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SPRING GROWTH OF AVOCADO TREES AS INFLUENCED BY PRUNING AND THE USE OF GROWTH REGULATORS TO STABILISE YIELD AND IMPROVE FRUIT QUALITY

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LIST OF ABBREVIATIONS

ABA	Abscisic acid
AI	Alternance index
DM	Dry matter
E.85	Experimental year 1985
E.86	Experimental year 1986
E.87	Experimental year 1987
FD	Fruiting density
GA (GA_3)	Gibberellin-like substance(s)
GA_3	Gibberellic acid
GA_{4+7}	Gibberellin A_4 + gibberellin A_7
I-Ado/I-Ade	Isopentenyl adenosine / Isopentenyl adenine
IAA	Indole-3-acetic acid
L/D	Length/diameter ratio
NAAm	Naphthaleneacetamide
PBZ	Paclobutrazol = (2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)pentan-3-ol
PBZ+GA_3	Paclobutrazol + gibberellic acid
PVP	Polyvinylpyrrolidone
RIA	Radioimmunoassay
YC	Yield capacity
Z/ZR	Zeatin / zeatin riboside

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1. INTRODUCTION

1.1 Problems of avocado cultivation

The avocado, Persea Americana Mill. is a member of the family Lauraceae and native to Central America. Since the beginning of the century, the avocado has also been cultivated outside its natural habitat in environments ranging from the tropics to subtropical winter-rainfall regions (POPENOE 1920). The most universally cultivated variety is the 'Fuerte' avocado. Its green coloured, pear-shaped fruits, averaging 300g in weight, are distinguished by their excellent flavour and good transportability (WOOD 1984b, GAILLARD 1987). Being the only acceptable early maturing variety with an extended harvesting season, 'Fuerte' fruit represent a valuable market commodity (COIT 1968).

Avocado trees grow vigorously and may attain a mature height of 20 metres (WILLIAMS et al. 1980), a fact which complicates fruit harvesting and tree management. Commercial varieties were until recently grown on fast-growing seedling rootstocks. In the meantime, vigorously growing but clonal rootstocks have become available which are less susceptible to root rot caused by Phytophthora cinnamomi (BROKAW 1987). However, in the long-term dwarfing rootstocks would be advantageous (WOLSTENHOLME 1987).

The two principal problems of commercial avocado cultivation are low fruit yields and the susceptibility of the trees to root rot. Low yields in avocado (BERGH 1967), particularly in the 'Fuerte' variety (POPENOE 1941, LAHAV et al. 1971, TROCHOULIAS and O'NEILL 1976), which is also alternate bearing (HODGSON 1947, ALEXANDER and SAROOSHI 1980, MONSELISE and GOLDSCHMIDT 1982) have been reported for virtually all the major cultivation regions of world.

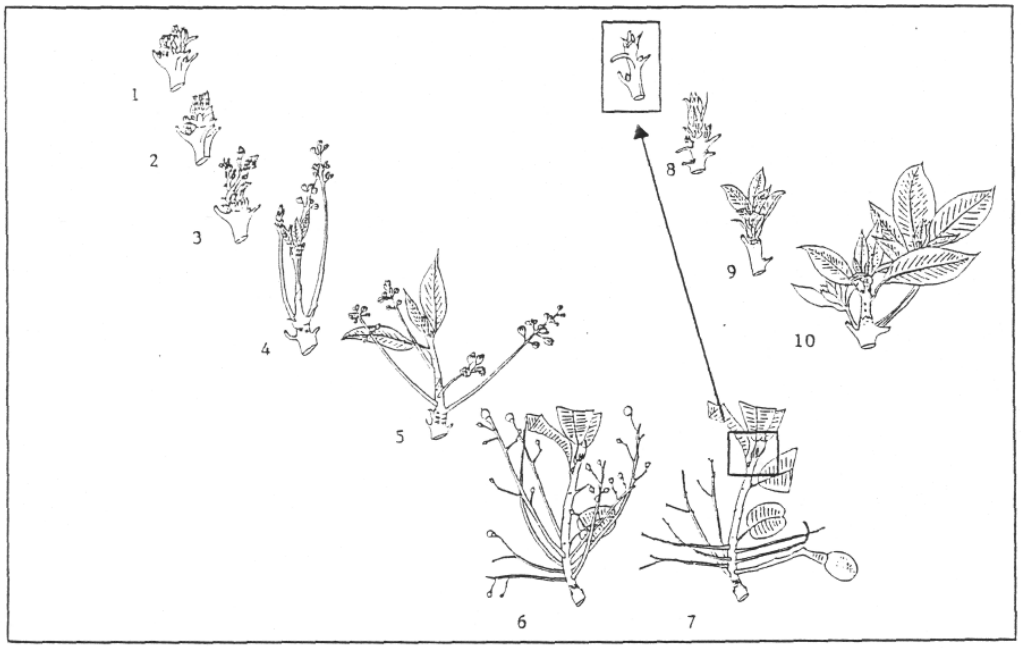
Phytophthora-infected trees wither, cease growing and die quickly (KRANZ 1979), By means of trunk injections of phosethyl-Al, DARVAS et al. (1984) succeeded in improving tree health and fruit yield of a heavily infected 'Fuerte' orchard. This since then widely adopted measure (PEGG and WHILEY 1987, WOOD et al. 1987) has increased yields to an average of eight tons per hectare (TUFFIN 1987); this has, however, been accompanied by increased vegetative growth, and the above mentioned problems.

In 1984, world avocado production was of the order of 1.6 million tons (GAILLARD 1987). The increasingly competitive export market has dictated ever higher quality standards, the production of high quality fruit is therefore of utmost importance.

1.2 Phenology

The phenological development of the avocado is represented in Figure 1. Flower bud differentiation proceeds in autumn in the terminal buds of the recently completed summer flush. Approximately two months later, without passing through a dormancy period the buds unfold (Stages 1 and 2), and a primary axis with lateral inflorescences emerges (Stage 3). Towards the end of the winter, the primary axis elongates to produce the spring flush (Stage 4) on the lateral branches of which a large number of hermaphrodite flowers come to bloom (Stage 5) (REECE 1942, ROBERTSON 1S69).

Figure 1 Phenological development stages of the avocado (modified after AUBERT and LOSSOIS 1972).



reproductive	Floral induction and differenti-ation in terminal buds of summer flush	Bud break (1-4)	Full flowering (5) Fruit set (6) Heavy fruit drop (7) Fruit size increase		Harvest
vegetative			Spring flush (4-7)	Summer flush (8-10)	
	Autumn	Winter	Spring	Summer	Autumn

The flowers show protogynous dichogamy with the female stage followed by a closed stage followed by the male stage. There are two flowering types; type A (e.g. 'Hass') with the female stage in the morning, and type B (e.g. 'Fuerte') with the female stage in the afternoon (SEDGLEY 1987).

There is a large number of flowers on an avocado tree (CHANDLER 1950). According to DAVENPORT (1982), in the 'Fuerte' variety 20-30% of the flowers develop into small fruits (Stage 6) whereof only 1-7% survive to the harvesting stage due to heavy fruit drop (Stage 7).

In avocado, vegetative growth occurs mainly in two flushes, the spring flush and the summer flush (SCHOLEFIELD et al. 1985). The spring flush (Stages 4-7) occurring at the time of flowering, is more vigorous than the summer flush (Stages 8-10) (VENNING and LINCOLN 1958).

1.3 Reproductive physiology

In avocado, more than 99% of the flowers and fruitlets drop (ADDICOTT 1983), resulting in low yields. As no anatomical reason for the high rate of fruit drop was observed by SEDGLEY (1980), a source and/or sink limitation, competition for assimilates or dominance could be possible reasons for low yields in avocado.

POSSINGHAM (1986) describes the foliage (source) of evergreen fruit trees as being exceptionally well developed; this also applies to the avocado (LAHAV et al. 1971). The assimilative capacity of avocado leaves has been investigated by SCHOLEFIELD et al. (1980), but no leaf:fruit ratio data are as yet available.

Assimilate import into a sink organ is a function of sink strength, which is determined by sink activity and sink size, which in turn are influenced by phytohormones (PATRICK 1982). The phytohormones present in the avocado seed play a crucial role in fruit development; seedless avocados, for example, are only one-tenth the size of seeded avocados (BLUMENFELD and GAZIT 1970). The shrivelling of the seed coat (testa) during early fruit development leads to fruitlet abscission (BLUMENFELD and GAZIT 1974). The seed and more especially the testa of avocado fruitlets contain auxins (GAZIT and BLUMENFELD 1972), gibberellin-like substances (BLUMENFELD and GAZIT 1972), cytokinin (BLUMENFELD and GAZIT 1970), ethylene, and abscisic acid (ADATO and GAZIT 1977).

A number of authors have proposed assimilate competition between spring flush and developing fruits as a causal factor in fruit drop in the avocado (SCHCLEFIELD et al. 1935. SEDGLEY 1337, ZILKAH et al. 1987). Investigations by BUCHHOLZ (1986) confirm that in the 'Fuerte' variety the spring flush temporarily acts as a sink, competing with the fruitlets for assimilates. After a month of development the young leaves of the spring flush begin to function as source organs. Since, however, the removal of young spring flush reduces fruit drop even after the commencement of assimilate export by the spring flush, assimilate competition does not appear to be the primary cause of the avocado's high fruit drop rate (BUCHHOLZ 1986).

Dominance effects range from very weak (e.g. tomato) to absolute, i.e. fruit abscission (e.g. cucumber, apple). Crucial for dominance is a temporal development advance of an

organ relative to the inhibited organ (BANGERTH 1989). On this hypothesis the developmental advance leads to a self-reinforcing IAA-transport from the dominant organ; this flow on meeting that from a later developing organ results in inhibition of the latter. This inhibited IAA transport of a suppressed fruit leads to reduced phloem development and thereby reduced nutrient supply.

The period of rapid spring flush growth largely coincides with the period critical for calcium supply of the avocado fruitlets. BOWER (1988) assumes that the weakly transpiring fruitlets are at a calcium disadvantage relative to the more strongly transpiring leaves. BANUELOS et al. (1987) have established that in addition to the transpiration: calcium linkage a further definite linkage exists in the fruit between IAA export and calcium import. In avocado, studies on IAA and calcium transport rates during the first five weeks after flowering have shown that the spring flush exports more IAA and imports more calcium than the flower and fruit peduncles (CUTTING and BOWER 1989). Calcium supply to fruits which plays an important role in reducing physiological disorders (BANGERTH 1979), influences both storability (TINGWA and YOUNG 1974, WILLS and TIRMAZI 1982) and the incidence of physiological disorders in avocado (CHAPLIN and SCOTT 1980). VELDMAN (1983) reports a significantly lower calcium content in 'Fuerte' fruit with pulp spot, a physiological disorder common in this variety, than in healthy fruit.

In apple, shoot tip removal shortly after full bloom results in reduced fruit drop and increased yield; this is attributable to reduced competition between fruit and vegetative growth (QUINLAN and PRESTON 1971). ZILKAH et al. (1987) report reduced fruit drop in avocado shortly after removal of the spring flush; this observation is however restricted to the period immediately after flowering and does not support extrapolation in terms of yield.

There are some studies on the effects of the use of growth regulators on fruit yield in avocado. STEWART and HIELD (1951) report an increased incidence of small, seedless fruits in response to auxin treatment but no effect on normal fruit yield. LAHAV and TOMER (1969) tested IAA, 2,4-D, 2,4,5-TP, TIBA, NAA, GA, Kinetin, CCC, Alar, Sevin, Ethrel, Amidthin and Fithin on avocado but report no yield increase in response to any of these treatments,

1.4 Definition of problem

In avocado, yields are low in comparison with many other fruit-bearing species (MONSELISE 1986, WOLSTENHOLME 1986). Moreover avocado trees exhibit marked alternation (MONSELISE and GOLDSCHMIDT 1982) and the fruits often show physiological disorders after storage (BOWER 1988). These manifestations may be connected with the vigorous growth of the spring flush (BOWER 1988). The present study therefore examines whether manipulating the spring flush may increase yield, reduce alternation and improve fruit quality.

To determine whether photosynthetic output (source) might limit fruit yield, the first step was to quantify performance in terms of leaf/fruit ratio. In a second experiment, the spring flush of 'Fuerte' trees was removed and re-growth inhibited by application of

Naphthylacetamide (NAAm). As pruning is very labour—intensive and impossible in large trees, attempts were then made to inhibit spring flush growth by the application of growth regulators. Good results were obtained using certain substances; triazole derivatives, in particular, were found to reduce fruit drop and to some extent to inhibit vegetative growth.

The triazole derivative paclobutrazol was singled out for testing in view of its availability and likelihood of registration. Paclobutrazol inhibits growth by blocking the gibberellin biosynthesis (DALZIEL and LAWRENCE 1984). In addition to paclobutrazol, gibberellins as well as a combination of paclobutrazol and gibberellin were applied. Thus the effects of a growth inhibition, growth stimulation and, in the case of the combination applied, a mutual canceling out of the effects could be studied. The treatments were part of a two-year in-depth field study, fruit retention and vegetative growth being monitored up to the time of harvesting. In view of the hypothesis that the conditions for physiological disorders are in a large measure laid down during the fruit growth period, the effects of the treatments on fruit mineral content at harvest and on the development of physiological disorders in stored fruits were also investigated.

To assess the effects of the endogenous hormones influencing fruiting and vegetative growth during the critical period of spring flush growth, the level of extractable gibberellins, IAA, ABA and cytokinins (Z/ZR and I-Ado/I-Ade) in the shoot tips and the levels of diffusible IAA and ABA in the fruits were determined.

2. MATERIALS AND METHODS

2.1 Environmental conditions and cultivation measures

All experiments were run in two adjacent avocado orchards on the Westfalia Estate. The Estate, with a total of 550 hectares under avocado cultivation, is situated in north-eastern South Africa (latitude 23.45 S, longitude 30.05 E, height above sea level 300 m).

The climatic conditions during the three years of the study varied mainly in rainfall (Figure 2).

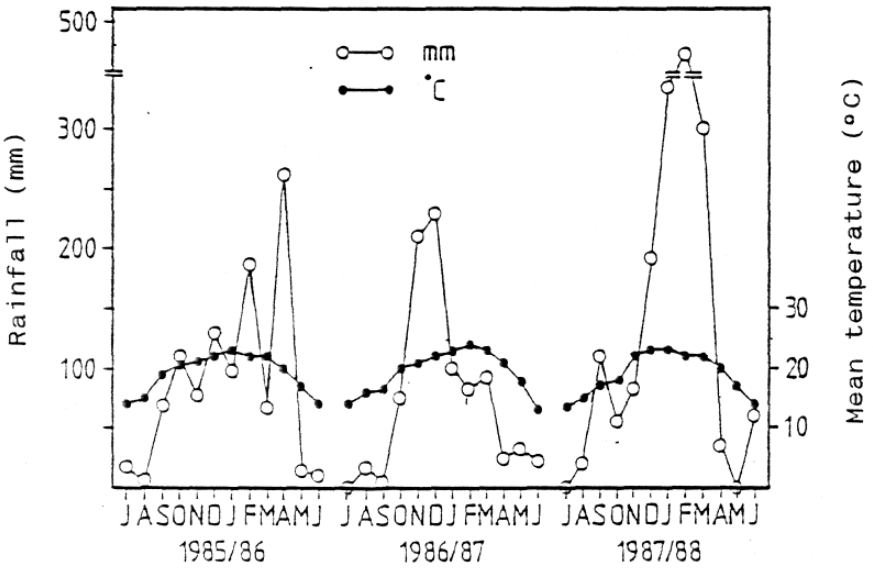


Figure 2. Rainfall and mean temperatures at Westfalia Estate over the 1985-1988 test period.

Soil quality was practically uniform throughout the test area: deep lateritic, averaging 43% sand, 23% silt, 33% clay. In 1987, soil analysis indicated 1,6 mg P, 13,2 mg K, 178,0 mg Ca and 16,8 mg Mg per 100 g of soil (average for depth 0-30 cm); pH 6,4. Yearly leaf analyses (N, P, K, Ca, Mg, Zn, B) only indicated a deficiency of the trace elements zinc and boron. Other than zinc and boron supplementation no fertilisers were applied. To eliminate water stress the trees were irrigated from 55 kPa ground capillary tension upwards (30 cm-subsurface tensiometer readings). Ground erosion protection was by seeding with an annual legume (Prosopis velutina).

Disease control was by two sprayings of copper against Cercospora spot (Pseudocercospora purpurea) and against root rot by thrice-annual trunk injections of phosethyl-Al.

2.2 Plant material

Leaf:fruit ratio determination was based on 12 six-year-old 'Hass' trees and 12 ten-year-old 'Fuerte' trees, all grown on seedling rootstocks.

The pruning tests were performed on 90 five-year-old 'Fuerte' trees grown on seedling rootstocks.

Three sets of plant growth regulator tests were carried out, in 1985, 1986 and 1987 (E.85, E.86 and E.87). Test E.85 was based on 'Hass' and 'Fuerte' trees; 19 six-year-old 'Hass' trees and 19 ten-year-old 'Fuerte' trees, all grown on seedling rootstocks, were used.

Test E.86 was based on 60 'Fuerte' trees grown on clonal rootstocks (Duke 6). The trees were five years old at the start of the study (September 1986). E.87 was carried out on the same 60 trees.

2.3 Leaf: fruit ratio

One hundred flowering branches each on 10 'Hass' trees and 10 'Fuerte' trees were girdled. A 1,0 cm wide strip was removed from the bark of one-year-old branches in 'Fuerte' on 15/9/1984 and in 'Hass' on 10/10/1984. To ensure the girdling effect, callus was removed from the bands at regular intervals up to the time of harvesting. By harvest in May 1985 only about one quarter of the girdled branches were bearing one fruit each. The leaf area and fruit weight distal to each girdle were recorded at this time.

Additionally, all the leaves and fruit were removed from four well-bearing, ungirdled trees two 'Hass' and two 'Fuerte' trees in May 1985. Yield and total leaf area were recorded for each tree,

Leaf area was determined using a sorting platter bearing 15 reference avocado leaves ranging in size from 5 cm^2 to 275 cm^2 measured with a planimeter (Ushikata 220P). The total leaf area was calculated by approximation by attributing to each leaf the area of the reference leaf nearest to it in size.

In the girdled branches, leaf area was assigned to the weight of fruit produced distal to the girdle. The ratios obtained for 25 girdled branches each of 'Hass' and 'Fuerte' were used to derive the correlation and regression indices of the two variables (ZÖFEL 1985). Leaf area was related to fruit yield for each stripped tree. The leaf:fruit ratio calculations were based on an average leaf area of 58 cm^2 and an average fruit weight of 285,5 g (the latter corresponding to the commonest avocado weight class, 266-305 g).

2.4 Pruning

Vigorous vegetative growth was observed over the 1985/86 period of the study. These trees had previously produced low yields: 2,4 t/ha in 1984 and 2,9 t/ha in 1985.

On attaining the phenological stage five (Fig. 1), thirty trees were treated by removing the spring flush distal to the flowers to optimize fruit setting conditions (Treatment: pruning).

A second group of 30 trees received the same treatment plus painting of the wounds with NAAm (1% naphthylacetamide dissolved in white pain); a third group of 30 trees were left untreated as the control.

To determine canopy size, the yield capacity (YC) of each tree was measured before treatment, at five months after treatment and at nine months after treatment. According to WINTER (1977), the yield capacity of a fruit tree corresponds to the surface of its silhouette, defined by mean diameter x mean height of the tree canopy. The increase in yield capacity was in each case referenced against the initial May 1985 value. Fruiting density, defined as the average number of fruit externally visible in a 0.36 m^2 canopy section (WINTER 1977), was determined for each tree at five and at nine months after treatment. An analysis of variance was conducted for the data obtained.

2.5 Tests with plant growth regulators

2.5.1 Treatments

The plant growth regulators listed in Table 1 were applied to branches of 'Hass' and 'Fuerte' trees in 1985 on attaining the phenological stage five (Fig. 1). One branch per tree was selected for each treatment. The plant growth regulators were applied to dripping wetness with a hand spray gun at the concentrations listed in Table 1. Contiguous branches were protected against contamination during spraying with sheets of plastic. Spraying was performed on 18/9/1985 on cv 'Fuerte' and on 30/9/1935 on cv 'Hass'. This screening of various plant growth regulators was undertaken with the aim of evaluating effects on spring flush growth and on fruit yield.

Table 1. Plant growth regulator treatments.

Product	Common name	Concentration (ppm)
Alar	Daminozide	1700
Alden	Piproctanylium bromide	100
Atrinal	Dikegulac sodium	2000
AVG	Aminoethoxyvinylglycine	500
BAS 106..W	Tetcylacis	4000
Berelex	Gibberellic acid (GA_3)	500
Cultar	Paclobutrazol (PBZ)	4000
Cultar + Berelex	As ingredients (PBZ + GA_3)	4000 + 500
Ethrel	Ethephon	480
Ethrel + Alar	As ingredients	480 + 1700
MB 25-105	Butylphenoxyacetate	400
Off-Shoot-O	Methyl esters of fatty acids	1350
RSW 0411	Triapenthenol	4000
Water (Control)	-	-

Of the plant growth regulators listed in Table 1, paclobutrazol (PBZ), gibberellin (GA_3) and their combination (PBZ+GA_3) were tested further in 1986 and 1987. Per treatment, 15 'Fuerte' trees were sprayed on attaining the phenological stage five (Fig. 1). The treatments were applied en 11/9/1986 and on 22/9/1987; in E. 86 four litres and in E. 87 five litres of spray solution were applied to each tree using a motorized knapsack

sprayer (Echo model DM-9). In E. 86 and E. 87 the same concentrations were used as listed in Table 1.

2.5.2 Determination of shoot growth

Flowering spring flushes were marked for determining shoot growth. In E. 85 and E. 86, growth of the primary axis of the marked flushes was measured at weekly to fortnightly intervals from the date of treatment until autumn (February) when vegetative growth ceased. As shoot growth in cv 'Fuerte' presented the same picture for the treatments PBZ, GA_3 and PBZ+GA_3 in E. 85 and E. 86, growth was not monitored continuously in E. 87. In E. 87, the length of the primary axis of the flushes was measured at the end of the spring growth period (December 1987) and again at the end of the summer growth period (February 1988).

In E. 85 the growth was measured on one shoot per treated branch. In E. 86 y E. 87 growth was measured on 10 shoots per treated tree.

2.5.3 Determination of fruiting density

In E. 85 and E. 86 the fruits developing on the branches previously marked for shoot growth determination were counted. In E. 85 counting was commenced at five weeks after full flowering, the fruitlets then being around 6 mm in diameter, and repeated fortnightly until the end of the vegetative period (February). In E. 86 counting was already commenced at one week from full flowering. As complete trees were treated in E. 86, the counting of fruit on the marked shoots was discontinued when only very few shoots were bearing fruit (70 days after treatment). At harvesting in E. 86 (25/5/1987) each tree was picked individually and the fruits counted and weighed.

In E. 87 fruiting density was determined at nine weeks from full flowering using the method described by WINTER (1977), and the diameter of 10 fruits per tree measured at the same time. At harvest in E. 87 (2/5/1988) each tree was stripped individually and the fruits counted and weighed. The harvest data were processed statistically by analysis of variance.

2.5.4 Hormone analyses

The effects of PBZ, GA_3 and PBZ+GA_3 en extractable hormone levels in shoot tips (GA_3, IAA, ABA and cytokinins) and on the levels of IAA and ABA diffusing cut of fruitlets were investigated. Random sampling was carried out at 2, 9, 19 and 30 days per from treatment in E. 86 and at 2, 6 and 28 days in E. 87.

2.5.4.1 Extractable hormones

For hormone analysis, a mixed sample of shoot tips (each about 20 g fresh weight) was taken from the 15 trees of each treatment per sampling date. The shoot tips consisted of the first 1-2 cm of the apical end of flowering and fruiting shoots respectively. The

shoot tips were frozen in liquid nitrogen immediately after removal from the tree and stored at -20°C until analysis.

For analysis 5 g of the frozen shoot tips were weighed out and homogenised in 30 ml of cold 100% methanol and extracted for 12 hours at 4°C. After filtration through G4 sintered glass frit the residue was washed with 10 ml of cold methanol. The combined filtrate was reduced under vacuum to the aqueous phase at 35°C (raw extract).

With one exception, further preparation of the samples immediately after extraction was not feasible. Therefore, the raw extract was applied to PVP columns (Polyclar AT, Serva) which were blow-dried with nitrogen, hermetically sealed and stored at -20°C until analysis. The methanol used for extraction contained 2,6-di-tert-butyl-4-methylphenol (BHT, 100 mg/l) as an antioxidant.

2.5.4.1.1 Gibberellin determination

In E. 86 two methods of quantitative gibberellin determination were used, viz. the lettuce hypocotyl test and a radioimmunoassay (RIA). In both cases, the raw extract was used immediately and not applied to PVP columns.

Raw extract pH was adjusted to 8,5 with 0,5 M K_2HPO_4 and the extract partitioned once with petroleum benzine (20 ml) and four times with ethyl acetate (15 ml). When partitioning with ethyl acetate an interphase emulsion formed often which complicated further preparation of the samples. The organic phase was discarded in each case. The pH of the aqueous phase was adjusted to 2,5 with 5 N HCl and the extract partitioned a further four times with ethyl acetate (15 ml). The combined ethyl acetate phases were then dried down under vacuum at 35°C. The residue was taken up in 2 x 2 ml phosphate buffer (0,1 M, pH 8), loaded on to a PVP column to remove phenolic compounds and eluted with the same buffer solution. Eluate pH was adjusted to 2,5 with 5 N HCl. After partitioning four times with ethyl acetate (15 ml) the combined ethyl acetate phases were dried under vacuum at 35°C. The purification procedure described is referred to as Sample Purification Procedure A in paragraph 3.3,4.1.1.

The residue was taken up in 0,3 ml methanol and an aliquot (0,1 ml) subjected to paper chromatography. The chromatograms were developed in a descending fashion with a solvent mixture propanol-(2): 30% NH_4OH: H_2O (10:1:1). The chromatogram was subdivided into 10 R_f zones and quantitative gibberellin determination performed using the lettuce hypocotyl test described by FRANKLAND and WAREING (1960). Lettuce seeds cv ‘Hildes Necckanniesen’ were pre-germinated for 24 hours under continuous light. After an incubation period of four days at 25°C and under continuous light hypocotyl length was measured.

To confirm the results of the lettuce hypocotyl test gibberellins were also determined with a RIA using one duplicate per sample after final concentration in the purification procedure described above. The RIA was carried out according to BOHNER and BANGERTH (1333). The antibody used recognized GA_1, GA_3 and GA_{20}.

The gibberellin levels determined with the RIA were slightly higher than those determined with the lettuce hypocotyl test although the latter responds less specifically to gibberellins and higher gibberellin levels would therefore have to be expected. This

finding may be attributable to insufficient sample purification and consequent false readings in the lettuce hypocotyl test due to substances which reduce the gibberellin activity. Therefore the remaining E.86 and the E.87 samples were analysed for gibberellins (GA_1, GA_3 and GA_{20}) by means of RIA. The method of purification of the PVP-bound raw extract was improved (Sample Purification Procedure B in paragraph 3.3.4.1.1.; Fig. 3).

2.5.4.1.2 Determination of IAA and ABA

Samples were prepared as shown in Figure 3. IAA losses during analysis were determined by addition of a radioactive standard (1^{-14}C-IAA). Recovery was between 60 and 75%; the results were corrected accordingly. IAA and ABA were determined by RIA according to BCHNER and BANGERTH (1988).

2.5.4.1.3 Determination of Z/ZR and I-Ado/I-Ade

Z/ZR and I-Ado/I-Ade were determined only in E.86 samples purified without separation of aqueous and organic phases. The extract was eluted from the PVP column using 50 ml of phosphate buffer solution (0,1 M, pH 8), acidified to pH 2,5 with 5 N HCl, put on a pre-wetted Sep-Pak cartridge and eluted with increasing methanol concentration (0, 20, 40 and 70%): The fraction obtained with 20% methanol (4 ml) contained Z/ZR, that obtained with 40% methanol (4 ml) contained I-Ado and that obtained with 70% methanol contained I-Ade. Z/ZR and I-Ado/I-Ade levels were determined by RIA according to BOHNER and BANGERTH (1988).

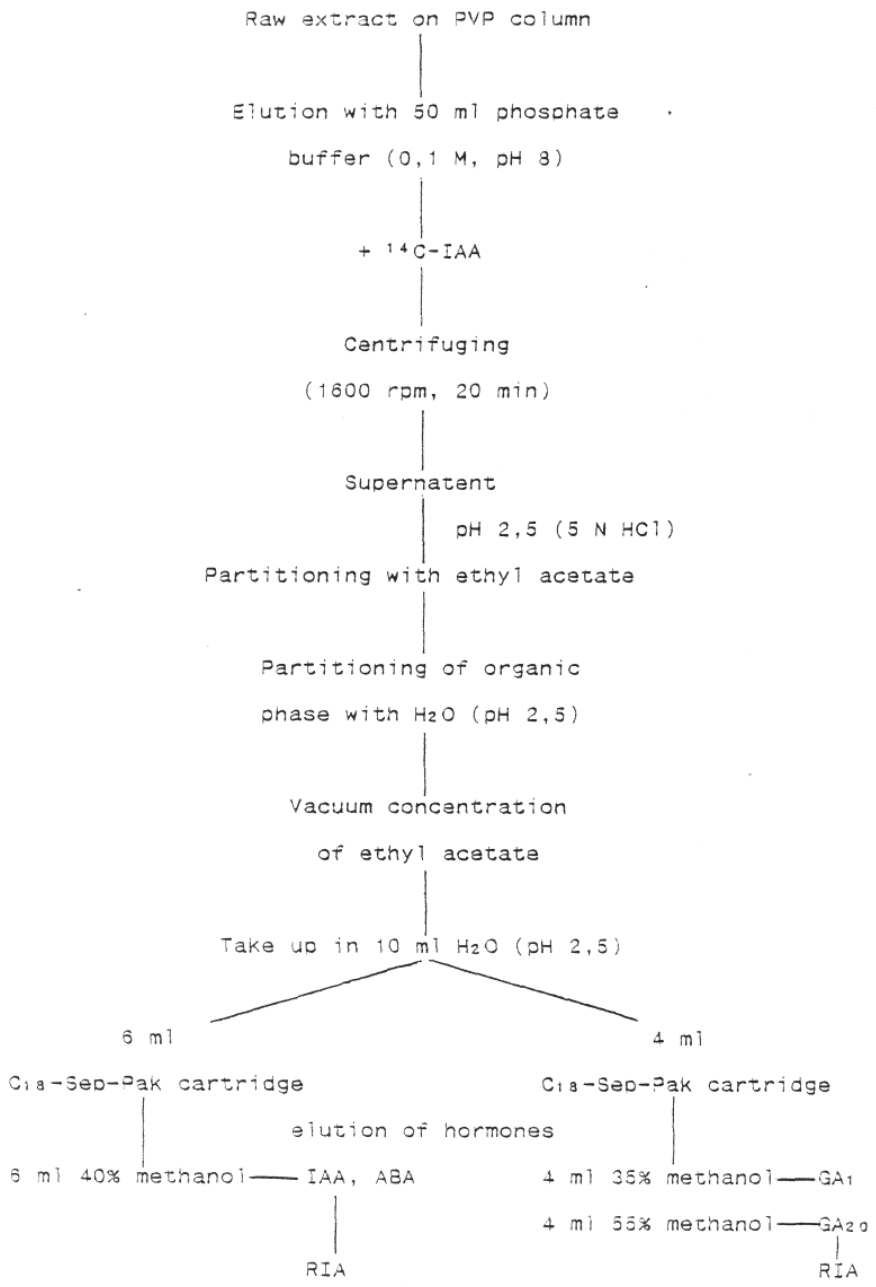
2.5.4.2 Diffusible IAA and ABA

A mixed sample consisting of 25 fruit was collected from the 15 trees of each treatment on each sampling date. Immediately after removal from the tree, the fruitlets were placed with the cut end of their pedicels in small vials containing 2 ml agar (1,5%) and insoluble PVP (1% Polyclar AT, Serva) to obtain diffusible IAA and ABA. After a diffusion period of 24 hours under continuous light (two neon lamps, Cool White, 30W) at 27°C the fruits were removed and the agar blocks frozen in liquid nitrogen and stored at -20°C until analysis.

For diffusible IAA and ABA determination the deep frozen samples each containing 25 agar blocks (=fruits) were extracted overnight at 4°C with 60 ml of 100% methanol. This was followed by filtration in round flasks through G4 sintered glass frits and the residue re-extracted twice with 10 ml of 100% methanol. The combined filtrate was reduced under vacuum to the aqueous phase at 35°C in a rotary evaporator; the residue was taken up in 5 ml of acetic acid (0.1 N). The sample was then transferred to centrifuge buckets and frozen overnight at -20°C. For lipids separation the thawed samples were centrifuged (20 minutes at 1600 rpm) and the supernatent transferred to a pre-wetted C_{1a}-Sep-Pak cartridge. The fraction obtained by elution with 6 ml of 40% methanol contained the IAA and ABA to be determined. Three aliquots were prepared from each sample and methylated with a few drops of diazomethane. After removal of the

diazomethane the IAA and ABA were determined by RIA according to BOHNER and BANGERTH (1988).

Figure 3. Schematic representation of extract preparation procedure for hormone determination (GA_1, GA_{20}, IAA, ABA) in avocado shoot tips by RIA.



2.5.5 Mineral analyses

In E.86 and E.87 the influence of PBZ, GA_3 and PBZ+GA_3 on calcium, magnesium and potassium levels in the ready-to-pick fruits were investigated. At harvest a mixed sample consisting of six fruits was taken from each tree. Fruits of uniform size (70-75

mm diameter) were picked from the outermost 50 cm of the tree canopy at a height of 1.5 to 2.5 metres from the ground.

The mesocarps were comminuted, dried for five days at 70°C and ground. Samples of 0.5 g each were mixed with 5 ml of concentrated HNC_3 and 2 ml H_2O_2 (30%) and left, overnight. To each sample 3 ml of $HClO_4$ (62%) was added and the mixture heated to 100°C for 30 minutes, then to 150°C for two hours. The samples were then filled up to 25 ml with distilled water and Ca, Mg and K determined by AAS (Techtron AA475). The results were processed statistically by analysis of variance.

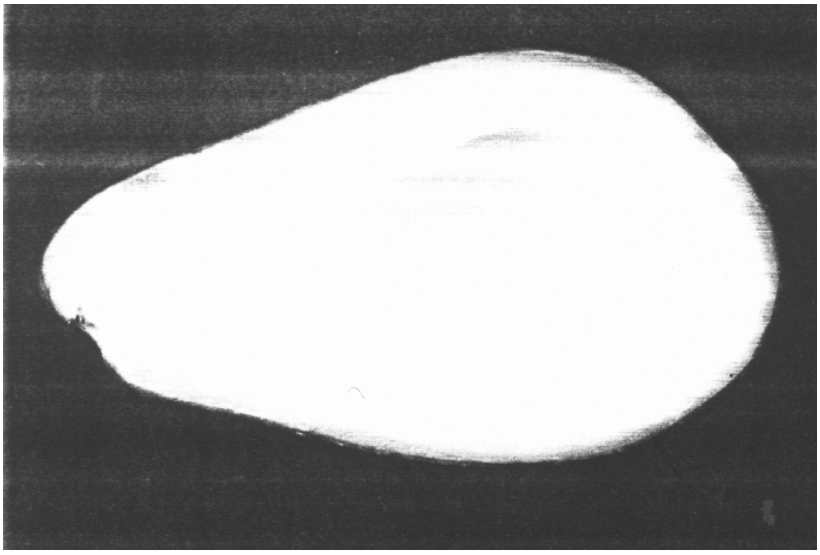
2.5.6 Fruit quality

After harvesting in E.86 and E.87 the fruits were sorted into various weight classes. Immediately after sorting, per treatment 210 fruit of uniform size (diameter 70-75 mm), average weight 285 g, were stored for 30 days at 5.5°C in a continuously ventilated chamber under conditions comparable to those of the commercial avocado trade.

After the 30 day storage period the fruits were kept at room temperature until they reached the eat-ripe stage. Fruits soft to the touch were deemed ripe for consumption and cut longitudinally into halves. After cutting the mesocarps were examined for signs of the physiological disorders grey pulp, pulp spot and vascular browning (Figure 4). For the rating, a scale of 0-3 was used (0 no visible damage, 3 = severe damage). The number of days of room temperature storage prior to attainment of table ripeness was recorded for each fruit.

Fifty fruits per treatment were measured for length and maximum diameter, and the pericarp and seed were weighed.

The fruit quality data were processed statistically by analysis of variance.



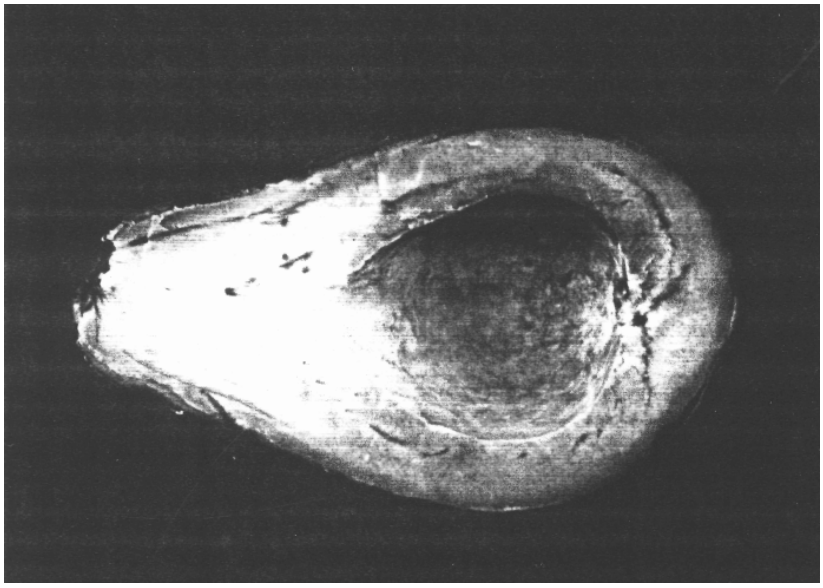
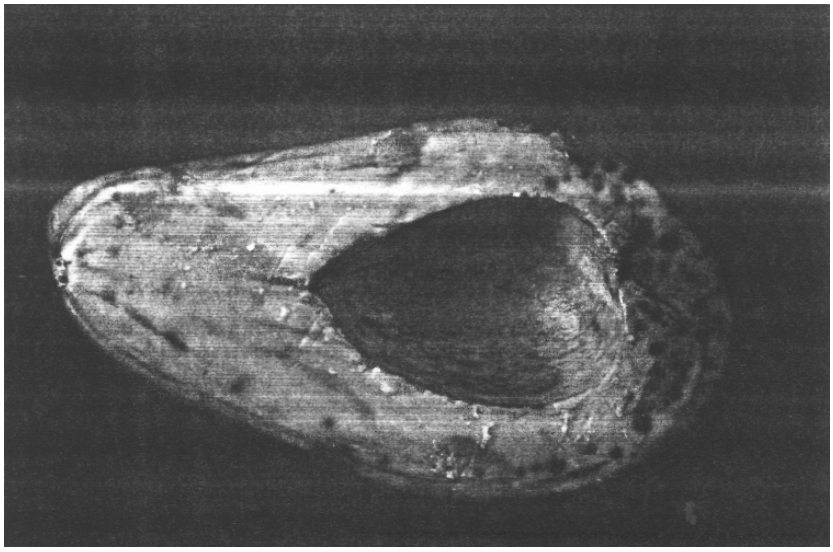


Figure 4a. Physiological disorders in ‘Fuerte fruit.
Top photo: healthy fruit. Bottom photo: grey pulp.



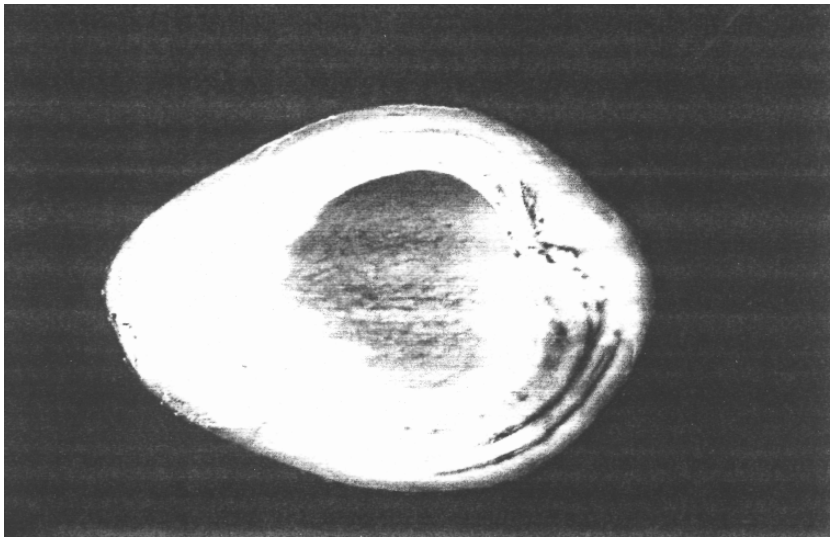


Figure 4b. Physiological disorders in ‘Fuerte fruit.
Top photo: pulp spot. Bottom photo: vascular browning.

3. RESULTS

3.1 Leaf: fruit ratio

Average single leaf area in 'Hass' and 'Fuerte' was 58 cm^2. For the girdled branches a close positive correlation was established ('Hass', r = 0.74; 'Fuerte', r = 0.71) between leaf area and fruit weight (Figure 5). In 'Hass', growth and leaf number of girdled branches were lesser than in 'Fuerte'. In 'Hass', the smaller leaf area per branch was accompanied by lower individual fruit weight as compared to 'Fuerte'; the two cultivars, however, produced the same fruit weight per unit leaf area.

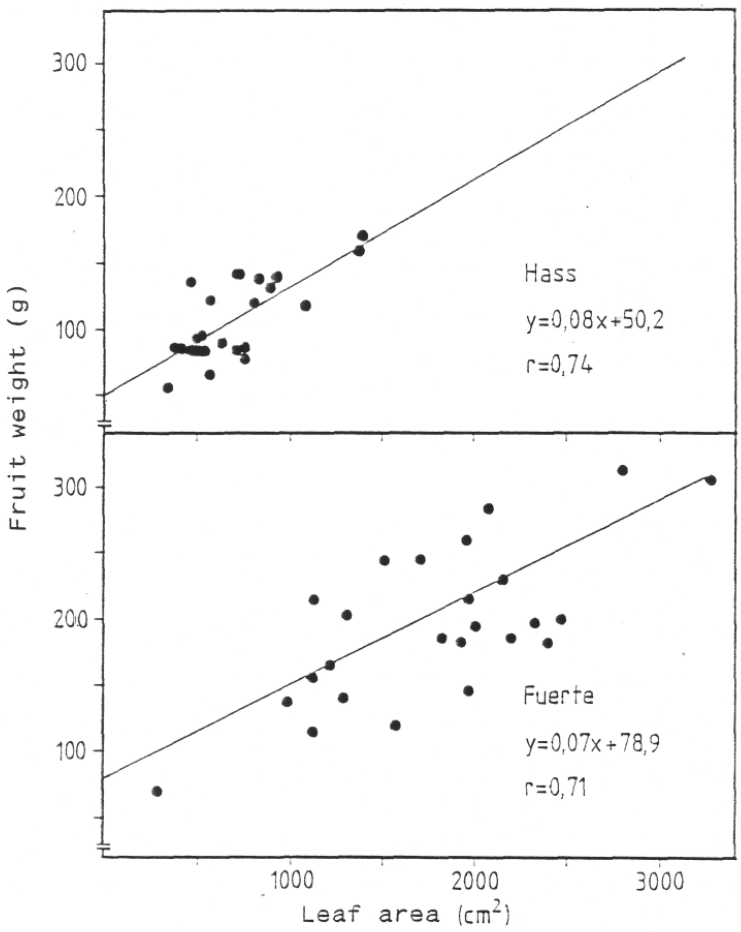


Figure 5. Relationship between fruit weight and leaf area for girdled 'Hass' and 'Fuerte' branches.

According to the equations in Figure 5 girdled 'Hass' branches require a leaf area of 2941 cm^2 to produce a 285.5 g fruit, as compared with 2951 cm^2 in 'Fuerte'. This works out at a leaf: fruit ratio of 51:1 for girdled branches of both cultivars.

Complete harvesting and defoliation of the six-year-old 'Hass' trees indicated a total fruit yield per tree of 52.8 kg and a total leaf area of 90.5 m^2 as compared with total fruit yield of 166.4 kg and total leaf area 347.1 m^2 for the ten-year-old 'Fuerte' trees, corresponding to a leaf fruit ratio of 84:1 in 'Hass' and 103:1 in 'Fuerte'.

3.2 Pruning

One month after pruning, regrowth was observed at the pruning sites. No regrowth occurred at the NAAm-treated wounds during the first three months after treatment. During the first five months after treatment the increase in yield capacity (YC) of the pruned trees (treatments: pruning and pruning+NAAm) was significantly lower than in the control; the treatment pruning+NAAm had the smallest increase in YC (Table 2). There were no statistically significant differences between treatments in fruiting density (FD) measured five months after treatment; the treatment pruning+NAAm had a tendency towards increased FD.

Nine months after treatment, YC was found to have increased for all treatments with the control having a higher increase than either pruning treatment. All treatments gave a lower fruiting density than that recorded at the preceding measurement. The FD of pruning+NAAm differed significantly from pruning and the control in terms of yield per tree, no significant differences were noted between the treatments, but pruning and pruning+NAAm tended to be higher than in the control.

Table 2. Yield capacity (YC), fruiting density (FD) and yield of 'Fuerte' trees as influenced by the treatments pruning and pruning + NAAm. Statistically confirmed differences identified by lowercase characters; error probability 5%

	5 months after treatment		9 months after treatment		
Treatment	Increase in YC (%)	FD	Increase in YC (%)	FD	kg/tree
Control	27.9 c	1.75 a	52.5 b	1.32 a	82.6 a
Pruning	15.7 b	1.61 a	41.7 a	1.34 a	91.6 a
Pruning + NAAm	5.8 a	2.17 a	32.4 a	1.77 b	99.7 a

3.3. Tests with plant growth regulators

3.3.1 Screening

The shoot growth and fruit retention observed during the screening of various plant growth regulators are set out in Table 3.

There were clear differences between the two cultivars 'Hass' and 'Fuerte'. Untreated 'Hass' shoots exhibited less growth and higher fruiting density than untreated 'Fuerte' shoots; this accords with multi-year comparisons between 'Hass' and 'Fuerte' yields at Westfalia Estate, The discussion of results that follows will concentrate on cv 'Fuerte', where yield increasing treatments would be especially profitable.

Table 3. Increase in shoot length and fruit set in cv. 'Hass' and 'Fuerte' following treatment with plant growth regulators, measured at the end of the vegetative period (E.85).

	Increase in shoot length (cm)		Fruit set/panicle	
	'Hass'	'Fuerte'	'Hass'	'Fuerte'
Alar	3.4	8.6	0.4	0
Alden	3.6	8.1	0.5	0.1
Atrinal	4.0	7.1	0.7	0.1
AVG	4.9	8.4	0.4	0.1
BAS 106..W	0.7	2.8	0.7	0.3
Berelex (GA_3)	7.4	12.6	0.5	0
Cultar (PBZ)	2.3	5.1	0.4	0.2
Cultar + Berelex (PBZ + GA_3)	6.2	10.2	0.6	0.2
Ethrel	1.2	7.6	0.3	0.1
Ethrel + Alar	3.9	7.9	0.5	0.1
MB 25-105	3.0	5.3	0.6	0.2
Off-Shoot-O	3.3	7.0	0.4	0.1
RSW 0411	3.5	8.2	0.6	0.3
Water (Control)	8.9	8.0	0.3	0.1

In 'Fuerte', treatments with BAS 106..W, PBZ, PBZ+GA_3, MB 25-105 and RSW 0411 increased fruit set as compared with the control. BAS 106..W, MB 25-105 and PBZ reduced shoot growth. Treatment with BAS 106..W and MB 25-105 led to phytotoxic manifestations (death of shoot tips, probably due to overdosing) while shoots treated with PBZ were characterized by shorter internodes and smaller, faintly striated dark green leaves.

3.3.2 Shoot growth

Over the three years of the study (E.85, E.86, E.87) shoot growth in 'Fuerte' occurred in two flushes from flowering (September) to early autumn (February), the spring flush and the summer flush. Especially the spring flush was influenced by the application of PBZ, GA_3 and PBZ+GA_3. Continuous shoot growth measurements in E.85 and E.86 (Figure 6) showed PBZ to reduce spring growth and GA_3 to increase it. In the PBZ+GA_3 combination PBZ failed to fully counteract the growth stimulating effect of GA_3. In E.87, the spring flush length measured were 28.4 cm (control) , 21.4 cm (PBZ), 32.3 cm (GA_3) and 27.5 cm (PBZ+GA_3).

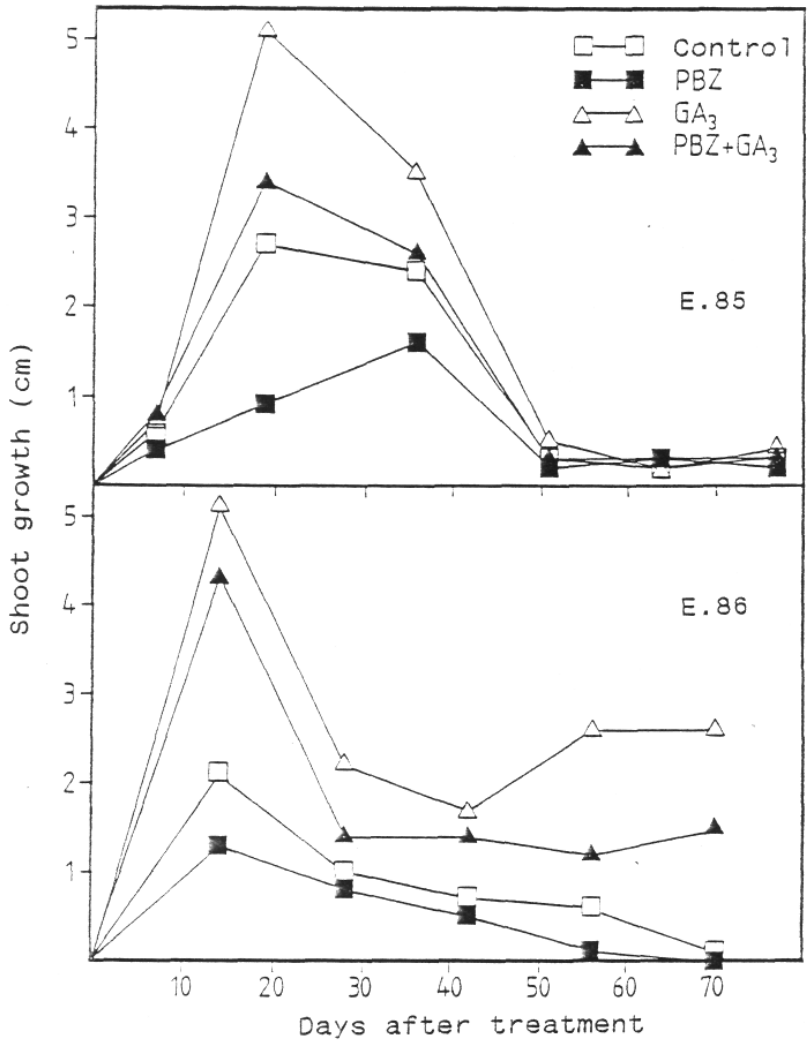


Figure 6. Spring flush growth in cv 'Fuerte' in E.85 and E.86.

Table 4. Increase in shoot length in cv. 'Fuerte' (E.85, E.86, E.87)

	Shoot length (cm)		
Treatment	E.85	E.86	E.87
Control	16.3	19.2	38.9
PBZ	13.4	18.2	30.7
GA_3	20.9	33.0	56.8
PBZ + GA_3	18.5	28.8	46.0

Shoot lengths measured after the end of the summer growth season are presented in Table 4. In all three test years PBZ was found to inhibit shoot growth as compared to the control, GA_3 increased it. In terms of shoot growth, the treatment PBZ+GA_3 was intermediate between the control and the GA_3 treatment.

3.3.3 Fruit retention

Figure 7 presents fruit retention data for 'Fuerte' avocado trees for E.85. Pronounced fruit drop was observed in the first 3-4 months after flowering. At the end of the observation period, the PBZ-treated and the PBZ+GA_3 treated shoots showed higher fruit retention than the control shoots. In the first three months after treatment, the fruit retention of the PBZ+GA_3 treated shoots was first lower, then higher than that of the PBZ-treated shoots. In the GA_3 treated shoots all the fruits had fallen by the end of the second month after treatment.

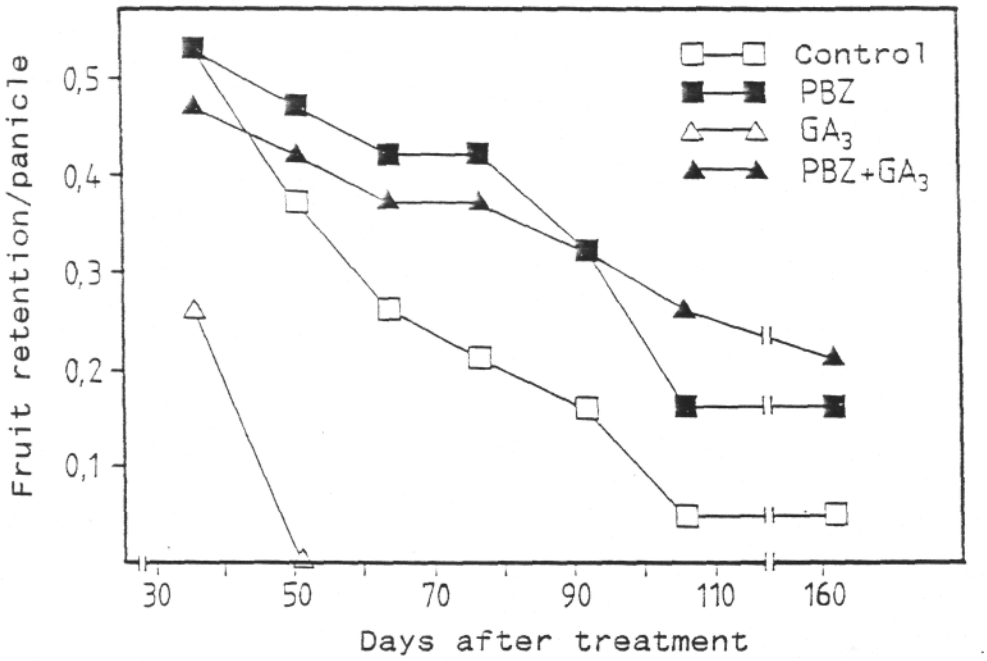


Figure 7. Fruit retention in cv 'Fuerte' (E.85).

Figure 8 shows the development of fruit retention in cv 'Fuerte' (E.86) during the first 70 days after treatment. Pronounced fruit drop was observed with all treatments during this period. During the first four weeks after treatment the PBZ group alone exhibited lower fruit drop than the control, while GA_3 produced the highest fruit retention followed by PBZ+GA_3. At the end of the observation period the PBZ+GA_3 treatment showed the highest fruit retention, followed in order by GA_3, PBZ and the control.

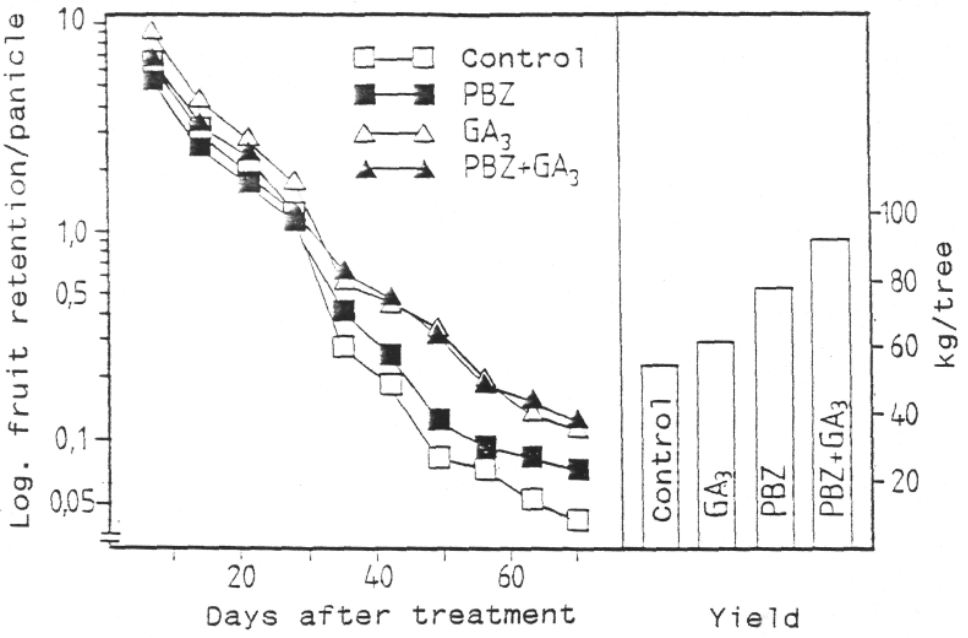


Figure 8. Fruit retention and yield of cv 'Fuerte' (E.86).

At harvest in E.86 the PBZ+GA_3-treated trees had the highest yield and the largest number of fruits, according with the last two fruit retention measurements (Figure 8). The second highest yield was obtained with PBZ, which however came in third place for fruit retention at 70 days after treatment. In yield and fruit number, PBZ+GA_3 and PBZ were followed by GA_3 which however was not significantly different from the control. The PBZ and PBZ+GA_3-treated trees had significantly higher yield and fruit number than the control, GA_3 gave significantly lower fruit weight than the other treatments.

After the high yields in E.86, the PBZ+GA_3 and the PBZ-treated trees flowered less strongly and slightly later than the control trees in E.87. The GA_3-treated trees showed the same tendency; the flowering intensity of the GA_3-treated trees being intermediate between that of the control (strong flowering) and the PBZ and PBZ+GA_3 treatment (both weakly flowering). This picture was confirmed by measurements of fruiting density and fruit diameter at nine weeks after treatment (fruiting density: control 4,7; PBZ 2.0; GA_3 2.9; PBZ+GA_3 2.1; fruit diameter: control 2.8 cm; PBZ 2.3 cm; GA_3 2.7 cm; PBZ+GA_3 2.5 cm).

In agreement with the high fruiting density at nine weeks after treatment, at harvest in E.87 the control trees had both the highest yield and the largest number of fruits per tree (Table 5). As in E.86 the second highest yield was from the PBZ-treated trees, followed by GA_3. The PBZ+GA_3-treated trees which had the highest yield in E.86, had the lowest in E.87. For yield and number of fruits per tree no significant differences were observed between the PBZ-, GA_3 and PBZ+GA_3-treated trees. Fruit weight was lower in E.87 than in E.86 for all treatments. As in E.86, the GA_3 group had the lowest fruit weight notwithstanding the significantly lower yield. The fruit weight of the PBZ and PBZ+GA_3 group was significantly higher than in the GA_3 and control groups.

The treatments did not differ significantly in cumulative yields of E.86 and E.87 (Table 5). Alternation was quantified by means of the alternation index (AI) (SCHUMACHER 1975). The treatment exhibiting the most marked alternation was the control. The trees showing the lowest AI, i.e. most uniform yield performance, were the PBZ+GA_3 treated trees; followed by the PBZ-treated trees. With regards to alternation, GA_3-treatad trees were intermediate between the control and the PBZ+GA_3 -treated trees.

Table 5. Yield of 'Fuerte' trees (E.86 and E.87). Statistically significant differences with 5% error probability designated by various lowercase characters.

	Yield (kg/tree)				Fruits/tree		Fruit weight (g)	
Treatment	E.86	E.87	E.86+E.87	AI	E.86	E.87	E.86	E.87
Control	54.4a	116.6b	170.9a	39.2a	174.2a	438.5b	312.2b	268.5a
PBZ	77.3b	83.5a	160.8a	26.7a	239.6bc	292.5a	326.9b	294.7b
GA_3	60.5a	76.5a	137.1a	31.6a	218.7ab	302.1a	281.7a	260.9a
PBZ+GA_3	91.7b	75.6a	167.2a	22.2a	290.7c	268.7a	317.5b	284.0b

Table 6. Extractable gibberellin levels in 'Fuerte' shoot tips (E.86) using various sample purification procedures and two detection procedures (lettuce hypocotyls test and radioimmunoassay).

		ng gibberellin/g of fresh weight		
		Purification A		Purification B
Treatment	Days after treatment	Lettuce hypocotyl	RIA	RIA
Control	2	1.8	2.4	5.0
PBZ	2	1.0	0.5	7.2
GA_3	2	>900.0*	>200.0*	2594.5
PBZ+GA_3	2	>800.0*	>200.0*	2038.0
Control	9	1.6	1.8	4.2
PBZ	9	0.7	1.0	2.7
GA_3	9	190.5	>200.0*	22.2
PBZ+GA_3	9	173.4	>200.0*	45.4
Control	19	1.6	3.0	-
PBZ	19	1.4	1.6	0.9
GA_3	19	93.0	100.0	24.6
PBZ+GA_3	19	26.1	100.0	14.1
Control	30	0.7	0.5	5.0
PBZ	30	-	0.2	1.8
GA_3	30	88.7	70.0	12.5
PBZ+GA_3	30	15.3	20.0	8.3

* Extrapolation

3.3.4 Hormone analyses

3.3.4.1 Extractable hormones

3.3.4.1.1 Gibberellins

In E.86 sample purification procedure A gave lower gibberellin levels in the control and PBZ treatment than did Procedure B (Table 6). In both purification procedures, gibberellin extraction was complicated by the high oil and phenol content of the avocado shoot tips. Samples taken from GA_3 and PBZ+GA_3-treated trees were not sufficiently diluted after sample purification procedure A. The gibberellin levels of these samples were accordingly estimated by extrapolation.

Irrespective of the purification and analysis procedures, the shoot tips of the PBZ treatment had distinctly lower gibberellin levels than untreated shoot tips (Table 6). Two days after treatment, the GA_3 and PBZ+GA_3-treated material showed very high gibberellin levels in the shoot tips. At 9, 19 and 30 days after treatment, the gibberellin levels of these two treatments were likewise distinctly higher than those in the control and the PBZ treatment, thereafter decreasing steadily up to the end of the experimental period. With one exception the PBZ+GA_3 treatment showed lower gibberellin levels than the GA_3 treatment.

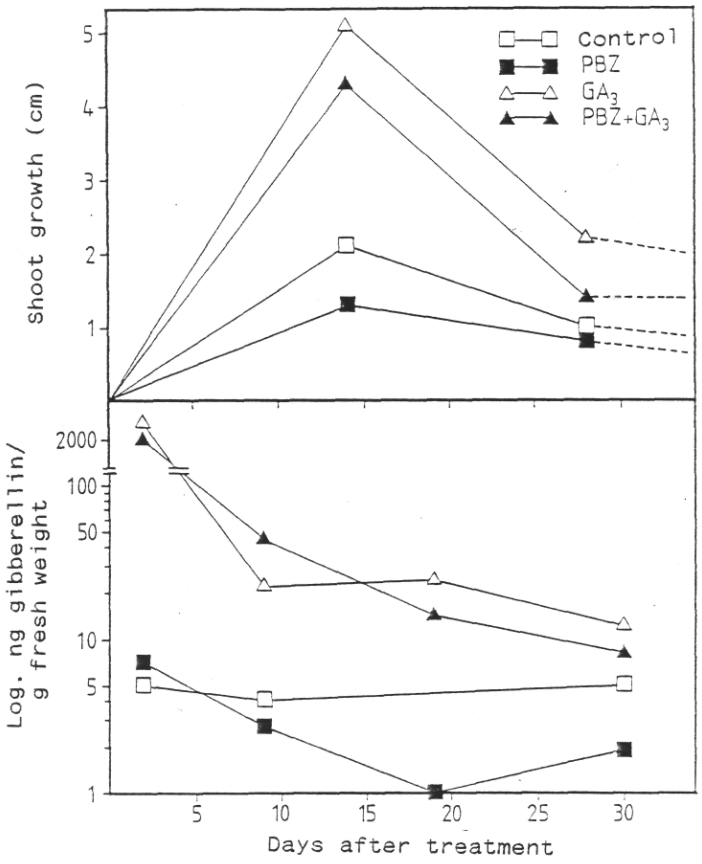


Figure 9. Spring flush growth and gibberellin levels in 'Fuerte' shoot tips (Purification Procedure B, RIA, E.86).

The gibberellin values for the shoot tips of the respective treatment largely corresponded to the spring flush growth (Figure 9). The PBZ treatment had both the lowest gibberellin levels and the least spring flush growth. The highest gibberellin levels and the strongest growth were observed after GA_3 treatment, and the PBZ+GA_3 treatment was intermediate between the GA_3 treatment and the control.

In E.87 the extractable gibberellin content in shoot tips of PBZ-treated trees was higher than that of the control two days after treatment, thereafter lower (Table 7). Six days after treatment, the shoot tips of GA_3 and PBZ+GA_3-treated trees had high gibberellin levels, with PBZ+GA_3 coming second in each case. Four weeks after treatment the PBZ treatment had a distinctly lower extractable gibberellin content than the control while GA_3 and PBZ+GA_3 had values barely distinguishable from the control. Comparison of the gibberellin concentration curves over the two years of the study reveals that although absolute gibberellin levels were lower in E.87 than in E.86 for all treatments, the curves are of similar shape.

Table 7. Extractable gibberellin levels in 'Fuerte' shoot tips (E.87) as determined by radioimmunoassay.

Treatment	Days after treatment	ng gibberellin/ g of fresh weight
Control	2	3.2
PBZ	2	5.5
GA_3	2	1368.0
PBZ+GA_3	2	480.5
Control	6	2.9
PBZ	6	2.9
GA_3	6	356.0
PBZ+GA_3	6	227.1
Control	28	4.3
PBZ	28	1.5
GA_3	28	4.8
PBZ+GA_3	28	4.0

3.3.4.1.2 IAA and ABA

In E.86 higher levels of extractable IAA were detected in all treatments at two days after treatment than in subsequent samplings (Table 8). Two days after treatment, the control had the highest level of extractable IAA, followed by GA_3, PBZ+GA_3 and PBZ. At all subsequent samplings, the IAA content was 4-5 times lower than at two days after treatment. Except for the second day after treatment, variation between treatments was minor.

In E.87 levels of extractable IAA showed smaller scatter than the E.86 figures. Relative to the control, all treatments tended towards higher IAA levels, with GA_3 initially producing the highest IAA level (Table 8).

As shown in Table 9, at two days after treatment in E.88 the highest level of extractable ABA was detected in the shoot tips of the control, the lowest in the GA_3 treatment. At 9, 19 and 30 days after treatment the PBZ treatment had the lowest level of ABA. In the

GA_3 treated group also, ABA was lower than in the control at 30 days after treatment while the PBZ+GA_3 group showed a marked increase in extractable ABA.

Table 8. Levels of extractable IAA in shoot tips of 'Fuerte' avocado trees (E.86 and E.87).

Days after treatment		ng IAA per g fresh weight			
		Control	PBZ	GA_3	PBZ+GA_3
E.86	2	81.1	54.7	66.7	61.5
	9	14.0	15.3	15.3	17.7
	19	-	-	16.3	10.7
	30	14.0	16.8	12.3	11.8
E.87	2	24.4	29.3	39.8	30.7
	6	23.7	26.9	29.9	31.9
	28	25.4	24.4	27.8	30.0

Table 9. Levels of extractable ABA in shoot tips of 'Fuerte' avocado trees (E.86 and E.87).

Days after treatment		ng ABA per g fresh weight			
		Control	PBZ	GA_3	PBZ+GA_3
E.86	2	3437	2277	1547	1673
	9	2063	1240	2288	1699
	19	-	899	1824	1404
	30	3256	2504	2634	4197
E.87	2	2721	1404	1304	1929
	6	1085	1840	1409	1012
	28	2219	2916	2428	2560

In E.87 at two days after treatment as in E.86 the highest level of extractable ABA was detected in shoot tips of the control while the GA_3 treatment again had the lowest ABA level. At two days after treatment, the PBZ and PBZ+GA_3 groups likewise had distinctly lower ABA levels than the control. At 6 and at 28 days after treatment, ABA levels in PBZ-treated shoot tips were higher than in the control and the other two treatments in contrast to the corresponding picture in E.86. Six days after treatment, the GA_3 group had slightly higher levels of extractable ABA than the control, the PBZ+GA_3 group marginally lower. At 28 days after treatment the control had the lowest level of extractable ABA.

3.3.4.1.3 Z/ZR and I-Ado/I-Ade

The levels of extractable cytokinins (Z/ZR and I-Ado/I-Ade) in PBZ-treated shoot tips were similar to those of the control, while the GA_3 and PBZ+GA_3 groups had higher levels at nearly all the sampling dates (Table 10).

Table 10. Levels of extractable Z/ZR and I-Ado/I-Ade in shoot tips of 'Fuerte' avocado trees (E.86).

Days after treatment		ng per g fresh weight			
		Control	PBZ	GA_3	PBZ+GA_3
Z/ZR	9	3.0	3.0	2.7	4.7
	19	2.0	-	2.4	3.6
	30	2.7	3.1	4.5	3.7
I-Ado/ I-Ade	9	1.9	1.7	2.1	2.1
	19	1.6	-	1.6	2.0
	30	1.8	2.2	3.9	1.7

3.3.4.2 Diffusible IAA and ABA

Due to thawing of samples as a consequence of a power failure, no diffusible IAA and ABA values are available for E.86. The E.87 figures for diffusible IAA in fruitlets after treatment with PBZ, GA_3 and PBZ+GA_3 are set out in Table 11. At two days after treatment, all the treated groups had lower diffusible IAA levels than the control. At six days after treatment, IAA was lower in the PBZ and GA_3 groups than in the control but higher in the PBZ+GA_3 group. At the last sampling date (28 days after treatment), levels of diffusible IAA were higher in the PBZ and PBZ+GA_3 that in the control, lower in the GA_3 group.

In E.87 at two days after treatment the PBZ+ GA_3 group had only half the diffusible ABA of the control; the PBZ and GA_3 treatments likewise had lower levels of diffusible ABA than the control (Table 11). At six days after treatment, all treatments had lower levels of diffusible ABA than at the preceding sampling date, with the control again having the highest figure. PBZ and PBZ+GA_3 had slightly lower levels of diffusible ABA than the control, the GA_3 group markedly reduced level. At the end of the experimental period all three treatments showed increased levels of diffusible ABA, with the control once again having the highest figure, followed in decreasing order by the GA_3 ,PBZ and PBZ+GA_3.

Table 11. Levels of diffusible IAA and ABA in 'Fuerte' fruit (E.87).

Days after treatment		ng per 25 agar blocks (=fruits)			
		Control	PBZ	GA_3	PBZ+GA_3
IAA	2	23.3	16.4	19.6	15.4
	6	17.8	9.9	13.8	19.0
	28	21.4	23.2	18.1	25.3
ABA	2	120	76	91	62
	6	62	58	34	55
	28	176	89	110	59

3.3.5 Mineral content

In all treatments in E.86 and E.87, the mesocarp potassium content of ready-to-pick

fruits was significantly lower than in the control (table 12), in E.86 the PBZ+GA_3 treatment and in E.87 the PBZ treatment had the lowest magnesium content, with the other treatments showing no statistically significant differences from the control. In E.86 the calcium content of the PBZ and PBZ+GA_3 treatments was significantly higher than in either the control or the GA_3 treatment. In E.87 there were no statistically significant differences in the calcium contents. In overall terms, potassium content was higher in E.86 than in E.87, calcium and magnesium contents, however, were lower in E.86 than in E.87.

Table 12. Potassium, magnesium and calcium levels in 'Fuerte' fruit (E.88 and E.87). Statistically validated differences, error probability 5%, designated by various lowercase characters.

Treatment		ppm in dry matter			
		K	Mg	Ca	(K+Mg)/Ca
E.86	Control	18500b	1093b	89a	231b
	PBZ	16073a	1107b	114b	156a
	GA_3	16333a	1141b	98a	183a
	PBZ+GA_3	15887a	1013a	115b	157a
E.86	Control	16987c	1281b	135a	137b
	PBZ	15273a	1103a	135a	123a
	GA_3	16167b	1258b	127a	139b
	PBZ+GA_3	16140b	1248b	134a	130ab

The (K+Mg)/Ca ratio was higher in E.86 than in E.87 in all the treatments. In both test years (E.86 and E.87) the PBZ treatment showed a significantly lower (K+Mg)/Ca ratio than the control, while the GA_3 and PBZ+GA_3 treatments differed significantly from the control only in E.86.

Table 13. Physiological disorders in 'Fuerte' fruit (E.86 and E.87). Statistically validated differences, error probability 5%, designated by various lowercase letters.

Treatment		Grey pulp	Pulp spot	Vascular browning
E.86	Control	0.42b	0.91b	0.21b
	PBZ	0.02a	0.41a	0.06a
	GA_3	0.08a	0.30a	0.08a
	PBZ+GA_3	0.05a	0.30a	0.08a
E.86	Control	0.30b	1.20d	0.18b
	PBZ	0.09a	0.54a	0.04a
	GA_3	0.27b	0.78b	0.06a
	PBZ+GA_3	0.15a	0.97c	0.09a

3.3.6 Fruit quality

At harvest in E.86 and E.87 the fruits of all treatments had a water content of 75%

corresponding to an oil content of 15% (HOLZAPFEL and KUSCHKE 1977). On attaining this oil content, Fuerte avocados are ripe for picking. Fruit quality appraisal revealed a lower incidence of physiological disorders in fruits after treatment with PBZ, GA_3 and PBZ+GA_3 (Table 13). In both years of the study (E.86 and E.87) a significantly lower incidence of grey pulp, pulp spot and vascular browning was observed in fruit treated with PBZ and PBZ+GA_3.

On average for the two years of the study, 75% of the control fruit showed signs of physiological disorders as compared with 50% in the GA_3 treatment. The best results against physiological disorders were obtained with PBZ, which reduced the disorder incidence by some 51%.

Table 14. Shape, pericarp weight and seed weight of 'Fuerte' fruit (E.86 and E.87). Statistically validated differences, error probability 5%, designated by various lowercase letters.

Treatment		Fruit length (cm)	Fruit diameter (cm)	L/D	Weight (g)	
					Pericarp	Seed
E.86	Control	11.4c	7.0b	1.6c	222.9a	43.0a
	PBZ	10.2a	7.0b	1.5a	229.4a	42.5a
	GA_3	11.5c	6.8a	1.7c	227.5a	41.2a
	PBZ+GA_3	11.0b	7.0b	1.6b	227.5a	38.8a
E.86	Control	12.1c	6.8a	1.8b	225.8a	40.0a
	PBZ	11.1a	7.0b	1.6a	225.1a	39.8a
	GA_3	12.6d	6.8a	1.9c	229.4a	38.4a
	PBZ+GA_3	11.4b	7.0b	1.6a	229.2a	40.4a

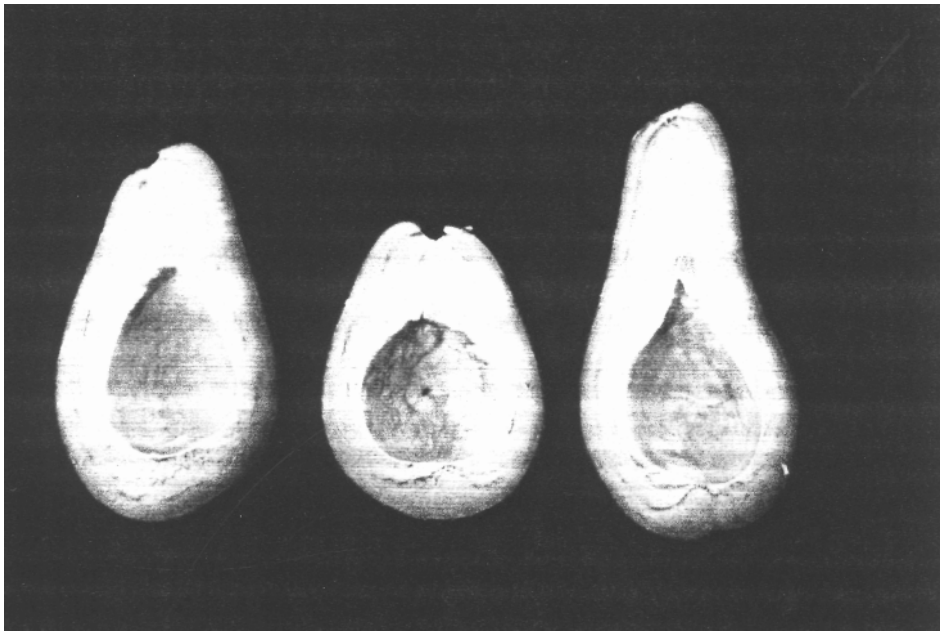


Figure 10. Shape changes in 'Fuerte; fruit. Left: control; centre: PBZ; right: GA_3

After removal from cold storage the fruit reached the soft-ripe stage within five to six days, with no differences emerging between the treatments. Treatment with PBZ, GA_3 and PBZ+GA_3 influenced fruit shape. In E.86 and E.87, fruit were shorter after PBZ and PBZ+GA_3 treatment when compared to the control, while fruit were longer after GA_3 treatment (Table 14). In both years of the study, the PBZ and PBZ+GA_3 treatments resulted in significantly larger fruit diameter than did the GA_3 treatment. In E.86 and E.87, the length/diameter ratios of fruits of the PBZ and PBZ+GA_3 groups were significantly smaller than those of the control and the GA_3 fruit. Fruits of the PBZ and PBZ+GA_3 groups were more compact than those of the control, while the GA_3 fruits tapered towards the peduncle (Figure 10). No seed or pericarp weight differences were observed between the treatments in either year of the study.

4. DISCUSSION

4.1 Assimilate production and partitioning

According to DAIE (1335;, assimilate partitioning within the plant is influenced by carbohydrate production in photosynthesizing organs (sources), phloem loading, transport and discharge into growing or storage organs (sinks). In apple, various sinks (growing shoot tips, developing fruit) are in competition for the available assimilates (PRIESTLEY 1987). In avocado, the young spring flush is in assimilate competition with the fruit until the new leaves begin to function as source organs after about four weeks (BUCHHOLZ 1986). Being almost completely dependent on the leaves for assimilate supply, the fruits are in assimilate competition with the new vegetative growth; this competition may adversely affect both fruit yield and fruit quality (BANGERTH 1983).

QUINLAN and PRESTON (1971) established in tests with $^{14}CO_2$ in apple that shoot tips have assimilate priority over early fruit growth, with only limited basipetal assimilate transport to the developing fruits. These authors successfully stimulated fruit set by removing new vegetative shoots and pinching the growing tips. At harvest, however, only the pinching treatment showed increased yield because the leaves of the originally competing growth subsequently contributed to fruit development.

While BYERS et al. (1985 attribute fruit drop to limitation of assimilate supply, GIFFORD and EVANS (1981) regard fruit sink capacity as the principal abscission determinant. This agrees with the observed existence of dominance effects in tomato and apple even when assimilate limitation can be ruled out as a causal factor in fruit drop (HO 1930, GRUBER and BANGERTH 1939). The sink capacity of fruits and hence assimilate partitioning, is significantly influenced by Phytohormones (BANGERTH 1334, DAIE 1985).Yield limiting factors and their identification are of vital interest in horticulture; in a given situation the question of whether yield would be increased by manipulating photosynthesis (source) or by manipulating sink capacity may be difficult to decide (MICHAEL and BERINGER 1980). This question is examined in the discussion below in the light of recent research into leaf/fruit ratio in avocado and with special reference to photosynthetic capacity as a possible fruit yield limiting factor.

4.1.1 Leaf: fruit ratio

The dependence of fruit weight on available leaf area is readily demonstrated in girdled branches (SILBEREISEN 1974, SNELGAR et al. 1986) since the branch distal to the girdle constitutes a closed system (SILBEREISEN and BUCHLOH 1970). In agreement with this finding the present study showed that a correlation between leaf area and fruit weight exists also in avocado; the below-average fruit weight is presumably a result of the limited assimilating area (Figure 5).

Girdled branches accumulate carbohydrates (WEAVER and McCUNE 1359) and gibberellin-like substances (SACHS and WEAVER 1968, GOREN et al, 1371) above the girdling cut, influencing yield characteristics. Girdled avocado branches give a higher yield than ungirdled branches in the girdling year but a lower yield in the following year (LAHAV et al. 1971), presumably indicating a negative influence of high fruit retention on flower bud formation. According to HOAD (1978), gibberellins

produced in the seed inhibit flower bud differentiation.

In most apple and pear cultivars good fruit development and adequate flower bud formation for the following year is regarded as a certainty if 15-30 leaves are present per fruit immediately after the June drop (SCHUMACHER 1975). In the present study, the leaf: fruit ratio of high-yielding Fuerte trees averaged 103:1. However, in view of the differences in size, lifetime and photosynthetic capacity of the leaves as well as in energy content and weight of fruit, these avocado findings can not be compared directly with the apple data.

With an average size of 58cm^2, avocado leaves are three to four times larger than apple leaves, Golden Delicious leaves having an area of 10-20cm^2 for example (PALMER 1987). SCHOLEFIELD et al. (1980) give the maximum photosynthetic capacity of avocado leaves as 0.30 mg CO_2 m^2s^{-1} , as compared to 0.97 mg CO_2 $m^{-2}s^{-1}$ in apple (AVERY 1977). However, it is reasonable to suppose that the comparatively low photosynthetic capacity of the avocado leaf is compensated for by its year-around photosynthetic activity (MONSELISE 1986). According to WOLSTENHOLME (1986), the energy content of avocado fruits with 17% oil content is 807.2 kJ 100 g^{-1}, three times that of the apple (262.8 kJ 100g^{-1}). In view of the high energy consent of the avocado fruit and its heavier weight in comparison with the apple, an avocado leaf: fruit ratio of around 100:1 may be regarded as equivalent to an apple leaf: fruit ratio of 15-20:1.

The yield obtained from the defoliated avocado trees in the present study was equivalent to 20 t per hectare, which is considered a good harvest. However, the writer's own observations indicate that the vigorous vegetative growth of the avocado and thereby rapid increase in leaf number, accompanied by marked fruit drop, may lead to leaf:fruit ratios well in excess of 100:1. Under-average fruit size due to assimilate limitation in the girdled branches was observed only after drastic reduction of the leaf:fruit ratio to below 50:1 (compare Figure 5).

Fruits influence both photosynthetic rate (AVERY 1977) and assimilate partitioning. FINAZZO and DAVENPORT (1986) found levels of radioactivity 300% higher in ^{14}C-labelled avocado leaves in the immediate vicinity of inflorescences containing only one fruitlet than in similarly labelled leaves in the immediate vicinity of inflorescences with several fruitlets; fruit drop was accompanied by a clear reduction in ^{14}C export from the marked leaves. Leaf assimilate production appears normally to proceed at a rate below the maximum possible rate, leaving a margin of reserve capacity available in case of necessity (MAGGS 1964 PRIESTLEY 1987). It is therefore unlikely that avocado yields (average < 10 t/hectare) are limited by photosynthetic capacity.

4.1.2 Fruit development, fruit drop and phytohormones

As regards fruit, type the avocado belongs to the single-seed berry category (FRANKE and GROSS 1984). The fruit growth curve is sigmoidal in form, in avocado, in contrast with many other fruits, mesocarp cell division continues as long as the fruit remains on the tree. For this reason avocado fruit growth up to the time of harvesting, involves cell division in addition to cell elongation (SCHROEDER 1953).

According to ROBERTSON (1969) the sigmoidal growth curve of the avocado fruit can be subdivided into three stages. The first stage lasts about 70 days during which growth is slow and well in excess of 90% of the fruit drop. The second stage is the longest, lasting 137 days marked by very rapid fruit growth and accounting for 80% of overall fruit growth. During the third stage at the beginning of which the seed reaches maturity, there is only a minor increase in fruit weight. This stage is also characterized by rapid oil accumulation in the mesocarp (BLUMENFELD and GAZIT 1974), and the avocado attains picking maturity.

In agreement with the findings of ROBERTSON (1969), the present study reports very marked fruit drop in Fuerte avocado trees during the first phase of fruit development (Figure 3). Similar observations are recorded by SLABBERT (1981), DAVENPORT (1982) and SEDGLEY (1987). Whether a fruitlet abscises or develops to ripeness appears to be decided very early in the first stage of fruit development.

Anatomical investigations of abscissed avocado fruitlets have revealed the majority of fruitlets dropped during the first few weeks after flowering to be unfertilized (SEDGLEY 1980). However, all fruits dropped from one month after flowering onwards were fertilized and showed normal embryo and endosperm development. These observations rule out fertilization defects as the causal factor for the high fruit drop rate.

Explanation in terms of high fruit energy content and consequent heavy assimilate demand is likewise unsatisfactory in view of the fact that 96% of the fruit abscise before the mesocarp has attained an oil content of 1% (ADATO and GAZIT 1977). Rapid oil accumulation in the mesocarp does not occur until five months after flowering (BLUMENFELD and GAZIT 1972). Clearly, the explanation of high fruit drop must be sought elsewhere.

Many growth and development processes in fruit are governed by phytohormones (GOODWIN 1978b), which also play an important regulatory role in fruit abscission (LUCKWILL 1948, 1953). Abscission appears to be triggered by changes in the hormone balance in the abscission layer, with the IAA/ethylene ratio having a decisive influence (JACKSON and OSBORNE 1972). In some plants (Lupinus, Gossypium) premature fruit drop is correlated with an increase in ABA, in others not (for example, Begonia) (DÖRFFLING 1982). Gibberellins and cytokinins have less direct influence on abscission layer formation but influence abscission indirectly by mobilizing nutrients and promoting sink activity (ADDICOTT 1982).

Determinations of endogenous phytohormones in avocado fruit have revealed high levels of auxin (GAZIT and BLUMENFELD 1972), gibberellin-like substances (BLUMENFELD and GAZIT 1972), cytokinin (BLUMENFELD and GAZIT 1970), ethylene (ADATO and GAZIT 1977) and ABA (CUTTING et al. 1986) in the seed coat. Shrinking and drying out of the seed coat coincides with fruitlet abscission during early fruitlet development as well as with fruit maturity at the end of summer. Drying of the seed coat marks the end of the influence of the seed on fruit development and leads to the cessation of fruit growth (BLUMENFELD and GAZIT 1974). Whereas in young avocado fruits much ethylene is produced in the seed coat and little in the pericarp, in older fruits ethylene production decreases abruptly with the drying of the seed coat leaving the pericarp as the sole site of ethylene synthesis (BLUMENFELD and GAZIT

1971). Marked dropping of young fruits is connected with high ethylene production in the seed coat (ADATO and GAZIT 1977, DAVENPORT 1982, SITRIT et al. 1987). The markedly increased ABA levels present in abscissed avocado fruit lead ADATO and GAZIT (1977) to postulate an important role for this hormone in fruit drop.

Abscission-promoting ethylene production in the abscission layer appears to be triggered by certain stimuli. Likely stimuli are falling IAA, rising ABA or falling IAA/ABA ratio in the abscission layer (ADDICOTT 1982). Research on apple trees points to a key role not for ethylene but for diffusible IAA as the correlative stimulus in fruit drop (BANGERTH 1986). Dominant apple and tomato have been shown to contain higher diffusible IAA levels than fruit with higher abscission potential (GRUBER and BANGERTH 1989).

In apple (GRUBER and BANGERTH 1989) and in avocado, the level of diffusible IAA is of the order of one ng per fruit shortly after flowering. Subsequently, the level of diffusible IAA increases to a maximum at four weeks after flowering in apple but remains approximately constant in avocado (Table 11). In apple, levels of diffusible ABA are generally lower than those of diffusible IAA (GRUBER 1985). In avocado, diffusible ABA levels are an order of magnitude higher than diffusible IAA levels (Table 11). Whereas in apple marked fruit drop occurs at four weeks after flowering (June drop) with mainly less-developed lateral fruits showing low IAA export being abscissed (GRUBER 1985), in avocado fruit drop continues throughout the first 70 days of fruit development (ROBERTSON 1969). In more than 90% of the panicles, all fruit abscise (Figure 8). In avocado, no differences in abscission tendency are observed between fruits at different stages of development but from the same panicle (DAVENPORT and MANNERS 1982).

Bearing in mind that the spring flush in avocado chronologically precedes fruiting, the spring flush may following BANGERTH (1989) be regarded as the dominant organ, inhibiting fruit development. According to this hypothesis, the developmental leap of the spring flush would lead to a self-reinforcing IAA export from the shoot tips; this IAA on encountering that produced by the subsequently developing fruits inhibits the latter. CUTTING and BOWER (1939) have found the IAA export of the avocado spring flush to exceed that of the flowers and young fruits. This finding, together with the very low IAA transport rates of young avocado fruits found in this study (Table 11) supports the spring flush dominance hypothesis.

Fruit trees in which the influence of dominant shoot tips has been reduced by pruning or the use of growth regulators exhibit improved initial fruit set and reduced fruit abscission (QUINLAN and PRESTON 1971, VARGA 1971, ZILKAH et al. 1987). Measures reducing spring flush dominance in avocado may accordingly be expected to result in improved fruit set and improved assimilate and calcium allocation to the developing fruits.

4.2 Influencing of shoot growth and yield

4.2.1 Pruning

Following on research by QUINLAN and PRESTON (1971) on apple trees, ZILKAH et

al. (1987) removed spring flush from avocado trees and observed increased fruit set shortly after flowering. These authors also used ^{15}N urea in their investigations but found no increase in nitrogen supply to the fruit on pruned branches. ZILKAH et al. (1987) attribute the improved fruit set to improved carbohydrate allocation and a more favourable hormone balance, GRUBER and BANGERTH (1989) have demonstrated increased IAA export from fruits in response to removal of nearby dominant or competing shoot tips,

In contrast to the above-mentioned avocado study by ZILKAH et al. (1987), confined to the period immediately after flowering, the present study recorded fruit retention at increasing intervals up to the time of harvest. Pruning did not result in higher fruit retention; pruning followed by application of NAAm had a tendency towards higher fruiting density. However, neither treatment produced a significantly higher yield than the control (Table 2). A possibly slightly reduced fruit drop rate immediately after pruning was soon compensated by renewed shoot growth and attendant fruit drop, whereas the trees with NAAm-treated pruning wounds showed the smallest yield capacity increase and the highest fruit retention (Table 2).

As the pruning measures tested in the present study are very labour intensive particularly in large avocado trees, they are impracticable. The same applies to repeated pinching-off of vigorous new growth and wound treatment with NAAm.

4.2.2 Plant growth regulators

Costs of manual removal of shoot tips even from apple or pear trees, slower growing than the avocado, are high. This has led to the concept of "chemical pruning" (VARGA 1971, JACKSON et al. 1978). These experiments showed that growth-inhibiting substances are to be preferred to shoot-killing preparations detrimental to fruit quality. The application of growth inhibiting plant growth regulators lead to results comparable to those obtained by pinching as regards yield and fruit quality (JACKSON et al. 1978)

Attempts to increase fruit set or reduce fruit drop in avocado by means of growth regulators have hitherto proved unsuccessful (MONSELISE 1982). However, screening of various plant growth regulators in the course of the present study has revealed that treatment of Fuerte avocado trees with certain recently developed gibberellin synthesis inhibitors (PBZ, RSW 0411, BAS 106..W) increases fruit set without a high degree of growth suppression (Table 3). Application of Alar and Ethrel, widely used in the cultivation of pome fruits (SCHUMACHER 1975), was without effect; Alden, Atrinal and Off-Shoot-O, growth inhibitors used in the cultivation of ornamentals (THOMSON 1983) were likewise unsuccessful. Also without effect was AVG, an ethylene synthesis inhibitor used to reduce fruit drop in apples (WILLIAMS 1980). Application of GA_3 over the three years of the present study resulted in substantially increased shoot growth (Table 3 and Figure 6) and resulted in a small yield increase in the first year of whole-tree application (Table 5). In the first year of application, both to branches and to whole trees, application of PBZ+GA_3 proved more successful in improving fruit set than application of PBZ or GA_3 alone (Figures 7 and 8). Similar results are reported by VARGA (1971) for pear trees using a combination of Alar and GA_{3+7} rather than Alar or GA_{3+7} alone.

In the quest for new growth regulators, two new types of compounds were discovered around 1980: triazole derivatives, e.g. PBZ and RSW 0411, and norbornenodiacetin derivatives, for example BAS 106..W. Both these groups of substances influence the gibberellin biosynthesis (NOGUCHI 1987). PBZ was developed primarily for intensive cultivation use and registered as early as 1984 in Great Britain for use in apple and pear cultivation (AMCNYMOUS 1984); RWS 0411 was developed for use of graminacecus crops, rape and leguminous crops (HACK et al. 1985); BAS 106..W is used as a growth inhibitor on rice (SCHOTT et al. 1984). In view of its ready availability and high probability of registration for use in avocado, PBZ was used as the sole growth inhibitor for the purposes of the present study.

GUINLAN and RICHARDSON (1984) sprayed PBZ on apple trees and report the necessity of thorough wetting of shoot tips and young shoots if effective growth inhibition is to be achieved. Application of PBZ to leaves and woody parts results in only insignificant growth inhibition. Being phloem-immobile and almost exclusively xylem-transported, PBZ accumulates in the leaves, with only limited amounts reaching the meristematic areas of cell division and expansion, the sites of its growth inhibitory action (DALZIEL and LAWRENCE 1984). In the present study, avocado trees were sprayed with PBZ at the start of spring flush growth (Figure 1, Stage 5). Reduced shoot growth was observed only a few days after application, with the length of the spring flush being reduced by approximately 50% (Figure 6). During the summer growing season, vigorous shoot growth resumed in the PBZ-treated trees, resulting in less pronounced flush length differences at the end of the summer growth period (Table 4). Pronounced growth inhibition shortly after application of PBZ followed by reduced subsequent action has been reported by various authors (WEBSTER and QUINLAN 1984, BAUSHER and YELENOSKY 1986).

Besides reduced internode length (ARON et al, 1985, MANTINGER et al 1988) the morphological effects of treatment with PBZ also observed in avocado, include the formation of small, often "wrinkled" dark green leaves (WAMPLE and CULVER 1983, STINCHCOMBE et al. 1984). WOOD (1984a) attributed the "wrinkling" of the leaves of pecan seedlings to a midrib inhibition. The dark green colouration of the treated leaves reflects a high chlorophyll content per cm^2 leaf area; this positive effect is however balanced by the smaller leaf size, rendering the treated leaves indistinguishable from the control as regards photosynthetic performance (DALZIEL and LAWRENCE 1984).

In addition to the above mentioned morphological effects, PBZ tests on apple seedlings grown in nutrient solution reveal far-reaching physiological and biochemical changes (STEFFENS et al. 1983, WANG et al. 1985). High carbohydrate levels were detected in all parts of the seedlings, indicating changes in assimilate allocation accompanying the PBZ-induced changes in growth.

SHEARING et al. (1986) attribute the frequently observed yield increases following PBZ treatment to changes in assimilate partitioning. Yield increases after application of PBZ have been reported in, among other crops, apples (TUKEY 1983, WILLIAMS 1984), pears (RÁESE and BURTS 1983, JAUMIEN et al. 1986), cherries (WEBSTER et al. 1986), peaches (MARTIN et al. 1987) and citrus (DELGADO et al. 1986).

In the present study, significant yield increases were achieved in avocado trees in the

first year of PBZ application (E.86). From approximately one month after treatment onwards the PBZ treated trees showed less fruit drop than the control. Since in E.86 no differences in flowering and fruit set were observed between trees of the different treatments before and at a few days after PBZ treatment, the increased yield of the PBZ treated trees must be attributed to its lower fruit drop rate in comparison with the control, After harvest the PBZ treated trees in E.87 flowered less strongly than the control. However, the number of flowers was sufficient to produce as good a yield as in the preceding year under the influence of a renewed PBZ treatment which reduced fruit drop. While the control yields showed marked alternation over the two years of the study, the PBZ treated trees showed steadier yield performance but no cumulative yield increase (Table 5).

According to DALZIEL and LAWRENCE (1984) PBZ inhibits gibberellin biosynthesis by blocking the oxidation of kaurene to kaurenoic acid. In the present study distinctly depressed gibberellin levels were detected in avocado shoot extracts as from about one week after PBZ treatment. At four weeks after treatment, at the end of the test period, the gibberellin levels in the PBZ treated shoots were still lower than in the control. The PBZ treatment had both the lowest gibberellin content and the least increase in shoot growth (Figure 9). BUCHENAUER et al. (1984) likewise reported retarded growth and reduced levels of glbberellin-like substances in barley shoot extracts after treatment with PBZ.

Triazole derivatives such as PBZ influence oxidative demethylation reactions in the synthesis of gibberellin and sterol. These reactions are catalysed by the microsomal cytochrome P 450 which interacts with triazole derivatives, inhibiting the demethylation of gibberellin and sterol precursors. The growth retarding influence of triazole derivatives is therefore attributable to interference with phytosterol synthesis as well as to inhibition of gibberellin synthesis (BUCHENAUER et al. 1984). WANG et al. (1988) report significantly lowered levels of β-sitosterol and campesterol which have a weak auxin activity or increase the effect of IAA (DÖRFFLING 1982), in PBZ treated apple seedlings.

The biosynthesis of other substances important for plant growth is also dependent on catalysis by cytochrome P 450. GROSSMANN et al. (1987) report lowered gibberellin and ABA levels as well as an influence on cytokinins in triazole treated soya seedlings. The leaves of PBZ treated apple seedlings were found to contain approximately one third less ABA (WANG et al. 1987) in addition to reduced ethylene synthesis (WANG and STEFFENS 1985). In the present study, equally marked reductions in extractable ABA in avocado shoot tips were found shortly after application of PBZ (Table 9). No clearly defined changes in extractable cytokinins and IAA were detected following PBZ treatment (Tables 8 and 10).

In the PBZ treated group, young avocado fruits showed first lower, then at four weeks after treatment slightly higher levels of diffusible IAA than the control; over the experimental period the diffusible IAA levels were lying below those of the control (Table 11). The PBZ treated group showed a higher IAA/ABA ratio than the control, probably reducing the fruit drop rate (see paragraph 4.1.2). BUBAN and NAGY (1985) report sharply reduced diffusible IAA levels in shoot tips after application of PBZ to apple trees. Such a spring flush dominance reducing effect may also have occurred in the PBZ

treated avocado trees, favouring fruit development.

In many species of fruit trees application of gibberellins results in improved fruit set (KREZDORN 1969, MOSS 1972, MONSELIsE 1979, SINGH and LAL 1980, HARTMANN 1984) and in some cases also to increased yield (SOOST and BURNETT 1961, MODLIBOWSKA and WICKENDEN 1977). Gibberellins can be used to reduce flowering and to increase vegetative growth (DENNIS 1973, HARTMANN 1984,-EL-MAHDY 1988).

Gibberellins produce increased growth of the shoot axis by stimulating mitotic activity in the subapical meristem area (DÖRFFLING 1982). In the present study, the application of 500 ppm of GA_3 greatly stimulated shoot growth in avocado trees in all tests (Tables 3 and 4; Figure 6). RAVIV et al. (1987) repeatedly sprayed avocado seedlings with 100 ppm GA_3 and likewise report increased shoot growth. Since the application of gibberellins results in increased growth of the shoot axis, levels of endogenous gibberellins may be a growth limiting factor (GOODWIN 1978a).

Based on analyses of young fruits after application of ^{14}C-GA_3 to pear, plum and cherry trees, KNIGHT and WEBSTER (1988) state that applied GA_3 is rapidly absorbed by active sinks and thereafter remains in situ. GARCIA-MARTINEZ and GARCIA-PAPI (1979) report increased sink activity of citrus fruit and reduced fruit drop after treatment with GA_3. In the present study, GA_3 treatment of avocado also reduced the fruit drop rate; this positive influence, still evident 2-3 months after treatment, decreased until Harvest (Figure 9). In the first year of application (E.86), the yield of the GA_3 treated trees was slightly higher than that of the control. Repeated application of GA_3 in the following year (E.87) resulted in increased yield relative to the preceding year, though significantly lower than in the strongly alternating control (Table 5).

Unlike PBZ, application of GA_3 to avocado trees shortly after full flowering had no effect on flowering intensity in the following year (see paragraph 3.3.3). In the apple, in contrast, application of GA_3 resulted in decreased floral initiation, while treatment with growth retardants favoured flowering (GROCHOWSKA et al. 1984, EL-MAHDY 1988). In apple, flower bud formation occurs during early fruit development and is presumably inhibited by gibberellins produced in the seeds of the young fruits (LUCKWILL 1970, MONSELISE and GOLDSCHMIDT 1982, EBERT and BANGERTH 1985). In avocado, however, floral induction does not occur until autumn after cessation of summer growth (DAVENPORT 1986); in the succeeding year flowering is exclusively from buds not yet in existence during early fruit development. It is probably for this reason that even heavy application of GA_3 to avocado trees shortly after flowering does not induce alternation but actually appears to have a yield stabilising effect (Table 5). The present study has established that increased growth of the spring flush does not result in marked fruit drop and low yields provided that fruit sink capacity can be increased, for example by application of GA_3.

Avocado shoot tips treated with GA_3 in two successive years (E.86 and E.87) showed extremely high levels of extractable gibberellins (Tables 6 and 7). As expected, extremely high gibberellin levels were recorded for the first sampling date but the possibility of residual GA_3 on the leaf surfaces being recorded could not be excluded. Approximately one month after application, the gibberellin content of the GA_3 treated

shoots approached that of the control (Figure 9). The increased IAA levels and reduced ABA levels in avocado shoot tips after GA_3 treatment (Tables 8 and 9) agree with EL-MAHDY's (1988) results with GA_3 treated apple shoots. In addition to gibberellin, the increased shoot growth may be attributable to the raised IAA levels or depressed ABA levels after the GA_3 treatment (LUCKWILL 1970). While the cytokinin content of avocado shoot tips was increased by GA_3 treatment (Table 10), GROCHOWSKA et al. (1984) and EL-MAHDY (1988) report depressed cytokinin levels in apple shoots after GA_3 treatment.

In citrus (GARCIA-MARTINEZ and GARCIA-PAPI 1979) and in apple (GRUBER 1985), GA_3 treatment resulted in raised IAA levels in diffusates from young fruits. In the present study, in contrast, application of GA_3 resulted in a reduction in diffusible IAA in young avocado fruits (Table 11); this test was however feasible in only one of the years of the study (E.87), in which the strongly alternating control trees showed very high fruit retention. The slightly delayed flower and fruit development of the treated trees may have resulted in a subsequent increase in diffusible IAA. The GA_3 treatment reduced the level of diffusible ABA (Table 11). Like the PBZ treated group, the GA_3 treated group showed a higher IAA/ABA ratio than the control group, presumably accounting for the reduced fruit drop (see paragraph 4.1.2)

A number of authors report that the growth inhibition of the shoot axis caused by PBZ treatment can be neutralised by treatment with gibberellins (GREENE and MURRAY 1983, WAMPLE and CULVER 1983). In agreement with the findings of these authors, no growth inhibition was observed in the present study after application of PBZ+GA_3. The GA_3 concentration selected for this combined treatment was higher than would have been required to balance the PBZ induced growth inhibition, with the result that the shoot growth of the PBZ+GA_3 group exceeded that of the control (Tables 3 and 4*;* Figure 6). Similar results were obtained by BALAMANI and POOVAIAH (1985) investigating the effect of PBZ and GA_3 on shoot growth in potato.

In avocado, the PBZ+GA_3 treated group showed reduced fruit drop and more uniform yield performance than the other two treatment groups and the control (Table 5; Figures 7 and 8). In the pear, simultaneous application of Alar and gibberellin (VARGA 1971) and PBZ and gibberellin (KNIGHT and BROWNING 1986) likewise favourably influenced cropping.

The influence of treatments with PBZ and GA_3 on endogenous hormone levels in the avocado has been discussed in the foregoing paragraphs. After PBZ+GA_3 treatment, levels of extractable gibberellins in shoot tips followed a similar curve to that observed in the GA_3 group, the values recorded during both test years on nearly all of the sampling dates were, however, distinctly lower than in the GA_3 group (Tables 6 and 7). The difference in the levels of extractable gibberellins between the GA_3 and the PBZ+GA_3 groups is higher than the difference between the PBZ group and the control. This in an unexpected finding in view of the fact that gibberellin biosynthesis is not stimulated by exogenous GA_3 application, and assuming that PBZ does not influence gibberellins directly but inhibits their biosynthesis (LEVER 1988).

In both years of the study, shoot tips treated with PBZ+GA_3 showed the same IAA and cytokinin levels as the GA_3 treated group (Tables 8 and 10). The extractable ABA levels

of the PBZ+GA_3 treated group were not the same as those of either the PBZ or the GA_3 treated group and did not appear to follow a consistent pattern (Table 9). Striking similarities in diffusible ABA were however observed in the PBZ+GA_3 and PBZ fruits; in terms of diffusible IAA likewise the PBZ+GA_3 and PBZ groups were very similar (Table 11).

SCHOLEFIELD et al. (1985) postulate that spring flush reduction could lead to more regular and higher avocado yields by increasing assimilate availability for reproductive development. While the PBZ results obtained in the present study appear to be compatible with this hypothesis, the same cannot be said of the GA_3 and PBZ-GA_3 results. In the GA_3 and PBZ+GA_3 groups improved assimilate availability to the fruits due to growth inhibition of the spring flush must be excluded as the cause of the reduced fruit drop since spring flush growth was more vigorous than in the control. Assuming that the yield increasing influence of PBZ is exclusively to spring flush growth reduction, application of PBZ-GA_3 might be expected to produce no yield increase relative to the GA_3 group. In this study, however, it was shown repeatedly that fruit drop was more reduced after PBZ+GA_3 treatment than after GA_3 treatment: the fruit drop reducing influence of PBZ must therefore be cue to a mechanism other than inhibition of spring flush growth. Possibly the IAA export from avocado shoot tips is reduced, analogous to that reported by SUBAN and NAGY (1985) in apple trees, and which would reduce spring flush dominance and attendant fruit drop.

4.3 Fruit quality

The calcium content of the Fuerte avocado fruits investigated in the present study is of the order of 110 ppm calcium in dry matter and within the range cited by BOWER (1985). GAILLARD (1987) cites higher values: Ca 530 ppm, Mg 1365 ppm, K 22640 ppm (In dry matter; compare Table 12).

In many fruits, including the avocado, the occurrence of physiological disorders is associated with a localized inadequacy of calcium (SHEAR 1975, BANGERTH 1979, VELDMAN 1983). The calcium content of the fruit plays a vital role in maintaining fruit quality after harvest (BRAMLAGE et al. 1980). Bitter pit in apple, an intensively studied physiological disorder, is known to be closely related to the calcium content and more especially the Ca/K and Ca/Mg ratios in the fruit (SCHUMACHER 1975). In agreement with the findings in apple, this study showed that avocados with low (K+Mg/Ca) ratio exhibit a lower incidence of physiological disorders.

The various browning effects observed in avocado fruits (pulp spot, grey pulp, vascular browning) are caused by increased membrane permeability leading to leakage of phenols out. of the vacuoles into the cytoplasm and their oxidation in the cytoplasm by polyphenoloxidases (BOWER 1988). In this regard the membrane stabilizing role of calcium is of fundamental importance. Membrane stabilization is dependent on calcium binding to certain membrane constituents; calcium under-saturation of these sites substantially increases membrane permeability. Depending on their concentration, other cations can displace calcium from the membrane. Potassium, magnesium and hydrogen are potentially antagonistic to calcium and may markedly increase membrane permeability (BANGERTH 1979).

The influence of application of PBZ and GA_3 on the incidence of physiological disorders has been investigated for a number of fruit species. PBZ in most cases reduces the incidence of physiological disorders (GREENE 1986, ELFVING et al. 1987, WANG and STEFFEMS 1987, MANTINGER et al. 1988), while gibberellins may be either beneficial (MARCELLE and CLUSTERS 1978, PAULSON et al. 1979) or unfavourable (LEH 1963, BANGERTH 1973).

In both years of the present study the PBZ results differed from those observed in the control in having significantly lower (K+Mg)/Ca ratios and appreciably lower incidence of pulp spot, grey pulp and vascular browning (Table 13). Significantly raised calcium levels in the fruits of the PBZ group were observed in only one year of the study (E.86) while potassium was significantly lower in both E.36 and E.87. In contrast, treatment with GA_3 which also reduced fruit potassium levels in both years of the study, had no statistically significant influence on fruit calcium content and produced a lower (K+Mg)/Ca ratio than the control in only one year of the study (E.86). The fruit of the GA_3 treated trees had a lower incidence of physiological disorders than those of the control trees; however, GA_3 was less effective than PBZ in reducing mesocarp discoloration. While both PBZ and GA_3 reduced the incidence of physiological disorders, PBZ+GA_3 tended towards a higher (K+Mg)/Ca ratio and consequent higher incidence of mesocarp discoloration than PBZ (Tables 12 and 13).

Because fruit of the same size category were studied in all treatments, fruit size differences did not influence mineral analysis and fruit evaluation results. The mechanism whereby the treatments applied in the present study resulted in higher fruit Ca levels relative to the levels of K and Mg is as yet poorly understood. In addition to influencing fruit calcium supply, governed by transpiration rate, the treatments may have favoured calcium uptake of the fruits by facilitating basipetal transport of IAA (BANUELOS et al. 1987).

As mentioned in the introduction, the period of rapid spring flush growth of the avocado largely coincides with the critical calcium uptake period of the young fruits (BOWER 1988). Calcium is almost entirely transported in the xylem; weakly transpiring fruits may be disadvantaged by calcium competition from the strongly transpiring leaves (BANGERTH 1979). WANG and STEFFENS (1985) report reduced transpiration in apple leaves in response to treatment with PBZ. In the present study, PBZ reduced transpiration in the leaves of the spring flush may have favourably influenced fruit calcium uptake. However, since application of PBZ+GA_3 and of GA_3 , with the attendant distinctly increased flush growth (Fig, 6), influences calcium content and (K+Mg)/Ca ratio in the same way as treatment with PBZ (Table 12), not only transpiration linked but also IAA linked calcium uptake must play a role.

Investigations by CUTTING and BOWER (1989) indicate that young avocado fruits export little IAA and constitute only weak sinks for the IAA linked calcium supply. Increased calcium uptake by the fruits as a result of raised levels of diffusible IAA after treatment with PBZ and PBZ+GA_3 could not be established in the present study because diffusible hormone analyses were not feasible during the test year (E.86) in which the fruit calcium levels resulting from the two treatments differed significantly from the control. It is however possible that the application of PBZ resulted in increased IAA transport from and calcium transport into the fruits by reducing IAA export from the

shoot tips (BUBAN and NAGY 1985).

Application of gibberellin in the early stages of fruit development frequently results in decreased fruit weight, reduced seed number, and parthenocarpic fruiting (SOOST and BURNETT 1961, KREZDORN 1969, BANGERTH 1983, HARTMANN 1984). In agreement with the findings of these authors, the GA_3 group in the present study also showed decreased fruit weight. Application of PBZ and PBZ+ GA_3 produced an increase in fruit weight (Table 5), indicating improved assimilate supply relative to the control. Interestingly enough, treatment with PBZ fully compensated for the negative influence of GA_3 on fruit size whereas simultaneous application of PBZ failed to reduce the GA_3 induced shoot growth to the level of the control (Figure 6). This suggests a fundamental difference between gibberellin regulated growth of reproductive and vegetative organs.

Development of parthenocarpic fruits was not stimulated by treatment with GA_3, PBZ+GA_3 or PBZ. In contrast, LAHAV and TOMER (1969) observed a very high set of parthenocarpic avocado fruits after bloom spraying with Alar and gibberellin.

GA_3 and PBZ are known to influence fruit shape in a number of fruit species. Fruit length/width ratio is typically increased after GA_3 treatment (SOOST and BURNETT 1961, STEMBRIDGE 1973, MODLIBOWSKA and WICKENDEN 1977, HARTMANN 1984) but reduced by PBZ (CURRY and WILLIAMS 1983, GREENE 1986, ELFVING et al. 1987, MANTINGER et al. 1988). In agreement with the findings of these authors, in the present study the length/width ratio of avocado fruits was increased by GA_3 and reduced by PBZ (Table 14, Figure 10). The fruit shape changes observed after application of PBZ is probably attributable to small amounts of PBZ reaching the young fruits via the xylem shortly after application. By blocking the gibberellin biosynthesis in the young fruits, PBZ may have so altered cell division and expansion rates in the mesocarp as to eventually produce a rounder fruit. Compared to the fruit from the PBZ treated trees, the fruits in PBZ+ GA_3 had a higher length/width ratio indicating that the influence of PBZ on fruit shape can be reduced by GA_3 (Table 14). This agrees with the findings of CURRY and WILLIAMS (1983) in apple.

A reduced length/width ratio is advantageous for packing Fuerte avocados even at the sacrifice of the familiar pear-like shape. None of the treatments influenced seed or pericarp weight within the weight category studied (Table 14). Storeability and ripening time were also unaffected.

These results clearly indicate that PBZ, GA_3 and PBZ+GA_3 can improve fruit quality in Fuerte avocados. All treatments produced definite reductions in the incidence of physiological disorders. The only side effects of the treatments were in the form of commercially acceptable changes in fruit shape.

5. SUMMARY

The objective of the present study was to increase yield and induce more regular bearing in vigorously growing and poorly producing avocado trees cv 'Fuerte' by pruning or applying plant growth regulators. In addition, the influence of plant growth regulators on fruit quality was studied, as 'Fuerte' fruit often show physiological disorders following storage.

The leaf:fruit ratio of 'Fuerte' trees was determined. It seems unlikely that low avocado yields are caused by a source limitation. Therefore, it should be possible to encourage reproductive growth.

On 'Fuerte' trees, reproductive growth was not encouraged by pruning the spring flushes. Regrowth occurred rapidly after pruning. Trees that were pruned and treated with NAAm to suppress regrowth had a tendency towards higher fruit set, but the yields did not differ significantly from the control.

The application of various plant growth regulators (screening) at the time of the most vigorous growth of the spring flush showed that mainly inhibitors of the gibberellin biosynthesis, such as paclobutrazol (PBZ), influenced both flush growth and fruit retention in avocado. In 'Fuerte', the growth of the spring flush was inhibited by the application of PBZ, strongly promoted by the application of GA_3 and to a lesser extent promoted by PBZ+GA_3.

Shoot tips of PBZ treated trees contained less endogenous gibberellins than shoot tips of the more vigorously growing control trees. The highest gibberellin levels in shoot tips were found after GA_3 treatment, while the gibberellin levels in shoot tips of PBZ+GA_3 treated trees lay between those of the GA_3 treated trees and the control. The levels of extractable indole-3-acetic acid (IAA) and those of the cytokinins zeatin/zeatin riboside (Z/ZR) and isopentenyl adenosine/ isopentenyl adenine (I-Ado/I-Ade) in shoot tips showed no clear changes after PBZ treatment, while there was a tendency towards higher levels after GA_3 and PBZ+GA_3 treatment as compared to the control. The levels of extractable abscisic acid (ABA) decreased shortly after all treatments.

In the first year of treating whole trees of the cv 'Fuerte', both the PBZ and the PBZ+GA_3 treated trees had significantly higher yields than the control trees. The GA_3 treated trees yielded slightly more than the control trees. In the following year the same treatments were repeated and in contrast to the previous year, PBZ, PBZ+GA_3 and GA_3 treated trees yielded significantly less than the control trees. The two-year cumulative yields of treated trees did not differ significantly from the control. The control trees showed the highest degree of alternation while the PBZ+GA_3 treated trees showed the lowest degree of alternation followed by the PBZ and the GA_3 treated trees.

The reduction in fruit drop observed in PBZ treated trees may be due to reduced dominance of the spring flush whereby the IAA export out of the fruits is increased. This could not be proved in the present study as diffusible IAA out of fruits could not be determined in the year in which the PBZ treated trees yielded significantly better than the control. The assimilate supply of fruits is improved by PBZ, leading to a higher fruit weight compared to that of the control fruit. Application of GA_3 appears to result in a temporary increase in the sink strength of young avocado fruits despite the fact that this

treatment is promoting shoot growth. After treatment with PBZ+GA_3, both PBZ and GA_3 effects were observed.

As an important practical result, all treatments (PBZ, GA_3 and PBZ+GA_3) significantly reduced physiological disorders in 'Fuerte' fruit in two successive years. On average, 75% of the fruit from the control trees developed physiological disorders, while only 37%, 45% and 50% of the fruit from the PBZ, PBZ+GA_3 and GA_3 treated trees respectively, developed physiological disorders. In both years, fruit from PBZ treated trees had a lower (K+Mg)/Ca ratio than fruit from control trees. The results indicate that 'Fuerte' fruit quality can be improved by spring application of PBZ.

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