

**Manipulation of avocado (*Persea americana* Mill.) trees  
for out of season fruit production**

**By**

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## CHAPTER 1

### General introduction

The main avocado production areas in South Africa are concentrated in the subtropical areas of the Northern and Mpumalanga provinces. The area planted to avocados covered some 12 500 ha in 1999, with 'Hass' and 'Fuerte' accounting for 33% and 42% of the area respectively (Derik Donkin, SAAGA, personal communication). Of the total avocado production, approximately 55% is exported to Europe from April to September, while 35% is consumed nationally and 10% is processed into oil. In recent years, the profitability of the avocado trade has been affected by increasingly unstable market prices, as the South African fruit are competing with fruit from Israel, Spain, Mexico and Kenya on the European market (Köhne, 1999).

Westfalia Estate, which forms part of the Hans Merensky Foundation, is a large-scale avocado producer with approximately 1000 ha of avocados. Avocados are produced from March until late October by farming in several areas with different climatic conditions, i.e. Mooketsi (warm, dry climate), Duivelskloof (warm, wet climate) and KwaZulu Natal (cool, wet climate). Westfalia Estate is mainly export orientated, however in an assessment of alternative market possibilities the supply of out of season fruit to the South African market was seen as an opportunity to increase profits.

Westfalia Estate has a contract to supply Woolworths with avocados for 12 months of the year. However, for a four-month period (November to February) avocados are not readily available in South Africa. In order to fulfill the contract, Westfalia Estate has to import fruit from neighboring countries. Besides the high costs incurred with importing, fruit quality is often below standard. Further, the introduction of early and late bearing cultivars to extend the avocado season holds promise but requires long-term research in order to find cultivars with superior qualities. Customer resistance to unknown cultivars poses a potential problem because of the time needed to create consumer confidence. Therefore, the ultimate solution would be to produce out of season fruit of a known cultivar on the Estate, through the manipulation of flowering and fruiting.

In mango, knowledge of the environmental links to floral induction and initiation is used in the tropics to enhance flowering during its normal season or to manipulate flowering to occur out of season (Davenport & Núñez-Elisea, 1997). In Thailand, paclobutrazol, a plant growth regulator, is used in combination with thiourea to produce out of season mango fruit (Nartvaranant, Subhadrabandhu & Tongumpai, 1999).  $KNO_3$  and  $NH_4NO_3$  sprays are used in Brazil (Ataide & Sao Jose, 1999), Mexico (Salazar-García, Perez-Barraza & Vazquaz-Valdivia, 1999; Perez-Barraza, Salazar-García & Vazquaz-Valdivia, 1999) and in the Philippines (Protacio, 1999) to induce earlier flowering in mango.

Avocado trees, grown in a sub-tropical region, have a cyclic growth pattern consisting of two to three vegetative flushes (spring, summer, autumn) and a reproductive flush in winter/early spring. Therefore, the normal growth and development cycles of the avocado tree must be disrupted to achieve out of season flowering and fruitset. There are two approaches to setting an out of season crop, viz. inhibiting normal season flowering or destroying the normal season flowers. In both cases, out of season flower induction will be the next step. This study was undertaken to investigate the production of out of season avocados according to the above mentioned approaches.



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## CHAPTER 2

### **The effect of gibberellic acid treatments on inflorescence development of avocado (*Persea americana* Mill.) cv. Fuerte, Hass and Ryan**

#### **2.1 Summary**

Gibberellins are known to have many regulatory functions on various processes in plants. In this study the effect of gibberellic acid (GA<sub>3</sub>) treatments on reproductive development of 'Hass' avocado trees was investigated at macroscopic and microscopic level. Bud swelling on avocado trees was observed in April and flowering occurred four to five months later in August/September. However, at microscopic level, the first signs of inflorescence development were already present in buds early in March, as secondary inflorescence axis meristems between the inner terminal bracts.

Concurrently, the effect of single and multiple GA<sub>3</sub> sprays (50 and 250 mg.L<sup>-1</sup>) on inflorescence development of potted 'Fuerte', 'Hass' and 'Ryan' trees was investigated. Depending on the timing of the application, single GA<sub>3</sub> treatments had the following effects on inflorescence development of avocado trees: none (February); delaying (mid March and early April); enhancing (late April and May). However, none of the single GA<sub>3</sub> application treatments had a significant effect on inflorescence development and flowering intensity.

Significant delaying and inhibition reactions on inflorescence development were observed with multiple GA<sub>3</sub> application treatments over a period of four months (February – May). The effect on inflorescence development tended to increase with increasing number of sprays applied and with an increase in the concentration used. Best results were obtained with a three-spray and a four-spray treatment. However, the additional inhibition or delaying effect obtained with a fifth spray was not significant when compared to the three-spray and four-spray treatments. Inflorescence development was almost completely inhibited on trees receiving a four-spray or five-spray treatment, as reflected by the flowering intensity at normal flowering time.

## 2.2 Introduction

The transition from vegetative growth to flowering is of paramount importance in agriculture, as it is the first step towards sexual reproduction (Bernier, Havelange, Houssa, Petitjean & Lejeune, 1993; Schwabe, 1987). In most tropical and subtropical fruit trees, flower initiation usually occurs after the termination of vegetative growth, during a period of cold weather or reduced water supply (Chaikiattiyos, Menzel & Rasmussen, 1994). However, it is known that plant growth regulators may be able to influence, replace or overcome these stimuli.

The gibberellins (GA's) are a group of natural plant growth regulators with many regulatory functions. GA's may stimulate cell division, cell elongation or both and they can control enzyme secretion (Hartman, Krofaneck, Rubatzky & Flocker, 1988). GA's are also involved in reproductive development which includes flower initiation, differentiation and all stages of the growth and development of the embryo, seed and fruit (Pharis & King, 1985).

GA application generally inhibits flowering in woody angiosperms such as apple, pear, peach, citrus and mango. In the deciduous fruit industry, the application of GA's to peach (Byers, Carbaugh & Presley, 1990; Southwick & Fritts, 1995), apricot, plum (Southwick & Fritts, 1995), nectarine (Southwick & Fritts, 1995; Coetzee & Theron, 1999) and apple trees (Greene, 1989) after full bloom,

inhibited the development of flower buds. This resulted in “fruit thinning” of the following year without the use of hand labour or chemicals.

Flower induction in citrus can occur after periods of water stress and / or low temperature stress. Gibberellic acid (GA<sub>3</sub>) treatment inhibited water stress-induced flowering in ‘Eureka’ lemon trees (Nir, Goren & Leshem, 1972) and ‘Tahiti’ lime trees (Southwick & Davenport, 1987). Flowering was decreased in the “on” year after GA application to Satsuma mandarins (Iwahori & Oohata, 1981) and *Citrus sinensis* (Lord & Eckard, 1987). An inhibitory effect on flowering of mango was also found (Tomer, 1984; Núñez-Elisea & Davenport, 1991). Tomer (1984), found double applications of GA<sub>3</sub> to exert a significantly higher inhibition on flowering of ‘Keitt’, ‘Tommy Atkins’ and ‘Palmer’ as compared to a single application. Núñez-Elisea & Davenport (1998) reported a delayed initiation of axillary buds, with increasing dosages of GA<sub>3</sub> applied to mango trees during cool, floral-inductive temperatures.

In avocado, competition between newly set fruit and developing spring flushes may influence fruit set and yield. As this competition is intense in indeterminate inflorescences, manipulation of flowering in order to reduce the competition may be successful in improving fruit set and yield. Therefore, a series of trials were conducted in California to investigate the effect of GA<sub>3</sub> treatments on the inflorescence phenology of avocado. Normal inflorescence phenology was altered by GA<sub>3</sub> application to selected branches of ‘Hass’ trees, causing the

vegetative shoot to develop precociously. After GA<sub>3</sub> treatment, development of the vegetative shoot occurred simultaneously with the elongation of the secondary and tertiary inflorescence axes (Salazar-García & Lovatt, 1998). A stimulation of vegetative shoot production, with a concomitant reduction in inflorescence production was also found after GA<sub>3</sub> was applied during the early stages of flower initiation. According to Salazar-García & Lovatt (1998), GA<sub>3</sub> applications before the beginning of inflorescence initiation and application to whole trees warrant further investigation.

Knowing exactly when each event leading to flowering occurs, is essential in order to manipulate flowering reliably. In avocado, floral induction which is the event triggering transcription and expression of flowering genes, evidently takes place sometime during the period between the last autumn vegetative flush and the time when initiation of inflorescence buds is apparent (Davenport, 1986). Robertson (1969) showed that under South African conditions, 'Fuerte' avocado buds collected early in February were completely vegetative as no differentiation of the meristematic areas in the bud scales had occurred. However, in March differentiation of the secondary meristems commenced. This is the first indication of differentiation from a vegetative to a reproductive phase. Salazar-García, Lord & Lovatt (1998) did similar research on the 'Hass' avocado in California. However, it is important to study these events under local conditions as climatic differences between regions can cause floral induction and initiation to occur at different times.

A field trial was conducted on Westfalia Estate to investigate inflorescence development at macroscopic and microscopic level on untreated and GA<sub>3</sub> treated 'Hass' trees. Concurrently, another trial was conducted in which the effect of single and multiple GA<sub>3</sub> sprays on inflorescence development of potted 'Fuerte', 'Hass' and 'Ryan' trees was investigated.



## 2.3 Materials and methods

### 2.3.1 Field trial

#### 2.3.1.1 Gibberellic acid treatments

Twenty three-year-old 'Hass' trees on Duke 7 rootstock, selected for uniform size and vigour, were treated with GA<sub>3</sub> (prepared from ProGibb® 4%, Abbott Laboratories). Treatments were applied during 1999 as single (9/3) or multiple (9/3 + 7/4 + 13/5) foliar sprays at two concentrations (50 or 250 mg.L<sup>-1</sup>). A motorised knapsack was used at a spray volume of 2.5 L.tree<sup>-1</sup>. Control trees were left untreated. Trees were evaluated for vegetative (early floral) and floral development every third week from March to June, every second week until August and then at monthly intervals until November, using a scale of 1-10 (Figure 2.1).

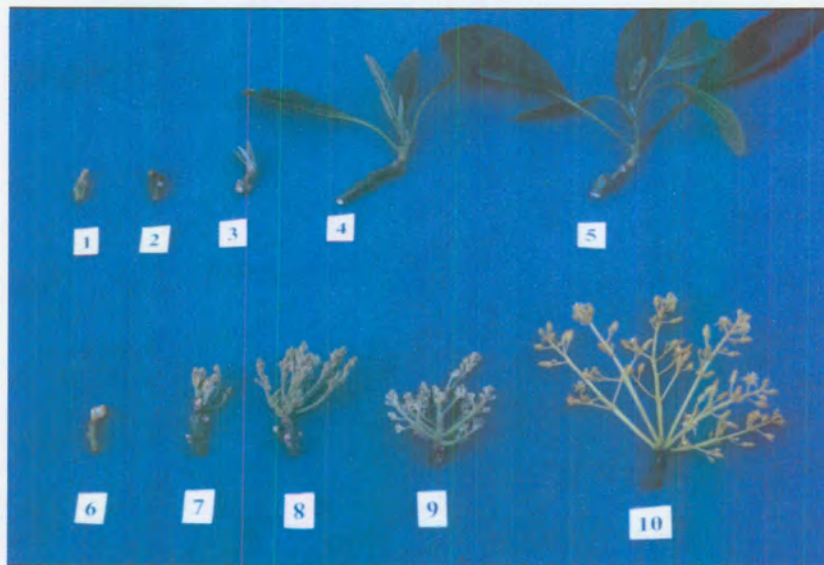


Figure 2.1. Evaluation scale of 1-10, where 1-5 represented vegetative (early floral) and 6-10 floral development.

Ratings of 1-5 (1 = unswollen vegetative bud, 5 = expanded flush) and 6-10 (6 = swollen flower bud, 10 = expanded inflorescence) represented vegetative and floral development respectively. Yield data were collected at harvest. No statistical analysis was performed on the evaluation data and yield data due to the limited number of trees used in the trial.

### **2.3.1.2 Anatomical study**

Five terminal buds from each of the 20 trees were collected every third week from March to May and every second week from June to August during 1999. Buds were fixed in FAA (5 formalin: 5 acetic acid: 90 ethanol solution, by volume) and dehydrated via sequential transfer through a series of aqueous ethanol solutions (70%, 96%, 100% ethanol), followed by a series of ethanol / xylene solutions (25%, 50%, 75%, 100% xylene) (Johansen,1940). Paraffin wax (paramat extra pastillated) was used for infiltration and embedding. Buds were sectioned with a rotary microtome at 12 $\mu$ m and stained with a safranin - fastgreen series. Sections were studied using a light microscope, and micrographs were taken with a Nikon DXM 1200 digital camera on a Nikon Optiphot microscope.

### **2.3.2 Trial on potted 'Fuerte', 'Hass' and 'Ryan' trees**

The trial consisted of 570 small trees (190 each of 'Fuerte', 'Hass' and 'Ryan') planted in 8 litres of Westfalia potting mixture. Trees were kept in the open and stems and exposed branches were painted with white PVA tree paint as

protection against sunburn throughout the duration of the trial. Irrigation was done on a daily basis. Trees received a slow release fertilizer (Multicote 8<sup>®</sup> at 5g.tree<sup>-1</sup>) every three months and a foliar feed Wuxal<sup>®</sup>, at a concentration of 3ml.L<sup>-1</sup> spray solution, was additionally applied every fortnight during winter to maintain good tree condition. An Aliette<sup>®</sup> stem paint at a concentration of 1000g.L<sup>-1</sup> of water was done regularly as protection against rootrot caused by *Phytophthora cinnamomi*.

GA<sub>3</sub>, prepared from ProGibb<sup>®</sup> 4% (Abbott Laboratories), was applied as a foliar spray. Two GA<sub>3</sub> concentrations (50 and 250 mg.L<sup>-1</sup>) were applied as single or multiple treatments. In order to evaluate the effect of GA<sub>3</sub> treatments made before the beginning of inflorescence initiation and during more advanced stages of inflorescence development, treatments started in February and ended in May 1999 (Table 2.1). Control trees were untreated.

Vegetative and floral development were evaluated every third week from February to May, and every second week from June to August, using the scale of 1-10 as shown in Figure 2.1. Eight terminal buds per tree were evaluated. In August 1999 the ratio between vegetative and floral development was quantified as a measure of flowering intensity, considering whole trees. A scale of 1-5 (1 = fully vegetative tree, 5 = tree in full bloom) was used (Figure 2.2).



Table 2.1. Application dates of single and multiple GA<sub>3</sub> treatments (50 and 250 mg.L<sup>-1</sup>) on 'Fuerte', 'Hass' and 'Ryan' trees as applied during 1999.

'Fuerte'	'Hass' and 'Ryan'
<b>Single application treatments (dates)</b>	
19/2	25/2
12/3	18/3
01/4	08/4
23/4	03/5
14/5	24/5
<b>Multiple application treatments (dates)</b>	
19/2 + 12/3	25/2 + 18/3
19/2 + 12/3 + 01/4	25/2 + 18/3 + 08/4
19/2 + 12/3 + 01/4 + 23/4	25/2 + 18/3 + 08/4 + 03/5
19/2 + 12/3 + 01/4 + 23/4 + 14/5	25/2 + 18/3 + 08/4 + 03/5 + 24/5



Figure 2.2. Evaluation scale representing flowering intensity (1= fully vegetative tree and 5= fully reproductive tree) as evaluated at full bloom in August 1999.

Evaluation of the trees continued for a second season (February – July 2000) in order to evaluate a possible carry-over effect from the 1999 treatments. However, no significant differences between treatments were observed and results will be presented for the 1999 season only.

The trial lay-out was a completely randomized design with 10 single tree replicates. Analysis of variance was performed on the data and least significant differences (LSD's) at the 5% significance level were used to compare treatments.



## 2.4 Results and discussion

### 2.4.1 Field trial

#### 2.4.1.1 Floral development on the tree

The first signs of bud swelling occurred in early March 1999. A month later (early April) swelling was observed in most of the buds. By early July, bud burst had taken place and subsequent flowering (anthesis) was observed in August / September 1999 (Figure 2.3).

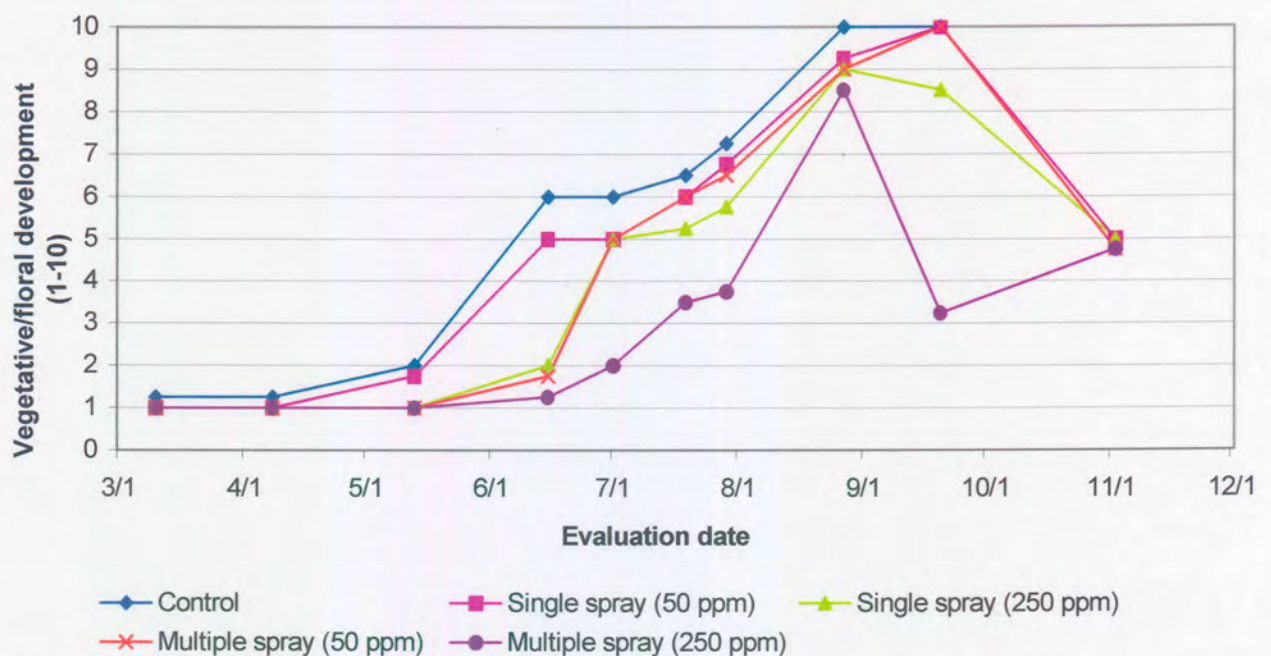


Figure 2.3. Average rating of vegetative (<5) to floral (>5) development (see Figure 2.1), as affected by single and multiple GA<sub>3</sub> treatments (50 and 250 mg.L<sup>-1</sup>) to 'Hass' trees during 1999. Values are means of 4 single tree replicates.

A single GA<sub>3</sub> treatment (50 mg.L<sup>-1</sup>) in early March, caused a slight delay in bud swelling and bud burst, but further development progressed similar to that of the

control trees, with anthesis (opening of the flowers) in August. Bud swelling on trees treated with a single GA<sub>3</sub> application (250 mg.L<sup>-1</sup>) in early March, was delayed by one month when compared to the untreated control trees (Figure 2.3). Although bud burst was also delayed by one month until late July, the trees flowered at the same time as the control trees. Bud swelling on trees subjected to a multiple GA<sub>3</sub> treatment at the low concentration of 50 mg.L<sup>-1</sup>, was delayed until June. However from then on, the rate of bud development increased and by the end of July bud burst occurred while anthesis started in August. GA<sub>3</sub> applied three times at the high concentration, almost completely inhibited flower development. Buds remained unswollen for the whole period (March - August) and then developed vegetative flushes in late September / October. However, the few inflorescences that did develop are reflected in Figure 2.3.

Although time of flowering was not greatly affected by the GA<sub>3</sub> treatments, the flowering intensity and subsequently yield were reduced on the treated trees. A 28% reduction in yield was obtained with a single GA<sub>3</sub> treatment (50 mg.L<sup>-1</sup>), whereas a multiple GA<sub>3</sub> treatment (250 mg.L<sup>-1</sup>) showed an 87% reduction in yield compared to untreated control trees. The reduction in yield for the other two treatments (single treatment at 250 mg.L<sup>-1</sup>; multiple treatment at 50 mg.L<sup>-1</sup>) was 58% when compared to the untreated control trees (Data not shown).

### 2.4.1.2 Anatomical study

The avocado has a compound inflorescence system, consisting of alternately borne secondary axes on a primary axis, with tertiary flower-bearing axes borne on the secondary axes. In most cases the primary axis does not end in a flower and retains its terminal bud, producing a vegetative flush (Figure 2.4).

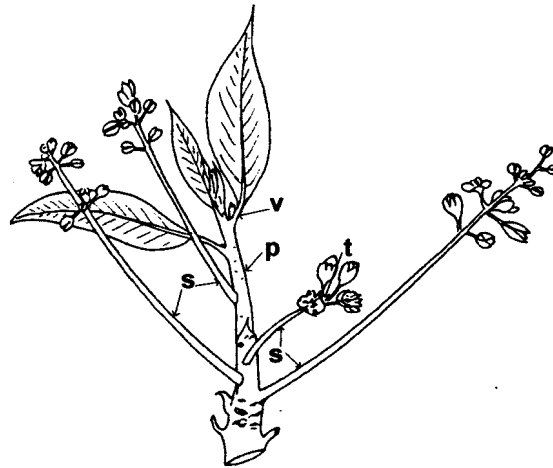


Figure 2.4. Diagram showing vegetative and reproductive growth of the avocado.

Abbreviations: p = primary inflorescence axis; s = secondary inflorescence axis; t = tertiary inflorescence axis; v = vegetative flush.

Already in early March, the first signs of inflorescence development were observed in bud sections studied under the microscope. Secondary inflorescence axis meristems were observed as small axillary meristems in the axils of the inner terminal bud bracts (Figure 2.5). These meristems developed during March - June forming the secondary axes of the inflorescence. This is in accordance with the results of Robertson (1969).



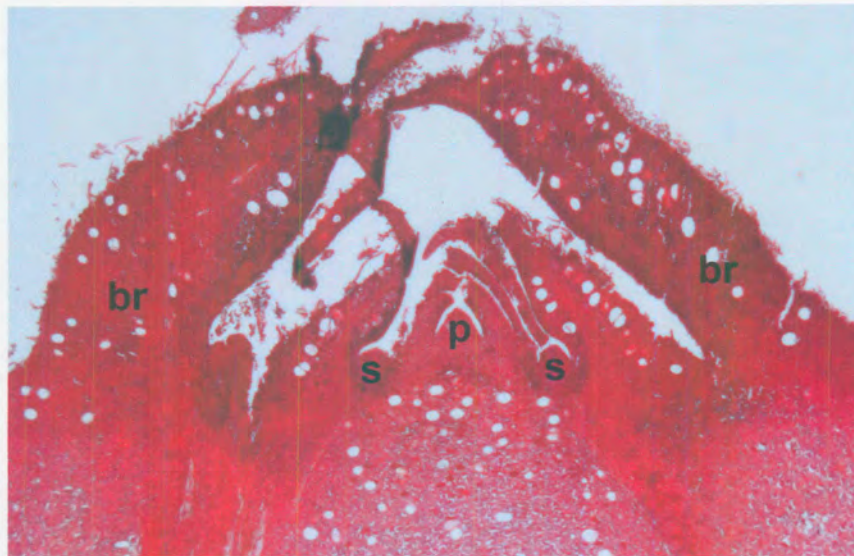


Figure 2.5. Two secondary inflorescence axis meristems present between the bracts of a bud sampled in mid March 1999. Abbreviations: p = primary inflorescence axis meristem; s = secondary inflorescence axis meristem; br = bract.

In the present microscopic study, the developing tertiary axes were visible by mid May. The first developing flower buds were microscopically observed by mid June and a month later the complete secondary axis with its flower buds could clearly be distinguished (Figure 2.6). From then on, the individual flower parts developed and anthesis occurred in August / September 1999.

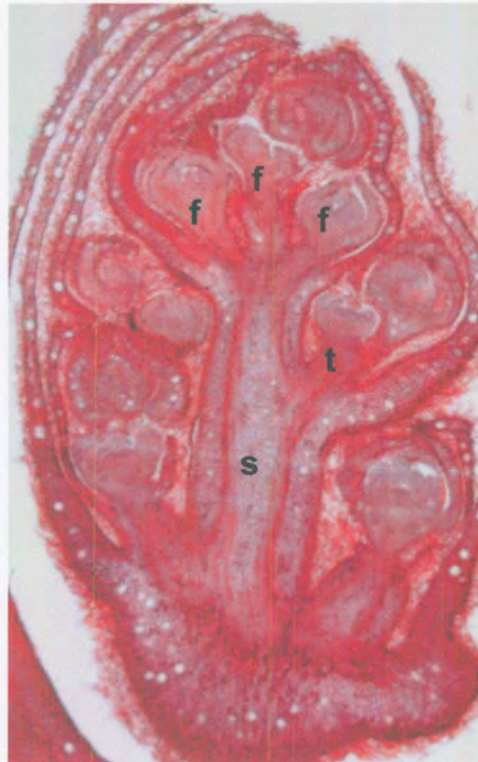


Figure 2.6. Elongated monopodial secondary inflorescence axis with sympodial tertiary inflorescence axes and developing flowers (mid July 1999).

Abbreviations: s = secondary inflorescence axis; t = tertiary inflorescence axis; f = flower.

As observed microscopically,  $GA_3$  ( $50 \text{ mg.L}^{-1}$ ) applied as a single treatment in March caused a delay in the early development of the secondary inflorescence axis meristems present in buds, until May / June. With  $GA_3$  ( $250 \text{ mg.L}^{-1}$ ) applied once in March, a greater delay in the early development of these meristems was observed. However from then on, flower development was quite rapid and flowering coincided with that of control trees. These results indicate that a single  $GA_3$  treatment was unsuccessful in inhibiting flowering and that the primary axis meristem was already committed to flowering in March. This is in accordance



with results obtained by Salazar-García, Lord and Lovatt (1999). Salazar-García *et al.* (1999) subjected avocado trees to a cold treatment known to induce floral development (3 weeks at 10°C/7°C + 1 week at 20°C/15°C). After cold treatment, buds were treated with GA<sub>3</sub> or trees were subjected to a temperature regime known to induce vegetative growth. This was done to determine the threshold developmental stage at which the primary axis meristem can no longer be reverted to vegetative growth and after which stage it is committed to flowering. From the results it seems as if the presence of three or more secondary inflorescence axis meristems can be indicative of a primary axis meristem committed to flowering. Macroscopically, this stage of development can be recognised by the partial senescence of the outermost bud scales of the apical buds (Salazar-García & Lovatt, 1998; Salazar-García *et al.*, 1999).

In this study, floral development was almost completely inhibited on trees treated with a multiple GA<sub>3</sub> treatment at the high concentration. Only a few inflorescences formed while the rest of the buds remained “dormant” (Figure 2.7). According to Salazar-García & Lovatt (2000), a monthly (September to January) application of 25 mg.L<sup>-1</sup> GA<sub>3</sub> to buds, which had already reached the threshold stage of four secondary inflorescence axis meristems, could not completely inhibit inflorescence development. A complete inhibition of inflorescence development, when GA<sub>3</sub> was applied to buds which have already reached the threshold stage, was only observed with a single GA<sub>3</sub> application (1000 mg.L<sup>-1</sup>) to axillary buds of ‘Hass’ trees (Salazar-García & Lovatt, 1998).

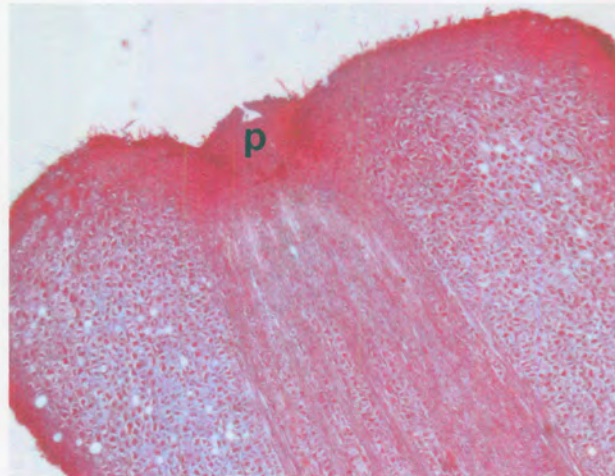


Figure 2.7. Vegetative bud collected early August from trees that received a multiple GA<sub>3</sub> treatment (250 mg.L<sup>-1</sup>). Abbreviation: p = primary axis meristem.

A conceptual model explaining the interaction between the flower stimulus perceived by trees and GA<sub>3</sub> sprays applied, can be derived from these results (Figure 2.8). In order for a tree to flower, the endogenous GA level must drop below a certain threshold level. By applying GA<sub>3</sub> sprays to trees, the GA level is maintained above the threshold level resulting in a shift towards vegetative growth. Once the trees have perceived more of the flower stimulus and the GA level falls below the threshold level, reproductive growth is favoured again. Therefore, a single GA<sub>3</sub> spray counteracts only a limited amount of the flower stimulus resulting in a temporary delay of flowering, whereas a multiple GA<sub>3</sub> treatment maintains the GA level above the threshold level resulting in inhibition of flowering.

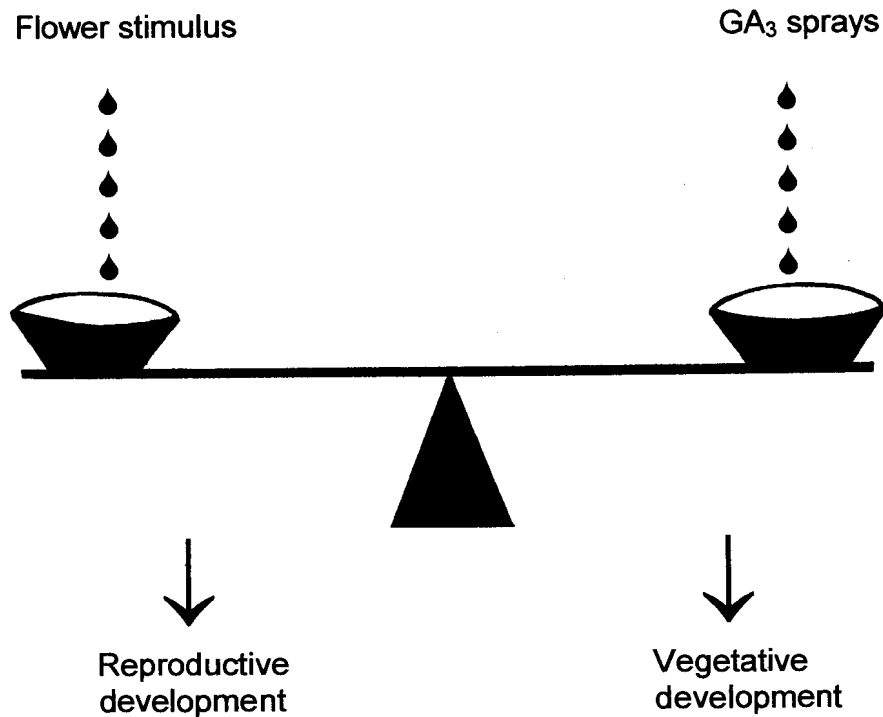


Figure 2.8. Conceptual model of the interaction between the flower stimulus perceived by trees, GA<sub>3</sub> applications to trees and the influence thereof on vegetative and reproductive development.

## 2.4.2 Trial on potted 'Fuerte', 'Hass' and 'Ryan' trees

### 2.4.2.1 Floral development on untreated trees

During the period late February until mid May, trees were in a "dormant" phase without visible signs of inflorescence bud development. Bud swelling on 'Fuerte' trees started in mid May, while bud swelling on 'Hass' and 'Ryan' trees was only observed two weeks later (early June). By mid July the first inflorescences were observed on all three cultivars and trees were in full flowering during August as reflected by the high mean ratings at the end of July (Table 2.2). Although bud swelling and subsequent inflorescence development were only observed from

May onwards, it was evident from the anatomical study (page 19) and that of Robertson (1969) that inflorescence development commenced much earlier.

#### **2.4.2.2 Floral development on trees that received a single GA<sub>3</sub> treatment**

Inconsistent results were obtained with the single GA<sub>3</sub> treatments at both concentrations. Inflorescence development was unaffected (February), delayed (mid March and early April) or enhanced (late April and May). However, none of the single GA<sub>3</sub> treatments differed significantly from the untreated control treatment (Table 2.2).

The inconsistent results obtained with single application treatments (especially the earlier treatments) can be attributed to the varying stages of bud development at the time of treatment. Iwahori & Oohata (1981) concluded that a GA treatment, at a time when most of the buds have not yet differentiated into flower buds, failed to affect flower development of Satsuma mandarins. The undifferentiated buds may have been too young to respond to treatment. According to Salazar-García & Lovatt (1998), flowering was advanced when GA<sub>3</sub> was applied to 'Hass' branches when one to three (stage 2), four (stage 3) and ten (stage 4) secondary inflorescence axis meristems were present at the time of treatment. Earlier applications and higher concentrations had a more pronounced effect. Similar results were observed on 'Fuerte' and 'Hass' trees in this study when GA<sub>3</sub> treatments were applied in February. The enhanced bud and inflorescence development observed on trees treated from late April to late

May when, according to the anatomical study, tertiary inflorescence axes were already present, are in accordance with the results of Salazar-García & Lovatt (1998). However, Salazar-García & Lovatt (1998, 2000), did not report a delay in inflorescence development as was experienced in this trial with GA<sub>3</sub> treatments applied in March and early April.

Table 2.2. Average rating of vegetative (<5) to floral (>5) development (see Figure 2.1) at the end of July 1999, as affected by single GA<sub>3</sub> applications (50 and 250 mg.L<sup>-1</sup>) to 'Fuerte', 'Hass' and 'Ryan' trees. Values are means of 8 buds from 10 single tree replicates.

Treatment dates <sup>1</sup>	'Fuerte'	'Hass'	'Ryan'
<b>Control</b>			
Untreated	5.3	5.6	5.8
<b>50 mg.L<sup>-1</sup> GA<sub>3</sub></b>			
Feb.	5.9	5.4	5.8
Mar.	4.3	5.4	5.1
Apr.	4.8	6.1	5.6
Apr./May	6.5	5.5	6.5
May	5.5	5.6	6.2
<b>250 mg.L<sup>-1</sup> GA<sub>3</sub></b>			
Feb.	5.9	6.0	5.2
Mar.	4.1	5.1	5.0
Apr.	4.5	5.6	4.9
Apr./May	5.5	6.5	6.2
May	5.7	6.6	6.3
F	<0.001	0.004	<0.001
e.s.e	0.495	0.3819	0.3608
LSD	1.383	1.066	1.0071

<sup>1</sup> See Table 2.1 for the exact date of treatment for each cultivar

### 2.4.2.3 Floral development on trees that received a multiple GA<sub>3</sub> treatment

Multiple GA<sub>3</sub> treatments at both the concentrations, caused a delay in bud and subsequent inflorescence development of 'Fuerte', 'Hass' and 'Ryan' trees. However on 'Hass' trees, a two-spray and a three-spray treatment (50 mg.L<sup>-1</sup>) did not delay development when compared to that of the untreated control trees. An increased delay in inflorescence development was found with increased number of sprays applied and with the higher GA<sub>3</sub> concentration used (Table 2.3).

Table 2.3. Average rating of vegetative (<5) to floral (>5) development (see Figure 2.1) at the end of July 1999, as affected by multiple GA<sub>3</sub> applications (50 and 250 mg.L<sup>-1</sup>) to 'Fuerte', 'Hass' and 'Ryan' trees. Values are means of 8 buds from 10 single tree replicates.

Treatment dates <sup>1</sup>	'Fuerte'	'Hass'	'Ryan'
<b>Control</b>			
Untreated	5.3	5.6	5.8
<b>50 mg.L<sup>-1</sup> GA<sub>3</sub></b>			
Feb. + Mar.	4.8	5.9	5.1
Feb. + Mar. + Apr.	4.2	5.7	5.0
Feb. + Mar. + Apr. + Apr./May	4.5	5.3	5.2
Feb. + Mar. + Apr. + Apr./May + May	4.8	5.1	4.7
<b>250 mg.L<sup>-1</sup> GA<sub>3</sub></b>			
Feb. + Mar.	5.0	5.4	4.9
Feb. + Mar. + Apr.	3.4	4.9	4.2
Feb. + Mar. + Apr. + Apr./May	3.5	4.5	3.6
Feb. + Mar. + Apr. + Apr./May + May	4.2	4.4	3.5
F	<0.001	0.004	<0.001
e.s.e	0.495	0.3819	0.3608
LSD	1.383	1.0660	1.0071

<sup>1</sup> See Table 2.1 for the exact date of treatment for each cultivar



A significant delay in inflorescence development was obtained with the high concentration applied as a three-spray treatment ('Fuerte' and 'Ryan'), a four-spray treatment (all three cultivars) and a five-spray treatment ('Hass' and 'Ryan'). On 'Ryan' trees inflorescence development was also significantly delayed when GA<sub>3</sub> at the low concentration was applied as a five-spray treatment. However, a fifth spray failed to increase the significant reaction obtained with the three-spray and four-spray treatments (250 mg.L<sup>-1</sup>). Porlingis & Boynton (1961) also reported that repeated applications of GA to strawberries resulted in a suppression of further flowering and Tomer (1984), reported an almost complete inhibition of flowering in mango after repeated GA applications.

There is considerable variation within an avocado tree's canopy concerning the stage of inflorescence development, and therefore shoots differ in their response to GA<sub>3</sub> treatment. GA<sub>3</sub> treatments spread over a three to four month period, should therefore affect a greater proportion of buds at a developmental stage sensitive to GA<sub>3</sub>. This explains the inconsistent results obtained with single and double application treatments. The failure of the fifth spray to further delay bud development, can be attributed to the delaying effect obtained with the GA<sub>3</sub> sprays applied during the preceding months. By late May most, if not all of the potential inflorescence buds would have been affected by the GA<sub>3</sub> sprays.

The first internode (hypopodium or scape) of inflorescences which developed on trees that received  $250 \text{ mg.L}^{-1} \text{ GA}_3$  in May (single and multiple treatments), was elongated and individual flowers were smaller when compared to that of the control trees (Figure 2.9). Salazar-García & Lovatt (1998) similarly observed inflorescences with elongated hypopodiums which appeared too weak to support fruit set, after a single  $\text{GA}_3$  treatment of  $1000 \text{ mg.L}^{-1}$ .



Figure 2.9. Elongated hypopodium of 'Hass' inflorescence on a tree that received  $250 \text{ mg.L}^{-1} \text{ GA}_3$  (left) in May (single and multiple treatment) as compared to an inflorescence from an untreated tree (right).

#### 2.4.2.4 Flowering intensity of untreated trees

The evaluation made at full flowering in August 1999, showed that the flowering intensity on 'Fuerte' control trees was low and trees produced slightly more vegetative than reproductive growth. 'Hass' control trees produced better flowering, with slightly more floral than vegetative development. Flowering intensity on 'Ryan' trees was very high with almost no vegetative development (Table 2.4).

Table 2.4. Average rating of vegetative and floral development (see Figure 2.2) after single GA<sub>3</sub> applications (50 and 250 mg.L<sup>-1</sup>) to 'Fuerte', 'Hass' and 'Ryan' trees, as a measure of flowering intensity at full flowering in August 1999. Values are means of 10 single tree replicates where 1=fully vegetative tree and 5=fully reproductive tree.

Treatment dates <sup>1</sup>	'Fuerte'	'Hass'	'Ryan'
<b>Control</b>			
Untreated	2.7	3.3	4.6
<b>50 mg.L<sup>-1</sup> GA<sub>3</sub></b>			
Feb.	3.1	3.0	4.3
Mar.	2.1	3.1	3.5
Apr.	2.0	3.7	4.2
Apr./May	4.1	3.4	3.6
May	3.6	2.9	3.6
<b>250 mg.L<sup>-1</sup> GA<sub>3</sub></b>			
Feb.	2.9	3.8	3.7
Mar.	2.7	2.7	3.8
Apr.	2.3	3.0	4.2
Apr./May	3.0	3.2	3.9
May	2.5	3.4	3.5
F	<0.001	0.019	<0.001
e.s.e	0.3729	0.3616	0.3230
LSD	1.0407	1.0092	0.9014

<sup>1</sup> See Table 2.1 for the exact date of treatment for each cultivar

#### 2.4.2.5 Flowering intensity of trees that received a single GA<sub>3</sub> treatment

Inconsistent results were obtained with GA<sub>3</sub> applied as single treatments to 'Fuerte', 'Hass' and 'Ryan' trees (Table 2.4). Some effect was observed on the flowering intensity of 'Fuerte' and 'Ryan' trees but the flowering intensity of 'Hass' trees was unaffected when compared to the untreated control trees. The

flowering intensity of 'Ryan' trees was significantly decreased with a single GA<sub>3</sub> treatment applied in mid March (50 mg.L<sup>-1</sup>), early May (50 mg.L<sup>-1</sup>) and late May (50 and 250 mg.L<sup>-1</sup>). A single GA<sub>3</sub> spray (50 mg.L<sup>-1</sup>) applied in late April, increased the flowering intensity of 'Fuerte' trees. An increase in flowering intensity of avocado with a GA<sub>3</sub> treatment has not been reported before.

Salazar-García & Lovatt (1998), reported a decrease in flowering intensity of 'Hass' trees which received a GA<sub>3</sub> application when one to three secondary inflorescence axis meristems were present. The decrease was associated with the production of partially formed inflorescences bearing fewer flowers. Winter trunk injections of GA<sub>3</sub> (10 secondary inflorescence axis meristems already formed) reduced flowering intensity by increasing bud abscission of young trees and increasing the proportion of inactive buds on mature trees (Salazar-García & Lovatt, 1999). In citrus, a reduction in flower number was also observed with GA<sub>3</sub> application to buds prior to, and after flower bud differentiation had occurred (Goldschmidt & Monselise, 1970; Guardiola, Monerri & Agusti, 1982).

#### **2.4.2.6 Flowering intensity of trees that received a multiple GA<sub>3</sub> treatment**

Multiple application treatments had a decreasing effect on flowering intensity, which tended to be more pronounced with the high concentration and increasing number of sprays applied (Table 2.5).

Table 2.5. Average rating of vegetative and floral development (see Figure 2.2) after multiple GA<sub>3</sub> applications (50 and 250 mg.L<sup>-1</sup>) to 'Fuerte', 'Hass' and 'Ryan' trees, as a measure of flowering intensity at full flowering in August 1999. Values are means of 10 single tree replicates where 1=fully vegetative tree and 5=fully reproductive tree.

Treatment dates <sup>1</sup>	'Fuerte'	'Hass'	'Ryan'
<b>Control</b>			
Untreated	2.7	3.3	4.6
<b>50 mg.L<sup>-1</sup> GA<sub>3</sub></b>			
Feb. + Mar.	2.7	3.3	3.7
Feb. + Mar. + Apr.	1.6	3.2	3.9
Feb. + Mar. + Apr. + Apr./May	1.8	2.6	3.4
Feb. + Mar. + Apr. + Apr./May + May	2.3	2.9	3.2
<b>250 mg.L<sup>-1</sup> GA<sub>3</sub></b>			
Feb. + Mar.	2.8	2.5	3.5
Feb. + Mar. + Apr.	2.3	2.8	3.5
Feb. + Mar. + Apr. + Apr./May	1.8	2.1	2.1
Feb. + Mar. + Apr. + Apr./May + May	1.6	1.9	1.4
F	<0.001	0.019	<0.001
e.s.e	0.3729	0.3616	0.3230
LSD	1.0407	1.0092	0.9014

<sup>1</sup> See Table 2.1 for the exact date of treatment for each cultivar

On 'Fuerte' trees a three-spray treatment reduced flowering intensity significantly when applied at the low concentration. Four-spray and five-spray treatments were most effective in reducing flowering intensity on all three cultivars, especially with the high concentration applied. Floral development was almost completely inhibited and no, or very few inflorescences developed on these trees when compared to untreated control trees. This is in accordance with Salazar-

García & Lovatt (2000) who found a significant increase in the percentage of vegetative shoots produced, with a concomitant decrease in the percentage of inflorescences produced, after five monthly GA<sub>3</sub> applications to avocado trees.



## 2.5 Conclusions

Foliar GA<sub>3</sub> treatments influenced inflorescence development of avocado, with the effect being more pronounced on 'Fuerte' and 'Ryan' than on 'Hass trees. Due to the heterogeneity of inflorescence development within an avocado tree's canopy, shoots differed in their response to GA<sub>3</sub> treatment. Therefore, inconsistent results were obtained with single GA<sub>3</sub> treatments applied at different dates. Depending on the time of application and consequently the morphological stage of the bud, different reactions to GA<sub>3</sub> treatment were obtained. The reaction of buds to GA treatments can best be explained using a model as presented in Figure 2.10.

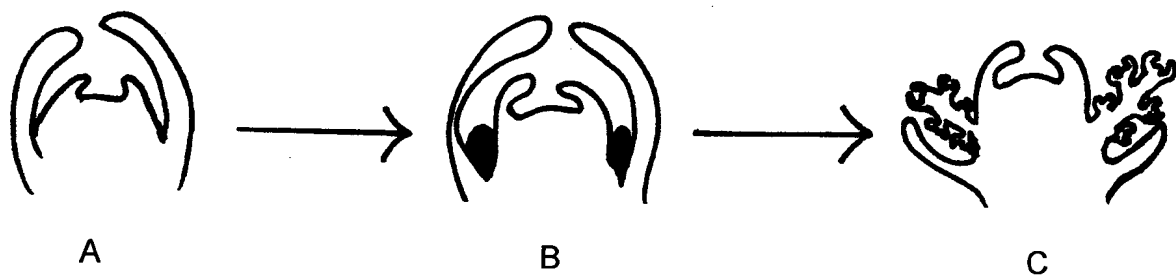


Figure 2.10. The effect of GA<sub>3</sub> treatments on inflorescence development when applied at different stages of bud morphology (A = undifferentiated bud not sensitive to GA<sub>3</sub> treatment, B = bud, with secondary inflorescence axes meristems differentiated between bud bracts, sensitive to GA<sub>3</sub> treatment C = bud, with elongated secondary inflorescence axes with tertiary axes, sensitive to GA<sub>3</sub> treatment).

Single GA<sub>3</sub> treatments applied when buds are still undifferentiated (Fig 2.10A), had no effect on floral development as buds are not sensitive to GA<sub>3</sub> treatment at this morphological stage. Single GA<sub>3</sub> treatments applied during early stages of inflorescence development (Figure 2.10B) delayed inflorescence development, by delaying the development of the secondary inflorescence axis meristems present in buds at the time of treatment. Development of the partially differentiated inflorescences was enhanced when single GA<sub>3</sub> treatments were applied during advanced stages (Figure 2.10C) of inflorescence development. This reaction is due to internode elongation as a result of the stimulation of cell elongation after GA<sub>3</sub> treatment. This reaction was observed as elongated hypopodiums in inflorescences that developed on trees treated during May (more advanced stage of inflorescence development microscopically).

When multiple GA<sub>3</sub> treatments were applied over a four month period, inflorescence development was significantly inhibited due to affecting more buds at a morphological stage sensitive to GA<sub>3</sub>. As a single treatment delayed development for a limited period only, each additional application made with the multiple GA<sub>3</sub> treatment “renewed” the delaying effect (see Figure 2.8), resulting in an inhibition of flowering. Therefore, multiple GA<sub>3</sub> treatments inhibited inflorescence development by keeping buds inactive during the normal floral development period. In conclusion, these results suggest that timely multiple GA<sub>3</sub> treatments may be an effective regulatory tool in the manipulation of avocado flowering.



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## CHAPTER 3

### Chemical and physical manipulation of 'Fuerte' avocado trees to produce an out of season crop

#### 3.1 Summary

The seasonality of avocado production leads to over-supplied markets during peak periods followed by shortages of fruit during other periods. Manipulation of flowering and fruiting are practiced worldwide to extend the season of various crops. In this study, 'Fuerte' avocado trees were manipulated (chemically and physically) to produce an out of season crop.

During the normal flowering period (August – September), an Ethrel<sup>®</sup> spray was applied as a de-flowering treatment. Trees were cinctured three months later to induce out of season flowering. Out of season flowering during January and February was weak compared to normal season flowering.

Normal season fruit were harvested in April (70% moisture) and out of season fruit in November (62% moisture). The out of season yield (17.7 kg.tree<sup>-1</sup>) was significantly lower than the normal season yield (45.8 kg.tree<sup>-1</sup>). Sunny<sup>®</sup> applied during full flowering to enhance fruit retention, had no effect on yield. However, the Sunny<sup>®</sup> application significantly increased the amount of fruit in the economically important count range 12-18 compared to the unsprayed out of season treatment.

Out of season fruit had a high incidence of the skin defect, “netting”, and lenticels tended to be conspicuously white. Post harvest fruit quality was evaluated on a sample of normal and out of season fruit stored for 28 days at 5.5°C and then ripened at 20°C. More lenticel damage was observed on the less mature normal season fruit than on the more mature out of season fruit. A significantly higher incidence of the physiological disorder pulpspot was observed in the normal season fruit than in the out of season fruit. In spite of lower yields, extremely good prices for out of season fruit make the manipulation of ‘Fuerte’ avocado trees a highly profitable venture.

### 3.2 Introduction

The seasonality of fruit limits a farmer's ability to be in production all year round. The fruit industries rely on breeding and selection to supply them with different cultivars or varieties to extend the season of a specific crop. In addition, the manipulation of flowering time and harvesting is carried out to extend the marketing season. In this regard, plant growth regulators are powerful manipulation tools in fruit production worldwide.

When applied to plant tissues, the active ingredient in Ethrel® (Rhône Poulenc), ethephon (2-Chloroethyl phosphonic acid), can cause a great burst of natural ethylene production – an autocatalytic effect. Ethylene is known to exert certain responses in plants and fruit. Trials done by Moore and Osgood (1989), showed that ethephon treated sugarcane fields had an 87% reduction in flowering when compared to untreated control fields. This reduction in flowering resulted in cane with higher fresh weights and sugar content than the controls. Ethephon applied to pineapple plants produces flower induction under noninductive conditions (Hartman, Kofranek, Rubatzky, Flocker, 1988). Ethephon is used on cherries to induce uniform ripening and loosening of fruit for easier harvesting and to improve colour development in citrus, apples, grapes and pineapples when applied as preharvest or postharvest treatments (Thomson, 1991). As ethylene gas is an important factor during fruit ripening and maturation, it is extensively used as a fruit ripening agent (Hartman *et al.*, 1988).



In avocado, vegetative growth takes place in two to three distinctive flushes throughout the year. Reproductive growth occurs once a year in late winter/spring (Davenport, 1986). Concomitant with flowering, many of the inflorescences initiate a vigorous terminal shoot which is likely to coincide with fruit set. Paclobutrazol and uniconazole, plant growth regulators from the triazole group, are known for their inhibitory effect on internode elongation. Paclobutrazol sprays on 'Fuerte' and 'Hass' avocado trees during full bloom reduced, on average, spring shoot length of fruiting and non-fruiting shoots by 40-44%, when compared with untreated trees (Wolstenholme, Whiley & Saranah, 1990; Whiley, Saranah, Wolstenholme & Rasmussen, 1991). Adato (1990), reported a significant yield improvement of 197% of the control when paclobutrazol was applied to 'Fuerte' trees at full bloom. This improvement in yield was due to improved fruit retention, as a result of the inhibitory effect on vegetative growth concomitant with the fruit setting process.

A positive effect on fruit size and fruit shape was also noticed. Treated fruits had lower length/diameter ratios (Wolstenholme *et al.*, 1990; Whiley *et al.*, 1991) and a significant increase of 20% in Hass fruit size was obtained compared to fruit from untreated control trees (Wolstenholme *et al.*, 1990). A comparison between paclobutrazol and uniconazole (Köhne & Kremer-Köhne, 1989) showed that, at the same concentration, uniconazole had a greater inhibitory effect than paclobutrazol. In South Africa, the standard commercial practice is to apply Sunny<sup>®</sup> (Sanachem (PTY)LTD), a uniconazole plant growth regulator, at full

bloom to enhance fruit retention. The European Union's tolerance of chemical residues on fruit has become extremely tight and zero residue levels are common for many plant growth regulators. A period of 84 days must pass between treatment with Sunny<sup>®</sup> and harvest of the fruit, in order to have zero residues. In this regard, the treatment at full bloom is safe, as avocado fruit need at least 200 days from full bloom to acquire picking maturity.

Apart from the application of plant growth regulators, girdling is a practice used to improve the cropping of fruit trees. During the 1970's, girdling avocado trees of low productivity was widely practiced in Israel. Girdling before the onset of flowering resulted in precocious flowering (Lahav, 1969; Lahav, Gefen & Zamet, 1972). A general increase in yield of avocado trees was observed in the first season after girdling. The best results were obtained when 'Fuerte' trees were girdled in autumn and spring (Lahav, Gefen & Zamet, 1971).

In the previous chapter, the use of GA<sub>3</sub> in order to delay or inhibit flowering during the normal season was discussed. However, success is dependant on the correct timing of the treatments and results are only available in the longer term. However, as Westfalia Estate is committed to supply Woolworths with fruit throughout the year, a method to produce out of season fruit in the shorter term was investigated. Therefore, this chapter deals with the production of out of season fruit through the destruction of normal season flowering. The abscission-inducing effect of ethylene was used to disrupt the normal growth and

development cycles of the trees. Cincturing, as a means to induce out of season flowering and the use of uniconazole foliar sprays to enhance fruit retention, were evaluated. These techniques and combinations thereof, have not previously been used to produce out of season fruit. As the South African market focuses mainly on green skin avocado cultivars, the trial was conducted on 'Fuerte' trees.

### 3.3 Materials and methods

This trial was conducted in a commercial avocado orchard with 10-year-old 'Fuerte' trees on Duke 7 clonal rootstock, planted at a spacing of  $7 \times 5$  m (200 trees.ha<sup>-1</sup>). The orchard consisted of 116 trees situated in one irrigation block and therefore receiving the same management practices including irrigation, fertilisation, pruning etc. Sixty trees were selected for uniform size, health and vigour and four treatments were allotted randomly to blocks of three trees each.

A de-flowering spray prepared from Ethrel<sup>®</sup> at a concentration of 0.2 % (product) was applied to three of the treatments at full bloom during the normal flowering time (August / September). A light canopy spray (no run-off) was applied using a spray-cart with a hand-held gun at a spray volume of 1200L.ha<sup>-1</sup>. Care was taken to prevent spray-drift onto untreated trees, by placing a protective barrier in between trees.

The trees from two of these treatments were cinctured two months after Ethrel<sup>®</sup> application to induce out of season flowering. A pruning saw was used to make a narrow cincture on all the main branches (0.3 - 0.5 m above ground), leaving one smaller branch as a breather branch (Figure 3.1).

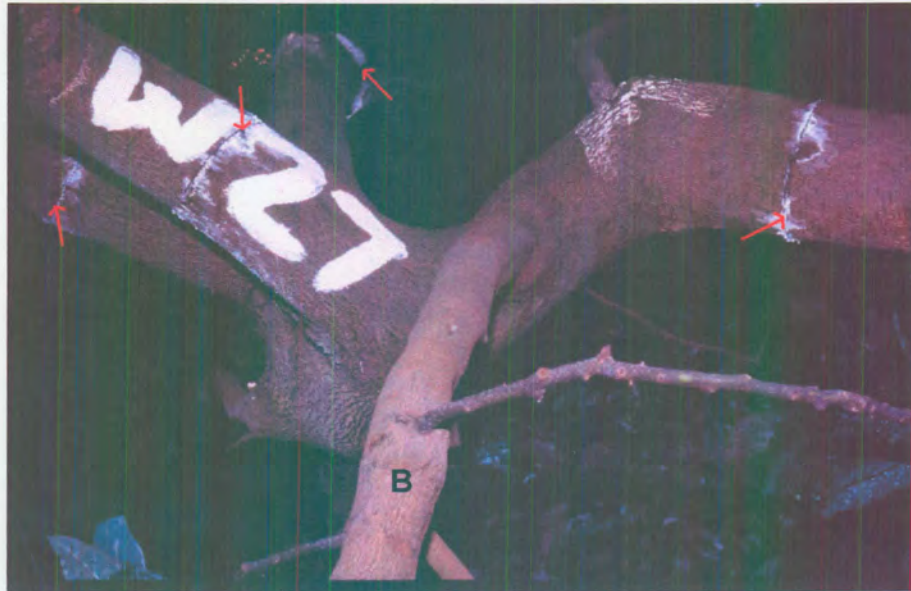


Figure 3.1. Cincturing of all the main branches (arrows) of trees at a height of 0.3 – 0.5 m above ground, leaving one smaller branch as a breather branch (B).

At full bloom one of these treatments was sprayed with the plant growth regulator Sunny<sup>®</sup> at a concentration of 1.0% (product) to enhance fruit retention. Bees were also introduced into the orchard to assist with pollination during the out of season flowering period.

As the trial was conducted in a commercial orchard, the non-data trees were also de-flowered and cinctured to facilitate management of the orchard. Control trees were left untreated. Vegetative and reproductive development were monitored at monthly intervals throughout the season on all the trees using the scale of 1-10 as described in chapter 2 (2.3.1.1 Figure 2.1).

Picking maturity of the fruit was determined using the commercial method of moisture determination. Individual tree yields were recorded at harvest. Fruit were pooled for each treatment and sent to the commercial Westfalia packhouse. Fruit were subjected to the normal packline procedures of washing (chlorinated water over brushes), sorting and waxing with Westfalia™ Biocoat (Hygrotech)-containing wax. The fruit count size distribution was determined gravimetrically, according to the number of fruit per 4 kg carton.

Normal season fruit destined for the export market, are subjected to a voyage of 28 days at a low temperature. The standard procedure for post harvest fruit quality evaluation, involves a simulation of this voyage to Europe. To test the possibility to further extend the avocado season, 70 fruit (count size 14) from the normal and out of season harvests were stored for 28 days at 5.5°C. Upon removal from cold storage, fruit firmness was measured with a densimeter on a sample of 25 fruit per treatment. The densimeter measures firmness by means of a small metal ball pressed onto the fruit and a reading of 100 (hard) to 0 shore (soft) is registered while being non-destructive (Köhne, Kremer-Köhne & Gay, 1998). Fruit were also evaluated externally and then ripened at 20°C. The number of days until the fruit ripened was recorded. Thereafter the fruit were evaluated internally. Evaluation was done on a scale of 0 – 3 where 0 depicted no symptom and 3 depicted a severe symptom. Parameters that were looked at included lenticel damage and the incidence of diseases and internal physiological disorders.



Results are only presented for the 1999/2000 season as this trial was not repeated in the same orchard for a second season. However, due to the success of this trial, a semi-commercial trial was conducted in another orchard during the 2000/2001 season.

Yield data were subjected to analysis of variance and least significant differences (LSD's) at the 5% significance level were used to compare treatments. As the fruit were pooled per treatment after harvest, a Chi-squared test ( $P = 0.05$ ) was used to compare treatment frequencies for the fruit count size distribution and the post harvest fruit evaluation parameters, lenticel damage and pulpspot. Firmness (densimeter readings) and days to ripen are presented as means with standard deviations.

### 3.4 Results and discussion

#### 3.4.1 Removal of normal season flowers

Two weeks after the Ethrel<sup>®</sup> application, first flowers started to drop while the secondary inflorescence axes were still strongly attached to the tree (Figure 3.2). During the following two weeks, the secondary inflorescence axes abscised. Some leaf abscission was also observed. Older leaves turned yellow and dropped within one month after the Ethrel<sup>®</sup> treatment. However, the emerging new flush was unaffected by the Ethrel<sup>®</sup> treatment and after expanding, protected the bare branches against sunburn.



Figure 3.2. Enhanced flower senescence, triggered by an Ethrel<sup>®</sup> spray during full bloom. Secondary inflorescence axes are still attached, but dropped within four weeks from the Ethrel<sup>®</sup> treatment.



As is the case with all plant growth regulators, the timing of the Ethrel<sup>®</sup> application is crucial. Ethrel<sup>®</sup> only enhances flower senescence and subsequent abscission when applied to fully expanded inflorescences with open flowers. The protracted flowering period and the subsequent variation in floral development within the canopy of avocado trees, impedes decision making with regard to the time of application and also affects the efficacy of the Ethrel<sup>®</sup> spray. Flowering and fruit set progressed normally on the untreated control trees.

### **3.4.2 Out of season flower induction and fruit retention**

Approximately eight to nine weeks after cincturing, swollen flower buds were observed on all the cinctured trees. The cincturing wounds healed within one to two months. As was observed by Trochoulis & O'Neill (1976) and Lahav *et al.* (1972) during the summer following girdling, girdled branches had paler leaves than the ungirdled branches or trees. However, the reduction in leaf colour was only temporary. Blumenfeld, Gazit, Tomer, Zakai & Biran (1975), attributed the yellowing of the leaves to the accumulation of starch and reducing sugars above the girdle and the decline in the nitrogen level in the crown of the trees, due to the disruption in translocation caused by the girdle. Starch grains form within the chloroplasts of leaves, thus masking the green colour (Tribe & Whittaker, 1972, Plate 3).

The cincturing treatment resulted in weak out of season flowering and produced less than half the number of flowers compared to normal season flowering. In

comparison with cincturing wounds, the wounds from a more harsh girdling treatment, will take two or more months to heal. Therefore, with girdling, the normal flow of carbohydrates and plant growth substances is disrupted for a longer period than with cincturing. This may lead to more intense flowering, than that achieved through cincturing but would have a more severe long term effect on tree health. In this trial, most trees were in full flowering during mid January and the treatment enhancing fruit retention was applied to one of the treatments. Flowering continued until March.

Avocado flowers exhibit a unique mechanism for alternation of sexes that enhances the opportunity for outcrossing (Davenport, 1986). Each flower opens twice, separated by an overnight period, with the first opening being functionally female and the second functionally male. Unfavourable environmental conditions and particularly temperatures, adversely affect this floral cycle (Lesley and Bringhurst, 1951; Sedgley, 1977). Sedgley (1977), found from growth cabinet studies performed with 'Fuerte', that certain temperatures may lead to a disruption in the normal floral cycle. Reduced pollen tube growth and no fertilisation and fruit development was observed at 17°C day/12°C night temperatures. A 25°C day and 20°C night resulted in the normal floral cycle with successful pollen tube growth, fertilisation and fruit development.

At Westfalia Estate the average day/night temperatures for January, February and March are much higher than for the months July to September (Figure 3.3).

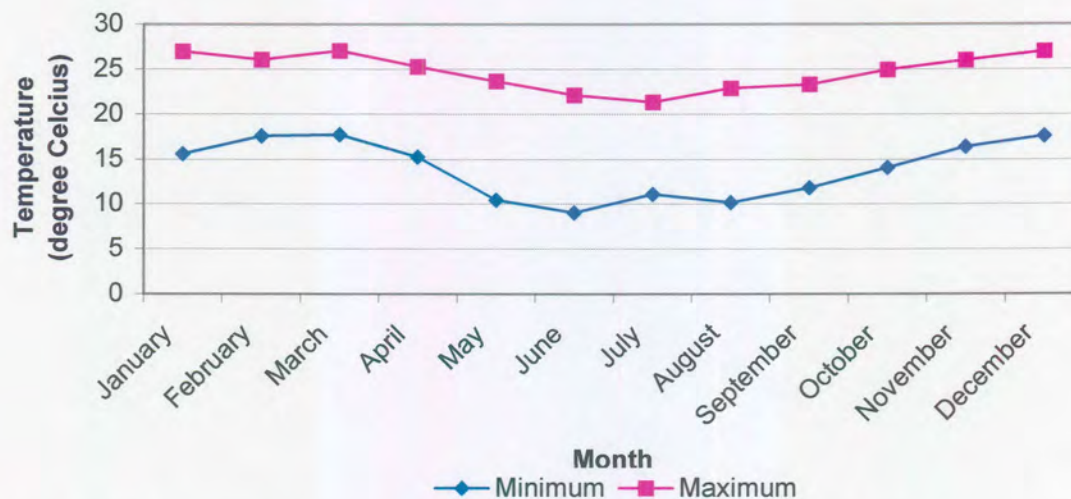


Figure 3.3. Average minimum and maximum temperatures for the period 1998 to 2000 as recorded at the Westfalia Estate weather station.

According to the results of Sedgley (1977) and the temperature data (Westfalia weather station), the period January to March would be more favourable for flowering and fruit set, than the much colder months, July to September. Unfortunately, flowering and fruit set are not only temperature dependant. Cool, cloudy weather may significantly disrupt the floral cycle (Whiley and Schaffer, 1994), and adversely affect bee activity (Lesley and Bringhurst, 1951). During February and March 2000, exceptionally high rainfall was recorded at the Westfalia weather station and the number of sunshine hours was considerably lower than that recorded for the same period in 1999 (Figure 3.4).



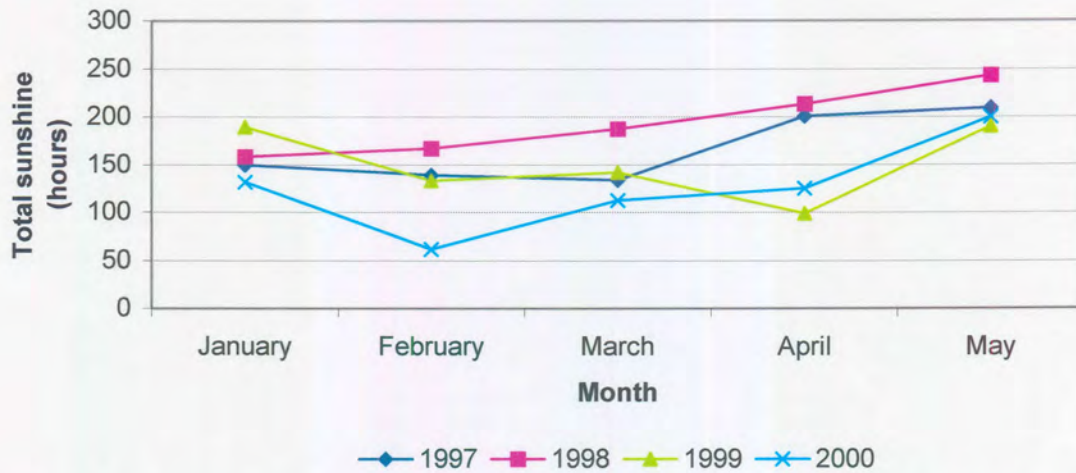


Figure 3.4. Sunshine hours for the months January to May (1997-2000) as recorded at the Westfalia Estate weather station.

The prevailing weather conditions during the out of season flowering period may therefore have disrupted the normal floral cycle with a subsequent negative effect on fruit set. However, a compensating effect would be the more favourable temperatures for pollen tube growth in those flowers that managed to be pollinated.

No out of season flowering was observed on the trees which only received the de-flowering Ethrel<sup>®</sup> spray or on the untreated control trees (Figure 3.5). These trees stayed in a vegetative state and only produced flowers at the normal flowering period (July/August 2000).





Figure 3.5. Induced out of season flowering on a cinctured tree (left) and a tree in a vegetative state (right) which received an Ethrel<sup>®</sup> spray only.

### 3.4.3 Fruit maturity and yield

As avocado fruit reach maturity, the oil content of the fruit increases with a concomitant decrease in the water content of the fruit. The maturity standard, according to the moisture content, differs between cultivars. In South Africa, 'Fuerte' fruit are picked at a maximum moisture content of 80%. Control fruit from the normal season, were picked at 70% moisture during April 2000. Out of season fruit were ready for picking in November 2000 when the moisture content of the fruit tested at 62%. An average normal season harvest of 45.8 kg.tree<sup>-1</sup> was obtained from the control trees. An average out of season yield of 17.7 kg.tree<sup>-1</sup> was obtained. There was no significant difference between the two out of season treatments with regard to yield data (Figure 3.6).



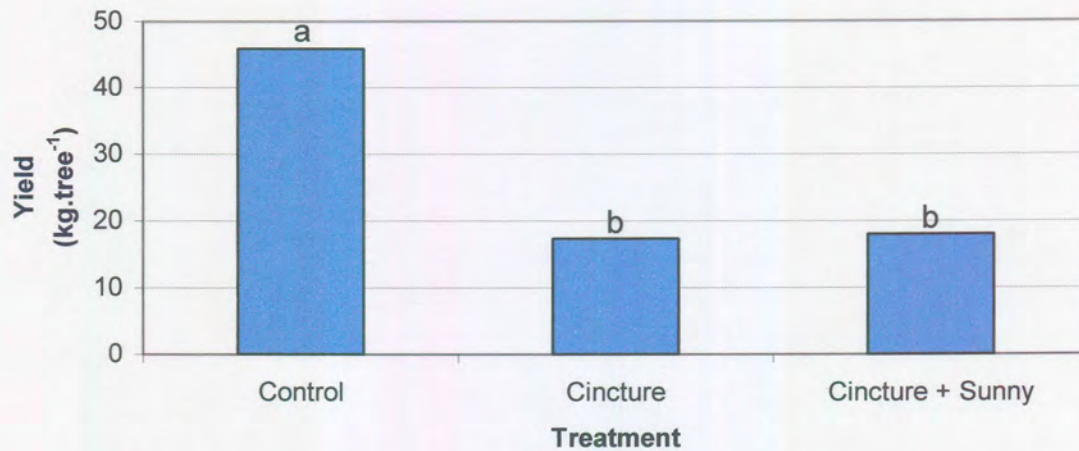


Figure 3.6. Yield (kg.tree<sup>-1</sup>) for the normal season harvest (Control) and for the out of season harvests (Cincture, Cincture + Sunny<sup>®</sup>). Means with the same letter are not significantly different at P=0.05.

Avocado trees are prone to alternate bearing, producing heavy flowering and yield in some years, followed by a very small crop the following year. Köhne (1989) and Adato (1990) reported that a paclobutrazol foliar application during “off” flowering will enhance fruit retention resulting in a higher yield, however, the effect was less pronounced during “on” flowering. This observation could not be confirmed for out of season flowering, possibly due to the exceptional climatic conditions during the out of season flowering period.



As no treatment was applied to inhibit flowering during the following normal flowering period (July/August 2000), the trees from all the treatments had swollen buds by June and first open inflorescences were observed by the end of July. The normal flowering period continued until September 2000. Having two sets of fruit on the trees (normal and out of season), creates problems concerning management practices. Spraying and pruning programs need to be adjusted, and fertilisation and irrigation must be carefully planned in order to benefit both sets of fruit.

#### **3.4.4 Fruit size**

The economically important fruit sizes are in the count range 12-18, whereas fruit from the count ranges 8-10 and 20-26 can be classed as too big and too small respectively. Although there was no difference in yield between the two treatments yielding out of season fruit, a significant difference in the fruit size distribution was observed (Figure 3.7). The treatment with Sunny<sup>®</sup> had 11% more fruit in the count range 12-18 when compared to the treatment receiving no Sunny<sup>®</sup> application. In addition, Sunny<sup>®</sup> had the advantage of lowering the amount of fruit in the too small category (count 20–26).

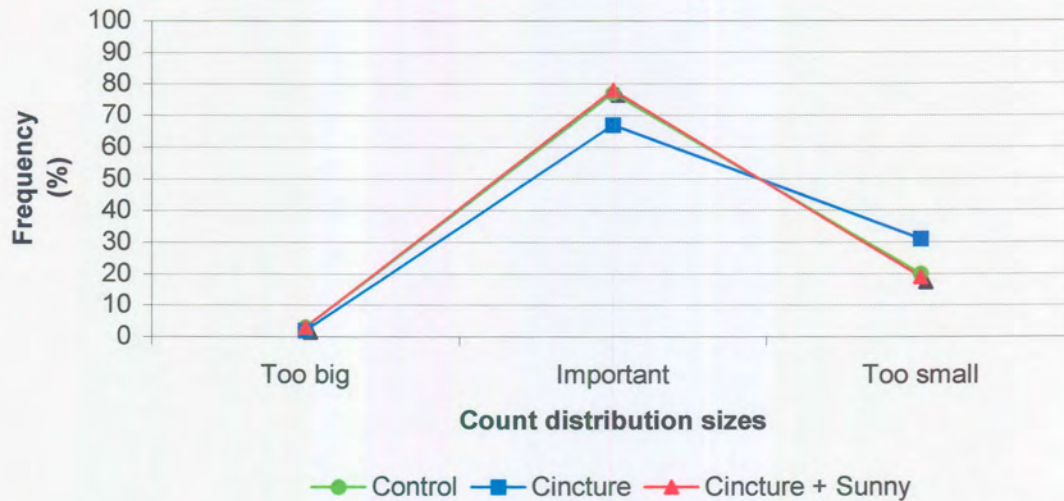


Figure 3.7. Fruit size distribution of normal season fruit (Control) and out of season fruit (Cincture, Cincture + Sunny<sup>®</sup>) classed as too big (count 8-10), important (count 12-18) and too large (count 20-26). ( $P = 0.05$ ).

This is in confirmation with results from Wolstenholme *et al.* (1990), who reported a significant increase of 10% in mean 'Fuerte' fruit size when paclobutrazol was applied twice in spring. Whiley *et al.* (1991) and Wolstenholme *et al.* (1990) found an increase in mean fruit size of 16% and 20% respectively, after a paclobutrazol spray at mid-anthesis on Hass trees.



### 3.4.5 Post harvest fruit quality

At picking, physical appearance of the out of season fruit differed from the normal season fruit. Out of season fruit tended to have a high incidence of the external skin defect, “netting” (Figure 3.8). “Netting” is caused by wind and does not negatively influence the internal quality of the fruit (Suter, 1997). In comparison with fruit from the normal season, lenticels on out of season fruit were conspicuously white (Figure 3.9).



Figure 3.8. Out of season 'Fuerte' fruit with “netting” marks on the fruit skin.



Figure 3.9. Conspicuous white lenticels of out of season fruit (left) and less prominent lenticels on normal season fruit (right).



After cold storage, the untreated control fruit took two days longer to ripen compared with fruit from the out of season treatments ( $4.2 \pm 0.8$  days vs.  $2.5 \pm 0.63$  and  $2.2 \pm 0.43$  days). The time from full bloom to picking was shorter for the untreated control fruit (Mid August – Mid April) than for the out of season fruit (Mid February – Mid November). The moisture determination at picking confirmed that the untreated control fruit were less mature (% moisture:70) than the out of season fruit (% moisture:62). This is in accordance with the results from Cutting and Wolstenholme (1992), who found a decrease in time to ripen with increasing fruit maturity. However, untreated control fruit were softer ( $82.2 \pm 4.38$  densimeter units) than fruit from the November out of season harvest ( $89.8 \pm 2.59$  and  $89.4 \pm 2.12$  densimeter units) when removed from cold storage.

Damage of the lenticels is associated with cold storage and involves the collapse or disintegration of the lenticels, resulting in black spots on the fruit skin (Figure 3.10).



Figure 3.10. Damage of the lenticels present as black spots on the fruit skin.

Although this problem is more prominent on Hass fruit due to the exposed nature of the lenticels, green skin cultivars are also affected (Suter, 1997). Fruit from the normal season were more prone to damage of the lenticels, although the severity was very low. The out of season treatments had 20-25% less fruit with lenticel damage compared with the untreated control treatment (Figure 3.11).

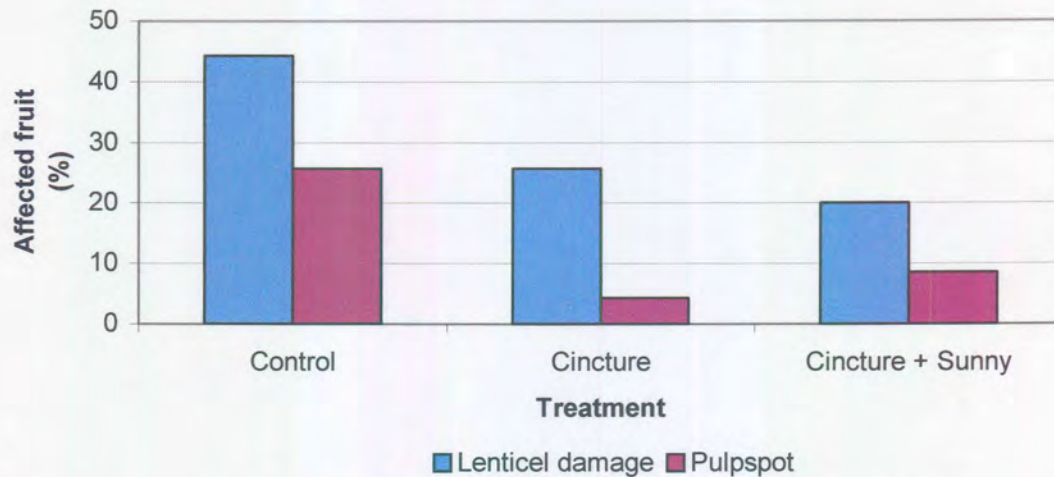


Figure 3.11. Incidence of lenticel damage and the physiological disorder pulpspot on fruit from the normal season (Control) and out of season (Cincture, Cincture + Sunny<sup>®</sup>) harvests. ( $P = 0.05$ ).

Early season 'Fuerte' fruit (high moisture content) are more sensitive to low temperatures. As the season progresses and fruit become more mature, fruit appear to be less sensitive to cold (Cutting & Wolstenholme, 1992). Out of season fruit were harvested at a lower moisture content which implies more mature fruit with lower susceptibility to lenticel damage. As both the normal season and out of season fruit were stored at 5.5°C, these low temperatures



may have led to the higher incidence of lenticel damage on the less mature control fruit.

The post-harvest diseases anthracnose and stemend rot were not observed in fruit from the normal and out of season harvests. With regard to physiological disorders, the incidence of pulpspot tended to be significantly higher in the less mature normal season fruit when compared to the more mature out of season fruit (Figure 3.11). Pulpspot is a physiological disorder which is associated with the vascular tissues of the fruit, developing black or grey spots a few minutes after fruit were cut (Figure 3.12).

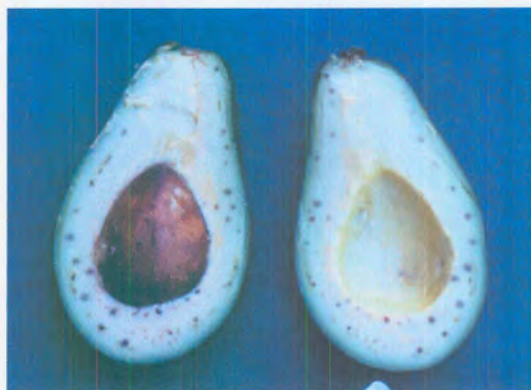


Figure 3.12. Fuerte fruit with the physiological disorder, pulpspot.

According to Cutting, Bower and Wolstenholme (1988), the potential and often the incidence of physiological disorders, increases with increasing fruit maturity. However, this is not the case with the disorder pulpspot. According to Swarts (1984), pulpspot is only evident at the beginning of the season. Quality assurance data over three years showed that the occurrence of pulpspot varies between years, but the disorder is more likely to be found early in the 'Fuerte'



season (Quality assurance reports, Merensky Technological Services, 1994-1996, Unpublished). In contrast to pulpspot, greypulp and vascular browning were only observed in a few fruit and at a very low severity.

A period of seven to nine months from full bloom is required for avocado fruit to reach maturity. Normal season fruit set occurs during spring/early summer (September/October) and fruit are growing through the hot summer months until they are ready for harvesting from as early as autumn through winter. However, out of season flowering occurred during mid summer (January to March) and subsequent fruit growth occurred during the colder winter months until November, when fruit were harvested. Low winter temperatures have a retarding effect on most physiological processes in plants, resulting in the slowing down of growth and development, until the warmer spring temperatures set in. Due to these climatic differences during fruit development, out of season fruit may differ physiologically from normal season fruit.

#### **3.4.6 Financial viability**

The production of out of season fruit involves a series of management practices in addition to the normal management practices of irrigation, fertilisation, pest and disease control and pruning. Although the out of season crop obtained in this trial was lower than the normal season crop, the extremely high prices obtained out of season makes this a profitable venture (Table 3.1). Disease pressure is highest during the rainy, hot and humid summer months. Out of season fruit

growth occurred through the cold and dry winter months until early summer and the expected disease incidence may therefore be lower on the out of season than on the normal season fruit.

Less rounds of disease preventative sprays required for the out of season fruit, cause a saving of approximately R860.ha<sup>-1</sup>. This saving can cover the costs of the de-flowering and flower induction treatments. Out of season fruit can fetch prices of 286%, 386% and 643% of the normal season's prices, resulting in an increased normal season profit.ha<sup>-1</sup> of 165%, 218% and 438% when harvested in November, December and January respectively (Table 3.1).

Table 3.1. Financial viability of out of season 'Fuerte' fruit production compared to normal season production (200 trees.ha<sup>-1</sup>).

Yield (t.ha <sup>-1</sup> )	Packout (%)	Marketable yield (t.ha <sup>-1</sup> )	Cartons <sup>1</sup> .ha <sup>-1</sup>	Price.carton <sup>-1</sup> (R)	Income (R.ha <sup>-1</sup> )	Costs (R.ha <sup>-1</sup> )	Profit <sup>2</sup> (R.ha <sup>-1</sup> )
<b>Normal season</b>							
9.16	86	7.88	1 970	R7	R13 790	(R7 292) <sup>3</sup>	R6 498
<b>Out of season</b>							
				R20 (Nov)	R15 900		R8 622
3.53	90	3.18	795	R27 (Dec)	R21 465	(R6 278) <sup>4</sup>	R14 187
				R45 (Jan)	R35 775		R28 497
				R45 (Feb)	R35 775		R28 497

<sup>1</sup> 4 kg cartons

<sup>2</sup> Profit.ha<sup>-1</sup> before production and packing costs

<sup>3</sup> Fruit retention enhancement: R4 700 + Disease preventative sprays (3 rounds): R2 592

<sup>4</sup> De-flowering treatment: R700 + Flower induction: R150 + Fruit retention enhancement: R4 700  
+ Disease preventative sprays (2 rounds) : R1 728

### 3.5 Conclusions

Chemical and physical manipulation of avocado trees to produce out of season fruit is a complex process and success depends on various factors. The disruption of the normal growth and development cycles is the first step necessary to induce out of season flowering. The de-flowering treatment, being non-selective, caused not only flowers, but also some leaves to abscise. The stimulus needed for floral induction was not perceived by trees receiving only a de-flowering Ethrel<sup>®</sup> spray. Therefore, cincturing is essential for out of season floral induction. Although out of season flowering was weak in comparison with normal season flowering, the yield was not increased by a Sunny<sup>®</sup> application. However, a significantly higher proportion of fruit in the economically important count range 12-18 was obtained with the Sunny<sup>®</sup> treatment.

Quality of out of season fruit proved to be equal to, or even better than that of normal season fruit, having less lenticel damage and pulpspot. Although additional costs are incurred for the de-flowering and cincturing treatments, the higher prices obtained out of season and the savings accomplished with fewer rounds of disease preventative sprays, makes the production of out of season fruit highly profitable.

Due to the limited number of trees in the test orchard and Westfalia Estate's need for out of season fruit, the best treatment (Ethrel<sup>®</sup> + cincture + Sunny<sup>®</sup>) was applied in another orchard during the following season (2000/2001) to produce out of season fruit on a semi-commercial scale. Out of season flowering on these trees was very good and favourable environmental conditions at the time of flowering and fruit set, contributed to a good out of season crop. These fruit will be ready for harvesting from December 2001 through February 2002.

However, the described method for producing out of season fruit is not ideal because of the stress inflicted on the trees through de-flowering and cincturing. Problems may also arise with the management of an orchard when two sets of fruit are present on the trees. In order to reduce the stress on the trees and avoid two fruit sets, the manipulation of flowering time by means of GA<sub>3</sub> treatments may be worthwhile to investigate.

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## CHAPTER 4

### General conclusions

Chemical and physical manipulation of avocado trees to produce an out of season crop as tested in this study, proved to be successful. Being the first step to out of season flowering and fruitset, the disruption of the normal phenological cycle by means of a de-flowering treatment or the inhibition of normal season flowering is crucial for success or failure. Unnecessary stress is inflicted onto trees by the de-flowering Ethrel<sup>®</sup> spray which is non-selective and results not only in flower but also leaf abscission. However, avocado trees have a prolonged flowering period, which reduces the effectiveness of the flower removal treatment. Therefore, the long term sustainability of the de-flowering method can be a limiting factor in out of season avocado production.

Theoretically, the inhibition of flowering during the normal season may replace the de-flowering treatment. In this regard, GA<sub>3</sub> treatments may be a powerful manipulation tool. Multiple GA<sub>3</sub> treatments applied to mature avocado trees inhibited flowering and resulted in a severely reduced yield. Significant inhibition of normal season flowering was also achieved by the application of multiple GA<sub>3</sub> treatments to potted avocado trees. Timing of GA<sub>3</sub> sprays is extremely important and it can be suggested that multiple treatments should be applied with the first treatment before or during early stages of floral initiation and the last treatment during advanced stages of inflorescence development in order to inhibit flowering

reliably. By using GA<sub>3</sub> sprays to inhibit the following year's normal season flowering, the problems associated with two sets of fruit on the trees will be eliminated.

Westfalia Estate now has the knowledge to produce high quality avocado fruit for the out of season niche market. However, it is important to note that all conclusions derived from this study were based on data from one season. More research is needed to investigate the sustainability of out of season avocado production and to refine the GA<sub>3</sub> treatment to inhibit flowering.

**Manipulation of avocado (*Persea americana* Mill.) trees**

**for out of season fruit production**

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**Summary**

The seasonality of avocado production leads to over-supplied markets during peak periods followed by shortages of fruit during other periods. The production of out of season avocado fruit was seen by Westfalia Estate as an opportunity to increase their profits. As the Estate is committed to supply Woolworths with fruit for 12 months of the year, this study was undertaken to investigate the production of out of season avocado fruit through chemical and physical manipulation of trees.

Gibberellins (GA) are known to have many regulatory functions on various processes in plants, including flowering. Trials were conducted during 1999 in which the effect of gibberellic acid (GA<sub>3</sub>) sprays on flowering of avocado was evaluated. A microscopic study revealed that the first signs of inflorescence development were already present in 'Hass' buds sampled in mid March. Secondary inflorescence axis meristems could be seen as small axillary



meristems in the axils of the inner terminal bud bracts. GA<sub>3</sub> sprays delayed the development of these secondary inflorescence axis meristems.

In a more comprehensive study, the effect of single and multiple GA<sub>3</sub> treatments (50 and 250 mg.L<sup>-1</sup>) on flowering of potted 'Fuerte', 'Hass' and 'Ryan' trees was evaluated. Depending on the timing of the application, single GA<sub>3</sub> treatments did not affect (February), delayed (mid March and early April) or enhanced (late April and May) inflorescence development of avocado trees. However, none of these effects were significant. Multiple GA<sub>3</sub> treatments applied over a four month period significantly inhibited inflorescence development. The effect on inflorescence development tended to increase with increasing number of sprays applied and with an increase in the concentration used. Timing of GA<sub>3</sub> sprays proved to be crucial and determined what kind of effect was obtained. In order to inhibit flowering reliably, the first application must be applied before floral initiation and the last application during advanced stages of inflorescence development.

In another trial, 'Fuerte' trees were de-flowered during the normal flowering period (August/ September 1999) by the application of an Ethrel<sup>®</sup> spray. Three months after the de-flowering treatment, out of season flowering was induced by cincturing the trees. Out of season flowering and fruit set were observed in January/February 2000. Normal season fruit were harvested in April 2000 at a moisture content of 70%, whereas the out of season fruit were harvested in



November 2000 at a moisture content of 62%. Although, the out of season yield ( $17.7 \text{ kg.tree}^{-1}$ ) was significantly lower than the normal season yield ( $45.8 \text{ kg.tree}^{-1}$ ), the extremely good prices obtained out of season makes this a highly profitable venture. Out of season fruit quality proved to be equal to or even better than the normal season fruit quality.

The de-flowering treatment resulted in unnecessary stress being inflicted onto the trees and may be a limiting factor in the long term sustainability of out of season fruit production. In this regard, the use of  $\text{GA}_3$  sprays to inhibit flowering during the normal season may eliminate the problems associated with the de-flowering treatment.

**Manipulation of avocado (*Persea americana* Mill.) trees**

**for out of season fruit production**

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**Opsomming**

Die seisoenaliteit verbonde aan avokadoproduksie lei tot oorvol markte tydens piek periodes, gevolg deur tekorte tydens ander periodes. Die produksie van buiteseisoen avokados is deur Westfalia Landgoed as 'n geleentheid gesien om hul winste te verhoog. Die Landgoed is kontraktueel daartoe verbind om Woolworths vir 12 maande van die jaar met avokados te voorsien. Hierdie studie is dus onderneem om die produksie van buiteseisoen avokados deur middel van chemiese en fisiese manipulasie van avokadobome te ondersoek.

Dit is algemeen bekend dat Gibberelliene (GA) verskeie prosesse, soos blomvorming, in plante kan reguleer. 'n Veldproef is gedoen op volwasse 'Hass' bome om die effek van gibberelliensuur (GA<sub>3</sub>) bespuitings op blomvorming van die avokado te ondersoek. 'n Mikroskoopstudie het getoon dat die eerste tekens van blomontwikkeling reeds teenwoordig was in groeiknoppe wat in die middel van Maart versamel is. Die sekondêre bloeiasmeristeme is waargeneem as klein

meristeme in die oksels van die terminale groeiknop, tussen die binneste skubblare. GA<sub>3</sub> bespuitings het die ontwikkeling van hierdie meristeme vertraag.

In 'n meer omvattende studie, is die effek van enkel en herhaaldelike GA<sub>3</sub> bespuitings (50 en 250 mg.L<sup>-1</sup>) op blomvorming van jong 'Fuerte', 'Hass' en 'Ryan' bome ondersoek. Enkel GA<sub>3</sub> behandelings het, afhange van die tyd van toediening, verskillende effekte op blomontwikkeling gehad. 'n Behandeling in Februarie het geen effek gehad, 'n behandeling in die middel van Maart of vroeg in April het blomontwikkeling vertraag, maar 'n behandeling teen die einde van April of in Mei het blomontwikkeling versnel. Geeneen van hierdie effekte was egter betekenisvol nie. Herhaaldelike behandelings, toegedien oor 'n periode van vier maande, het blomontwikkeling betekenisvol geïnhibeer. 'n Toename in die aantal bespuitings gedoen en die konsentrasie gebruik, het 'n sterker effek tot gevolg gehad. Hieruit kan afgelei word dat die tyd van GA<sub>3</sub> behandeling kritiek is en sal bepaal watter tipe effek verwag kan word. Om blomontwikkeling betroubaar te beïnvloed, moet die eerste bespuiting gedoen word voor blom inisiasie plaasgevind het, en die laaste bespuiting tydens 'n meer gevorderde stadium van blomontwikkeling.

In 'n volgende proef is volwasse 'Fuerte' bome se normale seisoen blom verwyder deur die toediening van 'n Ethrel<sup>®</sup> blaarbespuiting tydens volblom in Augustus/September 1999. 'n Buiteseisoen blom is drie maande later op hierdie bome geïnduseer deur middel van ringelering. Die bome het geblom en vrugte

geset in Januarie/Februarie 2000. Tydens April 2000, is die normale seisoen vrugte gepluk (voginhoud: 70%). Die buite seisoen vrugte is gepluk in November 2000 en het 'n voginhoud van 62% gehad. Die buite seisoen oes ( $17.7 \text{ kg. boom}^{-1}$ ) was betekenisvol laer as die normale seisoen oes ( $45.8 \text{ kg. boom}^{-1}$ ), maar die uitstekende pryse wat buite seisoen vrugte behaal, maak die produksie van buite seisoen vrugte 'n hoogs winsgewende onderneming. Die vrugkwaliteit van buite seisoen vrugte was gelykstaande of selfs beter as die vrugkwaliteit van normale seisoen vrugte.

Blaarval op bome veroorsaak deur die blomverwyderingsbehandeling asook die wonde veroorsaak deur ringelering, mag beperkende faktore wees in die volhoubaarheid van buite seisoen avokado produksie oor die langer termyn. Daarom kan die gebruik van  $\text{GA}_3$  bespuitings om blomontwikkeling tydens die normale seisoen te inhibeer, hierdie probleme uit die weg ruim.