DEVELOPMENTS IN PLANT GROWTH REGULATORS: MANIPULATION OF VEGETATIVE GROWTH, FLOWERING AND FRUITING OF AVOCADOS

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The goal

It has long been the goal of growers and researchers alike to be able to manipulate the vegetative and reproductive growth of crop plants. Avocado growers and researchers are no exception. Plant growth regulators at the present time are perhaps the most powerful tools available for achieving this goal. However, in the specific case of avocado production, the use of PGRs remains underdeveloped despite the enormous potential that PGRs offer for maximizing yield, solving fruit quality problems, and increasing net dollar return to the grower. In contrast, for a wide variety of tree and vine crops there are many examples of the successful use of PGRs to solve production problems. PGRs have been used successfully as foliar sprays to increase flowering, synchronize bloom, or change the time of flowering to avoid adverse climatic conditions or to shift harvest to a time when the market is more economically favorable. Foliar-applied PGRs are routinely used to improve fruit set, reduce June drop or to prevent pre-harvest drop to increase yield. PGRs sprays are applied to increase fruit size directly by stimulating cell division or to increase fruit size indirectly by decreasing fruit number through the application of PGRs that reduce the number of flowers formed or promote flower or fruit abscission. PGRs have been used as both pre- and post-harvest treatments to hasten or slow the ripening process, color development, and maturation of specific fruit tissues to improve the quality of the product sold in the market. More recently, success has been achieved using PGRs to even out alternate bearing and increase cumulative yield for multiple alternate bearing cycles. The emerging use of PGRs to overcome the adverse effects of abiotic stresses is increasingly successful. Surprisingly, these successes have been achieved with a modest number of commercial PGRs that are members of one of the five classic groups of plant growth regulators: auxins, cytokinins, gibberellins, abscisic acid and ethylene. The roles of these PGRs in plant development will not be reviewed here as the information can be found in an introductory biology or botany textbook if not already known by the reader and because the information is not critical to the discussion that follows.

The goal can be achieved

If PGRs can be used successfully to solve production problems in other tree crops, they can be used to solve these same problems in avocado production. There is no general formula for the rapid transfer of PGR technology that works in one crop to another. At best, the existing technology is a good starting point. Each avocado production problem to be solved requires an evaluation of what is already known and what additional information is required. Unfortunately, battle lines are often drawn over whether field or basic research should be conducted to obtain the required information. Field research conducted with what some might consider limited information is frequently criticized as “spray and pray”. Whereas basic research to identify the roles of endogenous PGRs might provide insight into the mechanism controlling a specific process, it is also without the guarantee of a useful outcome. We need to agree that all information is valuable and collect as much as we can. Even experiments that produce no significant results are informative. Their results should be published to help us hone in on what works and to save us from repeating what does not. Even with a good understanding of the roles of individual PGRs in a physiological process, a certain amount of “educated guessing” is required to initiate field research. This is because PGR treatments are only effective at specific stages of organ and tissue development and only produce the desired outcome within a narrow concentration range. Thus, a number of application times and concentrations typically must be tested to determine those that are optimal. I strongly believe that basic knowledge of the timing of key developmental events and an ability to predict when they occur each year based on avocado tree phenology and frequent evaluation of morphological markers, not calendar dates, is critical to obtaining reproducible responses to PGR treatments. In addition, reference to tree phenology makes it possible to transfer the technology from one avocado-growing area to another. Temperatures in the days following the application of the PGR can influence the result, as can the nutritional status and health of the tree. Hence, the technology must be tested in multiple orchards over several years in order to develop a commercial recommendation. Clearly, the more similar environmental and endogenous tree conditions are from year to year and orchard to orchard, the more reproducible and reliable the outcome will be. The time and cost associated with developing a PGR recommendation for an avocado industry could be considerable. Adding to the expense is the fact that many countries have rules restricting the use of PGRs on crops for which they are not registered. Hence, the crop on PGR-treated trees must be destroyed and the grower reimbursed for his/her financial loss. In California GA_3 is the only PGR that can be used on avocados without having to destroy the crop.

Consumers are increasingly concerned about how safe their food is. Use of synthetic PGRs in food production is coming under increased scrutiny. Rightly or wrongly, more and more ag-chemicals are being labeled as risks to human health and the environment. Algal extracts and fermentation products offer a “green” alternative to synthetic cytokinins that are worth investigating. Some, but not all, companies standardize the amount of cytokinin or cytokinin-activity in each batch. Keep in mind, however, that algal-based products contain other PGRs, e.g. the auxin indoleacetic acid (IAA) and abscisic acid (ABA), the concentrations of which are not typically monitored from batch to batch. Hence, the efficacy and reliability of individual products must be determined.

Despite the long-standing problems associated with PGRs enumerated by Cutting and Wolstenholme in 1993, the number of both synthetic PGRs and natural product bio-stimulants marketed has increased significantly. Unfortunately, manufacturers and distributors have limited information about how to best use their products, especially with regard to avocado. The good news is that the tools we need to manipulate avocado vegetative and reproductive growth are probably all available. Now, we have to figure out how to make them work to meet our needs. Our long-term goal should be nothing less than the ability to manipulate all the key physiological processes of the avocado. To this end our immediate objective must be to obtain knowledge of these processes and the response of the tree to available PGRs.

Small steps toward the goal

One step

Alternate bearing and low fruit set are two major problems associated with the commercial production of the 'Hass' avocado in California. Increases in the cost of water, labor and other inputs dictate that we achieve an increase in yield per ha. When avocado trees produce a heavy “on” bloom and crop, vegetative growth is “off” (Salazar-Garcia et al., 1998a). Thus, there are fewer shoots to bear flowers the next year. We (Salazar-Garcia and Lovatt, 2000) conducted a 2-year-long study with 10-year-old ‘Hass’ avocado trees on Duke 7 rootstock in a commercial orchard in Corona, CA, with the goal of the research to identify a strategy using GA_3 to even out alternate bearing and concomitantly increase cumulative yield. GA_3 (25 or 100 mg/liter plus Triton X-100 at 1 ml/liter; pH 5.5; 8 ± 1 liter/tree) was applied to separate sets of trees (20 individual tree replicates per treatment) in September (beginning of inflorescence initiation), November (end of inflorescence initiation), January (initial development of the perianth of terminal flowers), March (cauliflower stage), or monthly sprays from September through January. Control trees did not receive any treatment. September sprays with GA_3 at 100 mg/liter reduced production of inflorescences both years with a commensurate increase in vegetative shoot production. The research was initiated in September when the trees were carrying a heavy on-year crop (1995). November sprays with GA_3 at 100 mg/liter reduced the number of inflorescences produced in the off_{1996} year with a concomitant increase in production of vegetative shoots; there was no effect in the on_{1995} crop year. January and later applications had no effect on flowering or vegetative shoot production either year. Time of flowering was not affected by GA_3 treatment. Application of GA_3 (25 mg/liter) in November, January or March stimulated the precocious development of the vegetative shoot of indeterminate inflorescences which protected the young developing fruit from sunburn.

The research was initiated when trees were bearing an on_{1995} crop in an attempt to increase the subsequent off_{1996} year crop. During the on_{1995} year GA_3 at 25 mg/liter increased yield when applied in November, January, or March (34.8, 27.3, and 33.9 kg/tree, respectively), but no treatment was significantly better than the control which averaged 18.3 kg/tree. The second year GA_3 treatments were applied in the off_{1996} crop year. Control trees yielded 79.8 kg/tree. The November treatment with 25 mg GA_3/liter reduced yield 47%, whereas 25 mg GA_3/liter in September or March increased yield (106.7 and 89.3 kg/tree, respectively) but not significantly greater than the controls. Increase in yield was not at the expense of fruit size. GA_3 treatments tended to increase the number of commercially valuable fruit, those of sizes 70 (135-177 g), 60 (178-212 g), and 48 (213-269 g), when compared to control trees, during two consecutive years. Depending on the time of treatment, GA_3 delayed blackening of late-harvested fruit. GA_3 at 25 mg/liter in November reduced the alternate bearing index (ABI) by more than 50% but also reduced cumulative yield. The treatment with the highest cumulative yield (123.2 kg/tree), with the greatest effect on fruit size and in reducing fruit blackening of late hanging fruit, and with an intermediate ABI (40.7%) was obtained with 25 mg GA_3/liter in March.

In our present research, we have built on our new knowledge of ‘Hass’ avocado tree vegetative and floral shoot phenology and on what we learned about the use GA_3. The research of Salazar-Garcia et al. (1998a) demonstrated that ‘Hass’ avocados initiate inflorescence buds at the end of July beginning of August and documented that the failure of trees to produce sufficient summer vegetative shoot growth is the major factor leading to alternate bearing. We are determining the optimal time to apply GA_3 to increase summer vegetative shoot growth with the goal of overcoming alternate bearing. An additional treatment is the application of prohexadione calcium (Apogee$_{TM}$, BASF), an inhibitor of GA biosynthesis. The application is made towards the end of July to stop vegetative shoot growth and to increase the number of buds that transition to floral meristems. The treatment might also synchronize avocado flowering. We are continuing to test the efficacy of GA_3 applied in November (end of inflorescence initiation), January (initial development of the perianth of terminal flowers), March (cauliflower stage), and April (anthesis) in an additional orchard.

Another step

The work of Salazar-Garcia and Lovatt (1998b) demonstrated that branch studies make it possible to test many compounds at several concentrations applied at several different times in the phenology of the tree. Most importantly the results obtained in the branch studies were comparable to those obtained with whole trees (Salazar-Garcia and Lovatt, 2000). Branch studies are advantageous because we know little about the response of avocados growing in California to the various existing or new PGRs. Our goal is to determine optimal dose and application times. The best treatments identified in the branch study are selected for subsequent testing on whole trees in a commercial orchard. The PGRs we are currently testing and the reason we selected each follows.

Accel$_{TM}$ (Valent BioSciences Corp.), contains the cytokinin 6-benzyladenine (1.8%) plus $GA_{4,7}$ (0.18%). Cytokinins stimulate cell division, increases sink activity to improve the ability of fruit to compete for resources (Bower and Cutting, 1988), prevents leaf abscission and aging, maintains leaves as sources of photosynthetic carbon, nitrogen and other nutrients and endogenous PGRs. This may be important during flowering and fruit set, both of which rely on resources provided by mature leaves. High levels of cytokinin during early fruit development are critical for obtaining large size fruit (Cutting, 1993; Cowan et al., 1997). Accel is registered in California for use on apples, necessitating only efficacy data to add avocado to the label.

ProGibb$_{TM}$ (Valent BioSciences Corp.,) contains 4% GA_3, which is known to stimulate cell enlargement. GAs are important in the early stages of fruit development and fruit set. Work by Salazar-Garcia and Lovatt (2000) demonstrated that GA_3 increases fruit set and size when applied in March. Other application times need to be tested with these goals in mind to complement our GA_3 research testing the use of GA_3 to even out alternate bearing and increase cumulative yield. With sufficient efficacy data, some of which must be collected in California, avocado could be added to the ProGibb label and used in commercial avocado production in California.

Tryptophan (Sigma Chemicals) is a precursor of indoleacetic acid (IAA). Our previous research in a commercial orchard showed that avocado leaves take up and transport tryptophan to developing fruit and that fruit are capable of converting tryptophan to IAA. We are now ready to test the use of tryptophan as a safe and effective replacement for 2,4-D, an ag-chemical that is not considered safe to humans or the environment.

Tryptophan, like 2,4-D, has shown an ability to prevent fruit abscission and increase fruit size in citrus (Pillitteri, 1997). At high concentrations, tryptophan can serve as a flower or fruit thinning agent.

Retain$_{TM}$ (Valent BioSciences Corp.,) contains 15% aminovinylglycine (AVG), an inhibitor of ethylene biosynthesis. There is strong evidence that ethylene is involved in the abscission of young avocado fruit even after ovule fertilization (Davenport and Manners, 1982). Thus, inhibiting ethylene biosynthesis with AVG should increase fruit set and yield. Our recent research demonstrated in branch studies that AVG increased fruit set of the 'Washington' navel orange but decreased fruit size (Gonzalez, 1999). AVG is registered in California for use on apples. Thus, the use of AVG on avocados requires only efficacy data to add avocados to the existing label and does not require full residue studies.

Apogee$_{\mathbf{TM}}$ (BASF) contains 27% prohexadione calcium, a new GA biosynthesis inhibitor. Test results on apple show that prohexadione calcium at 250 ppm inhibits vegetative shoot growth for approximately four weeks. We plan to use Apogee to inhibit the growth of the vegetative shoot of indeterminate inflorescences to reduce the competition that exists between setting fruit and the developing flush. Previous work applying paclobutrazol to inhibit vegetative shoot growth during the fruit set period was successful in increasing total yield and total number of fruit of export size (Kremer-Kohne and Kohne, 1998). Apogee offers a relatively safe alternative to the growth inhibitors paclobutrazol or uniconizol (Sunny$_{TM}$ Sumitomo Chemical), which will not be registered for use in California (United States).

Two concentrations of each compound were tested. One based on the concentrations that are effective in other crops and one concentration significantly higher to insure that we get a response and/or detect possible phytotoxicity (personal communication from Charles W. Coggins, Jr.). Each compound at each concentration was applied at full bloom, during the fruit set period before June drop (30 days after full bloom), during the early part of June drop (60 days after full bloom), and in August to increase fruit size and to detect positive or negative effects on inflorescence initiation. In addition, there are five treatments, one for each PGR, in which the low concentration of each compound (with the exception of Apogee) is applied at each of these times. There are also treatments of multiple PGRs. The research was conducted in a commercial 'Hass' avocado orchard in Carpinteria, CA.

There were a total of 58 treatments. Each was replicated on 20 individual branches. Branches 1 meter in length were tagged and the number of indeterminate and determinate inflorescences and the number of flowers in each inflorescence type were determined at the beginning of the experiments. Remaining flowers or fruit were counted monthly and immediately before any treatment application is made. All applications were evaluated for their effects on the existing crop as well as the new developing crop.

This research was conducted through one full crop cycle to harvest (approximately 18 months). No treatment significantly increased yield. Apogee (prohexadione calcium at

250 mg/liter) applied at the cauliflower stage of inflorescence development, at anthesis and during fruit set as a single treatment significantly delayed the elongation of the vegetative shoot of indeterminate floral shoots and increased fruit retention during the early drop period (May). At this time fruit length to width ratio was significantly greater for the prohexadione-Ca treated fruit. Whether this effect will persist through harvest remains to be determined. Interestingly, this treatment did not affect vegetative shoot growth. Retain also successfully increased avocado fruit retention through this period. Accel at 25 mg 6-benzyladenine/liter increased fruit abscission during the fruit set period, especially by the end of June drop. In apples 50 mg 6-benzyladenine/liter applied when the fruit are 10 mm in diameter is an effective thinning agent used to increase fruit size. However, no accompanying increase in avocado fruit size was obtained with the Accel treatment. DL-tryptophan at 10^{-10} M also significantly increased fruit abscission. Treatments showing a beneficial effect were tested further on whole trees in a commercial orchard.

And another

A basic study to determine the role of endogenous PGRs in avocado fruit set and fruit development was undertaken. Previous work by Cutting (1993) established that cytokinin concentrations are high during early fruit development and are important to achieve good fruit size. Cowan et al. (1997) demonstrated that the cause of the “small fruit size problem” of ‘Hass’ avocados in South Africa was a low cytokinin content along with high abscisic acid (ABA) concentration both of which resulted from shriveling of the seed coat. A possible additional role for the ratio of ABA to cytokinin in fruit set warrants investigation. Gazit and Blumenfeld (1972) and Cutting et al. (1985) concluded that endogenous concentrations of IAA increased the sink strength of setting ‘Fuerte’ fruit. Whether this is the case in ‘Hass’ avocado fruit remains to be determined. Also in controversy is whether high IAA concentrations lead to increased ethylene biosynthesis and greater fruit abscission in avocado (Bower and Cutting, 1988). Perhaps this is the basis for the observed increase in fruit abscission in response to tryptophan treatment. Application of GA_3 (Salazar-Garcia and Lovatt, 2000) increases fruit size, but no reference of the regulatory function of endogenous GA could be found in the literature.

Differences in the relative amounts or absolute quantities of endogenous PGRs in setting and abscising fruit will provide information for optimizing the use of PGRs in avocado production. Knowing which PGRs are present and when in the process of fruit set and development will determine which combinations of PGRs should be applied and when. There are too many possible combinations to test by “spray and pray”. Basic information is the faster means to develop effective production strategies.

In this study, trees were shaken in the evening to remove all recently abscised fruit or the ones just ready to abscise. Sixteen hours later, the trees were shaken again and fruit that abscise (abscising fruit) collected in clean nets under each of 10 trees. A second set of fruit that did not abscise (persisting fruit) was picked at a height of 1.5 m around the canopy of the 10 trees. In this manner, fruit were collected weekly from petal fall through the end of the June drop period and then monthly through harvest. Each sampling date had 10 replications of abscising and persisting fruit. The two sets of fruit will be analyzed for their PGR content using radioimmonoassay for IAA, the cytokinins zeatin riboside and isopentyladenosine, GA_3, GA_4, ABA (Cutting et al., 1986), and ACC the precursor of ethylene biosynthesis (Lizada and Yang, 1979; McKeon et al., 1982). These data are not yet available.

The future of PGRs in avocado production

Concerns about the long-term availability of PGRs as horticultural tools were expressed by Cutting and Wolstenholme in 1993 and reiterated at the International Society of Citriculture (ISC) congress in 1996. Cutting and Wolstenholme (1993) wisely urged good stewardship, education of the public, development of “green” PGRs and a change in terminology to reflect the use of “natural plant growth modulators”. Two additional concerns were expressed at the ISC congress. One was the lack of financial support for PGR research at both the basic and applied level. Manufacturers and commodity boards each claim the other will be the financial benefactor of the research, so funding is minimal from either source. With neither group willing to invest in PGR technology, knowledge about the most fundamental processes of crop production advance slowly. The second concern was that PGR research would be replaced once genetically modified plants become a reality. At the present time, it would seem that genetically modified organisms are going to be the subject of harsher scrutiny by the consumer than PGRs. In the interim, as molecular biologists learn how to genetically modify plants to solve production problems, I am optimistic that a tremendous amount will be learned about how PGRs regulate fundamental processes that will be useful to the avocado industry. Considerable funding is available for research at the level of the gene. I personally look forward to the day when PGRs are simple, safe molecules that when applied to a crop turn on or off a gene of choice to elicit a desired physiological response.

In the meantime, I would like to encourage a dialogue and formation of a plan of action among avocado researchers that would facilitate more rapid progress in achieving the goal of being able to manipulate the key physiological processes of the avocado tree to solve production problems. The Australian and New Zealand Avocado Growers Conference “Vision 2020” provides an excellent opportunity to take the first step toward this goal.

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