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HortScience 22(3):467–469. 1987.

Sieve Tube Development in Strawberry Receptacles in Relation to Fruit Set and Initial Growth

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Additional index words. auxin, IAA, IAA ethyl ester, *Fragaria ×ananassa*, day-neutral

Abstract. **The development of phloem sieve tube files in receptacles of pollinated, nonpollinated, and auxin-treated strawberry flowers (*Fragaria ×ananassa* Duch. 'Fern') was examined from 0 to 120 hr after emasculation and treatment and correlated to fruit set and growth. The receptacle dry weight of nonpollinated flowers increased very little during the first 120 hr after emasculation. Conversely, receptacle dry weights of the pollinated and auxin-treated flowers began to increase within 24 hr after emasculation and continued to increase throughout the rest of the experimental period. No differences were found in the appearance of the phloem and xylem cells produced in the receptacles among the three treatments. Although the number of sieve tube files in all treatments increased with time, there was no consistent difference in the number of files between growing and nongrowing receptacles. These results indicate that there is no correlation between the degree of phloem sieve tube development and fruit set/initial growth of strawberry receptacles.**

The role of plant hormones in controlling fruit set has been studied extensively, with no clear picture emerging. Attempts to correlate differences in hormone levels with differences in fruit set have been largely unsuccessful. Analysis of gibberellin (GA), cytokinin, auxin, and abscisic acid (ABA) levels in receptacles of pollinated and nonpollinated 'Winter Nelis' pear revealed no differences in hormone concentrations between the two treatments from anthesis through 12 days after anthesis, although there were significant differences in set (12). Similarly, there were no significant correlations between strawberry receptacle growth and concentrations of either 1*H*-indole-3-acetic acid (IAA) (1, 5, 13), ABA (1), or their conjugates (1, 5) in achene or receptacle tissue.

There are numerous reports on the effects of plant hormones on vascular differentiation in tissue culture and in whole plants. IAA or 1-naphthaleneacetic acid (NAA) were found necessary for the induction and differentiation of phloem and xylem tissue in callus cultures of *Fraxinus, Salix, Helianthus,* and *Ligustrum* spp. (15). Application of (2,4-dichlorophenoxy)acetic acid (2,4-D) and *N*-(2-furanylmethyl)-1*H*-purin-6-amine (kinetin) to peach mesocarp callus induced incipient sieve tube and companion cell development (2).

Wound regeneration studies in intact *Coleus* plants suggest that auxins are responsible for regeneration of xylem and phloem tissues (3, 9). Recently, Ewers and Aloni (6) demonstrated that spray applications of NAA or GA_3 to developing needles of *Pinus brutia* and *P. strobus* doubled the phloem production, but had little or no effect on xylem production.

Hormones play a major role in determining the extent of phloem, and, to a lesser degree, xylem formation in plant tissues. The role of hormones in fruit set may be, in part, one of regulating the rate and/or amount of phloem differentiation in ovary and/or accessory fruit tissue. Development of the phloem transport system would be a prerequisite for translocation of raw materials required for growth. Exogenous hormones, which induce parthenocarpic set, or hormones released endogenously upon pollination or fertilization may lead to an accelerated rate or an increased amount of phloem development in immature fruit tissues, which, in turn, result in successful set and growth of fruit.

The present study was initiated to determine if pollination or auxin treatment, both of which induce fruit set in strawberry, influence the development of the phloem transport system within the receptacle during the fruit-setting period, as compared to a nonpollinated control. Fruit set, in these experiments, refers to the growth of the receptacle (the horticultural fruit) as opposed to the achene (the botanical fruit).

One-year-old day-neutral strawberry plants (cv. Fern) were planted in 15-cm pots in the greenhouse in 1 soil : 1 peat : 1 sand (by volume), and fertilized with 1 g of Osmocote (14N–6P–12K) slow-release fertilizer and 15 mg of iron and zinc chelate. Crown flower buds were removed for the first 30 days after planting to encourage vegetative growth. Subsequently, one inflorescence/plant was allowed to develop; however, the primary flower bud was removed from inflorescences as soon as it was identified. One day prior to treatment application, uniform plants were moved to a growth chamber equipped with mercury halide and fluorescent lamps maintained at a light intensity of 440 $\mu mol \cdot s^{-1} \cdot m^{-2}$. The temperature in the chamber was held at 21.6° ± 0.3°C during the 13-hr day cycle and 17.7° ± 0.2° during the night cycle. Secondary and teritiary flowers were emasculated 1 day prior to opening and the following treatments applied: a) hand-pollinated with pollen of the same cultivar; b) nonpollinated; or c) IAA ethyl ester (Et-IAA) application. Et-IAA (2.5×10^{-3} M) in aqueous solution containing 2% dimethyl sulfoxide (DMSO) and buffered at pH 6.8 was applied to the receptacles by dipping for 10 sec. The nonpollinated control consisted of dipping receptacles in a 2% DMSO aqueous solution buffered at pH 6.8.

Fruit growth measurements. Fruit set and initial growth in the three treatments were assessed by measuring receptacle dry weight gain during the fruit-setting period. Five rep-

Received for publication 17 Nov. 1986. We gratefully acknowledge Royce Bringhurst for his generous gift of plant material. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

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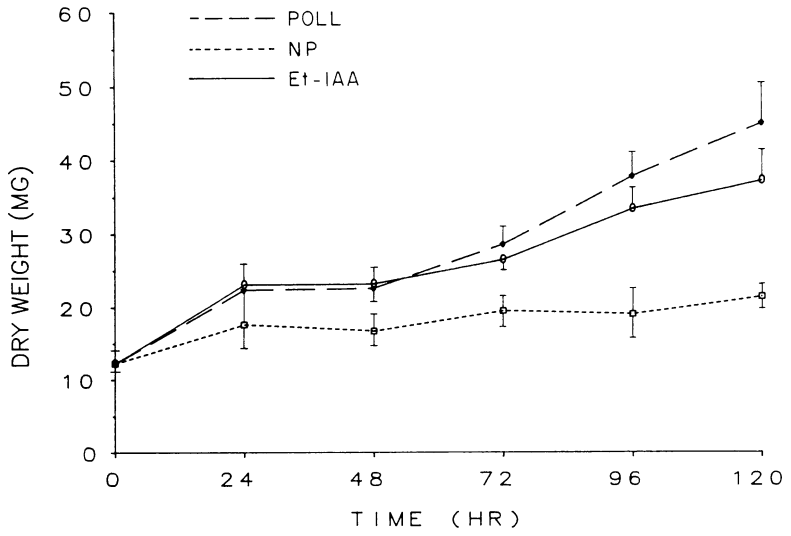

Fig. 1. Receptacle dry weights of pollinated (POLL), nonpollinated (NP), and Et-IAA-treated 'Fern' strawberry flowers from 0 to 120 hr after treatment (±SE, n = 5).

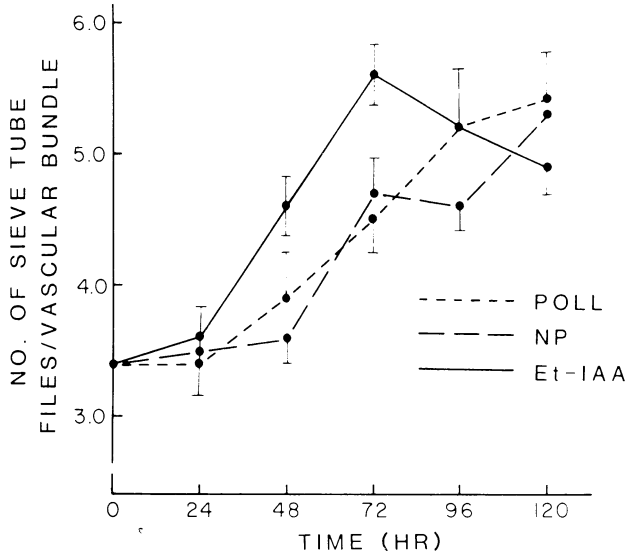

Fig. 2. Number of sieve tube files/lateral vascular bundle in receptacles of pollinated (POLL), nonpollinated (NP), and Et-IAA-treated 'Fern' strawberry flowers from 0 to 120 hr after treatment (±SE, n = 4).

lications of four receptacles from each treatment were sub-sampled at 0, 24, 48, 72, 96, and 120 hr after treatment, and dry weights were recorded after freeze-drying.

Anatomical evaluation. At 0, 24, 48, 72, 96, and 120 hr after treatment, receptacles were harvested into a solution of 50% 85 ethanol : 5 glacial acetic acid : 10 formalin (by volume), infiltrated under vacuum for 24 hr, dehydrated in a *t*-butyl alcohol : ethanol series, infiltrated with paraffin, and embedded (8). Receptacles were sectioned at 8-μm thickness, fixed onto slides, and stained using a modification of Currier's aniline blue staining technique (4). The modification consisted of increasing the pH to 11.8 by addition of K_3PO_4 in order to increase fluorescence intensity. Stained slides were mounted in Fluormount (14). Sections were examined and photographed under a Zeiss photomicroscope equipped with a fluorescence attachment. A Zeiss O2 filter set provided near-UV excitation wavelengths and passed emission wavelengths >420 nm. Phloem tissue in lateral vascular bundles (i.e., bundles connecting the achenes with the main bundles arising from the pedicel) was quantified by counting the number of sieve tube files per vascular bundle in the receptacle. Seven vascular bundles per receptacle were examined, and the entire experiment was replicated four times.

Receptacle dry weights of the pollinated and Et-IAA treated flowers began to increase within 24 hr after emasculation and treatment application and continued to increase throughout the rest of the experimental period (Fig. 1). There were no significant differences in dry weight accumulation between pollinated and Et-IAA treatments at any time. Significant differences in dry weight between the nonpollinated and either the pollinated or Et-IAA treatments were manifested by 48 hr after treatment, and these differences became more marked with time.

There were no differences in the appearance of phloem and xylem cells produced in the receptacles among the three treatments during the experimental period. The mean number of sieve tube files per lateral vascular bundle was 3.3 at anthesis, increasing to an overall mean among the three treatments of 5.2 at 120 hr after treatment (Fig. 2). Although the number of sieve tube files in all three treatments increased with time after treatment, there was no consistent difference in the number of files between the setting (i.e., pollinated and Et-IAA-treated flowers) and the nonsetting treatments during the fruit setting period. From 24 to 72 hr after treatment, Et-IAA-treated receptacles appeared to differentiate sieve tube files at a greater rate than the other two treatments. However, by 96 hr after treatment, there were no significant differences in sieve tube file number among the three treatments. The response observed in the Et-IAA treatment may be due to a supra-optimal auxin concentration during the early part of the experimental period, which encouraged a greater increase in the rate of phloem differentiation from 24 to 72 hr as compared to the other treatments. There have been previous reports of exogenous growth regulator application to plants resulting in initially accelerated growth responses (when compared to the "natural" growth responses induced endogenously) that decrease with time, presumably as the exogenous supply is depleted (11).

There was no correlation in this experiment between fruit set/initial growth and degree of phloem sieve tube development in the receptacle. Havis (7) reported that although the vascularization of strawberry receptacles increased with the size of the fruit, it was unclear as to whether there was a direct relationship between fruit size and degree of vascularization. The present study indicates that, at least during the first 5 days of growth, there is no direct relationship between receptacle size and phloem sieve tube development. On the other hand, the observation that receptacles of nonpollinated flowers continued to differentiate sieve tube files is surprising in light of the statement by Lis and Antoszewski (10) that vascular bundles in strawberry receptacles are initiated by developing achenes, which are known sources of auxin (5, 13). If auxin is required for phloem development, then apparently the receptacles of nonpollinated flowers contain sufficient auxin to induce and maintain differentiation during the first 5 days after treatment. Examination of auxin levels in nonpollinated strawberry flowers is required to determine if this is the case.

This study indicates there is no correlation between the degree of phloem sieve tube development and fruit set/per initial growth of strawberry receptacles. Whether auxin is required for the development of phloem in strawberry receptacles remains unclear.

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HortScience 22(3):469–471. 1987.

Effect of Spotted Tentiform Leafminer Injury on Ethylene Production and ACC Content in Apple Leaves

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Additional index words. *Malus domestica, Phyllonorycter blancardella*, premature fruit drop, leaf abscission

Abstract. **Apple leaves (*Malus domestica* Borkh.) injured by spotted tentiform leafminer (*Phyllonorycter blancardella* F.) released significantly higher levels of ethylene than control leaves. Leaves with tissue-feeding mines released the most ethylene, about eight times as much as control leaves. Leaves with tissue-feeding mines did not have increased levels of ACC, while some leaves with sapfeeding mines had increased levels of ACC. Chemical name used: 1-aminocyclopropane-1-carboxylic acid (ACC).**

One of the reported effects of spotted tentiform leafminer (STLM) injury to apple trees is premature fruit and leaf drop (7, 9, 10, 12). The cultivar McIntosh is particularly prone to premature fruit drop under a number of adverse conditions. Possible causes of premature fruit drop associated with STLM injury include changes in nutritional status (1), changes in levels of growth regulators other than ethylene (14), reduced photosynthetic capacity due to reduced leaf area (11), or increased ethylene from leaf wounding. Many tissues that normally evolve little or no ethylene produce large amounts when either stressed or wounded (17). There are many reports of increased ethylene production by plants injured by fungal, bacterial, and viral diseases (4, 13, 15). This increased ethylene production may be involved in wound-healing, production of phytoalexins, shedding of injured parts, increase in disease resistance, or promotion of plant growth under stress (17). Some reported responses of apple trees to STLM injury (premature ripening, fruit drop, and leaf drop) may be explained by the increased ethylene production from STLM-injured leaves.

The purpose of this study was to determine the relationship among STLM injury, ACC levels in apple leaves, and ethylene release from leaves injured by STLM.

Ethylene assays. The leaves for ethylene assay and ACC extraction were collected from either 40-year-old 'McIntosh' trees on seedling rootstock from a commercial orchard or from 13-year-old 'McIntosh' trees on M.26 rootstock in a research orchard. The mine stages included various stages of sapfeeding (SF) and tissue feeding (TF) mines. Control leaves were uninjured leaves from the same tree as the leaves with mines.

Two leaves were placed in each of six or ten 50-ml test tubes with petioles immersed in 2 ml of distilled water. The test tubes were left open for 1 hr, then flushed with ethylene-free air for 1 min and sealed with a rubber serum stopper. After a 4-hr incubation in ambient room light (14 $\mu mol \cdot s^{-1} \cdot m^{-2}$) and at room temperature (22° ± 2°C), 3 ml of head space gas was withdrawn with a syringe and analyzed for ethylene (C_2H_4) using a Hewlett Packard gas chromatograph (Model 5880A) (8).

The same leaves were used for the ethylene release and ACC data in Table 2. Once the air sample was taken for the ethylene assay, the leaves were weighed quickly and then frozen with liquid N_2 and placed in the freezer until ACC determinations were completed.

ACC extraction. The extraction method was modified from that of Mikitzel (8). Leaf samples (about 5 g, fresh weight) were frozen in liquid N_2 and held at −12°C until the extraction of ACC. The frozen leaves were then extracted in 95% ethanol.

ACC assay. ACC in apple leaf extracts was assayed by chemical conversion to ethylene in the presence of $HgCl_2$. A modified method (8) of the Lizada and Yang (6) assay was performed.

ACC was quantified using internal standards. For each extract tested, two equal volumes were taken, and one was spiked with 500 pmol of ACC. From the difference in ethylene produced, the yield of ethylene from the added ACC was calculated, and this amount used to determine efficiency of ethylene production from ACC in the unspiked sample. The mean conversion efficiencies for the two ACC extractions were 64% ± 1.7% and 56.2% ± 1.0%.

The Student *t* test or an analysis of variance was performed on the data. When the F test was significant, mean separation was by LSD.

Ethylene release. 'McIntosh' leaves injured by second generation sap-feeding (SF) or tissue-feeding (TF) mines released higher amounts of ethylene than control leaves (Table 1). Leaves with SF mines produced about twice as much ethylene as the control, whereas leaves with TF mines produced about eight times as much ethylene as the control.

Leaves with third generation TF mines from the research orchard released almost four times more ethylene than controls (Table 2). Leaves

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