

raised by the ethylene generation. As has been reported earlier (10, 11), ethylene production rose following early harvests of the berries (Fig. 5), but the rise was much slower and of much less magnitude than is usually associated with a climacteric (2). Conclusions about whether or not the cranberry is a climacteric-type fruit, and about the means by which ethephon stimulates anthocyanin accumulation, must await additional evidence.

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A Morphological and Histochemical Study of (2-Chloroethyl)Phosphonic Acid-Enhanced Abscission of Sour and Sweet Cherry Fruit¹

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Abstract. (2-Chloroethyl)phosphonic acid (ethephon) significantly reduced the fruit removal force (FRF) at the lower abscission zone of 'Montmorency' sour (*Prunus cerasus* L.) and 'Windsor' sweet (*Prunus avium* L.) cherry fruit near maturity. No qualitative differences were detected in abscission layer development as a result of ethephon treatment. The primary effect was an acceleration of fruit separation following a pattern similar to that observed in the control. Separation in both treated and control sour cherry fruit was preceded by a loss of pectin and polysaccharides and a loss of cellulose orientation in the walls of cells comprising the abscission layer. Although separation in treated sweet cherry fruit was more extensive than in the control at maturity, it was still localized as in nontreated fruit and was not preceded or accompanied by a change in pectin, cellulose, or polysaccharides in the abscission layer. No effect of ethephon was observed on the upper abscission zone for either species through fruit maturity. Ethephon caused a dramatic increase in ethylene evolution from cherry fruit.

The major barrier to efficient machine harvest of many tree fruits is the force required to remove the fruit (8, 9). A close relationship has been established for the sour cherry between abscission layer development and ease of fruit removal (7, 15). Generally, a well-defined abscission layer is present between the fruit and pedicel in the sour cherry at maturity (15, 16), and as the abscission layer develops in a higher percent of the fruit population, the mean fruit removal force (FRF) declines (15). In the sweet cherry only localized separation has been observed in the fruit-pedicel abscission zone and, even at maturity, no

well-defined abscission layer is present (18). This difference in abscission layer formation between the sour and sweet cherry is reflected, in part, in the ease of fruit removal (18).

Recently, we have demonstrated that the FRF of both the sour and sweet cherry can be dramatically reduced by chemical treatment (4, 5), ethephon being one of the more effective chemicals (4). Although the formation of the abscission layer appears to be quite different between the 2 species (15, 18), chemically induced reduction in FRF is pronounced in both (4, 5).

Our objectives were to: (a) establish the effects of ethephon on the anatomical and histochemical development of the abscission layer in the sour and sweet cherry and (b) to gain an insight on the mode of action of ethephon in reducing the FRF.

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²Wittenbach, V. A. 1970. Morphological and physiological aspects of cherry fruit abscission with reference to 2-chloroethylphosphonic acid. M.S. Thesis, Mich. State Univ. p. 114.

Materials and Methods

Plant material and treating procedure. *Sour cherry.* Three large branches on each of 3 mature sour (*Prunus cerasus* L. cv. Montmorency) cherry trees were sprayed with 0, 500, or 1000 ppm of ethephon approximately 10 days before maturity. Tween 20 (polyoxy ethylene-20-sorbitan monolaurate) was included at 0.1%.

Sweet cherry. Seven-year-old sweet (*Prunus avium* L. cv. Windsor) cherry trees were selected for uniformity of vigor and fruit load. Ethephon was applied as a foliar spray to whole trees at 0, 500, and 1000 ppm approximately 13 days prior to maturity. No wetting agent was included. Three trees were used per treatment.

Statistical. A randomized block design was employed.

FRF measurement. FRF was monitored so as to relate changes to abscission layer development. FRF measurements were made on the upper (peduncle:pedicel) and lower (pedicel:fruit) abscission zones by procedures previously described (4). For sour cherry, FRF was determined for all concn for the lower zone on a random sample of 20 fruit per treatment at 0, 4, 8, and 12 days after treatment. FRF for the upper zone was determined after 8 days for the 0 and 500 ppm concn. For sweet cherry FRF measurements were made on the lower zone on a random sample of 25 fruits from each treatment collected at time of treatment and thereafter every other day for 14 days. The FRF for the upper zone was determined only for the 0 and 1000 ppm concn at 12 days after treatment.

Anatomical and histochemical. Thirty fruits each of sour and sweet cherry were collected at time of treatment and every 2nd day thereafter until 1 week past maturity. The detached fruits were immediately killed and fixed in formalin-acetic acid-alcohol (13). Embedding, sectioning, and staining procedures have been described (18). Iron haematoxylin was used as a general stain for the cell wall. Histochemical reactions used to localize cell-wall constituents were those outlined by Jensen (10): ruthenium red and hydroxylamine ferric chloride for pectin and periodic acid-Schiff's reagent for polysaccharides. Disorientation of cell-wall cellulose was studied using plane-polarized light (18).

Ethylene evolution. Ethylene evolution of treated and nontreated fruit was determined at 0, 3, 6, 9, and 12 days after treatment. Fruits were detached from the tree and the pedicel was cut 3 to 4 mm above the fruit. Twenty fruits were sealed in a glass jar (volume of 264 ml) with a vaccine stopper fitted into the cover. A filter paper wick saturated with 10% NaOH was sealed in each container to absorb CO₂. Two to 4 replications were used. Sealed containers, including appropriate controls

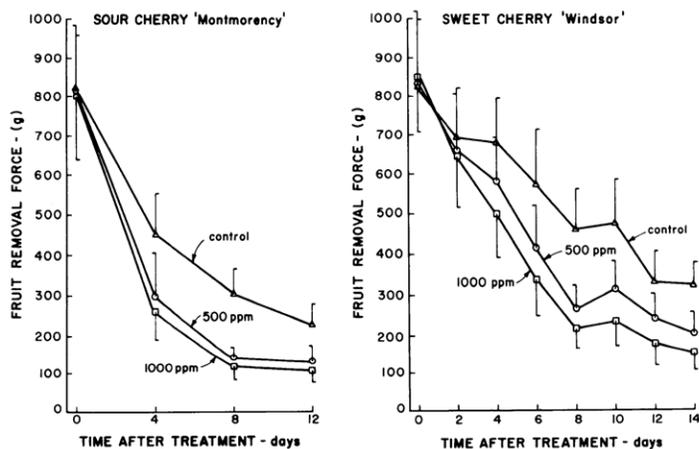


Fig. 1. Effect of a single foliar application of ethephon on FRF in 'Montmorency' sour and 'Windsor' sweet cherry. Vertical brackets indicate standard deviations for each point.

(lacking only fruit) were held at $25^{\circ}\text{C} \pm 1^{\circ}$ in a water bath. Oxygen was supplied, with a syringe every 2 hr, to maintain the O₂ level at $21\% \pm 3\%$. The CO₂ and O₂ levels were monitored using a Vapor Fractometer, Model 154B. Ethylene evolution was determined by assaying 1.0 ml of the gas phase taken after 8 hr according to the method of Lyons et al. (11). We used a Varian Aerograph, Series 1200, gas chromatograph equipped with a 76 cm column packed with Poropak Type R.

Results

Effect on FRF. Ethephon had no significant effect on FRF of the upper abscission zone for either the sour or sweet cherry, whereas the FRF of the lower zone was reduced to 52% of the control (Table 1). Ethephon caused a rapid and marked reduction in FRF of the lower zone of both the sour and sweet cherry within 8 days; thereafter, there was no significant decrease in FRF (Fig. 1). The FRF presented in Fig. 1 are representative of fruit used for anatomical and histochemical studies, and thus provide a comparison between change in FRF and abscission layer development. There was no significant difference in response to the 2 concn of ethephon.

Effect on abscission layer development. (a) *Anatomical.* We found no evidence that ethephon had any effect on the upper abscission zone of the sour or sweet cherry (Fig. 2). Further, the 500 and 1000 ppm treatments produced similar effects on the lower abscission zone; therefore, only data will be presented for the lower abscission zone following application of 500 ppm

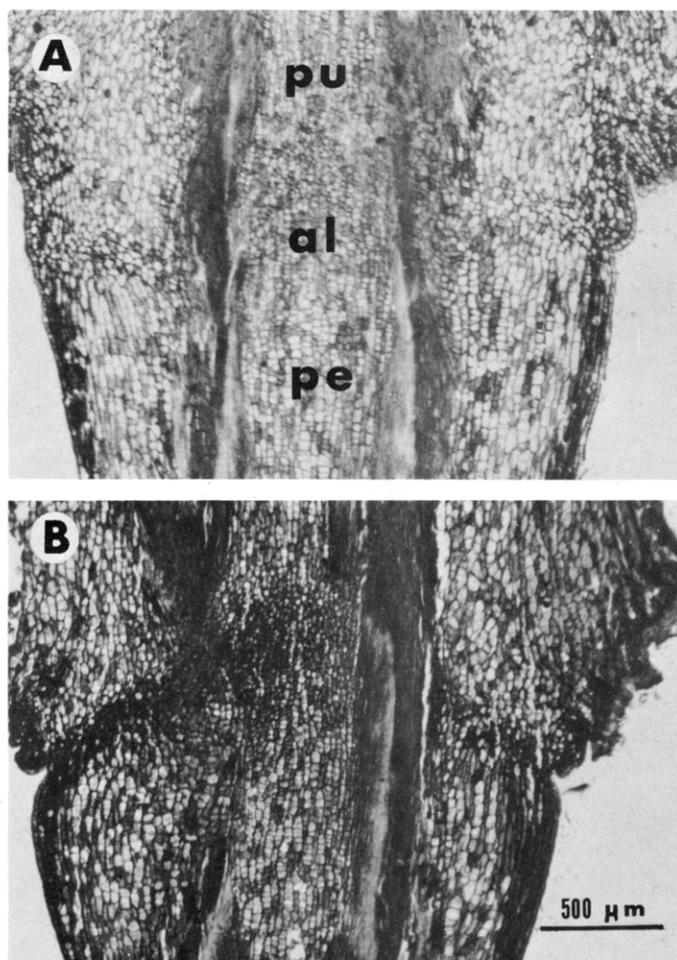


Fig. 2. Photomicrographs of longitudinal sections through the upper abscission zone of 'Montmorency' sour (A) and 'Windsor' sweet cherry (B) at 10 and 12 days, respectively, after treatment with 500 ppm ethephon. pu - peduncle, al - abscission layer, pe - pedicel

Table 1. Effect of ethephon on fruit removal force (FRF) at the upper and lower abscission zones of 'Montmorency' sour and 'Windsor' sweet cherry.

Abscission zone	FRF (% of control)	
	Montmorency ^z	Windsor ^y
Upper	109	107
Lower	52	52

^zFoliar application of 500 ppm approximately 10 days prior to maturity. FRF measured after 8 days.

^yFoliar application of 1000 ppm approximately 2 weeks prior to maturity. FRF measured after 12 days.

ethephon.

Separation in control (Figs. 3A, 3B) and ethephon-treated (Figs. 3C, 3D) sour cherry fruit occurred in a similar manner. The first evidence of abscission layer development in treated fruit was a loss in affinity for haematoxylin by the walls of cells comprising the abscission layer (Fig. 3C). Then cells all through the crescent shaped layer began to separate (Fig. 3D). Cells usually separated along the middle lamella leaving the cell walls intact; however, some cell walls were fractured (Fig. 3D). Anatomically the major difference was that in control fruit a few layers of cells next to the indentation failed to separate (Fig. 4A), whereas, in ethephon-treated fruit cell separation often proceeded through this region (Fig. 4C). The most striking effect of ethephon was the hastening of cell separation in the abscission layer (Fig. 3). Cells adjacent to the abscission layer in treated fruit exhibited greater plasmolysis and cellular distortion than did those in control fruit.

Fruit separation in ethephon-treated sweet cherry was similar to but more advanced than that in the control (Figs. 4B, 4D, and 5). The first evidence of abscission was formation of a cavity just above the stony pericarp, which resulted from separation and possibly degradation of cells (Figs. 4B, 4D). Later separation occurred at the fruit : receptacle indentation and proceeded in toward the vascular bundles (Fig. 5). Separation involved the fracturing of cell walls as well as separation along the middle lamella (Fig. 5D). More shearing of cells was evident in sweet than in sour cherry. Although the degree of separation was more advanced in ethephon-treated fruit at maturity (8 to 10 days after treatment), control fruit

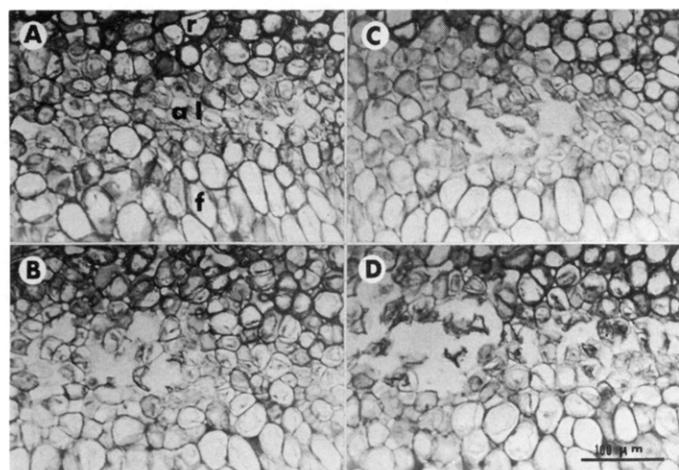


Fig. 3. Photomicrographs of longitudinal sections through the lower abscission zone illustrating development of the abscission layer between the indentation and vascular bundles of 'Montmorency' sour cherry. Control (A, B) and ethephon-treated (C, D) fruit 2 days (A, C) and 4 days (B, D) after treatment. Ethephon applied at 500 ppm. Sections were stained with iron haematoxylin. r - receptacle, al - abscission layer, f - fruit.

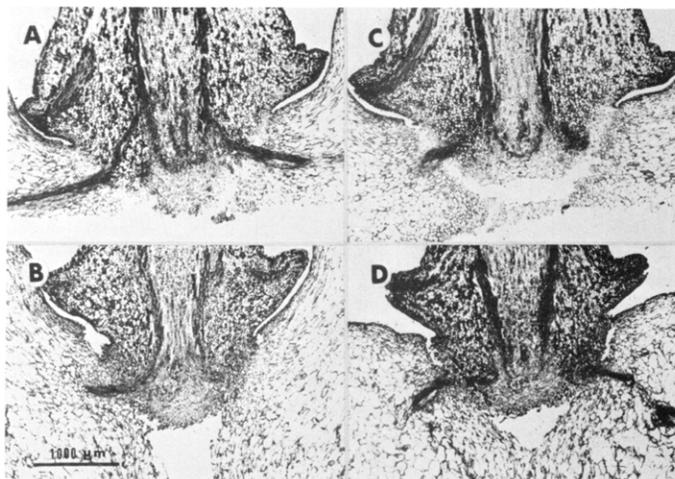


Fig. 4. Photomicrographs of longitudinal sections through the lower abscission zone of 'Montmorency' sour (A, C) and 'Windsor' sweet cherry (B, D) at maturity of control (A, B) and ethephon-treated (500 ppm) fruit (C, D).

attained the same degree of cell separation when held to over-maturity (7-10 days past maturity).

No separation was evident across the vascular bundles of treated or control fruit of either species. Some random cell division occurred proximal to the abscission layer but apparently was not directly involved in separation.

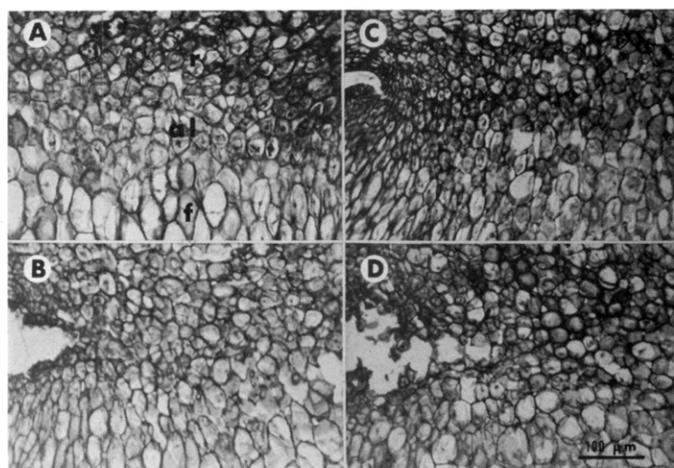


Fig. 5. Photomicrographs of longitudinal sections illustrating development of the abscission layer through the lower abscission zone between the indentation and vascular bundles of 'Windsor' sweet cherry. Control (A, B) and ethephon-treated (C, D) fruit 4 (A, C) and 8 (B, D) days after treatment. Ethephon applied at 500 ppm. r - receptacle, al - abscission layer, f - fruit.

(b) *Histochemical*. No qualitative differences were observed in the abscission zone as a result of ethephon treatment in either the sour or sweet cherry. However, in both the control and treated sour cherry, a loss of pectin and cell-wall polysaccharides and a loss in orientation of cell-wall cellulose were observed in the abscission layer. No comparable changes were observed in either the control or treated sweet cherry fruit during abscission.

Effect on ethylene evolution. Ethephon at 1000 ppm caused a marked increase in ethylene evolution by detached sour and sweet cherry fruit (Table 2). The levels remained high for at least 12 days after treatment.

Discussion

Although the mode of fruit separation is quite different between the sour and sweet cherry (18), ethephon caused a dramatic decrease in the FRF at the lower zone of both species.

Table 2. Ethylene evolution from 'Montmorency' sour and 'Windsor' sweet cherry fruit during an 8-hr period following detachment from nontreated or ethephon-treated (1000 ppm) trees.

Days after treatment	Montmorency		Windsor	
	0 ppm	1000 ppm	0 ppm	1000 ppm
	($\mu\text{l/kg/hr}$)			
0	.020	---	.018	---
3	.013	1.45	.033	1.07
6	.005	2.13	.015	2.05
9	.007	1.70	.017	1.65
12	.025	1.15	.028	.86

There was no detectable change in the FRF at the upper zone (Fig. 2). No qualitative changes in abscission were noted as a result of treatment with ethephon. The primary effect of ethephon was an acceleration of fruit separation following a pattern similar to that observed in the control (Fig. 4).

Stösser (14) reported that ethephon applied 8 to 10 days before maturity caused the induction of an abscission layer in the sweet cherry. Our data² indicated that induction, in a classical sense, probably occurs prior to Stage III of fruit growth, which is before histochemical changes or cell separation can be detected. Hence, any chemical applied less than 1 month before maturity would only advance or delay the progression of processes already induced. The separation of the abscission process of cherry fruit into induction and developmental stages is currently under study.

The source of the ethylene evolved from ethephon-treated detached fruit is not clear. Ethephon has been shown to degrade to ethylene on an equal molar basis (17, 19). The ethylene evolved could have been derived solely from the complete degradation of ethephon absorbed and accumulated in the fruit based on uptake and accumulation estimates made by Edgerton (personal communication). On the other hand, the ethylene released from ethephon may have induced ethylene formation by the fruit tissue (12, 18).

Regardless of the source, ethylene probably plays an important if not the primary role in accelerating cherry fruit abscission. Ethylene enhancement of leaf abscission is well documented (1, 2, 6), and the arguments proposed on mode of action are probably applicable to the sour cherry. In the sweet cherry, where physical forces appear to be involved (18), the role of ethylene may be one of stimulation of pericarp expansion and fruit ripening (5). It has been proposed that fruit abscission of avocado (3), mango (3), and sweet cherry (18) is simply a result of the fruit ripening process. Separation occurs at the juncture of the fruit and receptacle because this represents the weakest point of attachment, that is, between the softening cells of the maturing fruit and the woody receptacle tissue. The physiological nature of the abscission process must be elucidated before we can develop a general concept of cherry fruit abscission.

It should be stressed that ethephon was without effect at 500 or 1000 ppm in promoting abscission layer formation in the upper zone. The basis for this differential sensitivity between the upper and lower zones remains obscure. The relative insensitivity of the upper compared to the lower zone to ethephon (5) poses a problem in promoting cherry fruit abscission with stems attached by use of ethylene generating chemicals.

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