Project Title: Ethylene and Almond Development
Project Leader: J.M. Labavitch

ANNUAL REPORT TO ALMOND BOARD - 1985
PROJECT 85-I9

OBJECTIVES

To develop information about various aspects of almond fruit development:

1. The role of ethylene in embryo sac development and fruit set (Weis, Polito and Labavitch).

2. The role of ethylene in control of almond fruit maturation and application of this information for orchard practice (Weis and Labavitch).

3. The cytohistochemistry and anatomy of the dehiscence zone in maturing almond fruits and the enzymes involved in control of hullsplit (Weis, Polito and Labavitch).

INTERPRETIVE SUMMARY

Ethylene is a gas that is produced by many plants at various times in their development. This gas acts as a plant hormone in that its presence in or around the plant serves to regulate how the plant behaves. In many cases ethylene can act in combination with other plant hormones to regulate plant development and behavior. Project 85-I9 was designed to test whether the production of ethylene by almond flowers and/or fruits plays a role in regulating fruit set and/or nut maturation. A large portion of the work performed this year was done to confirm results of work done in 1983 and 1984.

1) Work in Spring, 1983 and 1984 showed a clear correlation betwe en successful pollination of 'Nonpareil' almond flowers and the production of ethylene by these flowers. In Spring, 1985 cross- and nonpollinated flowers were sprayed with AOA (aminooxyacetic acid), an inhibitor of ethylene synthesis. Sprays were applied in order to prevent ethylene production and thus see what effect an absence of ethylene would have on fruit set. While the sprays appear to have reduced ethylene production in cross-pollinated flowers, fruit set was not reduced. This suggests that an enhancement of flower ethylene production following cross-pollination is not essential for embryo sac development.

The possible role of auxin in postpollination phenomena was investigated using applications of NAA (napthaleneacetic acid) on cross- and nonpollinated pistils. Ovule development in these and GA-treated (gibberellic acid), nonpollinated pistils was evaluated for stage of embryo sac development and length of the embryo sac. In general, embryo sac development was enhanced by treatments with NAA and AOA, regardless of pollination treatment. GA treatment appeared to substitute for pollination to some extent.

Interpretation of results was complicated by the unusually favorable weather conditions which existed during the 1985 bloom period when orchard temperatures were unseasonably high. The data suggest a strong role for a combination of plant hormones in fruit set in almond and point out the need for repetition of these experiments in Spring, 1986.

2) The role of ethylene in almond fruit maturation and drop was studied in the summer of 1985. Continuous and intermittent applications of low doses of ethylene gas to bagged fruits in the field led to split and drop of full-sized nuts in a few days, confirming 1983 and 1984 data. However, weekly spray applications of ethylene-releasing chemicals (ethephon at 10 ppm and CGA 15281 at 20 ppm) did not result in increased rates of split or drop in 1985, when applied with spray bottles or a hand gun sprayer. Applications of STS, an agent which we have previously used to delay split, (presumably because it inhibits ethylene action), caused symptoms of phytotoxicity. Therefore, we are not yet able to conclude with confidence that ethylene production by the fruit occurs and controls natural maturation.

We plan to repeat relatively full-scale field trials of ethylene-releasing compounds in Summer, 1986 employing doses higher than those previously used but below levels that would produce phytotoxic effects. Because of reports of effects of late season ethylene applications on return bloom, those trees which were sprayed in 1985 will be followed in Spring, 1986 to determine if bloom has been affected.

3) Hullsplit presumably involves cell separation processes similar to those seen in almond fruit drop and gum duct formation. Samples of almond hull were taken throughout the maturation period (April-July, 1986) for investigation of the cellular and biochemical processes leading up to hullsplit. Biochemical tests for degradative enzymes involved in almond gum duct formation and ripening of many fruits indicated that pectinase (an enzyme that digests pectin - an intercellular "glue") may be a controlling factor in hullsplit. Histochemistry of almond hull samples confirmed these findings. 1985 results gave distinct evidence that almond fruits must carry out a limited self-digestion prior to hullsplit. Because the biochemistry of fruit ripening is similar to that of hull split we feel that hull split, too, may be ethylene controlled.

EXPERIMENTAL PROCEDURES

1. Methods for pollination treatments and gas analysis were similar to those used in 1983 and 1984, with the addition of spray treatments of the ethylene synthesis inhibitor, aminooxyacetic acid, AOA, and two plant growth regulators: NAA, (napthaleneacetic acid), an auxin, and GA (gibberellic acid).

2. Methods for gassing almond fruit clusters with ethylene were similar to those used in 1983 and 1984. Weekly spray applications were made on developing 'Nonpareil' almond fruits using a variety of chemicals. Applications with a hand gun sprayer were begun on June 20 in a commercial orchard in Durham. Spray materials used were:

a. Water
b. Ethephon (20 ppm in water)
c. CGA-15281 (20 ppm in water)

Hand-held sprayers were used in Davis in applications of chemicals on a weekly basis, with care taken to thoroughly wet fruit surfaces. Spray applications begun on July 5 included:

a. Water
b. Ethephon (10 ppm in water)
c. CGA-15281 (10 ppm in water)
d. STS (silver thiosulfate; 2 mM in water)

All spray treatments were compared to control groups of nuts that had received no spray treatments. The effects of spray treatments on nut dehiscence were assessed twice weekly in Davis and once weekly in Durham.

3. 'Nonpareil' almonds were sampled approximately once weekly, beginning April 18 and ending July 18. Tissue samples from almond hulls (dehiscence zone, or suture, and non-suture material) were extracted for cell wall-hydrolyzing enzymes. Enzyme assays of dehiscence zone and control tissue samples were compared thoughout the maturation process for presence and quantities of key enzymes.

Samples for anatomical and cytohistochemical studies of the dehiscence zone were processed at the same time and examined microscopically for changes in cell character and cell wall substituents.

RESULTS

1. Data from 1983 and 1984 showed that ethylene production by excised almond pistils increases with application of compatible pollen and that this increase parallels embryo sac development (Figure 1). Pollinated and nonpollinated flowers that had been sprayed with AOA in 1985 produced low levels of ethylene that were similar to those of non-pollinated, unsprayed controls (Figure 2). Embryo sac development in AOA-sprayed flowers was greater in cross-pollinated pistils than in nonpollinated pistils. Unlike the unsprayed pollination treatments of 1983 and 1984, however, no ovules aborted and all embryo sacs achieved at least the 7-celled, 8-nucleate condition. NAA appeared to substitute for cross-pollination, as more than 80% of all ovules of nonpollinated pistils exhibited fully differentiated, elongated embryo sacs. The GA treatment also resulted in a high degree of set and embryo sac development in these pistils was similar to that of those that were unsprayed and cross-pollinated.

2. Data from 1983 and 1984 indicated that hand-sprays of ethephon at low levels (5 and 10 ppm) may enhance the rate of hullsplit in almond and that endogenous ethylene probably is a natural regulator of almond fruit wall dehiscence and abscission. Similar experi-

ments performed in 1985, however, showed only a moderate effection on the rate of maturation when ethylene-releasing compounds (ethephon and CGA-15281) were used at 20 ppm (Figure 3). When entire young Nonpareil trees in Durham were sprayed with these same compounds at the same concentrations, no effect on rate of maturation was seen (Figure 4). When clusters on almond fruits were enclosed and gassed with 3-4 ppm ethylene, maturation and hullsplit rate were clearly enhanced (Figure 5). Indications for a role for ethylene as the natural regulator of almond fruit dehiscence remain strong.

3. Cytohistochemistry and anatomy of the dehiscence zone (DZ) of almond were characterized. The DZ is preformed as the suture in the flower bud. At anthesis the DZ is two cell-layers wide and the cells are already differentiated as unique layers of the fruit wall. During growth and development of the kernel and hull, the DZ expands in the endocarp region (that portion of the hull which will become the stony shell) to form a distinct region 4-5 cells wide. DZ cells in this region form thick stony walls and ultimately resemble other cells of the shell. The DZ in the mesocarp does not undergo this process of lignification and remains narrow with relatively thin-walled cells. Throughout the DZ, heavy layers of pectin are formed between the cells. The appearance and disappearance of pectins in various areas of the DZ were measured by microspectrophotometry and expressed as "% mean intensity" (Figure 6). A sharp decline in this value in the outer layers of the hull correlated with a relatively large amount of pectin-digesting enzyme detected in these tissues shortly before hullsplit. Levels of pectins were considerably lower in the outer layers of the hull (mesocarp) of fruits that were gassed with 3-4 ppm ethylene in mid-June than in those of non-gassed fruits collected on the same day.

DISCUSSION

1. Ethylene production by flowers has often been correlated with pollination, petal senescence, and flower drop. Ethylene emitted shortly after pollination seems to be responsible for many post-pollination effects. Because more ethylene is produced following cross-pollination than in nonpollinated flowers and because relatively high levels continue to be produced several days after pollination, we previously thought that the later-produced (5-9 days post-pollination) ethylene contributes to fruit set and development rather than floral degeneration. 1985 results, showing a reduction of ethylene production caused by AOA, but continued embryo sac development, seem to indicate, that the production of ethylene, in this case, is not required for control of embryo sac development. An enhancement of embryo sac development in non-pollinated flowers following applications of NAA and GA suggests that the reproductive process and fruit set in almond may be influenced by more than one growth hormone.

2. The data shown in Figure 5 confirm 1983 and 1984 findings that the almond fruit is responsive to ethylene gas at fairly low concentrations. Spray data from 1983-1985 show inconsistencies that may be due to year-to-year weather and orchard variations, differences among treatment starting dates relative to the onset of hull split, and inadequate sample sizes. 1985 spray experiments were designed to factor out as many of these inconsistencies as possible. Our results do not prove that ethylene controls dehiscence and abscission although we continue to believe that fruit ethylene production does play a part in regulating almond maturation. 1986 repetition of spray treatments with higher levels of ethylene-releasing compounds may give us a better indication of the likelihood of influencing these developmentally-important events by influencing the production of ethylene by almond fruits or manipulating ethylene levels in the orchard.

3. Anatomical investigation of the dehiscence zone in maturing almond fruits has provided a clearer picture of the nature of hullsplit in almond. This is an area in plant research which has had little attention in the past and results have led us to a better understanding of the processes involved. A specialized zone of cells develops sensitivity to endogenous controls during maturation. Specialized cell walls apparently break down in response to digestive enzymes that are in highest quantity in the dehiscence zone. Similar enzymes have been shown to be essential in abscission phenomena in other plant parts and in ripening of other fruits. Ethylene-treated almond fruits exhibited cellular changes in dehiscence zone cells which mimicked the cellular changes seen in DZ cells of ready-to-split, mature almond fruits. These results again imply an important role for the plant hormone, ethylene, in almond fruit maturation (dehiscence and abscission).

ABSTRACTS

Weis, K.G. and J.M. Labavitch. 1985. Control of almond fruit abscission. Proceedings of the 66th Annual Meeting of the Pacific Division of the American Academy of Science.

Weis, K.G. and V.S. Polito. 1985. Postpollination events in almond flowers: plant growth substances as related to embryo sac development. Acta Horticulturae; Proceedings of the 5th International Symposium on Growth Regulators in Fruit Production. (in press).

Weis, K.G. and J.M. Labavitch. 1986. Physiology and histochemistry of almond fruit dehiscence. XXIInd International Horticultural Congress. (submitted)

Weis, K.G. and V.S. Polito. 1985. Embryo sac development in almond as related to plant growth substances. XXIInd International Horticultural Congress. (submitted)

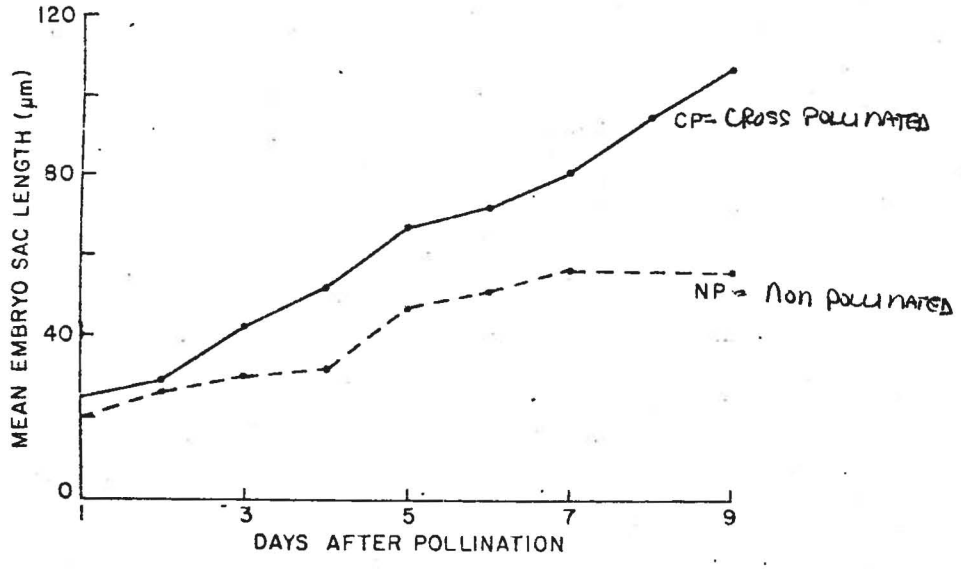

FIG. 1 Embryo sac length in pollinated and nonpollinated almond flowers.

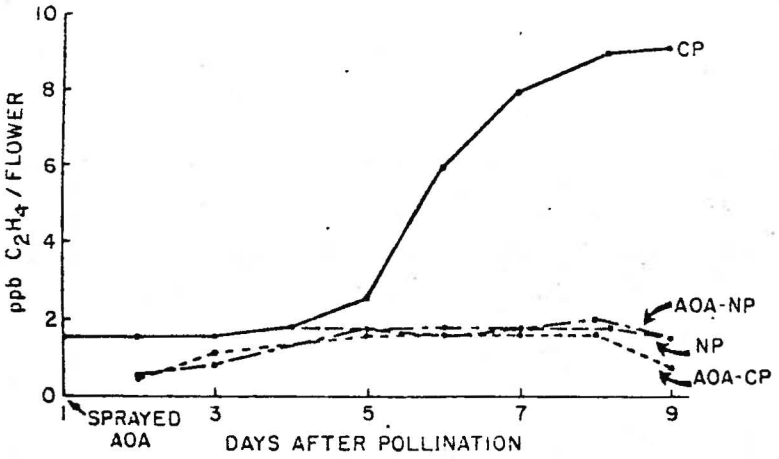

FIG. 2 Ethylene evolution by pollinated and nonpollinated almond flowers. Cross and nonpollinated flowers sprayed with 10 mM AOA, (aminooxyacetic acid, an ethylene synthesis inhibitor), produced amounts of ethylene similar to that seen in unsprayed, nonpollinated flowers. Embryo sac lengths in AOA-treated, cross-pollinated flowers were double that of AOA-treated, nonpollinated flowers (data not shown).

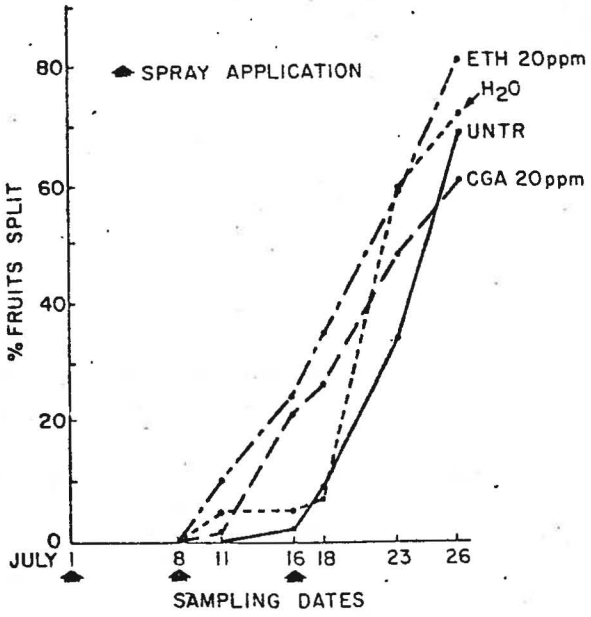

FIG. 3 In 1985, small-scale spray trials in Davis suggested a moderate effect of ethylene-releasing compounds on flower maturation.

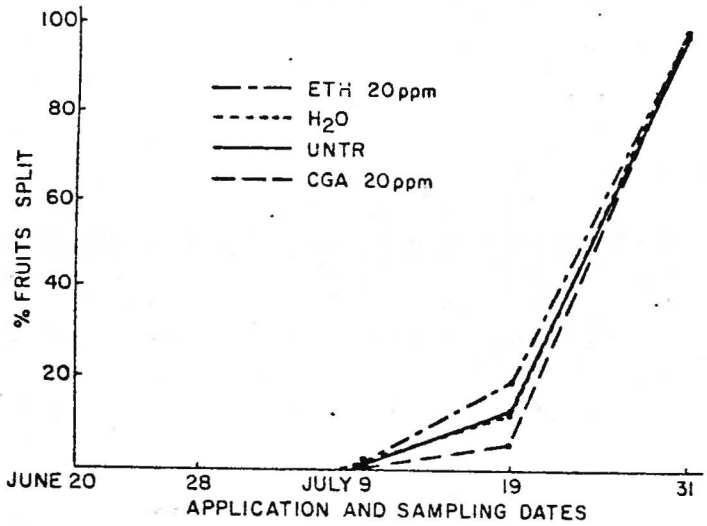

FIG. 4 1985 observations showed no effect on rate of maturation using 20 ppm CGA-15281 and 20 ppm ethephon on young Nonpareil trees in Durham when whole tree spray applications were made with a hand gun sprayer. (Co-operators: Joe Connell, Farm Advisor; Sam Lewis, Grower).

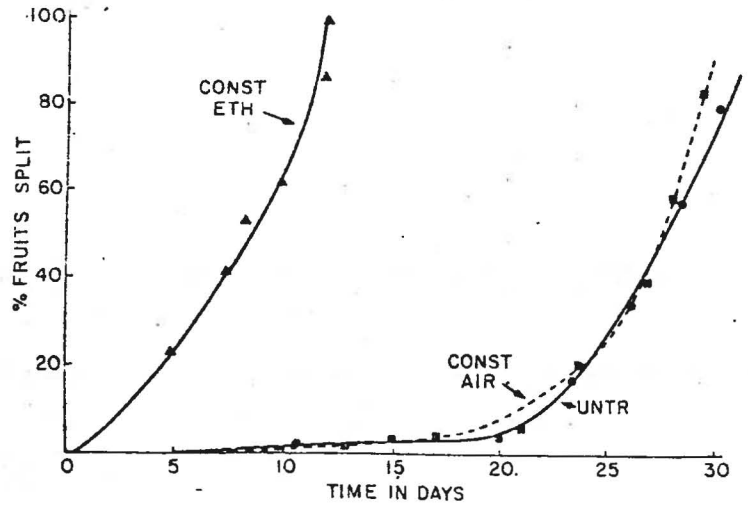

FIG. 5 Clusters of Nonpareil almonds were enclosed in bags which were flushed with air or air plus ethylene. Nuts were checked for dehiscence at regular intervals. Ethylene clearly promoted maturation.

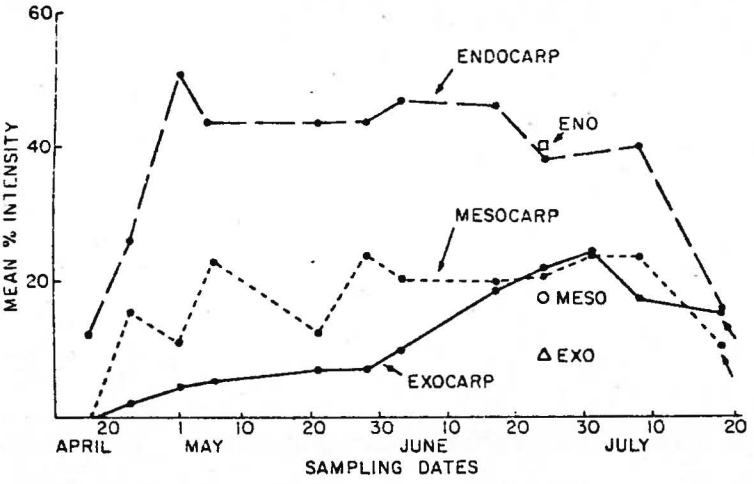

FIG. 6 Samples of almond hulls were analyzed throughout the maturation period (April-July, 1985). Biochemical tests for cell wall-degrading enzymes reported in gum duct formation and fruit ripening were made. The appearance and disappearance of pectins in the region of the dehiscence zone were measured as "Mean % Intensity" through microspectrophotometry. The sharp decline in this value in both exocarp and mesocarp regions coincides with a relatively large amount of pectinase measured in these tissues on July 18, 1985, (arrows). Levels of pectins were considerably lower in the exocarp and mesocarp of ethylene-gassed fruits sampled on June 24, 1985 (EXO, MESO), as compared to untreated samples collected on the same day. The level of pectin in the endo-carp (shell) tissue (ENO) was unaffected by the gassing.