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THEORETICAL AND PRACTICAL IMPLICATIONS OF PLANT GROWTH SUBSTANCE TRENDS IN DEVELOPING 'FUERTE' AVOCADO FRUITS

BN WOLSTENHOLME, PJ HOFMAN
DEPTS OF HORTICULTURAL SCIENCE AND ANIMAL SCIENCE, UNIVERSITY OF NATAL, PIETERMARITZBURG
JG CUTTING, AW LISHMAN
CITRUS & SUBTROPICAL FRUIT RESEARCH INSTITUTE, NELSPRUIT

OPSOMMING

Tendense in konsentrasies van vier plantgroeireguleerders (hormone) is in 'Fuerte'-vrugte deur middel van radioimmunobepalingsmetodes bestudeer. Die saad was 'n ryk bron van ouksien sowel as die sitokinien isopenteniel ademen (2iP), met hoër konsentrasies as in die pulp. Die peil van absisiensuur het met vruggroei toegeneem. Die belangrike reguleerende rol van die saad oor vruggroei is bevestig. Teoretiese en praktiese implikasies van die resultate is bespreek.

SUMMARY

Trends in the concentrations of four plant growth substances ("hormones") were studied in developing 'Fuerte' avocado fruits using radioimmunoassay techniques. The seed was a rich source of auxin, and the cytokinin isopentenyl adenine (2iP), and concentrations exceeded those in the flesh. The inhibitor ABA built up in the flesh as the fruit approached maturity. The study confirmed the vital regulatory role of the seed in fruit growth. Theoretical and practical implications are discussed.

INTRODUCTION

Plant growth substances (PGS), perhaps better known as plant "hormones", are widely accepted as having key roles in the regulation of growth processes, including those of fruits (Leopold & Kriedemann, 1975; Goodwin, 1978). Different fruit types vary in their dependence on seeds for normal fruit growth. Fruits such as strawberry (Nitsch, 1950) and avocado (Blumenfeld & Gazit, 1974), depend on seed(s) for virtually the entire period of fruit development. At the other extreme are vegetatively parthenocarpic fruits such as the cultivated banana, where even pollination is absent and seeds do not form, although ovules are present in the flower (Luck will, 1981).

A number of pioneering PGS studies on avocado fruits were reported from Israel in the 1970's. The paper chromatographic and bioassay techniques gave quantitative estimates of PGS activity, but were less accurate, sensitive and specific than modern physico-chemical and other techniques available today. Gazit & Blumenfeld (1972) found auxin activity in young fruit, and higher levels of auxin in seed and Testa at all

stages of fruit development than in the surrounding fruit flesh (mezoand endocarp). Blumenfeld & Gazit (1972) noted high levels of gibberellin activity in seed and Testa of developing avocados, the latter decreasing with fruit growth, but no measurable gibberellins-like activity in fruit flesh or in the embryo. They suggested that the Testa was the site of production of gibberellins in the avocado fruit.

High levels of cytokinin-like activity were found in both embryo and Testa of avocado, the levels declining as the fruit approached maturity (Blumenfeld & Gazit, 1970b). In another study, cytokinin-like activity in the flesh was found to be very low, decreasing with fruit development (Gazit & Blumenfeld, 1970). There is still uncertainty whether fruits and seeds in general synthesize cytokinins, or receive them from sap or roots (van Staden & Davey, 1979).

Bittner, Gazit & Blumenfeld (1971) detected an inhibitor in avocado flesh which they concluded was not ABA. Its level increased as fruit growth slowed. A later study (Gazit & Blumenfeld, 1972) detected three inhibitors, one of which had co-chromatographic properties similar to ABA and remained at a nearly constant level through fruit development. Adato, Gazit & Blumenfeld (1976) reported that ABA levels were nearly constant during avocado fruit maturation but rose during ripening (post-harvest) apparently as a result of synthesis rather than release from the bound form. According to Adato & Gazit (1977) there is a positive correlation between fruitlet and fruit drop and ethylene production, which occurred mainly in the seeds of young fruitlets with defective seeds. ABA levels in young abscissing fruit were 7 times higher than in normal fruits.

The many criteria which have been used to determine harvest maturity in avocado have recently been reviewed by Lee (1981). It is noteworthy that several researchers have pointed out the importance of the seed in avocado fruit development, influencing growth, size, shape and maturation of fruits. The threshold of avocado fruit maturity appears to correlate well with the maturity of the Testa (Erickson, 1966; Blumenfeld & Gazit, 1970a, 1974; Tomer & Gottreich, 1978).

Radioimmunoassays of PGS are now widely accepted. They have the prime advantages of short assay time, the potential for large sample throughput, reduced purification of the biological extract, high specificity based on the unique antigen: antibody response, and great sensitivity (Weiler, 1984).

The main objectives of this study were to apply RIA techniques which were developed, optimized and validated in our laboratory (Cutting, Lishman, van der Hoven & Wolstenholme, 1983; Cutting, Lishman, Hoffman & Wolstenholme, 1984; Cutting, Hoffman, Lishman & Wolstenholme, 1984) to avocado fruit development. We also believe that this is the first multi-PGS study using modern quantitive techniques conducted on avocado fruits. It was hoped that the information obtained would supplement the earlier studies, and provide a sound base for further investigations of avocado fruit problems, theoretical *as* well as practical.

MATERIALS AND METHODS

Samples of 'Fuerte' fruits were obtained from an inland, moist escarpment orchard at Claridge near Pietermaritzburg, representative of the Natal mist belt of Bioclimatic

Group 3. All samples were from a single early-flowering tree, considerably in advance of the average stage of development for this late-maturity orchard. Composite samples were collected at approximately two to four week intervals, depending on the stage of fruit development, from September, 1983 to early April, 1984. Samples were rapidly frozen and stored at -20ºC prior to extraction.

The first four fruit samples could not be meaningfully separated into the morphological components of interest, and were therefore pooled as whole fruit samples. All subsequent samples were divided into skin (exocarp), flesh (mesocarp plus endocarp), testa, and the remainder of the seed (embryo, consisting of cotyledons plus embryo axis). The mass and length of all fruits and components were determined.

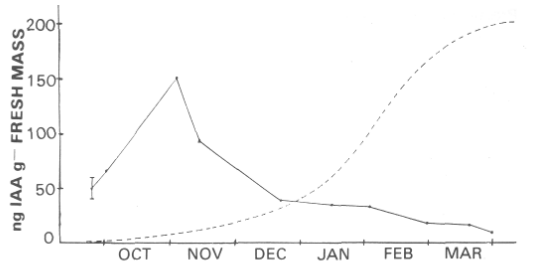

Fig. 1: Changes in IAA levels in developing 'Fuerte' fruit (solid line) in relation to growth (broken line). The results are for pooled whole fruit. The assay SE is presented.

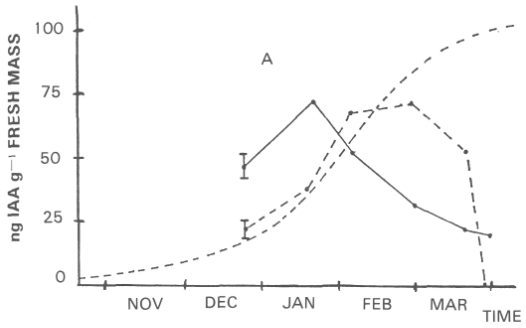

Fig. 2 A The levels of IAA in the 'Fuerte' seed minus testa (solid line) and testa (broken line) with the growth curve superimposed. The assay SE for seed and testa are given.

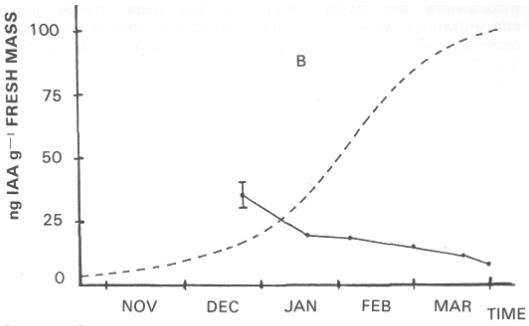

Fig. 2 B The levels of IAA in the 'Fuerte' flesh (solid line) with the growth curve superimposed. Assay SE for the flesh is presented.

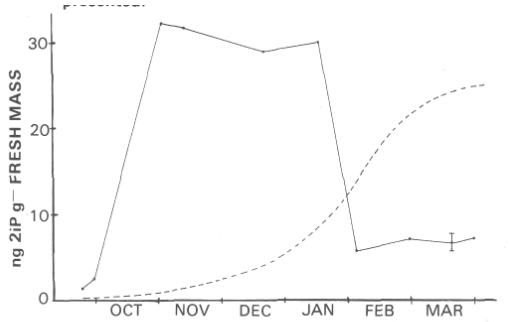

Fig. 3: The levels of the cytokin 2iP in 'Fuerte' fruit (solid line) in relation to growth (dotted line). Results are for pooled whole fruit. The assay SE is presented.

Radioimmunoassay for the four PCS investigated involved five major steps as summarized by Cutting *et al.* (1984). These were firstly the coupling of each PCS to the protein carrier bovine serum albumin (BSA). The production of anti-sera to the inoculated conjugate took place either in rabbits, for the two cytokinins isopentenyl adenine (2iP) and isopentenyl adenosine (IPA), and for the inhibitor abscisic acid (ABA); or in sheep for the auxin, indole-3-acetic-acid (IAA). The third step involved acquisition of tritium -labelled custom preparations (IPA, IAA) or catalogue items (ABA) of the PGS, and their purification by column or thin-layer chromatography (TLC) where necessary. The plant material (fruits) was then homogenized, extracted in 80% or 90% ethanol, centrifuged, and, for the two cytokinins, further purified by TLC. Details of the actual radio-immunoassays are provided by Cutting *et al.* (1983, 1984).

RESULTS

Fruit growth curve. The fruit growth curve (mass basis) is given in Fig. 1. A typical sigmoid growth curve was obtained in accordance with Val mayor (1964), Robertson (1971), Blumenfeld & Gazit (1974), and bar more (1977). About 26 weeks after the closed flower stage (i.e. early April) these fruits were adjudged to have reached minimum "legal maturity". This was largely based on the appearance of the testa (Erickson, 1966), which had changed from thick, white and fleshy to thin, dry and membranous, tightly enfolding the seed. Such fruits softened normally within 7 days at room temperature, and were organoleptically acceptable. It is stressed that these fruits were from a particularly early-flowering tree. More typical 'Fuerte' fruits from the same orchard continued slow growth through autumn and the following spring, with a winter (June/July) plateau, and reached equivalent legal maturity in mid-to late April (McOnie & Wolstenholme, 1982; van den Dool & Wolstenholme, 1983).

Auxin (IAA) Levels of IAA equivalents in pooled whole-fruit samples are given in Fig. 1, and for various fruit components in Fig. 2 A & B.

In whole-fruit samples, IAA peaked at about 150 ng g^{-1} in late October / early November when the fruit mass was about 10g. The concentration fell rapidly to less than 50 ng g^{-1} in December, and then more slowly to about 10 ng g^{-1} at the end of March. It was presumed that most of this activity was associated with seed components, which could not be separately assayed until mid-December

From mid-December, fruit components were separately analyzed. The seed (embryo, excluding testa) gave a high reading of about 70 ng g^{-1} in mid-January, declining steadily to 22 ng g^{-1} by the end of March — all these values exceeding those of the flesh. The testa, which was very prominent and fleshy until mid-March, peaked later than the seed at 70 ng g^{-1}, where after levels declined rapidly to zero coincident with testa maturity (drying and shriveling) (Fig. 2A).

Auxin levels in the fruit flesh (Fig. 2B) showed a steady decreasing trend from 30 ng g^{-1} in the mid-December sample to 10 ng g^{-1} at testa maturity at the end of March.

Cytokinins (2iP and IPA) of the two cytokinins analyzed, 2iP appeared to play the more prominent role in fruit development. 2iP equivalents a re given in Fig.3 for whole-fruit-samples, and Fig.4 A& B for fruit components.

From a very low level for the first fruitlet sample in September, 2iP equivalents rose rapidly to a peak of 32 ng g^{-1} in late October. A high level was maintained until the mid-January sample, followed by a marked decline to 5 to 6 ng g^{-1} for all subsequent whole-fruit samples (Fig. 3).

From Fig. 4A it is apparent that the seed (excluding testa) had a high level of above 30 ng g^{-1} 2iP for the December and mid-January samples, declining to 18 ng g^{-1} at the end of January, and about 14 ng g^{-1} by the end of March. Concentrations in the testa peaked later at over 30 ng g^{-1} in early February, thereafter falling rapidly, with no detectable 2iP in the mature, dried testa at the end of March.

Trends for IPA levels are given in Fig. 5 (whole fruit) and Fig. 6A & B (fruit components). In the whole fruit, levels were always below 5 ng g^{-1} (Fig. 5). Similar low levels were found in the seed (excluding testa) and especially the flesh (Fig. 6A & B). The testa was the only component to show a sharp peak during fruit development, this occurring in the mid-March sample (23 ng g^{-1}), with a decline to Zero in the dried testa two weeks later (Fig. 6A).

Free abscisic acid (ABA). The general trend was for ABA levels in whole-fruit samples to increase with time, although the mid-January sample had a lower level than the previous sample. At the time of testa maturity at the end of March, the level was 90 ng g^{-1} (Fig. 7).

ABA levels in the seed (excluding testa) varied between 35 and 65 ng g^{-1} from mid-December to the end of March, with an inconsistent but rising trend. For the testa, trends and concentrations were similar until mid-March, after which ABA declined to an undetectable level within two weeks (Fig. 8A). In the fruit flesh, ABA levels rose from the December early February plateau of around 40 ng g^{-1} to reach a high level approaching 100 ngg^{-1} at testa maturity (Fig. 8B)-these levels exceeding those of the seed in the last few weeks of fruit development.

DISCUSSION AND CONCLUSIONS

In most respects our results (summarized in Fig. 9) do not differ materially from the earlier Israeli studies, although the research is not always directly comparable due to differences in technique. Our measured values of IAA, 2iP, IPA and ABA are well within the ranges reported for fruits in the literature. Unlike Blumenfeld & Gazit (1970), who failed to detect endogenous cytokmin-like activity in the fruit flesh (as opposed to supposed "bound" cytokinins), we found both 2iP and to a lesser extent IPA to be present. Studies are also required on other physiologically-active cytokinins such as zeatin and zeatin riboside, but we have not been successful in developing RIA's for these.

This study also found a steady increase in ABA concentration in fruit flesh in late summer and autumn to levels in excess of those of the embryo (seed excluding testa). This contrasts with Gazit & Blumenfeld (1972) & Adato *et al's(* 1976) conclusion that ABA was constant in the flesh during fruit development. The explanation is possibly slight water stress in the non-irrigated orchard. Other inhibitors were not investigated. We also did not study gibberellins or ethylene in fruits.

Based on the local and Israeli PCS studies, supplemented by Blumenfeld & Gazit's (1974) study of seeded and seedless avocado fruit development, a tentative model is offered in Fig. 10. The objective is to summarize our present knowledge of avocado fruit and seed development and its presumed control by balances, synergisms and interactions of PGS, acting sequentially. The time axis is for atypical warm lowveld area with Fuerte fruit maturity in April, preceded by seed coat maturity (transition from thick, white and fleshy to thin, brown and membranous) in late March/early April. We believe that this is probably the best criterion of the onset of "legal maturity" in avocado fruits.

Our results again emphasize the vital importance of the seed in avocado fruit growth, in agreement with Blumenfeld & Gazit (1974). These authors pointed out that seeded fruits are larger than seedless fruits ("cukes") by a factor of about 10. Cummings & Schroeder (1942) noted that almost all the vascular bundles in the flesh join together to enter the seed coat at its distal end, from where they form a branched network in the testa. When the testa shrivels and dries (end of March for 'Fuerte' in warm conditions or in early-flowering trees), the seed is cut off from its nutrient supply, and the fruit growth rate drops markedly. Thus any fruit with a brown, membranous testa has the potential for only very slow subsequent growth. If this occurs early (eg in January) when the seed is still small, the fruit will remain relatively small with an elongated, thinner shape. Such fruits have up to twice the oil percentage of normal fruits, and soften very quickly once harvested.

It is generally accepted that seeds control fruit growth by synthesizing or attracting PGS, which then become available to the fruit flesh. PGS studies on avocado fruits appear to support this interpretation. For all promotive PGS studied, concentrations were higher in the seed than in the flesh. The nutritive endosperm of the seed is, according to Israeli studies, a potent source of IAA, gibberellins and cytokinins and probably the main source in the first two months after fruit set. When the endosperm has been used up (about January), the testa has become very prominent and a rich PGS source, together with the embryo of the seed. Testa concentrations of PGS peaked in March in this

study, and then rapidly declined to zero. At this stage the seed appeared to lose its controlling role over the fruit, which grew much more slowly and rapidly accumulated oil. Slower fruit growth was possibly also due to increase in ABA concentration in the fruit flesh.

The avocado seed must be thought of as a large storage organ which renders a service to the tree during the major part of fruit development. As such it is also a store of mineral elements, which change in concentration and in absolute amount during fruit growth. Haas (1951) pointed out that avocado seeds contain relatively small amounts of Ca and Mg. Perhaps significantly, Fuerte seeds appeared to have lower Ca and higher Mg levels, and a lower Ca: Mg ratio, than most other cultivars studies. The seed contained somewhat higher amounts of P, and high amounts of K. About half the K and substantial amounts of Ca and Mg were lost from the seed as the fruit matured.

Practical implications of these studies are the following:

1. In general terms, the larger the seed, the larger will be the fruit. Small fruits are more likely to be due to premature seed (testa) maturity than to later fruit set. The seed is a strong physiological "sink" from which the flesh benefits.

2. Conditions during the first few months after fruit set, when the potential for cell division in the whole fruit is greatest, are vitally important. If trees suffer physiological stress during this period, the "sink" capacity of the fruits is likely to be weakened-resulting in earlier seed maturity and smaller fruits (especially with the competition of a large crop).

3. Once the seed is mature (when its testa dries), the potential for further fruit growth is limited. Slow fruit growth certainly occurs, as many studies have shown. This is made possible by continued slow cell division as long as the fruit is firmly attached to the tree (Schroeder, 1953). This cell division in turn is probably made possible by the self-sufficiency of the flesh at this time for low concentrations of promotive PGS, especially cytokinins, which are however antagonized by the buildup of ABA particularly under moisture stress conditions.

4. The maturity of the testa is an excellent guide to the onset of overall fruit maturity (minimum legal maturity). Unfortunately testa maturity is likely to be affected by many variables, including the position of the fruit in the tree relative to competing sinks and sources of assimilate. "Intermediate" type fruits which are small and obviously thinner in shape should be harvested early, as they will never size up sufficiently and are prematurely high in oil (and dry matter). They are also likely to soften prematurely if exported by sea.

Further basic studies of this nature are needed, in particular a comparison of the PGS changes in large versus small but normal-shaped fruits in 'Fuerte' and also in 'Hass', which is renowned for its "small-fruit" problem in heavy-bearing older trees. Although significant progress has been made, our understanding of the PGS trends in avocado fruits is still very incomplete.

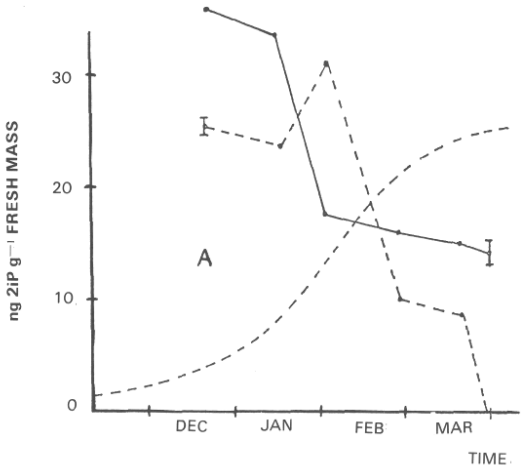

Fig. 4. A 2iP levels in seed (minus testa) (solid line) and testa (dotted line) in relation to stage of growth (dotted line). The assay SE is presented.

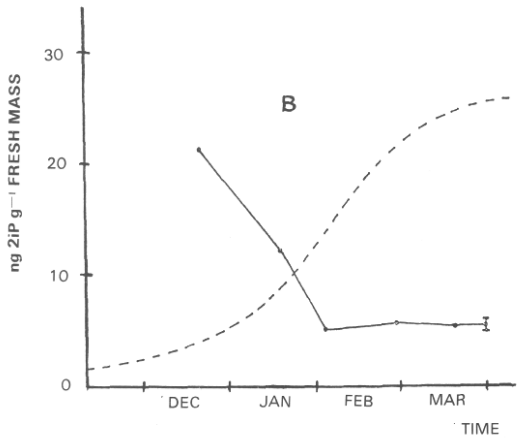

Fig. 4. B 2iP levels in the flesh (solid line) in relation to growth (dotted line) with the assay SE presented.

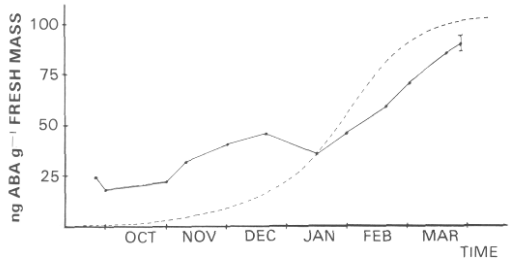

Fig. 5: IPA levels in 'Fuerte' fruit (solid line) in relation to growth (dotted line). Results are for pooled whole fruit. The assay SE is presented.

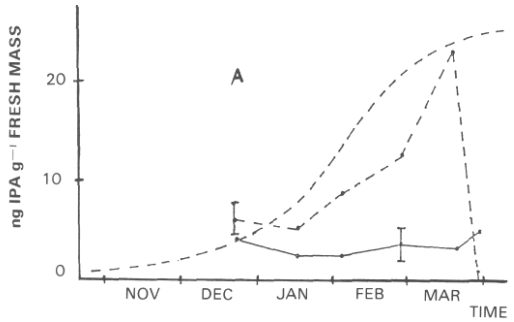

Fig 6 A IPA levels in seed minus testa (solid line) and testa (broken line) in relation to stage of growth (broken line). The assay SE is presented.

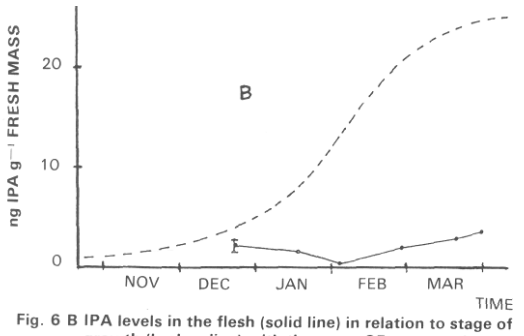

Fig. 6 B IPA levels in the flesh (solid line) in relation to stage of growth (broken line) with the assay SE presented.

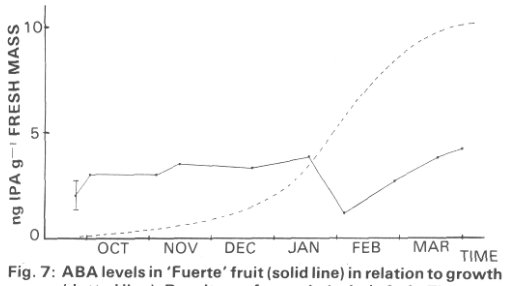

Fig. 7: ABA levels in 'Fuerte' fruit (solid line) in relation to growth (dotted line). Results are for pooled whole fruit. The assay SE is presented.

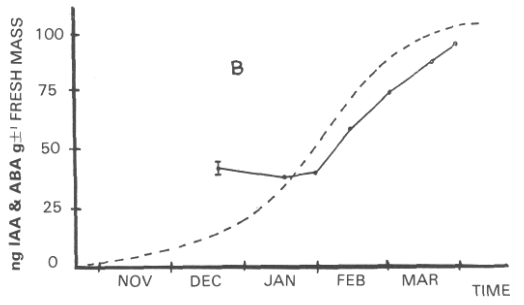

Fig. 8 A ABA levels in seed minus testa (solid line) and testa (broken line) in relation to stage of growth. The assay SE is presented.

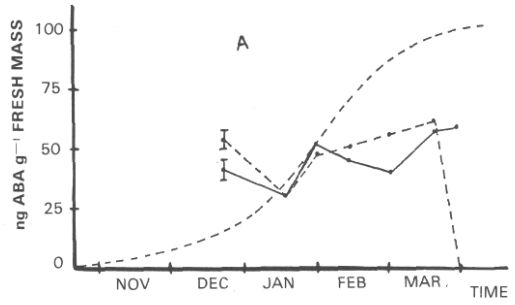

Fig. 8 B ABA levels in the flesh (solid line) in relation to stage of growth with the assay SE presented.

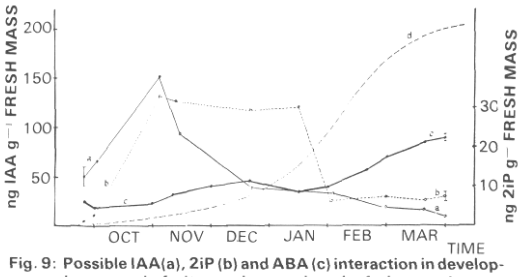

Fig. 9: Possible IAA(a), 2iP (b) and ABA (c) interaction in developing avocado fruit superimposed on the fruit growth curve (d).

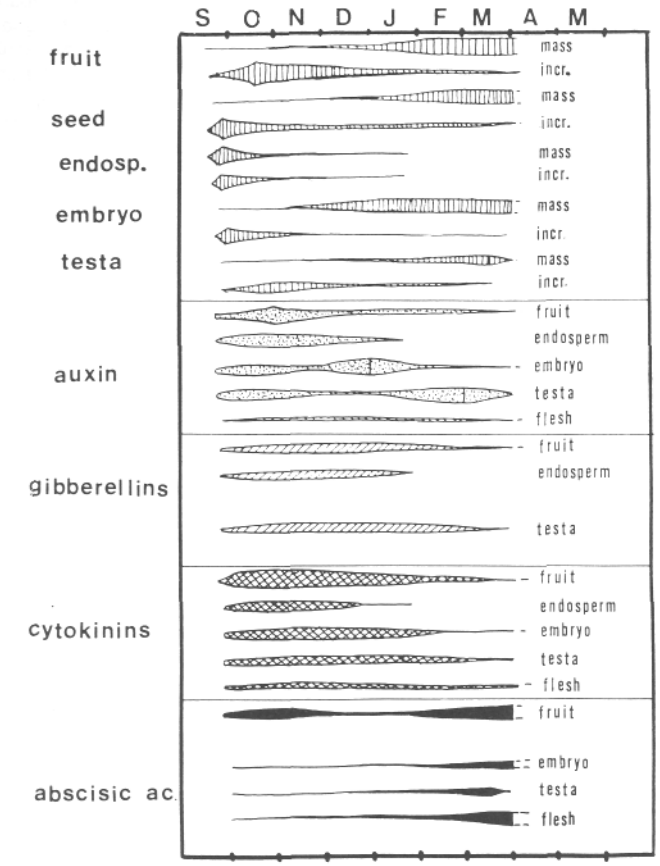

Fig. 10 Tentative representation of 'Fuerte' avocado fruit and seed growth patterns and PGS trends in a warm environment.

ACKNOWLEDGEMENT

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