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Fall Ethephon Delays Bloom in 'Redhaven' Peach by Delaying Flower Differentiation and Development during Dormancy

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Abstract. **Ethephon at 120 mg·liter^{-1} applied to hand-defoliated or nondefoliated trees in late Oct. 1984 delayed 'Redhaven' [*Prunus persica* (L.) Batsch.] full bloom by ≈5 days in 1985. The same treatment applied on 1 Nov. 1985 delayed full bloom by 9 days in 1986. Hand-defoliation alone was ineffective in delaying bloom in either season. Ethephon treatments increased abscisic acid (ABA) and ethylene levels in dormant buds collected throughout the 1985–86 dormant season. Starch and reducing sugar contents and total chilling requirement were not affected by the ethephon and hand-defoliation. Flower primordia were delayed in differentiation and growth during late fall following a 1986 spray of ethephon. A delay in flower development and growth may be caused by increased levels of ethylene and ABA. Chemical names used: (2-chloroethyl) phosphonic acid (ethephon); aminoethoxyvinylglycine (AVG).**

Delayed flowering in fruit trees has been achieved with fall ethephon sprays (5, 6, 10), but the mechanism is not clear. Ethephon may act either directly on bud physiology or indirectly by inducing early leaf abscission. Couvillon and Lloyd (3) reported bloom delay on peach induced by postharvest hand-defoliation. Similar results on bloom delay were reported on *Cornus sericea* L. after early hand-defoliation at the onset of rest (7, 8). However, Crisosto et al. (5) found that the effect of late fall ethephon application on bloom delay was independent of leaf abscission. Reduced carbohydrate reserves from leaf abscission has been suggested as the mode of bloom delay from ethephon (7). However, ethephon may affect flower bud development throughout the dormant period.

Martin and Nishijima (12) reported a possible relationship between ABA and ethylene, since they observed increased levels of ABA in peach fruit following ethephon sprays. ABA has been implicated in the regulation of dormancy in peach buds and seeds (1). Also, various stresses induce elevated ABA levels (1, 11). Thus, fall ethephon application may extend the chilling and/or heat-unit requirements for budbreak and development by altering the ABA level in peach buds.

The objective of these studies was to explore a possible mode of action for fall ethephon-induced bloom delay in 'Redhaven' peach by examining ethylene, ABA, and carbohydrate levels in flower-buds in relation to flower primordia development through the dormant period following fall ethephon application.

Materials and Methods

Experiment 1. The effects of fall ethephon spray and hand-defoliation treatments in 1984 and 1985 were studied in a factorial arrangement of three levels of ethephon (0, 60, and 120 mg·liter^{-1} adjusted to pH 5) and two levels of defoliation (with or without hand-defoliation) at the Lewis–Brown Horticultural Farm at Corvallis, Ore. Ethephon sprays were applied to run-off to five fully defoliated and to five control 'Redhaven' trees per treatment in a completely randomized design. The trees were on peach seedling rootstock and were 12 years old. In 1984, trees were hand-defoliated on 28 Oct. and treatments were applied on 1 Nov. at 10% natural defoliation. In 1985, defoliation and ethephon treatments were applied on the same trees as in 1984 on 24 and 28 Sept., respectively ≈2 weeks before initial leaf drop.

Experiment 2. In 1984, in a separate trial, ethephon at 300 mg·liter^{-1} and AVG, an ethylene inhibitor, at 500 mg·liter^{-1} were sprayed to run-off in aqueous solution with 0.01% (v/v) Tween-20 adjusted at pH 5 and 7, respectively. Controls were not sprayed. These chemicals were applied to five 12-year-old 'Redhaven' peach trees on peach seedling rootstocks in a completely randomized design after the chilling requirement was met (17 Dec. 1984).

Experiment 3. Ethephon at 120 mg·liter^{-1} was applied 28 Sept. 1986 to the same five 'Redhaven' trees previously treated with this concentration in Expt. 1 and to the control trees in a completely randomized design. The remaining trees were left unsprayed as controls.

Flower bud developoment (Expts. 1 and 2). Bloom delay was expressed as the difference in days to reach full bloom between treatments and the control. Daily counts were made during bloom to determine percent of bloom, with 80% open flowers rated as full bloom.

Flower primordia development (Expt. 3). In 1986, 'Redhaven' peach shoots were collected weekly, beginning 1 Sept. 1986, for trees in Expt. 3. Ten flower buds from each tree were dissected and the development of the various floral parts was determined under a stereomicroscope. After the point at which all floral bud primordia were distinguishable, fresh weight of floral primordia above the base of the pedicel was determined at monthly intervals between 20 Dec. and 20 Feb.

Chemical analysis (Expts. 1 and 2). During both seasons, 2.5-cm stem samples containing a floral bud were collected biweekly from 15 Oct. to 15 Mar. and analyzed for ethylene, ABA, reducing sugar, and starch contents. In Expt. 1, samples

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were taken only from control and the 120-mg·liter^{-1} ethephon treatments from both nondefoliated and defoliated treatments. The samples were weighed and stored in liquid N_2 for later analysis.

Ethylene determination (Expts. 1 and 2). Eight replications of 10-g (fresh weight) samples, each of stems and buds, were used for ethylene analysis each season. In the 1984–85 season, evolved ethylene was determined according to Siebel and Fuchigami (14).

In 1985–86, internal ethylene was determined by placing buds under water in a vacuum desiccator within 10 min after excision. An inverted funnel was placed over the tissue and the funnel stem was sealed with a rubber stopper. The desiccator was evacuated (about −100 kPa) until gas bubbles no longer were observed leaving the tissue. Gas extracted from the tissue was collected with a 1-ml plastic tuberculin syringe and assayed for ethylene by gas chromatography. In both cases, ethylene was assayed on a Carle-Analytical Gas Chromatograph model 311 (Hach Company, Loveland, Colo.) equipped with a flame ionization detector and activated alumina column at 55C.

Carbohydrate determination (Expt. 1). Four replications of 1-g (fresh weight) samples of fresh nodal stem and bud tissue per treatment were homogenized and extracted in 10 ml of 80% methanol with a Brinkmann polytron at 10,000 rpm for 2 min. Total reducing sugars and starch were determined from the same sample using the colorimetric glucose oxidase enzymatic method described by Sigma (15).

ABA determination (Expt. 1). Four replications of 1-g (fresh weight) samples of peach floral buds per treatment were collected during the 1985–86 dormant season and ground in a Brinkmann polytron homogenizer with 10 ml 80% distilled methanol, 100 mg diethyldithiocarbamic acid, and 10 mg butylated hydroxytoluene/liter. The homogenate was shaken in the dark for 60 min at 4C, centrifuged at 15,000× *g* for 15 min, and the supernatant adjusted to 70% by the addition of water. The extract was then passed over a pre-packed reversed-phase mini-column (Sep-Pak). One milliliter of the extract was diluted to 10 ml with distilled water and assayed by the Phytodetek immunoassay test (16) (Idetek, San Bruno, Calif.).

Statistics. Analyses of variance and regression were carried out using the Number Cruncher Statistical System Package (NCSS, Kaysville, Utah). When interactions were not significant, regression analysis on ethephon concentration was done on data of the combined defoliation treatments.

Results

Bloom delay (Expt. 1). Hand-defoliation in Expt. 1 had no significant influence on bloom delay at any ethephon concentration (Table 1). Extent of bloom delay in both hand-defoliated and nondefoliated plants increased with increasing ethephon concentration during the two seasons of this study. Ethephon at 120 mg·liter^{-1} delayed full bloom of nondefoliated 'Redhaven' peach by ≈5 days in 1985 and 9 days in 1986. Hand-defoliation on 28 Oct. 1984 and 24 Sept. 1985 alone or combined with ethephon did not significantly influence the time of bloom. These data disagree with those of Couvillon and Lloyd (3), who showed a small bloom delay by hand-defoliation in the late fall on 'Washington' peach. Fuchigami et al. (8, 9) delayed spring budbreak in red-osier dogwood only when hand-defoliation was done immediately after vegetative maturity, indicating that timing may be an important factor in bloom delay by fall defoliation.

Ethylene levels (Expt. 2). During quiescence, after the chilling requirement was satisfied, internal bud ethylene levels following ethephon treatment were consistently higher than in the control (Fig. 1). AVG reduced internal bud ethylene levels through the quiescent phase by ≈40% relative to the control. However, bud ethylene levels during quiescence did not appear to affect bloom time, fruit set, or yield (data not shown). The general pattern of internal ethylene throughout the dormancy period for

Table 1. Effect of hand-defoliation and ethephon treatment in fall on delay of full bloom of 'Redhaven' peach the following spring (Expt. 1).

Treatments	Delay in full bloom (days) Hand-defoliation	Non-defoliated
Application: 28 Oct. 1984		
Control	0.4	0.0
Ethephon at 60 mg·liter^{-1}	2.0	2.4
Ethephon at 120 mg·liter^{-1}	5.2	4.6
Significance:		
Defoliation	NS[z]	
Ethephon:		
Linear	*	
Quadratic	NS	
r^2	0.95	
Equation[z]	y = 0.1 + 0.04x	
Application: 24 Sept. 1985		
Control	2.0	0.0
Ethephon at 60 mg·liter^{-1}	7.0	6.3
Ethephon at 120 mg·liter^{-1}	9.5	9.0
Significance:		
Defoliation	NS	
Ethephon:		
Linear	*	
Quadratic	NS	
r^2	0.87	
Equation[z]	y = 0.16 + 0.07x	

[z]x = ethephon concentration, y = number of days delay in full bloom.
NS,*Nonsignificant or significant at $P = 0.05$.

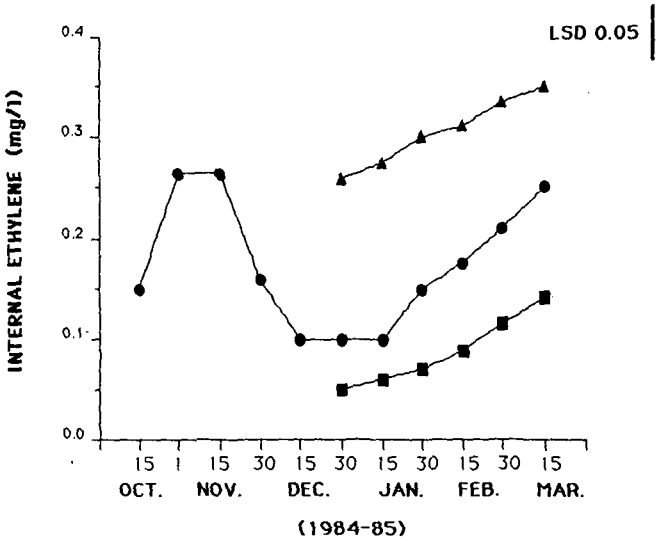

Fig. 1. Effect of 500 mg·liter^{-1} AVG (■) or 300 mg·liter^{-1} ethephon (▲) applied after rest, or (●) control onthe bud internal ethylene levels during the quiescent phase in 'Redhaven' peach; (Expt. 2). Each point is the mean of eight observations.

both years studied was similar (Fig. 2). The ethylene level was relatively high in the fall during leaf drop, then decreased and remained low during test, and was followed by an increase during quiescence. The levels of internal ethylene were greater during the rest and quiescent periods in ethephon-treated trees than in the other.

Carbohydrates (Expt. 1). No statistical differences in reducing sugars or starch levels of flower nodal buds were detected among the treatments throughout the dormant period (data not shown). Total sugar levels decreased at the onset of rest and reached a minimum at the end of rest for all treatments. At the end of rest, total sugar concentration reached a maximum and then decreased just before budbreak. Priestley (13) pointed out the fall defoliation was not related to apple bud carbohydrate content, but was highly correlated with root carbohydrate content.

Abscisic acid (Expt. 1). The pattern of flower bud ABA levels during the dormant season for all the treatments was similar (Fig. 3). ABA level was high at the onset of rest, decreased to a minimum during rest, reached the highest level at the end of rest, and again decreased during the quiescent phase. ABA levels were significantly higher in ethephon-treated buds than in the others during the onset and end of the rest period. Hand-defoliation increased ABA only in the unsprayed flower buds, but this level was significantly lower than those treated with ethephon. During quiescence, all treatments showed similar ABA levels.

Flower primordia development (Expt. 3). Ethephon applied during stamen differentiation (28 Sept. 1986) delayed pistil differentiation by 15 days (Table 2). After complete flower differentiation in the treated trees, the fresh weights of the floral-bud primordia during rest were only about half of those of the untreated controls (Table 3).

Discussion

In both years, high bud levels of ethylene during dormancy were related to bloom delay in 'Redhaven'. In an earlier study with peach (4) , manipulation of ethylene levels by ethephon and ethylene inhibitors in whole trees and cuttings after chilling accumulation was ineffective in bloom delay. Since ethephon and ethylene-biosynthesis inhibitors applied during quiescence increased or reduced bud ethylene levels without altering bloom time in 'Redhaven' peach trees, it is evident that ethephon action on bloom delay occurred between the onset of dormancy and completion of rest. The reduction in 'Redhaven' floral primordia development and fresh weight following fall ethephon treatment coincided with increased bud levels of ethylene and ABA. Apelbaum and Burg (2) reported an inhibition of deoxyribonucleic

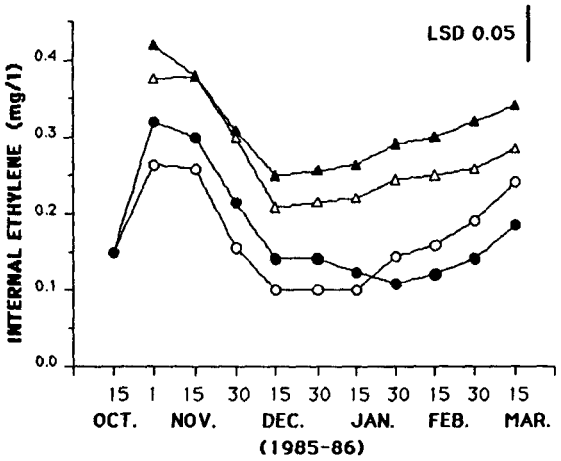

Fig. 2. Internal ethylene in 'Redhaven' flower buds during dormancy, 1985–86, after fall ethephon (29 Oct. 1985) and hand-defoliation (24 Oct. 1985) treatment (Expt. 1): ▲ = hand-defoliation and ethephon at 120 mg·liter^{-1}, △ = ethephon at 120 mg·liter^{-1}, ● = hand-defoliation, ○ = control. Each point is the mean of five observations.

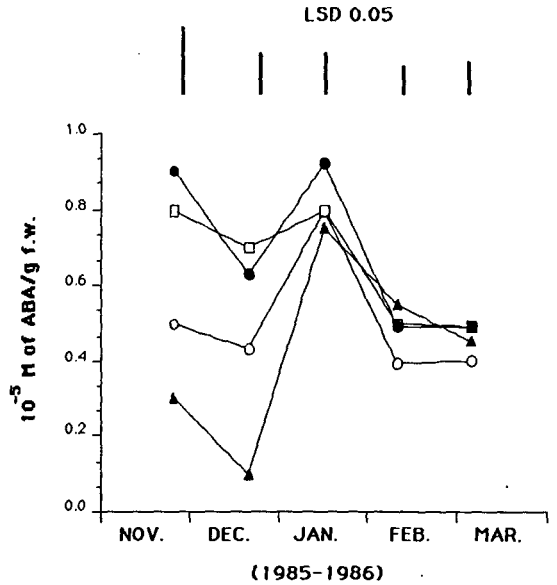

Fig. 3. Abscisic acid levels in 'Redhaven' flower buds during dormancy in 1985–86 after fall ethephon and hand-defoliation treatments (Expt. 1). Each point is the mean of five observations. (▲ = control; ○ = hand-defoliation; □ = ethephon at 120 mg·liter^{-1}; ● = hand-defoliation + ethephon at 120 mg·liter^{-1}.

Table 2. Effect of fall ethephon on floral bud differentiation and development in 'Redhaven' peach trees (Expt. 3).

Primordia structural stages	Date of first appearance of primordial stages, 1986 (80% of 50 live flower buds)	
	Untreated	Ethephon (120 mg·liter^{-1})
Flattened meristem	1 Sept.	1 Sept.
Sepal differentiation	1 Sept.	8 Sept.
Petal differentiation	23 Sept.	23 Sept.
Stamen differentiation	1 Oct.	30 Sept.
Pistil differentiation	29 Oct.	14 Nov.

Table 3. Effect of ethephon at 120 mg·liter^{-1} on 28 Sept. 1986 on flower bud fresh weight on three sampling dates (Expt. 3).

Sampling date	Floral bud fresh wt (mg)[z]	
	Control	Ethephon
20 Dec. 1986	16 a	9 b
20 Jan. 1987	21 a	12 b
20 Feb. 1987	32 a	18 b

[z]Means for each date followed by different letter differ at $P = 0.05$. Each mean represents five observations.

acid synthesis and cell division in the plumular hook of pea after ethephon application.

Elevated levels of ethylene and ABA in ethephon-treated 'Redhaven' flower buds apparently delayed bloom in part by slowing the rate of floral development, possibly through effects on cell division.

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