Hormonal systems in avocado fruits

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The role of the seed and the pericarp of the avocado fruit in supplying growth substances to the developing fruit was studied. We examined the relation between the development of the whole fruit, its various tissues and the changes in growth substance levels in these tissues. An attempt was also made to evaluate the status of growth substances in avocado mesocarp which differs in a number of important physiological factors from the mesocarp of other fruit, particularly cell division, which lasts as long as the fruit is on the tree. We compared the levels of growth substances in the mesocarp of seedless and normal fruit, as well as the growth rate of these fruit, and attempted to relate these differences to the presence or absence of an active seed.

In the first stages of the work, we studied the development of normal avocado fruit, the rate of growth of its different tissues, and at the same time, the rate of growth of seedless fruit. It was found that at the beginning of the development of normal fruit, the endosperm and seed-coats are larger than the embryo. Thereafter, the embryo grows at a very rapid rate, until the time it is severed from the source of food supply due to the drying of the seed-coats. The seed-coats shrivel before the diminution in growth rate of the mesocarp and the rapid accumulation of oil, which is the measure of fruit maturity.

It was found that wounding the seed at early stages of fruit development caused fruit drop, while a similar injury after seed-coat shrivelling did not cause abscission. Experiments in which we attempted to disconnect the seed-coats from the mesocarp, or to remove the embryo from the fruit, failed for this reason. It was found that fruit in which the seed-coats shrivelled earlier than usual, but after the wave of natural fruit drop occurring in July, was generally small and thin and tended to ripen early.

It seems that the difference between seedless and normal fruit is established very early on in fruit development since, from approximately two months after fruit set, the growth rate (additional growth relative to previous weight) of normal and seedless fruit was similar. though there was a large difference in their absolute weight.

During the very early stages of fruit development, cell number and diameter along the radius of a cross-section through the fruit was found to be similar in both types of fruit. Later, cell number and diameter increased at different rates in the two kinds of fruit. Growth in normal fruit was more rapid and the increase in cell number was greater than the increase in cell diameter.

Levels of growth substances were determined in samples collected at different dates during fruit development, beginning about one month after fruit set and ending after maturation. Parallel determinations of auxins, gibberellins, cytokinins and inhibitors were made on the same sample. The following fruit parts were examined: mesocarp, seed-coat endosperm and embryo.

The tissues were extracted with aqueous methanol, after which the active materials were fractionated between different solvents, at different pH values. These fractions were separated further by paper chromatography using an isopropanol, ammonia, water 10:1:1 solvent. Each

chromatogram strip was cut transversely into 10 segments, the biological activity of the segments being determined by bioassays.
Considerable effort went into testing and adapting different bioassay procedures to our conditions which have succeeded in improving techniques and apparatus so that the study could be conducted on a large scale.

Auxins: The activity of auxins in the extracts was studied by the wheat coleoptile elongation test. Two regions of activity were detected on the chromatograms. One was at Rf 0.3-0.5, corresponding to indole-acetic acid (IAA) in its chromatographic location and fractionation characteristics. A second zone of activity was found at Rf O.9. The active material in this zone behaves in a manner similar to indole-aceto-nitrile (IAN) on fractionation and chromatography. Further study is required for definite identification of these two substances.

The first promotive zone was found in all the fruit tissues. Its activity in embryo and seed coat extracts diminished during development of these tissues. No correlation was found between the level of this activity and the growth of the mesocarp. A causal relationship may exist between low auxin levels in the mesocarp and the wave of natural fruit drop occurring in the variety ‘Fuerte’ during July. The second promoter was detected in seed extracts only. It either does not exist in the mesocarp or is masked by inhibitors which we were unable to separate. Levels of the promoter decreased in the seed as the fruit developed.

Gibberellins: Gibberellin-like activity in the extracts was determined by the barley endosperm bioassay, which is known to respond to a large number of gibberellins. Levels of activity found in the endosperm and seed-coats were very high and high respectively during the early stages of fruit growth. As the fruit matured and the rate of growth of the mesocarp diminished, the gibberellin-like activity in the seed-coats gradually decreased. Activity was almost equally divided between the aqueous and the acidic ethyl acetate fractions. Part of the gibberellin activity can probably be attributed to the glycosides of the gibberellins. It was possible to obtain free gibberellin, soluble in ethyl acetate, from the gibberellin found in the aqueous fraction of the seed-coats, after acidic or enzymatic hydrolysis with b-gluco-sidase. The gibberellin activity found in the seed-coats was considerably higher than that found in adjacent tissues, i.e. the embryo and mesocarp. from this, we assume that gibberellins are produced in the seed-coats.

The level of activity in the embryo and mesocarp was very low, and close to the sensitivity limit of the test used. The significance of the fact that tissues very rich in gibberellins (endosperm and seed-coats) are located next to rapidly growing tissues in which gibberellin activity is difficult to detect, is not clear. The mesocarp and embryo may contain gibberellins in a "bound" form which does not exhibit any activity in our test methods. This may be a similar condition to the "bound" cytokinins to be described below and should be examined in the future.

Cytokinins: Cytokinin activity was tested in the soybean callus bioassay. In spite of its long duration, this test was chosen because it is specific for cytokinins and is based on cell division. Three zones of cytokinin activity were detected on the chromatograms at Rf 0.0-0.2, Rf 0.3-0.4, and Rf 0.6-0.7. Due to the different fractionation between solvents and their Rf values in different solvent systems in paper chromatography, we assume that the activity can be attributed to three forms of cytokinin: nucleotides, nucleosides, and free bases.

High levels of cytokinin activity were found in the seed-coats, and especially in the endosperm. Their activity in the seed-coats was very high in young fruit, and decreased as the fruit developed, similar to the changes observed in gibberellin-like activity. There was a positive correlation between the reduction in level of these two groups of growth regulators and the slowdown in growth rate of the seed-oats. In the embryo, the levels were high during the first three months of growth. Thereafter, they decreased somewhat, but still remained high, even during the period when the seed-coats shrivelled and the seed separated from the mesocarp.

Cytokinins were found in the mesocarp only during the first two to three months after fruit set. It was not possible to detect any cytokinin activity in mesocarp extracts from later samples, even though this tissue continued to divide. We did, however, find cytokinin activity in these extracts following acidic hydrolysis, or after they were passed through acidic ion exchange columns. We have designated the substances responsible for this activity as "bound cytokinins". Their level was found to be positively correlated with the rate of growth of the mesocarp. It is quite possible that bound cytokinins exist in other fruit, as well as in other plant tissues. Their presence may have been overlooked since in the methods employed to detect cytokinin activity in those tissues, acidic columns which release these bound cytokinins were used. The activity of extracts after passage through these columns was often attributed to the removal of inhibitors, without first examining whether such inhibitors were actually present before transfer through the columns. It would be of interest to clarify whether this bound form is the active state of cytokinins in the plant, and what is its chemical composition.

Inhibitors: Inhibitor activity was assayed in all extract fractions by all three bioassays. Three inhibitory zones at Rf 0-0.1, Rf 0.6-0.8 and Rf 0.8-1.0 were found on the chromatograms. We named these Inhibitors A, B and C, respectively. The last two were given more attention than the first one.

Inhibitor B is similar to abscisic acid (ABA) in its fractionation, chromatography and activity in the three bioassays. The level of its activity in the mesocarp did not greatly change during the season. We assume that if Inhibitor B is active in regulating growth processes, its effect is not due to changes in its absolute level, but rather to changing ratios between levels of the inhibitor and other growth substances. A new interaction between abscisic acid or Inhibitor B on the one hand and kinetin on the other was detected. We found that high levels of either abscisic acid or Inhibitor B together with high levels of kinetin, synergistically stimulated the growth of soybean callus.

On the basis of this finding and similar interactions of abscisic acid with other promoters, we assume that in certain biological systems, abscisic acid can act as a synergist for other growth substances. This may explain the presence of high levels of inhibitor in rapidly growing tissues. This phenomenon was previously left unexplained, or was interpreted as inhibitor regulating high levels of promoters present in those tissues.

Especially high levels of Inhibitor C were found. This is a non-specific inhibitor which inhibits the growth of wheat coleoptiles and soybean callus, but does not kill them. It acts as an anti-cytokinin in the soybean bioassay. Its inhibitory activity is almost completely cancelled out by

increasing the kinetin level in the soybean test. In the wheat coleoptile test however, addition of kinetin or IAA does not modify its activity.

The amount of Inhibitor C in the mesocarp increased as the fruit matured, and a negative correlation was found between inhibitor levels and the rates of fruit cell division. Possibly this substance, together with cytokinins, regulates the rate of cell division and mesocarp growth. It is also conceivable that it has no physiological task but only accumulates in the fruit as it matures. We are unable at the present to determine which of the above two is the case. Its function may be understood more fully if a way can be found to add inhibitor to young fruit exogenously (at a time when the endogenous level of the inhibitor is low) and subsequently fruit growth and cell division are followed.

By comparing growth substance levels in the mesocarp of seedless and normal fruit, we found that a marked difference only existed in the level of cytokinins. The greater cytokinin activity of normal mesocarp is in accordance with the more rapid cell division of this tissue. From this, as well as the lack of correlation between other growth substances and the growth of the mesocarp, we conclude that the growth of avocado mesocarp depends primarily on the presence of cytokinins.

We have proposed a number of hypotheses to explain the source of growth substances in fruit tissue. We found xylem sap centrifugates to contain auxins, gibberellins, cytokinins and Inhibitor B, whereas bark extracts contained the above regulators and Inhibitor C. From these findings we surmise that the fruit receives a supply of growth substances with the photosynthetic and transpiration streams. The fruit itself may be another source of these regulators. High levels in some tissues as opposed to low levels in adjacent tissues was used by others as evidence for the production of growth substances in certain tissues. We suggest that gibberellins and maybe other growth substances are formed in the endosperm and the seed-coats, because the level of gibberellins in these tissues is outstandingly high when compared to the level in adjacent tissues. We further suggest that cytokinins are produced in the embryo. This hypothesis is based on experiments with tissue cultures of avocado cotyledon callus in which we showed that this callus has the ability to produce cytokinins de novo. The production of growth substances in seed tissues can explain the higher levels found in the seed compared with those in the mesocarp. A second explanation could also be proposed: since the seed is located at the end of a transport system, substances may accumulate there. Our knowledge of the relatively rapid production and destruction of growth substances does not make this explanation plausible. Since we found no correlation between the high level of growth substances in the seed and the levels found in the mesocarp, we have no possibility of determining whether the seed supplies these substances to the fruit. There is, however, a possibility that the seed supplies regulators to the mesocarp, but that these become "bound" in the course of their activity. Another possibility which has been raised is that the seed acts as a sink for growth substances which are transported through the mesocarp in a manner similar to the transport of nutrients.

The presence and pattern of change in levels of the various growth regulators in the different tissues was repeatedly observed during the years over which the investigation was conducted. We conclude that no single growth substance regulates any specific stage of development in avocado fruit, but that the activity of several regulators in the different tissues is important.

In conclusion, we propose a scheme to describe the changes in level of growth regulators occurring during avocado fruit development, relating these changes to the growth and development of individual tissues and the fruit as a whole. This scheme is based on data which is more complete than that available for "fruit development schemes" found in the literature.

We know that changes in levels of endogenous growth regulators are different in various fruits and that these fruits respond differently to exogenous applications of growth regulators. It seems, therefore, that no universal scheme describing the changes in growth regulators occurring during fruit development can be devised. Our findings may only be applicable to the relation between growth substances and the development of avocado fruit, although certain details may be similar in other fruit.