1994 California Avocado Research Symposium pages 23-26
California Avocado Society and University of California, Riverside

# COMPARISON OF DETERMINATE (TOTALLY FLORAL) VS. INDETERMINATE (FLORAL WITH A TERMINAL VEGETATIVE FLUSH) INFLORESCENCES TO DETERMINE THE ROLES OF PGRS, CARBOHYDRATE, NITROGEN AND OTHER NUTRIENTS IN FRUIT SET OF THE 'HASS' AVOCADO

Carol J. Lovatt
Department of Botany and Plant Sciences
University of California, Riverside, CA

'Hass' avocado trees are outstanding with respect to the large number of flowers they produce (e.g., 1 to 2 million per tree) relative to the low number of fruit harvested; yield represents only 0.02% fruit set (Bergh, 1985). Flowering coincides with the spring vegetative flush. In indeterminate inflorescences (i.e., floral shoots ending with a vegetative flush), inflorescences leaves are reported to compete with flowers and setting fruit for assimilates until they are 2/3 full expansion and start to support the developing fruit (Whiley, 1990).

Recent avocado field studies of Blanke and Lovatt resulting from Dr. Blanke's visit in spring of 1992 provided three key results of importance to fruit set: (i) determinate and indeterminate inflorescences are comprised of a similar number of panicle branches (7.2+/-2.3 and 7.6+/-1.1 respectively, and bear a similar number of flowers (86.6+/-16.7 and 62.4+/-6.1, respectively; (ii) 80% of the leaves in a 'Hass' avocado indeterminate inflorescence were fully expanded and serving as exporters of photosynthate one week before anthesis; and (iii) due to consumption of H2O by inflorescence leaves, indeterminate inflorescences transpire 25 mls more $H_2O$ than determinate inflorescences both before and after anthesis. Thus, there are likely significant differences in the content of PGRs, carbohydrate, nitrogen and other nutrients in developing fruit borne on indeterminate vs. determinate inflorescences, one or more of which play a key role in avocado fruit set, fruit size, and postharvest fruit quality.

In the first year of this grant, we initiated three projects to characterize the similarities and differences between determinate and indeterminate inflorscences. The first is a field study to determine the distribution of each type of inflorescence in the cohort of early-opening vs. the cohort of later-opening inflorescences of the 'Hass' avocado and the potential of each to set fruit that survive to harvest. The second is to quantify differences in the flux of PGRs into (cytokinins, ABA or GA) , out of (IAA) , and in the distribution of PGRs within each type of inflorescence that are regulating to fruit set. In addition, the research will confirm that the differences are indeed due to the presence or absence of inflorscences leaves. In the third project,we are screening precursors of IAA to identify one which can effectively replace the application of IAA in avocado.

Dr. Isa Bertling has completed establishing and standardizing the methods for isolating and quantifying PGRs by both high performance liquid chromatography (HPLC) and radioimmunoassay (RIA). In addition, Dr.Bertling synthesized several radiolabeled

tracers for use in the RIAs.

**HPLC.** The following conditions for isolation and quantitation of PGRs were established. In some cases, as you will read below, these conditions are modified to give better separation for the purification of specific PGRs. PGRs are separated on a Zorbax ODS column, 250 x 9.4 mm equipped with a precolumn. The PGRs are eluted by a gradient solvent system. The initial conditions are 95% solvent A: 0.1 M acetic acid in 10% aqueous methanol adjusted to pH 3.4 with triethylamine and 5% solvent B: 100% methanol. The linear gradient shifts from 5% solvent B to 50% solvent B in 50 minutes at a flow rate of 2 ml/minute. Retention times for the PGRs are as follows:

| | |
|---|---|
| zeatin | 18-19 min |
| zeatinriboside | 20-21 min |
| indole-3-acetic acid | 31-32 min |
| gibberellic acid | 30-32 min |
| abscisic acid | 42-43 min |
| isopentyladenosine | 44-45 min |

To study the metabolism of trytophan to IAA a method slightly different from that for PGRs was established. In this case the conditions of the HPLC were changed as follows: solvent A was adjusted to pH 3.5 instead of 3.4 and the gradient shifts from 15 to 60 % solvent B (instead of 5 to 50%). Under these conditions the metabolites eluted at retention times in the following order:

| | |
|---|---|
| 5-Hydroxytryptamine | 8.3-8.5 |
| tryptophan | 12.4-12.6 |
| tryptamine | 13.9-14.2 |
| indole-3-lactic acid | 22.3-22.7 |
| indole-3-aldehyde | 27.3-27.8 |
| indole-3-acetic acid | 29.2-29.7 |
| indole-3-pyruvic acid | 34.4-35.2 |

**RIA.** The PI gratefully acknowledges Dr. Jonathan Cutting, University of Natal, for his generosity in supplying all antibodies needed for the RIAs.

**Cytokinins.** [$^3$H]Isopentyladenine, [$^3$H]isopentyladenosine, [$^3$H]zeatin, and [$^3$H] zeatinriboside were made from [$^3$H]adenine and [$^3$H]adenosine, respectively, by reaction with bromo-methyl-butene and dimethylamine to form, respectively, [$^3$H]isopentyladenine and [$^3$H]isopentyl-adenosine. These were reacted with tertiary butylhydroxiperoxide and dichloromethane to yield [$^3$H]zeatin and [$^3$H] zeatinriboside. [$^3$H]zeatin and [$^3$H]zeatinriboside were isolated from the reaction mixture and purified by

HPLC. [$^3$H]zeatinriboside was recovered in HPLC fractions 19 through 25. [$^3$H]isopentyladenine and [$^3$H]isopentyladenosine were purified in a similar manner.

The antibody to zeatinriboside was tested against its substrate to determine the dilution necessary for 50% binding (50% binding is desired to give a range of values over which the standard curve is accurate). Optimal dilution for the antibody to zeatinriboside is 1:2750.

Similarly, the optimal dilution of horse serum to be used in the assays was determined. Horse serum provides the extra protein necessary for precipitation of the antibody and the antigen. Optimal conditions are the lowest amount (based on dilution) of horse serum needed in an assay to still maintain 50% binding. This value was determined for each antibody.

[$^3$H]isopentenyl adenosine made from [$^3$H]adenosine as well as [$^3$H]isopentenyl adenine made from [3H]adenine were purified twice by HPLC. The fractions containing the highest activity were fraction 46 to 58. These fractions were tested for best binding with the antibody. Thereafter the optimal dilution of the antibody for isopentenyladenosine was established. This antibody can also detect isopentenyladenine. The cross reactivity was determined to be 85%.

**GA.** [$^3$H]$GA_3$ was partially purified and provided to us by Dr. Jonathan Cutting. $GA_3$ has been further purified by HPLC.

To increase the sensitivity and the stability of the acid PGRs (GAs, ABA, IAA) , the tracers as well as the standards have to be methylated. This is done with diazomethane which was produced by reaction of N-nitroso-N-methylurea. Fraction 34 to 37 of the methylated tracer yielded the best binding to the antibody. The optimal dilution of the antibody (50% binding of tracer) was determined at a dilution of 1:1000.

**IAA.** [$^3$H]IAA was purchased from American Radiolabeled Chemicals; no further purification was necessary.

The initial dilution of the antibody for IAA was 1:100. After a short while the antibody didn't provide 50% binding anymore and the problem was found to be the tracer. New tracer from Amersham was purchased. The tracer had to be ethylated with diazoethane which was produced by reaction of N-nitroso-N-ethylurea with 40% KOH. After this procedure the final dilution of the antibody was 1:100. The cross reactivity of the antibody with tryptophan was tested and found to be less than 1%.

Standard curves were set up for all the hormones, the sensitivity of the assays ranks for the following PGRs from:

| | |
|---|---|
| Z/ZR | 0.05-20ng |
| IAA | 0.25-5ng |
| $GA_3$ | 0.025-5ng |
| ABA | 0.01-lOng |
| IPA | 0.075-20ng |
| IAde(2iP) | 0.1-25ng (with IPA antibody) |

**Hormone purification.** A column system was set up for hormone purification. The system consists of a 5 ml polyvinyl pyrolidone column and attached to this a 5 ml DEAE ion exchange column. Equilibration of the system was done by washing it with 15 ml 0.01M ammonium acetate followed by 15 ml 1.0 M ammonium acetate. Thereafter a SepPak cartridge (C18) ( preconditioned by flushing with 10 ml methanol) was attached to the DEAE column. These 3 columns were washed with 15 ml ammonium acetate before the supernatant fraction of the sample (which was extracted in 80% methanol, dried down, taken up in 6ml 0.01 M ammonium acetate and adjusted to pH 7.0, and centrifuged at 12000 g) was loaded onto the system. The system was eluted with 5 + 25 ml 0.01 M ammonium acetate, the SepPak cartridge which contained the cytokinins removed and the PVP column discarded. Another SepPak cartridge was preconditioned by flushing with 10 ml methanol followed by 10 ml 0.1 M acetic acid and attached to the sephadex column. This system was eluted with 25 ml 1.5 M acetic acid and the SepPak containing the acidic hormones removed. Both SepPak columns were washed with water and eluted with increasing concentrations of methanol in water. The elution of the columns was done in the following way:

| **SepPak** | **% methanol** | **hormones eluted** |
|---|---|---|
| I= cytokinins | 10 (4ml) | discard |
| | 30 (4ml) | Z+ZR |
| | 60 (4ml) | IPA+2ip |
| II= acidic hormones | 20 (4ml) | discard |
| | 60 (4ml) | GAS, ABA, IAA |

Recovery for the different hormones was tested by running a sample of citrus leaf tissue with radioactive marker of each hormone. The following recoveries were obtained:

| | |
|---|---|
| ZR | 85% |
| IPA | 85% |
| $GA_3$ | 78-90% |
| IAA | 40-60% |
| ABA | 70% |