions

Alternate bearing in fruit crops disrupts continuity of supply to markets and reduces farm cash flow. Results reported herein indicate that harvesting time with respect to avocado fruit maturity, is an important criteria with respect to maintaining productivity on a yearly basis. Early removal of fruit from trees between 21 to 24% ('Fuerte') or 25 to 30% ('Hass') flesh dry matter sustained production levels in otherwise well managed orchards, but markedly delayed harvesting of fruit precipitated strong alternate cropping patterns. Early harvesting of 'Hass' where biennial bearing was entrenched, did not release the tree from this cropping pattern and more extreme practices such as fruit thinning or pruning may be needed to moderate the cycle. Indeed, where storm damage effectively removed crop load five months after fruit set, cropping patterns were modified in cv. Fuerte (see Childers data). In both cultivars the concentration flux of starch closely followed changes in phenological events and generally peaked in winter during an extended quiescent period. Although heavy fruiting depressed winter starch accumulation which resulted in reduced yield, production is more likely to be constrained by other environmental, resource and management bottlenecks at critical phenological stages.

CHAPTER 7

TRANSLATION OF PHENO/PHYSIOLOGY RESEARCH INTO MANAGEMENT STRATEGIES

The phenological and physiological aspects of avocado presented earlier in this thesis have been important in providing mechanisms for structured research to advance understanding of how the avocado tree grows, and more specifically defining its responses to the environment. However, ultimately the basic principles of tree mechanisms uncovered by research must be taken through to applied management strategies for their full potential to be realised. This chapter discusses two aspects of applied research which have utilised knowledge gained through the more basic areas of investigation. More specifically, the research methodology outlined in section 7. 1 was developed from phenology and carbon partitioning principles reported in Chapters 2 and 5, while the current investigations reported in section 7. 2 have been developed from the pheno/physiological studies detailed in Chapters 2 and 3.

7. 1 CONTROL OF PHYTOPHTHORA ROOT ROT WITH TRUNK-INJECTED PHOSPHONATE[‡]

7. 1. 1 Introduction

Phosphonates (viz. salts or esters of phosphonic acid) were the first commercially used ambimobile fungicides in plants (Zentmyer 1979; Lüttringer and De Cormis 1985). They are particularly effective in controlling diseases caused by Oomycetes such as the Phytophthora and Pythium species and downy mildews which cause severe economic losses in agricultural crops worldwide (Cohen and Coffey 1986). Due to effective translocation of phosphonates within plants several methods of application have been employed to control diseases. These include the traditional methods of foliar sprays and soil drenches (Pegg *et al.* 1985; Rohrbach and Schenck

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[‡] **Whiley, A.W., Hargreaves, P.A., Pegg, K.G., Doogan, V.J., Ruddle, L.J., Saranah, J.B. and Langdon, P.W., 1995.** Changing sink strengths influence translocation of phosphonate in avocado (*Persea americana* Mill.) trees. *Aust. J. Agric. Res.* Manuscript accepted 17 Oct 1994. **APPENDIX 4**

1985) and painting or sponge banding the trunks of trees with phosphonate formulations (Snyman and Kotzé 1983). The term phosphonate is widely used for the salts and esters of phosphonic acid (H_3PO_3). Once phosphonates are introduced into plant tissues they are rapidly hydrolysed to H_3PO_3 and subsequently ionised to the phosphonate anion, HPO_3^{-2} (Ouimette and Coffey 1990). This anionic form of H_3PO_3 is more correctly known as phosphonate (Ouimette and Coffey 1989).

Phytophthora cinnamomi Rands is a devastating root disease of avocado (*Persea americana* Mill.) in most countries which grow this crop. The fungus invades the unsuberised roots, and less frequently attacks the suberised woody tissue of major roots or the collar of the tree (Pegg *et al.* 1982). When injected into the xylem tissues of the trunk or major limbs, the phosphonate anion is ultimately translocated to the roots limiting colonisation by the pathogen (Schutte *et al.* 1988; Guest and Grant 1991). The development of trunk injection of phosphonates during the 1980s, was an unconventional application technique which has subsequently been shown to control phytophthora root rot in avocados (Darvas *et al.* 1984; Pegg *et al.* 1985). Initially, research with trunk-injected phosphonates for the control of avocado root rot, focused on curing diseased trees (Darvas *et al.* 1984; Pegg *et al.* 1985, 1987, 1990). It was demonstrated that trees rating 9 on the health scale of 0, healthy to 10, dead (Darvas *et al.* 1984) could be restored to full health within two years by a trunk injection program with phosphonate fungicides (Pegg *et al.* 1987). However, within a few years of the commercial development of trunk injection, the focus on tree health shifted from curative to preventative management procedures, which required more strategic and efficient use of the technology.

The symplastic distribution within plants of ambimobile herbicides such as 2, 4-D and glyphosate, and the nematicide oxamyl, has been shown to be source/sink related, with the respective compounds accumulating in organs with greatest sink strength at the time of application (Leonard and Crafts 1956; Crafts and Yamaguchi 1958; Tyree *et al.* 1979; Dewey and Appleby 1983). It is likely that the ambimobile phosphonate exhibits similar behaviour following entry into the symplast.

In studies with young, container-grown avocado trees, Whiley and Schaffer (1993) reported that shoot and root sink activity were temporally separated; leaves were the strongest sinks for ^{14}C-photosynthates during early shoot growth, and the sink strength of roots increased once shoots became quiescent (see also Chapter 5). The asynchronous pattern of shoot and root growth in avocado is illustrated in phenology models developed for avocado (Whiley *et al.* 1988a) which have since been confirmed by the observations of Ploetz *et al.* (1992) and Whiley and Schaffer (1994). The purpose of the research described in this Chapter was to relate the translocation of H_3PO_3 to roots, following trunk injection with a formulation containing mono- and di-potassium phosphonate (potassium phosphonate), to sink-strength dynamics within the tree at the time of application.

7. 1. 2 Materials and Methods

Trees selected for the experiment were 12-year-old, healthy 'Hass', approximately 12 m in canopy diameter, grafted to 'Velvick' Guatemalan seedling rootstock and growing in a site where *Phytophthora cinnamomi* was not present so that phosphonate fungicides had not been previously used. The trees were growing in a commercial orchard at Maleny in coastal S.E. Queensland (latitude 27° S, 650 m altitude), which has a cool, high rainfall subtropical climate.

Tree phenology was monitored by collecting fruiting shoots from each of the 9 experimental trees immediately prior to treatment, then subsequently at ≈ 30 day intervals following the first injection through to fruit maturity and harvest 296 days later. Three current seasons fruiting shoots were collected from each tree and oven dry mass of the stems, leaves and fruit were determined separately after 72 hrs at 90°C in a forced-draught oven. Trees were trunk-injected with potassium phosphonate at three different phases of tree phenology during spring and summer, thereby spanning the fluctuating relationships between shoot and root growth (Fig. 38). During each phenology phase, three trees were injected with the fungicide.

The first group of trees was trunk-injected with a 20% solution of potassium phosphonate towards the end of anthesis just as spring shoot growth commenced (4 October) (Fig. 38). Injections were carried out using Chemjet$^{(R)}$ tree injectors (Chemjet Trading Pty. Ltd., Caboolture, Australia) at

the rate of 15 mL m^{-1} of canopy diameter (Pegg *et al.* 1987). Each injection site was prepared by drilling 6 mm diameter holes into the trunk, penetrating the xylem tissue to a depth of 40 mm, thereby giving the injected H_3PO_3 direct access to the xylem tissue and the transpiration stream. Each injector was filled with 20 mL of the fungicide and injection sites were equally spaced around the circumference of the trunk; ca. nine injection sites per tree. Fresh samples of organs were collected from these trees before treatment and at intervals following injection, and analysed for H_3PO_3 content. Four sub-samples of bark and wood tissues (from tree trunks) were collected at each sampling time and care was taken to ensure that they were not selected from positions in close proximity to injection sites. Sub-samples of unsuberised roots were collected from each quadrant of the tree and 20 sub-samples of leaves, stems and shoots were taken from positions representative of the entire canopy at each sampling time. Sub-samples were bulked for H_3PO_3 analysis. For each tree, the amount of H_3PO_3 lost via fruit harvest and organ senescence was estimated over an eight month period. The senescence of flowers, leaves, twigs and fruit was monitored by placing 9 l containers in each quadrant of the canopy to collect a representative sample of material shed during the experiment. The containers were emptied at 14-day intervals, the material sorted into the various organs, dry mass determined and each component analysed for H_3PO_3 content. Fruit yield was recorded at harvest and H_3PO_3 content determined from samples from each of the three trees.

A second group of trees was trunk-injected when the spring shoots had completed extension growth and leaves were fully expanded (9 December), and a third group of trees was injected once the summer vegetative growth had matured (3 May) (Fig. 38). Following injection, fresh unsuberised root and leaf samples were collected at intervals from these two groups of trees and the H_3PO_3 content determined separately for each organ. The trunk injection treatments were repeated at the beginning of spring shoot growth and at the end of spring shoot maturity the following season and leaf and root samples collected and analysed over 96 days (data not presented).

To test lateral distribution of H_3PO_3, another group of three trees, each with trunks which formed two main vertical branches within 1 m of soil level was selected. At the beginning of spring shoot growth, only one of the main branches on each tree was injected with a 20% solution of

potassium phosphonate at a rate calculated to treat the whole tree based on 15 mL m^{-1} diameter of canopy. Leaf and root samples were collected at intervals from the treated and untreated sides of the tree and the H_3PO_3 concentration was determined in each organ.

Phosphonic acid analysis by GC

Concentrations of H_3PO_3 were measured in avocado tissues using an acid extraction and gas chromatography. Samples were extracted with dilute aqueous sulphuric acid and derivatised using diazomethane to form the dimethyl ester. This extract was injected into a gas chromatograph equipped with a glass chromatographic column (Carbowax 20M) and a flame photometric detector. Dimethyl phosphonate was detected as a peak on the chromatogram and the concentration determined by comparison with a known standard. Residue concentrations of H_3PO_3 were calculated and expressed as $\mu g\ g_{fw}^{-1}$ for each sample. This method allowed a rapid and quantitative analysis of phosphonic acid in a variety of tissues (leaf, root, fruit [including seed, skin and flesh], bark and wood) with low detection limits ($\leq 0.1\ \mu g\ g_{fw}^{-1}$) and recoveries of $\geq 80\%$ ([‡] P.A. Hargreaves, unpublished data).

Data analysis

The H_3PO_3 content in leaves was monitored for each injection time and the concentration flux fitted to the non-linear regression model derived by Wood (1967) where $y = ax^b e^{-cx}$. Linear and non-linear regression analyses were used to relate the concentration flux of H_3PO_3 in roots to the time elapsed after trunk injection.

[‡] P.A. Hargreaves, Agricultural Chemistry, Queensland Department of Primary Industries, Meiers Road, Indooroopilly 4068, Australia.

7. 1. 3 Results and Discussion

Distribution and loss of phosphonic acid from the tree

Prior to trunk injection, low levels of H_3PO_3 (< 3.0 μg g_{fw}^{-1}) were detected in all parts of the tree (Fig. 35), although there was no previous history of the use of phosphonate fungicides on the experimental trees or in the orchard. It is unlikely that these pre-treatment concentrations of H_3PO_3 were derived from natural sources (Hilderbrand 1983). Weeds were regularly controlled in the orchard by using glyphosate [N-(phosphono-methyl) glycine], an ambimobile phosphonate based herbicide (Dewey and Appleby 1983). Glyphosate is completely degraded to CO_2 by microorganisms in the soil with the main intermediary metabolite being aminomethylphosphonic acid (Carlisle and Trevors 1988; Pipke and Amrhein 1988). It is likely that H_3PO_3 is a metabolite from the degradation of aminomethylphosphonic acid, in which case it may have been taken up by the tree thereby accounting for its presence in tissues before treatments were applied.

Following trunk injection at the beginning of spring shoot growth, H_3PO_3 concentrations increased in all tissues. Within two days of treatment, substantial increases in shoot and leaf concentrations were measured: 2.2 ± 0.5 to 77.4 ± 6.9 and 1.1 ± 0.3 to 52.1 ± 7.20 μg g_{fw}^{-1}, respectively. The highest H_3PO_3 concentrations were measured in the spring shoots (stem and leaves) which were actively growing at the time of trunk injection (Figs. 35 & 38). The H_3PO_3 concentration in these tissues peaked eight days after injection and then rapidly declined in leaves (Figs. 35a & 35b). However, in the stems of spring shoots there was a high H_3PO_3 level until 96 days after injection when concentration in those tissues fell rapidly. This coincided with the beginning of summer shoot growth and the development of a new leaf sink (Whiley and Schaffer 1993).

Phosphonic acid levels in mature, over-wintered leaves peaked within eight days of treatment after which there was a rapid decline in concentration (Fig. 35b). The difference in maximum H_3PO_3 concentration between spring and over-wintered leaves was probably due to different sink strengths at the time of injection. Whiley and Schaffer (1993) showed that following exposure of a mature leaf to $^{14}CO_2$, 27% of the ^{14}C-photosynthate was recovered from actively growing leaves

at the terminal of the shoot while only 2.5% was found in mature leaves adjacent to the treated leaf. These mature leaves are a strong source of photoassimilates during spring shoot growth (Whiley 1990) and probably account for the more rapid loss of H_3PO_3 compared with spring leaves which remain strong sinks during development.

Ninety-six days after injection, sufficient summer shoot growth was present to allow sample collection. The concentration of H_3PO_3 in the stems of these new shoots was initially lower than in the stems of spring shoots at the same sampling time, but thereafter was similar despite temporal separation of their development in relation to the time of the trunk injection (Fig. 35a). During early growth, leaves on summer shoots had higher concentrations of H_3PO_3 than the adjacent spring leaves but by 195 days after trunk injection there was no significant difference between spring and summer leaves (Fig. 35b).

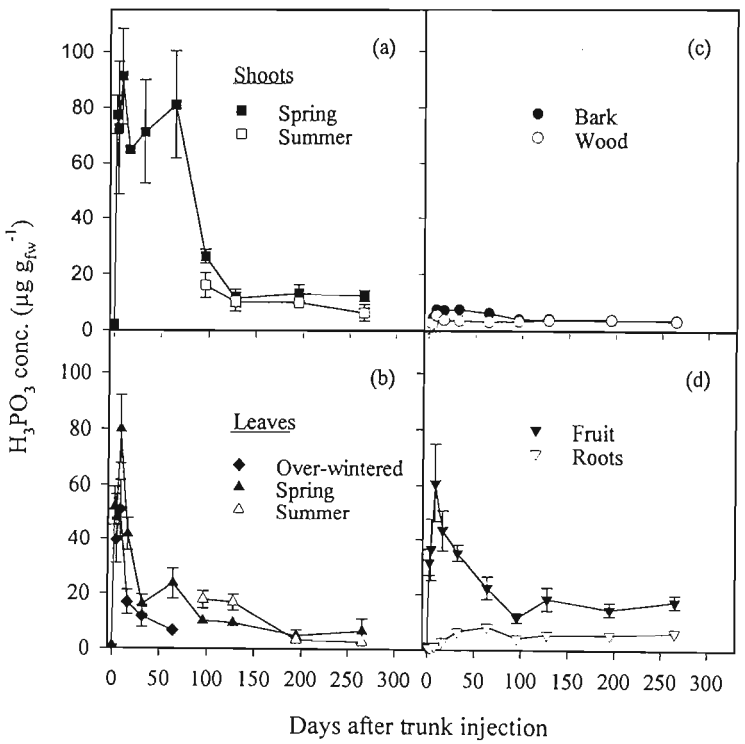

Fig. 35 Concentration flux of phosphonic acid (H_3PO_3) in avocado (a) shoots; (b) leaves; (c) trunk bark and wood; and (d) fruit and roots following trunk injection with 20% solution of di-potassium phosphonate at the beginning of spring shoot growth. Data points are mean values from three trees ± vertical SE bars which are obscured by symbols at some points.

Concentrations of H_3PO_3 in the bark and wood of trunks were low compared to other organs of the tree (Fig. 35c). In the bark an increase in H_3PO_3 concentration (from 1.3 ± 0.2 to 4.7 ± 0.7 μg g_{fw}^{-1}) was measured four days after trunk injection and peaked after 32 days (7.6 ± 1.2 μg g_{fw}^{-1}) while in the wood the maximum concentration (5.4 ± 0.5 μg g_{fw}^{-1}) was reached eight days after treatment.

Concentrations of H_3PO_3 in young fruit increased sharply following trunk injection, reaching 60.8 ± 14.0 μg g_{fw}^{-1} eight days after treatment. Thereafter, concentrations declined until stabilising (at ≈ 17 μg g_{fw}^{-1}) 64 days after injection (Fig. 35d). The considerable H_3PO_3 concentration in fruit at an early stage of their ontogeny was in contrast to < 1 μg g_{fw}^{-1} H_3PO_3 detected following trunk injection when fruit were mature (K.G. Pegg and A.W. Whiley, unpublished data). At the time of harvest, fruit (in this study) had maintained the highest H_3PO_3 concentration compared with other tissues, viz. 17.6 ± 2.4 μg g_{fw}^{-1} for fruit compared with 12.3 ± 2.0 μg g_{fw}^{-1} for stems of spring shoots. This was probably due to the comparatively strong sink status of the fruit throughout ontogeny (Cannell 1985), but is well below the maximum residue level of 100 μg g_{fw}^{-1} set for avocado fruit in Australia.

The accumulation of H_3PO_3 in roots was slower than in spring shoots and fruit, with no detectable increase until 16 days after treatment: from 1.4 ± 0.4 to 3.1 ± 0.5 μg g_{fw}^{-1}. The highest root concentration of H_3PO_3 was only 8.4 ± 1.9 μg g_{fw}^{-1}, measured 64 days after injection (Fig. 35d). Following this peak there was a slight decline in root concentration which remained relatively constant for the balance of the monitoring period. The pattern of both leaf and root accumulation of H_3PO_3 immediately following treatment was similar to that reported by Schutte *et al.* (1988). However, in their study, following a gradual increase in H_3PO_3 concentration in roots from 2 to 20 μg g_{fw}^{-1} during the first 35 days after injection, there was a 300% increase in H_3PO_3 between 35 and 42 days after treatment followed by a sharp decline in concentration.

At the time of treatment, 23 g of H_3PO_3 were injected into the trunks of each of the three trees. The residual H_3PO_3 concentration in senesced tree organs was highest in the inflorescence and fruitlets (50 to 80 μg g_{fw}^{-1}) with much lower concentrations in leaves (mature over-wintered) and twigs (10 to 20 μg g_{fw}^{-1}), thereby providing further evidence of the effect of sink strength on

distribution (Cannell 1985). It was estimated that 6.85 ± 0.98 g (≈ 30%) of H_3PO_3 were lost from each tree from the time of injection until fruit harvest (296 days later). Approximately 3.51 ± 0.28 g (≈ 15%) of the total amount lost was attributed to loss through the litter cycle while 3.34 ± 0.57 g (≈ 15%) was removed in harvested fruit. These estimates did not take into account other losses through root senescence and leakage (Ouimette and Coffey 1990) or possible oxidation in plant tissues to PO_4^{-} by bacteria as suggested by Bezuidenhout *et al.* (1987). The other significant factor responsible for declining tissue concentrations of H_3PO_3 was dilution by growth and its impact will largely depend on tree vigour.

Ouimette and Coffey (1990) concluded that symplastic entry of phosphonate across the plasmalemma occurs by active transport (Epstein 1973) which is dependent on metabolic energy. Several researchers using [^{14}C]sucrose as a standard for phloem-transported material, have demonstrated that translocation profiles of sucrose and phosphonate are almost identical (Martin and Edgington 1981; Dewey and Appleby 1983; Chamberlain *et al.* 1984). Following injection directly into the transpiration stream, the pattern of distribution of H_3PO_3 within avocado trees in this study provides further evidence of the ambimobility of phosphonate. Increased concentrations of H_3PO_3 were measured in stems and leaves of shoots and in fruit two days, and

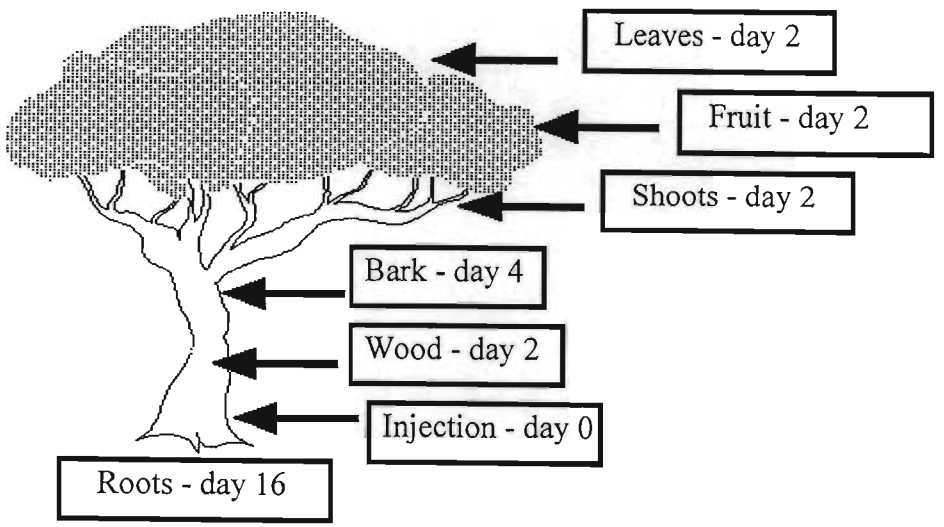

Fig. 36 Days after pre-spring shoot growth injection when significant increases in phosphonic acid concentration were measured in the different organs of the tree.

in bark of trunks, four days after treatment; however, there was no increase in root concentration until 16 days after trunk injection (Fig. 36). This time sequence suggests an apoplastic translocation pattern via the xylem to the leaves following trunk injection, whereafter symplastic entry into phloem resulted in basipetal movement to the roots.

Lateral distribution of phosphonic acid in the tree

This investigation showed that H_3PO_3 moved rapidly in an acropetal and basipetal direction, but had much less effective lateral translocation. Leaves and roots on the treated side of the tree showed a substantial increase in H_3PO_3 concentration within 8 (leaves) to 32 (roots) days after trunk injection (Fig. 37). However, the increase in concentration of H_3PO_3 in leaves and roots on the untreated side of the tree occurred more slowly, and only reached 2 and 35% of the peak concentrations of leaves and roots from the treated side of the tree, respectively. In contrast, studies with translocation of phosphonate fungicides in cocoa (*Theobroma cacao* L.) have shown that trunk injection into one site in the tree is sufficient to disseminate adequate levels of H_3PO_3 throughout the tree, thereby providing protection from pod rot (*Phytophthora palmivora* Butler) (Guest *et al.* 1994). This may be due to the less complex structure of cacao plants which have a central trunk (chupon) producing lateral plagiotropic branches at given intervals (the jorquette) (Purseglove 1968). In studies with trunk-injected phosphonate in monocotyledons, Darakis *et al.* (1985) found that there was an excellent distribution throughout the plant from a single injection site. This is likely due to the presence of many short xylem vessels with numerous cross-connections which facilitate both vertical and lateral translocation within these plants.

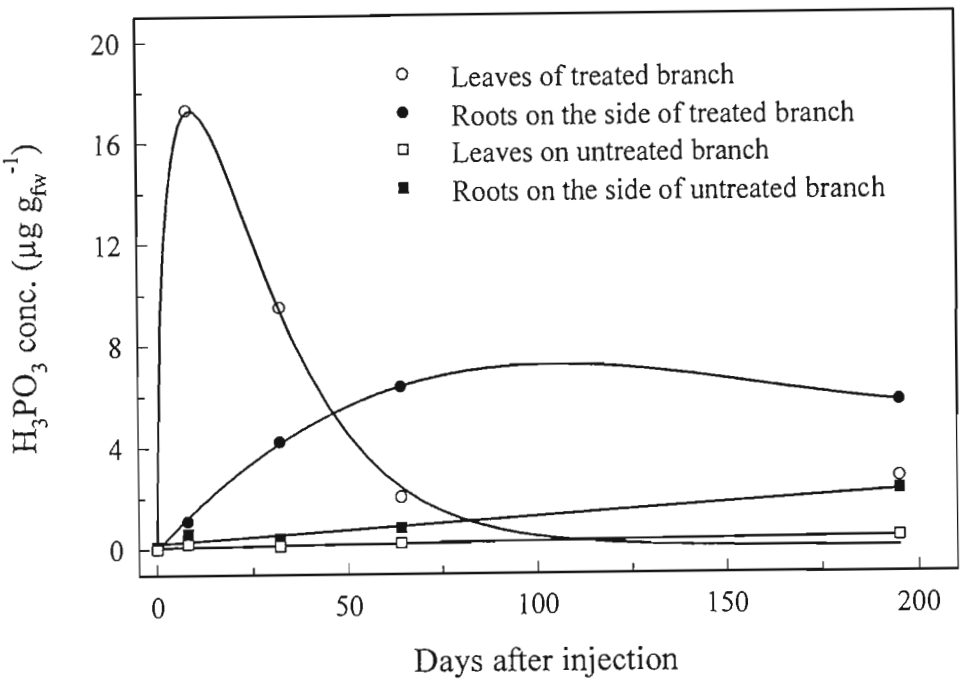

Fig. 37 Concentration flux of phosphonic acid (H_3PO_3) in avocado leaves and roots following trunk injection into one side of a dual-branched tree. The model for leaves on the treated branch is represented by $y = 9.7x^{0.48}\ e^{-0.053x}$, $r^2 = 0.88$ ($P < 0.17$); for leaves on the untreated branch by $y = 0.783 + 0.002x$, $r^2 = 0.88$ ($P < 0.05$); for roots on the side of the treated branch by $y = 0.0023 + 0.164x - 1.17e^{-4}x^2 + 2.44327e^{-6}x^3$, $r^2 = 0.99$ ($P < 0.05$) and for roots on the side of the untreated branch by $y = 0.22 + 0.01x$, $r = 0.97$ ($P < 0.05$). Data points are mean values of three trees.

Effect of sink strength and phosphonic acid root concentrations

The efficiency of translocation of H_3PO_3 to the roots appears directly related to the sink/source status of the leaves at the time of injection. In this study, shoot phenology measured by dry matter accumulation was similar to that previously reported for avocado (Whiley *et al.* 1988a; Ploetz *et al.* 1992; Whiley and Schaffer 1994). There were two major periods of shoot growth corresponding to spring and summer. Spring shoots grew vigorously for the first 32 days following bud-break, during which time they accumulated 66% of their final dry matter (Fig. 38). Thereafter, the growth rate declined with the maximum shoot dry matter attained 128 days after bud-break. Summer shoot growth began 96 days after spring shoot bud-break, at a time when

spring growth was relatively quiescent (Fig. 38). Dry matter accumulation in the summer shoots was not as rapid as in the spring shoots, taking ≈ 100 days to accumulate 66% of the dry matter and 190 days to maximum dry matter. There was a linear increase in fruit dry mass from fruit set to maturity, a period of ≈ 300 days (Fig. 38).

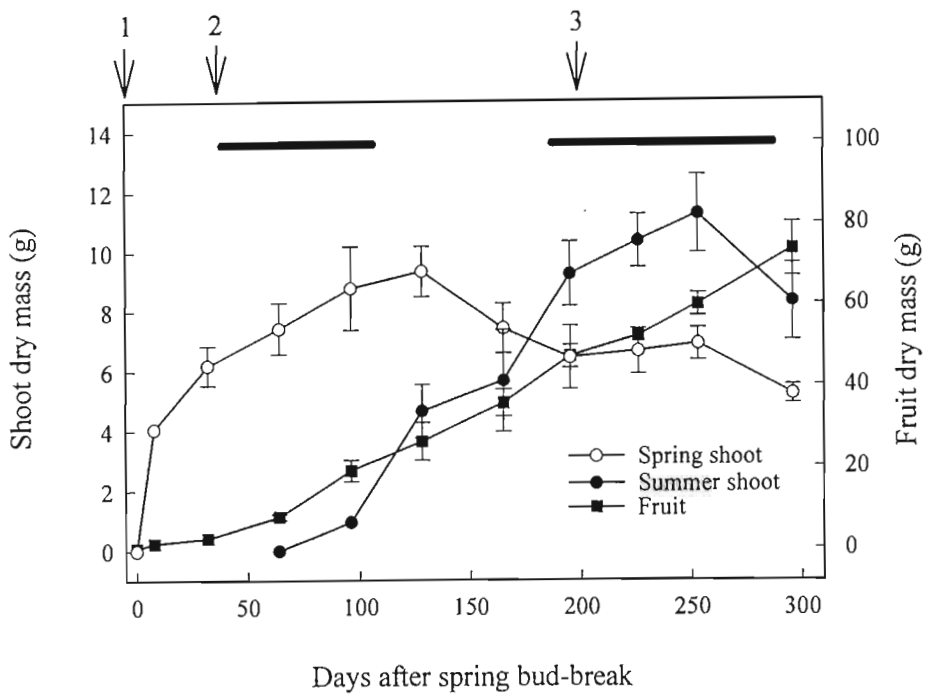

Fig. 38 Dry matter accumulation in spring and summer shoots and fruit, in cv. Hass trees during the period following trunk injection when phosphonic acid concentration fluxes in the tree were monitored. Trunk injection times are indicated by arrows where 1 = pre-spring shoot growth; 2 = spring shoot maturity; and 3 = summer shoot maturity. Horizontal bars indicate the major periods of root growth defined by Whiley *et al.* (1988a), Ploetz *et al.* (1992) and Whiley and Schaffer (1994). Data points are mean values from nine trees ± vertical SE bars which are obscured by symbols at some points.

While root growth data were not collected in this experiment, corroborating evidence from other studies (Whiley *et al.* 1988a; Ploetz *et al.* 1992; Whiley and Schaffer 1994) suggests that root growth (hence sink strength) was greatest when shoots were relatively quiescent, i.e. for a short

time ≈ 60 days following spring bud-break and for a longer period ≈ 200 days after the beginning of spring growth (Fig. 38). This is further substantiated by Whiley and Schaffer (1993) who reported that ^{14}C-photosynthate was largely retained in new, actively growing shoots (38% in shoots compared with 14.5% in roots) when trees were exposed to $^{14}CO_2$ shortly after new shoot growth had commenced. However, once all leaves on shoots were fully expanded, exposure to $^{14}CO_2$ resulted in a larger proportion of ^{14}C-photosynthate being translocated to the roots (32% in roots compared with 13% in the new shoots).

Concentration fluxes of H_3PO_3 in leaves and roots of trees trunk-injected at different stages of phenological development, mirrored the dynamics of temporal sink separation (Figs. 38 & 39). At each time following trunk injection of potassium phosphonate, there was a rapid increase in the leaf concentration of H_3PO_3 reaching between 50 and 70 $\mu g\ g_{fw}^{-1}$ within 8 to 12 days after treatment. The subsequent decline in leaf H_3PO_3 was also quite rapid, reflecting the exporting capacity of the leaves as H_3PO_3 crossed the symplast and became phloem-mobile. The decrease in leaf concentration was faster when trunk injection was given prior to spring growth (Fig. 39a) than at the other selected stages of phenology, and could be attributed to a combination of dilution by growth and translocation. However, prior to spring growth, the root sink was weak (Whiley and Schaffer 1994) which was reflected by the low concentration of H_3PO_3 that accumulated in the roots when trees were injected at that time. Conversely, leaf H_3PO_3 concentrations following treatment at summer shoot maturity, when vegetative and fruit sinks had weakened, took longer to decline (Fig. 39c). This was at a time when the root sink was strong, and resulted in the highest root concentration of H_3PO_3, which was sustained for a longer period than root concentrations resulting from injections at either the beginning or end of spring shoot growth (Fig. 39). Similar relationships with respect to leaf and root H_3PO_3 concentrations were obtained the following spring from different groups of trees treated in the same manner (data not presented).

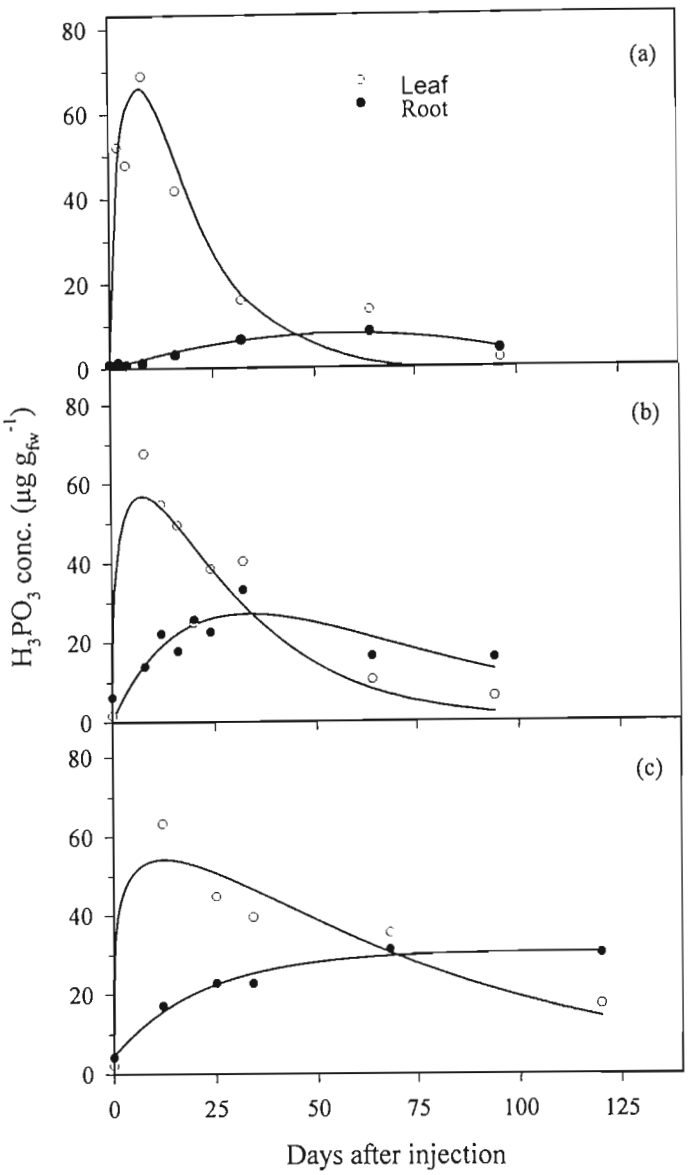

Fig. 39 Concentration flux of phosphonic acid (H_3PO_3) in avocado leaves and roots following trunk injection at (a) the beginning of spring shoot growth where the model for leaves is represented by $y = 36.0x^{0.649}e^{-0.093x}$, $r^2 = 0.69$ ($P < 0.05$); and for roots by $y = 2.94 + 0.24x - 0.0025x^2$, $r^2 = 0.87$ ($P < 0.01$); (b) maturity of the spring shoot growth where the model for leaves is represented by $y = 39.7x^{0.356}e^{-0.048x}$, $r^2 = 0.73$ ($P < 0.01$); and for roots by $y = 2.59x^{0.936}\ e^{-0.0281x}$, $r^2 = 0.58$ ($P < 0.05$); (c) during summer shoot growth where the model for the leaves is represented by $y = 39.7x^{0.205}e^{-0.0166x}$, $r^2 = 0.98$ ($P < 0.01$); and for roots $y = 30.7 - 26.02\ (0.955^x)$, $r^2 = 0.98$ ($P < 0.01$). Data points are mean values of three trees.

7. 1. 4 Conclusions

This study confirms previous reports that phosphonate is ambimobile in plants. Following trunk injection there is rapid acropetal movement in the xylem from the treatment site to the leaves. The dynamics of subsequent phloem translocation is determined by the strength of competing sinks when the H_3PO_3 enters the symplast. However, there was little redistribution of phosphonate to suggest lateral movement across the tree, demonstrating it to be slow and relatively inefficient compared with vertical movement. Phosphonate translocation to roots following trunk injection can thus increase three fold with correct timing. In subtropical Australia, disease pressure is greatest during the summer and autumn months when soil temperatures and moisture are optimum for growth and development of the pathogen, and when rapid fruit development imposes further stress on roots of heavily cropping trees. Strategically timed injections of phosphonate fungicides at either spring shoot growth maturity and/or during the mid to late summer months will protect the roots of the tree from colonisation by *P. cinnamomi* during this critical period. However, H_3PO_3 concentrations in plant tissues decrease over time due to several factors, and re-injection of phosphonate fungicides will be necessary to prevent phytophthora root rot. Further research is required to more closely define the optimum concentration of H_3PO_3 required for maximum root protection from fungal invasion, and to pre-empt a possible lowering of the MRL as a consequence of the "anti-pesticide" lobby.

7. 2 EFFECT OF SPRING APPLIED NITROGEN AND PACLOBUTRAZOL ON 'HASS' YIELD AND FRUIT SIZE

7. 2. 1 Introduction

In the last 10 years 'Hass' has become the major cultivar of the Australia avocado industry and is grown in all production regions from the highland tropics of the Atherton Tableland (latitude 17°S) to the cool Mediterranean climate of south Western Australia (latitude 32°S). Environmentally the cultivar is most suited to a cool subtropical climate such as that found at Maleny in S.E. Queensland (latitude 26.5°S, altitude 520 m) where sustainable average yields are > 24 t ha^{-1} (Whiley and Winston 1987). Production of 'Hass' in the warm subtropics of Australia results in reduced yield and smaller fruit compared with the cooler mesic conditions of Maleny (Whiley and Winston 1987; Chapter 6). A similar phenomenon occurs in South Africa when yield and fruit size is compared between the cooler production areas of Natal and warmer areas of the Eastern and Northern Transvaal (B. N. Wolstenholme, pers. comm.‡).

Chemical manipulation at critical phenological stages has resulted in a commercially significant increase in 'Hass' fruit size. In a warm, subtropical climate in South Africa, preliminary results showed an increase in fruit size of cv. Hass after dipping small fruitlets in forchlorfenuron (Köhne 1991), a chemical with cytokinin-like activity in some crops which potentially increases cell division. Wolstenholme *et al.* (1990) and Whiley *et al.* (1991) reported increased fruit size and yield of 'Hass' avocado following mid-anthesis foliar sprays of the growth retardant paclobutrazol (PBZ) which they attributed to reduced competition in spring from vegetative sinks. Paclobutrazol at 2.5 g a. i. l^{-1} resulted in larger fruit by the time spring shoot growth had fully matured and this advantage was maintained through to harvest (Wolstenholme *et al.* 1990) provided that the spring vegetative flush was not too severely retarded.

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‡ Professor B. N. Wolstenholme, Horticultural Science, University of Natal, Private Bag X01, Scottsville, Pietermaritzburg 3209, South Africa

Blanke and Whiley (1995) report that cv. Hass fruit have comparatively higher respiration (R_d and R_l) rates than cv. Fuerte, which is not affected by lower yields and smaller fruit in warm subtropical climates (Whiley and Winston 1987). They suggest that the higher energy requirement of 'Hass' may be responsible for smaller fruit size when grown in warmer climates (respiration is a direct function of temperature). Increased assimilate supply to 'Hass' fruitlets during early ontogeny may improve fruiting performance of this cultivar as this is the most rapid stage of cell division (Valmayor 1967) and fruit size of 'Hass' is directly related to cell numbers (Moore-Gordon *et al.* 1995). During this period assimilates are available from storage (starch), and current photoassimilates from over-wintered leaves which have lost photosynthetic efficiency due to photoinhibition and depleted nitrogen content (Chapter 2). Net CO_2 assimilation (A) is directly related to leaf nitrogen concentration (Lugg and Sinclair 1981; DeJong 1982; Syvertsen 1984). However, nitrogen fertilisation of avocados in spring reduced yield due to stimulation of competitive vegetative sinks during a critical stage of fruit development (A.W. Whiley, unpublished data).

The objective of this experiment was to improve both yield and fruit size of 'Hass', by increasing A of the over-wintered "source" leaves of the canopy during the early stages of fruit ontogeny with pre-bloom soil applications of nitrogen, while concurrently and temporarily suppressing vigour of the indeterminate spring shoots with mid-anthesis foliar sprays of PBZ. Forchlorfenuron (CPPU) was also included to evaluate its potential to increase fruit size. Data collected over a two year period are presented. However, the experiment has not been completed and will be continued for at least another year. Data presented in this thesis cover the period from July 1992 until October 1994.

7. 2. 2 Materials and Methods

Eight-year-old cv. Hass trees grafted to Guatemalan race seedlings and growing in a commercial orchard at Childers, S.E. Queensland (latitude 25°S, altitude 40 m) with a warm, subtropical climate were selected in 1992 for this study. Temperature and rainfall data are summarised in Table 14.

Table 14 Temperature and rainfall data for Childers, S.E. Queensland. Data are mean values for 1992 and 1993 and were collected with an automatic weather station (Monitor Sensors, Caboolture, AUST.) sited in the orchard.

Months	Rainfall (mm)	Temperature °C	
		Min.	Max.
Jan	51.6	20.5	32.5
Feb	272.8	20.6	29.6
Mar	19.8	18.3	27.4
Apr	11.0	16.5	25.0
May	9.4	13.5	23.9
Jun	12.5	10.3	21.9
Jul	15.6	9.3	20.4
Aug	4.7	10.0	23.1
Sep	68.2	12.5	24.7
Oct	66.4	16.1	29.6
Nov	56.2	18.1	30.1
Dec	200.0	19.3	31.3

For the duration of the experiment, trees were injected with 20% di-potassium phosphonate as recommended (Pegg *et al.* 1985, Whiley *et al.* 1995) to limit infection by Phytophthora root rot. Rainfall was supplemented with irrigation (micro-sprinklers) and scheduled with tensiometers to ameliorate the development of water stress (Whiley *et al.* 1988a; Banks 1992). All trees were fertilised with nitrogen, potassium and boron following recommendations from leaf analyses which were carried out on mature summer-grown leaves collected in May each year (Table 15). Leaf analyses were provided by a commercial laboratory (INCITEC, Brisbane, AUST.).

Table 15 Base fertiliser schedule for cv. Hass trees during 1993 and 1994.

Application Time	Nitrogen[1] (g tree^{-1})	Potassium[2] (g tree^{-1})	Boron[3] (g tree^{-1})
Feb 93	384	320	
Apr 93	96	160	22
May 93	96		
Sep 93			11
Jan 94	240	250	
Apr 94	144	200	11

[1] Nitrogen was applied as Urea (48% N)
[2] Potassium was applied as Muriate of Potash (50% K)
[3] Boron was applied as Solubor (22% B)

There were seven experimental treatments (Table 16) which were replicated five times in a randomised block design. The additional nitrogen was soil-applied as urea within the dripline of trees when inflorescence growth had begun on ≥ 80% of shoot terminals but before anthesis had commenced; 04/08/92 and 05/08/93, respectively. Immediately following application of urea, trees were irrigated for 5 hrs to move the fertiliser into the soil to reduce volatilisation of the nitrogen.

Paclobutrazol (as Cultar$^{(R)}$, ICI Australian Operations (Pty) Ltd, Melbourne, AUS.) was formulated with a non-ionic surfactant (Agral$^{(R)}$ at 0.05%) and applied at mid-anthesis (14/9/92 and 16/9/93, respectively) with a Stihl SG-17 motorised knapsack sprayer. Trees were sprayed to the point of run-off using between 5 and 7 l tree^{-1} (Wolstenholme *et al.* 1990; Whiley *et al.* 1991). CPPU (supplied by INCITEC, Brisbane, AUST.) was also formulated with 0.05% Agral$^{(R)}$ and foliar applied with the aforementioned sprayer unit (21/10/92 and 24/10/93, 21/11/93) to the point of run-off.

Table 16 Experimental treatments used on cv. Hass trees in 1992, 1993 and 1994.

1992	Treatments
1	Control (commercial practice - Table 15)
2	480 g $tree^{-1}$ of N at inflorescence emergence
3	Foliar PBZ (2.5 g a. i. l^{-1}) at mid-anthesis
4	Foliar PBZ (1.25 g a. i. l^{-1}) at mid-anthesis
5	480 g $tree^{-1}$ of N at inflorescence emergence with foliar PBZ (2.5 g a. i. l^{-1}) at mid-anthesis
6	CPPU foliar spray (10 mg a. i. l^{-1}.) 3 weeks after fruit set
7	CPPU foliar spray (20 mg a. i. l^{-1}.) 3 weeks after fruit set
1993‡	
1 to 5	As above
6	480 g $tree^{-1}$ of N at inflorescence emergence with foliar PBZ (1.25 g a. i. l^{-1}) at mid-anthesis
7	CPPU foliar spray (10 mg a. i. l^{-1}.) 3 and 7 weeks after fruit set

‡ In 1994 the CPPU treatment was discontinued.

Immediately prior to the emergence of the inflorescence (pre-anthesis), samples of the most recently matured leaves were collected for nitrogen analysis, and net CO_2 assimilation of three mature leaves from three trees of treatments 1 to 3 and 5 were measured with either a LI-COR 6200 portable photosynthesis meter in 1992 (LI-COR, Nebraska, USA) or a CIRAS-1 portable photosynthesis meter in 1994 (PP Systems, Herts, UK). Leaves for nitrogen analyses were dried and analysed following the procedures detailed in Chapter 2. Nitrogen leaf analyses and measurements of *A* were repeated on similar leaves from treatments 1 to 3 and 5 on fruiting shoots two weeks after the completion of anthesis (post-anthesis) when young fruitlets were growing at a rapid rate. Trees were harvested on 26/07/93 and 08/06/94 and yield and fruit size recorded. As nitrogen and foliar PBZ have the potential to affect fruit quality by regulation of tree vigour (Faust 1989; Witney *et al.* 1990), fruit from treatments 1 to 3, 5 and 7 were evaluated

for post-harvest storage performance from the 1992/93 crop. Twenty fruit from each tree of the five treatments were packed into standard industry fibre-board cartons immediately after harvest and stored in a cool room at 7°C. After 10 days of storage fruit were examined each day to detect softening. Samples were judged as being "sprung" when 50% of the fruit in each carton had softened (judged by hand), and the number of days post-storage was recorded.

Data analysis

Statistical analysis of yield data was by ANOVA and fruit size data were first analysed by covariance to account for the effect of crop load on each of the trees. Mean values ± SEs are presented for other data.

7. 2. 3 Results and Discussion

Although chlorophyll fluorescence was not determined, mean minimum night temperatures during July were below 10°C (Table 14) and photoinhibition of PS II could be expected, reducing photosynthetic efficiency of over-wintered leaves during the late winter and early spring (Chapters 2 & 3). Due to restricted research funding and the servicing requirements of equipment, nitrogen content and *A* of leaves were only measured at pre- and post-anthesis stages of growth in 1992 and 1994. In these years there were no significant differences among treatments in pre-anthesis leaf N concentrations and *A*. Nitrogen concentrations were between 2.0 and 2.2% and *A* determined during the first week of August across all treatments was ca. 12 μmol CO_2 m^{-2} s^{-1} (Table 17). Post-anthesis measurements taken 9 to 10 weeks later showed a substantial reduction in leaf N concentrations and *A* across all treatments. However, there were differences among treatments with leaf N concentrations higher than control where additional nitrogen was applied pre-anthesis. There were also differences between the two nitrogen treatments, with higher leaf N concentration on trees sprayed at mid-anthesis with PBZ. Paclobutrazol also maintained higher post-anthesis leaf N concentrations compared to control trees suggesting that where PBZ was applied, suppression of spring shoot growth reduced translocation of nitrogen from over-wintered leaves to the new growth. There are numerous other

Table 17 Effect of pre-anthesis urea and mid-anthesis paclobutrazol (PBZ) on N concentration and CO_2 assimilation (A) of over-wintered summer-grown leaves of cv. Hass. Data for N concentrations are mean values of five trees for each treatment and for A three trees for each treatment ± SEs.

Treatments	Leaf N concentrations (%) and A (μmol CO_2 m^{-2} s^{-1})							
	Pre-anthesis 1992		Post-anthesis 1992		Pre-anthesis 1994		Post-anthesis 1994	
	N‡	A	N	A	N‡‡	A	N	A
1. Control	2.05 ± 0.06	12.7 ± 0.6	1.56 ± 0.05	7.8 ± 0.6	1.98 ± 0.02	11.8 ± 0.4	1.56 ± 0.06	7.3 ± 0.2
2. Pre-anthesis N (480 g $tree^{-1}$)	1.99 ± 0.06	12.3 ± 0.5	1.78 ± 0.05	8.3 ± 0.4	2.08 ± 0.05	12.2 ± 0.7	1.73 ± 0.04	7.7 ± 0.4
3. Mid-anthesis PBZ (2.5 g a. i. l^{-1})	2.06 ± 0.04	12.6 ± 0.6	1.85 ± 0.06	9.7 ± 0.5	2.12 ± 0.07	11.7 ± 0.8	1.72 ± 0.10	8.5 ± 0.4
5. Post-anthesis N (480 g $tree^{-1}$) + mid-anthesis PBZ (2.5 g a. i. $tree^{-1}$)	2.01 ± 0.05	12.4 ± 0.5	1.99 ± 0.08	10.8 ± 0.4	2.10 ± 0.03	12.8 ± 0.8	1.90 ± 0.05	10.9 ± 0.2

‡ May 1992 leaf nitrogen concentration of trees in this experimental block was 2.10%.

‡‡ May 1994 leaf nitrogen concentration of trees in this experimental block was 2.52%.

reports documenting increased leaf N concentrations in fruit crops following applications of PBZ (Atkinson and Crisp 1982; Raese and Burts 1983; Wang *et al.* 1985). Post-anthesis *A* closely followed leaf N concentrations and was significantly higher in those treatments where additional nitrogen and PBZ were applied to trees. Net CO_2 assimilation was also higher in trees where only PBZ was applied compared with *A* of leaves on control trees (Table 17).

In studies with soil-applied PBZ on pecan seedlings, Wood (1984) was unable to show significant differences in *A* of treated trees compared with controls, although there was a trend in the data which showed increasing *A* as the concentration of applied PBZ increased. The lack of a significant response may be due to the study being carried out with container-grown trees which is known to restrict *A* (Arp 1991; Thomas and Strain 1991). Wood (1984) reported A_{max} of 11.4 μmol CO_2 m^{-2} s^{-1} which is considerably lower than the A_{max} measured by Anderson and Brodbeck (1988b) for pecan (22 μmol CO_2 m^{-2} s^{-1}).

Due to the high coefficients of variation typical of orchard trees (27.4% and 45.5%) there were no significant yield differences among treatments in either the 1992/93 or 1993/94 crops (Tables 18 and 20). However, in 1992/93 trees receiving additional nitrogen and PBZ produced on average ca. 33% more fruit than control trees suggesting improved performance of these trees (Table 18). The trend in yield of the PBZ treatments and their relationship to control trees and to each other is in line with results described by Whiley *et al.* (1991). From three consecutive years of data they reported that 'Hass' yield was significantly increased by mid-anthesis foliar sprays of PBZ and that 1.25 g a. i. l^{-1} gave higher yields than 2.5 g a. i. l^{-1}. However, the latter increased fruit size in relation to the control trees. The higher concentration of CPPU (treatment 7) gave mild phytotoxic symptoms in new shoot growth with chlorotic areas developing in some of the leaves and though not significantly, average yield was lower than controls. Following a covariance analysis adjusting for yield, there were no significant differences in mean fruit size among treatments (Table 18).

Table 18 Effect of soil-applied late winter N, mid-anthesis foliar paclobutrazol (PBZ), and CPPU applications on yield and fruit size of cv. Hass at Childers in 1992/93. Data are mean values from five trees for each treatment and fruit size data have been subjected to covariance analysis adjusting for yield. Fruit size values in parenthesis are unadjusted means. There were no significant differences between values in columns as tested by ANOVA.

	Treatments	Yield (kg tree^{-1})	Fruit size(g)
1	Control	114.0	244.5 (242.5)
2	480 g tree^{-1} of N at inflorescence emergence	102.5	275.7 (265.0)
3	Foliar PBZ (2.5 g a. i. l^{-1}) at mid-anthesis	112.9	245.5 (242.4)
4	Foliar PBZ (1.25 g a. i. l^{-1}) at mid-anthesis	123.9	231.7 (236.6)
5	480 g tree^{-1} of N at inflorescence emergence with foliar PBZ (2.5 g a. i. l^{-1}) at mid-anthesis	151.6	216.6 (241.8)
6	CPPU foliar spray (10 mg a. i. l^{-1}) 3 weeks after fruit set	112.1	231.9 (228.2)
7	CPPU foliar spray (20 mg a. i. l^{-1}) 3 weeks after fruit set	103.1	273.7 (263.4)
Regression coefficient			0.730**

With exception of treatment 2, there was no treatment effect on fruit shape. Additional nitrogen increased the fruit length/diameter ratio resulting in "neckier" fruit when compared with other treatments (Table 19). As fruit from control trees had similar length/diameter ratios as those from PBZ treatments it would appear that the latter had no effect on fruit shape. These results are in contrast to earlier reports where foliar applications of PBZ at mid-anthesis reduced the fruit length/diameter ratio of cvs. Hass and Fuerte avocados, effectively producing rounder fruit (Wolstenholme *et al.* 1990). The only major difference between these experiments were the environmental conditions, the latter being carried out in cooler, higher rainfall areas at more southerly latitudes than Childers.

Table 19 Fruit shape (length/diameter ratio) and storage life at 7°C until 50% of the fruit had detectable softening. Data for fruit shape and number of storage days are treatment means ± SEs of 20 fruit from each of five trees.

	Treatments	Fruit shape (l/d ratio)	‡N°. of days stored at 7°C
1	Control	1.36 ± 0.02	33.6 ± 1.1
2	480 g tree^{-1} of N at inflorescence emergence	1.42 ± 0.03	31.8 ± 1.3
3	Foliar PBZ (2.5 g a. i. l^{-1}) at mid-anthesis	1.35 ± 0.01	34.0± 1.3
5	480 g tree^{-1} of N at inflorescence emergence with foliar PBZ (2.5 g a. i. l^{-1}) at mid-anthesis	1.36 ± 0.02	37.4 ± 1.1
7	CPPU foliar spray (20 mg a. i. l^{-1}) 3 weeks after fruit set	1.35 ± 0.02	34.5 ± 1.7

‡ The author acknowledges the assistance of Dr P. J. Hofman and Mr L. Smith, Queensland Department of Primary Industries in the collection of these data.

The application of additional nitrogen and PBZ to trees significantly increased the post-harvest storage life of fruit when held at 7°C compared with all other treatments. Although not statistically significant, there was a trend for fruit from trees receiving only additional nitrogen (treatment 2) to soften earlier than other treatments. Witney *et al.* (1990) reported that suppression of vigour in avocado trees increased fruit calcium concentrations thereby improving the storage potential of fruit. In their studies an increase in fruit Ca concentration of 1000 mg kg^{-1} extended shelf life by six days. Whiley (unpublished data) found that mid-anthesis foliar applications of PBZ (1.25 g a. i. l^{-1}) to 'Hass' increased Ca concentrations of young fruitlets by up to ca. 20% during the first 12 weeks of ontogeny, a critical period for the development of cell wall and membrane integrity (Bower 1985; Cutting and Bower 1989). Cutting and Bower (1990) also report increased Ca concentrations in 'Hass' fruit after trunk injection of PBZ at mid-anthesis; a treatment which also reduced spring shoot vigour.

Unavoidable circumstances relating to grading and packing fruit from the experiment delayed the harvest in 1993 which occurred some time after floral induction and development had begun.

Treatment 5 trees were carrying very large crops and flowering was greatly reduced in this treatment which was reflected in the 1994 yield (Table 20). Post-harvest evaluations were not carried out in 1994 because some trees had too few fruit at maturity.

Table 20 Effect of soil-applied late winter N, mid-anthesis foliar paclobutrazol (PBZ), and CPPU applications on yield and fruit size of cv. Hass in 1993/94. Data are mean values from five trees for each treatment and fruit size data have been subjected to covariance analysis adjusting for yield. Fruit size values in parenthesis are unadjusted means. There were no significant differences between values in columns as tested by ANOVA.

	Treatments	Yield (kg tree^{-1})	Fruit size(g)
1	Control	108.2	194.9 (195.9)
2	480 g tree^{-1} of N at inflorescence emergence	93.2	200.7 (205.5)
3	Foliar PBZ (2.5 g a. i. l^{-1}) at mid-anthesis	131.0	216.2 (211.8)
4	Foliar PBZ (1.25 g a. i. l^{-1}) at mid-anthesis	144.0	206.4 (198.8)
5	480 g tree^{-1} of N at inflorescence emergence with foliar PBZ (2.5 g a. i. l^{-1}) at mid-anthesis	94.7	211.8 (216.2)
6	480 g tree^{-1} of N at inflorescence emergence with foliar PBZ (1.25 g a. i. l^{-1}) at mid-anthesis	111.0	215.6 (214.0)
7	CPPU foliar spray (10 mg a. i. l^{-1}) 3. and 7 weeks after fruit set	105.2	186.2 (187.7)
Regression coefficient			-0.243**

The 1993/94 yield data suggested a trend for mid-anthesis PBZ at 2.5 and 1.25 g a. i. l^{-1} to carry heavier crops than other treatments but differences were not significant. Once covariance adjustment had been made for crop load there were no significant differences among treatments in mean fruit size (Table 20).

With respect to the cumulative yield for the two years of the study there were significant differences among treatments (Table 21). Mid-anthesis PBZ at 1.25 g a. i. l^{-1} produced the most fruit on average, although not significantly more than PBZ at 2.5 g a. i. l^{-1} or nitrogen plus PBZ at 2.5 g a. i. l^{-1}. The cumulative yields of each of these three treatments were greater than those of the controls and trees where additional nitrogen alone was given. There were no significant differences in mean fruit size among treatments.

Table 21 Effect of soil-applied late winter N, mid-anthesis foliar paclobutrazol (PBZ), and CPPU applications on cumulative yield and mean fruit size of cv. Hass for 1992/93 and 1993/94. Data are mean values from five trees for each of the treatments. Values not having a common letter are significantly different ($P \leq 0.05$) as tested by ANOVA.

	Treatments	Yield (kg $tree^{-1}$)	Fruit size(g)
1	Control	194.7c	219.7a
2	480 g $tree^{-1}$ of N at inflorescence emergence	189.3c	238.2a
3	Foliar PBZ (2.5 g a. i. l^{-1}) at mid-anthesis	243.9ab	230.8a
4	Foliar PBZ (1.25 g a. i. l^{-1}) at mid-anthesis	267.9a	219.1a
5	480 g $tree^{-1}$ of N at inflorescence emergence with foliar PBZ (2.5 g a. i. l^{-1}) at mid-anthesis	246.3ab	214.2a
6	480 g $tree^{-1}$ of N at inflorescence emergence with foliar PBZ (1.25 g a. i. l^{-1}) at mid-anthesis‡	223.1bc	233.8a
7	CPPU foliar spray (10 mg a. i. l^{-1}.) 3. and 7 weeks after fruit set	208.2bc	229.9a

‡ In the first year of the study these trees received CPPU at 10 mg a. i. l^{-1}.

There was no significant effect of CPPU on fruit yield or mean fruit size (Table 21). This is in contrast to the earlier report of Köhne (1991) where mean fruit size of 'Hass' was significantly increased after dipping fruitlets in a 10 mg a. i. l^{-1} a. i. solution when about 3 to 5 mm in diameter. However, a more recent study where trees were sprayed with CPPU shortly after fruit set reported

a significant decrease in yield and no increase in fruit size from the treatment (Köhne *et al.* 1993). Wolstenholme (pers. comm.[‡‡‡]) was also unable to increase 'Hass' fruit size with forchlorfenuron sprays in an environment in Natal which is conducive to relatively large fruit size.

7. 2. 4 Conclusions

These preliminary results indicate that higher yield (averaged over two seasons) and improved storage life can be achieved for 'Hass' growing in warm, subtropical climates. Nitrogen applied before flowering in combination with mid-anthesis foliar sprays of PBZ gave increased net photosynthesis, yield and storage life, while PBZ alone also increased fruit yield compared with trees receiving standard commercial recommendations. There was no improvement in fruit size from any of the chemical treatments. However, despite higher yields these treatments were able to maintain fruit size in relation to lower producing treatments. The pre-anthesis nitrogen treatment plus mid-anthesis PBZ in 1993 produced extremely heavy crops which led to failure of flowering the following season. The latter may be due to insufficient root growth during the late summer and early winter at the time of floral induction as discussed in earlier chapters. These trees have flowered and set heavily again in 1994 and large crops are expected in 1995. The combination of pre-flowering nitrogen and mid-anthesis PBZ on fruit set and retention is probably causing over-cropping in years when trees flower normally. While no direct evidence has been produced from this study, it is likely that the increased A at a critical stage of development together with restricted shoot growth has improved the opportunity for fruit retention. Further research is required to see if reliable management strategies can be developed to effectively utilise the concept being tested. It is clear that a high level of management expertise would be necessary to reliably assess tree condition and the likely response to treatment. Finally, in view of the practical impossibility of applying CPPU exclusively to young fruits, further research with this growth regulator would appear to be contra-indicated

[‡‡‡] Professor B. N. Wolstenholme, Horticultural Science, University of Natal, Private Bag X01, Scottsville, Pietermaritzburg 3209, South Africa

GENERAL DISCUSSION AND CONCLUSIONS

DISCUSSION

Internationally, avocados are grown across a wide range of environments (Whiley and Schaffer 1994), however for the most part research reported in this thesis was carried out in humid subtropical regions. Although the attributes of phenological modelling assist in the translation and interpretation of growth responses to changing environments, caution is necessary when translating specific principles across diverse climatic conditions. While it is thought that some results from this research will be of benefit to avocado production in semi-arid cooler climates, its greatest impact will be felt in subtropical regions.

Low average yields are a feature of avocado production in most countries which grow this tree as a commercial orchard crop (Monselise and Goldschmidt 1982; Wolstenholme 1987). There is little doubt that unfavourable climatic conditions in some situations (southern Australia, California, Chile, Israel) are largely responsible for continued poor performance which leads to economically unsustainable production of avocado, e.g. California (L. Francis, pers. comm.[‡]) and Israel (Dr O. Reuveni, pers. comm.[‡‡]). Strong alternate bearing cycles precipitated by either climatic stress or poor management (Monselise and Goldschmidt 1982) introduce economic uncertainty into avocado production which impacts on the confidence of producers and their ability to reliably service markets. The present rate of planting of new production areas in Australia will inevitably increase pressure on domestic markets where most of the fruit is currently sold. For sustainable economic growth new markets, both processing and export, must be developed. Reliable and more cost-effective production is needed to achieve this goal which may be realised through improved production technology and its implementation in the orchard.

[‡] L. Francis, Research Coordinator, California Avocado Society, California, USA.
[‡‡] Dr O. Reuveni, Volcani Institute, Bet Dagan, Israel.

Phenology and Rootstocks

Since the late 1970s the author has been developing phenology models for avocados which have become increasingly sophisticated with time. These models have been used to gain a greater understanding of the synchrony of growth, resource competition within the tree and genotypic responses to diverse environments (Whiley and Winston 1987; Whiley *et al.* 1988a). Results presented in this thesis have extended this holistic approach with the development of a pheno/physiological model which integrates related growth events and physiological changes in trees. As a research approach, modelling growth and key physiological changes assists with identification of factors limiting growth and production, spatial and temporal relationships of sinks and sources (vegetative:reproductive competition), and critical pheno/physiological events which may have a large impact on yield and fruit quality.

The seasonal flux of new root growth and related starch concentrations in roots and scions are the first long-term studies reported for avocado trees. The extension of root growth through winter, at least under the mesic high rainfall conditions at Maleny, has not been previously identified and has implications for long-term tree health and performance. The substantial loss of "feeder" roots from the onset of flowering through to maturation of the spring flush which this study has highlighted, occurs during the critical period of fruit set and early ontogeny. The related decline in starch content suggests that the flower sink either diverts assimilates or remobilises carbon products from roots, which in either case leads to assimilate starvation of this organ. Loss of root surface at a time of competitive reproductive:vegetative growth (Biran 1979; Wolstenholme 1990) potentially reduces the uptake of water and minerals and may limit cytokinin production. This is also a time of the year when Phytophthora root rot stress is greatest as roots may have been damaged in late summer (Pegg *et al.* 1982) thereby reducing the potential volume approaching flowering. The objective of the fertiliser program recommended by Whiley *et al.* (1988a) was to optimise the requirements of the tree during summer and autumn so that recyclable nutrients are available to support spring growth from endogenous sources (e.g. leaves and stems), at a time of reduced root volume. Subsequent studies reported in this thesis have validated and reinforced the concepts on which the recommendations were based.

Differences between the two rootstock/scion combinations were pronounced with cropping efficiency comparatively increased for the cloned 'Velvick'/'Hass' which also carried a higher percentage of fruit on determinate inflorescences. The seedling 'Velvick'/'Hass' trees produced more roots which accumulated higher concentrations of starch than the cloned 'Velvick'/'Hass' trees, which accumulated more in the scion. Rootstock manipulation of growth and cropping is highly developed in apples (Lockard and Scheider 1981). Dwarfing of avocado trees (cv. Fuerte) has been reported by Barrientos Priego *et al.* (1987) who found that using Colin V-33 as an interstock reduced growth by ca. 30%, although no yield data were supplied to indicate tree performance. Results with the 'Velvick' rootstocks give a clear indication that genetic manipulation of tree performance with avocado can be achieved and is worthy of further investigation in the future. Both rootstock and scion require attention, and in view of the paucity of horticultural research on avocado rootstocks, it is likely that investment in this area will be more rewarding in the short and medium term.

Reproductive:vegetative competition

Reproductive:vegetative competition during spring shoot development has been previously reported (Biran 1979; Blumenfeld *et al.* 1983; Köhne and Kremer-Köhne 1987; Adato 1990; Cutting and Bower 1990; Wolstenholme *et al.* 1990). Shoot tipping and chemical manipulation have both increased fruit retention on spring shoots. Results from studies reported in this thesis have contributed to knowledge on photoassimilate supply, presumably one of the limiting factors, during early fruit and shoot ontogeny. Increased sample size in some studies would improve confidence in the results reported, however there were limitations with respect to resources (labour) available to assist with the detailed field aspects of the research. Despite these limitations some assurance can be taken from the repeatability of the studies and support given by previous research (Biran 1979; Blumenfeld *et al.* 1983; Köhne and Kremer-Köhne 1987; Adato 1990; Cutting and Bower 1990; Wolstenholme *et al.* 1990).

The dynamics of carbon dioxide efflux of fruit during ontogeny showed that a small contribution is made from fruit photosynthesis towards the fruits own carbon requirements for growth. On a g_{dw}^{-1} basis fruit photosynthesis was highest during early ontogeny and

progressively declined as fruit mass increased. While carbon fixation from fruit photosynthesis is negligible in terms of total fruit requirement for growth it may be a significant factor during the first few weeks of ontogeny - a period of strong competition for assimilate resources.

The importance of retention of over-wintered (previous spring and summer flush) leaves during anthesis through to sink:source transition of the renewal spring shoots was clearly demonstrated with 'Hass' trees and is probably due to low starch reserves in heavily cropping trees. Pre-anthesis starch concentrations determined in trees growing in the subtropics were much less (ca. 8%) than reported in similar tissues by Scholefield *et al.* (1985) (ca. 18%) where avocados were growing in a cool, semi-arid Mediterranean climate. These differences are likely due to successive heavy crops on trees in the subtropics which reduce investment into carbohydrate reserves (Whiley *et al.* 1992) and the longer period of quiescence and the smaller crops carried on trees growing in cooler interior climates which promote the accumulation of starch (Scholefield *et al.* 1985; Whiley and Winston 1987; Whiley *et al.* 1988a). In cool, semi-arid Mediterranean climates there is significant leaf loss during anthesis (Scholefield *et al.* 1985; Bergh 1986; Sampson 1986) and it is likely that the remaining over-wintered leaves have irreversible physiological damage due to prolonged exposure to mean minimum temperatures of $\leq 4^{\circ}C$. In southern California, starch accumulation in avocado trees during winter is much higher than citrus and more akin to that expected in deciduous fruit tree species (Chandler 1958). It is suggested that avocados in these more stressful cool climates are dependent on the "storage pool" of carbohydrates to sustain fruitlets from set through to the sink:source transition of the spring shoot growth as the supply of current photoassimilates during this period would be negligible. Furthermore, premature leaf senescence would be more likely to be aggravated by salinity stress in semi-arid areas.

With respect to the importance of the over-wintered canopy to yield of avocados in subtropical climates brought to light in this study, knowledge on leaf dynamics in orchard situations is limited. Longevity, rate of turn-over, acclimation to changing PPFs and stress recovery cycles are areas of leaf dynamics worthy of further research, with the potential to develop management strategies promoting higher yield. In particular, the determination of genotypic responses to

photo-inhibiting winter temperatures, common to all subtropical regions, has the potential to identify sources of cold tolerance useful in future plant improvement programs.

Preliminary results from research which increased the photosynthetic efficiency of over-wintered leaves following anthesis (pre-anthesis N plus mid-anthesis paclobutrazol) were encouraging in that a trend for a substantial increase in cumulative yield was demonstrated. Prior recommendations have targeted summer as opposed to pre-anthesis applications of nitrogen to avoid invigorating renewal spring shoot growth at the expense of developing fruitlets (Whiley *et al.* 1988a). Results indicate that these recommendations remain valid in the absence of growth inhibition treatments to counter the effects of nitrogen applied during late winter. However, it remains to be seen if pre-anthesis nitrogen with appropriate suppression of shoot growth can be developed into manageable strategies at the production level. Further research should be pursued in this area in respect to improving the photosynthetic efficiency of the over-wintered canopy with increased partitioning of photoassimilates to developing fruitlets.

Influence of harvest time on sustainable production

Results in this thesis are the first long-term studies reporting on the effects of harvest time on sustainable yield with experiments continuing from three to six consecutive years. It was conclusively shown that long-delayed harvest of 'Fuerte' caused significant yield reduction the following season, with trees driven into alternate bearing cycles. However, 'Hass' appeared more tolerant of late harvesting and took longer to develop alternate bearing cycles. Once biennial cropping patterns had developed they could not be broken by early harvesting of fruit, and more severe strategies will be required to moderate cropping patterns, e.g. early fruit thinning or strategic pruning.

Ecological significance of phenological and physiological characteristics of avocado trees

The high net CO_2 assimilation (A) for avocado reported in this thesis (> 17 μmol CO_2 m^{-2} s^{-1}) is contradictory to the common held rubric for evergreen fruit trees ($A_{max} \leq 12$ μmol CO_2 m^{-2} s^{-1}) in the literature. A probable explanation is that leaf longevity and the dynamics of canopy

replacement suggest that many aspects of avocado phenology and physiology are more typical of deciduous than evergreen species.

Avocados evolved in the subtropical and highland tropical rainforests of Central American and have many features identifying them as climax species (Whitmore 1990), colonising small gaps when the forest canopy is disturbed. Successful capture of canopy space by a forest species is initially dependant on the presence of viable seed or seedlings when gaps are formed; the latter being a feature of the abundance and distribution of the reproductive propagule and its ability to germinate and survive for a considerable time in low light regimes (Denslow 1987; Whitmore 1990). Avocado seed is large, 40 to 80 g_{fw} and represents 20 to 35% of the fresh fruit mass (A.W. Whiley, unpublished data). Due to the large fruit size (300 to 700 g), seed dispersal would require large mammals such as were present in the Americas during the Pleistocene age (Janzen and Martin 1982), with the energy-rich pulp a substantial attractant and reward. Germination of the seed can occur in darkness or at low light intensity and the rich store of energy in the fleshy cotyledons (Wolstenholme 1986, 1987) provides reserves for the initial establishment of seedlings in understorey strata. Light environments of understorey strata are characterised by irradiance levels of 0.4 to 3% of full sunlight (Björkman and Ludlow 1972; Pearcy 1983; Chazdon and Fletcher 1984). Plants permanently occupying this niche are extremely shade tolerant (light saturation level for $A \approx 3$ to 4 μmol quanta m^{-2} s^{-1}; A at saturating PPFs from 3 to 4 μmol CO_2 m^{-2} s^{-1}), and are able to regularly flower and fruit despite minimal interception of light (Chazdon 1986). As the Q_0 for avocado is ca. 30 μmol quanta m^{-2} s^{-1}, PPFs penetrating to the understorey strata would preclude growth once seed reserves were depleted.

The plasticity of the light response of avocado is more typical of small gap colonisers which are usually deciduous trees or those evergreens with leaves which live for less than a year (Grubb 1992). While tolerant of shade, for these species A at saturating PPFs is ≥ 10 μmol CO_2 m^{-2} s^{-1}, allowing moderate to fast growth and capture of space when opportunities arise (Langenheim *et al.* 1984; Oberbauer and Strain 1984; Pearcy and Francheschi 1986; Denslow 1987). As rainforest environments are at least in part resource-rich, trees grow rapidly in the vegetative phase and usually produce several cohorts of leaves in a season. Leaf redundancy is rapid as they quickly become shaded by successive cohorts, senesce and fall to the forest floor. Thus, plant

investment in short-lived leaves is returned by higher A when compared with the "true" evergreen species with extended longevity of leaves (Chabot and Hicks 1982), e.g. 2 years for citrus; several years for mango (Erickson 1968; Verheij 1991). The relatively high A_{max} of avocado compared with other rainforest species (although A_{max} for the pioneer species *Ochroma lagopus* (balsa) is ≈ 27 μmol CO_2 m^{-2} s^{-1}; Whitmore 1990), is likely due to its spatial and temporal organisation of reproductive and vegetative sinks (described in this thesis) and its comparatively large investment into high-energy fruit (Wolstenholme 1987).

CONCLUSION

In the past 20 years or so, probably the most important limiting factor to higher avocado yields was Phytophthora root rot. The application of fungicide technology based on trunk injection of phosphonates has largely removed this potent threat. Yet even in this instance the principles on tree manipulation developed in pheno/physiological modelling were found to be eminently relevant for strategic timing of fungicide injections. This thesis has mainly highlighted the horticultural aspects of tree manipulation, and in several respects the research represents pioneering work on this often recalcitrant crop. The payoff in funds invested has undoubtedly been high, but much remains to be done.

The application of pheno\physiological principles developed throughout this research program and implemented as management strategies, have demonstrated the ability to sustain orchard yields > 20 t ha^{-1} for an extended number of years in subtropical environments. This level of production is double that of the current Australian industry average for bearing avocado orchards and indicates opportunities for substantial improvement for the majority of growers. However, it is acknowledged that in some areas of Australia more severe environmental conditions will limit this yield potential. Despite this considerable improvement in crop production, sustainable yield remains lower than the potential target yield of ca. 32 t ha^{-1} estimated for avocados by Wolstenholme (1986, 1987).

It is the authors opinion that current sustainable production of 23 to 25 t ha^{-1} has reached the genetic limit of cultivars and rootstocks currently available to the industry. Significant

improvement in yield is likely from breeding new cultivars and the development of superior rootstocks and rootstock/cultivar combinations. By their nature, breeding programs are high risk and long-term in respect to committed resources. Future developments in biotechnology areas may improve the potential for results, but to date no serious investment has been made in this field for avocados. In the short-term, rootstock improvement offers the greatest opportunities for improving tree performance (Whiley 1991) and this neglected area deserves an investment of resources so that its potential may be realised.

SUMMARY

Avocados in Australia are grown across a diverse range of environments ranging from warm, humid subtropical to semi-arid Mediterranean. At present more than 95% of production is consumed on domestic markets. Currently the industry is expanding rapidly and new markets will be required to sustain this growth at profitable levels. Geographical isolation and strong competition from countries with lower production costs (labour) have in the past made export unattractive. However, due to the limited domestic market the industry will need to develop export outlets to ensure future prosperity. To compete effectively the efficiency and reliability of production and quality of fruit must be improved. This can be achieved by the development and adoption of advanced technology to give the Australian industry a competitive edge in export markets.

Pheno/physiological studies of cv. Hass growing in a cool mesic subtropical climate have indicated bimodal periodicity for shoot and root growth over a typical 12 month period. After a period of quiescence during winter and following flowering, indeterminate inflorescences and shoots grew vegetatively for a short time in spring. Cessation of spring shoot growth was followed by a period of root growth (measured at the soil-panel interface of rhizotrons) which then declined prior to an extended period of vegetative flushing during summer through to autumn. Strong root growth occurred from late summer until just before flowering when there was a substantial and rapid decline in white "feeder" roots. The magnitude of root growth, particularly during the winter months was dependant on crop load, with heavy crops significantly reducing root growth. Periods of marked fruit abscission occurred at the end of flowering and again during early summer when fruit had reached 30 to 40% of their final mass.

Rootstock/scion combinations influenced the accumulation of starch in roots and the scion (trunk of the tree). 'Hass' grafted to seedling 'Velvick', a Guatemalan race rootstock, accumulated double the starch content in roots compared with 'Hass' grafted to cloned 'Velvick'. This relationship was reversed in the scion with the greatest starch content

accumulating in 'Hass' grafted to cloned 'Velvick'. Mild incompatibility occurred between the rootstock and scion of the 'Hass'/cloned 'Velvick' combination which probably accounts for these differences in starch content. Higher starch concentrations in roots were directly related to greater root lengths measured at the soil-panel interface of the rhizotrons. The 'Hass'/cloned 'Velvick' combination produced ca. 20% more of its fruit on determinate terminals, as opposed to indeterminate and had a more efficient fruiting index when compared with the 'Hass'/seedling 'Velvick' combination; viz. 3.57 ± 0.31 kg m^{-3} compared with 2.12 ± 0.42 kg m^{-3}, respectively. These values appear to be associated with the reduced root growth and vegetative vigour of the 'Hass'/cloned 'Velvick' combination.

Seasonal changes in nitrogen, starch and chlorophyll concentrations and CO_2 assimilation (A) of summer flush leaves and their relationship to tree phenology were studied. There was an increase in all of these parameters as leaves matured during late summer and autumn, a period of relative quiescence in the tree. As inflorescences developed from late June leaf nitrogen content dropped sharply, though it partially recovered during the latter stages of anthesis. Starch concentrations of leaves increased rapidly once leaves matured in late summer until just before anthesis, when there was a gradual decline through to leaf senescence in spring. Net CO_2 assimilation was highest during late summer and autumn but declined rapidly when mean minimum temperatures were $< 10^{\circ}C$, probably due to photoinhibition. Chlorophyll concentrations followed a similar pattern to A with both making a partial recovery after anthesis.

In studies of leaf and shoot ontogeny of fruiting cv Hass trees it was found that leaves reached full expansion ca. 30 days after bud-break and underwent the sink to source transition when 80% expanded. Maximum A of leaves was reached when they were ca. 50 days old. With respect to whole shoot ontogeny, for the first 27 days after bud-break there was a net carbon loss (sink phase) after which net gains in CO_2 assimilation were made. With respect to fruit retention 86% of fruits initially set had abscised by the time that shoots had reached their CO_2 assimilation compensation point, i.e. respiration loss equalled photosynthetic gains, and a further 6% abscised from this point until the shoot had reached its maximum photosynthetic capacity.

Earlier studies of the gas exchange response of avocado leaves to irradiance have largely been made on container-grown trees and gave relatively low A values. Light saturation of A (Q_A) of mature leaves on field-grown 'Hass' trees was measured at 1270 μmol quanta m^{-2} s^{-1} when A was 16.12 ± 0.26 μmol CO_2 m^{-2} s^{-1}, a much higher value than for container-grown trees. The light compensation point (Q_0) for these leaves was ≈ 30 μmol quanta m^{-2} s^{-1}. Prolonged exposure of leaves to minimum mean night temperatures of < 10°C caused photoinhibition of photosystem II, reduced Q_A to 1040 μmol quanta m^{-2} s^{-1} and increased Q_0 to 50 μmol quanta m^{-2} s^{-1}. Cold temperatures also reduced the quantum yield of leaves from 0.0545 to 0.0336. Photoinhibition was reversible with A in leaves making a partial recovery in spring once minimum mean temperatures increased above 10°C.

Photosynthates and possibly their metabolism from current assimilation had a significant impact on the retention of fruitlets on indeterminate spring shoots. Removal of this resource by defoliation reduced final yield by ca. 400%. Strong competition between the closely coupled reproductive and vegetative sinks on fruiting shoots was also shown in studies on the fruit retention dynamics of indeterminate and determinate flowering terminals. Determinate inflorescences initially set 30% more fruit than indeterminate inflorescences and retained significantly more fruit at the maturation of the indeterminate shoot. Fruit on determinate terminals also grew more rapidly and were larger at maturity than those on indeterminate shoots. Control of the vigour of the spring vegetative flush has been intuitively recommended in the past - these results reinforce the importance of orchard management with respect to spring shoot growth.

Net efflux of CO_2 from attached avocado fruit was measured periodically from three weeks after anthesis to fruit maturity. Net CO_2 exchange was determined in the daylight (light respiration, R_l) at a PPFs > 600 μmol quanta m^{-2} s^{-1}, and in the dark (dark respiration, R_d). Dark respiration and R_l were highest during the early cell division stage of fruit growth (about 25 and 22 nmol CO_2 g_{dw}^{-1} s^{-1} respectively) and decreased gradually until fruit maturity to about 1 and 0.5 nmol CO_2 g_{dw}^{-1} s^{-1}, respectively. Fruit photosynthesis, calculated from the difference between R_d and R_l, ranged from 0.5 to 3.1 nmol CO_2 g_{dw}^{-1} s^{-1}. Net CO_2 assimilation on a fruit

dry weight basis was highest during early stages of fruit growth and reached the lowest rate at fruit maturity. Net CO_2 assimilation of fruit exposed to light was 0.4 to 2.5% of that for fully expanded leaves. Light interception in the fruiting zone of the tree canopy showed sufficient irradiance levels for fruit photosynthesis from fruit set to the end of spring shoot growth. Although the relative amount of carbon assimilated by the fruit was small compared with the total amount assimilated by the leaves, the data indicate that avocado fruit contribute at least marginally to their own carbon requirement, especially in the early critical stages, by means of CO_2 assimilated in the light. However, data were insufficient to quantify this on a percentage basis.

The influence of shoot age on partitioning of ^{14}C in container-grown avocado trees was determined. The oldest leaf of actively growing shoots and the youngest leaf of the previously matured shoots were exposed to $^{14}CO_2$ 18 and 34 days after bud-break (DABB) of new shoots. At these times, treated leaves had a positive net CO_2 assimilation rate and, therefore, were considered to be net C exporters. Sixteen days after ^{14}C exposure, separate plant tissues were harvested, dried, weighed, and oxidised. The percentage of ^{14}C in each tissue was determined by liquid scintillation spectrometry. Photoassimilates were translocated acropetally and basipetally from all treated leaves. However, at 18 DABB, developing leaves of the actively growing shoots appeared to be the strongest sink for carbon assimilated by the oldest leaf of these shoots, whereas the roots were the strongest sink for carbon assimilated by the youngest leaf of the previously matured shoot. By 34 DABB, roots were the strongest sink for carbon assimilated by leaves of both new and previously matured shoots. These data are useful in developing improved management strategies for controlling Phytophthora root rot (caused by *Phytophthora cinnamomi* Rands) in avocados by systemic phosphonate fungicides translocated via the photoassimilate pathway.

Translocation of the fungicide phosphonic acid (H_3PO_3) in cv. Hass avocado trees was studied after trunk injection with 20% H_3PO_3, formulated as potassium phosphonate, at three stages of tree phenology during the growing season. Initially, translocation was solely acropetal in the xylem, and H_3PO_3 was detected in the leaves 24 hours after treatment. Several days after injection, H_3PO_3 concentration in the bark of trunks and in roots increased, indicating basipetal

phloem transport of H_3PO_3 from leaves. The rate of accumulation and the final concentration of H_3PO_3 in the roots was directly related to the sink: source relationship of the shoot at the time of injection. For example, trunk injection at the beginning of spring growth flush, when renewal shoots were strong sinks, resulted in low H_3PO_3 root concentrations (< 9 mg g_{fw}^{-1}) which peaked about 45 days after treatment. When potassium phosphonate was injected after the transition of spring-grown shoots from sinks to sources, or at summer shoot maturity, root concentrations of H_3PO_3 increased to ≥ 25 mg g_{fw}^{-1} by 30 days after treatment. These results, supported by ^{14}C studies, suggest that strategic timing of injections according to phenological events may greatly improve fungicide efficacy when targeting specific organs for protection.

The effect of harvest time in relation to subsequent yield and fruit size, and the seasonal dynamics of starch concentration flux were studied for an early ('Fuerte') and late ('Hass') maturing cultivar over 3 to 6 consecutive seasons. Harvesting fruit at or shortly after reaching legal maturity sustained yield while delayed harvesting resulted in alternate bearing patterns. Delayed harvesting resulted in a significant reduction of accumulated yield over a number of years with both early and late maturing cultivars. When strong alternate bearing patterns had developed they could not be alleviated by early-harvesting fruit at minimum legal maturity. There was a tendency for delayed harvesting of 'Fuerte' to significantly ($P \leq 0.05$) increase fruit size but this did not occur every year. This effect was less pronounced with 'Hass' with almost significant ($P \leq 0.06$) increases in size only recorded in the "off" year of cropping. Seasonal starch concentration fluxes in the trunks of 'Fuerte' trees were small (< 30%) and gave no indication of potential cropping in subsequent seasons. Starch fluxes were much greater in 'Fuerte' shoots (600 to 700%) but were also a poor indicator of yield in the following season. Seasonal starch concentration fluxes in shoots and trunks of 'Hass' trees were greater than in 'Fuerte' and followed more predictable patterns. July concentrations of starch in 'Hass' were directly related to subsequent yield and have potential for use as a prediction index for crop estimation.

Reduced fruit size and yield are problems with 'Hass' growing in warm, subtropical climates. Low *A* of over-wintered leaves was identified at flowering and fruit set, a time of assimilate

demand by competing reproductive and vegetative sinks on indeterminate terminals. Enhancement of nitrogen content of these leaves while simultaneously restricting shoot growth with the growth inhibitor paclobutrazol (PBZ), was investigated over two seasons as a means of increasing the assimilate supply to developing fruitlets during the critical first 12 weeks of ontogeny. Forchlorfenuron (CPPU) a chemical with cytokinin-like activity with the potential to increase cell division was also evaluated for its potential to increase fruit size. Pre-anthesis soil nitrogen applications together with mid-anthesis foliar sprays of PBZ (2.5 g a. i. l^{-1}) or mid-anthesis foliar sprays of PBZ alone (2.5 g a. i. l^{-1}) significantly increased *A* (ca. 10.8 and 9.0 μmol CO_2 m^{-2} s^{-1}, respectively) compared with control trees (ca 7.5 μmol CO_2 m^{-2} s^{-1}). These treatments and mid-anthesis foliar sprays of PBZ at 1.25 g a. i. l^{-1} significantly increased accumulative fruit yield over two consecutive years. Nitrogen plus PBZ also increased the storage life of fruit held at 7°C by six days. CPPU sprayed on trees at either 10 or 20 mg a. i. l^{-1} when fruit was in the first two weeks of ontogeny had no effect on fruit size or yield. Further research is needed before possible orchard adoption of this manipulative strategy.

The plasticity of the light response of avocados identified in this thesis together with morphological characteristics reported in the literature provide evidence that ecologically avocado has evolved as a small gap species in the subtropical and highland tropical rainforests of Central America. The tree has normally been classified and usually behaves as an evergreen species, albeit with remarkably short-lived leaves. However, many of the physiological characteristics of avocado identified in research reported herein are more typical of those described for deciduous species. In subtropical climates the avocado exhibits wintergreeness, replacing the over-wintered canopy after a cohort of new leaves in spring. However, in harsher environments and under Phytophthora root rot or other severe stresses the tree may become "near deciduous", thereby relieving stress until more favourable conditions return.

The detailed pheno-physiological studies reported in this thesis involve research conducted since 1989 and in particular over the period 1991 through 1994. However, the conceptual framework was developed over a longer period. The author's studies have thrown more light on the ecological, physiological and ultimately genetic constraints on the performance of currently available cultivars/composite trees, and helped to explain relatively low commercial orchard

yields. However, the resultant more sophisticated understanding of the tree has led to opportunities for tree manipulation to improve leaf (and fruit) photosynthetic efficiency, increased carbon partitioning to roots and fruits, control of vegetative:reproductive competition at critical periods in the phenological cycle, and improved efficacy of translocated fungicides. The data base for managing the avocado tree has been considerably expanded.

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APPENDICES

Appendix 1

Whiley, A. W., Schaffer, B. and Lara, S.P., 1992. Carbon dioxide exchange of developing avocado (*Persea americana* Mill.) fruit. *Tree Physiol.* 11, 85-94.

Appendix 2

Whiley, A.W. and Schaffer, B., 1993. ^{14}C-Photosynthate partitioning in avocado trees as influenced by shoot development. *HortScience* 28, 850-2.

Appendix 3

Whiley, A.W., Hargreaves, P.A., Pegg, K.G., Doogan, V.J., Ruddle, L.J., Saranah, J.B. and Langdon, P.W., 1995. Changing sink strengths influence translocation of phosphonate in avocado (*Persea americana* Mill.) trees. *Aust. J. Agric. Res.* Manuscript accepted 17 Oct 1994.

Appendix 4

Supporting Publications

APPENDIX 1

Whiley, A. W., Schaffer, B. and Lara, S.P., 1992. Carbon dioxide exchange of developing avocado (*Persea americana* Mill.) fruit. *Tree Physiol.* 11, 85-94.

Tree Physiology 11, 85–94

Carbon dioxide exchange of developing avocado (*Persea americana* Mill.) fruit

A. W. WHILEY,[1,2] B. SCHAFFER[1] and S. P. LARA[1]

[1] *University of Florida, Tropical Research and Education Center, 18905 S.W. 280 Street, Homestead, Florida, USA*

[2] *On leave from Department of Primary Industries, Maroochy Horticultural Research Station, P.O. Box 5083, Sunshine Coast Mail Centre, Queensland, Australia*

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Summary

Net efflux of CO_2 from attached avocado (*Persea americana* Mill.) fruit was measured periodically from three weeks after anthesis to fruit maturity. Net CO_2 exchange was determined in daylight (light respiration, R_l) at a photosynthetic photon flux (PPF) greater than 600 μmol m^{-1} s^{-1}, and in the dark (dark respiration, R_d). Dark respiration and R_l were highest during the early cell division stage of fruit growth (about 25 and 22 nmol CO_2 g_{dw}^{-1} s^{-1}, respectively) and decreased gradually until fruit maturity to about 1 and 0.5 nmol CO_2 nmol CO_2 g_{dw}^{-1} s^{-1}, respectively. Fruit photosynthesis, calculated from the difference between R_d and R_l, ranged from 0.5 to 3.1 nmol CO_2 g_{dw}^{-1} s^{-1}. Net rate of CO_2 assimilation on a fruit dry weight basis was highest during the early stages of fruit growth and reached the lowest rate at fruit maturity. Net rate of CO_2 assimilation of fruit exposed to light was 0.4 to 2.5% of that for fully expanded leaves. Although the relative amount of carbon assimilated by the fruit was small compared with the total amount of carbon assimilated by the leaves, the data indicate that avocado fruit contribute to their own carbon requirement by means of CO_2 assimilated in the light.

Introduction

Crop yield increases have been achieved largely by increasing the proportion of assimilates partitioned to the harvested organs of plants (Evans 1976). For example, in avocado (*Persea americana* Mill.), a substantial yield increase was obtained in response to a reduction in vegetative growth as a result of treatment with paclobutrazol foliar sprays (Wolstenholme et al. 1990, Whiley et al. 1991). Other key factors determining fruit yield are the respiratory cost of growth and the seasonal photosynthesis efficiency of the crop (Amthor 1984, Cannell 1985).

Respiratory losses from fleshy fruit, during growth and ripening, have been documented for several crop species (Kidd and West 1925, Jones et al. 1964, Blanke et al. 1985). However, the contribution of fruit to their own carbon economy should not be ignored. Previous studies with young green fruit have established their photosynthetic contribution to the carbon requirement for growth and maintenance (Bean and Todd 1960, Todd et al. 1961, Kriedemann 1968, Bazzaz et al. 1979, Flinn et al. 1977, Jones 1981). For example, pods of pea (*Pisum sativum*) exhibited a net photosynthetic gain during the first 30 days after anthesis, but thereafter respiration losses exceeded CO_2 assimilation (Flinn et al. 1977). For oranges and lemons (*Citrus sinensis* and *Citrus limon*), grape (*Vitis vinifera* cv. sultana), and apple (*Malus*

domestica cvs. Jonathan and Golden Delicious), fruit respiratory losses of CO_2 during a diurnal cycle exceeded photosynthetic gains throughout fruit ontogeny (Clijsters 1969, Bean et al. 1963, Kriedemann 1968, Jones 1981). However, Clijsters (1969) demonstrated a 36% reduction in the growth of apples when photosynthetic activity was inhibited by excluding light from developing fruit.

Wolstenholme (1986, 1987) calculated that the oil-accumulating avocado fruit has a high energy requirement for growth (807.2 kJ 100 g^{-1} for cv. Fuerte at 17% oil content, compared with 262.8 and 292.5 kJ 100 g^{-1} for apples and citrus, respectively). Avocado fruit are climacteric (Eaks 1980), and that respiratory sequence is initiated by detachment from the tree. Previous studies on avocado fruit respiration have been conducted exclusively with detached fruit at various stages of development and to our knowledge, there are no reports on net CO_2 exchange of avocado fruit attached to the tree throughout ontogeny. Avocado fruit remain green from setting until maturity and have a high stomatal density (50–75 stomata mm^{-2}, shortly after fruit set), with active stomata similar to those of the leaves, facilitating gas exchange (Blanke and Bower 1990). Total chlorophyll concentration in the mesocarp is only 12 to 30% that of the peel concentration (Cran and Possingham 1973). Thus, a fruit has the potential for photosynthetic activity, thereby contributing to its own carbon requirements during growth. Refixation of respiratory CO_2 within fruit by phosphoenolpyruvate carboxylase (PEPC) may be a significant contributor to fruit photosynthesis (Blanke and Lenz 1989). This mode of CO_2 refixation in fruit may also be present in avocado, because PEPC has been observed in avocado fruit (Blanke and Notton 1991).

The purpose of this study was to determine the dynamics of CO_2 efflux from avocado fruit from post anthesis to fruit maturity and to assess the contribution of fruit to their own carbon economy from the fixation of atmospheric CO_2.

Materials and methods

Avocado trees (*Persea americana* var. *americana* × *P. americana* var. *guatemalensis*, cv. Booth-7) planted at the University of Florida, Tropical Research and Education Center, Homestead, Florida (25° N latitude) were used in this study. Trees were on 'Waldin' or 'Lula' seedling rootstocks and were 35 years old at the beginning of the experiment. Trees were maintained with standard fertilization, irrigation, and pest control practices recommended for avocado (Malo and Campbell 1983).

From 3 weeks after anthesis (early April 1989) to fruit maturity (mid-September 1989), CO_2 efflux from attached fruit was determined in the field at 14-day intervals for three fruit on each of five trees. Because fruit were harvested after each measurement, different fruit were used on each measurement date. However, fruit growth rates within and among trees were fairly uniform, and flowers were tagged at anthesis to be certain that test fruit were the same age. Net CO_2 exchange of fruit was determined from CO_2 fluxes by enclosing individual small fruit in a Parkinson's leaf chamber (Analytical Development Co., Hoddesdon, Herts., England), or larger fruit in a 14 × 14 × 13 cm Plexiglas chamber containing a battery-powered fan and a

thermocouple. Net CO_2 exchange was determined with an LCA-2 field portable open gas exchange system (Analytical Development Co.) as described by Schaffer and O'Hair (1987). Flow rate of ambient air into the chamber was maintained at 400 ml min^{-1} for the first five measurement dates and at 600 ml min^{-1} for the later dates. Net CO_2 exchange was calculated using equations described by Jarvis (1971) and Von Caemmerer and Farquhar (1981). Light respiration (R_l) of fruit was determined by measuring CO_2 efflux throughout the day at a minimum photosynthetic photon flux (PPF) of 600 μmol m^{-2} s^{-1}, which exceeds the light saturation for CO_2 assimilation of avocado leaves (Scholefield et al. 1980). Immediately following measurements made in the light, the chamber was covered with two layers of black polyethylene. Dark CO_2 efflux, i.e., dark respiration (R_d), was then determined. Chamber air temperature was monitored, but not controlled, during CO_2 exchange determinations, and ranged from 31 to 45 °C during the study. Respiration data were standardized to 30 °C by using temperature response data from each sampling date. This same method has been used to standardize temperatures for peach respiration data (DeJong et al. 1987). Data were not collected until the CO_2 flux in the chamber had stabilized (about 5 minutes for small fruit during the first measurement date, and up to 2 hours for large, mature fruit). Immediately after each CO_2 exchange measurement, the fruit used was harvested and its dry weight determined after slicing and drying at 60 °C.

Fruit R_d and R_l was expressed on a g_{dw}^{-1} and a $fruit^{-1}$ basis. Statistical models determining fruit growth over time and comparing fruit dry weight to fruit R_d and R_l were constructed by linear and nonlinear regression analysis using SAS software (SAS Institute, Inc., Cary, NC, USA). Fruit photosynthetic activity was calculated from the difference between fruit R_l and fruit R_d at each point on the regression line (Bean and Todd 1960, Clijsters 1969, Jones 1981).

Light interception by the avocado tree canopy was defined in a separate study carried out on a 5-m diameter tree (cv. Hass) in subtropical southeastern Queensland (lat. 27° S). During flowering, which on avocado is mostly terminal to the last vegetative flush (Whiley et al. 1988*a*), and early fruit set, spot measurements of PPF were made with a quantum sensor (Model LI-190 SA, Li-Cor, Inc., Lincoln, NE, USA) within the fruiting zone and compared to the full sunlight position. At the completion of spring shoot growth, 1-m line sensors (Model LI-191 SA, Li-Cor, Inc.) were positioned in the fruiting zone of the tree as well as inside the canopy at distances of 0.5 and 1.0 m interior to the fruiting zone. The sensors were aligned as closely as possible to an angle of 90° to the midday sun on the northern side of the tree. A fourth quantum sensor (Model LI-190 SA, Li-Cor, Inc.) was positioned outside the tree canopy in full sunlight. The PPF was integrated hourly during the light period of each day using a datalogger (Model LI-1000, Li-Cor, Inc.) and the accumulated quanta at each line sensor expressed as a percentage of full sunlight. Mean values of the percentage of light intercepted at each point in the canopy were calculated for a 1-week period. The quantum sensors were left positioned in the tree and PPF measurements were collected again about 8 weeks later, after summer shoot growth had occurred.

Results

Fruit dry weight increased exponentially with time (Figure 1). The increase was relatively slow during the first 10 weeks after anthesis and then increased rapidly from Week 10 to fruit maturity (20 weeks after anthesis).

As fruit dry weight increased over time, R_d and R_l showed similar CO_2 efflux patterns on a dry weight basis (Figure 2a). Dark respiration and R_l were highest three weeks after anthesis, 25 and 22 nmol CO_2 g_{dw}^{-1} s^{-1}, respectively. As fruit ontogeny progressed, R_d and R_l decreased and were lowest at fruit maturity, about 1.0 and 0.5 nmol CO_2 g_{dw}^{-1} s^{-1}, respectively. The difference between R_d and R_l decreased as fruit weight increased (Figure 2a). This was concomitant with a reduction in the calculated fruit photosynthetic rate, from about 3.1 nmol CO_2 g_{dw}^{-1} s^{-1} during early fruit growth to about 0.5 nmol CO_2 g_{dw}^{-1} s^{-1} at fruit maturity (Figure 2b).

The pattern of fruit respiration expressed on a per fruit basis was similar in the dark and the light (Figure 3a). Until fruit dry weight reached 10 g, R_d and R_l per fruit increased linearly as the fruit developed (Figure 3a). When fruit were about one-third of their mature weight (20 g dry weight), respiration per fruit approached an asymptote and increased little until fruit were harvested (Figure 3a). Dark respiration was always greater than R_l, and these differences were greatest when fruit dry weight was between 20 and 55 g (Figure 3a). Respiration rates were highest at fruit maturity and were about 208 and 152 nmol CO_2 $fruit^{-1}$ s^{-1} or 34 and 25 mg CO_2 h^{-1} for R_d and R_l, respectively. Calculated fruit photosynthesis, expressed on a per fruit basis, increased linearly as fruit dry weight increased from 0 to 20 g, then leveled off when fruit reached approximately half of their maturation weight (Figure 3b). There was little increase in calculated fruit photosynthesis as fruit weight increased from 30 to 60 g.

Photosynthetic photon flux measurements taken during flowering and early fruit

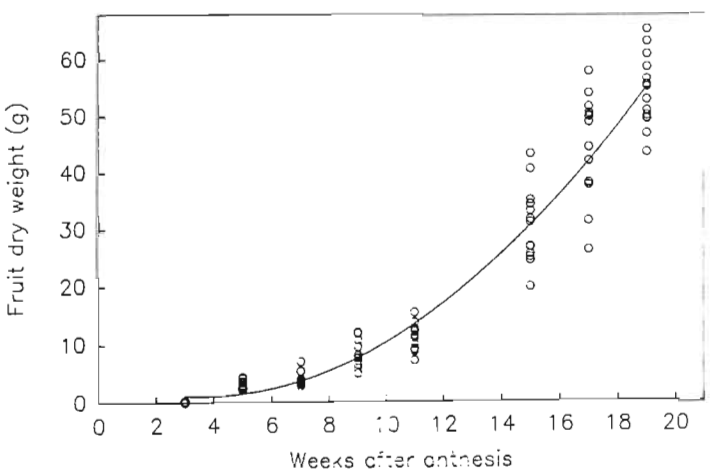

Figure 1. Fruit dry weight of 'Booth-7' avocados during fruit development. The regression line is defined by the equation: $y = 3.94 - 1.618x + 0.227x^2$, $R^2 = 0.99$.

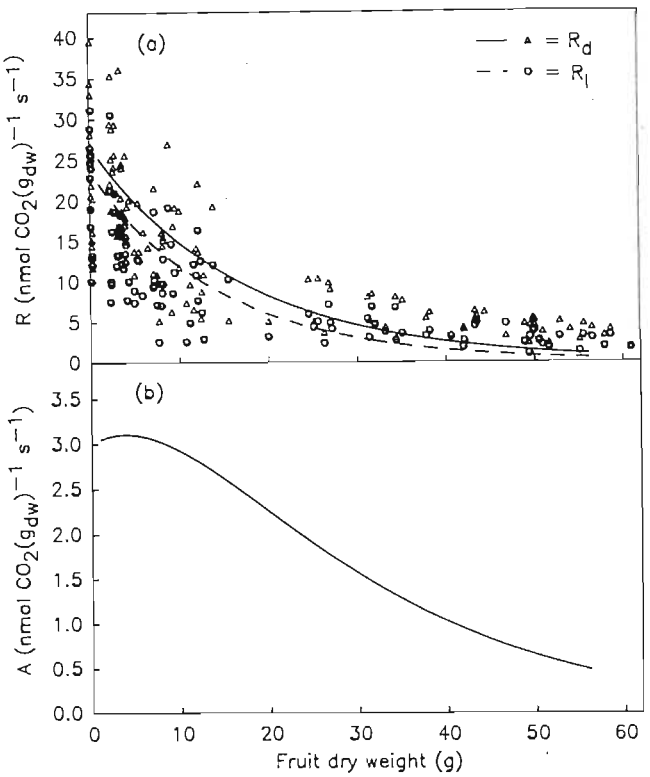

Figure 2. (a) Respiration of developing 'Booth-7' avocado fruit in the dark (R_d) and in the light (R_l) expressed on a gram dry weight basis, where the regression line for R_d is defined by the equation: $y = 26.55e^{-0.057x}$, $R^2 = 0.60$, and the regression line for R_l is defined by the equation: $y = 19.98e^{-0.0067x}$, $R^2 = 0.63$. (b) Net CO_2 assimilation (A), determined from $R_d - R_l$ of developing 'Booth-7' avocado fruit, expressed on a gram dry weight basis.

set indicated that most young fruit were exposed to full sunlight for the first four weeks of their development (data not shown). During the two periods when light interception data were integrated, daily PPF ranged from 15.5 mol m^{-2} on overcast days to 59.5 mol m^{-2} on cloud-free days. By the end of spring shoot growth, light transmission to the fruiting zone had been reduced to 35.9% of full sunlight, and at distances of 0.5 and 1.0 m inside the canopy from the fruiting zone it had been reduced to 13.7 and 9.7%, respectively (Figure 4). By the end of the summer shoot growth, light transmission to the fruiting zone had further declined to 13.1% of full sunlight and to 9.7 and 6.3% of the respective internal monitoring positions.

Discussion

The dynamics of R_d and R_l observed for attached, developing avocado fruit were similar to those observed for other crops (Clijsters 1969, Jones et al. 1964, Jones 1981, DeJong et al. 1987, DeJong and Walton 1989). The highest respiration rates were observed during the early stage of fruit growth, from the first measurement date

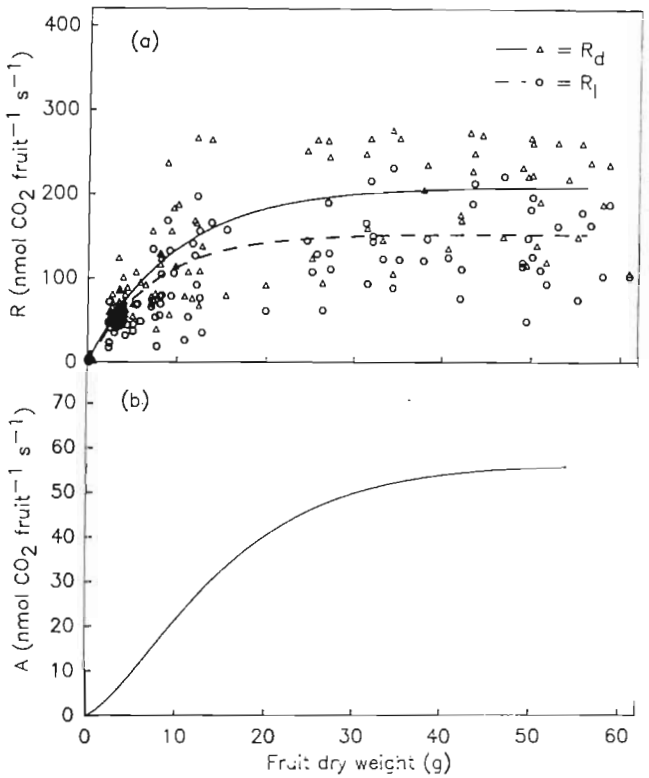

Figure 3. (a) Fruit respiration of developing 'Booth-7' fruit in the dark (R_d) and in the light (R_l) expressed on a $fruit^{-1}$ basis, where the regression line for R_d is defined by the equation: $y = 209.01\ (1 - e^{-0.107x})$, $R_2 = 0.71$, and the regression line for R_l is defined by the equation: $y = 140.60(1 - e^{-0.117x})$, $R^2 = 0.66$. (b) Net CO_2 assimilation (A), determined from $R_d - R_l$ of developing 'Booth-7' avocado fruit, expressed on a per fruit basis.

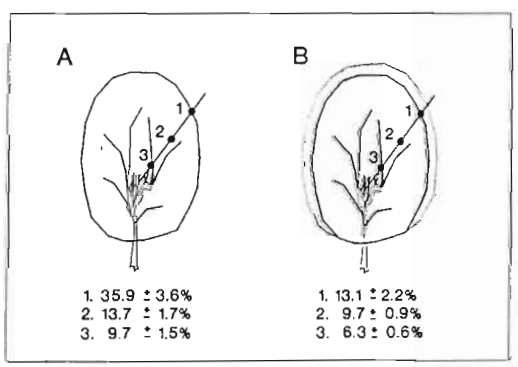

Figure 4. Light transmission in an avocado tree canopy: (A) when spring shoot growth had stopped and (B) at the end of summer shoot growth. Photosynthetic photon flux (PPF) was measured in full sunlight, (1) in the fruiting zone, (2) 0.5 m interior to the fruiting zone and (3) 1.0 m interior to the fruiting zone. Data are mean values ± SE ($n = 7$) of the percentage of full sunlight measured at each point. The PPF in full sunlight ranged between 15.5 and 56.6 mol m^{-2} (A) and 19.8 to 59.5 mol m^{-2} (B) over each 7-day period.

to about 12 weeks after anthesis, then decreased to the lowest rates at fruit maturity. The period when the highest respiration rates were observed corresponds to the time that cell division is greatest in avocado fruit (Valmayor 1967). Similar patterns have been reported previously for avocado (Todd et al. 1961), apple (Clijsters 1969, Jones 1981) and peach (DeJong et al. 1987, DeJong and Walton 1989). The maximum R_d value measured at 30 °C for avocado fruit of about 25 nmol CO_2 g_{dw}^{-1} s^{-1} was similar to R_d measured at 20 °C for apple fruit, about 26 nmol CO_2 g_{dw}^{-1} s^{-1} (recalculated from Jones 1981) and peach fruit at 20 °C, about 28 nmol CO_2 g_{dw}^{-1} s^{-1} (DeJong et al. 1987). Jones (1981) reported that the greatest difference between R_d and R_l for apple was during the early phase of fruit growth and the smallest difference was at fruit maturation. We observed a similar response for avocado fruit. When the R_d and R_l of avocado were expressed on a per fruit basis, respiration at 30 °C was highest at fruit maturity, about 34 and 25 mg CO_2 h^{-1} $fruit^{-1}$ for R_d and R_l, respectively. These values were somewhat lower than the value of 50 mg CO_2 h^{-1} $fruit^{-1}$ measured at 23 °C reported for avocado by Blanke (1991). The difference between values may be due to experimental or genotypic differences. The cultivar used in Blanke's (1991) study was *P. americana* var. *drymifolia* cv. Fuerte, whereas we used the hybrid *P. americana* var. *americana* × *P. americana* var. *guatemalensis* cv. Booth-7. The oil concentration of 'Booth-7' fruit (about 8%) is lower than that of 'Fuerte' (about 12–14%) at maturity (C.W. Campbell, personal communication). Presumably this leads to lower energy demands for growth and development for 'Booth-7' than for 'Fuerte' (Wolstenholme 1986), resulting in lower respiratory activity in 'Booth-7' fruit.

At all stages of fruit development, fruit photosynthesis was substantially less than dark respiration. However, the calculated photosynthetic rate of developing avocado fruit (i.e., the difference between R_d and R_l; Todd et al. 1961, Jones 1981), was highest during early fruit growth, about 3.0 nmol CO_2 g_{dw}^{-1} s^{-1}. The photosynthetic rates for developing avocado fruit were 42 times less than those for mature leaves, about 126.0 nmol CO_2 g_{dw}^{-1} s^{-1} (Schaffer and Whiley, unpublished data).

Although chlorophyll concentrations in the peel of avocado fruit are similar to concentrations in the leaves (Cran and Possingham 1973, Schaffer et al. 1991), the difference in the maximum CO_2 assimilation rates between the two organs may be attributed to the difference in the chlorophyll a/b ratio, which is 1–2/1 in fruit (Cran and Possingham 1973) and 2–3.3/1 in leaves (Schaffer et al. 1991). However, the difference in the amount of CO_2 assimilated between the organs is more likely to be a result of the greater surface area to volume ratio in leaves than in fruit, which results in a severe decline of light penetration into fruit tissue (only 0.02% of incident light penetrates more than 2 mm into an avocado fruit (Cran and Possingham 1973)), and a change in the spectrum of photosynthetically active radiation (Blanke 1990). This relationship is further expressed by the declining net CO_2 assimilation (expressed as nmol CO_2 g_{dw}^{-1} s^{-1}) as fruit increase in size.

Vu et al. (1985) suggested that reproductive organs fix little atmospheric CO_2 by means of ribulose-bisphosphate carboxylase (RuBPC) in the respiratory pentose phosphate (RPP) pathway. They reported that the CO_2 assimilation (PEPC/RuBPC)

ratio was 4–5/1 and 0.1/1 for citrus flowers and leaves, respectively. Furthermore, Blanke and Lenz (1989), Blanke (1990), and Blanke and Notton (1991) concluded that refixation of respiratory CO_2 by the PEPC pathway provides a significant contribution of carbon by the fruit for its own growth requirements. The data from the present study indicate that avocado fruit contribute to their own carbon requirement by means of CO_2 fixation in the light and that the relative contribution of fruit photosynthesis to the total energy requirement is greatest during the early stages of fruit development. This may be a significant factor influencing fruit retention because it is concomitant with the period of photoassimilate competition between reproductive and vegetative sinks (Biran 1979, Blumenfeld et al. 1983, Wolstenholme et al. 1990, Whiley et al. 1991), which extends for about 42 days after spring shoot growth commences (Whiley 1990). In addition, the over-wintered leaf canopy has lost photosynthetic efficiency (Whiley, unpublished data) at a time of critical assimilate demand. During this period young developing fruit are in full sunlight with the opportunity to maximize their photoassimilate contributions to growth. Our data show that up to the end of spring shoot growth, when fruit have attained a size between 12 and 15 g dry weight, there is sufficient light during cloud-free conditions to support fruit photosynthesis within the fruiting zone of the canopy. However, by the time the summer growth flush is complete (Whiley et al. 1988*b*), the light environment in the fruiting zone is unlikely to support photosynthetic activity in the fruit. At this stage of fruit ontogeny the renewed and photosynthetically efficient leaf canopy would meet all photoassimilate requirements of fruit growth.

Acknowledgments

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APPENDIX 2

Whiley, A.W. and Schaffer, B., 1993. ^{14}C-Photosynthate partitioning in avocado trees as influenced by shoot development. *HortScience* 28, 850-2.

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^{14}C-Photosynthate Partitioning in Avocado Trees as Influenced by Shoot Development

Anthony W. Whiley[1]
Maroochy Horticultural Research Station, Queensland Department of Primary Industries, P.O. Box 5083 Sunshine Coast Mail Centre, Nambour 4560, Australia

Bruce Schaffer
Tropical Research and Education Center, Institute of Food and Agricultural Sciences, University of Florida, 18905 Southwest 280th Street, Homestead, FL 33031

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Abstract. **The influence of shoot age on ^{14}C partitioning in potted avocado (*Persea americana* var. *americana* Mill.) trees was determined. The oldest leaf of actively growing shoots and the youngest leaf of previously matured shoots were exposed to $^{14}CO_2$ 18 and 34 days after budbreak (DABB) of new shoots. At these times, treated leaves had a positive net CO_2 assimilation rate and, therefore, were considered to be net C exporters. Sixteen days after ^{14}C exposure, separate plant tissues were harvested, dried, weighed, and oxidized. The percentage of ^{14}C in each tissue was determined by liquid scintillation spectrometry. Photoassimilates were translocated acropetally and basipetally from all treated leaves. However, at 18 DABB, developing leaves of actively growing shoots seemed to be the strongest sink for C assimilated by the oldest leaf of these shoots, whereas the roots were the strongest sink for C assimilated by the youngest leaf of the previously matured shoots. By 34 DABB, roots were the strongest sink for C assimilated by leaves of new and previously matured shoots. These data are useful in developing improved management strategies for controlling phytophthora root rot (incited by *Phytophthora cinnamomi* Rands) in avocados by systemic phosphonate fungicides translocated in the photoassimilate pathway. Thus, phosphonates should be applied after shoots have matured and most of the canopy is in a quiescent state for maximum translocation to the roots.**

A major consideration in the management of avocado orchards in most avocado-producing countries is phytophthora root rot caused by *Phytophthora cinnamomi* (Darvas and Bezuidenhout, 1987; Zentmyer, 1971). This disease is controlled effectively by foliar sprays or trunk injections of systemic phosphonate fungicides (Darvas et al., 1984; Pegg et al., 1985), which are transported acropetally in the xylem and basipetally along with photoassimilates in the phloem (Guest and Grant, 1991). To be effective, these fungicides must be moved basipetally from the leaves to the roots in sufficient concentrations to suppress disease development.

Architecturally, the avocado is defined as a polyaxial species with a usually synchronous growth pattern characterized by alternating shoot and root growth (Verheij, 1986; Whiley et al., 1988). The movement of systemic fungicides in the tree is related to the dynamics of photoassimilate partitioning (unpublished data), which varies with the activity of competing sinks, often temporally separated. The relationship between vegetative flushing and photoassimilate partitioning in the tree indicates the stage of vegetative growth at which systemic fungicides are likely to be transported most effectively to the roots. The objective of this study was to determine the influence of shoot development on photoassimilate partitioning in avocado trees.

Two-year-old 'Simmonds' avocado trees, grafted on 'Waldin' seedling rootstocks, were planted in a peat–perlite potting medium (Promix; Premier Brands, Stamford, Conn.) in 12-liter plastic pots. Plants were fertilized at 14-day intervals with an 8N–3P–9K granular fertilizer (Atlantic–Florida East Coast Fertilizer and Chemical Co., Homestead, Fla.) and a 7N–56P–14K soluble fertilizer with minor elements (SOL-U-GRO; Miller Chemical and Fertilizer Corp., Hanover, Pa.) in the irrigation water. Trees were trained to a single leader and, to synchronize growth, were topped at ≈15 to 20 cm above the graft union, leaving 10 to 15 mature leaves per tree, and placed in an air-conditioned glasshouse in May 1989. The glasshouse was maintained at 30 ± 2C (day), and 20 ± 2C (night). The axillary bud in the terminal position on each tree was allowed to develop into a new shoot; all other axillary buds were removed (Fig. 1).

Eighteen days after budbreak (DABB) of the new shoot, the oldest leaf on this shoot and the youngest leaf of the previously matured shoot were exposed to $^{14}CO_2$ (Fig. 1). Sixteen days later (34 DABB), when all of the leaves of the actively growing shoot were fully expanded, the oldest leaf on this shoot and the youngest leaf of the previously matured shoot on a different set of trees were exposed to $^{14}CO_2$. Thus, there were two treatments based on the position of the leaf exposed to $^{14}CO_2$: the oldest leaf of the new shoot (T-1) and the youngest leaf of the previously matured shoot (T-2). Each treatment consisted of six single-plant replications at each exposure time in a completely randomized design.

T-1 leaf areas were measured in situ with a leaf area meter (model LI-3000; LI-COR, Lincoln, Neb.) at the time of exposure and at shoot maturity to ascertain their stage of physiological maturity. In addition, net CO_2 assimilation was determined for T-1 and T-2 leaves immediately before treatment to ensure that leaves to be exposed were primarily net C exporters. Net CO_2 assimilation was determined with a portable infrared gas analyzer (Analytical Development Corp., Haddesdon-Herts, England) at a photosynthetic photon flux (PPF) >600 $\mu mol \cdot m^{-2} \cdot s^{-1}$, which is above the light saturation level for avocado (Scholefield et al., 1980).

Trees were labeled with ^{14}C by exposing leaves to $^{14}CO_2$ in a sealed transparent plastic chamber attached to a CO_2 generator, as described by Schaffer et al. (1985). The $^{14}CO_2$ was produced by adding 1 N HCl to 1 ml of $NaH^{14}CO_3$ (18.5×10^{10} Bq·ml^{-1}) in an Erlenmeyer flask. The gas was circulated continuously through the leaf chamber for 10 min at a flow rate of 2 liters·min^{-1} by a pump attached to the flask and chamber with plastic tubing. Excess $^{14}CO_2$ was absorbed by bubbling the gas through 1 liter of a saturated $Ba(OH)_2$ solution for 3 min to avoid contaminating the environment with $^{14}CO_2$ and prevent nontreated leaves from being exposed to residual $^{14}CO_2$ once the leaf chamber was removed. At the time of $^{14}CO_2$ exposure, PPF in the glasshouse

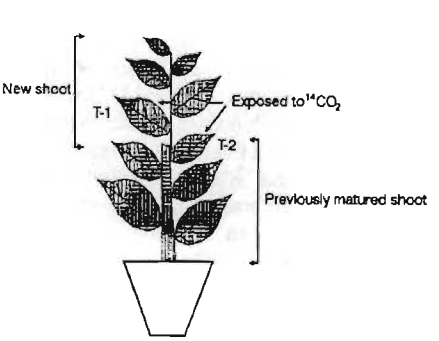

Fig. 1. Schematic diagram of a potted avocado tree illustrating the various shoots and relative positions of leaves exposed to $^{14}CO_2$.

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[1]On leave at the Tropical Research and Education Center, Institute of Food and Agricultural Sciences, Univ. of Florida, Homestead.

was >600 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Sixteen days after leaves were exposed to $^{14}CO_2$, trees were harvested, organs were separated and oven-dried at 65C, and their dry weights were determined. Material from each organ was ground finely in a spice mill (Black and Decker, Shelton, Conn.), a measured amount of tissue was oxidized in a sample oxidizer (model 306; Packard Instruments, Downersville, Ill.), and ^{14}C from each sample was placed in 20 ml of 1 Carbosorb II : 2 Permaflor 5 (v/v) (Packard Instruments). Scintillation fluid (10 ml) was added to the samples for counting. The radioactivity of each sample was determined by radioassay with a liquid scintillation spectrometer (model 5801; Beckman Instrument Co., Fullerton, Calif.). Five nonradio-labeled samples of each tissue also were prepared and assayed for use as standards. The percentage of ^{14}C in each organ was calculated from disintegrations per minute multiplied by organ dry weight and is reported as percentage of total recovered ^{14}C in the plant.

At 18 DABB, the $^{14}CO_2$-exposed T-2 leaf was fully expanded, whereas the $^{14}CO_2$-exposed T-1 leaf was 88% expanded. Leaf area measurement of T-1 at 34 DABB indicated that all leaves of the new shoot were fully expanded, thus the new shoot was mature.

The mean net CO_2 assimilation rates of T-1 and T-2 leaves were 6.1 and 9.2 $\mu mol \cdot m^{-1} \cdot s^{-1}$, respectively, at 18 DABB, as determined with the infrared gas analyzer. The lower assimilation rate for T-1 probably was related to the fact that these leaves had not attained maximum photosynthetic capacity, which is reached after full expansion (Schaffer et al., 1991).

More ^{14}C remained in exposed T-1 than T-2 leaves at 18 and 34 DABB (Fig. 2). This result most likely was due to the photoassimilate requirement for leaf expansion and dry-matter accumulation in the younger leaf. Although Schaffer et al. (1991) observed that avocado leaves reach full expansion in ≈28 days, dry-matter accumulation continues to increase beyond this point. There was no difference between treatments in ^{14}C partitioning to the stem of the new and mature shoots and to the leaves of mature shoots at either treatment time. At 18 DABB, T-1 accumulated a higher proportion of absorbed ^{14}C in the leaves of the new shoot than T-2 (Fig. 2a). This result indicates that more of the assimilates for current shoot growth were provided by the oldest leaf of the same shoot than leaves of the previously matured shoot. At 18 and 34 DABB, more ^{14}C photoassimilates were partitioned to the roots from the T-2 than the T-1 treatment (Fig. 2), a result that is consistent with ^{14}C translocation patterns in orange [*Citrus sinensis* (L.) Osb.] (Kriedemann, 1969b). However, the difference in assimilate partitioning to the roots between the T-2 and T-1 treatments was greater at 18 DABB.

When ^{14}C-assimilate transport from the T-1 and T-2 leaves was averaged, the developing leaves of the new shoot were a stronger photoassimilate sink than the roots 18 DABB (Fig. 3). However, by 34 DABB, the roots had become a stronger sink. These results agree with those reported for grape (*Vitis vinifera* L.)

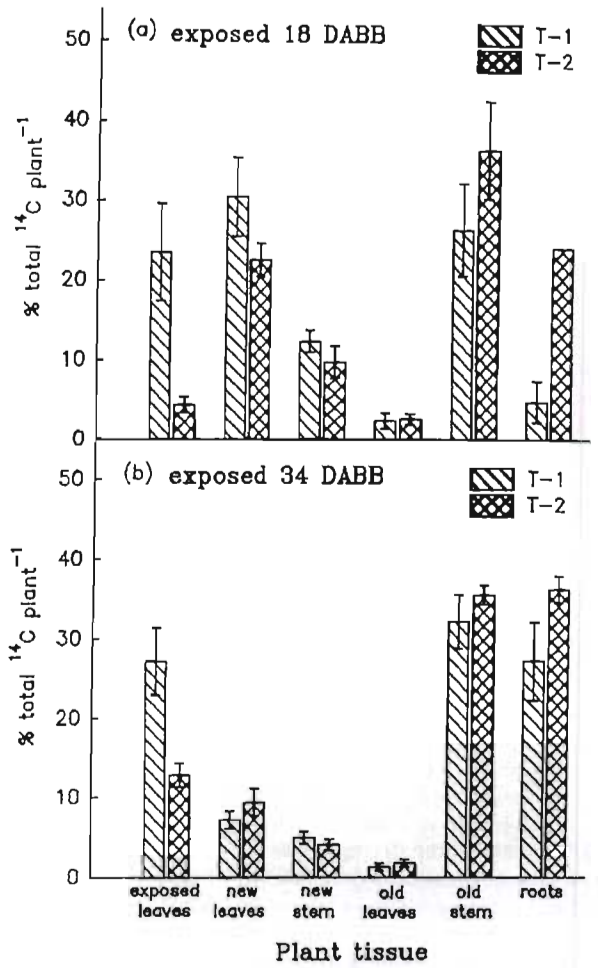

Fig. 2. Partitioning of ^{14}C in avocado trees supplied as $^{14}CO_2$ to the tree (a) 18 and (b) 34 days after budbreak (DABB) of the newest shoot. Various tissues were analyzed 16 days after exposure to $^{14}CO_2$. T-1 = the oldest leaf of the new shoot that was exposed and T-2 = the youngest leaf of the previously matured shoot that was exposed to $^{14}CO_2$. Exposed leaves = leaves exposed to $^{14}CO_2$, new leaves = all leaves of the new shoot, new stem = stem of the new shoot, old leaves = all leaves of the previously matured shoot, old stem = stem of the previously matured shoot. Vertical lines represent ±SE, where n = 6.

(Hale and Weaver, 1962), *Citrus* (Kriedemann, 1969a, 1969b), and pecan [*Carya illinoinensis* (Wangenh.) C. Koch] (Davis and Sparks, 1974), where new shoots were the strongest photoassimilate sink during their growth and development. Spring shoot growth in avocado trees is predominantly from terminal vegetative buds of indeterminate panicles and is synchronized strongly by low winter temperatures, which induce flowering (Davenport, 1982; Venning and Lincoln, 1958; Whiley et al., 1988). This shoot growth occurs at a time

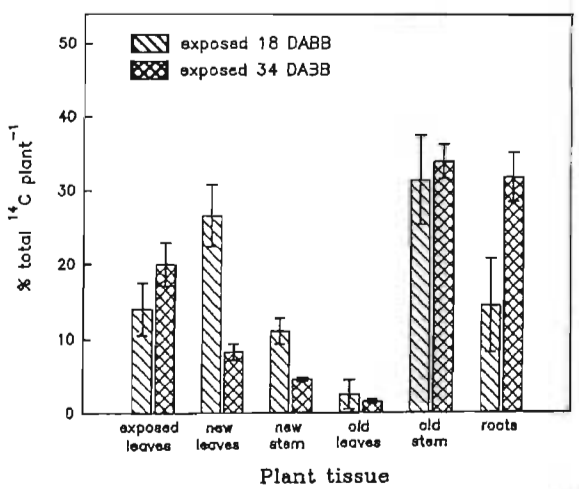

Fig. 3. Partitioning of ^{14}C in avocado trees. Leaves were exposed to $^{14}CO_2$ at 18 or 34 days after budbreak (DABB) of the new shoot and various tissues were analyzed 16 days later. The percentage of ^{14}C in each tissue was calculated by averaging the percentage translocated from the oldest leaf of the new shoot and youngest leaf of the previously matured shoot at each time. Vertical lines represent ±SE, where n = 6.

when the overwintered canopy is losing its photosynthetic efficiency and is approaching senescence (unpublished data), and rising soil temperatures promote the activity of *P. cinnamomi* (Pegg et al., 1982). Growth flushes during summer are typically asynchronous; portions of the canopy remain quiescent, while other areas are in active growth (Whiley et al., 1988). The results from our research indicate that treating trees with phosphonate in spring likely will be most effective after new shoots are mature and will maximize fungicide translocation to the roots. Timing of phosphonate application in summer is not likely to be as critical, since at any one time large portions of the canopy remain in a quiescent state and leaves on mature shoots favor photoassimilate translocation to the roots. This hypothesis requires substantiation using phosphonate treatments at various stages of canopy development.

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APPENDIX 3

Whiley, A.W., Hargreaves, P.A., Pegg, K.G., Doogan, V.J., Ruddle, L.J., Saranah, J.B. and Langdon, P.W., 1995. Changing sink strengths influence translocation of phosphonate in avocado (*Persea americana* Mill.) trees. *Aust. J. Agric. Res.* Manuscript accepted 17 Oct 1994.

Aust. J. Agric. Res., 1995, 46, 1–0

Changing Sink Strengths Influence Translocation of Phosphonate in Avocado (*Persea americana* Mull.) Trees

A. W. Whiley,[ADE] *P. A. Hargreaves,*[B] *K. G. Pegg,*[B] *V. J. Doogan,*[C] *L. J. Ruddle,*[B] *J. B. Saranah,*[A] *and P. W. Langdon*[A]

[A] Maroochy HRS, PO Box 5083, SCMC, Nambour, Qld 4560.
[B] Department of Primary Industries, Meiers Rd, Indooroopilly, Qld 4068.
[C] Department of Primary Industries, Locked Bag 4, Moorooka, Qld 4105.
[D] Submitted as part of a Ph.D. Thesis, Department of Horticultural Science, University of Natal, South Africa.
[E] Corresponding author.

Abstract

Translocation of phosphonic acid (H_3PO_3) in cv. Hass avocado trees was studied after trunk injection with 20% H_3PO_3, formulated as potassium phosphonate, at three stages of tree phenology during the growing season. Initially, translocation was solely acropetal in the xylem, and H_3PO_3 was detected in the leaves 24 h after treatment. Several days after injection, H_3PO_3 concentration in the bark of trunks and in roots increased, indicating basipetal phloem transport of H_3PO_3 from leaves. The rate of accumulation and the final concentration of H_3PO_3 in the roots were directly related to the sink strength of the shoot at the time of injection. For example, trunk injection at the beginning of spring growth flush, when renewal shoots were strong sinks, resulted in low H_3PO_3 root concentrations ($<9\ \mu g\ g_{fw}^{-1}$) which peaked about 45 days after treatment. When potassium phosphonate was injected after the transition of spring-grown shoots from sinks to sources, or at summer shoot maturity, root concentrations of H_3PO_3 increased to $\geq 25\ \mu g\ g_{fw}^{-1}$ by 30 days after treatment. These results suggest that strategic timing of injections according to phenological events may greatly improve fungicide efficacy when targeting specific organs for protection.

Keywords: avocado, phosphonates, phosphonic acid, *Phytophthora cinnamomi*, sink strength.

Introduction

Phosphonates (viz. salts or esters of phosphonic acid) were the first commercially used ambimobile fungicides in plants (Zentmyer 1979; Lüttringer and De Cormis 1985). They are particularly effective in controlling diseases caused by oomycetes such as the phytophthoras, pythiums and downy mildew which cause severe economic losses in agricultural crops worldwide (Cohen and Coffey 1986). Owing to effective translocation of phosphonates within plants several methods of application have been employed to control diseases. These include the traditional methods of foliar sprays and soil drenches (Pegg *et al.* 1985; Rohrbach and Schenck 1985) and painting or sponge banding the trunks of trees with phosphonate formulations (Synman and Kotzé 1983). The term phosphonate is widely used for the salts and esters of phosphonic acid (H_3PO_3). Once phosphonates are introduced into plant tissues they are rapidly hydrolysed to H_3PO_3 and subsequently ionized to the phosphonate anion, HPO_3^{-2} (Ouimette and Coffey 1990). This anionic form of H_3PO_3 is more correctly known as phosphonate (Ouimette and Coffey 1989).

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Phytophthora cinnamomi Rands is a devastating root disease of avocado (*Persea americana* Mill.) in most countries which grow this crop. The fungus invades the unsuberized roots, and less frequently attacks the suberized woody tissue of major roots or the collar of the tree (Pegg *et al.* 1982). When injected into the xylem tissues of the trunk or major limbs, the phosphonate anion is ultimately translocated to the roots limiting colonization by the pathogen (Schutte *et al.* 1988; Guest and Grant 1991). The development of trunk injection of phosphonates during the 1980*s*, was an unconventional application technique which has subsequently been shown to control Phytophthora root rot in avocados (Darvas *et al.* 1984; Pegg *et al.* 1985). Initially, research with trunk-injected phosphonates for the control of avocado root rot, focused on curing diseased trees (Darvas *et al.* 1984; Pegg *et al.* 1985, 1987, 1990). It was demonstrated that trees rating 9 on the health scale of 0, healthy to 10, dead (Darvas *et al.* 1984) could be restored to full health within two years by a trunk injection program with phosphonate fungicides (Pegg *et al.* 1987). However, within a few years of the commercial development of trunk injection, the focus on tree health shifted from curative to preventative management procedures, which required more strategic and efficient use of the technology.

Symplastic distribution within plants of ambimobile herbicides such as 2,4-D and glyphosate, and the nematicide oxamyl, has been shown to be source/sink related, with the respective compounds accumulating in organs with greatest sink strength at the time of application (Leonard and Crafts 1956; Crafts and Yamaguchi 1958; Tyree *et al.* 1979; Dewey and Appleby 1983). It is likely that the ambimobile phosphonate exhibits similar behaviour following entry into the symplast.

In studies with young, container-grown avocado trees, Whiley and Schaffer (1993) reported that shoot and root sink activity were temporally separated; leaves were the strongest sinks for ^{14}C-photosynthates during early shoot growth, and the sink strength of roots increased once shoots became quiescent. The asynchronous pattern of shoot and root growth in avocado is illustrated in phenology models developed for avocado (Whiley *et al.* 1988) which have since been confirmed by the observations of Ploetz *et al.* (1992) and Whiley and Schaffer (1994). If translocation of both photoassimilates and phosphonate is subject to similar controls, then natural variation (asynchrony) in root vs. shoot growth may well influence distribution patterns of trunk-injected H_3PO_3. Accordingly, we set out to establish whether H_3PO_3 movement to roots was influenced by sink-strength dynamics within trees at the time of application.

Materials and Methods

Trees selected for the experiment were 12-year-old, healthy 'Hass', approximately 12 m in canopy diameter, grafted to 'Velvick' Guatemalan seedling rootstock and growing in a site where *Phytophthora cinnamomi* was not present so that phosphonate fungicides had not been previously used. The trees were growing in a commercial orchard at Maleny in coastal S.E. Queensland (latitude 27° S., 650 m altitude), which has a cool, high rainfall subtropical climate.

Tree phenology was monitored by collecting fruiting shoots from each of the nine experimental trees immediately prior to treatment, then subsequently at *c.* 30 day intervals following the first injection through to fruit maturity and harvest 296 days later. Three current seasons' fruiting shoots were collected from each tree and oven-dry weights of the stems, leaves and fruit were determined separately after 72 h at 90°C in a forced-draught oven. Trees were

trunk-injected with potassium phosphonate at three different phases of tree phenology during spring and summer, thereby spanning the fluctuating relationships between shoot and root growth (Fig. 4). During each phenology phase, three trees were injected with the fungicide.

The first group of trees was trunk-injected with a 20% solution of potassium phosphonate towards the end of anthesis just as spring shoot growth commenced (4 October) (Fig. 4). Injections were carried out using Chemjet® tree injectors (Chemjet Trading Pty Ltd, Caboolture, Qld) at the rate of 15 ml m^{-1} of canopy diameter (Pegg *et al.* 1987). Each injection site was prepared by drilling 6 mm diameter holes into the trunk, penetrating the wood to a depth of 40 mm, thereby placing the injected H_3PO_3 into the xylem tissue and directly into the transpiration stream. Each injector was filled with 20 ml of the fungicide, and injection sites were equally spaced around the circumference of the trunk; about nine injection sites per tree. Fresh samples of organs were collected from these trees before treatment and at 2–30 day intervals following injection, and analysed for H_3PO_3 content. Four subsamples of bark and wood tissues (from tree trunks) were collected at each sampling time and care was taken to ensure that they were not selected from positions in close proximity to injection sites. Subsamples of unsuberized roots were collected from each quadrant of the tree and 20 subsamples of leaves, stems and shoots were taken from positions representative of the entire canopy at each sampling time. Subsamples were bulked for H_3PO_3 analysis. For each tree, the amount of H_3PO_3 lost via fruit harvest and organ senescence was estimated over an 8 month period. The senescence of flowers, leaves, twigs and fruit was monitored by placing 9 L containers in each quadrant of the canopy to collect a representative sample of material shed during the experiment. The containers were emptied at 14-day intervals, the material sorted into the various organs, dry weights determined and each component analysed for H_3PO_3 content. Fruit yield was recorded at harvest and H_3PO_3 content determined from samples from each of the three trees.

A second group of trees was trunk-injected when the spring shoots had completed extension growth and leaves were fully expanded (9 December), and a third group of trees was injected once the summer vegetative growth had matured (3 May) (Fig. 4). Following injection, fresh unsuberized root and leaf samples were collected at intervals from these two groups of trees and the H_3PO_3 content determined separately for each organ. The trunk injection treatments were repeated at the beginning of spring shoot growth and at the end of spring shoot maturity the following season, and leaf and root samples collected and analysed over 96 days (data not presented).

To test lateral distribution of H_3PO_3, another group of three trees, each with trunks which formed two main vertical branches within 1 m of soil level was selected. At the beginning of spring shoot growth, only one of the main branches on each tree was injected with a 20% solution of potassium phosphonate at a rate calculated to treat the whole tree based on 15 ml m^{-1} diameter of canopy. Leaf and root samples were collected at intervals from the treated and untreated sides of the tree and the H_3PO_3 concentration was determined in each organ.

Phosphonic Acid Analysis by Gas Chromatography

Concentrations of H_3PO_3 were measured in avocado tissues using an acid extraction and gas chromatography. Samples were extracted with dilute aqueous sulfuric acid and derivatized using diazomethane to form the dimethyl ester. This extract was injected into a gas chromatograph equipped with a glass chromatographic column (Carbowax 20M) and a flame photometric detector. Dimethyl phosphonate was detected as a peak on the chromatogram and the concentration determined by comparison with a known standard. Residue concentrations of H_3PO_3 were calculated and expressed as mg g_{fw}^{-1} for each sample. This method allowed a rapid and quantitative analysis of a variety of tissues (leaf, root, fruit [including seed, skin and flesh], bark and wood) with low detection limits ($\leq 0{\cdot}1$ μg g_{fw}^{-1}) (P. A. Hargreaves, unpubl. data).

Data Analysis

The H_3PO_3 content in leaves was monitored for each injection time and the concentration flux fitted to the non-linear regression model derived by Wood (1967) where $y = ax^b e^{-cx}$. Linear and non-linear regression analyses were used to relate the concentration flux of H_3PO_3 in roots to the time elapsed after trunk injection.

Results and Discussion

Distribution and loss of Phosphonic Acid from the Tree (Pre-spring Shoot Growth Injection)

Prior to trunk injection, low levels of H_3PO_3 (<3·0 mg g_{fw}^{-1}) were detected in all parts of the tree (Fig. 1), although there was no previous history of the use of phosphonate fungicides on the experimental trees or in the orchard. It is unlikely that these pre-treatment concentrations of H_3PO_3 were derived from natural sources (Hilderbrand 1983). Weeds were regularly controlled in the orchard by using glyphosate [*N*-(phosphono-methyl) glycine], an ambimobile phosphonate based herbicide (Dewey and Appleby 1983). Glyphosate is completely degraded to CO_2 by microorganisms in the soil, with the main intermediary metabolite being aminomethylphosphonic acid (Carlisle and Trevors 1988; Pipke and Amrhein 1988). It is likely that H_3PO_3 is a metabolite from the degradation of aminomethylphosphonic acid, in which case it may have been taken up by the tree, thereby accounting for its presence in tissues before treatments were applied.

Following trunk injection at the beginning of spring shoot growth, H_3PO_3 concentrations increased in all tissues. Within 2 days of treatment, substantial increases in shoot and leaf concentrations were measured: 2·2±0·5 to 77·4±6·9 and 1·1±0·3 to 52·1±7·20 $\mu g\ g_{fw}^{-1}$, respectively. The highest H_3PO_3 concentrations were measured in the spring shoots (stem and leaves) which were actively growing at the time of trunk injection (Figs 1 and 4). The H_3PO_3 concentration in these tissues peaked 8 days after injection and then rapidly declined in leaves (Figs 1*a* and 1*b*). However, in the stems of spring shoots there was a high H_3PO_3 level until 96 days after injection, when concentration in those tissues fell rapidly. This coincided with the beginning of summer shoot growth and the development of a new leaf sink (Whiley and Schaffer 1993).

Phosphonic acid levels in mature, over-wintered leaves peaked within 8 days of treatment, after which there was a rapid decline in concentration (Fig. 1*b*). The difference in maximum H_3PO_3 concentration between spring and over-wintered leaves was probably due to different sink strengths at the time of injection. Whiley and Schaffer (1993) showed that following exposure of a mature leaf to $^{14}CO_2$, 27% of the ^{14}C-photosynthate was recovered from actively growing leaves at the terminal of the shoot, while only 2·5% was found in mature leaves adjacent to the treated leaf. These mature leaves are a strong source of photoassimilates during spring shoot growth (Whiley 1990), and probably account for the more rapid loss of H_3PO_3 compared with spring leaves which remain strong sinks during development.

Ninety-six days after injection, sufficient summer shoot growth was present to allow sample collection. The concentration of H_3PO_3 in the stems of these new shoots was initially lower than in the stems of spring shoots at the same sampling time, but thereafter was similar despite temporal separation of their development in relation to the time of the trunk injection (Fig. 1*a*). During early growth, leaves on summer shoots had higher concentrations of H_3PO_3 than the adjacent spring leaves, but by 195 days after trunk injection there was no significant difference between spring and summer leaves (Fig. 1*b*).

Concentrations of H_3PO_3 in the bark and wood of trunks were low compared to other organs of the tree (Fig. 1*c*). In the bark an increase in H_3PO_3 concentration

was measured 4 days after trunk injection (from 1·3±0·2 to 4·7±0·7 $\mu g\ g_{fw}^{-1}$), and peaked after 32 days (7·6±1·2 $\mu g\ g_{fw}^{-1}$) while in the wood the maximum concentration (5·4±0·5 $\mu g\ g_{fw}^{-1}$) was reached 8 days after treatment.

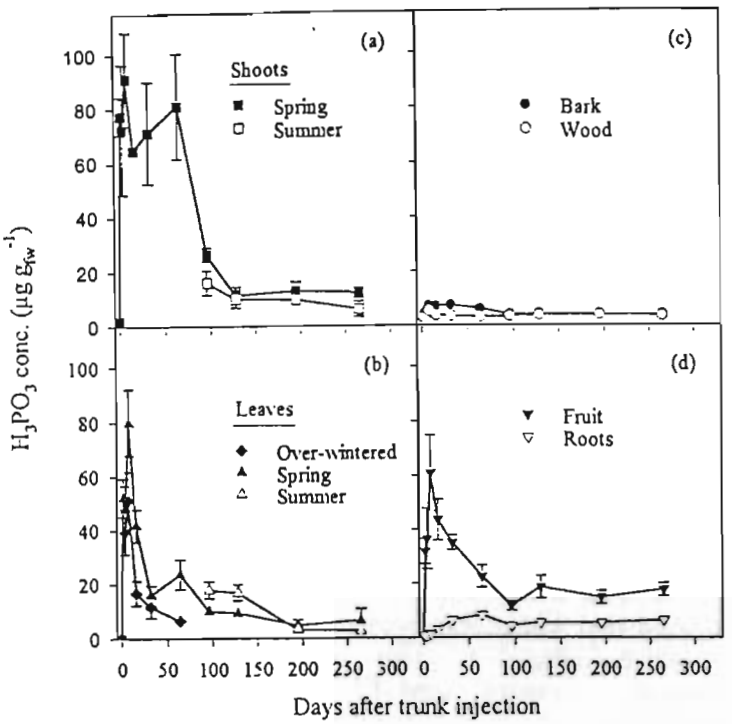

Fig. 1. Concentration flux of phosphonic acid (H_3PO_3) in avocado (*a*) shoots, (*b*) leaves, (*c*) trunk bark and wood, and (*d*) fruit and roots following trunk injection with 20% solution of potassium phosphonate at the beginning of spring shoot growth. Data points are mean values from three trees±s.e. bars (s.e. bars are obscured by symbols at some points).

Concentrations of H_3PO_3 in young fruit increased sharply following trunk injection, reaching 60·8±14·0 $\mu g\ g_{fw}^{-1}$ eight days after treatment. Thereafter, concentrations declined until stabilizing (at ≈17 $\mu g\ g_{fw}^{-1}$) 64 days after injection (Fig. 1*d*). The considerable H_3PO_3 concentration in fruit at an early stage of their ontogeny was in contrast to <1 $\mu g\ g_{fw}^{-1}$ H_3PO_3 detected following trunk injection when fruit were mature (Pegg and Whiley, unpublished data). At the time of harvest, fruit (in this study) had maintained the highest H_3PO_3 concentration compared with other tissues, viz. 17·6±2·4 $\mu g\ g_{fw}^{-1}$ for fruit compared with 12·3±2·0 $\mu g\ g_{fw}^{-1}$ for stems of spring shoots. This was probably due to the comparatively strong sink status of the fruit throughout ontogeny (Cannell 1985), but is well below the maximum residue level of 100 $\mu g\ g_{fw}^{-1}$ set for avocado fruit in Australia.

The accumulation of H_3PO_3 in roots was slower than in spring shoots and fruit, with no detectable increase until 16 days after treatment: from 1·4±0·4 to 3·1±0·5 $\mu g\ g_{fw}^{-1}$. The highest root concentration of H_3PO_3 was only 8·4±1·9 $\mu g\ g_{fw}^{-1}$, measured 64 days after injection (Fig. 1*d*). Following this

peak there was a slight decline in root concentration which remained relatively constant for the balance of the monitoring period. The pattern of both leaf and root accumulation of H_3PO_3 immediately following treatment was similar to that reported by Schutte *et al.* (1988). However, in their study, following a gradual increase in H_3PO_3 concentration in roots from 2 to 20 $\mu g\ g_{fw}^{-1}$ during the first 35 days after injection, there was a 300% increase in H_3PO_3 between 35 and 42 days after treatment followed by a sharp decline in concentration.

At the time of treatment, 23 g of H_3PO_3 were injected into the trunks of each of the three trees. The residual H_3PO_3 concentration in senesced tree organs was highest in the inflorescence and fruitlets (50–80 $\mu g\ g_{fw}^{-1}$) with much lower concentrations in leaves (mature over-wintered) and twigs (10–20 $\mu g\ g_{fw}^{-1}$), thereby providing further evidence of the effect of sink strength on distribution (Cannell 1985). It was estimated that 6·85±0·98 g (≈30%) of H_3PO_3 were lost from each tree from the time of injection until fruit harvest (296 days later). Approximately 3·51±0·28 g (≈15%) of the total amount lost was attributed to loss through the litter cycle, while 3·34±0·57 g (≈15%) was removed in harvested fruit. These estimates did not take into account other losses through root senescence and leakage (Ouimette and Coffey 1990) or possible oxidation in plant tissues to PO_4^- by bacteria as suggested by Bezuidenhout *et al.* (1987). The other significant factor responsible for declining tissue concentrations of H_3PO_3 was dilution by growth, and its impact will largely depend on tree vigour.

Ouimette and Coffey (1990) concluded that symplastic entry of phosphonate across the plasmalemma occurs by active transport (Epstein 1973), which is dependent on metabolic energy. Several researchers, using [^{14}C]sucrose as a standard for phloem-transported material, have demonstrated that translocation

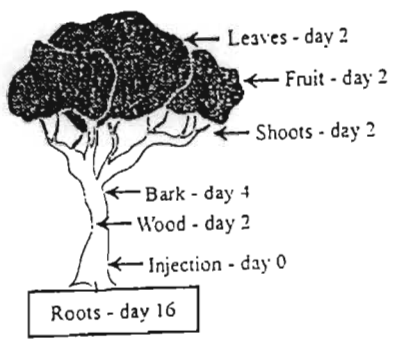

Fig. 2. Number of days after pre-spring shoot growth injection when significant increases in phosphonic acid concentration were measured in the different organs of the tree.

profiles of sucrose and phosphonate are almost identical (Martin and Edgington 1981; Dewey and Appleby 1983; Chamberlain *et al.* 1984). In our study the pattern of distribution of H_3PO_3 within avocado trees provides further evidence of the ambimobility of phosphonate. Following injection directly into the transpiration stream of trees, increased concentrations of H_3PO_3 were measured in stems and leaves of shoots and in fruit 2 days, and in bark of trunks, 4 days after treatment; however, there was no increase in root concentration until 16 days after trunk injection (Fig. 2). This time sequence suggests an apoplastic translocation pattern via the xylem to the leaves following trunk injection, whereafter symplastic entry into phloem resulted in active basipetal movement to the roots. The speed of the initial distribution within each canopy will thus vary with time of day that the injection is made owing to differences in transpirational flux.

Lateral Distribution of Phosphonic Acid in the Tree

Our studies showed that H_3PO_3 moved rapidly in an acropetal and basipetal direction, but had a much less effective lateral translocation. Leaves and roots on the treated side of the tree showed a substantial increase in H_3PO_3 concentration within 8 (leaves) to 32 (roots) days after trunk injection (Fig. 3). However, the increase in concentration of H_3PO_3 in leaves and roots on the untreated side of the tree occurred more slowly, and reached only 2 and 35% of the peak concentrations of leaves and roots from the treated side of the tree, respectively. In contrast, studies with translocation of phosphonate fungicides in cacao (*Theobroma cacao* L.) have shown that trunk injection into one site in the tree is sufficient to disseminate adequate levels of H_3PO_3 throughout the tree, thereby providing protection from pod rot (*Phytophthora palmivora* Butler) (Guest *et al.* 1994). This may be due to the less complex structure of cacao plants which have a central trunk (chupon) producing lateral plagiotropic branches at given intervals (the jorquette) (Purseglove 1968). In studies with trunk-injected phosphonate in monocotyledons, Darakis *et al.* (1985) found that there was an excellent distribution throughout the plant from a single injection site. This is likely due to the presence of many short xylem vessels with numerous cross-connections which facilitate both vertical and lateral translocation within these plants.

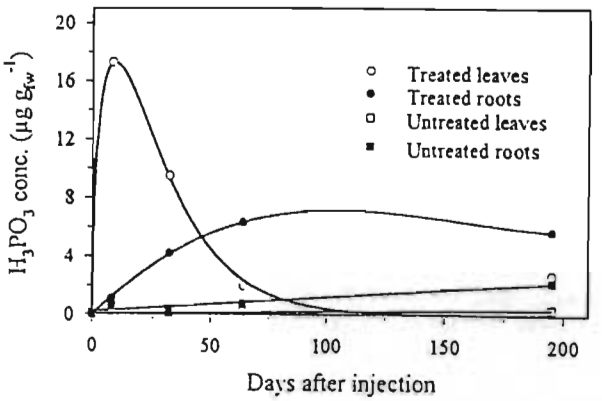

Fig. 3. Concentration flux of phosphonic acid (H_3PO_3) in avocado leaves and roots following injection into one branch of a forked tree. The model for leaves on the treated branch is represented by $y = 9{\cdot}7x^{0{\cdot}48}e^{-0{\cdot}053x}$, $r^2 = 0{\cdot}88$ ($P < 0{\cdot}17$); for leaves on the untreated branch by $y = 0{\cdot}783+0{\cdot}002x$, $r = 0{\cdot}88$ ($P < 0{\cdot}05$); for roots on the side of the treated branch by $y = 0{\cdot}0023+0{\cdot}164x-1{\cdot}17e^{-4}x^2+2{\cdot}44327e^{-6}x^3$, $r^2 = 0{\cdot}99$ ($P < 0{\cdot}05$) and for roots on the side of the untreated branch by $y = 0{\cdot}22+0{\cdot}01x$, $r = 0{\cdot}97$ ($P < 0{\cdot}05$). Data points are mean values of three trees.

Effect of Strategic Timing of Injections on Phosphonic Acid Root Concentrations

The efficiency of translocation of H_3PO_3 to the roots appears directly related to the sink/source status of the leaves at the time of injection. In our study, shoot phenology measured by dry matter accumulation was similar to that previously reported for avocado (Whiley *et al.* 1988; Ploetz *et al.* 1992; Whiley

and Schaffer 1994). There were two major periods of shoot growth corresponding to spring and summer. Spring shoots grew vigorously for the first 32 days following bud-break, during which time they accumulated 66% of their final dry matter (Fig. 4). Thereafter, the growth rate declined with the maximum shoot dry matter attained 128 days after bud-break. Summer shoot growth began 96 days after spring shoot bud-break, at a time when spring growth was relatively quiescent (Fig. 4). Dry matter accumulation in the summer shoots was not as rapid as in the spring shoots, taking *c.* 100 days to accumulate 66% of the dry matter and 190 days to maximum dry matter. There was a linear increase in fruit dry weight from fruit set to maturity, a period of *c.* 300 days (Fig. 4).

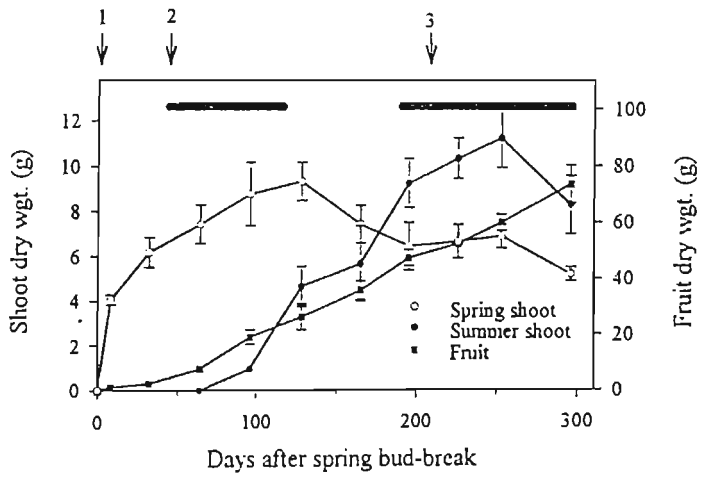

Fig. 4. Dry matter accumulation in spring and summer shoots and fruit, in cv. Hass trees during the period following trunk injection when phosphonic acid concentration fluxes in the tree were monitored. Trunk injection times are indicated by arrows where 1 = pre-spring shoot growth, 2 = spring shoot maturity, and 3 = summer shoot maturity. Horizontal bars indicate the major periods of root growth defined by Whiley *et al.* (1988), Ploetz *et al.* (1992) and Whiley and Schaffer (1994). Data points are mean values from nine trees±s.e. bars (s.e. bars are obscured by symbols at some points).

While root growth data were not collected in this experiment, corroborating evidence from other studies (Whiley *et al.* 1988; Ploetz *et al.* 1992; Whiley and Schaffer 1994) suggests that root growth (hence sink strength) was greatest when shoots were relatively quiescent, i.e. for a short time *c.* 60 days following spring bud-break and for a longer period *c.* 200 days after the beginning of spring growth (Fig. 4). This is further substantiated by Whiley and Schaffer (1993), who reported that ^{14}C-photosynthate was largely retained in new, actively growing shoots (38% in shoots compared with 14·5% in roots) when trees were exposed to $^{14}CO_2$ shortly after new shoot growth had commenced. However, once all leaves on shoots were fully expanded, exposure to $^{14}CO_2$ resulted in a larger proportion of ^{14}C-photosynthate being translocated to the roots (32% in roots compared with 13% in the new shoots).

Concentration fluxes of H_3PO_3 in leaves and roots of trees trunk-injected at different stages of phenological development, mirrored the dynamics of temporal

sink separation (Figs 4 and 5). At each time following trunk injection of potassium phosphonate, there was a rapid increase in the leaf concentration of H_3PO_3 reaching between 50 and 70 $\mu g\, g_{fw}^{-1}$ within 8 to 12 days after treatment. The subsequent decline in leaf H_3PO_3 was also quite rapid, reflecting the exporting capacity of the leaves as H_3PO_3 crossed the symplast and became phloem-mobile.

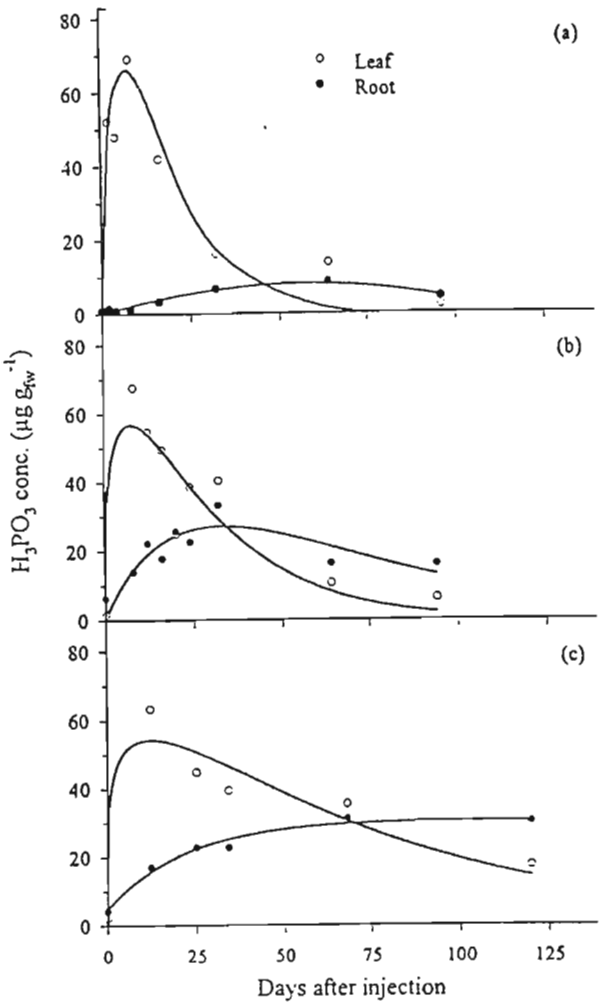

Fig. 5. Concentration flux of phosphonic acid (H_3PO_3) in avocado leaves and roots following trunk injection at (*a*) the beginning of spring shoot growth where the model for leaves is represented by $y = 36{\cdot}0x^{0{\cdot}649}e^{-0{\cdot}093x}$, $r^2 = 0{\cdot}69$ ($P < 0{\cdot}05$); and for roots by $y = 2{\cdot}94 + 0{\cdot}24x - 0{\cdot}0025x^2$, $r^2 = 0{\cdot}87$ ($P < 0{\cdot}01$); (*b*) the maturity of the spring shoot growth where the model for leaves is represented by $y = 39{\cdot}7x^{0{\cdot}356}e^{-0{\cdot}048x}$, $r^2 = 0{\cdot}73$ ($P < 0{\cdot}01$); and for roots by $y = 2{\cdot}59x^{0{\cdot}936}\, e^{-0{\cdot}0281x}$, $r^2 = 0{\cdot}58$ ($P < 0{\cdot}05$); (*c*) the maturity of summer shoot growth where the model for the leaves is represented by $y = 39{\cdot}7x^{0{\cdot}205}e^{-0{\cdot}0166x}$, $r^2 = 0{\cdot}98$ ($P < 0{\cdot}01$); and for roots $y = 30{\cdot}7 - 26{\cdot}02\ (0{\cdot}955^x)$, $r^2 = 0{\cdot}98$ ($P < 0{\cdot}01$). Data points are mean values of three trees.

The decrease in leaf concentration was faster when trunk injection was given prior to spring growth (Fig. 5*a*) than at the other selected stages of phenology, and could be attributed to a combination of dilution by growth and translocation. However, prior to spring growth, the root sink was weak (Whiley and Schaffer 1994), which was reflected by the low concentration of H_3PO_3 that accumulated in the roots when trees were injected at that time. Conversely, leaf H_3PO_3 concentrations following treatment at summer shoot maturity, when vegetative and fruit sinks had weakened, took longer to decline (Fig. 5*c*). This was at a time when the root sink was strong, and resulted in the highest root concentration of H_3PO_3, which was sustained for a longer period than root concentrations resulting from injections at either the beginning or end of spring shoot growth

(Fig. 5). Similar relationships with respect to leaf and root H_3PO_3 concentrations were obtained the following spring from different groups of trees treated in the same manner (data not presented).

Conclusions

Our study confirms previous reports that phosphonate is ambimobile in plants. Following trunk injection there is rapid acropetal movement in the xylem from the treatment site to the leaves. The dynamics of subsequent phloem translocation is determined by the strength of competing sinks when H_3PO_3 enters the symplast. However, there was little redistribution of phosphonate to suggest lateral movement across the tree, demonstrating it to be slow and relatively inefficient compared with vertical movement. Hence, for effective protection of roots, injection sites must be spaced around the full circumferance of the trunk and with correct timing phosphonate translocation to roots may be increased by threefold. In subtropical Australia, disease pressure is greatest during the summer and autumn months when soil temperatures and moisture are optimum for growth and development of the pathogen, and when rapid fruit development imposes further stress on roots of heavily cropping trees. Strategically timed injections of phosphonate fungicides at either spring shoot growth maturity and/or during the mid to late summer months will protect the roots of the tree from colonization by *P. cinnamomi* during this critical period. However, H_3PO_3 concentrations in plant tissues decrease over time due to several factors, and reinjection of phosphonate fungicides will be necessary to prevent phytophthora root rot. Further research is needed to more closely define the optimum concentration of H_3PO_3 required for maximum root protection from fungal invasion.

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Manuscript received 16 August 1994, accepted 9 February 1995

APPENDIX 4

SUPPORTING PUBLICATIONS

Publications listed below were written during the term of enrolment for the Doctor of Philosophy (Agriculture) degree at the University of Natal (1991-94) and cover research activities related to that presented in this thesis.

Blanke, M.M. and Whiley, A.W., 1995. Bioenergetics and water relations of developing avocado fruit. *J. Plant Physiol.* (In Press).

Guest, D.I., Pegg, K.G. and Whiley, A.W., 1994. Control of *Phytophthora* diseases of tree crops using trunk-injected phosphonates. *Hort. Rev.* 00, 00-000. In Press.