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Avocado Fruit Abscission

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AVOCADO FRUIT ABSCISSION

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ABSTRACT

Research was conducted to better understand the complex, interactive roles of hydrolytic enzymes, phytohormones, pollination, and assimilate partitioning in fruit abscission. Complications arising from interfering interactions in fruitlet tissue limited our ability to consistently assay for tissue protein using the Bradford and Lowry procedures and thus our ability to unequivocally evaluate the roles of hydrolytic enzymes on the senescence of seed coat. After much effort to correct the problem, it is still unresolved. Substantial headway was made in developing immunoassay procedures for analysis of AbA and GA's in avocado tissue. Successful development of these techniques now enables us to study the phytohormones in great detail. Pollination of the cultivars grown in South Florida were found to occur primarily in the second flower opening, heretofore considered to be strictly male in function. This discovery helps explain many of the inconsistencies in our understanding of avocado pollination and its role in fruit set. It also impacts South Florida growers who have historically interplanted cultivars to provide ample cross pollination.

We have discovered that in avocado seven carbon sugars are main translocated sugars. In the spring reserve carbohydrate serve as a source of carbohydrates for the developing leaves and fruits. One month old leaves export more carbohydrate than they import and contribute to the developing fruits but only to which they are connected by the transport system. Fruits are strong sinks, where the seed tissues are the strongest but not largest sink. Before abscission sink strength decreases. It was found that determinated inflorescences are more productive than indeterminated ones, but based on pinching experiments it does not seem to us that a competition on carbohydrates exist between vegetative and reproductive growth, and among-fruits.

Synthesis of Acc limits ethylene production while SAM and EFE are present in attached fruits. Harvesting, Wounding or senescence of seed coat cause a climacteric ethylene production which result in fruit abscission and softening.

Objectives of the research

Yields of avocado depend on an undisturbed chain of events such as differentiation, flowering, pollination fertilization, fruit abscission. Problem in each of the above mentioned, can reduce yields. The situation is even more complicated due to interaction with other growth phenomena such as vegetative growth. In the research proposal, a wide program was submitted in order to study simultaneously different aspects of the problem. The original objectives can be grouped:

1. To study patterns of fruit abscission in various cultivars, and to determine interactions between fruit abscission and other phenomena such as the senescence of seed coats or the relationships with vegetative growth.
2. Gain a better understanding of the mechanisms involved in fruit abscission and its control. Such as the enzyme systems which take place in the senescence of seed coats and the role of phytohormones and partitioning of carbohydrates in controlling fruit abscission.
3. To find ways to reduce fruit abscission.

During the research it was needed to have information on factors influencing the initial fruit set in Florida, therefore studies on pollination and fertilization were conducted.

Research Report

Because of the differences between the various subjects which were treated in the project, the research report is arranged according to the various subjects. The report is based on publications some of which are not included like theses.

Hydrolitic enzymes and hormones in senescence of seed coat, and the initial fruit set in Florida.

The research results to be reported here will be divided into four general areas. Based upon previous research, we know that seed coat senescence and the coincidental production of large quantities of ethylene by this tissue precede abscission of developing avocado fruitlets (4). The separation response is dependent upon the production of ethylene by the seed coat of the fruitlets. We were able to separate ethylene production from the senescence events since ethylene fumigation does not induce the events and the senescence events can proceed in the presence of ethylene synthesis inhibitors. Thus, it appears that

the senescence event is the first biochemical marker that we have to indicate that separation of the fruit from the tree is eminent.

One of the objectives of the present study was to investigate the roles of oxidative and hydrolytic enzymes as well as the changes in total and specific proteins to see if we could identify specific events occurring earlier in the senescence sequence and to elucidate the possible roles of these enzymes in the senescence process. Furthermore, we were interested in identifying any changes in the levels of two classes of phytohormones, AbA and gibberellins, to see if there was possibly a role for them in the induction of fruitlet, seedcoat senescence.

The third area of investigation centered on the characteristics of carbohydrate partitioning into developing fruits and the role that carbon loading into the fruit plays, either directly or indirectly in the longevity of those fruit.

The fruit setting habits of avocado cultivars grown in South Florida have been classified into two types: Type I fruit setting heavy, typified by many flowers setting many developing fruitlets which abscise over time and Type II fruit setting heavy with few fruit being set initially, but these fruit tend to remain on the tree until harvest (3). Because of the great difference in the numbers of fruitlets initially set by these two habits, it is possible that pollination and fertilization of the flowers may be a limiting factor in this initial fruit set period. We, therefore, set up several field experiments to investigate whether the first step, pollination, had a role to play in this phenomenon.

Materials and Methods

Fruit samples for enzyme and hormone analysis. Branches bearing clusters of fruitlets were harvested from 'Simmonds' or 'Peterson' trees in a commercial orchard and transferred to humid chambers until sampled. It has previously been shown that this procedure synchronizes the onset of seedcoat senescence in the avocado fruitlets (4). Over the three years this study was conducted, great care was taken to minimize biological variability due to sampling. It was determined that the optimum protocol was to harvest the fruit from a single scaffolding branch each on one of three or more trees so that any variability from tree to

tree could be observed. Branchlets from a single scaffolding branch bearing the harvested fruit were placed in 60-liter plexiglass chambers supplied with humidified air to purge any fruit-produced ethylene from the enclosed space. Sampling of the fruit from the chambers was done by randomly selecting fruit from each of the small branches so that the sample consisted of an even distribution of fruit from the entire branch population. Samples were taken twice daily after harvest of the branches from the trees for four days. Those fruit to be sampled for specific enzyme activity and protein concentration were homogenized in chilled acetone, filtered on Watman 3 paper in a Buchner funnel with chilled acetone, freeze dried, and stored at -10°C until analysis. Those fruitlet samples destined for hormone analysis were frozen in liquid nitrogen, freeze dried, and stored at -10°C until analysis. Extraction procedures are described by Southwick and Davenport (Appendix 3 & 4).

Enzyme and protein analysis. Peroxidase activities in the pericarp, seed coat, and embryonic tissues were determined using the procedures of Brochert (2). Proteolytic activities were estimated by measuring the timed hydrolysis of hemoglobin as reflected by free amino acid analysis according to Yemm and Cocking (4). Total proteins were assayed using techniques of Bradford (1) and Lowry (3). Descriptions of other specific experiments involving protein analysis are included in Appendix 1.

Hormone analysis. details of the derivitization and analysis of AbA using gas-liquid chromatography are described in Tietz and Tietz (Appendix 2). Inoculation of rabbits for anti-AbA and anti-GA antibodies and development of the radioimmunoassay for these phytohormones were conducted as described by Southwick and Davenport (Appendix 3 and 4). The glucose ester of AbA, a potential cross reactant of the competitive binding radioimmunoassay for free AbA, was synthesized according to Southwick, et al (Appendix 4). Novel approaches to the synthesis of GA_3 - and GA_4 - protein conjugates for use in developing gibberellin antibodies were conducted and described by Southwick and Davenport (Appendix 5).

Pollination studies. Initial experiments were conducted by sampling avocado styles at the end of the Stage I and II openings using micro forceps and placing them on a gel composed of carboxymethyl cellulose (H. Melamud, personal communication). Seven cultivars interplanted with several complementary cultivars in seven locations were sampled throughout the flowering period.

In the following year, branches of avocado trees of two cultivars, Simmonds and Hardee, were covered with cheese cloth to prevent pollination by flying insects during Stage I opening, Stage II opening, or both openings. Styles and flowers from these were collected from stage I and II avocado flowers immediately prior to flower closing using fine forceps. Styles were placed on a gel-covered microscope slide and covered with a cover slip, and flowers were also collected at 24 and 48 hr later. All were later analysed for the number of flowers pollinated and the length of pollen tube growth in the styles. Other parameters such as the times of floral opening, the condition of the stigmatic surface, availability and types of pollinators in the grove, and the weather conditions were also noted during the study.

Assimilate partitioning. Source leaves were radiolabeled with $^{14}\text{CO}_2$. Leaves and fruit were sampled to determine translocation rates. The distribution of the translocated carbon into various fruitlets and fruitlet tissues as well as to other parts of the branch was examined by dissection and extraction of components and assayed by liquid scintillation. Photosynthetic rates of source leaves and fruitlets were also determined in situ using an instrument supplied by Analytical Development Co. Ltd.

Results and Discussion

Enzyme and protein analyses. Our efforts to investigate activities of peroxidase and protease in embryonic, seedcoat, and pericarp tissues were hampered by our inability to obtain consistent protein determinations in avocados sampled at various times after harvest (Appendix 1). Work in this area will continue because of its importance in a number of studies.

Hormone analyses. Much time was spent in developing techniques of analysis of both AbA and gibberellins. Responsibility for these duties were shared among three individuals over the period of the grant. Results of initial experiments to develop a convenient method of AbA-methyl esterification for gas chromatographic analysis were reported in the enclosed publication by Tietz and Tietz (Appendix 2). The accompanying correspondence explains why BARD was not recognized as the granting agency for the research reported in this article.

Early on, it was decided to utilize immunoassay techniques to take advantage of the opportunity to use small amounts of tissue when analyzing

avocados. A doctoral graduate student working on another project involving hormone analysis assumed responsibility for developing the radioimmunoassay and enzyme-link immunoassay procedures for analysis of AbA and gibberellins. This research was successful and resulted in the enclosed publication (Appendix 5) and abstracts (Appendix 6 & 7). Additional publications on development of these techniques are in preparation (Appendix 3 & 4).

Attempts to analyse avocado tissues using these techniques were successful; unfortunately however, inclement weather and grove management problems created difficulties in our ability to sample fruitlets from where the experiments were conducted. The situation results in suspect data. Thus, no results from these experiments are included here. Thus studies are continuing.

Pollination study. Experiments conducted during the first year of the study suggested the surprising result that most of the pollination was occurring, in stage II flowers, i.e. during the second anthesis in those avocado cultivars examined (see Table in Appendix 8). This period has been generally considered strictly male in function; yet, we were observing fruit set in cultivars where there was no possibility of pollination occurring in the first (functionally female) opening, Stage I flowers.

A second year of more detailed observations using cheesecloth nets to enclose Stage I or II flowers on the trees in two cultivars, Simmonds and Hardee, thus preventing pollination by flying insects, clearly verified these conclusions. Manuscripts are in preparation for publishing this information. Several important questions arising from this study require further investigation as indicated in the review article (Appendix 9).

Assimilate partitioning Study. A doctoral graduate student is presently in the process of completing her analysis of the data obtained over two seasons of research on assimilate partitioning in avocado. A preliminary report is included in Appendix 10. Preliminary analysis of the data suggests that carbohydrate partitioning is determined by the demand of the various sinks and that photosynthates are not limiting to fruit load or demand. To the contrary, it appears that leaves typically do not operate at their maximum potential efficiency, the photosynthetic rate being dependent upon the fruit load.

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Biosynthesis of ethylene in avocado fruit

Ethylene is a plant hormone which initiates and regulates abscission and ripening of many fruits including avocado. Attached avocado fruit produce only trace amounts of ethylene. It does not abscise nor does it ripen unless high amounts of ethylene (climacteric) are produced.

In this section we describe the biosynthesis of ethylene in avocado fruit.

Materials and Methods

Avocado fruits of the cvs. Fuerte and Hass were used. Biosynthesis of ethylene by the various tissues was carried out as described in appendix 11.

Ethylene was determined by gas chromatography, ACC was extracted and assayed according to Iizada and Yang. ACC synthase was determined according to the ability of the extract to convert SAM to Acc. EFE activity was determined in vivo according to the ability of fruit discs to convert Acc to ethylene (Appendix 11).

Biosynthesis of ethylene in various fruit tissues was done after separation of the tissues (Appendix 12).

When analyzing young fruits some of them were already induced to abscise while other not, which introduce a big variability among fruits. Therefore harvesting of the fruits served as a common trigger for ethylene production. This ethylene resulted in ripening of the fruits as well as their pedicel abscission.

Results and discussion

Ethylene is involved in avocado abscission but generally, even in mature attached fruit, it is not produced in sufficient amounts to cause abscission. In order to find out whether the limiting factor is the endogenous levels of SAM (S-adenosylmethionine), we treated attached fruit and pericarp discs with SAM and examined their ethylene production. No acceleration of ethylene production was found in SAM-treated tissues, indicating that SAM levels are not the limiting factor in the production of ethylene. Levels of ACC (1-aminocyclopropane-1-carboxylic acid) and ACC-synthase activity were very low in attached fruit. Inhibitors of RNA and protein synthesis which inhibited the development of ACC-synthase activity in preclimacteric pericarp discs prevented the development of the climacteric (appendix 11). These results led us to conclude that in order to reach climacteric there must be a de novo synthesis of ACC-synthase. Furthermore, we assume that the low rate of ethylene production in an attached fruit is not a result of inhibition of ethylene activity but due to the lack of enough ACC-synthase in the fruit.

Preclimacteric fruit tissues contain low but measureable activity of EFE (Ethylene Forming Enzyme), as indicated by the immediate rise in ethylene production after ACC application. The fact that cycloheximide did not inhibit EFE activity in these tissues within the first 2 hrs of discs incubation, further support the above conclusion. The activity of EFE was raised with the beginning of the climacteric ethylene. In the postclimacteric stage, EFE

activity decreased to a level similar to that found in the preclimacteric stage. Infusion of ACC into fruit attached to the tree caused a rise in ethylene production, followed by fruit drop. The results indicate that although EFE activity in preclimacteric fruits is low, it is sufficient to produce enough ethylene for inducing abscission and ripening when ACC is available.

Significant level of MACC (1-(malonylamino)-cyclopropane-1-carboxylic acid)' was found in the preclimacteric fruit. It increased by 2-3 fold during the climacteric peak. Conjugation of ACC during the climacteric peak seems to have no significant role in regulating ethylene biosynthesis at this stage, because the rate of conjugation was relatively low in comparison to ACC synthesised at this stage. However, in the preclimacteric stage conjugation may play an important role in regulating ethylene biosynthesis. The low amount of ACC that is produced during this stage is probably entirely conjugated to MACC.

We also examined the possibility that ethylene might inhibit ethylene. Exposure of pericarp discs to ethylene caused inhibition of ACC and ethylene production. The concentration of exogenous ethylene, which caused autoinhibition were high in comparison to levels of endogenous ethylene at the preclimacteric stage. Therefore, autoinhibition is not likely to be the mechanism responsible for the inhibition of ethylene production in preclimacteric avocado fruit (appendix 11). In young avocado which abscises easily, seed coat and endosperm exist, while in mature fruit which generally does not abscise these tissues are degenerated. Most ethylene production in young fruit is biosynthesised by the seed coat. When ACC was applied to endosperm and cotyledons, there was very little conversion of ACC to ethylene in these tissues. On the otherhand, application of ACC to seed coat and pericarp tissues resulted in, almost immediate ethylene production. Twenty four hours after application of ACC we found in the seed coat autocatalytic ethylene production. This phenomenon was not found in the other tissues (Appendix 12).

In all the tissues, except endosperm, wounding of the tissues increased the activity of ACC-synthase. In cotyledons the rise in ACC production was accompanied by conjugation of ACC and production of MACC. The highest level of conjugation of ACC and production of MACC. The highest level of conjugation was found in cotyledons seemingly because in this tissue the conversion of ACC to ethylene is almost nonexistent. In wounded cotyledons of mature avocado fruit the levels of ACC and MACC formed are much lower than in cotyledons of young

fruit. In contrast pericarp tissue of mature fruit produce more ACC and MACC than pericarp of young fruit. (Appendix 12).

We had some difficulties in the determination of ACC in pedicels, where the separation of abscising fruit occurs. We found that phenolic compounds which are present in the extract interfere in ACC conversion to ethylene. A procedure which overcomes most of these problems was developed and will be used in future studies. (Appendix 13).

The results shown in this study are in agreement with the theory that a repressor exists which inhibits synthesis of ACC-synthase and consequentially inhibits ACC and ethylene production. The low levels of ACC which are produced before the climacteric are converted to MACC therefore although, EFE is present in there is no ethylene production. In destruction of the repression either by wounding, or by harvest enables ACC to synthesise and ethylene (Appendix 14). A scheme describing the changes occurring after removing the repression was prepared (Fig 1 appendix 14).

Partitioning of carbohydrates as a possible factor in avocado fruit abscission

Introduction. Many approaches has been used during the study of avocado productivity. Only the removal of the young vegetative growth of 'Fuerte' during and short time after fruit set resulted in increased yields. (Biran, Gazit, Blumenfeld unpublished). It was therefore suggested that competition on carbohydrates exist between the developing vegetative and the reproductive growth of avocado.

To study the nature of the carbohydrate partitioning in avocado in relation to fruit abscission forced us to deal with subjects such as identification and quantification the translocated sugars in avocado, the development and maturation of avocado leaves. Sink/source relationships between different organs and tissues etc. This part of the work was limited only to carbohydrates.

Materials and Methods. Observations and measurements were carried out mainly in three orchards: Bet Dagan, Naan and Givat Brenner. Labeling with C^{14} was done only in Bet Dagan orchard. Labeling of stored carbohydrates was done by repeated treatments by CO_2^{14} 2 hrs each every ten day in the autumn of 3 years old 'Ettinger' grafted on the same vegetative propagated rootstock. The plants were enclosed in a special devise which was developed for the purpose (Fig. 1).

Fig. 1. System for application of $C^{14}O_2$ to whole plants.

Labeling transported carbohydrates was done by treating desired source leaf with C^{14} which was generated in enclosing bag (Fig. 2). Preliminary studies showed us the connections between various leaves and fruits. Collection of plant material was done 96 hrs after treatment. This time was chosen after studying the changes of carbohydrates with time. Plant material was frozen by liquid air then dried by freeze drying and was kept in $-18^{\circ}C$ until analysis.

Sugars were extracted by metanol: chloroforms: water 12:5:3. the alcohol-water phase was evaporated, and filtered with sep-pak C-18. Sugars were separated by Varian Vista 5500 HPLC. Solvent used was acetonitril: water 86:14. column NH_2-5 15 cm. Temp $40^{\circ}C$ flow rate 1.5 ml/min. Sample volume 0.01 ml. Running time 15 min. Radioactivity in sugars was detected by Contron Betamatic I centilation counter. Samples for radioactivity detection were collected at the outlet of the HPLC detector.

Because of the huge variability in CO_2 uptake by different plants and even leaves, the C^{14} distribution is given in relative values: Either as percents or as Relative Specific Activity - R.S.A. this value is a good indication for sink strength of various parts in one system.

Results and discussion

Translocated sugars in avocado. Five sugars were detected as the main sugars in avocado: Fructose, Glucose, mannoheptulose, sucrose and perseitol (See also appendix 15). The absolute and relative levels are given in Fig. 3. It was surprising to find that while in most trees the sucrose is the main translocated sugar, in avocado the 7 carbon sugars are also very important together with it. a kinetic of CO^{14} movement out of new mature leaf is depicted in Fig 4. We see that major movement out of the leaf takes place only 24 hrs. after the C^{14} treatment. The distribution of the CO^{14} between the various sugars is given in Fig 5. Two changes are very prominent during the first 96 hrs: after treatment. The decrease in the level of sucrose from 60% to 25% and the increase in perseitol from 60% to 40%. This could point that first sucrose is synthesized while later, part of it changes to perseitol. This assumption needs further study.

We have measured the levels of sugars in organs where translocation is carried out. We found (Table 1) that more than 60% of the labeling appears in seven carbon sugars while only 20% as sucrose. Considering that sucrose is a

Fig. 2. Method of treating source leaf with $C^{14}O_2$

Fig. 3. Differences in sugars in various tissues.
 A - amount
 B - relative level

Fig. 4. Export of C^{14} labeled sugars out of mature leaf. shown as % of total C^{14} which was found out of the source leaf.

Table 1. Distribution of applied C^{14} in various organs (% of total). Different letters show significant difference (5%).

Organ	Sucrose	Perseitol	manoheptulose	Glucose	Fructose
Petiol, source leaf	22.7	44.1	29.8	1.3 b	2.1 b
Twig	18.5	36.3	32.7	4.5 a	8.3 a
Inflorescence axis	23.0	31.4	33.1	4.1 a	8.3 a
Pedice1	24.2	36.4	29.5	2.4 ab	9.3 a

disaccharide and has therefore two C^{14} in each molecule, means that actually the amount of the sucrose is less than the amount shown and that the amount of the others, monosacharids is actually higher.

In many plants the main translocated sugar is the sucrose, a sugar that its metabolism has been extensively studied. There are however plants in which other sugars such as the sorbitol (in apple) takes this function. We found that in avocado in the spring there is a period when all the old leaves drop and the new growth depend on sugars coming from the reserves in the permanent parts of the tree: The branches trunk and roots. Then, main translocated sugars are the perseitol and mannoheptulose, These sugars are important as translocated sugars also later on together with sucrose. It is very interesting to know why the avocado has this special translocated sugars, is it a system special to avocado or also to other members of the family, and what is the metabolism.

Interaction between vegetative and reproductive growth

Introduction. Observations in the 'Fuerte' showed that vegetative growth develops in the end of many inflorescences, during fruit set period and thereafter. Removal of all the new growth in this period resulted in higher yield. A competition on essential substances between the vegetative and reproductive growth was suggested. This part of the work deal with description of growth phenomena of the inflorescences and the relationships to fruit abscission and the productivity.

Materials and Methods. During three years growth phenomena of the main cvs grown in Israel were recorded. Preliminary study was conducted in 1984 and intensive during 1985 and 1986. We followed various stages of vegetative growth and their timing as well as time of fruit set in 'Ettinger', 'Fuerte', 'Nabal', 'Reed' and 'Hass'.

The amount of determinated inflorescences - DI and 'indeterminated inflorescences' -I.I, abscission of fruits in the two types and their final yield was recorded in 'Hass' and 'Fuerte'. Distribution of fruits on D.1 and I.1 before harvest was done also in 'Nabal', 'Reed' and 'Pinkerton'.

In order to influence the interaction between vegetative growth and developing fruits we removed by pinching, beginning in the early spring all the vegetative growth of branches of three year old 'Ettinger' plants, and of branches of mature 'Fuerte' trees. Each desired time, number of fruits on the

A

	24	48	72	96 (h)
○- Fro.	c	c	c	c
○- Glo.	c	c	c	c
□- Menth.	b	b	a	b
◇- Pers.	c	c	b	a
△- Suc.	a	a	b	bc

B

	24	48	72	96 (h)
○- Fro.	b	ab	a	a
○- Glo.	b	b	b	b
□- Menth.	c	c	ab	b
◇- Pers.	d	d	b	a
△- Suc.	a	a	ab	b

Fig. 5. Changes in distribution of C^{14} between different sugars during 96 hrs after C^{14}O_2 application.
A. percent
B. relative specific activity

treated branches and controls were recorded. We have also removed the leaf which interacts with a certain fruit. This was done after preliminary study of the relationships between, certain leaves and fruits on a developing inflorescence. Growth of leaves was done by measuring and weighting developing 'Ettinger' leaves at desired intervals.

Results and Discussion

Productivity of D1 and II. Time of bud swelling, elongation of the inflorescence, flowering fruit set, and vegetative growth at the end of the inflorescence is depicted in fig. 6. In 'Fuerte' there is a great variability between trees. Flowering occurs from the autumn until spring and fruit can set when temperatures are high enough. In other cvs flowering and vegetative growth are more uniform. In 'Nabal' and 'Reed' the vegetative growth begin before the elongation of the inflorescence. In 'Ettinger' and 'Hass' vegetative growth is fully developed at flowering and fruit set period.

The amount of the two types of the inflorescences In-avocado I.D, and I.I various in different cvs. As can be seen in table I. In 'Fuerte', 'Hann' and 'Pinkerton' the ratio is 1.1 while in 'Nabal' and 'Reed' D.I. are rare; and in 'Ettinger' (not shown) they were not observed at all.

In 'Hass' although most of the fruits abscisse later in both types there is a significant higher number of fruit at harvest on D.I. Figs. 7,8 This Fig also shows the pattern of avocado fruit abscission.

Leaf growth and development. A developing leaf is a big sink while young and becomes later on a strong source of carbohydrates. There is a period when a leaf import and export carbohydrates at the same time therefore studies of carbohydrate movement in and out of a leaf are required in addition to determination of its dry weight. Growth in length, increase in total dry weight, and increment of growth are depicted in Fig. while growth in length is almost linear there are changes in the rate of dry weight accumulation. The increment accumulation of dry weight shows some picks which need clarification. A young leaf can double its weight within 2-3 days. After 30 days it has arrived to its final size, while the length width ratio remains constant throughout its growth. The change in dry weight accumulation is suggested to be related to the change of a leaf from a sink to a source. At the same time, leaf color also changes from copper-brown to green.

Table 2: Frequency of determinated or indeterminated inflorescences and their productivity at harvest. The number of trees for each cv. and location varies between four to twenty trees.

Cv.	Locat.	<u>Indeterminated inflorescences</u>		<u>determinated inflorescences</u>			
		% of Tot. fruits	Number of fruits	% of Tot. Inflor.	% of Tot. fruits	Number of fruits	% of Tot. Inflor
'Fuerte'	Na'an*	38	945	53	62	1534	47
"	" **	35	1253	58	65	2302	42
"	Brener*	30	256	55	70	594	45
"	" **	26	671	60	74	1911	40
"	K. Yaroq*	28	439	-	72	1131	-
'Hass'	Na'an*	37	492	55	63	866	45
"	" **	30	314	59	70	732	41
"	Brener**	32	481	61	68	1017	39
"	K. Yaroq*	33	451	-	67	912	-
'Nabal'	Bet-Dagan*	98	1470	100	2	30	0
"	" **	96	1630	95	4	71	5
'Reed'	" *	96	672	100	4	28	0
"	" **	95	814	97	5	44	3
'Pinkerton'	" *	27	19	-	73	52	-
"	" **	31	28	-	69	63	-
"	Yad Mord.*	49	185	-	51	190	-

* - 1985

** - 1986

Fig. 6. Time of various growth phenomena in different cultivars

Fig. 7. Fruit abscission in determinated or indeterminated 'Hass' inflorescences.

Fig. 8. Fruit abscission in determined or indetermined 'Hass' inflorescences.

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Fig. 9. 'Ettinger' leaf growth.
A. Accumulative length and dry weight.
B. Increment growth.

Changes in total amount of sugars in a leaf as well as the ratio between various sugars is depicted in fig.10. The sugars in petioles of the same leaves is also shown. From day 7 to 30 of leaf age the total amount of sugars is doubled to be 14 mg/gr.f.w. This is further increased (not shown) to 25 mg/gr.f.w. in 2 month old leaf. There are prominent changes also in the ratio between the various sugars. Perseitol which is 60% of total sugars in the first ten days goes down to 30% when its one month old. While sucrose which is very low at the beginning goes up to 30% when the leaf is 3 weeks old.

By measuring the appearance of stored carbohydrates in developing leaves we could observe that they decrease rapidly and almost do not appear when a leaf is one month old. A leaf becomes a source to sugars when it is larger than 12 cm when it is about one month old. The increase of sugar export from a leaf as a function of leaf age-size is summarized in Fig. 11. As mentioned before highest export is 96 hr after CO_2 treatment. (Longer periods were also tested - not shown). The change in distribution of applied CO_2 between the various sugars in leaves of different age is shown in Fig.12.

Intervention in sink source relationships. One of the ways to study the relationships between sources and sinks is to remove or add one of them. We were able to remove a sink - the developing leaf and to obtain an influence on the other. By picking the new growth more fruits survived in the treated branches, compared to controls. Fig. 13. Similar results were obtained when the vegetative growth was removed from branches of mature trees. There too more fruits remained in the treated branches. The results presented in three ways are depicted in Fig. 14.

Fig. 1a Export of C^{14} labeled sugars as related to leaf age (presented by leaf size) and time after CO_2 application.

Fig. 2 Distribution of C^{14} among sugars as a function of leaf length (leaf age). A - 24 hrs after treatment. 96 hrs after treatment.

Fig. 13 Remaining fruitlets on Pinched or control inflorescences. 'Ettinger' young plants. Vegetative growth was pinched immediately after sprouting.

Fig. 14. Survival of fruitlets on pinched or girdled branches presented in various ways.
 A - number of fruits per branch
 B - percent remaining in each week
 C - percent of first count

The developing fruit as a sink for carbohydrates.

Fruit interacts with developing vegetative growth which occurs at the end of the inflorescence. It was interesting to learn how strong is the fruit as a sink, what is the importance of its different tissues, and what changes in carbohydrates occur toward fruit abscission.

Experiments were conducted with fruit of early maturing seedling of the Mexican race and with "Ettinger" fruits. Fruits were divided to: pericarp, embryo, endosperm, and seed coats which also contain the nucellus.

Results and discussion - In a young (one month old) fruit, seed tissues are a big sink for carbohydrates. They contain 6% of applied C^{14} when they are only 20% of fruit weight. Change in the weight of fruit tissues and of C^{14} distribution in these tissues is depicted in fig 15. The amount of different sugars in these tissues is shown in Fig 16.

We found a great variability in C^{14} distribution between fruits of the same age. This variability was much smaller after the main wave of abscission. We observed that in some fruits the sink strength (RSA) is much higher in the seed than in the pericarp and in others it is lower. We have concluded that the variability in a sample of fruits is due to mixture of fruits with different tendencies to abscise, and therefore after the main wave of abscission all the fruits have the same tendency to abscise and the variability diminish.

To test this hypothesis we divided the fruits to three groups according to their tendency to abscise: Normal fruits, fruits which abscise easy by touch and dull fruits. All fruits were with no signs of damage, including the seed coat. It was observed that levels of carbohydrates were four times higher in normal fruits than in easy to abscise ones. A difference in the relative amounts of the various sugars also occurred: The level of reducing sugars in the embryo of normal fruits was high and perseitol was low, and the level of seven carbon sugars in seed coats of normal fruits was low Fig. 17. A decrease of the level of sugars occurred before the abscission. We have some reservation as to the methods we used, however we concluded that a change in levels and in the relative quantities of various sugars occurs before abscission. We think that these changes are secondary and may be a result of some regulatory signal in fruits toward abscission. To clarify this further work is needed.

Fig. 15. Tissues weight and distribution of C^{14} during fruit growth.

Fig 16 Sugar content in fruit tissues.

Fig 17. Sugar levels in tissues of fruits with different tendency to abscise.

4. Description of cooperation

The objectives of the research were very wide, each covering a different research research area. We did not had any clue what research direction will yield the best results, therefore the work was divided between the two cooperating teams. In Florida work was conducted with enzymes related to senescence of seed coats and with two hormones which are supposed to have a regulatory role in abscission: ABA and giberellins. Some work dealing with partitioning of carbohydrates was also carried out.

In Israel the work was concerned with the description of abscission patterns of various cultivars, with relationships with other growth phenomena such as vegetative growth, with study of carbohydrates of the developing fruits in relation to abscission, with sink source relationships and with metabolism of ethylene.

Visits of the cooperating scientists in each others laboratory enabled us to study the differences in plant material which is grown in the two countries, and to coordinate the future research based on the results obtained in the previous year. The new information generated by each team complemented the other.

MAIN ACHIEVEMENTS OF THE RESEARCH

We feel that we have been moderately successful in achieving our proposed research goals. Preliminary data suggests that peroxidase and protease enzyme activities may play a role in the seed coat senescence events involved in fruitlet abscission; however, until we can resolve the important issue of reliable protein analyses, it will be impossible to verify such a claim. This conclusion applies to all research being conducted on the biochemistry of avocado, and it certainly leaves past work in this area in suspect.

The role that phytohormones play in fruitlet abscission remains to be determined. We have made significant headway during the course of this study to develop the techniques necessary to investigate this problem and will continue this line of research in the future.

The discovery that pollination occurs primarily in the second floral opening was a surprise to us. Clearly this is the case, however, in many Florida-grown cultivars, and it is clear that some cultivars are self-pollinated. The significance of this discovery is immediately obvious considering that virtually all growers in South Florida interplant trees to promote cross pollination. Further studies will determine whether Stage II flowers are receptive to this pollen, the environmental conditions which make this possible, and whether this process plays a significant role in subsequent fruit set.

The above mentioned finding will be tested also in Israel for cvs. are grown there where pollination occur already in the first female opening of avocado flowers.

The work which dealt with carbohydrates showed that in avocado, most of the transported sugars (7 carbon sugars) are different than in many plants (sucrose). We now understand much better part of the carbohydrate cycle in avocado. We have good information on maturation of avocado leaves and on relative sink strength of developing leaves and fruits. In fruits we know the relative sink strength of its tissues. We were able to show that although an interaction between vegetative to reproductive growth exist, there is no competition on carbohydrates between them, neither between developing fruits. The reason for the interaction should be looked for in other areas such as growth regulators or something else.

We have done a great step forward in understanding the biosynthesis of the hormone which is involved in avocado fruit abscission - the ethylene since this hormone regulates also fruit ripening a process which served us as a model for ethylene biosynthesis, we gained also an answer to the old question why avocado does not ripe on the tree.

We expect to generate fifteen or more publications from this effort within the next year. Five have already been published. Copies of the others will be sent to BARD upon receipt of reprints.

List of Publication

1. Blumenfeld, A., Sitrit, Y. and Riov, J. Avocado fruit ripening and ethylene production Acta Hort. 179:787-791.
2. Buchholz, A. 1986. Carbohydrate partitioning between fruitlets and young vegetative growth as a possible factor involved with fruitlet abscission in avocado. M.Sc. Thesis. Hebrew univ. of Jerusalem.
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6. Davenport, T.L. 1986. Efficacy of Triazol Growth retardants on tropical fruit tree growth. Proc. 13th Ann. Meet. Plant Growth Reg. Soc. Amer. (in press).
7. Davenport, T.L. 1986. Unknown factors contributing to variability in protein determination in some plants. Plant Physiol. 80S:42. (Abstract).

8. Finazzo, S. and T.L. Davenport. 1986. Assimilate partitioning in avocado, *Persea americana*. Plant Physiol. 80S:133 (Abstract).
9. Sitrit, Y. 1964. Regulation of ethylene biosynthesis in avocado. M.Sc. Thesis. Hebrew Univ. of Jerusalem.
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11. Sitrit, Y., Riov, J. and A. Blumenfeld. 1987. Interference of phenolic compounds in the 1 aminocyclopropane 1 carboxylic acid Assay. Submitted to plant Physiol.
12. Sitrit, Y., Blumenfeld and Riov, J. 1987. Ethylene biosynthesis in tissues of young and mature avocado fruits. Physiol. Plant. 69:504-510.
13. Southwick, S.M. 1986. Development of radioimmunoassays for measurement of abscisic acid and gibberellins during floral inductive treatments in *Citrus latifolia* Tan. PhD Diss., Univ. of Florida, Gainesville, Fla.
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16. Southwick, S.M., T.L. Davenport, and A.L. Castro. 1984. Development of radioimmunoassays for GA₃ and (+) ABA for use in the study of flower induction in 'Tahiti' lime (*Citrus latifolia* Tan.). Proc. 11th Ann. Meet. Plant Growth Reg. Soc. Amer. p.59.
17. Tietz, D and A. Tietz. 1985. Less hazardous derivitization procedure for gas chromaography of plant hormone abscisic acid. J. Chrom. 325:425-429.