*J. Amer. Soc. Hort. Sci.* 104(2):185–188. 1979.

# Abscisic Acid in Pear Seed, Fruit, and Fruit Exudate[1]

**George C. Martin and C. Nishijima**
*Department of Pomology, University of California, Davis, CA 95616*

*Additional index words. Pyrus communis,* parthenocarpy, fruit set, abscission, embryo

*Abstract.* **Large differences in abscisic acid (ABA) concentrations were found among persisting fruit of 'Winter Nelis', seeded 'Bartlett' and parthenocarpic 'Bartlett' pear (*Pyrus communis* L.) even though fruit set and fruit growth rates were similar. Concentration of ABA was positively correlated with rate of fruit and seed growth in these 3 pear types. The concentration of ABA was greater in the seed than in fruit flesh, and in the integuments plus endosperm than in the embryo.**

At anthesis the ovule, ovary, and receptacle of the pear flower are delicately poised and receptive to the stimulus of pollination and fertilization. With most pear cultivars, growth will not continue without pollination and fertilization. An exception is 'Bartlett' pear in the Sacramento River delta area of California, which produces commercial crops of parthenocarpic fruit when planted in solid blocks. After many years of investigation, the cause of parthenocarpy remains unknown, although climatic factors appear to be involved.

The developing seed appears to control fruit growth in apple (1, 9), and many studies have correlated seed hormone levels with persistence of fruit. Parallel variations do not establish cause and effect and these correlations may have been fortuitous (13). Zucconi (17) suggests using multiple systems to avoid chance correlations.

In pear we have a multiple system, for 'Winter Nelis' must be pollinated and fertilized for fruit persistence, while 'Bartlett' may be either pollinated and fertilized, thus seeded, or non-pollinated and remain seedless (6). Using the multiple system of 'Winter Nelis' and 'Bartlett' we have accumulated the following information:

a) At 5 to 7 weeks after full bloom (AFB) exudate from parthenocarpic 'Bartlett' fruit contained more growth promoting activity (wheat coleoptile assay) than did that from seeded 'Bartlett' or 'Winter Nelis' fruit (7).

b) From FB to 20 days AFB greater amounts of a GA-like promoter, as judged by the dwarf rice assay, diffused from seeded 'Winter Nelis' than either parthenocarpic or seeded 'Bartlett' fruit (5).

c) As judged by bioassay, no differences occurred in concentration of auxin-, GA- and ABA-like compounds in developing seed or fruit of the 3 types of pear (4).

d) $GA_{17}$, $GA_{25}$, $GA_{45}$, ABA, phaseic acid, c,t-dihydrophaseic acid and t,t-dihydrophaseic acid were identified in immature seed of 'Winter Nelis', and both seeded and seedless 'Bartlett' (10), but no major differences in their concentration could be established among the types at 25 or 40 days AFB (11).

The purpose of the present investigation was to measure ABA in flower and fruit exudate, seeds, and fruit during the entire growing season, using electron capture gas chromatography.

## Materials and Methods

Twenty flowers or fruits of seeded and seedless 'Bartlett' and seeded 'Winter Nelis' were collected at intervals from anthesis until harvest from the Sacramento River delta region of California. These samplings were done subjectively, taking the freshest flowers and largest fruits with green pedicels – the fruits judged to be persisting. Upon selection the pedicels were immediately inserted into small vials each containing 2 ml of 20 mM ethylenediaminetetraacetic acid (EDTA) at pH 7. Initially, 5 flowers or fruits were used per vial and remained in solution for 6 hr. As fruit enlarged in size, fewer specimens were used per vial and the solution was increased to 3 ml per vial.

Following the 6 hr diffusion period, diffusates from each type of fruit were combined and 3 volumes of water were added. The pH was adjusted to 3.0 with 1 N HCl and the solution partitioned 3 times with one-third volume ethyl acetate. The bulked ethyl acetate was backwashed with one-fourth volume $H_2O$ and the $H_2O$ discarded. The ethyl acetate was evaporated under $N_2$ gas, and the residue methylated for gas chromatography as previously described (16).

At the termination of the 6 hr diffusion period the fresh weight was determined and in 1977 fruit diameter was measured (Fig. 8). In 1977 only, the fruit were extracted in 80% methanol. As the seeds enlarged they were separated from the fruit, weighed, and extracted with 80% methanol. With further enlargement the embryo and integuments with endosperm were separated, weighed, and extracted. Methanol extracts were concentrated to the aqueous phase. The pH was adjusted to 8 with 1 N NaOH and the solution partitioned 3 times with one-third volume ethyl acetate. The pH of the aqueous phase was readjusted to pH 3 with 1 N HCl and processed as described above for diffusates prior to gas chromatography. In all cases and for both years the final aqueous phase was treated with 11 N NaOH for 1 hr at 60°C then cooled, the pH adjusted to 3, the solution partitioned 3 times with ethyl acetate, and the ethyl acetate processed for ABA.

In 1977 only separate limbs on trees samples for flowers and fruit were tagged and flowers counted; thereafter fruit

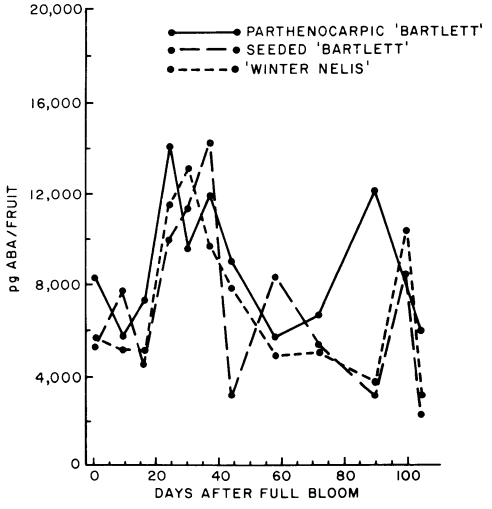


Fig. 1. ABA in pear fruit exudate in 1976.

[1]Received for publication July 24, 1978.
The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper must therefore be hereby marked *advertisement* solely to indicate this fact.

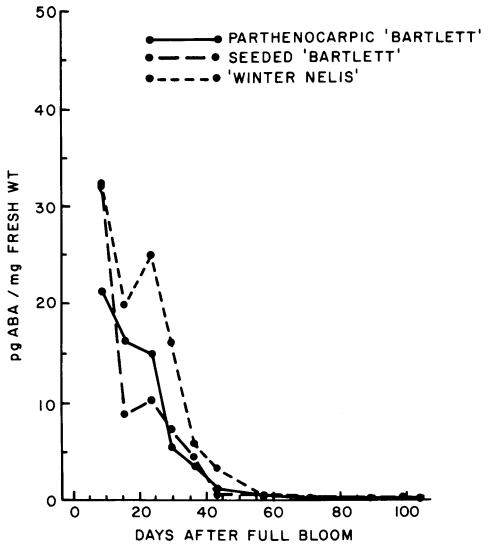


Fig. 2. ABA in pear fruit exudate in 1976.

counts were made at 5 and 25 days AFB and at harvest to account for abscission.

## Results

*ABA in fruit exudate.* During the 1976 season ABA in the diffusate ranged from 2 to 14 ng/fruit (Fig. 1). At anthesis parthenocarpic 'Bartlett' exuded 8 ng/fruit, seeded 'Bartlett' and 'Winter Nelis' 5.5 ng/fruit. From 24 to 37 days AFB, the quantity ranged from 10 to 14 ng/fruit. The exudate also contained large amounts of ABA 99 days AFB, just prior to harvest. The greatest differences among pear types occurred at 44 and again at 89 days AFB, but the seasonal patterns were remarkably similar for all types. No bound ABA was found.

Weight data for flowers in 1976 were lost, hence ABA per mg fresh weight could not be determined. However, the concentration of ABA in all types of pear declined from 9 to 58 days AFB, remaining virtually unchanged thereafter (Fig. 2). Notable differences among cultivars occurred 9, 16, 24 and 30 days AFB.

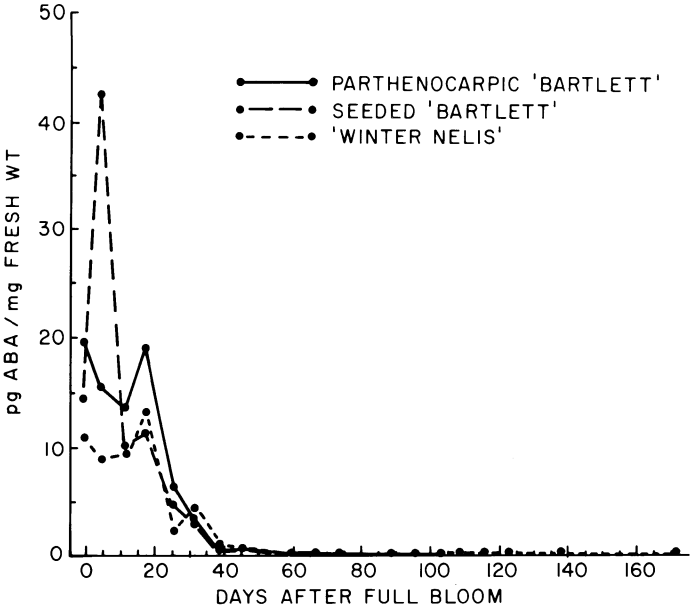


Fig. 4. ABA in pear fruit exudate in 1977.

In 1977, ABA in the exudate ranged from 0.5 to 19 ng/fruit during the growing season (Fig. 3). Quantities were relatively low at anthesis and rose to a maximum of 11 to 19 ng/fruit between 17 and 31 days AFB. The greatest differences among types occurred at 5, 25, 59, 73 and 103 days AFB. As in 1976 the seasonal patterns of the 3 types were remarkably similar. No bound ABA was found.

In 1977 striking differences in ABA occurred 5 days AFB, with seeded 'Bartlett', 'Winter Nelis' and parthenocarpic 'Bartlett' containing 43, 9 and 15 pg ABA/mg fresh weight, respectively (Fig. 4). Slight differences among types occurred at anthesis, and at 11 and 17 days AFB. As in 1976, concentration declined until 59 days AFB with little change thereafter.

*ABA in fruit flesh 1977.* Maximal ABA concentration occurred from anthesis to 31 days AFB in each cultivar (Fig. 5). The concentration then declined until harvest except for 'Winter

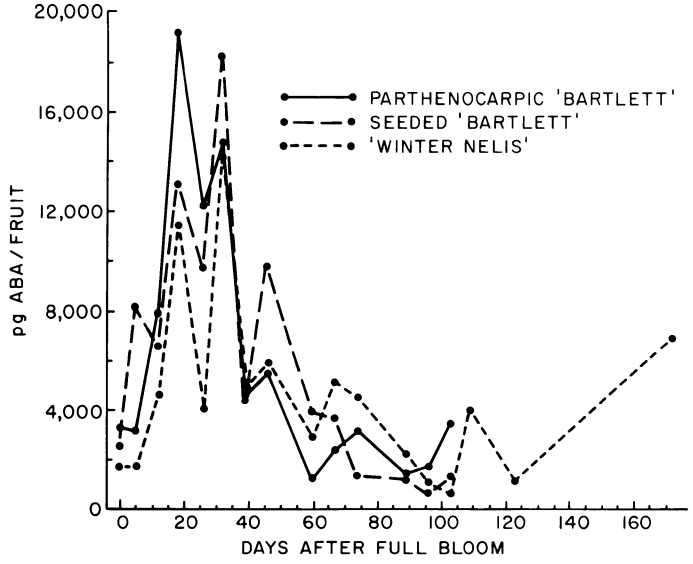


Fig. 3. ABA in pear fruit exudate in 1977.

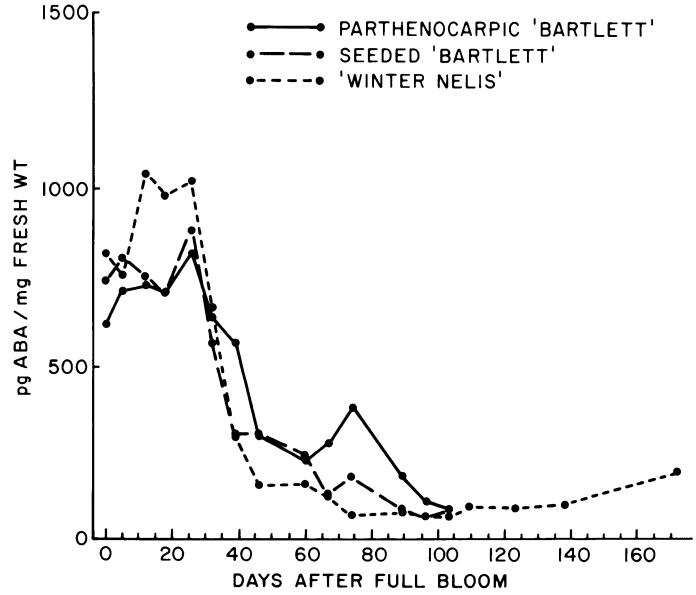


Fig. 5. ABA in pear fruit flesh in 1977.

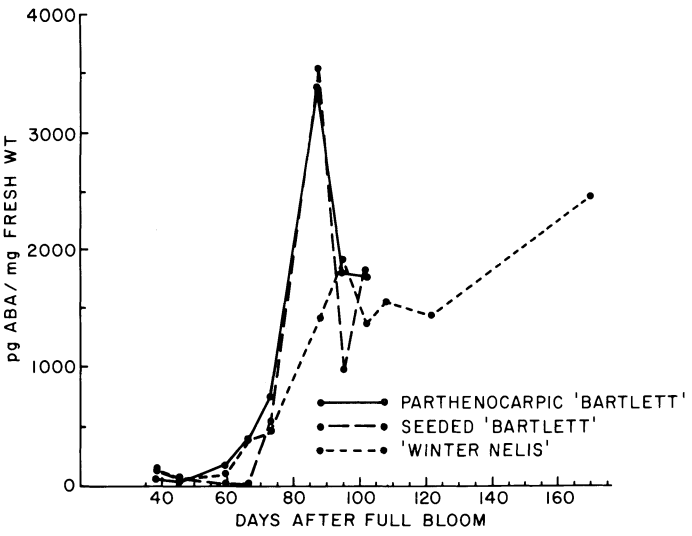


Fig. 6. ABA in 'Winter Nelis' and 'Bartlett' pear seed and parthenocarpic 'Bartlett' ovules in 1977.

Nelis', which increased slightly prior to harvest, and parthenocarpic 'Bartlett', which exhibited a small rise 73 days AFB. Differences among the types was greatest at 11, 17, 45 and 73 days AFB.

*ABA in seed 1977.* Seeds could not be readily separated from fruit until 38 days AFB (Fig. 6). ABA concentration remained low until 73 days AFB, then increased dramatically during the period of rapid growth of the integuments and embryo. The larger 'Winter Nelis' seed contained less than half as much ABA per unit wt as did 'Bartlett' at 88 days AFB. ABA declined in both 'Bartlett' types between 88 days AFB and harvest, but increased in 'Winter Nelis' seed from 123 days AFB until harvest, at which time the concentration was maximal for that cultivar. The concentration of ABA was much greater in the seed than in the fruit. As the former increased, the latter decreased (Figs. 5, 6).

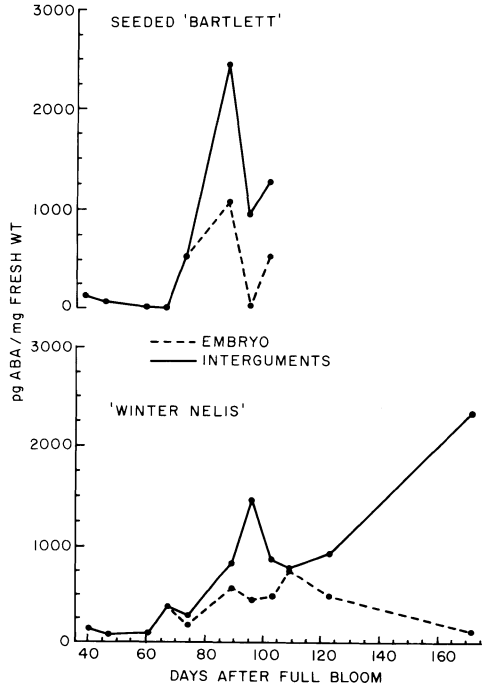


Fig. 7. ABA in pear embryo and integuments (including endosperm) in 1977.

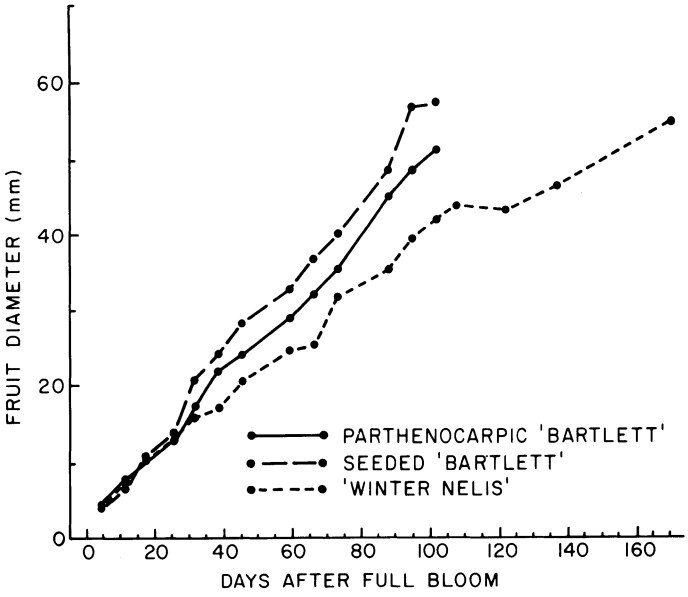


Fig. 8. Average pear fruit diameter in 1977; 20 fruits per sample.

ABA content of the embryo and integuments was evaluated only in seeded 'Bartlett' and 'Winter Nelis', as parthenocarpic 'Bartlett' ovules contain no embryos. The embryo could be excised 73 days AFB in 'Winter Nelis' and 88 days AFB in seeded 'Bartlett' (Fig. 7). Concentration of ABA was greater in the integuments plus endosperm than in the embryo, and changes were more dramatic in the former. Large increases in ABA occurred 73 to 88 days AFB in seeded 'Bartlett' and 123 to 172 days AFB in 'Winter Nelis' (Fig. 7).

*Fruit growth 1977.* From 5 to 25 days AFB fruits of all 3 types were similar in size and growth rate (Fig. 8). Between 25 and 31 days AFB seeded 'Bartlett' grew fastest followed by parthenocarpic 'Bartlett' and 'Winter Nelis'. Between 31 and 38 days AFB 'Winter Nelis' grew least rapidly. Between 38 days AFB and harvest both 'Bartlett' types and 'Winter Nelis' grew at similar rates.

## Discussion

In both 1976 and 1977, exuded ABA per fruit was greatest 17 to 37 days AFB, ranging from 10 to 17 ng/fruit. This period of maximum ABA production coincided with a period of heavy abscission in the general fruit population and a doubling of fruit diameter in 2 weeks. The temptation is strong to attribute abscission to these high concentrations of ABA in the exudate. However, the fruit selected were those judged most likely to persist and were the largest fruits on each tree; we were not comparing persisting vs. abscising fruit. Further, if the relationship between ABA exuded per fruit and abscission were direct, one would expect large differences in abscission among the 3 types of pear 17 to 25 days AFB in 1977, when 300% differences occurred in ABA (Fig. 3). When calculated on a fruit weight basis, ABA content of the exudate declined during the first 4 weeks AFB – the period of active abscission in the general fruit population (Fig. 2, 4). Also, maximum differences in ABA exuded per fruit among the 3 fruit types occurred at 44 and 89 days AFB in 1976, after the period of initial heavy fruit drop. Moderate bloom occurred in 1977 with heavy set in the test orchards. Maximum differences among types in ABA/fruit occurred at 5, 25, 59 and 73 days AFB. Although considerable abscission occurred at 5 and 25 days AFB, little or none occurred at 59 or 73 days. In all 3 types of pear 55% of the abscission occurred within 25 days AFB, only 5% thereafter. Therefore, these data do not provide

evidence for a correlation between high concentration of ABA and fruit abscission.

The large differences between years in concentration of ABA in the exudate just prior to harvest (10 to 12 ng/fruit in 1976 vs. only 1 to 4 ng/fruit in 1977) are puzzling (Figs. 1, 3). When calculated on a fruit fresh weight basis these differences were not evident (Figs. 2, 4). Although not critically evaluated, fruit density was greater in 1976 than in 1977. However, we know of no data to suggest that fruit density affects ABA content.

ABA content is more closely related to growth rate than to rate of abscission (3, 14, 15, 17). With plum, Letham (8) found greatest inhibitory activity, as judged by bioassay, during the cell division period. After cell division the inhibitor level declined. In *Pyrus communis*, cell division in the flesh and rapid growth occur for 42 to 56 days AFB (2), yet the ABA concentration in the exudate and flesh is highest during this period of time, particularly through 30 days AFB. Fruit diameter doubled from 11 to 25 days AFB in 1977 (Fig. 8). This was associated with a sharp increase in total exuded ABA/fruit (Fig. 3) and a high concentration of extractable ABA per unit weight of fruit flesh (Fig. 5). However, ABA content of the exudate per unit fruit weight was declining (Fig. 4). Similarly, both rate of embryo enlargement and ABA content per unit weight were high 65 to 85 days AFB (Figs. 6, 7). The integument of parthenocarpic 'Bartlett' accumulated an amount of ABA similar to that of 'Bartlett' and 'Winter Nelis' seeds containing embryos on a fresh weight basis (Fig. 6), and many times more ABA on a dry weight basis (11). The dry weight change in ovules of parthenocarpic 'Bartlett' is minimal between 40 days AFB and harvest, while major changes occur in seed dry weight in seeded pears during the same period of time. This explains the large differences in ABA when calculated on a dry weight basis (11). We have been impressed by the similarity in seasonal trends of ABA whether exuded from fruit or extracted from fruit or seed of the pear types.

That ABA is associated with rapid periods of growth may not be surprising. During periods of maximum growth higher concentration of an inhibitor like ABA may be required to bring growth to a halt (12). Although bioassays are constantly maligned, our data parallel both bioassay measurements (4, 5) and data obtained by gas chromatography-mass spectrometry (11).

### Literature Cited


1. Abbott, D. L. 1958. The effects of seed removal on the growth of apple fruitlets. Annu. Rpt. Long Ashton Res. Sta. for 1957, p. 52-56.
2. Bain, J. M. 1961. Some morphological, anatomical and physiological changes in the pear fruit (*Pyrus communis* var. Williams Bon Chretien) during development and following harvest. *Austral. J. Bot.* 9:99-123.
3. Davison, R. M., R. M. Rudnicki, and M. J. Bukovac. 1976. Endogenous plant growth substances in developing fruit of *Prunus cerasus* L. V. Changes in inhibitor (ABA) levels in the seed and pericarp. *J. Amer. Soc. Hort. Sci.* 101:519-523.
4. Gil, F. G., G. C. Martin, and W. H. Griggs. 1972. Fruit set and development in the pear: extractable endogenous hormones in parthenocarpic and seeded fruits. *J. Amer. Soc. Hort. Sci.* 97:731-735.
5. _____, _____, and _____. 1973. Fruit-set and development in the pear: diffusible growth substances from seeded and seedless fruits. *J. Amer. Soc. Hort. Sci.* 98:51-54.
6. Griggs, W. H. and B. T. Iwakiri. 1954. Pollination and parthenocarpy in the production of 'Bartlett' pears in California. *Hilgardia* 22: 643-678.
7. _____, G. C. Martin, and B. T. Iwakiri. 1970. The effect of seedless versus seeded fruit development on flower bud formation in pear. *J. Amer. Soc. Hort. Sci.* 96:243-248.
8. Letham, D. S. 1963. Regulators of cell division in plant tissues. *New Zealand J. Bot.* 1:336-350.
9. Luckwill, L. C. 1953. Studies of fruit development in relation to plant hormones. *J. Hort. Sci.* 28:14-24.
10. Martin, G. C., F. G. Dennis, Jr., P. Gaskin, and J. MacMillan. 1977. Identification of gibberellins $A_{17}$, $A_{25}$, $A_{45}$, abscisic acid, phaseic acid, and dihydrophaseic acid in seeds of *Pyrus communis*. *Phytochemistry* 16:605-607.
11. _____, _____, J. MacMillan, and P. Gaskin. 1977. Hormones in pear seeds. I. Levels of gibberellins, abscisic acid, phaseic acid, dihydrophaseic acid, and two metabolites of dihydrophaseic acid in immature seeds of *Pyrus communis* L. *J. Amer. Soc. Hort. Sci.* 102:16-19.
12. Powell, L. E., Jr. 1973. Naturally occurring plant growth regulators and their physiological roles in fruit trees. *Acta Hort.* 34:33-40.
13. _____ and C. Pratt. 1966. Growth promoting substances in the developing fruit of peach (*Prunus persica* L.). *J. Hort. Sci.* 41: 331-348.
14. _____ and S. D. Seeley. 1970. The distribution of abscisic acid in apple shoots: does it really play a role in terminal bud formation? *HortScience* 5:327.
15. Ramsey, J. and G. C. Martin. 1970. Seasonal changes in growth promoters and inhibitors in buds of apricot. *J. Amer. Soc. Hort. Sci.* 95:569-574.
16. Shaybany, B. and G. C. Martin. 1977. Abscisic acid identification and its quantitation in leaves of *Juglans* seedlings during waterlogging. *J. Amer. Soc. Hort. Sci.* 102:300-302.
17. Zucconi, F. 1975. Reassessment of the relationship between hormonal and developmental changes during abscission with particular reference to peach [*Prunus persica* (L.) Batsch] fruit. Ph.D. thesis. Michigan State Univ.