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FACTORS INFLUENCING THE OCCURRENCE OF PREMATURE AND EXCESSIVE LEAF ABSCISSION IN THE AVOCADO (PERSEA AMERICANA MILL.) CULTIVAR 'RYAN' AND POSSIBLE PREVENTATIVE MEASURES

By

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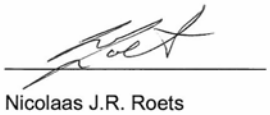
DECLARATION

I, undersigned, hereby declare that the dissertation submitted herewith for the degree M.Sc. (Plant Science) to the University of Pretoria, contains my own independent work and has not been submitted for any degree at any other university.



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Abstract

Premature and excessive leaf abscission during flowering time in the late avocado (*Persea americana* Mill.) cultivar 'Ryan' is a considerable problem for avocado growers. They are especially concerned that premature and excessive leaf abscission will have a negative effect on yield. No previous investigations have been performed where premature and excessive leaf abscission in avocado has been studied in detail. This study therefore aimed to investigate the pattern of premature and excessive leaf abscission in 'Ryan', and compare it with two other important commercial cultivars, 'Fuerte' and 'Hass', which do not display this phenomenon. Time course studies of leaf abscission in the orchard were performed during 2006 and 2007 to determine the pattern of leaf abscission on 'Ryan', Fuerte' and 'Hass'. This also included anatomical studies to determine the time of leaf abscission zone formation. Possible stress factors, which accelerate leaf abscission were also investigated, namely unfavourable climatic conditions (temperature, solar radiation, rainfall, relative humidity and evapotranspiration), nutrient imbalances, excessive flowering and leaf area. The possible impact leaf abscission may have on yield was then assessed by determining levels of reserve carbohydrates in the bark of the tree. In addition, practical solutions, i.e. the application of fertilizers, plant growth regulators (PGRs) and kaolin, were investigated in order to reduce or eliminate premature and excessive leaf abscission. This study was carried out over the period 2005 until 2007, with experiments being modified on an annual basis as information was gathered on the phenomenon.

Experiments began in 2005 with a study on the pattern of leaf abscission in 'Ryan', which revealed an increase in leaf abscission just prior to flowering. However, this increase was not significant. During 2006, the leaf abscission pattern for 'Ryan' was compared with the leaf abscission patterns of 'Fuerte' and 'Hass'. Leaf abscission for 'Ryan' was significantly higher than for 'Fuerte' and 'Hass' during 2006. During 2006 'Ryan' displayed two periods of high leaf abscission, namely the spring flush between bud dormancy and bud swell, and a drastic increase in spring and summer flush leaf abscission between inflorescence development and full bloom. These periods of increased leaf abscission were absent during the 2007 season. In addition, 'Fuerte' and 'Hass'

did not display these peaks of high leaf abscission, with leaf abscission occurring in these cultivars at higher rates from full bloom onwards. Premature and excessive leaf abscission is therefore not an annual event in 'Ryan' and is in all likelihood influenced by external factors. Anatomical studies did not reveal any results in terms of initiation of leaf abscission, with only the protective layer of the abscission zone being visible after leaf yellowing occurred.

During 2006, two peaks of extremely low temperatures (<4°C) occurred just prior to the acceleration of leaf abscission. During the second period of low temperatures, the solar radiation:temperature-ratio was also considerably higher. These periods of low temperatures were absent during 2007, indicating that cold and light stress could be contributing to premature and excessive leaf abscission in 'Ryan' in 2006. In addition, 'Ryan' flowered excessively during 2006, which could have been triggered by low temperature stress just prior to flower initiation. A significant correlation was found between excessive flowering and excessive leaf abscission in 'Ryan' during 2006. The occurrence of reduced flowering in 'Fuerte' and 'Hass' may possibly be due to these two cultivars being more tolerant to stress, and it is possible that 'Ryan' is genetically more prone to excessive flowering than 'Fuerte' and 'Hass'. Excessive flowering could accelerate leaf abscission by causing an unusually high demand for water, nutrients and carbohydrates, resulting in the acceleration of leaf abscission. No significant relationship between nutrient levels and excessive leaf abscission was found for either 2006 or 2007. In addition, no significant correlation could be found between leaf abscission on a branch and the total leaf area of that branch during the 2007 season. During 2007, leaf abscission was low and it is possible that a significant correlation could be found in a season with excessive leaf abscission.

During 2005, chemical applications to reduce leaf abscission did not yield any significant improvement in leaf retention. In fact, the 50 g/tree Solubor® and 50 g/tree Solubor® in combination with 2 kg/tree dolomitic lime had a significant negative effect on fruit set, possibly because too high concentrations were applied too close to fruit set. Chemical applications during 2006 were therefore made at bud dormancy and bud swell, as it was found that leaf abscission occurred before flowering time. However, no effect was observed on leaf

retention or fruit set. During 2007, emphasis was placed on treatments that might reduce stress, as it became evident that stress could be responsible for premature and excessive leaf abscission in ‘Ryan’. Most treatments showed a slight positive effect on leaf retention, but no significant results were obtained possibly because that particular season was a season of low leaf abscission. Further research on application of stress-reducing treatments is therefore recommended. Best farm management practices including optimal fertilization and irrigation is therefore vital to prevent stress, accelerating leaf abscission.

Chapter 1: Introduction

The avocado (*Persea americana* Mill.) is one of the most important subtropical crops in South Africa. With three different cultivars ('Fuerte', 'Hass' and 'Ryan') available to the grower, the period during which avocado fruit are available for marketing is extended (Bijzet, 2001). 'Ryan' is a late season cultivar, which comes into production after 'Fuerte' (February to June – depending on the production area) and 'Hass' (May to August, depending on the production area) (Bijzet, 2001). 'Ryan' comes into production from June to September, thereby extending the marketing period for South African avocados by another month (Bijzet, 2001). 'Ryan', has a considerable problem with excessive leaf abscission during flowering time. According to growers, leaf abscission is so severe that the trees are almost completely deciduous. This problem does not appear to occur in 'Fuerte' or 'Hass'. The leaves that drop during flowering time are important in that they should provide energy for fruit set and early fruit growth, which follows the leaf drop period (Wolstenholme, 2001). Leaf drop during this period may therefore have a negative effect on fruit set and yield, and this has been reported in Israel (Aviles, 1995). The potential negative effect of excessive leaf abscission on yield is also a major concern to South African avocado growers.

Although excessive leaf abscission is known to occur during flowering time in avocado, no literature has been published where this phenomenon was investigated in detail. In all reports where excessive leaf abscission of avocado was mentioned, it would usually consist of a descriptive paragraph based on observations. In all these reports the causes of excessive leaf abscission in avocado were mostly speculative, and included excessive flowering (Davenport, 1982), a combination of excessive flowering and other stress factors (e.g. photo-inhibition, nutrient stress and moisture stress) (Whiley and Schaffer, 1994), and semi-arid environmental conditions and photo-inhibition (Wolstenholme, 2001). A study investigating leaf abscission on avocado and the factors affecting or accelerating this phenomenon was therefore deemed necessary.

Growers have not indicated if excessive leaf abscission in 'Ryan' is an annual event or only occurs under certain conditions (e.g. stressful conditions) in South Africa, and this is still unknown. If excessive leaf abscission on 'Ryan' is not an

annual event and only occurs under stressful conditions, the question is whether or not 'Fuerte' and 'Hass' also display higher leaf abscission under the same conditions. It would therefore be important to determine if excessive leaf abscission can be linked to extreme environmental conditions, or perhaps excessive flowering, all of which may result in stress. In the event of excessive leaf abscission during flowering, the processes of fruit set, early fruit development and new spring flush growth will have to depend heavily on stored carbohydrate reserves, as there will be almost no functional leaves to provide these processes with carbohydrates (Wolstenholme, 2001). Thus, when stored carbohydrate levels are low, excessive leaf abscission may have a negative effect on yield. The effect of excessive leaf abscission on 'Ryan' carbohydrate reserve levels and yield therefore needs to be investigated. Lastly, if excessive leaf abscission is a major problem in terms of production, a solution should be found to prevent excessive leaf abscission on 'Ryan' in order to improve production. Such a solution should be practically applicable to growers.

It can therefore be hypothesized, based on the above discussion, that premature and excessive leaf abscission on 'Ryan' occurs under stressful environmental conditions and can be eliminated if stress-reducing treatments are applied. This study therefore had the following aims:

1) To determine the pattern of leaf abscission in 'Ryan' over more than one season and compare it with leaf abscission in 'Fuerte' and 'Hass', in order to determine if leaf abscission in 'Ryan' is premature and excessive, and if leaf abscission in 'Ryan' is an annual event or not;

2) To link leaf abscission in 'Ryan' with possible stress factors (unfavourable climatic conditions, nutrient imbalances, excessive flowering, and disease) and its effect on starch reserve levels and ultimately yield;

3) To find practically applicable solutions to reduce or prevent excessive leaf abscission in 'Ryan'.

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Chapter 2: Literature Review

2.1 The avocado and its importance for South Africa

The avocado (*Persea americana* Mill.) belongs to the Lauraceae family. This is the same plant family to which the well known South African Black Stinkwood (*Ocotea bullata* (Burch.) Baill.) belongs. Two other well known and economically important plants that belong to this family are the cinnamon tree (*Cinnamomum zeylanicum* Breyn.) and camphor tree (*Cinnamomum camphora* (L) Nees et Eberm) (Robbertse, 2001).

The avocado is native to Central America where it grows as a rainforest substory species (Whiley and Schaffer, 1994). At first, in the 1920's, the avocado was divided into three distinguishable races, namely the Mexican, West-Indian and Guatemalan races based on their area of origin (Bijzet, 2001). However, later Bergh *et. al.* (1973) classified these three races as separate subspecies based on differences in essential oil components in leaves. These were *P. americana* Miller *var. drymifolia* Schlecht & Cham. (Mexican race), *P. americana* Miller *var. americana* (West-Indian race) and *P. americana var. guatemalensis* (Guatemalan race). Today, other clear differences have been described between the races and the Guatemalan race is now classified as *Persea nubigena* L. Wins *var. guatamalensis* (Gaillard and Godefroy, 1995).

It is unknown when the first avocado tree was brought into South Africa, but the introduction of improved cultivars probably occurred during the 1950's (Gaillard and Godefroy, 1995). Today the avocado industry is an economically important industry, with orchards established in most of the tropical and subtropical areas of South Africa (Sippel, 2001). Statistics from the South African Avocado Growers' Association (Figures 1 & 2) show a general increase in the production of avocados over the past four decades. Since 1979, approximately two thirds of the avocados produced in South Africa have been exported, while the remaining one third has been sold on the local or domestic market. Exported avocados earn valuable foreign exchange for the country. Since the beginning of the new millennium, foreign exchange earned as a result of exported avocados exceeded R100-million annually, with a record of R179-million during the 2005 season. Currently South Africa produces 12% of the 3 200 000 tons of avocados

produced worldwide (Imbert, 2008). The avocado industry therefore makes a valuable contribution to the South African economy and to the economy of the areas in which they are produced. It further aids the creation of numerous jobs, which improves the quality of life for people and communities.

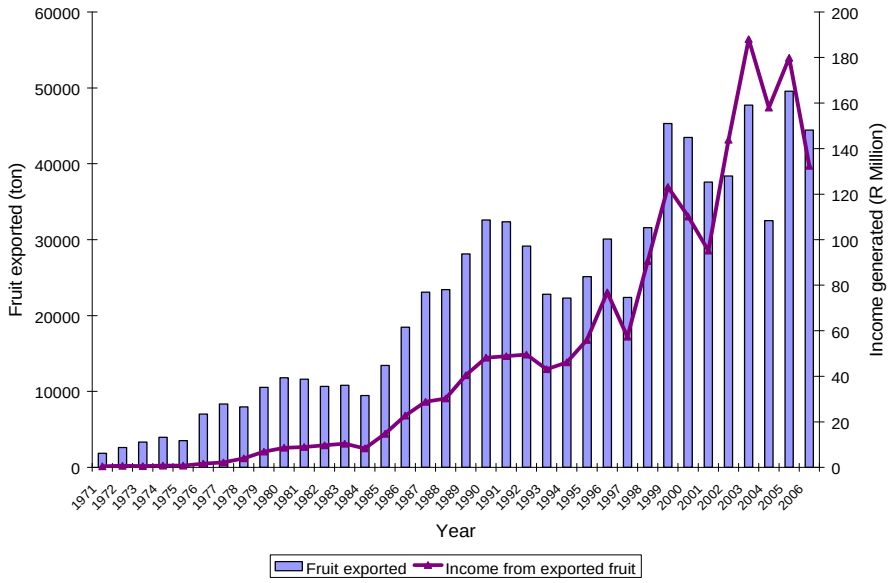

Figure 1: The South African avocado export market during the period 1971 until 2006 (South African Avocado Growers' Association, 2006)

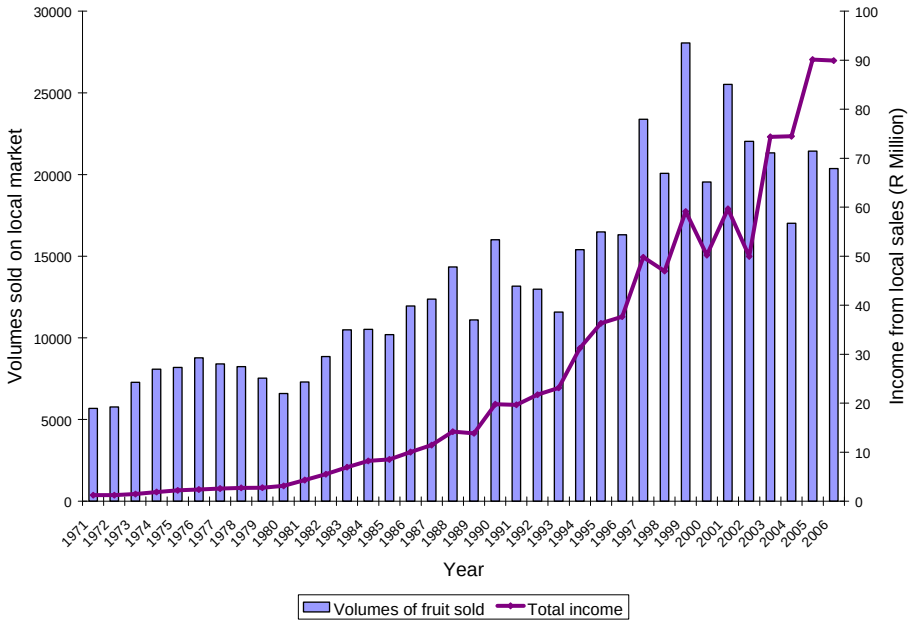

Figure 2: The local South African avocado market from 1971 until 2006 (South African Avocado Growers' Association, 2006)

However, to remain internationally competitive it is vital that the industry improve and keep up to date with the latest agricultural technology. Many problems are experienced with avocado production extending from orchard management to post-harvest problems. As a result of this, the South African Avocado Growers' Association was established in 1967, with one of the aims being to initiate research dealing with the problems of commercial avocado growing and exporting in South Africa (Sippel, 2001).

It is important for the grower to earn an income for as long as possible throughout the year, and preferably throughout the whole year. A single avocado cultivar only produces fruit for a short time during the year (approximately one to three months) and therefore it would be ideal for the grower to have a number of cultivars producing at different times of the year, which would extend the period avocado fruit are available for harvesting, thereby increasing the farmers' income. There are currently four important commercial cultivars available to the South African avocado grower. These are 'Fuerte' (early season), 'Hass' (mid to late season), 'Pinkerton' (harvesting time between 'Hass' and 'Ryan') and 'Ryan' (late season) (Bijzet, 2001). Early season and late season cultivars are especially

important, as they not only extend the period avocado fruit is available for harvesting, but also come into production when fruit supply to the market (locally and international) is low. There will therefore be a higher demand for avocado fruit at that time and growers can expect higher prices for fruit from early and late season cultivars. This study focuses mainly on the late season cultivar 'Ryan'. 'Ryan' fruit can earn higher prices than mid-season cultivars as fruit are in high demand during harvest.

2.2 The late season avocado cultivar 'Ryan'

The late season cultivar 'Ryan' was identified by Edward R. Ryan on the farm Whittier in California (Bijzet, 2001). This cultivar is believed to be a seedling offspring of the rough skinned, purple fruit cultivar, 'Amigo' and belongs to the Guatemalan race (Gaillard and Godefroy, 1995; Bijzet, 2001). It displays medium productivity (approximately 15 to 20 ton/ha) and has a fairly upright growth habit. Fruit are ellipsoid in shape with an average mass of 300-400g, containing a single large seed (Figure 3). Fruit pulp has a cream colour and high oil content (±25%) with a bland watery flavour. On-tree storage of fruit is very poor (Bijzet, 2001).

In areas where 'Ryan' trees are cultivated in South Africa, growers have reported excessive leaf abscission in early spring during flowering. This leaf drop appears to occur mostly from the previous spring and summer flushes. It was further reported by growers that excessive leaf abscission during this time has a negative effect on fruit set and retention and therefore production (W. Retief, personal communication).

Figure 3: Ryan fruit are ellipsoid in shape, green at maturity with cream-coloured fruit pulp and a single large seed (photo courtesy of Z. Bijzet)

Little is known about the conditions under which natural leaf senescence and abscission occurs in avocado and how different flushes affect one another with regard to leaf senescence and abscission. Heath *et al.* (2005) conducted a study on flush development and leaf abscission in the avocado cultivar 'Hass'. They found that when leaf abscission occurred in avocado, it usually started during the early stages of the new flush growth cycle. They also hypothesized that leaf abscission in avocado is triggered mainly by plant hormones and by illumination. They postulated that the primary trigger that initiates leaf abscission is provided by the plant, and is hormonal in nature and that a secondary trigger, such as a reduction in light or a reduction in the total production from a branch, is necessary to cause leaf abscission. Excessive leaf abscission has been reported to occur in avocado, especially under semi-arid conditions (Wolstenholme, 2001) and after long periods of low temperatures (minimum temperatures below 8°C) (Whiley and Schaffer, 1994). It would therefore appear that leaf abscission in avocado can be accelerated by unfavourable environmental factors. To date no scientific study has been conducted on excessive leaf abscission in 'Ryan' and the mechanisms underlying this phenomenon. It is also not known if excessive leaf abscission occurs before the start of the next flush cycle.

2.3 Leaf abscission in plants

Abscission can be defined as the process by which various plant organs are shed (Taiz and Zeiger, 2006). The term abscission should not be confused with senescence, which refers to the death of an organism or part thereof, in which case abscission can follow senescence or abscission can occur without senescence (Taylor and Whitelaw, 2001). Leaf senescence, followed by abscission, is a natural phenomenon that is genetically programmed and highly coordinated in all individual plant species (Thomas and Stoddart, 1980; Sexton and Roberts, 1982; Brown, 1997; Taylor and Whitelaw, 2001; Roberts *et al.*, 2002).

Differences in genetic make-up of individual plants (genotype) results in considerable variation in leaf lifespan between different plant species and individual plants within a specie. Chabot and Hicks (1982) grouped plants in four broad categories based on leaf life span, viz: 1) summergreen plants, which retain leaves for most of the growing season, but for less than one year, such as deciduous plants; 2) wintergreens, where leaves are retained during the growing season and winter where after they are replaced (one year); 3) evergreens, which retain leaves for more than one year, and 4) ephemerals, which maintain leaves for a very short period during the growing season where after they are shed and replaced.

Due to the rainforest origin of avocados, trees are adapted to shade conditions. Leaves are characterized by a relatively short lifespan (9 to 12 months on average) and high leaf turnover rates by means of episodic growth flushes, in which case leaves of older flushes are eventually replaced by leaves of new flushes (Whiley *et al.*, 1988; Whiley and Schaffer, 1994). In addition, leaves also have low photosynthetic efficiency, light saturation at 20 to 25% sunlight and C3-photosynthesis (Wolstenholme, 1987). Due to the formation of episodic growth flushes, where new flushes replace older flushes, and the relatively short leaf lifespan of leaves, avocados are typically wintergreen plants, according to the classification of Chabot and Hicks (1982). Avocado trees are, however, considered by most authors as evergreen and it is possible that leaves can be retained for more than one year (Heath *et al.*, 2005). However, based on average leaf lifespan, avocados should be considered wintergreens. This aspect is

important when leaf abscission and the causes thereof are studied, as leaf lifespan may influence the leaf abscission pattern and the number of leaves abscised by natural aging versus the number of leaves abscised by other factors (e.g. unfavourable environmental factors).

When cultivated under South African conditions, avocado trees display two vegetative growth cycles. The first occurs during or just after fruit set and is known as the spring flush, and the second flush, which occurs during mid-summer, is known as the summer flush (Wolstenholme, 2001) (Figure 4). Both of these flushes are vital for optimal production of carbohydrates for the tree and in supplying an adequate amount of energy for all metabolic processes (Wolstenholme, 2001). Since leaves are the sites of production of primary carbohydrates, excessive and premature abscission of leaves will have a negative effect on carbohydrate accumulation and the total energy budget of the plant, with a subsequent negative effect on production.

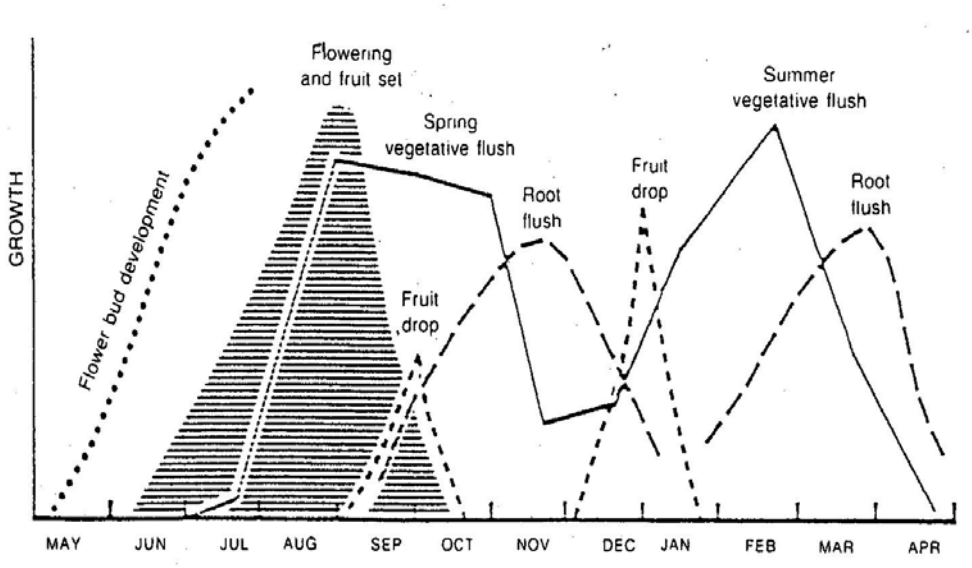

Figure 4: Phenological cycle of avocado trees grown in the southern hemisphere (Whiley *et al.*, 1988)

2.3.1 The abscission zone and differentiation of the abscission zone

Abscission of leaves and other plant organs takes place at predetermined positions called abscission zones (Figure 5) (Sexton and Roberts, 1982; Roberts

et al., 2002). Differentiation of the abscission zones can occur many months before the actual abscission of the organ occurs (Roberts *et al.*, 2002). The number of cells making up the abscission zone varies considerably between species and organs (Taylor and Whitelaw, 2001). Cells of the abscission zone may also differ in their morphology from their neighbouring cells and react differently to hormonal signals than their neighbouring cells. Usually cells in the abscission zone are isodiametrically flattened, more densely cytoplasmic, less vacuolated, have smaller intercellular spaces, contain large starch deposits, and have highly branched plasmodesmata (Sexton and Roberts, 1982; Huberman *et al.*, 1988; Brown, 1997; Roberts *et al.*, 2002). Deposition of suberin and lignin and accumulation of calcium oxalate and tannins were found to occur in cells of the abscission zone of citrus leaf and fruit explants (Huberman *et al.*, 1988). In general, the abscission zone consists of two discernable layers, namely the separation layer at the distal end, where actual abscission takes place, and a protective layer which protects the abscission scar after abscission at the proximal end of the abscission zone (Sexton & Roberts, 1982).

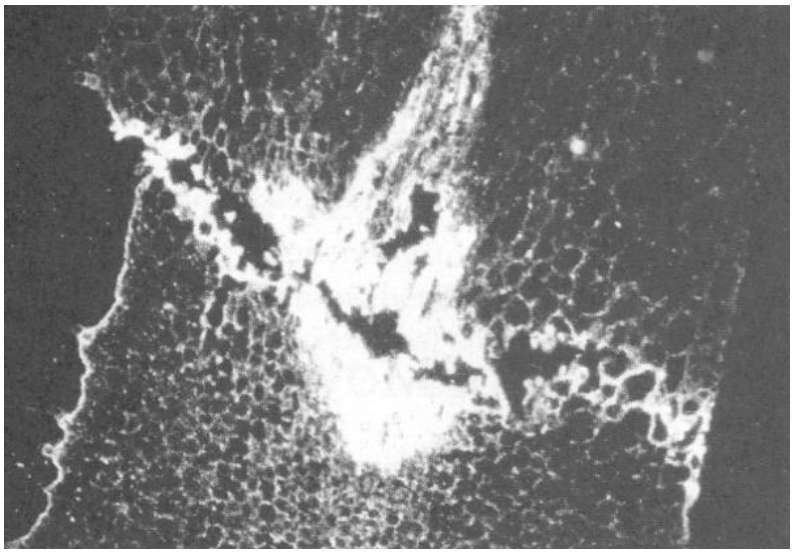

Figure 5: Immunochemcial localization of cellulase mRNA in the leaf abscission zone of a bean plant, showing that abscission takes place only at predetermined positions, which are the abscission zones (Salisbury and Ross, 1994)

2.3.2 Hormonal control of leaf abscission

Abscission of any plant organ is mostly under hormonal control (Salisbury and Ross, 1992; Brown, 1997). There are five major groups of plant hormones (Bearder, 1984) (Figure 6), of which four are involved in abscission (Taiz and Zeiger, 2006). It was shown in early experiments that auxins and ethylene play a major role in abscission, with a high auxin to ethylene ratio preventing abscission and a low auxin to ethylene ratio enhancing abscission (Hall, 1952). This is still the most widely accepted theory and many experiments have illustrated that ethylene application enhances abscission and auxin application delays abscission (Brown, 1997). Some of these experiments include prevention of abscission by application of the synthetic auxin, naphthalene acetic acid (NAA) (Abeles and Rubinstein, 1964), and inhibition of auxin flow to the abscission layer with ethylene application, which subsequently promoted abscission (Beyer Jr. and Morgan, 1971; Beyer Jr., 1973). In another experiment, confirming the role of ethylene and auxin in abscission, Chatterjee and Leopold (1965) showed that as the leaf aged, its sensitivity to auxin decreased and leaf abscission was promoted. These authors also showed that there are two stages of hormonal control of leaf abscission by auxins during leaf aging: 1) where auxin inhibited abscission (younger leaves); and 2) where auxin promoted abscission (older leaves). Following the experiments performed from the 1950's to the 1970's, the following two-stage model for the regulation of leaf abscission by auxin and ethylene was proposed by Brown (1997): Stage 1: The abscission zone is unresponsive to ethylene. As auxin flux from the leaf blade through the petiole declines, ethylene sensitivity increases. Stage 2: The abscission zone is sensitive to the accelerating effect of ethylene and insensitive to the inhibiting effect of auxin. The abscission zone becomes sensitive to ethylene as the auxin flux from the leaf blade declines with leaf age. Ethylene is, however, not always a requirement for abscission and it is rather the balance between different plant hormones that may be the key factor in regulating the timing of abscission (Roberts *et al*., 2002). It remains unclear if ethylene is an inducer of abscission or just a potent accelerator of abscission (Gonzalez-Carranza *et al.*, 1998). In addition, it is only the cells of the separation layer that are responsive to ethylene (Taylor and Whitelaw, 2001), and the expression of cell wall degrading enzymes is regulated by ethylene at the m-RNA level (Brown, 1997).

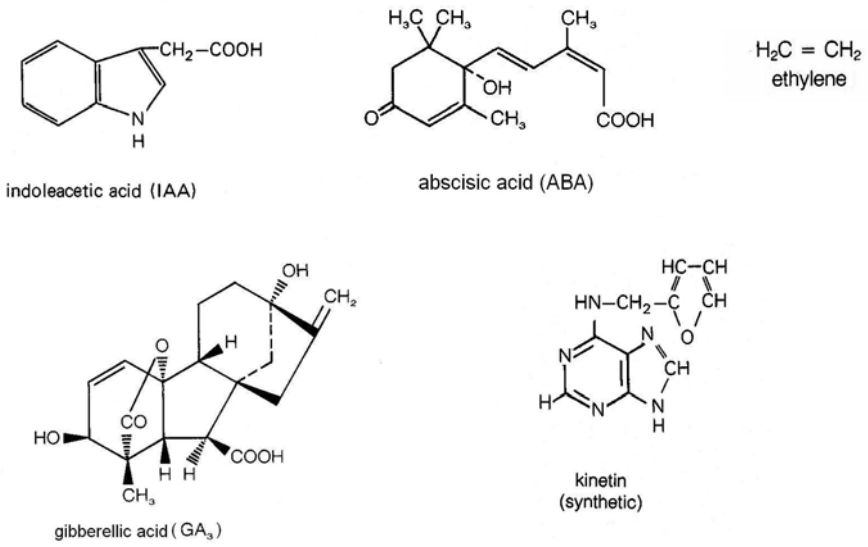

Figure 6: The five major groups of plant hormones (auxins, e.g. indole acetic acid; abscisic acid; gibberellins, e.g. gibberellic acid (GA_3); cytokinins, e.g. kinetin; and ethylene) (Salisbury and Ross, 1994)

Cytokinins are apparently also an important plant hormone controlling organ senescence in plants, including leaf senescence (Sakakibara, 2006). The exact role of cytokinins in the control of abscission is, however, uncertain as cytokinins neither induce abscission (Munné-Bosch & Alegre, 2004) nor regulate cell separation directly (Sexton & Roberts, 1982). Cytokinin application improved chlorophyll retention and delayed senescence in bean explants, but had no effect on intact plants (Jacoby & Dagan, 1970). This delay was due to maintaining high cytokinin levels, as cytokinin levels decrease with age (Taiz & Zeiger, 2006), which is apparently necessary for progression of leaf senescence and abscission (Munné-Bosch & Alegre, 2004). However, in a study performed on lemon (*Citrus limon* (L.) Burm.) it was found that leaf aging did not accompany a decrease in cytokinin levels (Ilan & Goren, 1979). It therefore appears that there is no general trend in cytokinin levels that can be linked with the prevention of leaf senescence and abscission and it can be concluded that cytokinins are not always involved in leaf senescence and abscission. On the other hand, it was shown that application of high levels of exogenous applied cytokinins can promote abscission, possibly by stimulating ethylene production (Grossmann & Hansen, 2001). Enhanced

fruitlet abscission in litchi (*Litchi chinensis* Sonn.) by the exogenous application of high concentrations of cytokinins was demonstrated by Roets (2003).

A third plant hormone, abscisic acid (ABA) is involved in leaf senescence, especially under unfavourable climatic conditions (Gonzalez-Carranza *et al.*, 1998; Lim *et al.*, 2007). Leaf senescence is enhanced by ABA, probably by its direct stimulation of senescence genes or by increased generation of reactive oxygen species (ROS), especially H_2O_2 (Lim *et al.*, 2007). Although this still remains open to debate, it was proposed that ABA stimulates higher production of ethylene and in this way may trigger abscission (Gonzales-Carranza *et al.*, 1998; Roberts *et al.*, 2002). Abscisic acid is therefore clearly involved in the enhancement of leaf senescence, but its role in triggering leaf abscission is not clear at this stage.

On the basis of hormonal control, the process of leaf abscission occurs in three distinct phases viz, 1) leaf maintenance phase, 2) shedding induction phase, and 3) shedding phase (Taiz and Zeiger, 2006). The leaf maintenance phase occurs prior to any stimulating signal that initiates abscission and the leaf remains healthy and functional. At this point the auxin gradient between the stem and leaf blade maintains the abscission zone in a non-sensitive stage (Taiz and Zeiger, 2006). During the shedding induction phase the leaf receives the signal for abscission (Munné-Bosch and Alegre, 2004; Taiz and Zeiger, 2006). This phase is usually entered when photosynthesis drops below a certain threshold level and the leaf costs the tree in terms of water and nutrients (Taylor and Whitelaw, 2001). As a result there is a reduction in the stem to leaf blade auxin gradient, which increases the sensitivity of the abscission zone to ethylene (Brown, 1997). During the shedding induction phase, there is also a decrease in cytokinin and gibberellin levels in the leaf blade (Taylor and Whitelaw, 2001). In the shedding phase the sensitized cells in the abscission zone respond to low endogenous levels of ethylene, resulting in the subsequent production of cell wall degrading enzymes and abscission (Roberts *et al.*, 2002; Taiz and Zeiger, 2006). During leaf abscission, plant hormones may trigger biochemical events in the abscission zone that eventually result in cell separation.

2.3.3 Biochemical and molecular changes associated with abscission and the shedding process

A common feature of all cell separation processes, including leaf abscission, is the degradation of cell walls by cell wall degradation enzymes (Roberts *et al*., 2002). Plant cells are different to animal cells in that they possess cell walls, which are mainly composed of cellulose (Taiz and Zeiger, 2006). Plant cells are further connected to each other by the middle lamella, which is composed primarily of pectin and protein and contains no cellulose, as found in the primary cell wall (Roberts *et al.*, 2002; Jarvis *et al.*, 2003). To keep the pectin molecules in an insoluble state, they are cross-linked and kept together by covalent ester, amide or glycosidic-like bonds, or by cations, such as calcium (Jarvis *et al.*, 2003). The cellulose-containing cell wall and cells connected by the middle lamella provide important support to the plant.

After leaf abscission is initiated in the cells of the separation layer, wall degrading enzymes are produced. Firstly, ethylene binds to receptor proteins in the cell membranes of cells in the separation layer, which results in a reaction cascade that leads to the transcription of genes coding for enzymes involved in abscission, namely cellulases (β-1,4-glucanases), polygalacturonases and possibly expansins (Roberts *et al.*, 2002; Jarvis *et al.*, 2003). It is proposed that there is also an increase in ethylene receptors in cells of the separation layer as it becomes more sensitive to ethylene (Taylor and Whitelaw, 2001). Gene coding of cellulases is repressed by exposure to indole acetic acid (IAA) and upregulated, specifically in the abscission zone, by ethylene (Roberts *et al.*, 2002). From experiments performed on tomato, different isoenzyme forms of cellulase were identified (Gonzales-Carranza *et al.*, 1998), but it is not certain if all the different isoenzyme forms contribute to cell wall separation processes (Roberts *et al.*, 2002), or if they act at different sites of abscission (Gonzales-Carranza *et al.*, 1998). Cellulase is probably not directly involved in cell separation but possibly causes hemicellulose turnover, which allows for cell expansion, which in turn generates the mechanical forces necessary for cell separation (Brown, 1997). Hemicelluloses, such as xyloglucan, are responsible for linking microfibrils in the cell wall together (Cosgrove, 1999). Polygalacturonase hydrolyzes pectins in the middle lamella, which then become soluble resulting in the disappearance of the middle-lamella (Brown, 1997; Gonzales-Carranza *et al.*, 1998). Expansins

possibly also play a role in cell separation by disrupting polysaccharide adhesion in the cell walls, which results in cell wall loosening and cell expansion or cell enlargement (Cosgrove, 1999; Cosgrove, 2000). Expansins are therefore important in cellular enlargement, but their role in cell wall loosening, that results in cell separation, remains to be assessed (Cosgrove, 2000). However, the same principle as for cellulase might possibly also apply here, where cell expansion may generate the mechanical force necessary for cell separation. The action of all these enzymes results in cell enlargement in order to contain the turgor force in the protoplast, with subsequent rounding of cells perpendicular to the abscission zone. The stress created as a result may force a break in the separation layer, and together with mechanical forces, such as wind and gravity, cells in the separation layer are forced away from each other and the leaf is shed (Brown, 1997).

During the abscission process enzymes are produced to protect the exposed surface against pathogen invasion after shedding. These include peroxidases, uronic acid oxidases, chitinases, β-1,3-glucanases and pathogen-related proteins (Brown, 1997; Gonzales-Carranza *et al.*, 1998; Roberts *et al*., 2002). Enzymes of the jasmonic acid biosynthesis pathway, which are associated with plant defense, are also produced at higher levels in the abscission zone during abscission (Roberts *et al.*, 2002). The genes that transcribe the above mentioned protective proteins and enzymes are activated at the same time as the genes coding for wall degrading enzymes (Taylor and Whitelaw, 2001).

As mentioned, senescence is usually followed by abscission. During the senescence process, changes at a cellular level in the leaf to be abscised, occur. These include a decrease in photosynthesis, degradation of proteins, lipid peroxidation, increase in mitochondrial respiration, nucleic acid degradation and conversion of lipids to sugars (Munné-Bosch and Alegre, 2004; Lim *et al.*, 2007). The degradation of lipids and nucleic acids releases phosphorus, while protein degradation releases nitrogen. These two mobile elements are transported out from the leaves for redistribution to other plant organs prior to leaf abscission (Aerts, 1996). During the senescence process, approximately 50% of the total leaf nitrogen and phosphorus gets reabsorbed and redistributed (Aerts, 1996). There are also other mobile elements which get exported from leaves and

redistributed to other plant organs prior to abscission. These include potassium, magnesium, sodium and chlorine (Mengel and Kirkby, 1978; Thomas and Stoddart, 1980). An increase in the levels of reactive oxygen species (ROS) also occurs (Kunert and Ederer, 1985; Munné-Bosch and Alegre, 2004), with a subsequent increase in the levels of antioxidants, especially vitamin C and E (Kunert and Ederer, 1985). However, as abscission progresses, the levels of these antioxidants decrease and levels of ROS increase substantially, resulting in lipid peroxidation and oxidative damage (Kunert and Ederer, 1985), and eventually cell death or necrosis (Munné-Bosch and Alegre, 2004). Loss of cellular integrity due to lipid degradation of cellular membranes results in disruption of cellular homeostasis, which also results in cell death (Lim *et al.*, 2007). Other metabolic changes include the breakdown of chlorophyll, which can be seen as leaf yellowing as carotenoids are unmasked. Carotenoids will also eventually be degraded (Munné-Bosh and Alegre, 2004).

Environmental factors can accelerate leaf senescence and cause premature abscission. The primary role of leaves is to produce primary carbohydrates during photosynthesis, which provides the energy for all other growth processes in the plant (Geiger and Servaites, 1994; Taiz and Zeiger, 2006). Premature leaf senescence and abscission will therefore affect carbohydrate production and crop yields negatively (Lim *et al.*, 2007). Environmental factors that may accelerate leaf abscission or induce leaf abscission prematurely, include extremes in temperature, reduced quality or quantity of light, drought stress, nutrient deficiency, wounding and pathogen infection (Woeste *et al.*, 1999; Taylor and Whitelaw, 2001).

2.4 External or stress-related factors associated with leaf abscission

2.4.1 Climatic stress (light-induced stress and photo-inhibition)

Under cold and drought conditions, as during winter months, light stress may occur in evergreen and wintergreen species. This is because cold or drought results in a decrease in the overall rate of photosynthesis (Öquist and Huner, 2003). When light intensity is high under these conditions, photon flux through the photosynthesis system exceeds that which can be utilized, with subsequent inhibition of photosystem II and to a lesser extent photosystem I (Demmig-Adams

and Adams, 1992) (Figure 7). This condition is known as photo-inhibition. In addition, high light intensity and photo-inhibition may result in increased free radical production (Powles, 1984; Leshem *et al.*, 1986; Demmig-Adams and Adams, 1992; Foyer *et al.*, 1994; Niyogi, 1999). The plant employs a number of mechanisms and compounds to protect itself against free radicals, which include carotenoids, tocopherols, ascorbate, glutathione, dismutase and possibly catalase enzymes, and the xanthophyll cycle which neutralizes free radicals and protects the photosynthetic system against damage (Leshem *et al.*, 1986; Demmig-Adams *et al.*, 1997; Noctor and Foyer, 1998; Møller, 2001). However, it might happen that the production of free radicals may exceed the protection offered by the above mentioned compounds and enzymes, with subsequent damage to the ultrastructure of leaves (Leshem *et al.*, 1986; Demmig-Adams and Adams, 1992). Under cold stress situations that lead to light stress, it was found that auxin transport from the leaf blade to the petiole decreased resulting in increased sensitivity of the abscission zone to ethylene, which subsequently initiated leaf abscission (Michaeli *et al.*, 2001). Therefore, cold and light stress can result in premature leaf abscission.

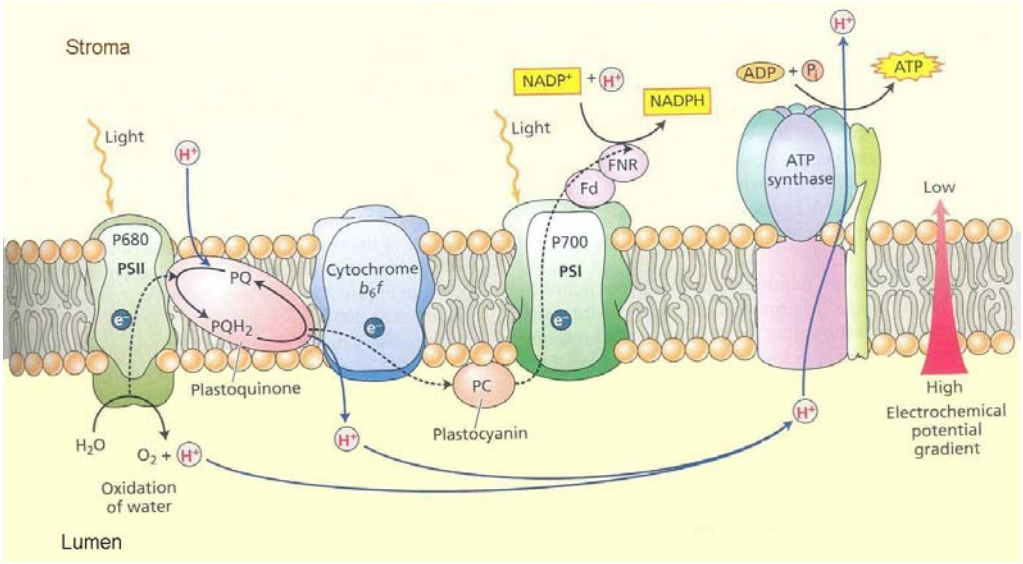

Figure 7: The light reaction of photosynthesis, which is influenced by high light intensity during light stress (PSII = photosystem II; PSI = photosystem I; Fd = ferrodoxin; FNR = ferrodoxin-NADP reductase) (Taiz and Zeiger, 2006)

Photo-inhibition is a readily observed phenomenon in overwintering plants (Öquist & Huner, 2003), including avocado. During winter conditions where average minimum temperatures fall below 8°C, it was found that avocado leaves were photo-inhibited and chlorophyll photo-oxidation took place (Whiley and Schaffer, 1994). This was found to accelerate leaf abscission during flowering, especially when additional stress was experienced. Accelerated leaf abscission results in trees being dependent on stored carbohydrates during flowering and fruit set, as the spring flush that replaces the abscised leaves is still a net user of carbohydrates during this period (Whiley and Schaffer, 1994). Low levels of stored carbohydrates will therefore possibly result in low fruit set and production, if a high rate of leaf abscission occurs during flowering. Keeping summer flush leaves healthy and photosynthetically active until the time the leaves of spring flush become net exporters of carbohydrates will possibly reduce the impact of leaf abscission on fruit set and result in better production.

To prevent leaves from suffering the consequences of photo-inhibition and damage and to keep them photosynthetically active, it is important to protect them against excessive sunlight during winter. Several cultural practices have been developed to aid in protection of several fruit trees against excessive sunlight, including shade nets, evaporative cooling and kaolin based particle films (Cohen *et al.*, 2005; Gindaba and Wand, 2005; Glenn, 2005). Kaolin applications reduced fruit and leaf temperature with subsequent reduction in solar damage to fruit and leaves in apple [*Malus sylvestris* (L.) Mill. *var domestica* (Borkh) Manst.] (Glenn *et. al.*, 2001; 2002; Gindaba and Wand, 2005). Glenn *et al.* (2002) also reported that damaging UV-radiation was highly effectively reflected by kaolin. In addition to the protection of the leaves against excessive sunlight, these cultural practices also reduced transpiration, and improved water use efficiency and photosynthesis when the chosen crops were subjected to high light intensities (Cohen *et al.*, 2005; Glenn, 2005).

2.4.2 Water stress

Drought is known to affect plant productivity negatively and may also cause leaf abscission under extreme conditions (Brown, 1997; Munné-Bosch and Alegre, 2004). During the development of water stress a number of physiological events occur over time, which may result in drought-induced leaf senescence.

The first visual symptom when a plant experiences water stress is wilting, which is the result of water loss by cells (Grobbelaar *et al.*, 1987). The onset of water stress will also induce changes in gene expression, which elicits a physiological response to the water stress. During this response to water stress, drought tolerance genes and genes involved in ABA-biosynthesis, namely the 9-*cis*-epoxycarotenoid dioxygenase (NCED) genes, are activated (Agusti *et al.*, 2007). NCED-genes code for the enzyme catalyzing the rate limiting step in ABA biosynthesis (Agusti *et al*., 2007) (Figure 8). Abscisic acid biosynthesis occurs in the roots (Zeevaart and Creelman, 1988; Nambara and Marion-Poll, 2005), chloroplasts of the leaves (Sembdner *et al.*, 1984) and the guard cells in leaves (Zeevaart and Creelman, 1988). The ABA synthesized in the roots is transported to the leaves via the transpiration stream (Zeevaart and Creelman, 1988; Nambara and Marion-Poll, 2005), and thus serves as a root-to-shoot signal. This results in a significant increase in leaf ABA levels, which induces stomatal closure thereby protecting the plant against excessive water loss (Hsiao, 1973; Leshem *et al.*, 1986; Leung and Giraudat, 1998). However, because CO_2 needed for CO_2-assimilation mainly enters through the stomata, stomatal closure for prolonged periods will affect CO_2-assimilation negatively (Hsiao, 1973). In addition, prolonged stomatal closure may also result in an increase in leaf temperature, which may affect other biochemical processes in the leaf, since enzymes are temperature dependent (Hsiao, 1973).

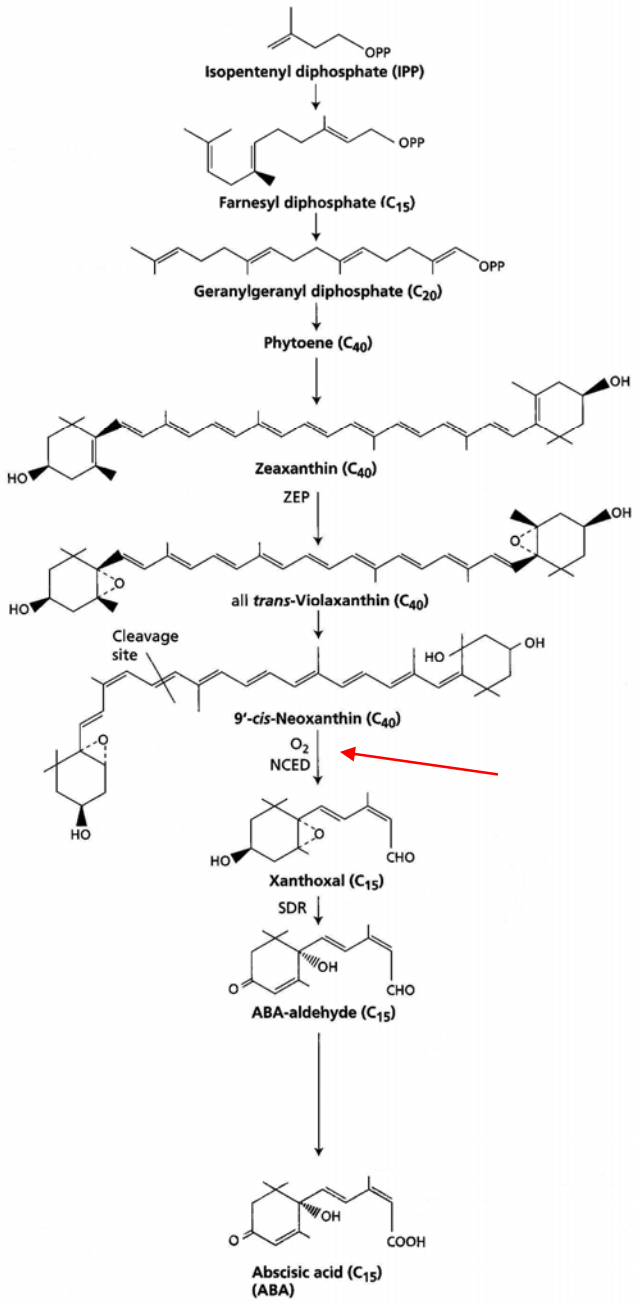

Figure 8: The abscisic acid (ABA) biosynthesis pathway where the rate-limiting reaction, indicated by the red arrow, is the cleavage of 9'-cis-neoxanthin by NCED to yield xanthoxal (Taiz and Zeiger, 2006)

Other hormonal changes include a rapid increase in levels of ethylene during the onset of water stress (Hsiao, 1973; El-Beltagy and Hall, 1974; Evans, 1984; Brown, 1997; Taylor and Whitelaw, 2001). It is speculated that the increase in ethylene is caused by increased levels of ABA, as ABA is believed to stimulate ethylene production (Chen *et al.*, 2002). It is not certain how ABA stimulates ethylene production, but it was found that when leaves of wheat (*Triticum aestivum* L.) and leaves and roots of loblolly pine (*Pinus teada* L.) were subjected to water stress, there was an increase in the rate of conversion of S-adenosyl-methionine (SAM) to 1-aminocyclopropane-1-carboxylic acid (ACC), the rate-limiting step in ethylene biosynthesis (Figure 9). Higher ACC levels resulted in a higher rate of ethylene production in both plant species (Apelbaum and Yang, 1981; Stumpff and Johnson, 1987). There was, however, no direct evidence that ABA influenced the conversion of SAM to ACC. Alternatively, it may also be possible that drought stress directly stimulates higher production of ethylene by increasing the rate-limiting reaction of ethylene biosynthesis (Grossmann and Hansen, 2001). In addition, the sensitivity of cells in the abscission zone to ethylene may also increase during water stress (Chen *et al.*, 2002). Ethylene production therefore increases during water stress either as a result of the effect of ABA or as a direct result of the impact of drought stress on ethylene production. The increase in ethylene levels and higher sensitivity of abscission zone cells to ethylene during drought stress might therefore accelerate leaf abscission under water stress conditions. A decrease in the levels of cytokinins also occurs during water stress (Itai and Vaadia, 1971; Taylor and Whitelaw, 2001; Munné-Bosch and Alegre, 2004), but it is not evident if decreased cytokinin levels contribute to accelerated leaf abscission during water stress.

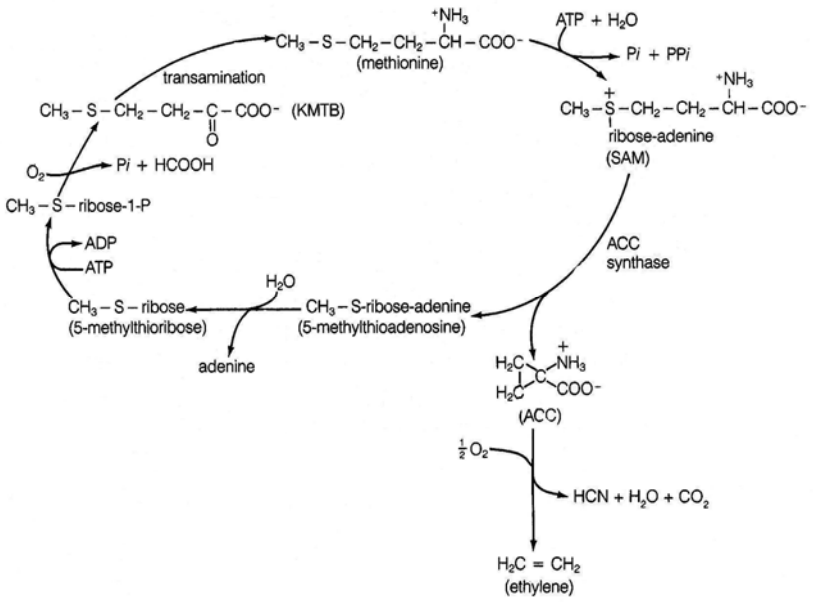

Figure 9: The ethylene biosynthesis pathway: conversion of S-adenosyl-methionine (SAM) to 1-aminocyclopropane-1-carboxylic acid (ACC), catalysed by ACC synthase, is the rate-limiting reaction in this pathway (Salisbury and Ross, 1994)

When the plant is exposed to drought stress for prolonged periods, drought-induced senescence of leaves occurs. Drought-induced senescence displays the same symptoms as normal senescence, which includes chlorophyll breakdown, nutrient mobilization, increased levels of ROS and antioxidants, loss of cellular integrity, cell starvation, and eventually cell death (Munné-Bosch & Alegre, 2004). Abscission will follow cell death and excessive leaf abscission may result under prolonged drought stress conditions. This, in turn, will have a negative effect on carbohydrate production, accumulation and crop production.

A plant's response to water stress is influenced by genotype, possible acclimation to previous stresses, phase of growth and the part of the plant exposed to stress (Tobin *et al.*, 1999; Kozlowski and Pallardy, 2001). The avocado is not regarded as a drought tolerant plant. Although the avocado plant has effective epicuticular structures on the shoots and reproductive organs (Whiley and Shaffer, 1994), the stomatal density on leaves and petals of flowers is very high (Wolstenholme, 1987; Whiley and Schaffer, 1994) and water loss through transpiration may

therefore be high. In addition, the avocado has a shallow, relatively inefficient root system with poor water uptake and hydraulic conductivity (Whiley and Schaffer, 1994). Plants with shallow root systems may experience water stress more rapidly and easily than plants with deep root systems, because roots are closer to the soil surface and the closer the soil is to the surface the quicker it will dry out as a result of evaporation (Tobin *et al.*, 1999). Avocado trees with their shallow root system will therefore experience water stress relatively easily. Water stress has a negative effect on a range of physiological processes in avocado, including photosynthesis, protein synthesis, solute accumulation, leaf anatomy and gas exchange (Ramadasan, 1980; Chartzoulakis *et al.*, 2002). Proper irrigation management is therefore crucial to prevent water stress and ensure optimal tree performance and production. On the other hand, avocado is also flood-sensitive because avocado roots need highly aerobic conditions for survival and functioning and cannot survive in anaerobic conditions created by flooding (Whiley and Schaffer, 1994). In addition, flooding may also increase the risk of *Phythophthora cinnamomi* infection (Gaillard and Godefroy, 1995), as free soil water is required for the reproduction, spread and growth of *P. cinnamomi* (Manicom, 2001).

2.4.3 Nutrient stress

After water stress, nutrient disorders are the most important limiting factor to crop production. Deficiencies of nutrients or nutrient imbalances (macro- and micronutrients) have a negative effect on crop production as well as on tree performance and development (Rashid and Ryan, 2004). It is, however, unclear if nutrient deficiencies can induce leaf abscission. Evergreen and wintergreen plants occur in most cases in nutrient-poor environments and nutrient redistribution from older leaves to new leaf flushes and reproductive organs occurs naturally after leaf senescence of the older leaves is induced (Aerts, 1996; Wright and Cannon, 2001; Franklin and Agren 2002). Redistribution of nutrients under nutrient-poor environments is an adaptation to increase plant fitness, competitiveness and chance for survival under these conditions (Aerts, 1996). Drossopoulos *et al.* (1988) found that nutrient deficiencies in tobacco were responsible for chlorophyll degradation but not necessarily for induction of leaf abscission. On the other hand, under conditions of low nutrient availability competition between leaves and reproductive organs increases and leaf senescence which will be followed by abscission, of especially the older leaves, could possibly be induced to allow distribution of the limited nutrients to developing and reproductive organs (Thomas and Stoddart, 1980; Rabe, 1990; Munné-Bosch and Alegre, 2004). It is only the mobile nutrients, namely nitrogen, phosphorus, potassium, sodium and chlorine that can be distributed to other organs in need of them (Mengel and Kirkby, 1978; Thomas and Stoddart, 1980; Aerts, 1996). There is therefore a possibility that leaf abscission of only the older leaves could take place under nutrient deficient conditions.

Nutrient deficiencies were also found to cause a decrease in the production of cytokinins (Munné-Bosch and Alegre, 2004), however, as discussed previously cytokinins are not always linked to leaf abscission, but could play a role in preventing leaf senescence. It was also shown in cotton that nitrogen deficiency, in particular, increased the sensitivity of plants to water stress by increasing ABA levels and inducing stomatal closure at higher water potentials (Radin, 1981). Higher sensitivity to water stress may therefore exacerbate the impact of nutrient stress on leaf abscission. It is therefore possible that leaf abscission may be increased under conditions of nutrient stress, due to the impact it has on a plant's sensitivity to water stress conditions, which contribute towards leaf abscission.

Leaf nitrogen levels decline during the winter in avocado (Wolstenholme, 2001), which may be due to nitrogen redistribution in response to induction of leaf senescence, or nitrogen redistribution in response to low availability of nitrogen from the soil. Usually nitrogen deficiency symptoms in avocado are characterized by pale, small-sized leaves and premature or early leaf abscission of these leaves (Bar *et al*., 1987). There may therefore be a link between premature leaf abscission and nitrogen levels in avocado. Nitrogen fertilization in tobacco delayed leaf maturation and senescence (Drossopoulos *et al.*, 1980). If a nitrogen deficiency is the cause of excessive leaf abscission, it could be delayed by application of nitrogen during summer (Whiley and Schaffer, 1994).

In general, it is always important to ensure proper fertilization of tree crops to avoid nutrient stress situations and ensure optimal production (Rashid and Ryan, 2004). As for most other agricultural crops, avocado tree nutrient levels are determined by analyzing the nutrient content of leaves. Time of sampling of leaves for nutrient analysis and the position of the leaves sampled are critical to obtain accurate levels. The correct time, (April, after the summer flush has hardened off) and leaf to be sampled for avocado were determined by Koen and Du Plessis (1991) (Figure 10). Optimum levels for avocado nutrition were published by the South African Avocado Growers' Association (SAAGA Research and Technical Committee, 1990) (Table 1). Through the determination of leaf nutrient levels, fertilizer applications can be made accordingly to rectify any nutrient disorders and deficiencies.

Figure 10: The position of the leaf to be sampled (black coloured leaf) for nutrient analysis of avocado trees (Koen and Du Plessis, 1991), which is the first fully expanded leaf on the most recently hardened-off flush.

Table 1: Leaf analysis norms for avocado (SAAGA Research and Technical Committee, 1990)

Element	Shortage	Below normal	Normal	Above normal	Excess
Nitrogen (Hass) (% of DM)	1.40	1.41-2.19	2.20-2.40	2.41-2.69	2.70
Nitrogen (Fuerte) (% of DM	1.30	1.31-1.69	1.70-2.00	2.01-2.49	2.50
Nitrogen (Ryan) (% of DM)	1.30	1.31-1.89	1.90-2.20	2.21-2.49	2.50
Phosphorus (% of DM)	0.05	0.06-0.07	0.08-0.15	0.16-0.24	0.25
Calcium (% of DM)	0.50	0.51-0.99	1.00-2.00	2.01-2.99	3.00
Magnesium (% of DM)	0.25	0.26-0.39	0.40-0.80	0.81-0.99	1.00
Sodium (% of DM)			0.01-0.06	0.06-0.24	0.25
Sulphur (% of DM)	0.05	0.06-0.19	0.20-0.60	0.61-0.99	1.00
Chlorine (% of DM)			0.07-0.23		0.25
Copper (ppm of DM)	3	4	5-15	16-24	25
Iron (ppm of DM)	40	41-49	50-150	151-249	250
Manganese (ppm of DM)	19	20-49	50-250	251-749	750
Molybdenum (ppm of DM)	0.01	0.02-0.04	0.05-1.00		
Zinc (ppm of DM)	20	21-24	25-100	101-299	300
Boron (ppm of DM)	14	15-49	50-80	81-149	150

* *DM = dry mass*

2.4.4 Diseases

Pathogen infection may either retard or accelerate leaf abscission (Thomas and Stoddart, 1980). After pathogen infection occurs, gene expression is triggered to cause cell death (programmed cell death) of the cell infected, thereby isolating the area of infection and preventing spread of infection. During this event large amounts of ROS are produced (oxidative burst) that possibly result in cell death (Greenberg, 1997; Van Camp *et al*., 1998). It is, however, not evident if this reaction is specifically for pathogen infection or if it occurs as a general stress reaction (Van Camp *et al.*, 1998).

Ethylene and ABA levels are also often elevated during infection (Leshem *et al.*, 1986; Grossmann and Hansen, 2001). Ethylene production has been shown to promote lesion formation around the infected site, and also regulates the accumulation of jasmonates, which regulate defense-related gene expression (Van Camp *et al.*, 1998; Taylor and Whitelaw, 2001). In addition, it was shown by Weidhase *et al.* (1987) that addition of jasmonic acid methylesters accelerated leaf abscission in barley (*Hordeum vulgare* L.). In another case, infection of cotton with the pathogen *Verticillium albo-atrum* resulted in leaf abscission and increased levels of ABA (Taylor and Whitelaw, 2001). In tobacco, levels of ethylene and ABA were also shown to be higher in tissue infected by the tobacco mosaic virus (TMV) than in the surrounding healthy tissue (Grossmann and Hansen, 2001). It is therefore evident that pathogen infection can stimulate or accelerate leaf abscission by increasing levels of ethylene, ABA or jasmonates.

The most important disease in avocado globally is the root rot disease, *Phytophthora cinnamomi* (Zentmyer, 1972). When trees are infected with the disease, leaves are often small and yellowish, and they typically abscise during flowering time. Trees die back gradually and show a decline in production (Pegg, 1991; Gaillard and Godefroy, 1995; Manicom, 2001) (Figure 11). Avocados in South Africa are mostly grafted on ‘Duke 7’ rootstocks, which are to a large extent tolerant to the disease (Zentmyer, 1972) and very effective chemical control can be achieve with stem-injections of fosetyl-Al (Aliette®) and soil drench, foliar sprays and trunk injections of a partially neutralized solution of phosphorous acid (Guest *et al.*, 1995). Therefore, the probability that *Phytophthora* root rot could be the primary cause of leaf abscission in ‘Ryan’ is unlikely, as the disease

is a general problem in all avocado cultivars (i.e. if present, the other cultivars will also abscise leaves excessively) and South African avocado growers chemically controls the disease with Aliette® and phosphorous acid. Since Aliette® and phosphorous acid has been used for the past 20 years the possibility of development of resistance to these chemicals cannot be excluded as less sensitive isolates of root rot to Alliette® were found by Duvenhage (1994). If resistance against these chemicals could develop in future it will have detrimental effects on the South African avocado industry, and therefore alternate control measures need to be evaluated. Research has been initiated on the use of water soluble silicon as an alternative against the disease (Bekker *et al.*, 2006). However, this research is still in the early experimental stages. There are no other diseases documented on avocado that are known to cause excessive leaf abscission.

Figure 11: Typical symptoms of an avocado tree infected with *Phythophthora cinnamomi*, which include small yellowish leaves, leaf abscission, a wilted appearance, tree decline and branch die-back (Manicom, 2001).

2.5 The effect of excessive leaf abscission on tree carbohydrate levels and yield

Carbohydrates in all plants are primarily produced in the leaves via photosynthesis (Hatch and Slack, 1970), from where it is transported, mainly as sucrose, via the phloem (Eschrich, 1970; Oparka and Cruz, 2000) to organs where it is utilized or stored, mostly as starch, for later use (Turner and Turner, 1975; Daie, 1985). The build-up of reserve carbohydrates in a plant increases as the rate of photosynthesis increases. Photosynthetic rates are usually optimal if there are sufficient clean, healthy, insect free leaves, exposed to the required light intensity and if conditions, such as temperature, humidity, water and wind are optimal (Janse van Vuuren *et al.*, 1997). Many of these factors are unable to be controlled by the grower. Avocado leaves have a very low photosynthetic capacity (Wolstenholme, 1987) and with the possibility of photo-inhibition during winter (Wolstenholme, 2001), it places a restriction on photosynthesis and carbohydrate production. If a heavy crop occurred during the previous season, carbohydrate levels can be expected to be low during flowering in spring. If carbohydrate levels are low and excessive leaf abscission occurs during flowering, a negative effect on yield is expected. This is because the new spring flush and young fruit that have just set compete for the available carbohydrates, while there is limited carbohydrate production (Whiley and Schaffer, 1994). Competition for low levels of carbohydrates will result in low fruit set and production (Scholefield *et al.*, 1985).

Certain environmental factors can influence or even trigger initiation of alternate bearing in avocado trees. When a tree is in an alternate bearing cycle, it yields a high crop during one season, followed by a low crop yield the following season with this cycle being repeated. During the season with a high crop yield, most reserve food (starch) is used for the fruit, leaving very little accumulated starch to provide for the next season's crop, resulting in a low crop load the next season. During the season with a low crop load, starch reserves build up or increase again to levels favouring a high crop yield for the next season (Wolstenholme, 2001) (Figure 12). Unfavourable climatic and soil conditions, as well as diseases, which result in plant stress, may initiate alternate bearing behaviour, which the avocado is well known for. Once the alternate bearing cycle is initiated it will continue for several years because of its self-perpetuating nature and it will even

increase with age (Monselise and Goldschmidt, 1982). Excessive leaf abscission as a result of environmental stress may possibly initiate alternate bearing. There are a number of horticultural practices that can be employed to reduce alternate bearing, such as the use of plant growth regulators, girdling, pruning and fruit thinning during years of high production (Monselise and Goldschmidt, 1982). In South Africa, Ethephon® (2-chloroethylphosphonic acid) is used very successfully to prevent alternate bearing in litchi (Kift and Roets, 2001; 2002). Usually during an ”off” season, litchi trees display delayed flush due to low carbohydrate levels after a large crop of the pervious season. For successful flowering, the terminal buds must initiate growth when temperatures decline during the onset of winter (stimulus for flower induction) as flower initiation only occurs in growing buds less than 10 mm in length (Olesen *et al.*, 1996). Delayed flush during an “off” season results in buds being dormant during the onset of winter and therefore poor flower initiation with subsequent low yields. Ethephon® is used to control late flush by destroying the apical bud of the branch to stimulate bud growth during the onset of winter, thereby improving flowering and resulting in more constant annual yields.

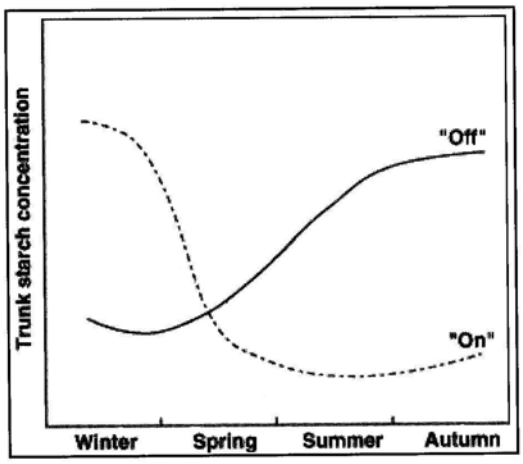

Figure 12: Fluctuation in starch reserve levels during a season of a high crop load (“on” year) and a season of a low crop load (“off” year) (Wolstenholme, 2001)

2.6 Preventing excessive leaf abscission through the application of plant growth regulators

Abscission of different plant organs can be delayed by the use of plant growth regulators (PGRs). The synthetic auxin, NAA was used in a number of experiments to delay or prevent organ abscission, e.g. delayed abscission of 'Red Kidney' bean cotyledons (Abeles and Rubinstein, 1964) and improved fruit retention in litchi (Kift *et al.*, 2002). NAA was also used in combination with other PGRs, such as cytokinins, to prevent or delay abscission, e.g. the use of NAA in combination with the cytokinin 6-benzylamino purine (BA) to improve leaf retention, as well as chlorophyll retention in leaves of avocado cuttings (Raviv and Reuveni, 1984). Other experimental uses of PGRs include the use of ABA to enhance drought tolerance by maintaining higher photosynthetic rates and higher water use efficiency in Jack Pine (*Pinus banksana* Lamb.) (Rajasekaran and Blake, 1999). However, it is important to take into account that PGRs may also enhance abscission. NAA was found to be an effective fruit thinning agent on 'Satsuma' mandarins (*Citrus unshiu* Marc.) (Iwahori and Oohata, 1976). The synthetic cytokinin, CPPU (N-(2-chloro-4-pyridyl)-N'-phenylurea), also enhanced fruitlet abscission in litchi when applied during fruit set (Roets, 2003). Synthetic PGRs have been extensively used as fruit and flower thinning agents for deciduous fruit crops, especially apples (Williams, 1979; Honeyborne, 1993; Costa, 2004; Robinson, 2004) and also pears (Marais, 1987). It is therefore important to bear in mind that PGRs function in very limited concentration ranges, and as a result abscission will either be favoured or prevented depending on the concentration applied. The time or phenological stage at which PGRs are applied is also critical to either favour or prevent abscission. This was demonstrated by Chatterjee and Leopold (1965) on 'Red Kidney' beans, where they showed that NAA application on relatively young leaves prevented abscission, whilst NAA application on older leaves promoted abscission. The time of application, the concentration of the PGR applied, and the specific PGR formulation used is therefore crucial to determining the outcome of a PGR application (Sachs & Hackett, 1972). Although silicon is not a PGR or an essential plant nutrient, it may also potentially reduce leaf abscission. This is because silicon is known to reduce the impact of abiotic and biotic stress that may be experienced by the plant, by strengthening the plant's resistance against stress, thereby enabling the plant to cope better with stress (Epstein, 1999). Therefore, if a number of factors

contribute to leaf abscission, silicon application may strengthen the plant's resistance against these stress factors, thereby reducing leaf abscission.

Apart from timing and concentration, there are other factors that may affect the uptake and mode of action of foliar-applied PGRs. These factors include the structure (e.g. presence of a cuticle) and age of the leaf, climatic conditions (temperature, humidity, light intensity, wind and occurrence of rain), the chemical formulation of the PGR, the surfactant used, and the spraying equipment used. These factors combine to influence the uptake and mode of action of PGRs (Bukovac, 2005; Stover and Greene, 2005).

2.7 Concluding remarks

Leaf abscission is a natural phenomenon that is genetically predetermined and under hormonal control. It is beneficial for any plant to abscise old unproductive leaves as they cost the plant in terms of nutrients, water and carbohydrates. In addition, these old leaves also contain stored nutrients that can be made available to actively growing regions and reproductive organs in the plant. Leaf abscission takes place at predetermined areas called abscission zones, which may differentiate many months before actual abscission. This abscission zone consists of two discernable layers, namely the separation layer, where actual cell separation takes place, and a protective layer that protects the wound left after abscission. Cells of the separation layer are responsive to hormonal signals controlling leaf abscission. Auxin (IAA) and ethylene are the two major plant hormones controlling leaf abscission, with IAA an inhibitor of abscission and ethylene an accelerator of abscission. As the leaf ages, auxin flux from the leaf blade to the abscission zone decreases and the sensitivity of the abscission zone for the accelerating effect of abscission by ethylene increases. At the same time the sensitivity of the abscission zone for the inhibiting effect of abscission by auxins decreases. Leaf abscission is then induced. However, since leaf abscission is under hormonal control and environmental factors can influence hormonal balances in the plant, premature and excessive leaf abscission can occur under environmentally stressful conditions. These stress factors may include one or more of the following: light, temperature, nutrient and water stress, as well as diseases. Although the effect of each stress factor on excessive and premature leaf abscission was discussed, it is highly likely that in an orchard situation more than one stress factor can be present. The combined effect of all the stress factors present may yield a completely different result than the sum of each individual stress (Mitler, 2006). Therefore, if any plant, including avocado trees of the cultivar ‘Ryan’, experiences excessive and premature leaf abscission, it is important to firstly determine which environmental or stress factors contribute towards premature and excessive leaf abscission and consider a combined effect if more than one stress factor or unfavourable condition is present. Thereafter, orchard management techniques should be developed, e.g. application of PGRs and/or adaptations in fertilization and irrigation programmes, to reduce plant stress and prevent excessive and premature leaf abscission.

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Chapter 3: The pattern of leaf abscission in the avocado cultivar 'Ryan'

3.1 Abstract

Excessive and premature leaf abscission was reported by avocado growers to occur for the late season avocado cultivar 'Ryan', with the concern that it may affect yield negatively. Due to a lack of detailed investigations on excessive and premature leaf abscission in avocado, the reasons for this phenomenon remain mostly speculative. This study therefore investigated the timing, pattern and severity of leaf abscission in 'Ryan' over three seasons, by recording abscission of leaves on the most recent spring and summer flushes for 'Ryan', 'Fuerte' and 'Hass'. During 2005, leaf abscission was only investigated on 'Ryan' and most leaves tended to abscise between three weeks before flowering (inflorescence development) and three weeks after flowering (fruit set). During the 2006 and 2007 seasons, leaf abscission in 'Ryan' was compared with 'Fuerte' and 'Hass'. For both seasons overall spring and summer flush leaf abscission was significantly higher for 'Ryan' than for 'Fuerte' and 'Hass'. For all cultivars spring flush leaf abscission was significantly higher than summer flush leaf abscission. As leaves of the spring flush were older than leaves of the summer flush, natural senescence could also play a role in abscission of the spring flush. For 'Fuerte' and 'Hass' during 2006 spring flush leaf abscission tended to increase from full bloom onwards, whilst high spring flush leaf abscission for 'Ryan' already started at bud dormancy and tended to peak between inflorescence development and fruit set. Abscission of the summer flush in 2006 peaked between inflorescence development and full bloom for 'Ryan', whilst it tended to increase from inflorescence development for 'Fuerte' and 'Hass'. The pattern of spring flush leaf abscission during 2007 was different for all cultivars with most leaves abscising between full bloom and fruit set for all cultivars. Summer flush leaf abscission was very low for all cultivars during 2007. Excessive and premature leaf abscission in 'Ryan' is therefore not an annual event and not a characteristic of the cultivar. This therefore leads to the conclusion that excessive and premature leaf abscission in 'Ryan' is most probably triggered by stress.

3.2 Introduction

Excessive leaf abscission during flowering time in the avocado (*Persea americana* Mill.) cultivar 'Ryan' is a major concern to growers. This is because the leaves that abscise should provide carbohydrates for fruit set, early fruit development and new flush growth, all of which follow the period of leaf abscission (Wolstenholme, 2001). Avocado growers in South Africa are therefore concerned that excessive leaf abscission may have an adverse effect on fruit set and production.

Although there have been reports on premature and excessive leaf abscission in avocado (Davenport, 1982; Whiley and Schaffer, 1994; Wolstenholme, 2001), it was in all cases just mentioned and not regarded as a major problem. No detailed quantitative study can be cited where premature and excessive leaf abscission was investigated in avocado. In fact, little is understood about the physiology of natural leaf abscission in avocado and how flushes of different ages influence each other (Heath *et al.*, 2005).

Studies on leaf abscission in avocado should consider the native environment of the avocado in order to understand the adaptations it underwent over time. This should aid in the understanding of the genetic characteristics of the avocado with regard to leaf abscission and the conditions to which leaves are adapted. The avocado is native to central and southern America where it grows as a substory, evergreen species in rainforests, and is therefore adapted to cool and moist forest conditions (Whiley *et al.*, 1988; Whiley and Schaffer, 1994). Avocado trees display episodic growth flushes, resulting in leaves of varying ages and efficiencies being found on the same tree and even on the same branch at any one time (Wolstenholme, 1987; Heath *at el.*, 2005). Leaf turnover is high, and compared with other evergreen species, leaf lifespan of avocado trees is short, with an average lifespan of 9 to 12 months (Whiley and Schaffer, 1994). As a result of evolution under forest conditions, avocado leaves are shade-adapted, having a low light compensation point (Whiley *et al.*, 1988), which increases the probability of light stress under orchard conditions. In addition, leaf abscission in avocado, as in other plant species, may be accelerated by unfavourable climatic conditions and stress (Woeste *et al.*, 1999; Taylor and Whitelaw, 2001). It was reported that where avocado trees are cultivated in semi-arid environments, trees

may be semi-deciduous (Whiley and Schaffer, 1994), but any other stress condition (e.g. nutrient imbalances, diseases, and unfavourable light conditions) may also result in premature and excessive leaf abscission.

Under South African conditions avocado trees produce two leaf flushes per season. The first follows flowering (September) and is called the spring flush, and the second occurs during late summer (February) and is called the summer flush (Wolstenholme, 2001). Although growers reported that excessive leaf abscission on 'Ryan' occurs during flowering time, it is still unknown at this stage when leaf abscission is initiated on each flush and how long it takes for all the leaves to abscise on a given flush. It is also unknown if excessive leaf abscission, observed by growers, is an annual event on 'Ryan' or if it only occurs during certain years. In addition, it would also be important to compare leaf abscission in 'Ryan' with other commercial cultivars in order to ascertain if premature and excessive leaf abscission is specific to 'Ryan', or if it is a general response of avocado trees to stress.

The aim of this study was therefore to determine the leaf abscission pattern of the cultivar 'Ryan' from the time the summer flush hardened off (bud dormancy) to four weeks after fruit set, over three seasons (2005 to 2007) and compare it with the leaf abscission patterns of 'Fuerte' and 'Hass' over the same period. From these leaf abscission patterns it should be possible to explain if leaf abscission in 'Ryan' is excessive and premature and an annual phenomenon, or if it only occurs during certain years. The leaf abscission pattern for 'Ryan' trees can then be placed in context with the rest of the phenological cycle of the tree.

3.3 Materials and Methods

3.3.1 Experimental site

The trial was conducted on the farm Langspruit in the Hazyview area (25°07'S and 31°05'E) during a three year period from 2005 to 2007. The cultivars evaluated were 'Ryan', 'Hass' and 'Fuerte', which were planted in adjacent blocks. For the cultivar 'Ryan', two blocks were selected during the 2005 season: one that was reported by the grower to have high leaf abscission (block 1) and another that was reported to have low leaf abscission (block 2). During the second and third year, only one 'Ryan' block was used since the preliminary study showed that the two blocks did not differ in overall leaf abscission rates. All orchard management practices performed on the farm were the same for all four blocks.

3.3.2 Experimental design

During the 2005 season eight trees (replicates) in each of the 'Ryan' blocks were selected in a random design and eight branches were marked per tree (four on the eastern and four on the western side of each tree) for leaf counts. For the 2006 and 2007 season, 'Fuerte' and 'Hass' were also included with one 'Ryan' block. Sixteen trees (replicates) were selected in a random block design for each orchard and four branches were marked per tree (two on the eastern and two on the western side of each tree) for leaf counts.

3.3.3 Determination of the time and pattern of leaf abscission

During the 2005 season, leaf counts were carried out on the selected branches on each tree at three week intervals, starting from six weeks before full flowering to six weeks after full flowering. Spring and summer flush leaves were not counted separately. During the 2006 season, leaf counts were performed at six phenological stages (Table 1 and Figure 1) for each of the selected branches, for each tree and for each cultivar. In order to compare the two seasons, the same phenological stages were used during the 2007 season. Leaves of the spring and summer flushes were recorded separately during the 2006 and 2007 season. The percentage leaf abscission for each flush and between each phenological stage was calculated by using the following formula:

$$\% LA = (L_i - L_f)/L_i \times 100 \quad (1)$$

where, % LA is the percentage of leaves that abscised between two consecutive phenological stages, L_i is the number of leaves at the first of the two phenological stages, and L_f is the number of leaves at the second of the two phenological stages.

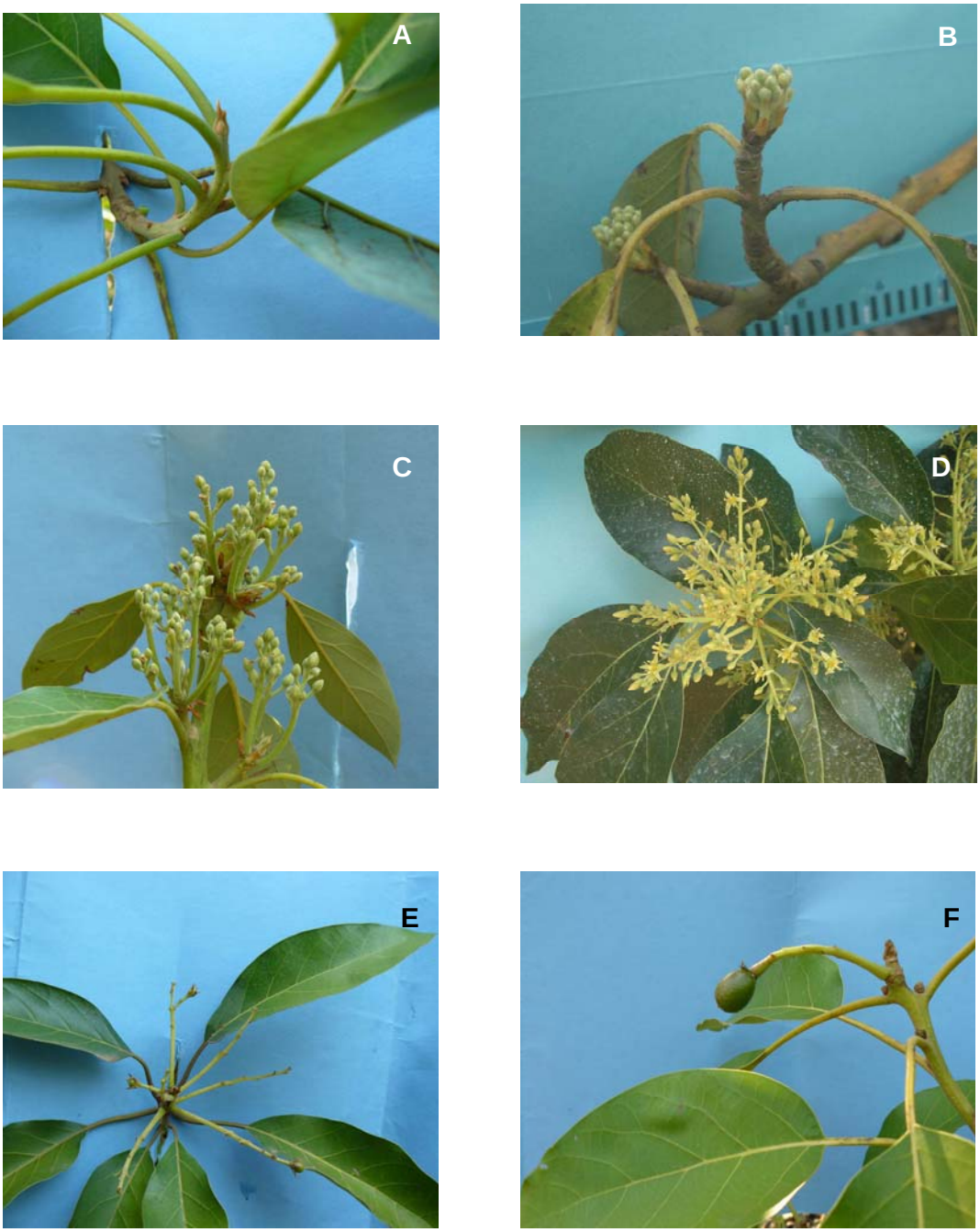

Figure 1: *Phenological stages used for data collection on leaf abscission for three avocado cultivars (A: bud dormancy, B: bud swell, C: inflorescence development, D: full bloom, E: fruit set, F: four weeks after fruit set)*

Table 1: *Phenological stages for data collection on three avocado cultivars. The approximate dates of each of these stages for 2006 and 2007 are indicated*

Phenological stage	Date
Bud dormancy	End-April to Mid-June
Bud swell	Mid-June to Mid-July
Inflorescence development	Mid-July to End-August
Full bloom	End-August to End-September
Fruit set	Mid-October
4 weeks after fruit set	Mid-November

**Note: at bud dormancy spring flush leaves were approximately 8 months old and summer flush leaves approximately 2 months old*

3.3.4 Statistical analysis of data

Data was analysed using the Statistical Analysis System (SAS), version 8.0 (SAS Institute Inc., 1999). For the 2005 season, differences between three week intervals and 'Ryan' orchards (block 1 and 2) were obtained using the General Linear Model (GLM) procedure of SAS. For both the 2006 and 2007 seasons, the differences in leaf abscission between cultivars were also obtained by using the GLM procedure of SAS. Separate analyses were performed for spring and summer flush leaf abscission. Friedman's two way analysis of variance test was used to compare the different phenological stages within each cultivar. The 2006 and 2007 seasons for the spring and summer flushes were analysed separately. The GLM procedure was further used to compare leaf abscission between the two flushes for a given year for each cultivar and phenological stage, and between the 2006 and 2007 season for each cultivar and phenological stage.

3.4 Results and Discussion

3.4.1 Determination of the pattern of leaf abscission

3.4.1.1. Leaf abscission for the 2005 season

When leaf abscission for the two 'Ryan' blocks was compared, a higher percentage of leaves abscised between three and one week before flowering for 'Ryan' block 2 (Figure 2). No significant differences between the two blocks were observed for any of the other weekly counts and finally (six weeks after flowering) there were no significant differences in leaf abscission between the two 'Ryan' blocks (Figure 2). Leaf abscission for both blocks increased from three weeks before flowering (which corresponds with the time of inflorescence development) to three weeks after flowering (fruit set) (Figure 2). From this season's data, it is evident that leaf abscission in 'Ryan' occurred during flowering time as observed by avocado growers. However, in order to determine if leaf abscission in 'Ryan' is premature and excessive, it should be compared with leaf abscission in 'Fuerte' and 'Hass'.

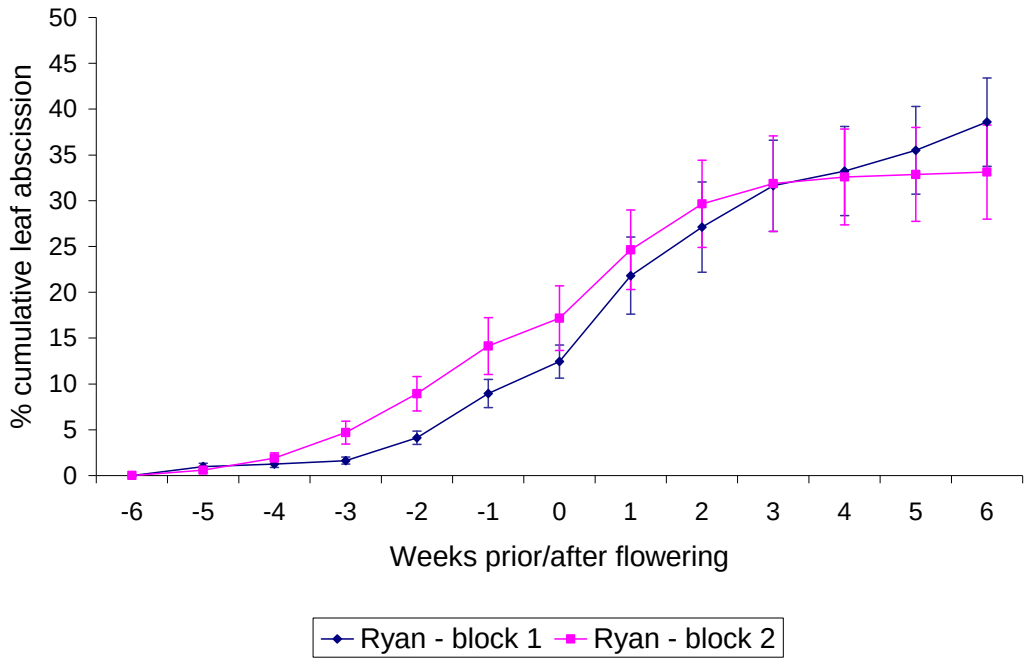

Figure 2: *Leaf abscission pattern for the two blocks of 'Ryan' during the 2005 season*

3.4.1.2 Leaf abscission for the 2006 and 2007 season

During the 2006 and 2007 seasons, leaf counts were performed from when the most recent summer flush had hardened off (Bud dormancy stage – Table 1). At this stage spring flush leaves were approximately 8 months old, and leaf abscission was not evident from both the latest spring and summer flushes.

During the 2006 season, spring flush leaf abscission between bud dormancy and bud swell and bud swell and inflorescence development was significantly higher for 'Ryan' than for 'Fuerte' and 'Hass' (Figure 3A). Between bud dormancy and bud swell, leaf abscission for 'Ryan' was 16.22% higher than leaf abscission in 'Fuerte' and 18.89% higher than in 'Hass'. In addition, between bud swell and inflorescence development, spring flush leaf abscission in 'Ryan' was 13.12% higher than in 'Fuerte' and 14.09% higher than in 'Hass' (Figure 3A). 'Ryan' therefore displayed much earlier abscission of the spring flush leaves than 'Fuerte' or 'Hass'. Spring flush leaf abscission for 'Ryan' was also significantly higher between inflorescence development and full bloom, than for 'Fuerte' and 'Hass', but at this time leaf abscission started to increase for 'Fuerte' and 'Hass', such that between full bloom and fruit set there were no significant differences in spring flush leaf abscission between the three cultivars (Figure 3A). Spring flush leaf abscission was significantly lower for 'Ryan' than for 'Fuerte' and 'Hass' between fruit set and four weeks after fruit set because close to 90% of all spring flush leaves on 'Ryan' had already abscised by this time. Overall, 'Ryan' abscised significantly more spring flush leaves during the 2006 season than 'Fuerte' and 'Hass'.

From the results presented in Figure 3A and B, it is evident that an increase in spring flush leaf abscission occurred from inflorescence development until four weeks after fruit set for both 'Fuerte' and 'Hass' for both 2006 and 2007. For 'Ryan', spring flush leaf abscission tended to peak between inflorescence development and full bloom during 2007. This leaf abscission for 'Ryan' between inflorescence development and full bloom differs significantly from leaf abscission between bud swell and inflorescence development, and fruit set and four weeks after fruit set (Figure 3B).

During the 2007 season, the pattern of spring flush leaf abscission differed from the 2006 season, and total spring flush leaf abscission was significantly lower for the 2007 season when compared with the 2006 season. The high rate of spring flush leaf abscission observed for ‘Ryan’ relative to ‘Fuerte’ and ‘Hass’ between bud dormancy and bud swell, and bud swell and inflorescence development during the 2006 season, did not occur for the 2007 season, resulting in no significant differences in spring flush leaf abscission between the cultivars at these phenological stages (Figure 3B). However, between inflorescence development and full bloom ‘Ryan’ displayed significantly higher spring flush leaf abscission than ‘Fuerte’ and ‘Hass’ (Figure 3B). Between full bloom and fruit set no significant differences in spring flush leaf abscission were obtained between cultivars, while ‘Hass’ displayed significantly higher leaf abscission than ‘Fuerte’ and ‘Ryan’ between fruit set and four weeks after fruit set (Figure 3B). During the 2007 season spring flush leaf abscission in ‘Ryan’ tended to peak between full bloom and fruit set (Figure 3B), which differed from the 2006 season where it tended to peak between inflorescence development and full bloom (Figure 3A). Although spring flush leaf abscission was lower during the 2007 season for all cultivars, ‘Ryan’ still abscised significantly more of its spring flush leaves than ‘Fuerte’ and ‘Hass’ (‘Ryan’ abscised 65.83% of its spring flush leaves, comparing to ‘Fuerte’ which abscised 29.59% and ‘Hass’ which abscised 49.04% of its spring flush leaves).

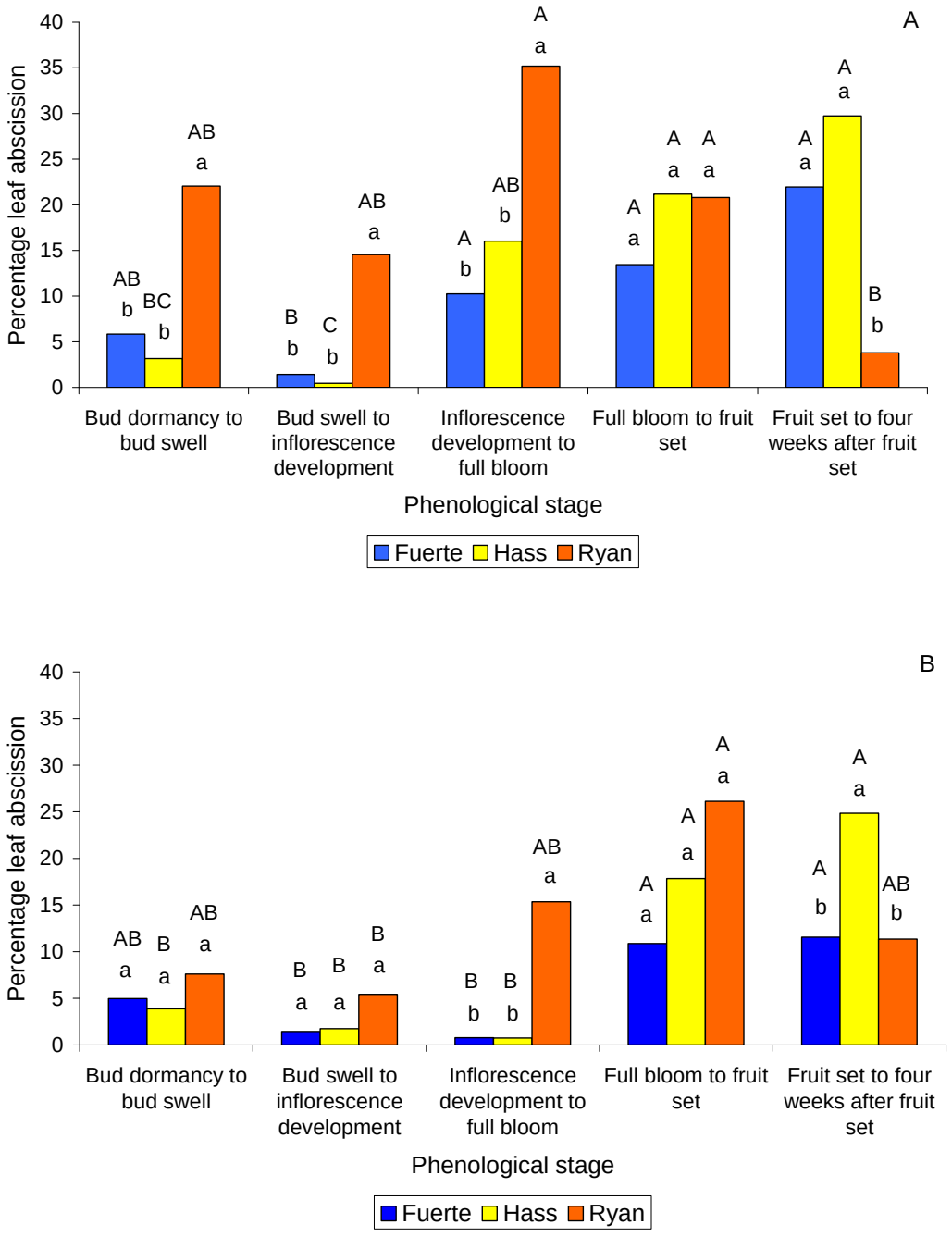

Figure 3: Spring flush leaf abscission for three avocado cultivars for the 2006 (A) and 2007 (B) seasons (upper case letters indicate significant differences between different phenological stages for each cultivar, while lower case letters indicate significant differences between different cultivars for each phenological stage at P = 0.05)

For the summer flush of 2006, no significant differences in leaf abscission between the cultivars were obtained from bud dormancy up until inflorescence development (Figure 4). 'Ryan' displayed significantly higher abscission of its summer flush leaves between inflorescence development and full bloom (Figure 4A), when 32.72% and 28.32% more of its leaves were abscised than for 'Fuerte' and 'Hass' respectively. It was also observed that most leaves that abscised from the summer flush between inflorescence development and full bloom were still green. The percentage of leaves abscised green was not determined. Significantly higher summer flush leaf abscission was also noted between full bloom and fruit set for 'Ryan', as compared with 'Fuerte' (Figure 4A). Between fruit set and four weeks after fruit set, abscission of the summer flush leaves of 'Ryan' decreased again, with 'Hass' displaying significantly higher leaf abscission than 'Fuerte' and 'Ryan' (Figure 4A). Overall in 2006, 'Ryan' displayed significantly higher leaf abscission of its summer flush than 'Fuerte' and 'Hass', with 'Ryan' abscising 77.04% of its summer flush leaves as compared with 19.74% in 'Fuerte' and 39.37% in 'Hass'.

When differences between phenological stages in 2006 are considered for each cultivar separately, 'Ryan' displayed significantly higher leaf abscission between inflorescence development and full bloom, and full bloom and fruit set when compared with the other phenological stages (Figure 4A). Summer flush leaf abscission for 'Ryan' therefore peaked between inflorescence development and fruit set. For 'Fuerte' and 'Hass' summer flush leaf abscission followed the same trend as abscission of the spring flush, with leaf abscission tending to increase from full bloom to four weeks after fruit set. Leaf abscission for these cultivars was significantly higher between fruit set and four weeks after fruit set than any other period (Figure 4A).

Although summer flush leaf abscission during the 2007 season for 'Ryan' was higher than for 'Fuerte' and 'Hass' between inflorescence development and full bloom, and between full bloom and fruit set, values were very low when compared with the 2006 season (Figure 4A and B). The total leaf abscission for all cultivars was significantly lower during the 2007 season when compared with the 2006 season, and therefore no excessive leaf abscission of summer flush leaves occurred for any of the cultivars during the 2007 season. No significant

differences in summer flush leaf abscission were obtained between phenological stages within each cultivar. Overall, ‘Ryan’ only displayed significantly higher summer flush leaf abscission than ‘Fuerte’ during the 2007 season.

The higher leaf abscission obtained for the 2006 season when compared with the 2007 season is an indication that excessive leaf abscission in ‘Ryan’ is not an annual event and may be influenced by external factors. It is also evident that this phenomenon is not restricted to ‘Ryan’, as higher rates of leaf abscission were also observed in ‘Hass’ and ‘Fuerte in 2006. In 2006, 83.42% of all ‘Ryan’ leaves abscised, with ‘Hass’ abscising 56.78% and ‘Fuerte’ abscising 33.97% of its leaves. ‘Hass’ is less tolerant to stress than ‘Fuerte’ (Gaillard and Godefroy, 1995) and this could explain the higher leaf abscission in ‘Hass’. For ‘Ryan’, the peak in leaf abscission observed during the 2006 season is also of importance because at that time summer flush leaves were only six to seven months old, compared with the normal leaf lifespan of avocado leaves of approximately 9 to 12 months (Whiley and Schaffer, 1994). Summer flush leaves therefore dropped prematurely during the 2006 season on ‘Ryan’. Summer flush leaf abscission for ‘Fuerte’ and ‘Hass’ was also significantly higher during the 2006 season when compared with the 2007 season, and premature leaf abscission of the summer flush leaves therefore also occurred for these two cultivars, although to a much lesser extent than for ‘Ryan’.

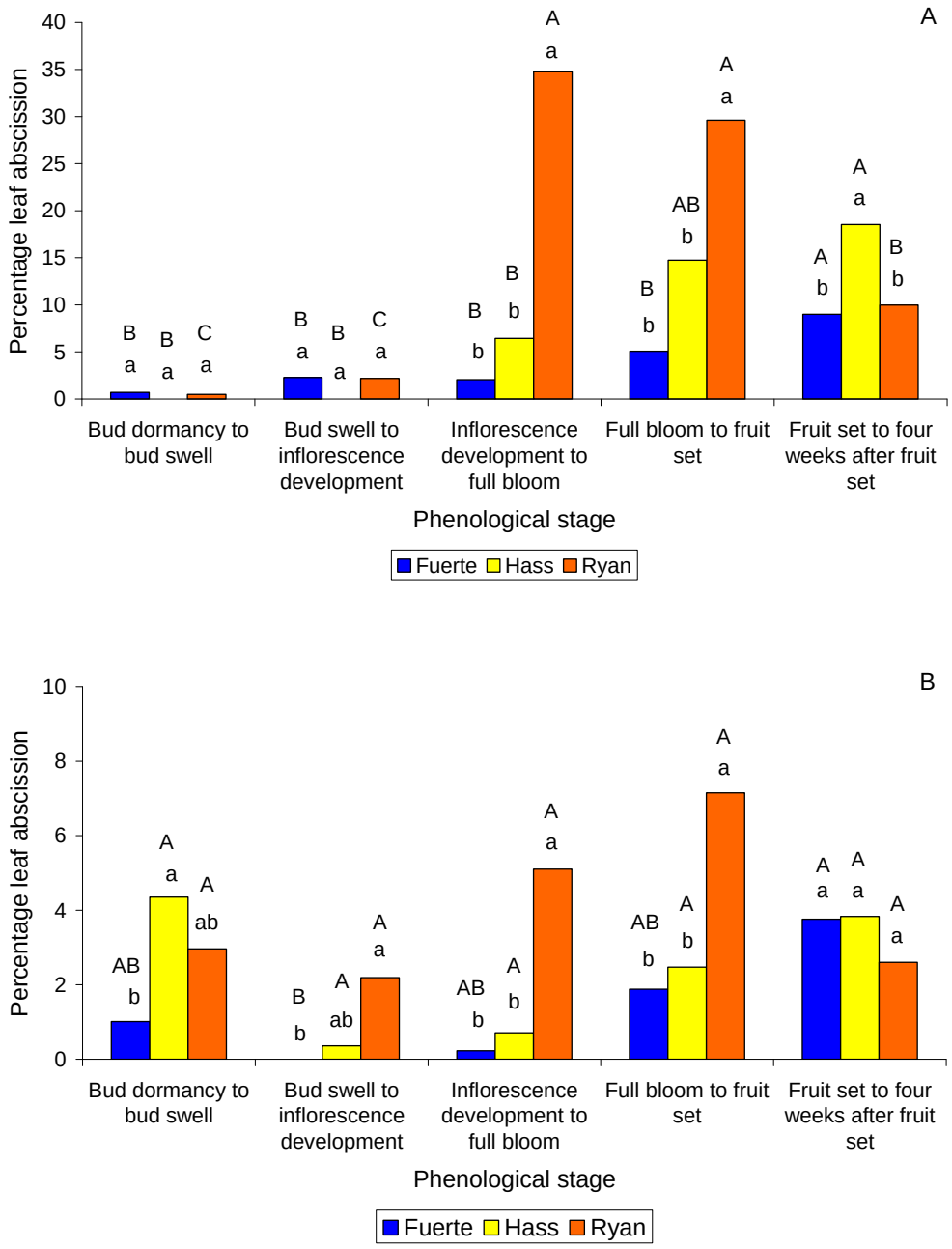

Figure 4: Summer flush leaf abscission for three avocado cultivars for the 2006 (A) and 2007 (B) seasons (upper case letters indicate significant differences between different phenological stages for each cultivar, while lower case letters indicate significant differences between different cultivars for each phenological stage at P = 0.05)

Spring flush leaf abscission was significantly higher for both seasons and all three cultivars than summer flush leaf abscission (Table 2). Although all the evidence suggests that leaf abscission was accelerated during the 2006 season, higher leaf abscission of the spring flush, when compared with the summer flush, should not only be attributed to factors accelerating leaf abscission but also to natural ageing. This argument is strengthened when considering spring and summer flush leaf abscission of the 2007 season, during which leaf abscission was low. The trend in leaf abscission for both flushes during the 2007 season were similar (Figures 3B and 4B), but the spring flush abscised significantly more leaves then the summer flush. These spring flush leaves, which appeared in September the previous season, were approximately nine to ten months old, and therefore five months older than the summer flush leaves, which compares with the average lifespan of avocado leaves (Whiley and Schaffer, 1994). Therefore, it could be possible that the spring flush leaves were approaching natural senescence at this point, with a subsequent decline in efficiency and productivity (Lim *et. al.*, 2007), and increased sensitivity to ethylene, which promotes abscission (Brown, 1997). A large number of summer flush leaves abscised green during 2006 for ‘Ryan’, while a small number of leaves abscised for 2007. This indicated that the whole senescence process was bypassed during the 2006 season for a large number of the leaves. It is therefore likely that a severe combination of stresses could cause leaf abscission of especially the summer flush leaves of 2006 and this stress combination was absent in 2007, resulting in lower leaf abscission when compared with 2006. On the other hand, ‘Fuerte’ and ‘Hass’ did not display abscission of green leaves, which could be an indication that these two cultivars were more tolerant to the stresses that cause premature and excessive leaf abscission in ‘Ryan’ and could therefore retain leaves better.

Table 2: *Comparison of mean total spring and summer flush leaf abscission for three avocado cultivars for the 2006 and 2007 seasons respectively*

	Fuerte		Hass		Ryan	
	Mean % leaf abscission[#]	STD	Mean % leaf abscission	STD	Mean % leaf abscission	STD
Year			***2006***			
Spring	52.93a	23.75	70.57a	17.89	96.40a	6.73
Summer	19.74b	11.26	39.73b	20.09	77.04b	16.87
Year			***2007***			
Spring	29.59a	16.91	49.04a	22.12	65.83a	26.22
Summer	6.88b	4.79	11.72b	8.27	20.00b	9.81

[#]*Means in the same column followed by the same letter do not differ significantly at P=0.05*
Different years and cultivars are not compared. The spring and summer flushes for each cultivar and year were compared separately

The timing of the occurrence of premature and excessive leaf abscission in 'Ryan' is also important. Leaf abscission was observed during the onset of the winter (end of April), when temperatures started to drop. As temperatures declined, photosynthesis decreases substantially, with an increase in photo-inhibition and higher occurrence of light stress (Whiley and Schaffer, 1994; Wolstenholme, 2001). During this leaf abscission period, flower initiation and flower development also occurred, processes that place a demand for nutrients, reserve carbohydrates and water on the tree. Should flowering in 'Ryan' be excessive then it may impose additional stress on the tree, due to an unusually high demand for nutrients, water and reserve carbohydrates, which could result in the acceleration of leaf abscission (Davenport, 1982; Whiley and Schaffer, 1994). The acceleration of leaf abscission during the flowering period is supported by the finding that summer flush leaf abscission for 'Ryan' peaked during inflorescence development and full bloom. Other external stress factors, such as extremely low temperature events (Wolstenholme, 2001), nutrient imbalances (Rabe, 1990), water deficits (Munne-Bosch and Alegre, 2004) and diseases (Thomas and Stoddart, 1980), alone or in combination, could also have contributed to the observed premature and excessive leaf abscission and should therefore also be considered. Excessive and premature leaf abscission may

therefore be the result of stress imposed on trees as a result of unfavourable external factors, which can be more severe during certain years. This could explain why this phenomenon is not an annual event. In addition, differences in genetic make-up between cultivars may explain variation in their response to unfavourable external factors causing stress, resulting in the cultivars differing in the severity of leaf abscission (low or excessive and premature). The determination of the impact of stress factors on leaf abscission could therefore be important and based on these findings, practical solutions to prevent excessive and premature leaf abscission could be evaluated.

3.5 Conclusion

Excessive and premature leaf abscission, as reported by growers, was confirmed to occur in the avocado cultivar 'Ryan'. However, it was observed that this phenomenon is not an annual event, as 'Ryan' abscised significantly more leaves during 2006 than during 2007. Most leaves of 'Ryan' trees abscised between inflorescence development and full bloom (including the summer flush leaves, which abscised green), although the older spring flush leaves started to abscise from bud dormancy. The dramatic increase in leaf abscission between inflorescence development and full bloom recorded for 'Ryan' during 2006 did not occur during 2007. In addition, 'Ryan' abscised significantly more of its leaves than 'Hass' and 'Fuerte' although 'Hass' and 'Fuerte' also displayed significantly higher leaf abscission during 2006 than during 2007. The observation that premature and excessive leaf abscission in 'Ryan' is not an annual event, and that leaf abscission in 'Fuerte' and 'Hass' is also higher at the same time, would indicate that external factors, resulting in stress, possibly accelerated leaf abscission. These stress factors could include low temperature, resulting in light stress, nutrient imbalances, drought and diseases. These stress factors could alone or in combination gave rise to a stress response resulting in premature and excessive leaf abscission in 'Ryan' and higher leaf abscission in 'Fuerte' and 'Hass'. The effect of possible stress factors on excessive and premature leaf abscission in 'Ryan' therefore needs further investigation. Genetic differences between the three cultivars might explain the differences in leaf abscission between cultivars, in which case 'Ryan' could be more sensitive to stress than 'Fuerte' and 'Hass' and therefore displayed higher leaf abscission. On the other hand, most leaves abscised between inflorescence development and full bloom for 'Ryan' and thus flowering itself could also affect leaf abscission. It is therefore necessary to investigate differences in flowering intensity between the cultivars as well in order to determine whether flowering (on its own or in combination with other stress factors) also plays a role in excessive and premature leaf abscission in 'Ryan'.

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Chapter 4: Anatomical changes in the leaf base associated with leaf abscission in the avocado cultivar 'Ryan'

4.1 Abstract

In many instances leaf abscission is initiated before any visual indications that the leaf will drop can be observed, e.g. leaf yellowing. However, a distinct abscission zone is usually formed after initiation of leaf abscission. If a leaf abscission zone can be observed before leaf yellowing is evident on 'Ryan' leaves, it will enable a much more accurate determination of when leaf abscission is initiated and thus give an indication of the possible factors responsible for excessive and premature leaf abscission in 'Ryan'. Leaf samples, which included the petiole, petiole base and a piece of the stem to which the petiole was attached, were sampled at five phenological stages for light microscopy studies to determine the time of leaf abscission zone formation. No anatomical changes could be observed at the base of the leaf petiole before leaf yellowing, and it was therefore not possible to determine with anatomical studies if leaf abscission was initiated before leaf yellowing occurred. However, from 50% yellowing of leaves a distinct zone, consisting of three cell layers with thickened cell walls, was formed. Starch deposits were also clearly visible in these cells. This zone of thick-walled cells formed the protective layer of the abscission zone. The separation layer probably formed adjacent to the protective layer and was distinguishable from the rest of the cells in the leaf petiole.

4.2 Introduction

Cell separation is an important event during abscission and is genetically programmed in cells of plants (Roberts *et al.*, 2000). Abscission of any plant organ takes place in defined areas called abscission zones (Sexton and Roberts, 1982; Roberts *et al.*, 2000; Roberts *et al.*, 2002). However, actual abscission of an organ may occur many months after differentiation of the abscission zone, meaning abscission does not always immediately follow abscission zone differentiation (Roberts *et al.*, 2002).

Usually the abscission zone can be distinguished from the surrounding cells. In peach the abscission zone consists of two distinct bands, one band containing small cells with small intercellular spaces and another band having larger cells

with large intercellular spaces (Rascio *et al.*, 1985). The abscission zone of *Sambucus nigra* L. consists of 30 to 40 rows of flattened cells, which were responsive to ethylene (Osborne and Sargent, 1976). In *Quercus cerris* L. cells of the abscission zone are again thin-walled parenchyma cells with many intercellular spaces (Bellani and Bottacci, 1995). It is therefore evident that anatomical characteristics of the abscission zone differ between different plant species, but that, especially in deciduous plants, the cells of the abscission zone are mostly distinguishable from surrounding cells. However, in some instances the abscission zone is not easily distinguishable from surrounding cells. Cells of the abscission zone of the secondary ovary of 'Navel' oranges were similar to surrounding tissue and not easily distinguishable prior to abscission (Lima and Davies, 1984).

Apart from visible differences between the abscission layer and surrounding cells, depositions or disappearance of different compounds may also be evident in cells of the abscission layer, which after staining with different staining techniques for microscopy, render these cells distinguishable from surrounding cells. It was found that phenolic compounds occurred in higher amounts in abscission zone cells than in surrounding cells of peach (Rascio *et al.*, 1985). In *Quercus cerris* L. starch grains are present in the cells surrounding the abscission zone but absent in cells of the abscission zone (Bellani and Bottacci, 1995). Starch was also found to disappear from leaf abscission zone cells of citrus with the progression of abscission (Huberman *et al.*, 1988). High amounts of starch and calcium oxalate crystals also occurred in abscission zone cells of the fruit peduncle (AZ-A) of citrus (Huberman *et al.*, 1988). Abscission zone cells of apple fruit were also darkly stained, but no information on the compound being stained was given in the study (Pandita and Jindal, 1991).

Cell separation in the abscission zone occurs in a specific area, namely the separation layer. It is the cells of the separation layer that respond to hormonal changes, which result in abscission (Esau, 1977; Sexton and Roberts, 1982; Taylor and Whitelaw, 2001). In general, cellular enlargement, degradation of the middle lamella and loosening of the cell wall is observed during cell separation (Valdovinos *et al.*, 1972; Osborne and Sargent, 1976; Sexton, 1976; Brown,

1997). It might be possible that only cell wall loosening will be visible with light microscopy studies.

The aim of this study was to determine the time of leaf abscission zone formation in the leaf petioles of 'Ryan' avocado trees by means of light microscopy. Anatomical differences between cells and the deposition of some compounds within these cells were investigated at five phenological stages and correlated with visible symptoms of leaf senescence. Cell wall loosening, and the stage at which it occurred, was also investigated with light microscopy. As the initiation of leaf abscission is usually followed by the formation of an abscission layer, it was hypothesised that by determining when this layer was formed, conclusions could be made as to what factors influence premature and excessive leaf abscission in 'Ryan'.

4.3 Materials and Methods

This study was performed during the 2007 season on 'Ryan' only. The samples consisted of a piece of stem containing the node, the bud and the lower part of the leaf petiole (Figure 1). Samples were collected at five of the six phenological stages used in Chapter 3, namely: 1) green leaves subtending dormant inflorescence buds; 2) green leaves subtending inflorescences at the bud swell stage; 3) leaves starting to turn yellow at the full bloom stage; 4) leaves showing 50% yellowing at the fruit set stage; and 5) leaves showing 100% yellow discolouration four weeks after fruit set. Ten samples were collected from five trees (two samples per tree), selected randomly in the first three rows of the orchard. At the fist two phenological stages, no leaf yellowing was observed, while at the last three phenological stages a mixture of yellow and green leaves was present on trees. Yellow leaves were in different stages of senescence (Figure 2). After leaves were sampled, they were stored in FAA-solution (ethanol: acetic acid: formaldehyde, 9:1:1 v/v/v). Before sectioning, the samples were thoroughly rinsed in running water for approximately five minutes. A sliding microtome (Reichert-Jung 2040) fitted with liquid carbon dioxide freezing stage was used for making the sections. Sections were between 20 and 30 μm thick and included a longitudinal section of the stem, the base of the petiole and the bud. The sections were stained with toluidine blue and potassium iodate. The

stained sections were mounted in glycerol and viewed under the light microscope (Leitz-Biomed) fitted with a digital camera (Olympus Camedia C4000).

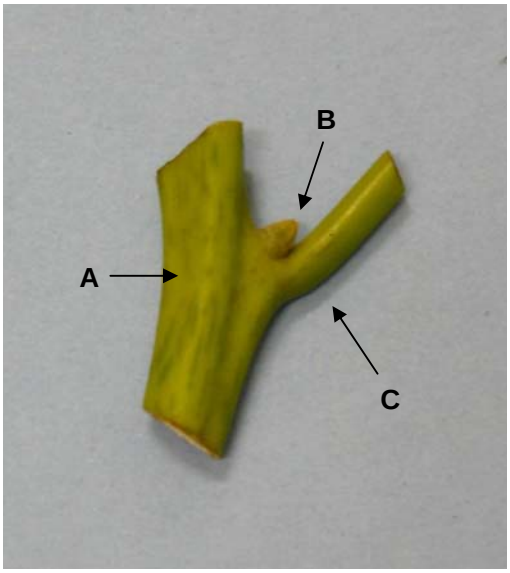

Figure 1: Example of samples taken for making sections for investigating the abscission layer in 'Ryan' avocado. Samples contained the node (A), the bud (B) and lower part of the leaf petiole (C)

Figure 2: A mixture of green and yellow leaves on ‘Ryan’ trees at the full bloom stage (stage 3)

4.4 Results and Discussion

Green leaves were sampled during bud dormancy and inflorescence development to determine if a leaf abscission zone would be formed before visible symptoms of leaf abscission were evident. Green leaves sampled at the bud dormancy stage, showed no anatomical evidence that an abscission layer had formed. At this stage all cells at the base of the petiole were uniform in appearance after staining with toluidine blue or potassium iodate (Figure 3 – A1 to A3). A similar trend was observed when green leaves were sampled at the stage of inflorescence development (Figure 3 – B1 to B3). There was therefore no anatomical evidence of the formation of an abscission layer at any of the stages when leaves were still green. At this stage there might be two possible reasons: On the one hand, anatomical evidence of the abscission layer may only become evident once leaf yellowing had started. On the other hand, this study was performed in a season during which excessive and premature leaf abscission did not occur for ‘Ryan’, and there was therefore a chance that the green leaves sampled would not abscise during that season. In addition, during

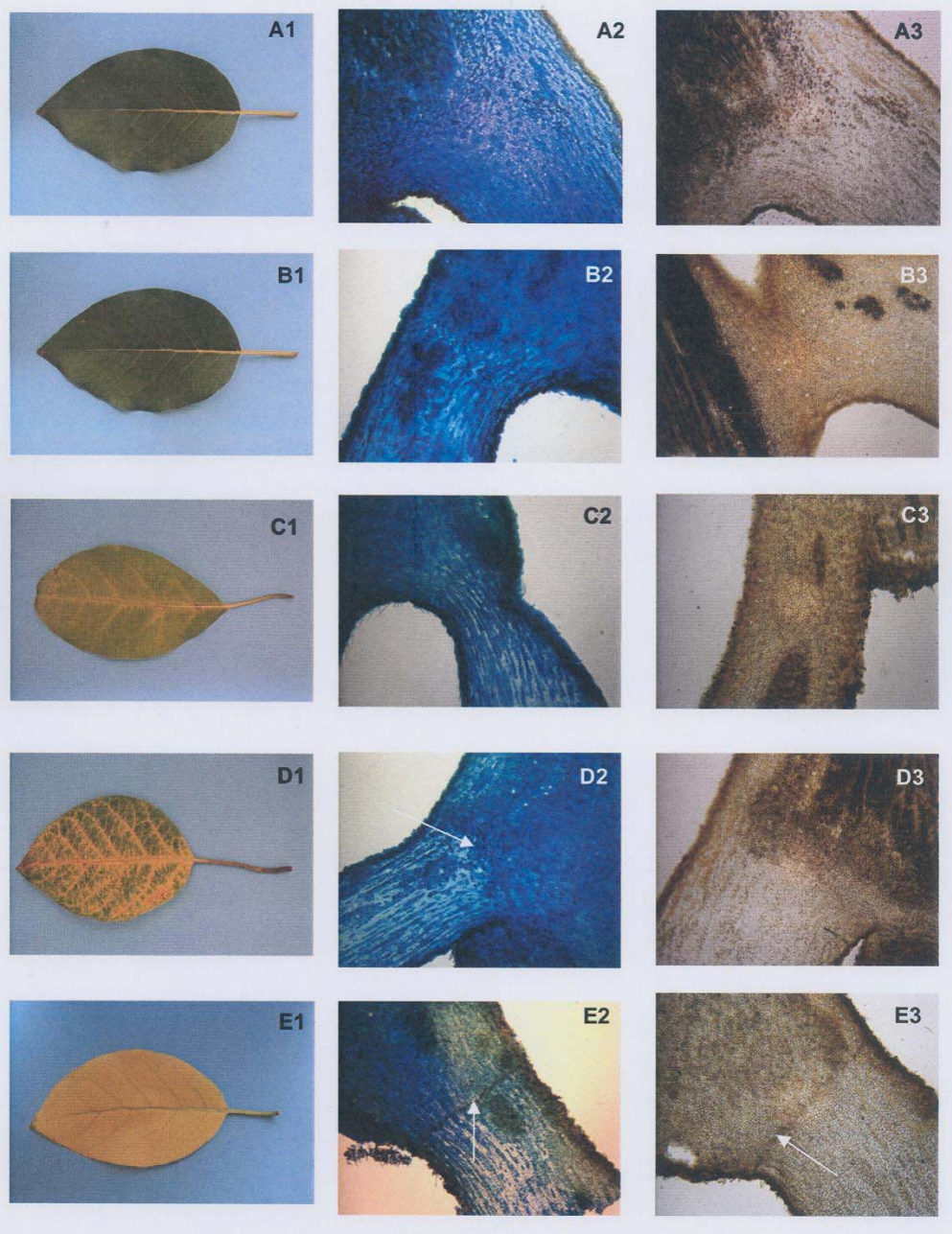

Figure 3: *Different stages of abscission zone development in 'Ryan' avocado leaves. A) Dormant bud stage and green leaves; B) Inflorescence development and green leaves. C) Yellow colouration of leaves started (full bloom); D) 50% yellow colouration of leaves (fruit set); E) 100% yellow colouration of leaves (four weeks after fruit set). All stages contain three photos, with the first show the progression of yellow colouration of the leaves (A1 to E1), then the attachment of the leaf petiole to the main stem at a magnification of 4X stained with toludine blue (A2 to E2) and potassium iodate (A3 to E3). The arrows in Figure D2, E2 and E3 indicate the three layered zone identified at 50 and 100% yellowing of leaves*

the 2007 season no abscission of green leaves was observed as was the case for the 2006 season. The anatomical characteristics of the leaf abscission layer

should therefore be clarified firstly in leaves that already started to turn yellow and whether cells in this area are distinguishable from the surrounding cells.

When leaf yellowing started, cells at the base of the leaf petiole still appeared uniform in appearance with no visible anatomical changes when stained with toluidine blue or potassium iodate (Figure 3 – C1 to C3). Thus, even when leaf yellowing had already started there was still no anatomical evidence for the formation of a leaf abscission layer. However, leaf yellowing had already started to take place, and the abscission layer therefore should have already started to develop. It might be that the abscission layer is not distinguishable from the surrounding cells in the leaf petiole with the microscopic techniques used in this study.

When leaf yellowing proceeded to cover approximately 50% of the leaf surface, a three cell layer zone with slightly thickened cell walls was discernable (Figure 3 – D1 to D3) and became more prominent in sections made from samples taken at 100% leaf yellowing (stage 5) (Figure 3 – E1 to 3, and Figure 4). At this stage, staining with potassium iodate revealed that the concentration of starch grains, in this zone of thick-walled cells was higher than in the surrounding cells (Figure 3 – E3). In general the abscission zone consists of two discernable layers, namely the separation layer and the protective layer (Esau, 1977). The zone of thick-walled cells observed in the leaf base of the 'Ryan' avocados for this study, conforms to the protective layer of the abscission zone. The separation layer may be one or more cell layers thick and is the actual site at which cell separation takes place (Sexton and Roberts, 1982), which, in this case, could form adjacent to the protective layer, but was not distinguishable from the rest of the cells in the base of the petiole. According to Fahn (1982) the protective layer could be either primary, in which case cell walls of primary parenchyma cells become lignified or suberized, or secondary where a periderm is formed. For the avocado cultivar 'Ryan', the protective layer can be regarded as a primary type where the parenchyma cells in the leaf base become thick-walled to form the protective layer. In addition, this protective layer forms shortly before leaf abscission. The time at which the protective layers appear also differ in different plant species and may form just prior to leaf fall (Mauseth, 1988), which was the case for 'Ryan' in this study.

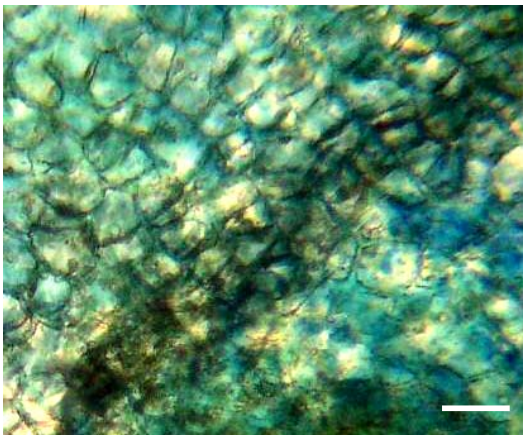

Figure 4: Micrograph showing the abscission layer of a yellow leaf of the avocado cultivar 'Ryan'. The thick-walled protective layer is visible, while the separation layer, which is not visible will be between the protective layer and the rest of the leaf (bar = 1mm)

4.5 Conclusion

There is no anatomical evidence to suggest that leaf abscission zone formation occurs before visible signs of senescence (leaf yellowing) are apparent and therefore there is no indication when leaf abscission was initiated before leaf senescence or yellowing occurred. The formation of a protective layer prior to leaf abscission (from 50% leaf yellowing) was the only anatomical evidence that leaf abscission would take place. This protective layer consisted of three layers of thick walled cells (primary protective layer). Therefore, the first anatomical evidence for the initiation of leaf abscission only became visible when leaf yellowing proceeded to 50%. The separation layer probably formed adjacent to the protective layer but was not distinguishable from the rest of the cells in the leaf base. There is therefore a possibility that the abscission zone formed before leaf yellowing but it was not distinguishable from the rest of the cells in the base of the petiole, as was the case for the abscission zone of the secondary ovary of 'Navel' oranges. This study can further be expanded to include electron microscopy studies to determine changes on ultracellular level in the leaf base in an attempt to define the position and formation of the leaf abscission zone.

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Chapter 5: Factors causing excessive leaf abscission

5.1 Abstract

Premature and excessive leaf abscission is not an annual event in the avocado cultivar 'Ryan' and it is therefore likely that stress-related factors play an important role in this phenomenon. Climatic factors (temperature and light), nutritional status of the tree, flowering and leaf area were investigated in relation to leaf abscission in an attempt to determine which factors might play a role in accelerating leaf abscission. Irrigation and diseases were not investigated because these factors were managed according to best management practices by the grower. The effect of excessive leaf abscission on starch levels and yield was also determined. From the data obtained, no single factor investigated could be identified as the cause of excessive and premature leaf abscission in 'Ryan'. During the 2006 season, increased leaf abscission in 'Ryan' followed two periods of low temperatures (<4°C). In addition, nutrient imbalances (excessively high copper levels and deficient zinc and boron levels) occurred for all cultivars. Nutrients were more balanced during 2007, although excessively high copper levels still occurred. In addition, in contrast to 'Fuerte' and 'Hass', 'Ryan' flowered excessively during 2006. This excessive flowering could place a high demand for nutrients, water and reserve carbohydrates (energy) on the plant. The effect of flowering, together with nutrient and temperature stress, could result in a stress response that caused excessive and premature leaf abscission in 'Ryan' in 2006, when compared with 2007. Excessive flowering and leaf abscission in 'Ryan' resulted in a sharp decrease in starch reserve levels prior to fruit set, and negatively affected fruit set and yield for the next season.

5.2 Introduction

Excessive leaf abscission for the avocado cultivar 'Ryan' is a phenomenon that does not occur on an annual basis (Chapter 3), and could therefore be influenced by external factors or unfavourable environmental conditions. It was reported for other plant species that unfavourable environmental conditions that result in stress can accelerate leaf abscission and cause premature leaf abscission (Taylor and Whitelaw, 2001). It has even been reported that avocado trees can be semi-deciduous during flowering time under semi-arid environmental conditions, where water stress is most likely the trigger for premature and

excessive leaf abscission (Whiley and Schaffer, 1994; Wolstenholme, 2001). Excessive flowering in avocado trees was also reported to impose stress on the trees, thereby triggering premature and excessive leaf abscission (Davenport, 1982; Whiley and Schaffer, 1994).

It is a well known fact that water stress results in premature leaf abscission in many plant species (Munné-Bosch and Alegre, 2004). This is because drought-induced leaf abscission contributes to plant survival under drought stress, as it allows for early diversion of resources from vegetative to reproductive development, remobilisation of nutrients from senescing leaves, and reduction of water loss (Munné-Bosch and Alegre, 2004). As avocado trees are adapted to cool moist forest conditions (Whiley *et al.*, 1988) it could be expected that when grown under semi-arid conditions, premature leaf abscission will occur, resulting in semi-deciduous trees.

The possibility exists that stress factors, other than water stress, may also play a role in the acceleration of leaf abscission in 'Ryan' and these factors should not be excluded. Light and low temperature stress result in photo-inhibition of photosynthesis, which is a phenomenon known to occur in overwintering evergreen trees (Öquist and Huner, 2003). Photo-inhibition is the inhibition of the light reaction of photosynthesis under conditions of high light intensities in relation to low temperatures (Demmig-Adams and Adams, 1992). In avocado, it was reported that during winter when temperatures are low, photosynthesis drops to approximately 50% of its optimal photosynthetic rate (Whiley and Schaffer, 1994). Avocado trees experience cold stress when minimum temperatures drop below 10°C, with cold-induced damage to photosystem II and photo-inhibition (Schaffer and Whiley, 2003). Leaves can be severely damaged when minimum temperatures drop below 4°C, possibly as a result of the production of free radicals (which damage cell walls and cellular components) and chlorophyll oxidation, which could result in premature leaf abscission (Whiley and Schaffer, 1994).

Nutrient stress occurs when nutrients are present in insufficient quantities or when nutrient imbalances occur in the soil. Trees subjected to nutrient stress display similar physiological responses to other forms of stress (Rabe, 1990).

Although nutrient deficiencies do not always result in premature leaf abscission (Drossopoulos *et al.*, 1988), redistribution of the mobile nutrients from older leaves to younger developing organs may induce leaf abscission, especially of the older leaves (Aerts, 1996; Wright and Cannon, 2001; Franklin and Agren, 2002). Nutrient toxicities should also be avoided as it may contribute to excessive and premature leaf abscission (Fernandes and Henriques, 1991). Orchard fertilizer programmes should therefore always be optimally managed to prevent nutrient stress, because apart from premature leaf abscission, nutrient deficiencies also negatively affect yield and tree performance (Rashid and Ryan, 2004).

Diseases often cause excessive and premature leaf abscission (Thomas and Stoddart, 1980). This is because in most cases pathogen infection results in higher ethylene production (Leshem *et al.*, 1986), which accelerates leaf abscission. The root disease, *Phytophthora cinnamomi,* is the most important disease affecting avocado trees globally (Zentmyer, 1972), and is associated with excessive leaf abscission during flowering time (Pegg, 1991; Manicom, 2001). However, very effective control of *Phytophthora cinnamomi* can be achieved with stem-injections of fosetyl-Al (Aliette®) and soil drench, foliar sprays and trunk injections of a partially neutralized solution of phosphorous acid (Guest *et al.*, 1995) and tolerant rootstocks e.g. ‘Duke 7’ and ‘Dusa’. When commercial cultivars are grafted on resistant rootstocks and fosetyl-Al application is done effectively on the farm, it is highly unlikely that *P. cinnamomi* could be a major contributor to excessive and premature leaf abscission in ‘Ryan’.

At present, it can be postulated that factors contributing to or causing premature leaf abscission in ‘Ryan’ avocado trees consist of one or more of the stress factors discussed above. It is, however, most likely that in an orchard situation a combination of environmental stresses, and not just a single factor, act to cause premature leaf abscission in ‘Ryan’ avocado trees. It would therefore be important to investigate more than one stress factor, because the combined effect of many stresses can yield a completely different outcome than the effect of each factor alone (Mitler, 2006). The aim of this study was therefore to determine the causes of premature and excessive leaf abscission in ‘Ryan’ by investigating climatic parameters, tree nutrient status, flowering intensity, and

total leaf area per branch in relation to leaf abscission patterns in 'Ryan'. In addition, the effect of excessive and premature leaf abscission on tree carbohydrate levels and yield was also investigated.

5.3. Materials and Methods

This study was conducted at the same site and on the same trees as described in Chapter 3. This study was conducted over the 2006 and 2007 seasons, and the experimental design for this study was the same as for the leaf abscission study (Chapter 3). Yield data for each orchard and season was obtained from the grower.

5.3.1. Effect of different climatic parameters on leaf abscission

Weather data was obtained from the Hazyview weather station (25°03'S, 31°09'E), which was closest to the farm. Minimum, maximum and average daily temperatures, rainfall, relative humidity, evapotranspiration (ETo) and solar radiation data were obtained for the 2006 and 2007 seasons. The 2006 and 2007 seasons were compared for differences and linked with differences in leaf abscission for the spring and summer flushes between the two seasons.

5.3.2. The relationship between tree nutrient status and leaf abscission

Leaf samples were collected on the same cultivars ('Fuerte', 'Hass' and 'Ryan'), trees (16 trees per cultivar) and phenological stages, as was used for leaf abscission determinations (Chapter 3 – Table 1). The leaf chosen for analysis at each phenological stage, and for both seasons, was the first fully expanded green leaf, after the point where the summer flush and spring flush are connected (Figure 1) (Koen and Du Plessis, 1991). Twenty leaves were sampled per tree, (i.e. 320 leaves per cultivar). Leaf samples were washed with phosphorus-free soap to remove all dirt and substances sprayed on the leaves by the grower. The leaf samples were then oven-dried at 60 °C for approximately 24 h. The dried leaf samples were passed through a 0.5 mm sieve, where after they were prepared for mineral analysis. For determination of P, K, Ca, Mg, Zn, Cu, Mn, Fe and B, samples were prepared by using the wet ashing method. Using the wet ashing method, 0.5 g of sample was placed in a 50 mL calibrated flask, and 1 mL perchloric acid – sulphuric acid mixture (7:1) and 2 mL of nitric acid was added. Pre-digestion was carried out overnight, where after flasks were placed on a hot

plate and slowly brought to boil until a clear solution was obtained (Adrian, 1973). For nitrogen determination, samples were prepared by wet digestion, adding 4 mL 98% sulphuric acid and 3 mL 30% hydrogen peroxide to 0.75 g sample (Novozamsky *et al.*, 1983). The levels of K, Ca, Mg, Zn, Cu, Mn and Fe in the extracts was determined using atomic absorption spectrometry (Varian, SpectrAA 250 Plus atomic absorption spectrometer), while the levels of N, P and B in the extracts was determined colorimetrically with a continuous-flow analyser (Bran & Luebbe, Auto Analyser 3). Leaf nutrient analysis for this study using the above-mentioned methodology was performed by the Soil Science Laboratory of the Agricultural Research Council – Institute for Tropical and Subtropical Crops (ARC-ITSC). Correlations between the levels of elements for each phenological stage and leaf abscission were determined. Nutrient levels at bud dormancy were also compared with the optimal norms determined for avocado (See Table 1, Chapter 2).

Figure 1: Position of the leaf for nutrient analysis, which was the first fully expanded green leaf on the most recent flush (Koen and Du Plessis, 1991)

5.3.3. The relationship between flowering intensity and leaf abscission

The number of flowers per inflorescence and the number of inflorescences per branch were determined just prior to full flowering for the same cultivars, trees and branches as for the leaf abscission study (Chapter 3). The number of flowers per branch was then calculated by multiplying the number of flowers per inflorescence with the number of inflorescences per branch. The mean number of flowers per branch was determined and for each given branch the number of flowers was correlated with leaf abscission on that branch. In addition, flower samples were taken just prior to flower opening and the nutrient levels of the flowers were determined using the same method as described for the determination of leaf nutrient levels. The same nutrients were quantified in flowers as for leaves.

5.3.4. The relationship between total leaf area and leaf abscission

This experiment was only performed during the 2007 season. Five hundred leaves (100 leaves from five trees, 50 on the eastern and 50 on the western side of each tree) from each cultivar were collected after the summer flush had hardened off and the leaf area of all leaves was measured with a leaf area meter (LiCor Model 3100 Area Meter). Thereafter the length and width of each leaf was measured and a relationship established between leaf dimensions (leaf length, leaf width and leaf length x width) and leaf area. This was done in order to establish a non-destructive method of determining total leaf area of a branch, without removing the leaves from the branch, to allow for other measurements to be made on that branch, such as leaf abscission rates. At bud dormancy, the length and width of each leaf on the labelled branches (same branches on which leaf abscission was measured in Chapter 3) were measured, before any leaf abscission occurred. The same cultivars and trees were used as for leaf abscission determinations (Chapter 3). Leaf area of each leaf was then determined using the equation obtained for the relationship between leaf area and leaf length x width. The total leaf area for each labelled branch was then calculated as the sum of the area of all leaves on that branch. The total leaf area on a branch was then correlated with spring flush leaf abscission that occurred on that branch.

5.3.5. The effect of leaf abscission on starch reserve levels and yield

For each of the selected cultivars and trees, samples for starch analysis were collected. Each sample consisted of two bark discs collected from four scaffold branches on each tree (eight bark discs per tree) using a 25 mm bell punch. Bark samples were collected in order to study the fluctuations in starch content over the phenological stages, as Kaiser and Wolstenholme (1994) noted that fluctuations in starch are easier to identify in the bark than in the wood. Bark discs were taken at all the phenological stages at which leaf counts were performed. Thereafter samples were dried in an air-forced oven at 60 °C for 72 h, ground and passed through a 0.5 mm sieve. Starch content of each sample was determined using the modified enzyme-chromogen method for determination of low levels of starch in plant material by Davie (1997).

Ground sample (0.5 g) was placed into a 50 mL centrifuge tube, followed by the addition of 30 mL 80% (v/v) ethanol. The tubes were placed in a water bath at 70 °C for 1 h and shaken every 25 min. After 1 h, the tubes were centrifuged for 20 min at room temperature at 770 *g*. The supernatant was discarded and 30 mL of 80% (v/v) ethanol was added. The tubes were placed again in a water bath at 70 °C for 1 h and centrifuged again for 20 min at room temperature at 770 *g*. After the supernatant was discarded for the second time, the remaining pellet was dried overnight at 60 °C.

The following day the dry pellets were rinsed with 30 mL distilled water and transferred into clean test tubes. Three blanks containing 30 mL distilled water were also added. The test tubes, containing the samples in the distilled water, and the blanks were capped and autoclaved at 115 °C and 70 kPa for 2 h. The test tubes were removed from the autoclave and placed in a water bath at 60 °C. After the samples were allowed to cool down to 60 °C, three drops of toluene, 2.5 mL 2 M acetate buffer (pH 4.6) and 2.5 mL of amyloglucosidase (E.C. 3.2.1.3, Sigma A3042), diluted to 1:65 with distilled water before addition, were added. These test tubes were incubated at 60 °C in the water bath for 4 h and shaken every 20 min, after which they were placed in an incubator overnight at 60 °C.

The following day the test tubes were allowed to cool and the contents decanted and carefully rinsed with distilled water into 50 mL volumetric flasks. The flasks

were made up to volume with distilled water. Approximately 15 mL of the sample was taken from each volumetric flask and 40 mg casein added. The samples were shaken thoroughly and placed in a water bath at 60 °C for 1 h. Thereafter, the samples were filtered through filter paper (Advantec no. 2). Sample (50 μL) was then placed into a clean test tube and 2.65 mL enzyme chromogen-reagent added. The enzyme-chromogen reagent consisted of 0.0874 M disodium hydrogen orthophosphate, 0.0517 M sodium dihydrogen orthophosphate, 0.0164 M benzoic acid, 0.0109 M p-hydroxybenzoic acid and 0.492 mM 4-aminoantipyrine (Sigma A2814). 20 mg L^{-1} glucose oxidase (E.C. 1.1.3.4., Sigma G6641) and 5 mg L^{-1} peroxidase (E.C. 1.11.1.7., Sigma P8125) were also added to the colour reagent. Glucose standards equivalent to 5, 10, 20, 40 and 80 mg g^{-1} dry sample matter were prepared. Standard solution (200 μL) was added to 2.50 mL enzyme-chromogen reagent. Distilled water samples (200 μL) were also added to 2.50 mL enzyme-chromogen reagent to serve as water blanks. All samples, standards and water blanks were placed in a water bath at 40 °C for 15 min after the enzyme-chromogen reagent was added. Thereafter the samples were left at room temperature for 1 h for developing a colour complex. After development of the colour complex, absorbance values were read at 505 nm on a spectrophotometer (Shimadzu Corporation UV265). A standard curve was obtained using the glucose standards and the following formula used to calculate starch content:

$$S = [(A'-b)/a][V/50 \times 1000][0.500/m \times 2] \times 2.4624 \quad (2)$$

where, S, is the starch concentration (mg g^{-1}); A' = Absorbance reading (A) after subtraction of the blank prepared on the second day (bl) (A' = A – bl); b is the y-intercept on the standard curve; a is the slope of the standard curve; V is the volume diluted to in the volumetric flask and m is the mass of sample.

5.3.6 Statistical analysis of data

Differences in minimum temperatures, relative humidity and evapotranspiration between the 2006 and 2007 seasons were obtained by using the paired t-test. The rest of the data was analysed using the Statistical Analysis System (SAS), version 8.0 (SAS Institute Inc., 1999). The General Linear Model (GLM) procedure of SAS was used to compare nutrient and starch levels between phenological stages and between cultivars. The GLM procedure was also used to compare flowering between cultivars and seasons. The Kruskal-Wallis one way analysis of variance test was used to compare nutrient levels within each cultivar between seasons, and to compare the total leaf areas per branch between the three different cultivars. The CORR procedure of SAS was used to determine correlations between leaf abscission and flowering, and leaf abscission and leaf area. This procedure was also used to obtain the best correlations between leaf area and leaf length X leaf width, leaf area and leaf length, and leaf area and leaf width. In all cases where correlations were obtained, Pearson correlation coefficients were used.

5.4 Results and Discussion

5.4.1. Effect of different climatic parameters on leaf abscission

Abscission of the spring flush leaves for 'Ryan' started to occur from the end of April for both the 2006 and 2007 seasons, with spring flush leaf abscission being significantly higher between bud dormancy and bud swell, bud swell and inflorescence development, and inflorescence development and full bloom for the 2006 season when compared with the 2007 season (Figure 2). For the summer flush in 2006, a dramatic increase in leaf abscission occurred between inflorescence development and fruit set. Such an increase was not observed during the 2007 season (Figure 3). Prior to inflorescence development leaf abscission was very low and not significantly different between the two seasons (Figure 3). If leaf abscission is accelerated by stress it would be most likely that this stress would occur prior to the start of leaf abscission.

When the daily minimum temperatures are considered for 2006 and 2007 there were occasions when temperatures dropped below 4°C. For 2006, two such events could be observed, the first occurred between day 80 and 90 (at the stage of bud dormancy) and the second between day 170 and 180 (during inflorescence development) (Figure 2 & 3). For the 2007 season, a single low temperature event occurred between day 70 and 80 (at bud dormancy) (Figure 2 & 3). For both 2006 and 2007, leaf abscission of the spring flush occurred after these periods of low temperature between day 80 and 90 for 2006 and day 70 and 80 for 2007 (Figure 2). The dramatic increase in leaf abscission of the summer flush during 2006 followed directly after the second low temperature period between day 170 and day 180 (Figure 3). During the same time a significant increase in spring flush leaf abscission also occurred (Figure 2).

It was observed in the field that spring flush leaves first turned yellow or senesced before abscission, while most leaves of the summer flush abscised whilst still green during 2006. The finding that increased leaf abscission followed a period of low temperatures, indicated that low temperature stress could play a role in accelerating leaf abscission in 'Ryan' trees. This is further supported by the abscission of green summer flush leaves directly following the second period of low temperatures (days 170-190) during 2006, and contrasting low rates of leaf

abscission in 2007 in the absence of low temperatures during this period (Figure 3). A similar trend is also observed when the ratio between solar radiation and temperature is considered, with a high solar radiation to temperature ratio between days 60 and 90 and between days 170 and 180 for 2006, and the absence of these high ratios during 2007 (Figure 4). An increase in this ratio increases the chances of photo-inhibition and light stress (Wolstenholme, 2001), which could be especially prominent between days 170 and 180 during 2006, possibly contributing to increased leaf abscission after this period in 2006. This abscission was possibly triggered by a decrease in auxin transport from the leaf blade to the abscission zone and an increased sensitivity to ethylene. This phenomenon was noted in *Ixora coccinea*, in that leaf abscission, as a result of low temperature stress (and possibly light stress), was caused by a decrease in auxin transport and increased sensitivity of the abscission zone to ethylene (Michaeli *et al.*, 2001). The higher leaf abscission of the spring flush (especially after the first drop in temperature below 4°C during 2006), when compared with the summer flush, could possibly be attributed to higher sensitivity of the spring flush leaves to low temperature and light stress. The spring flush leaves were approximately five months older than the summer flush leaves and as leaves age, their activity decreases (Heath *at al.*, 2005). With lower physiological activity, it might be possible that older leaves experience stress more severely than younger, more active leaves.

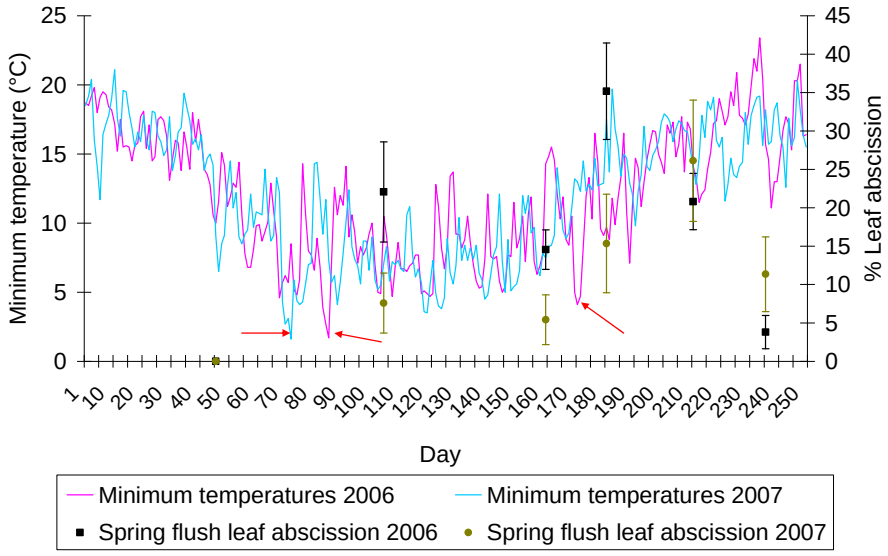


Figure 2: Daily minimum temperatures of the experimental site and spring flush leaf abscission for the avocado cultivar 'Ryan' in 2006 and 2007 (Red arrows indicate events when temperatures dropped below 4°C; day 1 on the x-axis is 15 March with day 250, 19 November)

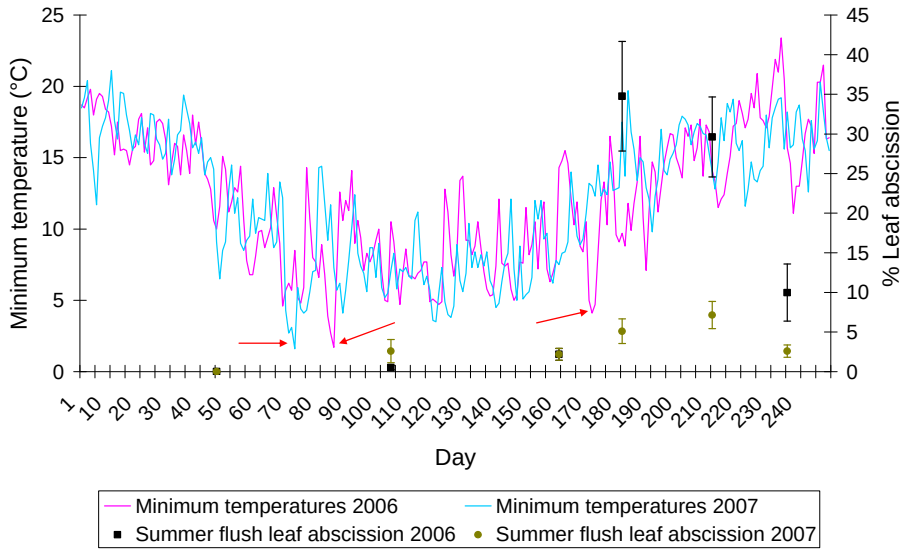


Figure 3: Daily minimum temperature for the experimental site and summer flush leaf abscission for the avocado cultivar 'Ryan' in 2006 and 2007 (Red arrows indicate events when temperatures dropped below 4°C; day 1 on the x-axis is 15 March with day 250, 19 November)

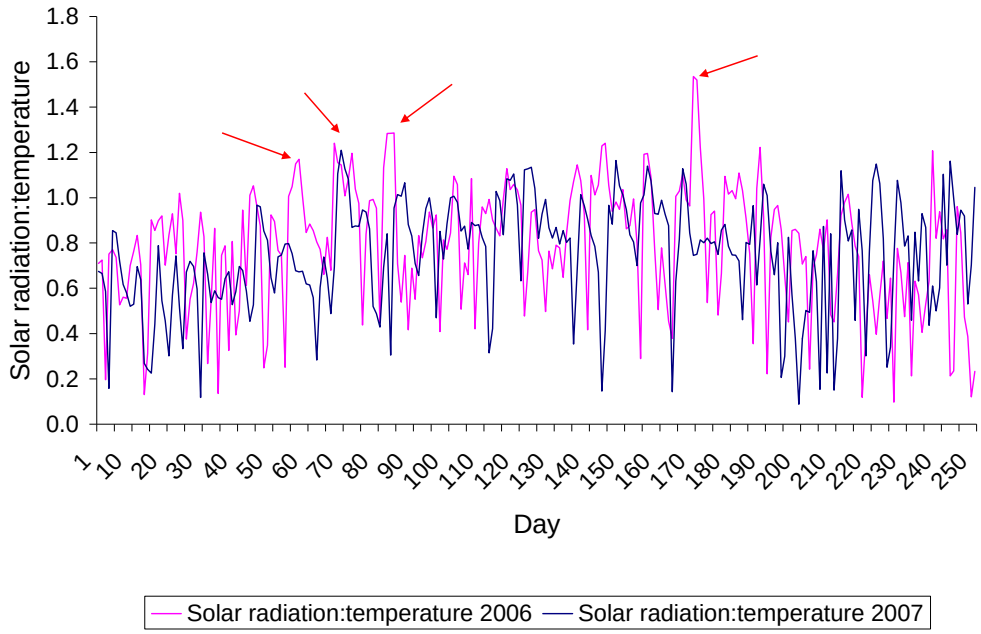


Figure 4: Daily solar radiation to temperature ratios for the experimental site during 2006 and 2007 (Red arrows indicate the periods of high solar radiation in relation to temperature; day 1 on the x-axis is 15 March with day 250, 19 November)

The excessive leaf abscission observed in 'Ryan' during 2006 did not occur in 'Fuerte' and 'Hass'. Although total leaf abscission for all cultivars was higher during 2006 than during 2007 (Chapter 3), indicating that low temperature stress could have contributed to higher leaf abscission in all cultivars during 2006, this does not explain why 'Ryan' trees abscised significantly more leaves when compared with 'Fuerte' and 'Hass'. One explanation could be genetic differences between cultivars. 'Fuerte' is a Mexican-Guatemalan hybrid, while both 'Hass' and 'Ryan' are Guatemalan cultivars (Bijzet, 2001). The Mexican races are more tolerant to cold than the Guatemalan races (Whiley and Schaffer, 1994) and as 'Fuerte' contains "Mexican genes" it is more tolerant to low temperature and light stress and abscises less leaves during low temperature periods. 'Hass' and 'Ryan' would be expected to display similar leaf abscission because both belong to the same race. However, genetic differences between the two cultivars can still cause 'Ryan' to be more cold susceptible than 'Hass', explaining higher leaf

abscission in 'Ryan' when compared to 'Hass'. However, the role of stress factors, other than low temperature stress, should also be considered, especially in an orchard situation where more than one stress factor is present.

5.4.2. The relationship between tree nutrient status and leaf abscission

The nutritional status of avocado trees and most other crops is determined by analysing nutrient levels of the leaves. In South Africa, leaf samples for avocado are taken after the summer flush has hardened off and fertilizer applications are then made in cases of nutrient shortages (Koen and Du Plessis, 1991). It is documented that nutrient shortages or imbalances result in similar physiological responses and negative effects as other forms of stress (Rabe, 1990), and excessive leaf abscission might therefore be a result of nutrient deficiencies or imbalances. In order to determine if nutrient levels were within the recommended norms, leaves were sampled for all three cultivars, after the summer flush had hardened off, in both 2006 and 2007 (Table 1). In addition, leaf samples were taken at the different phenological stages to determine nutrient withdrawal of the mobile nutrients prior to leaf abscission.

When comparing the nutrient levels of the three different cultivars during the 2006 season, all macronutrients, except calcium which was slightly lower, were within the recommended norms for 'Fuerte' (Table 1). For 'Hass', all macronutrients, with the exception of nitrogen, were within the recommended norms (Table 1). For 'Ryan', of all the macronutrients, only phosphorus (although deficient in the soil – 11.36 $mg.kg^{-1}$ compared with the norm of 30 to 90 $mg.kg^{-1}$) and calcium were within the recommended norms (Table 1). With regard to the micronutrients, for all cultivars, zinc and boron levels were below the recommended norms, with zinc deficiencies prevalent during 2006. In addition, for all cultivars during 2006, copper levels were in excess, with high manganese levels and sufficient iron levels (Table 1). Copper was in excess for all cultivars, by a factor of 10 to 14. In 2007, only calcium, zinc and boron were lower than the recommended norms for all cultivars (Table 1). Copper was in excess for 'Fuerte' and 'Ryan' only, while it was in sufficient quantities for 'Hass' (Table 1).

Zinc plays an important role in the anti-oxidative defence of plants against reactive oxygen species (viz. O_2^-, H_2O_2, and $\cdot OH$), and it was found that higher

levels of these reactive oxygen species were found in leaves of wheat plants that were zinc-deficient (Sharma *et al.*, 2004). Fernandez and Henriques (1991) reported that excess or toxic copper levels inhibit a large number of enzymes, resulting in negative effects on photosynthesis, pigment synthesis, membrane integrity, fatty acid and protein metabolism and respiration, as well as inhibition of the electron transport chain of photosynthesis, resulting in oxidative stress. The excessively high copper levels in the trees could be as a result of continuous use of copper oxychloride from October to January to control the fungus, *Pseudocercospora purpurea* (Cercospora spot) on avocado fruit. The negative effects that copper may have on avocado trees are recognised by growers and researchers, and research on alternatives for copper oxychloride is ongoing. Boron deficiency was reported to influence photosynthesis negatively in cotton (Zhao and Oosterhuis, 2003). In avocado, leaf yellowing and abscission during flowering time is one of the symptoms of advanced (less than 30 $mg.kg^{-1}$) boron deficiency (Whiley *et al.*, 1996). For both seasons, boron levels were higher than 30 $mg.kg^{-1}$ (Table 1) for 'Ryan' and thus although boron was deficient in 'Ryan', this deficiency was not very advanced. Therefore, it would be unlikely that boron could be a major contributor to premature and excessive leaf abscission in 'Ryan'. The combined negative effect of excess copper and deficiencies of zinc and boron on the physiology of the plant (which may exacerbate the impact of photo-inhibition during winter) could therefore result in nutrient stress, which in combination with the observed low temperature stress, could influence excessive and premature leaf abscission in 'Ryan'. Overall, there was also a tendency that nutrient levels for 'Fuerte' and 'Hass' were more balanced (within recommended ranges) than 'Ryan', and therefore 'Ryan' trees could experience stress caused by nutrient imbalances during the 2006 season. During 2007, nutrient levels were also closer to, and more within, the recommended ranges than during 2006 for all cultivars, which made nutrient stress less likely to occur during 2007 than during 2006.

The extrapolation of this nutrient data from this specific experimental site to other orchards exhibiting premature and excessive leaf abscission is, however, very difficult. Different growers use different fertilization programmes, based on recommendations from fertilizer companies and other laboratories and therefore the nutrient status of trees varies between orchards and farms. Premature and

excessive leaf abscission on ‘Ryan’ was not only limited to the experimental site, but also occurred on other farms in the vicinity of the experimental site. Due to the possible variation in nutrient levels between farms and orchards, nutrient stress cannot be regarded as a major cause of excessive and premature leaf abscission in ‘Ryan’ at this stage. However, it is evident from the data presented in this study that imbalances in tree nutrient levels may be a contributing factor to premature and excessive leaf abscission in ‘Ryan’.

Changes in mobile nutrients (N, P, K and Mg) were monitored in ‘Ryan’ trees from bud dormancy until four weeks after fruit set in order to determine mobilization of these nutrients from the older leaves. Again, the first fully expanded leaf on the most recent flush was sampled. However, as time progressed (from one phenological stage to the next) leaves became older, but leaves were not sampled if they showed visual signs of senescence or yellowing. Therefore, only green leaves were sampled. The pattern of change in nutrient levels over time did not differ between the 2006 and 2007 seasons, but in many instances the levels were different (Figures 5 to 8). For both nitrogen and phosphorus, a significant decrease in the leaf nutrient levels occurred between bud swell and fruit set, which corresponded with increased leaf senescence of the spring and summer flushes (Figures 5 and 6). Leaf potassium levels decreased significantly between bud dormancy and inflorescence development for the 2007 season, while no substantial changes occurred during the 2006 season (Figure 7). Minimal changes in leaf magnesium levels occurred over time during both seasons (Figure 8).

Table 1: Leaf nutrient levels for three avocado cultivars during the 2006 and 2007 seasons from samples taken after summer flush hardening (April) and the recommended norms for each cultivar

Nutrient	Fuerte	Hass	Ryan	Recommended norms[#]
2006				
Nitrogen (%)	1.90	2.13	1.76	1.70 to 2.00 (Fuerte) 2.20 to 2.40 (Hass) 1.90 to 2.20 (Ryan)
Phosphorus (%)	0.12	0.13	0.11	0.08 to 0.15
Potassium (%)	0.89	0.94	0.74	0.90 to 1.00*
Calcium (%)	0.82	1.08	1.06	1.00 to 2.00
Magnesium (%)	0.61	0.70	0.90	0.40 to 0.80
Zinc ($mg.kg^{-1}$)	16.7	15.3	17.6	25 to 100
Copper ($mg.kg^{-1}$)	203.2	149.7	207.1	5 to 15
Manganese ($mg.kg^{-1}$)	292.7	302.5	325.1	50 to 250
Iron ($mg.kg^{-1}$)	66.8	73.7	110.6	50 to 150
Boron ($mg.kg^{-1}$)	32.2	28.7	36.3	50 to 80
2007				
Nitrogen (%)	1.76	2.21	1.94	1.70 to 2.00 (Fuerte) 2.20 to 2.40 (Hass) 1.90 to 2.20 (Ryan)
Phosphorus (%)	0.11	0.14	0.13	0.08 to 0.15
Potassium (%)	1.26	1.25	1.34	0.90 to 1.00*
Calcium (%)	0.63	0.69	0.64	1.00 to 2.00
Magnesium (%)	0.47	0.52	0.58	0.40 to 0.80
Zinc ($mg.kg^{-1}$)	19.4	16.6	23.8	25 to 100
Copper ($mg.kg^{-1}$)	147.4	20.3	195.3	5 to 15
Manganese ($mg.kg^{-1}$)	169.9	219.1	206.1	50 to 250
Iron ($mg.kg^{-1}$)	96.8	77.8	80.8	50 to 150
Boron ($mg.kg^{-1}$)	33.1	25.3	33.1	50 to 80

[#]*SAAGA Research and Technical Committee, 1990*

**Norms for potassium according to Koen and Du Plessis, 1991*

Nutrient resorption takes place from the leaves when they start to senesce (Thomas and Stoddart, 1980; Aerts, 1996). During leaf senescence, nucleic acids, phospholipids (major contributor of phosphorus) and proteins (major contributor of nitrogen) are hydrolysed to release nitrogen and phosphorus, which get resorped and redistributed to other sinks by the plant (Aerts, 1996). The decline in nitrogen and phosphorus levels observed in the green leaves sampled between bud swell and fruit set could possibly indicate that leaf senescence of those leaves could already have been initiated. However, this could not be verified as green leaves were destructively harvested and thus it is impossible to determine when these leaves would have senesced. The decline in nitrogen and phosphorus levels over time, on the other hand, corresponded with inflorescence development. At that stage, the developing inflorescences can be expected to be stronger sinks for nutrients than the leaves, and nutrients absorbed by the plant would be preferentially transported to the developing inflorescences. This follows the nutrient diversion theory of Sachs and Hackett (1983), which stated that transport of assimilates, and possibly also nutrients will be limited (in this case to the leaves) as a result of enhanced activity of a competing sink (in this case the developing inflorescences). It can therefore also be argued that the declining nitrogen and phosphorus levels in the leaves were to supply the developing inflorescences with nutrients and not as a response to the initiation of leaf abscission, because the levels of other mobile nutrients (with the exception of potassium during 2007) did not change substantially. It is therefore not certain if the decline in the levels of nitrogen and phosphorus was in response to the initiation of leaf abscission, or to meet the needs of the developing inflorescences.

Large differences in leaf magnesium and potassium levels were observed between the 2006 and 2007 season. Leaf potassium levels were significantly lower during 2006 than during 2007 (Figure 7), and leaf magnesium levels were significantly higher during 2006 than in 2007 (Figure 8). It was reported that high levels of potassium suppress the uptake of magnesium (Mengel and Kirkby, 1978), and it could be expected that when magnesium levels are high, potassium levels will be low and vice versa. In addition, it was reported that magnesium deficiency in general (Mengel and Kirkby, 1978) or a high K:Mg ratio, as found for sweet chestnut (*Castanea sativa* Mill.) (Portela *et al.*, 2003), resulted in

premature and excessive leaf abscission. Portela *et al.* (2003) reported further that a K:Mg ratio higher than 9:1 indicated magnesium deficiencies, and sufficient quantities of magnesium are important in preventing leaf abscission (Mengel and Kirkby, 1978). In this study, according to the norms described by Koen and du Plessis (1991) for avocado, magnesium was not deficient in the 2006 and 2007 seasons (Figure 11 and 12) and the K:Mg-ratio for 2006 was significantly lower than for 2007 (Figure 9), with potassium slightly deficient during 2006 and slightly high during 2007 (Figure 7) at all phenological stages. Therefore, a high K:Mg-ratio does not seem to contribute to excess and premature leaf abscission in 'Ryan' avocados, but it is possible that imbalances in potassium and magnesium levels (higher or lower than the optimal ranges) could influence leaf abscission.

The levels of all mobile nutrients between bud dormancy and bud swell, with the exception of magnesium were lower during 2006 than during 2007 (Figures 5 to 8). This is particularly evident for leaf potassium levels, which were much lower during 2006 than during 2007 (Figure 7). These differences could be as a result of alternate bearing experienced in avocado trees. 2005 was an "on" year (40.7 t) and the high yield would demand a large amount of nutrients, while 2006 was an "off" year (8.9 t), which would result in a smaller demand for nutrients. It follows that the levels of mobile nutrients following an "on" year will be lower than those following an "off" year, as seen for nitrogen (Figure 5), phosphorus (Figure 6), and potassium. These levels were all significantly lower at bud dormancy in 2006 than in 2007. Avocado fruit are especially high in potassium (Pieterse *et al.*, 2003) and this explains the large difference between leaf potassium levels for the 2006 (which followed an "on" season) and 2007 (which followed an "off" season) seasons. Similar results were also found for olive (*Olea europaea* L.) (Fernandez-Escobar *et al.*, 1999). Importantly, no correlations were found between nutrient levels of mobile nutrients between bud dormancy and bud swell and leaf abscission for 'Ryan', for both the 2006 and 2007 season, which implied that initial nutrient levels (between bud dormancy and bud swell) had no effect on excessive and premature leaf abscission.

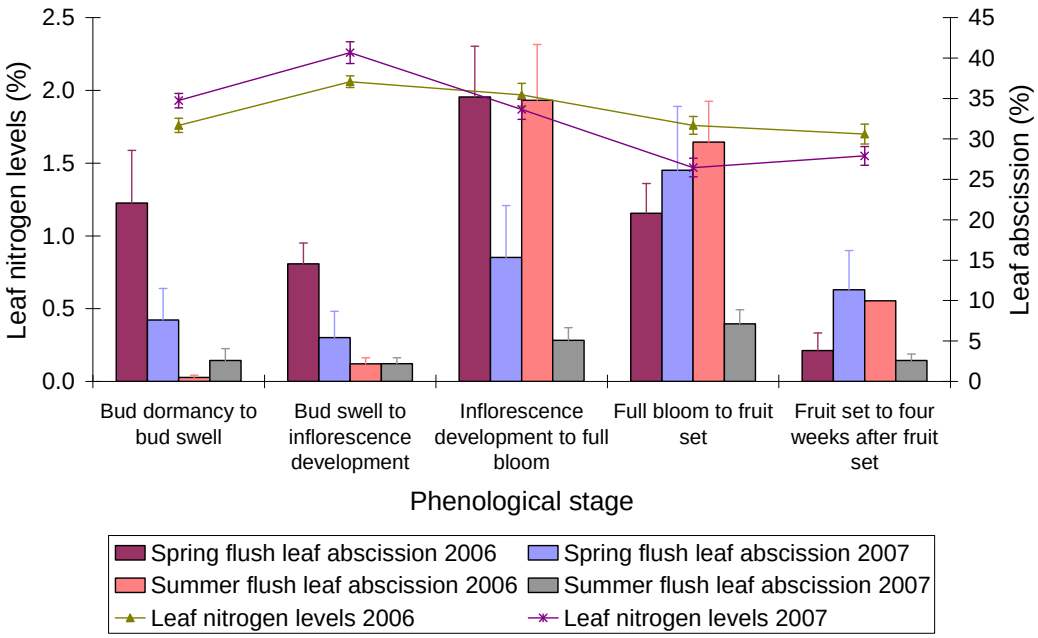


Figure 5: Changes in leaf nitrogen levels and leaf abscission over six phenological stages for the avocado cultivar 'Ryan' for the 2006 and 2007 seasons

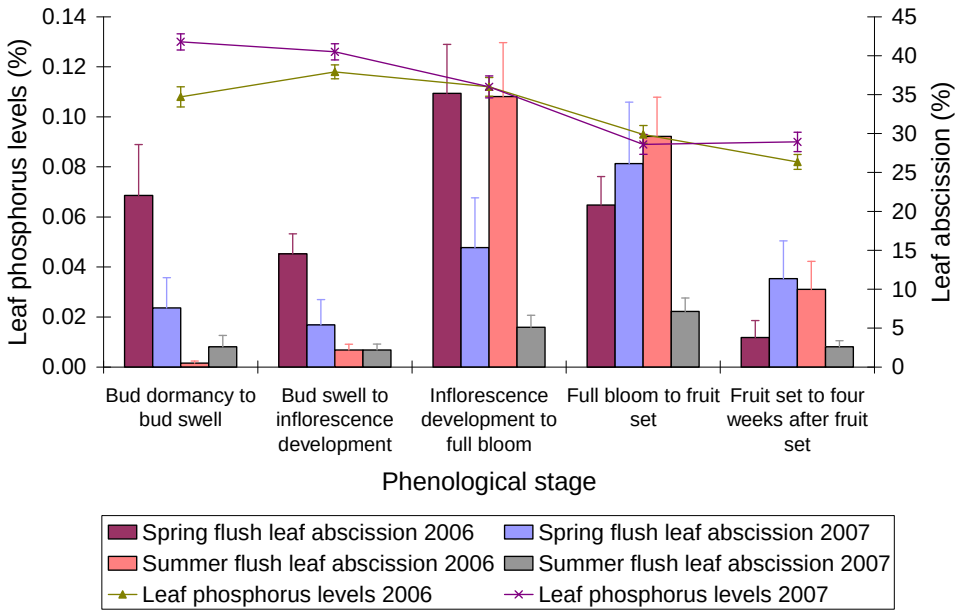


Figure 6: Changes in leaf phosphorus levels and leaf abscission over six phenological stages for the avocado cultivar 'Ryan' for the 2006 and 2007 seasons

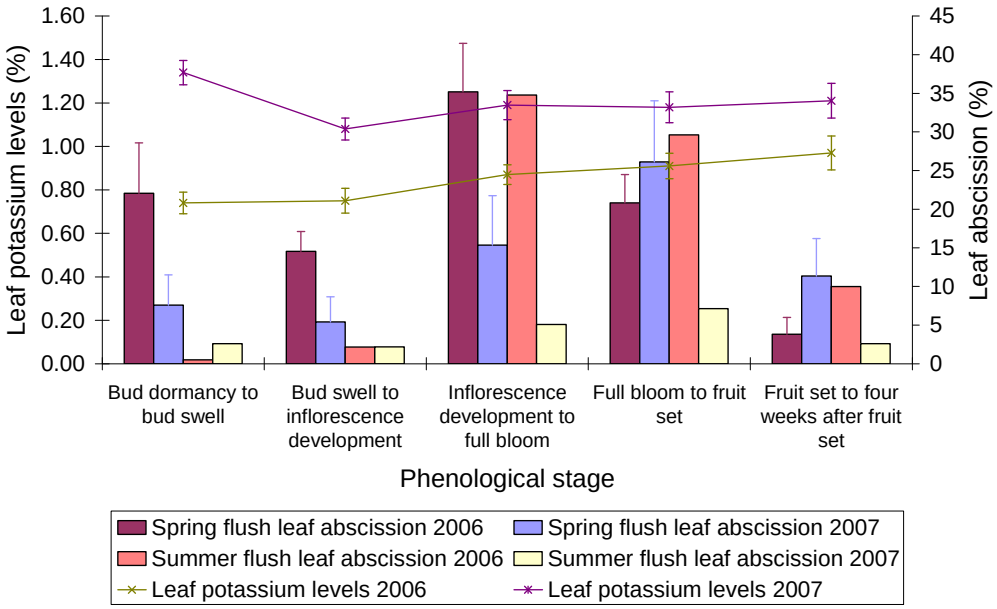


Figure 7: Changes in leaf potassium levels and leaf abscission over six phenological stages for the avocado cultivar 'Ryan' for the 2006 and 2007 seasons

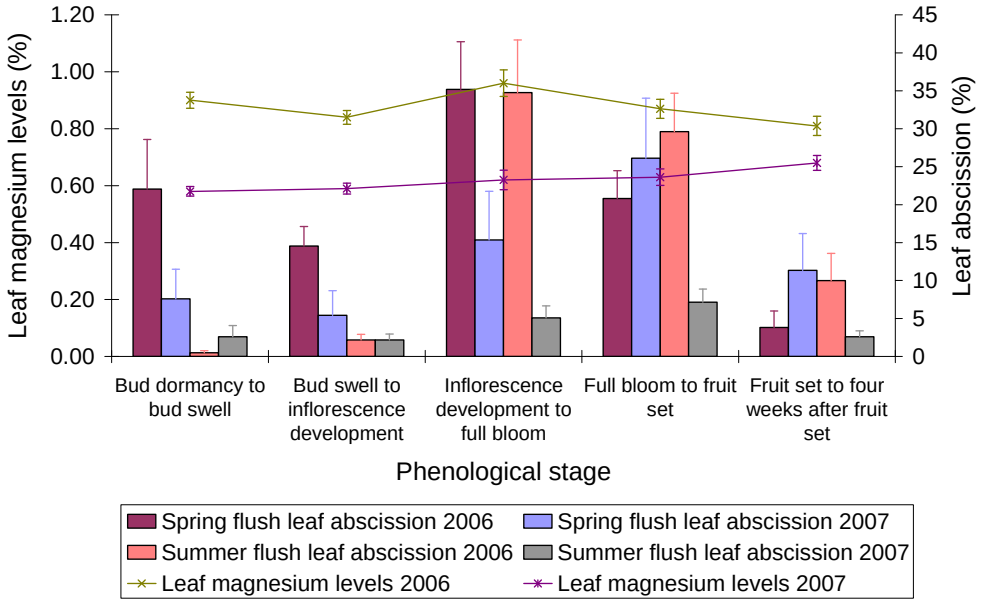


Figure 8: Changes in leaf magnesium levels leaf abscission over six phenological stages for the avocado cultivar 'Ryan' for the 2006 and 2007 seasons

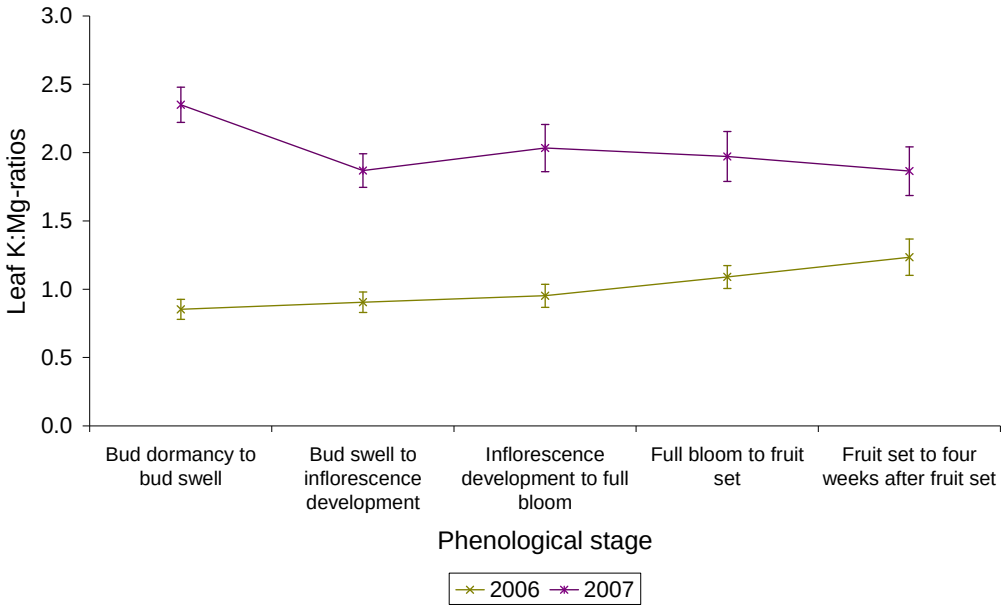


Figure 9: Changes in leaf potassium to magnesium (K:Mg) ratios over six phenological stages for the avocado cultivar 'Ryan' for the 2006 and 2007 seasons

5.4.3. The relationship between flowering intensity and leaf abscission

The mean number of flowers per branch was compared for the three different cultivars and between the 2006 and 2007 seasons. For both seasons, 'Ryan' flowered significantly more than 'Fuerte' and 'Hass', with 'Ryan' flowering profusely during the 2006 season (Table 2). It was also during the 2006 season that flowering and leaf abscission were significantly correlated for 'Ryan' (Table 2). No correlations between leaf abscission and flowering were found for 'Fuerte' and 'Hass' during 2006, and for any cultivar during 2007 (Table 2). Although the correlation between leaf abscission and flowering for 'Ryan' was significant during 2006 the r^2-value was relatively low (Table 2), indicating that profuse flowering was another contributing factor (with the possible factors already mentioned) to premature and excessive leaf abscission in 'Ryan'. In an earlier report, heavy flowering resulted in excessive leaf abscission in the avocado cultivar 'Lula' and it was suggested that leaf yellowing and abscission were controlled by the flowers (Davenport, 1982). However, in this instance the correlation between flowering and leaf abscission was relatively low and would it not be suggested that flowering was the major contributing factor to premature and excessive leaf abscission. Whiley and Schaffer (1994) also reported that excessive leaf abscission in avocado (no specific cultivars were mentioned) could be associated with heavy flowering and it was proposed that heavy flowering imposed stress on the tree, which resulted in premature and excessive leaf abscission.

Table 2: Flower intensity and its correlation with leaf abscission for three avocado cultivars during the 2006 and 2007 seasons

Year	Cultivar	Mean number of flowers per branch			Total leaf abscission (%)
		Mean[#]	STD	r^2	
2006	Fuerte	142 cd	270	0.049	33.97
	Hass	72 d	147	0.257	56.78
	Ryan	1 147 a	1 045	0.359*	83.42
2007	Fuerte	155 c	208	0.219	17.37
	Hass	206 c	213	0.108	29.54
	Ryan	319 b	296	0.036	33.03

[#]*Means followed by the same letter do not differ significantly at P=0.05*

**Significant correlations at P=0.05*

Stress may be imposed on the tree by heavy flowering, as a result of the demand the excessive amount of flowers place on the tree for nutrients, water and carbohydrates (energy). In addition, flowers of 'Ryan' trees appear to have a higher demand for nutrients than 'Fuerte' and 'Hass' flowers, as indicated by higher nutrient levels in 'Ryan' flowers (Table 3). In addition, the levels of mobile nutrients, with the exception of magnesium occurred in higher amounts in the flowers than in the leaves (Table 3). For the immobile elements, zinc and boron, higher levels were found in the flowers than in the leaves, while calcium, copper, manganese and iron levels were lower in the flowers than in the leaves (Table 3), indicating a higher demand for zinc and boron during flowering. Higher boron levels in flowers are to be expected because boron is an important nutrient needed for successful pollination and fertilization of flowers, by playing a crucial role in pollen tube germination and elongation (Blevins and Lukaszewski, 1998). Data in the previous section indicated the possibility that nitrogen and phosphorus were being remobilised from leaves to flowers. However, there is no indication if the nutrients remobilised from the leaves were to supply the nutrient needs of the flowers or if it was remobilised in response to natural leaf senescence. It is therefore uncertain if stress imposed by excessive flowering is the result of insufficient available nutrients only or if it is the result of the total demand that flowers placed on the tree for nutrients, water and carbohydrates (energy).

Table 3: Nutrient content of flowers, sampled just prior to full bloom during the 2006 season for three avocado cultivars

Nutrient	Fuerte	Hass	Ryan
Nitrogen (%)	1.88 b*	2.22 b	3.05 a
Phosphorus (%)	0.23 b	0.24 b	0.36 a
Potassium (%)	1.66 a	1.67 a	1.85 a
Calcium (%)	0.31 ab	0.25 b	0.38 a
Magnesium (%)	0.45 a	0.46 a	0.37 b
Zinc (mg/kg)	25.92 b	22.83 b	48.44 a
Copper (mg/kg)	25.92 a	20.83 a	28.25 a
Manganese (mg/kg)	105.00 a	90.67 a	116.25 a
Iron (mg/kg)	61.92 b	66.33 ab	76.13 a
Boron (mg/kg)	31.08 b	42.40 ab	48.56 a

** Means followed by the same letter in each row do not differ significantly at P=0.05*

It is also not certain why 'Ryan' flowered excessively during the 2006 season. Flower induction in the Mexican and Guatemalan races of avocado is stimulated by low temperatures after the summer flush has hardened off and buds are dormant (Whiley and Schaffer, 1994), which will be from the end of April to the end of May under South African conditions. The effect of low temperatures on flowering of 'Hass' (a cultivar from the Guatemalan race) avocados was established through glasshouse experiments with temperature regimes set at 18°/15°, 23°/18° and 29°/25° day/night cycles (Chaikiattiyos *et al.*, 1994). Flower induction only occurred at 18°/15°C and 23°/18°C (Chaikiattiyos *et al.*, 1994).

Chaikiattiyos *et al* (1994) found no relationship between flowering and starch content for 'Hass' avocados, implying that fluctuations in starch reserve levels, as a result of alternate bearing, may not exert an effect on excessive flowering. There was also no evidence that photoperiod affects flower induction in avocado (Whiley and Schaffer, 1994). It would therefore appear that low temperatures are mainly responsible for synchronised flower induction in avocado. For both 2006 and 2007, temperatures during April and May were favourable for flower

induction, with no dramatic differences between the years (Figure 10). However, stress prior to the period of floral induction may also result in heavier flowering, and it could be possible that low temperature stress, as discussed in section 5.4.1, during bud dormancy could result in excessive flowering in 'Ryan'. It is unlikely that drought stress could trigger excessive flowering, because 2006 was not significantly drier than 2007. The autumn period (March to May) prior to flower induction had more rain during 2006 than 2007 (423.4 mm for 2006 and 114.4 mm for 2007), and the relative humidity for 2006 was significantly higher than for 2007 (average of 75.8 % for 2006 and an average of 66.5% for 2007). In addition, no significant differences in evapotranspiration (Et) for the period March to May were recorded between the 2006 and 2007 seasons (242.7 mm for 2006 and 255.3 mm for 2007). However, a single event of extremely high evapotranspriation could result in increased moisture stress as a result of a higher rate of transpiration. But, no single event of extremely high evapotranspiration was observed for 2006, and daily evapotranspiration did not differ substantially between the 2006 and 2007 seasons (Figure 11). Trees were also irrigated according to a scheduled programme and managed according to best farm management practices. The remainder of the winter (May to August) was wetter during 2006 (38.6 mm) than during 2007 (22.3 mm), with the average relative humidity during winter for 2006 also being significantly higher than for 2007 (63.6% in 2006 and 57.5% in 2007). During the same period, no significant differences in total or daily evapotranspiration between the 2006 and 2007 seasons (196.4 mm for 2006 and 200.6 mm for 2007 and Figure 11) were noted. Taking the rainfall, relative humidity and evapotranspiration into account, it may be expected that trees had a much lower rate of water usage and transpiration prior to and during winter in 2006 than 2007, resulting in a lower chance of trees experiencing water stress during 2006 than during 2007. It is therefore highly unlikely that drought stress could induce excessive flowering in 'Ryan' trees. Another possibility is that alternate bearing cycles could be related to flowering, but no long term yield data was available from the grower to establish a link between excessive flowering and alternate bearing.

The dramatic increase in summer flush leaf abscission and increased spring flush leaf abscission recorded between inflorescence development and full bloom for 'Ryan' during 2006, could therefore be strongly influenced by excessive

flowering, especially taking into consideration that 'Fuerte' and 'Hass', which did not display excessive leaf abscission during 2006, did not flower excessively. Excessive flowering could therefore be an important contributor to excessive and premature leaf abscission in 'Ryan' during 2006.

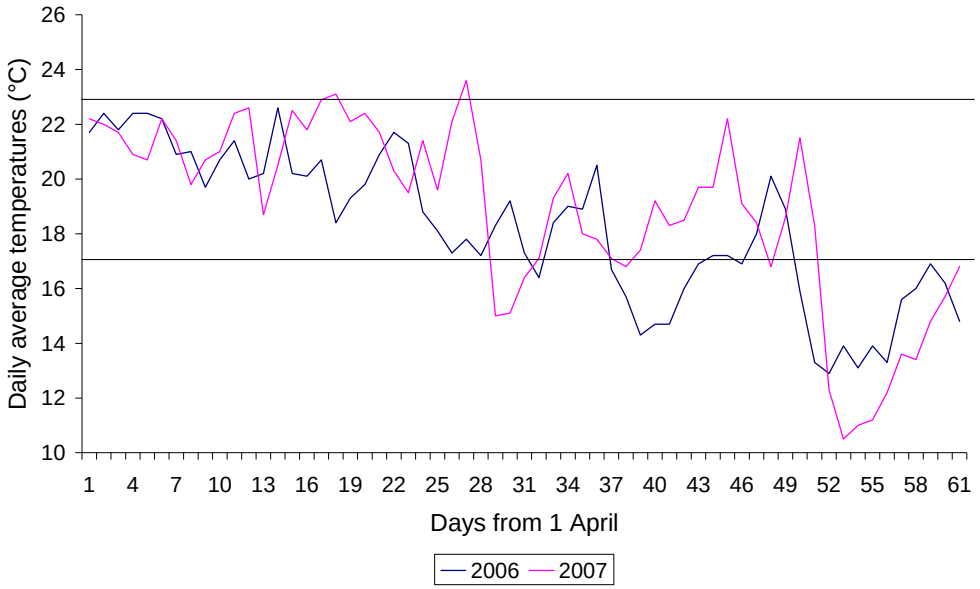


Figure 10: Average daily temperatures for the experimental site during April and May for 2006 and 2007 (black lines indicate the temperature regimes for floral induction in avocado)

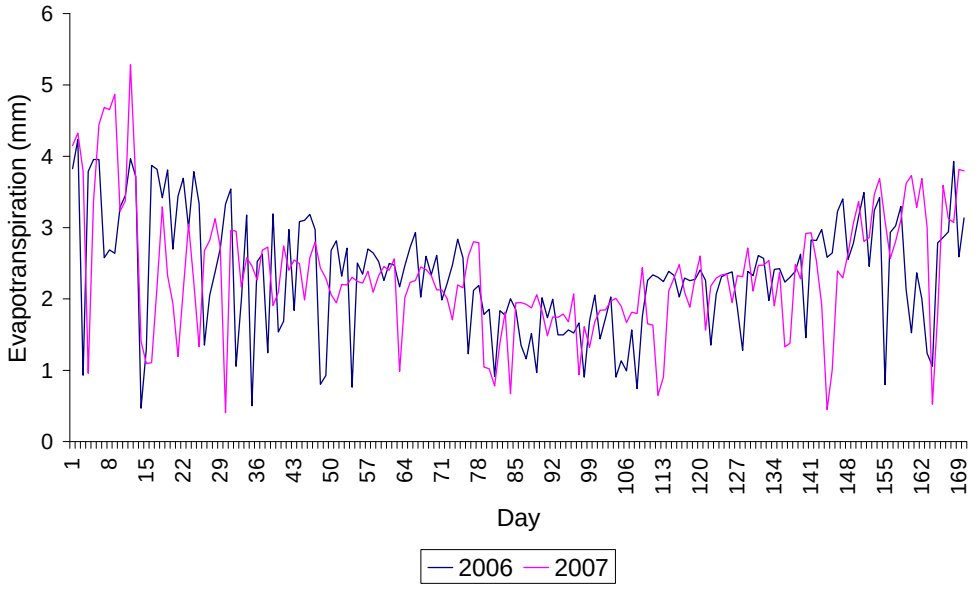
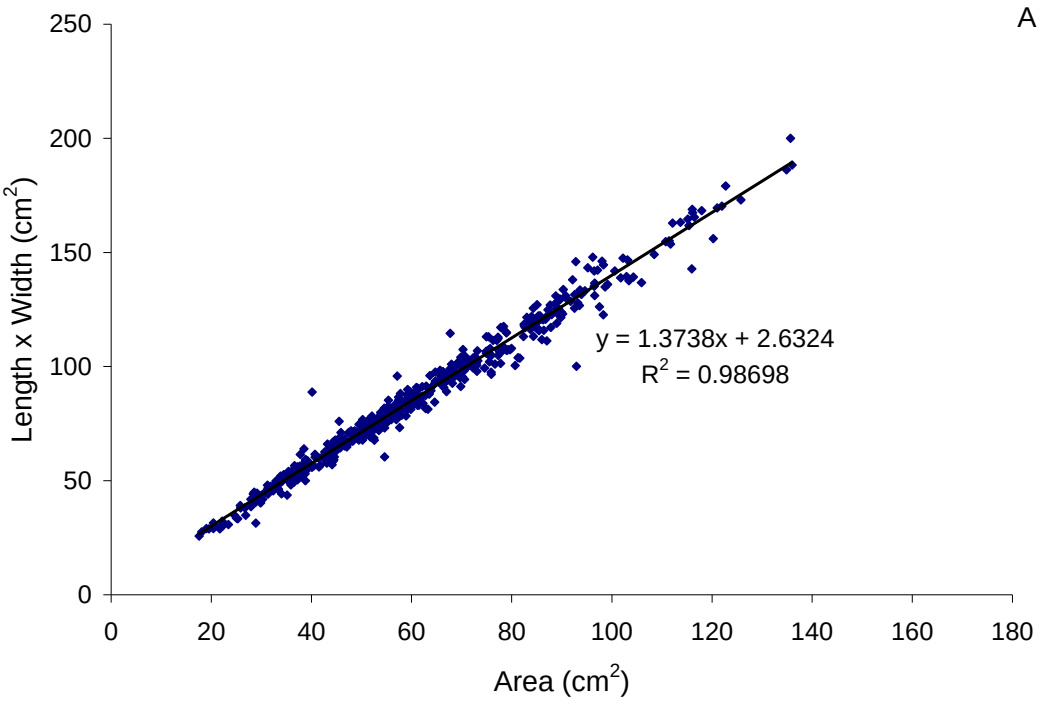


Figure 11: Daily evapotranspiration of the experimental site during 2006 and 2007 (Day 1 on the x-axis is 1 March with day 170 being 31 August)

5.4.4. The relationship between total leaf area and leaf abscission

In this study, a relationship between 1) leaf area and leaf width, 2) leaf area and leaf length, and 3) leaf area and leaf width X length was determined in order to develop a non-destructive estimation of the total leaf area of each of the selected branches. The best linear relationship was found between leaf area and leaf length X width for each cultivar (Figure 12). Correlations between leaf area and leaf length and leaf area and leaf width had R^2-values between 0.75 and 0.90 for all cultivars.



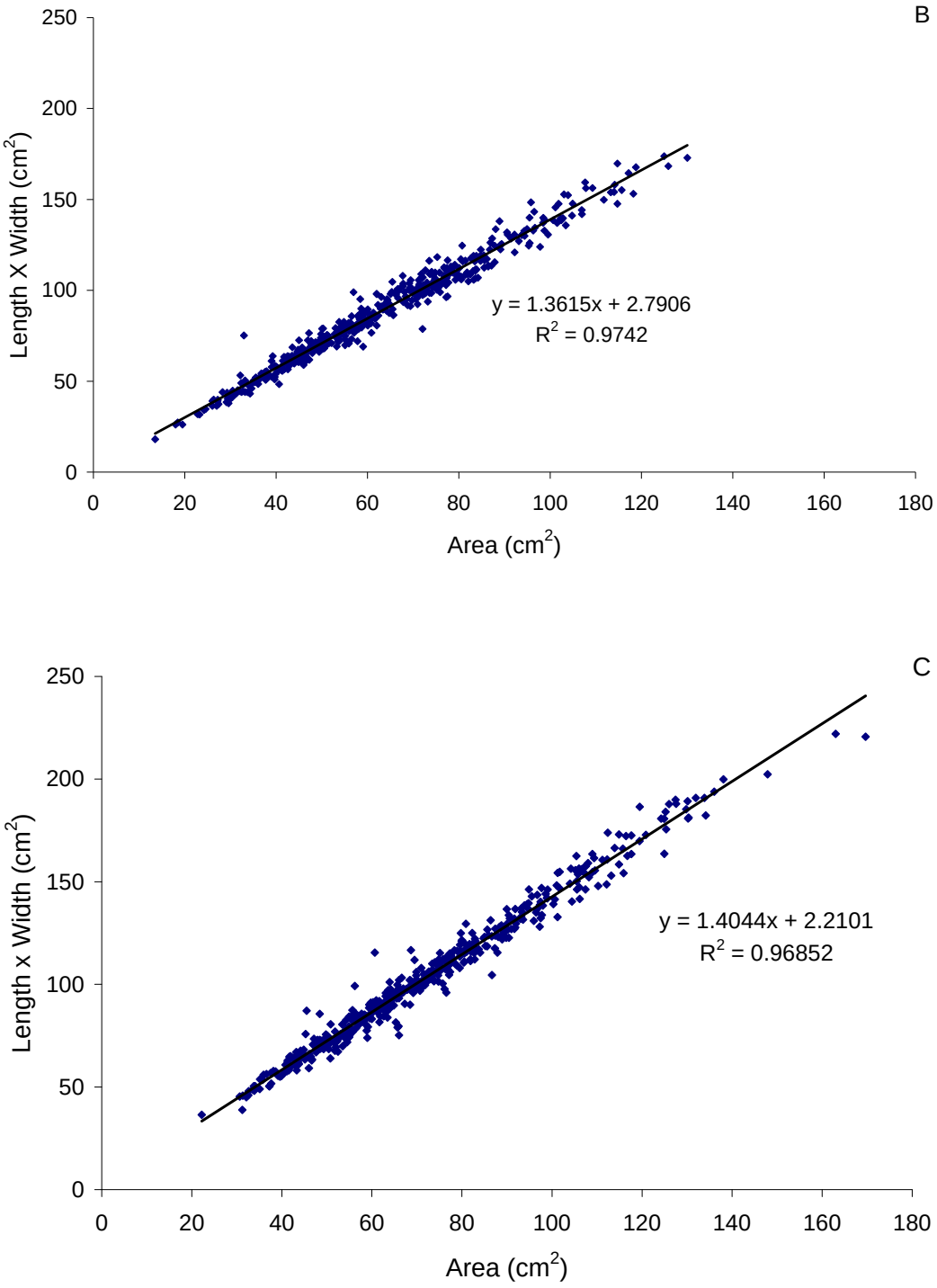


Figure 12: Relationship between leaf area and leaf length X width for A) 'Fuerte', B) 'Hass', and C) 'Ryan' during the 2007 season

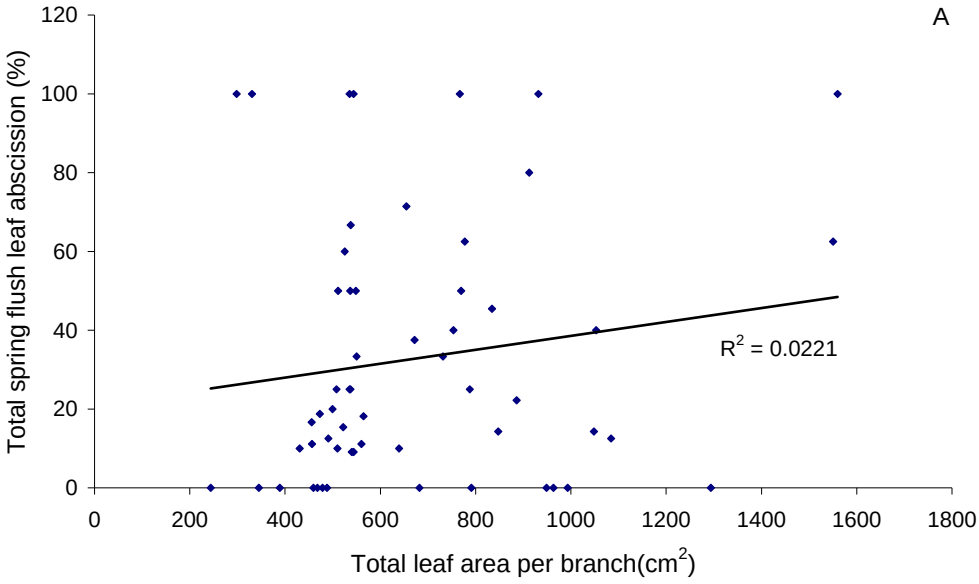
The formulas obtained in Figure 12 were used to calculate the leaf area from length and width measurements of each leaf made in the field. From this the

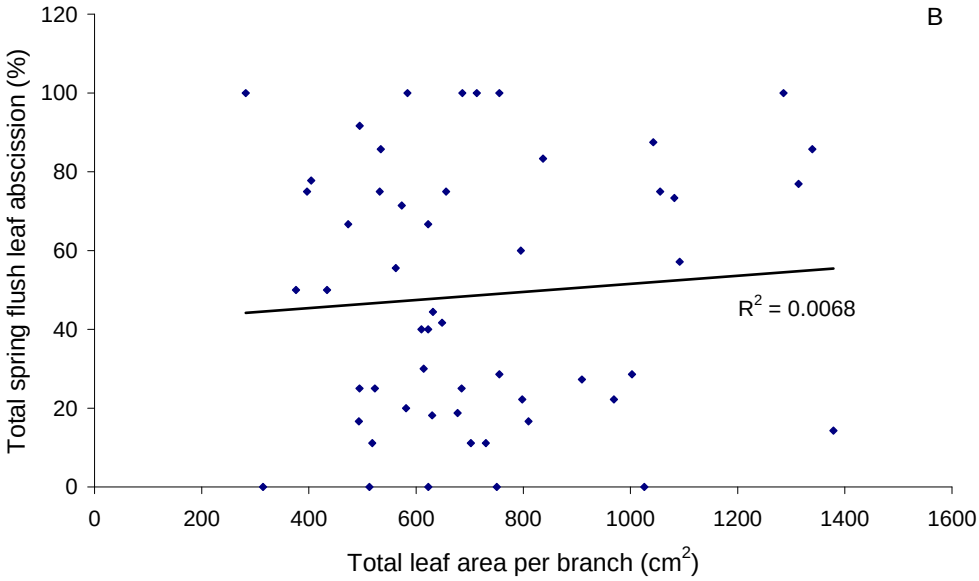
mean total leaf area per branch was calculated. For 'Ryan' the total leaf area per branch was significantly lower than for 'Hass' (Table 4). Although the mean total leaf area per branch for 'Fuerte' and 'Hass' was the same, 'Fuerte' did not differ significantly from 'Ryan' due to larger variation in the data for 'Fuerte' (Table 4). Correlations were then obtained between the total leaf area and spring flush leaf abscission during the 2007 season for all cultivars (Figure 13). Heath *et al.* (2005) found that a relationship exists between leaf abscission of the flush prior to the most recent flush (which would be the spring flush for this study) and the total leaf area of the branch. They found that if the total leaf area on the branch is equal to or higher than 1 000 cm^2, no leaf abscission will occur, suggesting that the assimilatory support is high enough to prevent leaf abscission. This therefore implies that leaf abscission may also be influenced by the total productivity of the leaves on a branch. In this study, very weak correlations were found between total leaf area and spring flush leaf abscission, suggesting that the impact of other stress factors on leaf abscission may be more influential than the total productivity of leaves on the branch. However, during 2007 leaf abscission was very low, and it is possible that the correlations obtained for the 2007 season (low leaf abscission) may differ from a season with high leaf abscission.

Table 4: Total leaf area per branch of three avocado cultivars measured at bud dormancy for the 2007 season

Cultivar	**Mean total leaf area per branch**	
	Mean total leaf area (cm^2)	STD
Fuerte	713ab[#]	187.6
Hass	713a	126.9
Ryan	599b	127.8

[#]*Means followed by the same letter do not differ significantly at P=0.05*


A
Total spring flush leaf abscission (%)
120
100
80
60
40
20
0
0
200
400
600
800
1000
1200
1400
1600
1800
Total leaf area per branch(cm2)
R2 = 0.0221


B
Total spring flush leaf abscission (%)
120
100
80
60
40
20
0
0
200
400
600
800
1000
1200
1400
1600
Total leaf area per branch (cm2)
R2 = 0.0068

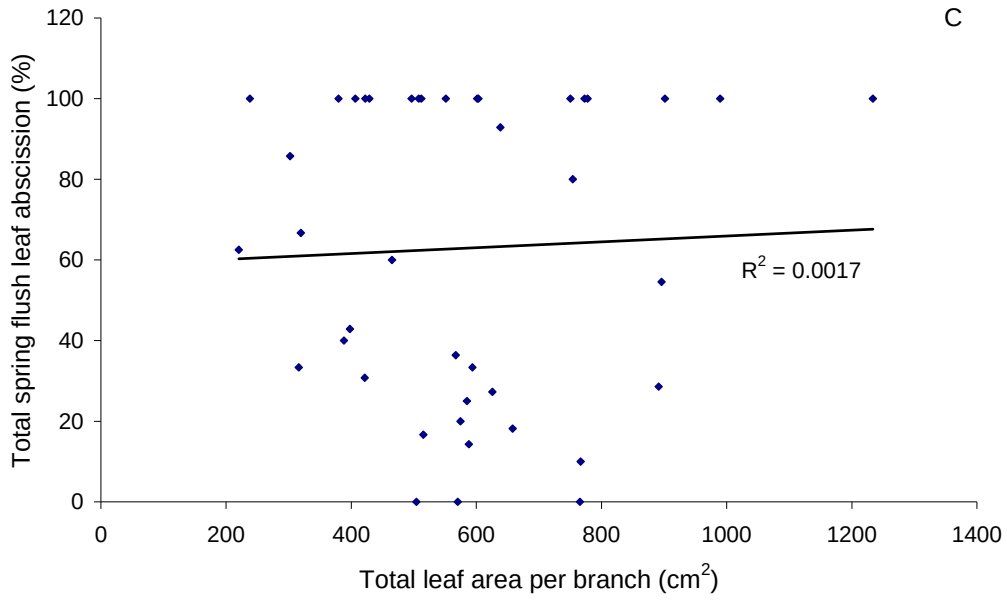


Figure 13: Relationship between spring flush leaf abscission and total leaf area per branch for A) 'Fuerte', B) 'Hass', and C) 'Ryan' during the 2007 season

5.4.5. The effect of leaf abscission on starch reserve levels and yield

Bark carbohydrate levels of the scaffold branches differed substantially between the 2006 and 2007 seasons (Figure 14). During 2006, starch levels increased from bud swell to full bloom, followed by a significant decrease between full bloom and fruit set (Figure 14). During 2007, starch levels increased significantly between bud dormancy and bud swell, where after the levels remained approximately constant until four weeks after fruit set (Figure 14). During 2006, 'Ryan' trees abscised a larger number of their leaves (96% of the spring flush leaves and 77% of the summer flush leaves), especially between inflorescence development and fruit set, whilst in 2007 this was not the case and the trees retained a large proportion of their leaves (65% of the spring flush leaves and 20% of the summer flush leaves were abscised) (Figure 14 and Chapter 3).

Leaves produce primary carbohydrates via photosynthesis for its energy demand (Hatch and Slack, 1970). In perennial trees, primary carbohydrates that are produced in excess of the plant's needs are stored as starch, mostly in the wood of branches (Turner and Turner, 1975; Daie, 1985), although in avocado also in the bark (Kaiser and Wolstenholme, 1994). With high leaf abscission, such as that occurring during the 2006 season, lower starch production would be

expected, due to reduced photosynthesis. In addition, 'Ryan' trees also flowered excessively during the 2006 season (see section 5.4.3), placing a large energy demand on the trees. This energy demand for flowering, coupled with the reduced photosynthesis due to excessive leaf abscission, would in all likelihood result in the trees being more dependent on stored starch to meet energy demands. This could explain the significant decrease in starch levels between full bloom and fruit set for the 2006 season. In addition, the decrease in starch levels between bud dormancy and bud swell during 2006 (Figure 14) could be to supply the energy demand for the formation of the large number of flower initials.

Leaf abscission and flowering were significantly lower during the 2007 season, which possibly resulted in less dependence on stored starch reserves for energy demands for flowering and thus higher starch levels could be maintained. During fruit set, starch levels were approximately 6% lower during the 2006 season when compared with the 2007 season. Photosynthates produced by the leaves are not enough to supply the energy demands of fruit growth and fruit development is dependent on stored carbohydrate levels as well. The lower starch levels during the 2006 season as well as a lower rate of photosynthate production due to excessive leaf abscission could have a negative effect on fruit set. It should also be taken into consideration that during fruit set, the new developing spring flush acts as a sink. Fruit set and new spring flush development would therefore compete for stored starch reserves (Wolstenholme, 2001) since there are enough leaves on the tree to meet the energy demand for both new flush development and fruit set. It is possible that low starch reserve levels at fruit set may result in poor fruit set and yield while higher starch levels will result in higher yields. In a study done be Scholefield *et al.* (1985) on 'Fuerte' they concluded that biannual bearing appeared to be closely related carbohydrate reserves (starch). It is therefore possible that the low starch levels during fruit set in the 2006 season could result in an almost three times reduction in yield during the 2006 season (8.9 t), when compared with the 2007 season (23.7 t) when starch reserve levels were higher at fruit set. Excessive leaf abscission therefore had a potential negative effect on production, as was found in Israel (Aviles, 1995), due to the decline in starch levels prior to fruit set. Alternate bearing is a major problem in avocado (Monselise and Goldschmidt, 1982; Wolstenholme, 2001), and it appears that alternate bearing in 'Ryan' (and

possibly other avocado cultivars) can be exacerbated by excessive and premature leaf abscission. Due to the absence of yield data from the grower over a number of seasons, this link between alternate bearing and excessive and premature leaf abscission in 'Ryan' could not be established in this study. On the other hand, the stress factors causing excessive and premature leaf abscission in 'Ryan' and higher leaf abscission in 'Fuerte' and 'Hass' may also contribute to alternate bearing. This aspect should therefore be investigated over a number of alternate bearing cycles, in order to establish a definite link between excessive leaf abscission, the factors responsible for excessive leaf abscission, and alternate bearing.

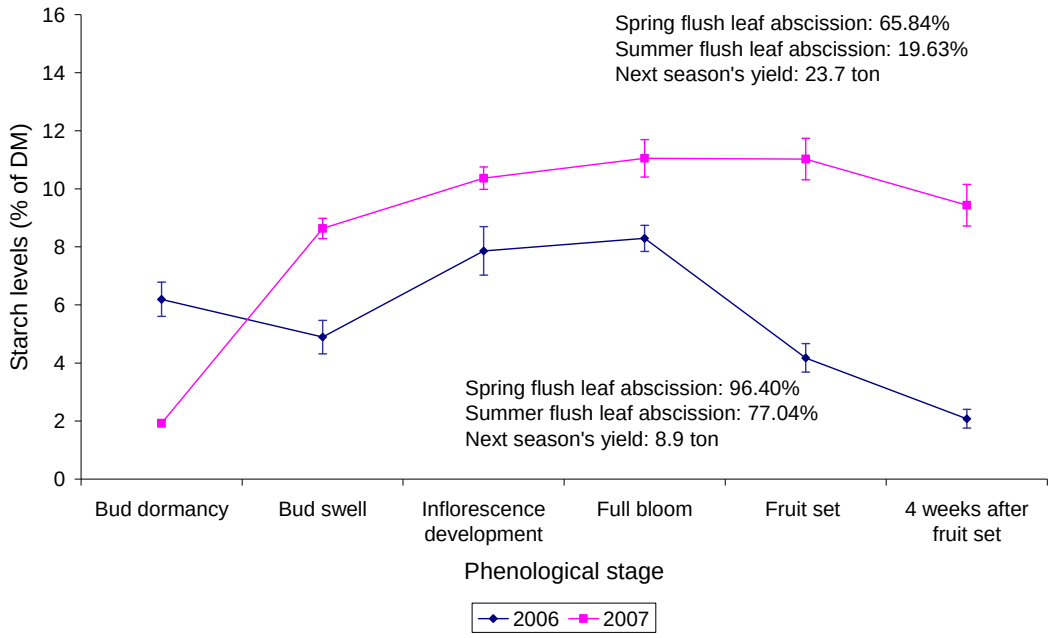


Figure 14: Changes in bark starch levels for 'Ryan' over six phenological stages for the 2006 and 2007 seasons

5.5 Conclusion

From this study, it is evident that no single environmental factor or stress-related factor can be highlighted as the cause of excessive and premature leaf abscission in 'Ryan'. Rather, a combination of factors appears to act together to cause premature and excessive leaf abscission in 'Ryan'. It is evident that an extreme climatic event, in this case minimum temperatures, is a contributing factor, since increased leaf abscission occurred directly after periods of low temperature. Leaf abscission in this case could be as a result of low temperature stress or as a result of light stress (photo-inhibition and photo-oxidative damage to leaves), caused by high light intensity in relation to low temperatures. In this instance 'Ryan' could be more sensitive to the effect of low temperatures and high light intensities than 'Fuerte' and 'Hass'. However, further research is necessary to establish the impact climate alone has on leaf abscission, because it is evident that climate alone is not solely responsible for the acceleration of leaf abscission in 'Ryan'.

In all cultivars, the excessively high levels of copper and deficient levels of zinc and boron could also contribute to excessive and premature leaf abscission as a result of nutrient stress. However, nutrient stress alone is unlikely to account for the observed excessive and premature leaf abscission in 'Ryan', since 'Fuerte' and 'Hass' also displayed similar nutrient imbalances with no premature and excessive leaf abscission. Low temperature stress (possibly resulting in light stress) and to a lesser extent, nutrient stress, could therefore perhaps explain the higher leaf abscission for the 2006 season when compared with the 2007 season, but not why 'Ryan' lost significantly more leaves than 'Fuerte' and 'Hass' during 2006, because these factors were similar in all cultivars.

In 2006 most of the leaves on 'Ryan' trees abscised after flowering, including the observation that green leaves of the summer flush abscised. During 2006 'Ryan' also flowered excessively, whilst Fuerte' and 'Hass' did not. The excessive flowering in 'Ryan' possibly placed a high demand for nutrients, water and energy on the tree, which when combined with low temperatures and nutrient imbalances, resulted in a stress reaction that caused premature and excessive leaf abscission. Genetic factors might also play a role as 'Ryan' may be more sensitive to stress and more prone to excessive flowering, but the possibility

exists that excessive and premature leaf abscission could be prevented if excessive flowering is prevented. Correlations obtained between total leaf area on a branch and spring flush leaf abscission were very weak for the 2007 season. This suggested that the impact of other stress factors on leaf abscission could be more influential than total leaf area. However, 2007 was a season of very low leaf abscission, and a better correlation could possibly be found during a season of high leaf abscission. The effect of total leaf area on leaf abscission therefore needs further investigation during years of high leaf abscission. The high demand that flowering places on reserve starch levels and the subsequent decrease in these levels, together with a low number of leaves to produce carbohydrates, results in a very limited amount of reserves available for fruit set. This results in low yield for the following year. It is therefore important to find measures to prevent excessive flowering and premature and excessive leaf abscission in order to ensure good production. Water requirements, especially during flowering, deserves further attention, as water stress during this period could trigger excessive flowering. New recommendations regarding soil water depletion may be necessary during this period.

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Chapter 6: Prevention of excessive leaf abscission

6.1 Abstract

Premature and excessive leaf abscission in 'Ryan' may possibly be prevented by application of fertilizers, plant growth regulators (PGRs), and stress-alleviating treatments, such as silicon. Plant growth regulators, viz. auxins and cytokinins, kaolin, additional fertilizer applications and silicon (Nontox-Silica®), were applied over a three year period from 2005 to 2007, in an attempt to reduce excessive leaf abscission and improve production of 'Ryan' trees. During the 2005 season, applications were made just after bud break and no significant reduction in leaf abscission was observed. Solubor®, Solubor® in combination with dolomitic lime, and benzyladenine (BA) treatments had a significantly negative effect on fruit set, possibly because boron and BA application rates were too high and were made too close to fruit set. During 2006, premature and excessive leaf abscission of 'Ryan' could not be reduced with applications of PGRs and kaolin. Premature and excessive leaf abscission during 2006 was most likely caused by low temperature stress and excessive flowering and it is possible that PGR applications could not negate the effect of these stresses on leaf abscission. During 2007, the application of treatments aimed at reducing stress showed no significant improvement in leaf retention, because leaf abscission in the controls was low and the effect of treatments could have been masked by the low leaf abscission. Although not significant, some positive effects were observed, and further investigation is necessary to determine optimal application rates and timing of these treatments.

6.2 Introduction

Results in Chapter 5 indicated that excessive and premature leaf abscission prior to flowering in 'Ryan' has a negative effect on the following season's yield. This was also found in Israel where excessive and premature leaf abscission affected yield in avocado (no specific cultivars mentioned) negatively (Aviles, 1995). This is because the leaves that drop are responsible for producing carbohydrates for flowering, fruit set and new spring flush growth (Wolstenholme, 2001). As a result of excessive leaf abscission prior to flowering, flowering must depend heavily on stored starch reserves, leaving a limited amount of these stored resources available for fruit set and new flush growth (Chapter 5). New spring flush growth and fruit set compete for the limited stored carbohydrates, which results in lower fruit set and yield (Wolstenholme, 2001). The prevention of leaf abscission is therefore very important in order to ensure that leaves remain active and provide carbohydrates required for good fruit set and thus production.

A number of horticultural practises may be employed in an attempt to prevent excessive leaf abscission in 'Ryan' avocado trees. The application of plant growth regulators, including auxins and cytokinins, successfully prevented abscission in a number of crops. The auxins used included IAA (indole acetic acid), IBA [γ-(indole-3)-n-butyric acid], IPA [β-(indole-3)-propionic acid], NAA (naphthalene acetic acid), 2,4-D (2,4-dichlorophenoxyacetic acid), TP (2,4,5-trichlorophenoxypropionic acid), and TPA (3,5,6-trichloro-2-pyridyloxyacetic acid). The cytokinins used were mostly BA (benzyladenine) and CPPU [N-(2-chloro-4-pyridyl)-N'-phenylurea]. Examples of such use of growth regulators included the prevention of fruitlet abscission in litchi with 2,4-D, IAA, NAA, TP, TPA (Hoda *et al.*, 1973; Singh and Lal, 1980; Stern *et al.*, 1995; 2000; Kift *et al.*, 2002), reduction of preharvest fruit drop in citrus using 2,4-D (Anthony and Coggins Jr., 1999), prevention of cotyledon abscission in bean with IAA, IPA, IBA and NAA (Abeles and Rubinstein, 1964), and prevention of seedling leaf abscission in avocado, using a combination of NAA and BA (Raviv and Reuveni, 1984). The correct concentration, application rate and timing of PGR application is crucial in order to obtain the desired effect, because PGRs can also enhance abscission when applied at too high a concentration or at non-optimal times. The application of PGRs is used extensively in the deciduous fruit industry to thin apples (Williams, 1979; Honeyborne, 1993; Costa, 2004; Robinson, 2004) and pears

(Marais, 1987). Fruit thinning was also achieved in litchi with the application of high concentrations of the synthetic cytokinin, CPPU [N-(2-chloro-4-pyridyl)-N'-phenylurea] (Roets, 2003). It is therefore crucial to determine the optimum application rate and time of application of PGRs in order to retard leaf abscission in 'Ryan' avocados.

The damaging effect of high light intensities during winter and the resulting photo-inhibition of 'Ryan' leaves may be reduced with the application of the reflective substance, kaolin. Kaolin application reduced solar damage on apple (Glenn *et. al.*, 2001; 2002; Gindaba and Wand, 2005), reduced transpiration and improved water use efficiency and photosynthesis in different crops subjected to high light intensities (Cohen *et al.*, 2005; Glenn, 2005).

Silicon is in many cases neglected as an important element in plants, although it is present in relatively high concentrations. Silicon has, however, recently been described as an important element in increasing a plant's tolerance to stress, such as salinity stress (Zuccarini, 2008), oxidative stress (Gong *et al.*, 2008) and metal and boron toxicities (Epstein, 1999; Gunes *et al.*, 2007). Silicon application on nursery avocado trees showed positive results in lowering *Phytophthora* root rot and improving root density (Bekker, *et al.*, 2007). Silicon application could therefore also be investigated as a method for increasing tolerance against stresses other than *Phytophthora*, thereby having a positive effect on leaf retention in avocado trees.

Other stresses that may accelerate leaf abscission, such as nutrient and water stress, excessive flowering and diseases, all of which the grower has a certain degree of control over, must also be prevented. Annual leaf and soil sampling should be performed in order to determine tree nutrient requirements whereafter fertilizer programs should be adapted accordingly (Koen and Du Plessis, 1991). Effective pest and disease control, as well as optimal irrigation scheduling is crucial to optimize production.

The aim of this study was to identify a practical method to reduce excessive leaf abscission in 'Ryan'. This included the application of plant growth regulators, fertilizers, kaolin, silicon and flower thinning agents. The effect of these

treatments on fruit set was also investigated as this has a major impact on final yield.

6.3 Materials and Methods

6.3.1 2005 season

Two blocks of 'Ryan' trees were used during this season. One block was expected by the grower to have high leaf abscission (Block A) and one block to have low leaf abscission (Block B). For each block, eight trees (replicates) per treatment were selected in a complete random design and applications of dolomitic lime (H. Pistorius (Pty.) Ltd.), Solubor® (Hortichem, Ocean Agriculture (Pty.) Ltd., SA), naphthalene acetic acid (NAA) (Rhône-Poulenc Agrichem SA (Pty.) Ltd.), benzyladenine (BA) (Sigma, SA), Kelpak® (Kelp Products (Pty) Ltd.), sodium nitroprusside (Merck, SA) and kaolin (Engelhard, USA) were made (Table 1). The treatments were made during July 2005 just after bud break (Figure 1). For the foliar applications, a handgun was used to apply the spray mixture (pressure of 2 bar or 200 kPa) until runoff (approximately 8 to 10 L per tree). For the soil applications, the chemicals were mixed with 20 L of water and applied in the root zone or drip area of the tree. A 20 L mixture of the soil treatment was prepared for every tree. Applications were made early in the morning when it was still cool, to prevent evaporation of the spray mixture. The wetting agent Sanawet® (Dow Agro Sciences, SA) was used at a concentration of 0.1% (v/v) for each spray mixture and chemical used. Thereafter, eight branches were selected (four on the eastern and four on the western side of the tree) on each tree for leaf abscission measurements The number of leaves on the day of application was recorded, and again four weeks after fruit set. The percentage leaf abscission was calculated using the following formula:

$$\% \text{ Leaf abscission} = (L_i - L_f)/L_i \times 100 \quad (1)$$

where L_i, the number of leaves on the day of chemical applications, and L_f, is the number of leaves four weeks after fruit set

The number of fruit per branch was recorded four weeks after fruit set on the same branches that were used for leaf counts.

Table 1: Treatments applied just after bud break (July) in an attempt to prevent excessive leaf abscission in 'Ryan' trees during the 2005 season

Chemical applied	Concentration
Control	-
Dolomitic lime	2kg/tree
Solubor®	50g/tree
Combination of Dolomitic lime and Solubor®	2kg/tree & 50 g/tree
Naphthalene acetic acid (NAA)	30mg L^{-1}
Benzyladenine (BA)	10mg L^{-1}
*Kelpak®	5mL L^{-1}
Sodium nitroprusside	20mg L^{-1}
Kaolin	30g L^{-1} (3%)

**An extract from seaweed containing 0.031mg L^{-1} cytokinin and 11mg L^{-1} auxin*

Figure 1: Applications of fertilizers, plant growth regulators and kaolin were made just after bud break during the 2005 season.

6.3.2 2006 season

The trial for this season was laid out in a random block design with 16 trees (replicates) per treatment. Applications of growth regulators and kaolin were made earlier than during the 2005 season, at the bud dormancy and bud swell phenological stages (Table 2). Kelpak®, kaolin and NAA were included again, and during this year applications of Ethephon® (Kynoch Agrochemicals (Pty.) Ltd.), ProGibb® (containing 4% GA_3, Valent BioSciences, Philagro SA (Pty.) Ltd) and LB urea (Hortichem, Ocean Agriculture (Pty.) Ltd., SA) were made (Table 2). The wetting agent, Sanawet® (0.1%, v/v), was used again for all treatments. On each tree, four branches were marked, two on the eastern and two on the western side of the tree. The number of leaves on each branch was counted at the bud dormancy stage and again four weeks after fruit set for both application times. The percentage leaf abscission was calculated, using formula (1). The number of fruit on each branch was recorded four weeks after fruit set and recorded as fruit set.

Table 2: Treatments applied with the aim of preventing of excessive leaf abscission in 'Ryan' during the 2006 season

Chemical	Concentration	Application time[#]
Control	-	1
Kelpak®	5mL L^{-1}/L (1:200)	1,2
NAA	30mg L^{-1}	1,2
Ethepon®	250mg L^{-1}	1,2
ProGibb® (GA_3)	50mg L^{-1}	1,2
Kaolin	3% (m/v)	1,2
LB urea	0.5% (m/v)	1

[#]*1: Bud dormancy stage; 2: Bud swell stage*

6.3.3 2007 season

The 'Ryan' orchard used, and the trial layout for this season, was the same as those used during the 2006 season. However, the treatments differed from those applied during the 2006 season as applications were made earlier than in 2006 and different products and treatment combinations used (Table 3). Products added for this season that were not used during the previous seasons were limestone ammonium nitrate (LAN) (Sasol Chemical Industries Ltd., SA) and Nontox-Silica® (Plant Bioregulators (Pty.) Ltd.), a product containing 14% (w/w) silicon. Again, the wetting agent, Sanawet®, was used for all treatments. For each branch, the number of leaves was recorded during March 2007 just after the latest summer flush hardened off, and again four weeks after fruit set. The percentage leaf abscission was calculated using formula (1). The number of fruit that set on the same branches on which leaf counts were performed, was recorded four weeks after fruit set. In addition, fruit retention data was obtained for this season by recording the number of fruit per tree after the November fruit drop period.

Table 3: Treatments applied with the aim of preventing excessive leaf abscission in 'Ryan' during the 2007 season

Chemical and concentration	Application time
Control	March 2007
A combination of 150g LAN, 100g Solubor®, 5mL L^{-1} Kelpak® (soil application), and 3% Kaolin (foliar application)	LAN, Solubor® and Kelpak® - March and repeated in April 2007. Kaolin – monthly from March to July 2007
5mL m^{-2} Nontox-Silica® (soil application)	March 2007 and repeated during inflorescence development (July 2007)
2mL L^{-1} Nontox-Silica® (foliar application)	March 2007 and repeated during inflorescence development (July 2007)
75 mg L^{-1} ProGibb® (foliar application)	March 2007 and repeated in April 2007
Inflorescence thinning (removal of approximately 33% of inflorescence by hand pruning)	During inflorescence development (July 2007)

6.3.4 Statistical analysis of data

Data was analyzed using the Statistical Analyzing Systems (SAS), version 8.0 (SAS Institute Inc., 1999) software program. For the 2005 and 2006 seasons, significant differences between treatments with regard to leaf abscission and fruit set were obtained using the General Linear Model (GLM) procedure of SAS. For the 2007 season, treatments were compared for differences in leaf abscission using the GLM procedure of SAS, while differences in fruit set and fruit retention between treatments were obtained using the Kruskal-Wallis analysis of variance test.

6.4 Results and Discussion

6.4.1 2005 season

Although leaf abscission in Block A (suspected high rates of abscission) was marginally higher than block B (suspected low abscission rates), this was not statistically significant. Leaf abscission was, on the whole, increased by the various treatments, but this increase was not significant, partly due to high variation within the orchard (Table 4). The treatments in which a slight reduction in leaf abscission was recorded for the 2005 season was the 50g/tree Solubor® treatment in both blocks and the 2kg/tree dolomitic lime treatment in Block B (Table 4). Although no significant differences were observed in leaf abscission rates for the various treatments, there were significant differences in fruit set for Block B in 2005. In this block, the 50g/tree Solubor® treatment, 50g/tree Solubor® in combination with 2kg/tree dolomitic lime, 10mg L^{-1} BA and 3% kaolin treatments all significantly lowered fruit set compared with the control (Table 5).

Table 4: Effect of chemical applications on leaf abscission of 'Ryan' trees during the 2005 season in two orchards with different expected rates of leaf abscission

Treatment	High abscission suspected (Block A)		Low abscission suspected (Block B)	
	Mean % leaf abscission[#]	Standard deviation (STD)	Mean % leaf abscission[#]	STD
Control	40.45a	12.72	33.53a	14.31
50g/tree Solubor®	36.51a	5.64	32.01a	14.46
2kg/tree Dolomitic lime	39.49a	17.78	34.60a	17.43
50g/tree Solubor® & 2kg/tree Dolomitic lime	45.74a	14.79	37.04a	12.89
30mg L^{-1} NAA	43.50a	14.52	53.12a	16.09
10 mg L^{-1} BA	44.69a	12.23	44.26a	16.10
5mL L^{-1} Kelpak®	47.79a	17.49	36.33a	11.60
20mg L^{-1} Sodium nitroprusside	42.12a	16.59	44.35a	12.30
3% Kaolin	46.15a	4.22	46.44a	12.32

[#]*Means in the same rows and columns followed by the same letter do not differ significantly at P=0.05*

Table 5: Effect of chemical applications on fruit set of 'Ryan' trees during the 2005 season

Treatment	High abscission suspected		Low abscission suspected	
	Mean no. of fruit per branch [#]	STD	Mean no. of fruit per branch [#]	STD
Control	1.13a	0.86	1.41a	1.34
50 g/tree Solubor®	0.73a	1.32	0.48b	0.40
2 kg/tree Dolomitic lime	0.98a	0.84	0.60ab	0.41
50 g/tree Solubor® & 2 kg/tree Dolomitic lime	0.75a	0.69	0.27b	0.26
30 mg L^{-1} NAA	0.89a	0.61	0.76ab	0.77
10 mg L^{-1} BA	0.80a	0.60	0.44b	0.20
5 mL L^{-1} Kelpak®	1.27a	1.52	0.59ab	0.67
20 mg L^{-1} Sodium nitroprusside	1.02a	0.70	0.80ab	0.49
3% Kaolin	0.83a	0.73	0.52b	0.29

[#]*Means in the same columns followed by the same letter do not differ significantly at P=0.05*

The 2005 investigation served as a pilot study, where treatments were chosen based on field observations and observations by growers. Dolomitic lime (containing calcium and magnesium) and Solubor®, (containing 250g/kg boron), were applied as magnesium and boron deficiency symptoms were observed on trees. However, although both Solubor® treatments reduced leaf abscission slightly, they had a negative effect on fruit set. It was found that high concentrations of boron applied to melons (*Cucumis sativus* L.) had a negative effect on fruit set (Goldberg *et al.*, 2003), but there are no reports that high boron application on avocado will have the same effect. It is, however, recommended that low quantities of boron are applied regularly to the soil in avocado orchards, while monitoring leaf boron levels to prevent toxicity (Whiley *et al.*, 1996). Two to three foliar applications of 100 g Solubor® per 100 L on new flush is currently recommended, or a 10 to 20 g m^{-2} Borax soil application at the same time (Abercrombie, 2001). It is therefore possible that the excessively high

concentrations of boron were applied to trees in this trial, which could result in the observed negative effect on fruit set. Therefore, although the 50 g/tree Solubor® application reduced leaf abscission it will not be recommended as it influenced fruit set negatively.

Naphthalene acetic acid, BA and Kelpak® (a natural product containing auxin and cytokinins) were included due to their possible inhibiting effect on leaf abscission (Raviv and Reuveni, 1984). However, application time and concentration of the growth regulators applied are crucial and leaf abscission may be accelerated or no effect may be observed, depending on the stage of leaf senescence. PGRs may also influence fruit set and retention, as was found for litchi with the application of synthetic auxins, which improved fruit retention when applied just prior to natural fruit abscission (Stern *et al.*, 1995; Wittmann, 2002). Application of the synthetic cytokinin, CPPU, at a concentration of 10 and 20 mg L^{-1} at an early stage of fruit set in litchi enhanced fruitlet abscission (Roets, 2003). Results from this trial on 'Ryan' avocados suggest that 10 mg L^{-1} BA, applied during flowering, enhanced fruitlet abscission, whilst having no effect on leaf retention. It is also possible that the concentration of BA applied close to fruit set was too high and resulted in enhanced fruitlet abscission, as it was reported that high concentrations of exogenous applied cytokinins stimulate ethylene production, which will enhance abscission (Grossmann & Hansen, 2001). It is therefore possible that the young avocado fruit at fruit set could be more sensitive to BA than the leaves, resulting in fruit set being negatively affected with no effect on leaf retention. As BA did not have a desirable impact on leaf retention and a negative effect on fruit set it would not be recommended.

Kaolin applications were performed with the aim of protecting leaves against photo-inhibition (Glenn, 2005). However, kaolin application during July did not reduce leaf abscission, possibly because photo-inhibition was already initiated before kaolin applications were made in early winter. The damaging effect of photo-inhibition could therefore already have occurred and earlier applications should be considered. Another negative effect of kaolin during this season was that fruit set was significantly reduced, which could be attributed to a negative effect on pollination, as the trees were flowering when kaolin applications were made. The honeybee (*Apis mellifera* L.) is the main pollinator of avocado flowers

(Van den Berg, 2001), and the kaolin coat on the trees and flowers could create an unfavourable environment for these pollinators (the kaolin is reported to stick to the insects), preventing them from visiting the flowers for pollination (P.H. Joubert, personal communication), with a subsequent negative effect on pollination. In addition, during kaolin application some flowers could be open in which case the pistils of these flowers could be covered with kaolin, making pollen attachment difficult, thereby further negatively affecting pollination.

The application of sodium nitroprusside was performed in an attempt to protect leaves against photo-oxidative damage. During occurrence of photo-inhibition free radical production (eg. ·OH and $O_2^{\cdot-}$) may occur at higher rates (Niyogi, 1999), which will result in photo-oxidative damage to plant cells. Sodium nitroprusside is a nitric oxide (NO) donor, and NO is reported to protect the plant against photo-oxidative stress by detoxifying reactive oxygen species (Beligni and Lamattina, 2002). However, in this trial on 'Ryan', no significant effect was observed on leaf retention with this treatment, possibly because photo-inhibition could already be present when the applications were made.

6.4.2 2006 season

Based on results achieved during 2005, treatment application times were altered and new treatments added. Results during 2005 indicated that earlier applications of the various treatments could be beneficial and thus, in 2006, applications were made at the bud dormancy and/or bud swell stages.

During the 2006 season excessive and premature leaf abscission occurred for 'Ryan' with up to 85% of leaves abscising in the control treatments (Chapter 3). However, none of the treatments significantly reduced excessive leaf abscission (Table 6), despite applications occurring at both the bud dormancy and bud swell stages and the inclusion of additional treatments. However, no adverse effects on fruit set were noted during this season (Table 7), indicating that earlier applications were less deleterious on fruit set.

Table 6: Effect of chemical applications on leaf abscission for 'Ryan' for the 2006 season applied at two different phenological stages, viz. the dormant bud and bud swell stages and recorded 4 weeks after fruit set

Treatment	Dormant bud stage		Bud swell stage	
	Mean[#]	STD	Mean	STD
Control	82.85 a	13.13	82.85 a	13.13
5 mL L^{-1} Kelpak®	76.77 a	15.27	89.92 a	8.34
30 mg L^{-1} NAA	85.89 a	14.90	81.69 a	14.27
250 mg L^{-1} Ethephon®	86.58 a	12.64	77.40 a	15.15
0.5% LB urea	80.78 a	13.85	n/a*	n/a
3.0% Kaolin	86.30 a	18.88	86.08 a	14.39
50 mg L^{-1} GA_3	84.60 a	11.67	83.43 a	14.49

[#]*Means in the same rows and columns followed by the same letter do not differ significantly at P=0.05*

**n/a = no application*

Table 7: Effect of chemical treatments on fruit set for 'Ryan' during the 2006 season applied at different phenological stages, viz. the dormant bud and bud swell stages

Treatment	Dormant bud stage		Bud swell stage	
	Mean[#]	STD	Mean	STD
Control	0.34 a	0.53		
5 mL L^{-1} Kelpak®	0.20 a	0.26	0.23 a	0.45
30 mg L^{-1} NAA	0.06 a	0.14	0.20 a	0.36
250 mg L^{-1} Ethephon®	0.16 a	0.29	0.27 a	0.38
0.5% LB urea	0.13 a	0.27		
3.0% Kaolin	0.12 a	0.28	0.48 a	0.95
50 mg L^{-1} GA_3	0.13 a	0.19	0.16 a	0.33

[#]*Means in the same rows and columns followed by the same letter do not differ significantly at P=0.05*

Applications of PGRs and kaolin were done at two phenological stages (bud dormancy and bud swell), because it was uncertain when leaf abscission would be triggered. This also followed the observation in 2005 that leaf senescence was already evident before application of the 2005 treatments during bud break, and to prevent an adverse effect of the PGRs on fruit set. From the 2006 season's data it became evident that leaf abscission occurred predominantly during two periods of low temperature. Firstly, spring flush leaf abscission started to increase after the first low temperature event at the beginning of June 2006 (at bud dormancy), and secondly, a large number of spring flush and summer flush leaves dropped at flowering time after a second low temperature event during inflorescence development (in which case green abscission of some summer flush leaves occurred) (Chapter 5). In addition, 'Ryan' flowered excessively and a weak positive correlation was obtained between excessive leaf abscission and excessive flowering in 'Ryan' (Chapter 5), indicated that excessive flowering could also be a contributing factor to premature and excessive leaf abscission, possibly by placing additional stress on the tree. It is therefore possible that the application of PGRs and kaolin could not negate the effect of these stress factors on premature and excessive leaf abscission, and had therefore no effect in improving leaf retention. GA_3 applications were made in an attempt to prevent

excessive flowering. This is based on a study done by Salazar-Garcia and Lovatt (1999), where they found that trunk injections of GA_3 at concentrations of 25 and 50 mg per tree reduced the total number of inflorescences as a result of increased flower bud abscission. Although flower bud abscission was not quantified in this study, the foliar application of 50 mg L^{-1} GA_3 had no effect in reducing premature and excessive leaf abscission in 'Ryan'. The application of Ethephon, meant to accelerate leaf abscission, served as a negative control, but had no significant effect on leaf abscission. This could be as a result of the naturally high abscission rates of leaves during this season overriding the effect Ethephon could have had on leaf abscission.

6.4.3. 2007 season

Data from the 2006 season indicated that a combination of stress factors were responsible for premature and excessive leaf abscission in 'Ryan' (Chapter 5). Applications for the 2007 season were therefore focussed on stress relief. Combinations of additional fertilizers, PGRs and kaolin were therefore applied in an attempt to increase tolerance towards stress and prevent premature and excessive leaf abscission (Table 3). In addition, Nontox-Silica®, containing 14% (w/w) silicon, was included as a foliar and soil application (Table 3). Chemical and mechanical flower thinning treatments were included to lessen the effect of excessive flowering on leaf abscission, since excessive flowering has been identified as a possible contributing factor to premature and excessive leaf abscission (Chapter 5). All treatments (except the mechanical flower thinning treatments) were repeated during inflorescence development in an attempt to improve the effectiveness of the treatments (Table 3).

None of the treatments had any significant effect on leaf abscission, although mean leaf abscission for all treatments tended to be lower than the control (Table 8). Fruit set of the combination treatment (fertilizer, kaolin and PGRs) and the two Nontox-Silica treatments tended to be higher than the control (Table 9). However, there were no significant differences in fruit set between the control and the treatments or between treatments. The 75 mg L^{-1} GA_3 treatment and mechanical flower thinning treatment, aimed at lowering stress by preventing excessive flowering, also had slightly lower leaf abscission than the control, but it was not

statistically significant. Fruit set for these two treatments was also not statistically significant.

Table 8: Effect of chemical applications on leaf abscission on 'Ryan' during the 2007 season

Treatment	**Percentage leaf abscission**	
	Mean[#]	STD
Control	29.61a	22.17
150g LAN, 100g Solubor®, 5mL L^{-1} Kelpak® & 3% Kaolin (March, April and May 2007)	24.10a	14.78
5mL m^{-2} Nontox-Silica® – soil application (Bud dormancy & Inflorescence development)	22.61a	13.37
2mL L^{-1} Nontox-Silica® – foliar application (Bud dormancy & Inflorescence development)	18.79a	13.06
75mg L^{-1} ProGibb® – foliar application (Bud dormancy & bud break)	22.01a	12.07
Inflorescence thinning (Inflorescence development)	23.12a	17.58

[#]*Means followed by the same letter do not differ significantly at P=0.05*

Table 9: Effect of chemical applications on fruit set and retention on 'Ryan' during the 2007 season

Treatment	Mean number of fruit set per branch		Mean number of fruit per tree after the November fruit drop period	
	Mean[#]	STD	Mean[#]	STD
Control	0.50a	0.76	44a	46.7
150g LAN, 100g Solubor®, 5mL L^{-1} Kelpak® & 3% Kaolin (March, April and May 2007)	1.65a	2.12	52a	47.6
5mL m^{-2} Nontox-Silica® – soil application (Bud dormancy & Inflorescence development)	0.69a	0.67	53a	31.4
2mL L^{-1} Nontox-Silica® – foliar application (Bud dormancy & Inflorescence development)	1.93a	2.07	72a	58.2
75mg L^{-1} ProGibb® – foliar application (Bud dormancy & bud break)	0.70a	1.48	37a	46.4
Inflorescence thinning (Inflorescence development)	0.48a	0.69	36a	27.2

[#]*Means followed by the same letter do not differ significantly at P=0.05*

6.5 Conclusion

During the 2005 season it became evident that chemical applications aimed at preventing excessive leaf abscission should be made before inflorescence development, since leaf abscission could be triggered before inflorescence development and to prevent a negative effect of chemical applications on fruit set. However, earlier applications during 2006 and 2007 showed no significant improvement in leaf retention, and no definite conclusion could therefore be

reached on the use of chemical products, concentrations and application times for the prevention of premature and excessive leaf abscission in 'Ryan'. However, from the investigation and results described in Chapter 5 it became evident that events, such as extreme low temperatures and possibly excessive flowering, can result in stress and accelerate leaf abscission. These stress-causing events are unpredictable and might even be absent during certain seasons, which creates difficulty in determining the optimal timing of chemical applications to prevent premature and excessive leaf abscission. This could be a possible reason why no significant improvement in leaf retention was made in this study. It is therefore suggested at this stage that growers must ensure that the best farm management practices are always followed, ensuring optimal irrigation, fertilization and pest and disease management to prevent stress. However, it would be necessary to provide additional protection to leaves against photo-inhibition and the subsequent damage (which will trigger leaf abscission) caused by excessive sunlight during winter. It is therefore important that further research be carried out on the use of kaolin, and possibly sodium nitroprusside, to determine the optimal time of application and application rates, and also to determine the effect of these substances on photo-inhibition. The use of silicon as a stress reducing treatment also needs further attention, based on the positive trends obtained during the 2007 season on leaf retention and fruit set and retention. More detailed investigation is needed on flower thinning to prevent excessive flowering whilst at the same time ensuring that this thinning has no adverse effect on yield.

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Chapter 7: Conclusion

The observation by avocado growers that premature and excessive leaf abscission occurs on 'Ryan' trees during flowering time was confirmed quantitatively in this study. The leaf abscission pattern of 'Ryan' was studied over three seasons (2005 to 2007) and it has became evident that premature and excessive leaf abscission is not an annual event for 'Ryan' trees, as was speculated by some growers. In addition, during 2006 when excessive and premature leaf abscission occurred, there was a dramatic increase in spring, as well as summer flush, leaf abscission for 'Ryan' between inflorescence development and fruit set, with most of the leaves dropping during this period. This dramatic increase in leaf abscission observed for the 2006 season did not occur during 2005 and 2007. Premature and excessive leaf abscission was not observed for 'Fuerte' and 'Hass' trees, although leaf abscission was significantly higher for 'Fuerte' and 'Hass' during 2006 when compared with 2007. Anatomical studies did not shed any light on the exact time of formation of the abscission layer, and only the protective layer of the abscission zone was visible just prior to leaf abscission. As premature and excessive leaf abscission was found not to be an annual event in 'Ryan', it has created speculation that environmentally stressful conditions (e.g. unfavourable climatic conditions, nutrient imbalances, drought stress, diseases and excessive flowering) could cause this phenomenon. This speculation was strengthened by the fact that even 'Hass' and 'Fuerte' displayed higher leaf abscission when 'Ryan' trees abscised their leaves prematurely and excessively. Different environmental conditions were therefore studied and compared during the 2006 and 2007 seasons.

During 2006, two distinct periods of extremely low temperatures occurred, which did not occur during 2007. Increased leaf abscission directly followed these periods of low temperatures. During the second low temperature event during 2006, the solar radiation to temperature ratio was also very high when compared with the rest of the measuring period. This period was followed by a dramatic increase in leaf abscission, with most leaves abscising after this period, including green summer flush leaves. Low temperature and light stress, which could result in photo-inhibition and photo-oxidative damage to leaves, could therefore be a major factor inducing premature and excessive leaf abscission in 'Ryan' during

2006, especially taking the abscission of green summer flush leaves into consideration. However, the impact of such low temperature events on photo-inhibition, light stress, and premature and excessive leaf abscission needs further investigation with the inclusion of photosynthesis and chlorophyll fluorescence studies. In addition, low temperature stress at the beginning of winter in 2006 could have triggered the excessive flowering observed in 'Ryan' during that season, as it occurred at the time of flower initiation. Excessive flowering places a much higher demand for nutrients, water and energy on the tree than would be the case under normal flowering circumstances. Excessive flowering could therefore also cause stress that could further contribute to excessive and premature leaf abscission in 'Ryan'. The absence of excessive flowering and premature and excessive leaf abscission in 'Hass' and 'Fuerte' strengthens the argument that excessive flowering could be a contributing factor to excessive and premature leaf abscission in 'Ryan'. However, the low correlation between excessive flowering and excessive leaf abscission obtained in this study, suggested that excessive flowering was not a major contributing factor to excessive leaf abscission. Excessive flowering has not previously been reported to occur in 'Ryan' and it is possible that it would only occur under environmentally stressful conditions. Other environmental factors, which included rainfall, relative humidity and evapotranspiration, did not differ substantially between the 2006 and 2007 seasons and drought stress is therefore not considered to play a major role in excessive and premature leaf abscission in 'Ryan' for this study. However, it should be kept in mind that drought stress will cause premature and excessive leaf abscission and irrigation management should therefore always be optimal. It is suggested that further investigation into the water requirements for 'Ryan' be conducted in order to establish if current irrigation scheduling is optimal and if irrigation scheduling for 'Ryan' should differ from 'Hass' and 'Fuerte'. Although nutrients in extreme deficiencies were shown to cause premature and excessive leaf abscission in other plant species, nutrients did not play a role in premature and excessive leaf abscission in 'Ryan' in this study. However, nutrients should also be managed optimally to ensure that they are within the recommended norms. The cultivar 'Ryan' is possibly more sensitive to stress than 'Fuerte' and 'Hass' due to genotypic differences, and thus under the same stressful conditions, 'Ryan' will abscise more leaves.

This study was conducted in a single orchard and conditions may vary across different orchards and areas and the factors resulting in premature and excessive leaf abscission in one orchard could therefore be different from those in another orchard. In addition, in an orchard situation, more than one environmental factor impacts a tree at any given time. The combined effect of more than one factor could result in a different response by the plant than the sum effect of each factor acting alone. The impact of environmental factors in an orchard situation on a tree may therefore be complex and a large variation may even occur within a single orchard.

Due to the complexity of the causes of excessive and premature leaf abscission in 'Ryan', and the fact that different orchards may experience different conditions, there is no single application or chemical that can be recommended to reduce excessive and premature leaf abscission in 'Ryan'. However, stress that results in premature and excessive leaf abscission can be managed to a certain extent. Although climatic stress factors cannot be managed, nutrition, irrigation and pest and disease control should be optimally managed in order to prevent stress caused by nutrient imbalances, drought or waterlogging, and pests and diseases. In addition, flower thinning may be performed in the event of excessive flowering, although more thorough research is still needed with regard to determining the exact timing and number of flowers to be thinned. In the long term, it would be important that cultivar development programmes focus on developing an alternative late season cultivar (to extend the harvesting period and ensure that fruit are available for longer periods), which is more cold tolerant and more tolerant to other stress factors that could result in premature and excessive leaf abscission. It is recommended that growers considering planting 'Ryan', should identify the warmest areas on their farms for planting this cultivar, and avoid areas where extreme low temperatures and frost occurs.