Regulation of Growth, Flowering and Fruit Abscission with 2-Chloroethanephosphonic Acid[1]

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Abstract. The new growth regulator 2-chloroethanephosphonic acid (Ethrel)[3] was applied to apple and peach branches or young trees at several stages of development from prebloom to harvest. Application at 1000 to 2000 ppm during the prebloom to early postbloom stages on several apple cultivars virtually eliminated all fruit with little or no phytotoxicity. Peach foliage and shoots were more sensitive to the chemical. Vegetative growth was checked and flower bud formation promoted in some cases. From one week after bloom to 4 weeks after bloom, fruit set could be reduced but it was not possible to eliminate the fruit in all cases without phytotoxicity. Application from then until a few weeks before maturity had little effect on abscission. Fruit abscission at maturity was promoted with application of Ethrel.

[1]Received for publication August 29, 1968.
[2]The authors wish to express their appreciation to Amchem Products, Inc., Ambler, Pa. for supplying the chemicals used in this study.
[3]This chemical had been released as G-996 in a formulation identified as Amchem 66-329. It contained, in addition to the acid, the mono-2-chloroethyl ester of the acid and the anhydride.

Introduction

IN a study in 1967 on materials to delay flower bud opening of apple and to promote cold resistance during bloom, 2-chloroethanephosphonic acid (Ethrel) was included with several other materials because of its reported effect on flowering of pineapple and growth regulatory properties (2). In preliminary trials it was found that application at prebloom and full bloom stages, while appearing at first to delay flower development, resulted in extensive flower drop, depending on the concentration. Complete elimination of flowers and fruits occurred at a concentration of 2000 ppm. This was accomplished without phytotoxicity on the apple cultivars. Similar effects were noted from applications on the peach cultivars 'Redhaven' and 'Golden Jubilee', although leaf and twig injury appeared at the higher concentrations. With this background, tests were planned on cultivars of apple and peach at various intervals after petal fall to evaluate the effects of Ethrel on fruit set, vegetative growth and flower bud formation. This paper presents the results of tests on these 2 species in New York in the spring and summer of 1967 and in Australia during the 1967–68 season.

Materials and Methods

The Ethrel applications for these tests were prepared either from the original formulation, 66-329, or from the technical material G-996. The composition and characteristics of 66-329 have recently been described by Cooke and Randall (3). Tween 20 was added to all sprays at 0.1%.

Individual limbs were selected for uniformity on mature trees of the various cultivars at the University orchard at Ithaca and at the Agricultural Research Station in Bathurst. The treatments were replicated on 3 to 5 trees depending on the cultivar and location. Limbs were marked and flowers counted on the treated portions. Sprays were applied to run off with a suitable hand sprayer. Fruit set data were obtained on these limbs after fruit drop was completed. Fruit size was determined at various intervals and at maturity. Shoot growth measurements were obtained during the dormant season in some cases, and bloom counts were made on some of the apple cultivars the following spring to determine the effect of the treatments on repeat bloom.

Table 1. Effect of Ethrel on fruit set and size of 3 apple cultivars. Ithaca, N. Y. 1967.

Variety	Treatment (ppm Ethrel)	Date applied	Stage of development	Fruit set[a] (%)	Fruit diam.[b] (in)
McIntosh	0	May 1	Del. dorm.	36.2	2.65
	200	May 1		4.6	2.70
	2000	May 1		0	—
	0	May 17	Pink	31.4	2.67
	200	May 17		10.1	2.61
	2000	May 17		0	—
	0	May 23	Full bloom	28.3	2.74
	1000	May 23		2.9	2.62
	0	June 2	F.b. + 10 days	28.0	2.63
	50	June 2		29.2	2.58
	250	June 2		10.6	2.47
	500	June 2		5.8	2.42
	0	June 20	F.b. + 28 days	32.6	2.70
	250	June 20		30.5	2.59
	0	July 6	F.b. + 44 days	31.7	2.73
	75	July 6		29.8	2.77
	250	July 6		33.6	2.69
R. I. Greening	0	May 17	Pink	37.3	—
	200	May 17		19.5	—
	2000	May 17		0	—
E. McIntosh	0	June 1	F.b. + 8 days	70.2	2.48
	500			8.1	2.40
	1500			0	—
	50	June 6	F.b. + 13 days	54.5	2.45
	150			13.6	2.37
	450			0	—

[a]Number of fruits per 100 blossom clusters as determined August 1.
[b]Average of fruits on treated and control limbs at harvest.

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Table 2. Effect of Ethrel on 'Democrat' apples. Bathurst, Australia. 1967–68.

Treatment[a] (ppm Ethrel, Oct. 24)	Fruit[b] set (%)	Fruit diam. (in) Dec. 6	Fruit diam. (in) Apr. 2	Seeds[c] per fruit	Firmness[c] (lb.)	Soluble[c] solids (%)
100	30.7	1.35	2.56	6.40	10.8	14.0
200	11.5	1.19	2.55	6.56	10.8	13.5
300	4.7	1.07	2.27	6.48	10.9	14.6
Control	62.3	1.57	2.72	5.32	11.0	13.4
L.S.D. (.05 level):	14.8	0.11	n.s.	0.79	n.s.	n.s.

[a]Treatment date was full bloom + 10 days.
[b]Fruit per 100 blossom clusters Dec. 6.
[c]Firmness, soluble solids and seeds per fruit obtained at harvest April 2, 1968.

Results

Apples. Reduction in fruit set was obtained when Ethrel was applied at 200 ppm during bloom or prebloom stages on 2 apple cultivars (Table 1). Complete elimination of fruit occurred with an application of 2000 ppm. Ethrel at 200 ppm also reduced set when applied 10 days after full bloom. However, concentrations up to 250 ppm were ineffective in reducing set on 'McIntosh' in this test on June 20 and July 6, 28 and 44 days respectively after full bloom. In spite of the reduction in set from the earlier applications, fruit size at harvest was generally smaller than the controls (Table 1). The prebloom application on 'R. I. Greening' and the postbloom applications on 'Early McIntosh' affected set reduction or crop elimination similar to that of 'McIntosh' (Table 1).

In the Bathurst test with 'Democrat', fruit thinning was also accomplished with applications of Ethrel from 100 to 300 ppm 10 days after full bloom (Table 2). Fruit size measurements both in midseason and at harvest also showed the depressing effect on growth rate of Ethrel even though set reduction was achieved. Fruits from the treated limbs were found to contain on the average a higher seed content than the controls. Fruit firmness and soluble solids at harvest were not significantly altered on this variety.

Ethrel was also applied to limbs of 'Granny Smith' about 1 week before the beginning of harvest for this cultivar. The fruit was not harvested until the end of the normal harvest period which was 1 month after treatment. Fruit abscission, as measured by drop at harvest, was significantly accelerated at 250 ppm (Table 3). However, fruit firmness and soluble solids were not affected by the treatment under these conditions.

Peaches. Ethrel applied 1 month after full bloom in 1967 at 50 and 150 ppm effectively reduced fruit set of 'Redhaven' peaches in the New York test (Table 4). At 450 ppm all fruits were eliminated in this test. Some leaf yellowing occurred with abscission of about 20% of the leaves on limbs receiving this concentration. Yellowing of a few of the basal shoot leaves took place at the 150 ppm rate with no apparent effect on shoots or leaves at the lowest rate.

In contrast to the applications on apple cultivars, the size of the peaches at harvest on the limbs treated at the 50 and 150 ppm rates were larger than fruits on control limbs which were hand thinned in late July after fruit set data were obtained (Table 4). Shoot growth was retarded in this test with the 150 and 450 ppm applications.

Similar results on fruit set were obtained in the Bathurst test on 3 varieties (Table 5). 'Earlyvee' and 'Reid's Seedling' were thinned more heavily at the higher concentrations than the heavy setting 'Success' cultivar. The applications were made October 24, approximately 4 weeks after full bloom. Some leaf yellowing and abscission occurred on all varieties at the 400 ppm rate similar to that observed in the 'Redhaven' test. While fruit size measurements were not recorded at harvest, size was only slightly improved as a result of the reduction in set.

Table 3. Effect of Ethrel on harvest drop and quality of 'Granny Smith' apples. Bathurst, Australia. 1968.

Treatment[a] (ppm Ethrel, April 23)	Fruit drop May 24 (%)	Firmness[b] (lb.)	Soluble solids[b] (%)
250	61.0	7.0	15.7
500	87.2	7.2	15.9
1000	99.4	7.2	15.9
Control	11.2	7.1	15.9
L.S.D. (.05 level):	14.5	N.S.	N.S.

[a]Mature trees with 8 replicate branches on separate trees per treatment.
[b]At time of treatment April 23 the fruits had an average pressure of 8.5 pounds and soluble solids of 14.8%. Values in table obtained on samples harvested May 24.

Table 4. Effect of Ethrel on fruit set and fruit size, and on shoot growth of 'Redhaven' peaches. Hamlin, N. Y. 1967.

Treatment[a] (ppm Ethrel, June 21)	Fruit set[b] (%)	Fruit wt[c] (g)	Shoot length (cm)
50	29.7	147.3	30.4
150	7.5	152.8	23.6
450	0	—	16.6
Control	67.2	126.0	30.8

[a]Mature trees, full bloom May 20; 5 replicate branches per treatment.
[b]Per cent of fruits present June 21 remaining July 19.
[c]Harvest August 21.

Table 5. Effects of Ethrel on three peach cultivars. Bathurst, Australia. 1967.

Treatment[a] (ppm Ethrel, Oct. 25)	Per cent fruit set[b] Earlyvee	Success	Reid's Seed.
50	47.6	48.5	50.8
100	42.7	46.1	36.3
200	13.8	24.9	16.1
400	1.0	9.5	0
Control	55.8	58.2	37.9

[a]Application date about 4 weeks after full bloom.
[b]Averages of 6 to 19 replicate limbs for each variety with about 150 flowers per limb.

Discussion

Plant responses to applications of Ethrel include flowering of pineapple (3), abscission of apple leaf petioles and mature fruits (4), ripening of bananas (6) and swelling of pea stems (7). These effects have generally been related to either the direct release of ethylene within the tissue or to a stimulated ethylene synthesis in the treated tissues. It has been demonstrated that Ethrel solutions are direct sources of ethylene (3). Only small amounts of ethylene are released from aqueous solutions of Ethrel in the 3 to 4 pH range while rapid release occurs in the more alkaline range (5). Thus the degradation of Ethrel with release of ethylene which occurs in plant tissue is controlled at least in part by the pH of the cytoplasm.

Exposure of developing 'Golden Delicious' fruits to ethylene a few days after fertilization resulted in abscission of fruits, depending on concentration of ethylene in the air and duration of exposure.[4] Ethylene is known to promote abscission of leaves (1, 9) and fruits (8) and it is apparently through this mechanism that the reduction in set is affected. The apple fruits with the fewer developing embryos would appear to be the more susceptible as evidenced by the seed content of mature fruits from treated and control branches (Table 2).

The slower growth rate of apple fruits following treatment is probably a result of the gradual release of ethylene in the tissues. Ethylene may act as growth retardant if the tissue is exposed to the compound for extended periods. Shoot growth of both apple (5) and peaches (Table 4) was checked in these tests. Because of the reduced growth rate of young apple fruits following the application of Ethrel the present formulation of this compound

[4]Unpublished data, Cornell University Agr. Exp. Station. 1968.

Changes in Endogenous Growth Substances in the Embryos of *Juglans regia* During Stratification[1]

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Abstract. Kernels from *Juglans regia* walnuts stratified at 0°C were sampled at weekly intervals and extracted with methanol. The extracts were partitioned into 4 phases which were water, neutral ether, acidic ether and acidic butanol, then bioassayed for cytokinins, gibberellins, auxins and inhibitors. No cytokinins nor gibberellins were found in the tissue. There was activity analogous to that from auxins. An inhibitor which diminished during stratification was found. This inhibitor is believed to be abscisic acid, on the basis of UV absorption spectrum, Rf values established by co-chromatography on paper and silica gel plates, and derivatives analyzed by gas liquid chromatography.

Introduction

IN most cases, deciduous fruit tree seeds are inherently protected from adverse environmental conditions such as low temperature by the state of dormancy. While protected from winter cold by dormancy the low temperatures play the important role of providing the requirement for winter chilling which in time breaks dormancy.

Though unproven, it appears plausible to ascribe the primary and directive mechanism of dormancy to the balance of growth promoting and inhibiting substances rather than to such factors as phosphate metabolism (20, 22) and respiration (12, 21, 24). The literature supporting the role of growth promoter and inhibitor balance in dormancy is voluminous (1, 2, 3, 8, 10, 23). The data from these works support the contention that the levels of promoters remain fairly constant while the levels of inhibitors decrease as the low temperature requirement is fulfilled. Also, the increase in endogenous gibberellins has been ascribed as a factor overcoming dormancy (6).

Naylor and Simpson (17) established that a gibberellin type seed component increased during the latter stages of dormancy. Others have shown that the application of gibberellin breaks rest in certain seeds (11). Yet another promoting factor, cytokinin, has been reported to break dormancy (16).

In order to regulate dormancy, more knowledge of its internal control mechanism is needed. The research reported in this paper was initiated to gain greater insight about the mechanism of dormancy by studying the changes in growth promoting and inhibiting compounds of walnut kernels during stratification.

Materials and Methods

Extraction. 'Payne' walnuts, *Juglans regia* L., with their hulls previously removed but unshelled, were stratified at 0°C in moist vermiculite. At weekly intervals, the nuts were taken out of the stratifying medium. The shells were removed and the fresh weight of the kernels was determined. The kernels were then cut into pieces and placed in a flask containing 100% methanol and stored at 0°C. After 4 days, the methanol was filtered off and replaced by 80% methanol; then the process was repeated on the 9th and the 14th day. The filtrates from each methanol change were combined and stored at 0°C. The alcohol insoluble substances (AIS) hereinafter called extracted dry weight, were dried in an oven maintained at 86°C. All calculations were made on the basis of the extracted dry weight (AIS).

A modification of Milborrow's (15) scheme, Fig. 1, was used for separating plant acids from the methanol extracts. The methanol extracts were concentrated to the aqueous phase under reduced pressure. The sample was centrifuged to remove any water insoluble debris and the supernatant decanted. Sodium bicarbonate at 5% was added to the water insoluble precipitate which was then triturated and centrifuged. The soluble fraction was decanted and stored for later analysis.

The aqueous supernatant was adjusted to pH 2.8 with dilute H_2SO_4. On adding NaCl to each sample, it was partitioned 3 times with ether. The ether phases were combined and concentrated to 50 ml. The aqueous-phase 1 was taken to dryness and stored in a freezer for later measurement of cytokinin.

The combined ether fraction was treated with 5% $NaHCO_3$ and water alternately. The aqueous and alkaline fractions were combined. The neutral ether-phase 2 (Fig. 1) containing the neutral and weakly acidic compounds was concentrated to dryness under reduced pressure and stored in a centrifuge tube in the freezer.

[1]Received for publication September 23, 1968.

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would not appear promising as an apple thinning agent. On the other hand, it offers considerable promise as a treatment from prebloom to early postbloom stage where complete elimination of fruit is desired.

The loosening of the fruit at harvest as shown in Table 3 would be of particular value with cultivars to be harvested mechanically as pointed out previously (4). It would also benefit conventional hand harvesting where firm attachment slowed the operation and contributed to excessive removal of stems from the fruits or to broken spurs and bruising.

While the majority of these tests were conducted on unit branches rather than on entire trees the trials were duplicated under widely differing conditions and on a range of cultivars. Nevertheless, some variations are to be expected with applications made on entire trees.

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