Effects of Preharvest Application of Ethephon on ‘Early Black’ Cranberries

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Abstract. Application of (2-chloroethyl) phosphonic acid (ethephon) at a rate of 0.92 kg/ha stimulated preharvest anthocyanin accumulation in ‘Early Black’ cranberries. A 10-fold increase in ethylene production followed application; however, within 10 days there was little difference in ethylene production between control and treated fruit. The increase in pigment that occurred during this time persisted well beyond this period. Ethephon did not affect the rate of CO2 production from the fruit. Whether or not anthocyanin stimulation occurred via accelerated ripening was not resolved.

The American cranberry (Vaccinium macrocarpon Ait.) owes much of its commercial value to the red color imparted by 4 anthocyanin pigments (16, 20) which form as the fruit mature. Many berries are poorly colored when harvested, since it is usually necessary to harvest the fruit while shaded berries are still white or pink.

Malathion (5, 7, 8, 17) and (2-chloroethyl) phosphonic acid (ethephon) (4, 9, 14) enhance red color of cranberries. In Massachusetts, Devlin, and Demoranville (4) found that 100-1000 mg/liter, 2800 liters/ha of ethephon applied 2 weeks before harvest sharply increased the anthocyanin content of berries at harvest.

Since ethephon may stimulate ripening of fruit (15), the anthocyanin promotion in cranberry might be due to accelerated ripening. A sensitive index of ripening in many fruit is the time of occurrence of the respiratory climacteric. No clearly discernible respiratory climacteric has been reported in cranberry, but 2 reports (6, 10) have indicated a minor rise in respiration during ripening. However, a distinct increase in ethylene production occurs during cranberry ripening (10, 11).

Our objectives were to characterize the effects of preharvest application of ethephon on cranberries, and to determine whether or not fruit ripening is accelerated by treatment.

Materials and Methods

Tests were conducted during 1969, 1970, and 1971 using the cv. Early Black grown at the University of Massachusetts Cranberry Experiment Station, East Wareham. In all tests ethephon was applied at a concn of 1.0 g/liter and at a rate of 0.92 kg active ingredient/ha (5 lbs./A).

1969. On August 13, 9 plots were sprayed with ethephon, and on each of 3 dates (August 27, September 3, and September 10) 3 of the sprayed plots and 2 untreated plots were scoop-harvested. Nine additional plots were sprayed on September 10 with ethephon, and on each of 3 dates (September 24, October 1, and October 8) 3 of these sprayed plots and 2 untreated plots were harvested. Samples of fruit were thus obtained over a period extending from prior to commercial harvest to later than commercial harvest, and the samples varied from mostly white and pink berries (on August 27) to nearly all dark red berries (on October 1 and 8). Following harvest the berries were hand-sorted to remove imperfect or bruised fruit and stored at 0°C, 90% RH until assayed for CO2 production.

1970. Based on 1969 results, berries were harvested only 3 times, on September 8, September 22, and October 6. Ethephon was applied 2 weeks before each harvest, and each treatment was replicated 3 times. The harvested berries were hand-sorted and CO2 production of samples was immediately determined at 21°C.

1971. A time-course study was conducted with 3 replicates. Plots sprayed on August 24 were harvested 3, 6, 10, and 14 days later. Plots sprayed on September 7 were harvested 3, 6, 10, and 13 days later. All samples were hand-sorted and stored at 0°C, 90% RH until assayed for CO2 and ethylene production.

Each year, immediately following harvest, fruit samples were analyzed for anthocyanin content using a slight modification (4) of the method of Fuleki and Francis (13). In 1969 samples were also indirectly assayed for pectin composition by the viscometric method of Baker and Kneeland (1).

Respiration rate was assessed by CO2-analysis of constant-flow effluent air with a MSA Model 200 infrared-gas analyzer. Fruit samples were sealed in a 9 liter desiccator at 21°C and a continuous flow of air (10 liters/min) was monitored daily for CO2 content. In 1969, sample size was 500g (ca. 500 berries) and in 1970 and 1971, sample size was 1000g. Since cranberries have an RQ near 1.0 throughout their development (6), O2 uptake was not measured.

Ethylene was determined by sealing 100g of fruit in a 250 ml Erlenmeyer flask covered by a vaccine cap after equilibrating 24 hr at 21°C. The flasks remained sealed for 24 hr at 21°C after which duplicate or triplicate 1-ml samples of head space were analyzed with a Varian Aerograph Model 600-D gas chromatograph equipped with a flame ionization detector. A 6 ft long, 1/8 in ID stainless steel column containing 60-80 mesh activated alumina was employed at 70°C. Flasks were then aerated and resealed.

Results

1969. Ethephon increased the anthocyanin content of cranberries 33-49% at the first 4 harvests (Table 1), resulting in a clearly distinguishable color difference at the first 3 harvests. At the last 2 harvests, which were beyond the desirable harvesting period, the percent increase over the control fruit was less and samples were no longer visually separable. Juice viscosity declined with maturation, but the treatments had no apparent effect on rate of decline (Table 1).

The postharvest respiratory behavior of berries was similar among the first 3 harvests; in Fig. 1a response of the third harvest is illustrated. Respiration dropped sharply after harvest, as had been found previously (6, 10, 11), but then underwent a distinct but brief rise that clearly resembled a respiratory climacteric. Ethephon affected neither the time of occurrence nor the magnitude of this rise. Berries from the last 3 harvests underwent only small and erratic rises in respiration, and again there appeared to be no distinct difference between control and treated fruit. Fruit from the last 3 harvests may have passed through the climacteric prior to harvest.

1970. At each of the 3 harvest dates, the application of ethephon 2 weeks earlier significantly increased the anthocyanin content of berries (Table 1). The percent increase was greatest
Table 1. The effects of preharvest application of ethephon on anthocyanin content of ‘Early Black’ cranberries and viscosity of extracted juice.

<table>
<thead>
<tr>
<th>Date of application</th>
<th>Days from application to harvest</th>
<th>Anthocyanin content (mg/g FW)</th>
<th>Juice viscosity (Centipoise units)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td></td>
<td></td>
<td>% increase</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td>8/13</td>
<td>14</td>
<td>0.10 ± .00</td>
<td>0.14 ± .01</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>0.15 ± .01</td>
<td>0.20 ± .01</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>0.17 ± .02</td>
<td>0.26 ± .02</td>
</tr>
<tr>
<td>9/10</td>
<td>14</td>
<td>0.43 ± .03</td>
<td>0.57 ± .03</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>0.55 ± .01</td>
<td>0.65 ± .01</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>0.77 ± .01</td>
<td>0.81 ± .05</td>
</tr>
<tr>
<td>8/26</td>
<td>13</td>
<td>0.11 ± .01</td>
<td>0.24 ± .01</td>
</tr>
<tr>
<td>9/22</td>
<td>14</td>
<td>0.43 ± .01</td>
<td>0.49 ± .02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1970</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.58 ± .02</td>
<td>0.69 ± .01</td>
<td></td>
</tr>
</tbody>
</table>

*Rate of application, 0.92 kg/ha.

at the first harvest, but the absolute increase in pigment (mg/g FW) was similar at both the first and last harvest.

Berries from the first (September 8) harvest exhibited a 4-fold increase in respiration after harvest (Fig. 1b). This increase was very brief and occurred simultaneously in both control and treated fruit. Berries from the last 2 harvests underwent no postharvest increase in respiration, remaining in a steady state whether or not they had been treated with ethephon before harvest. These fruits may have produced a climacteric prior to harvest.

1971. Three days after an early (August 24) application, ethephon had no effect on anthocyanin content of berries, but 6, 10, or 14 days after application treated berries contained about 50% more pigment and were clearly redder in appearance than the controls (Fig. 2). Application to more mature fruit (September 7) resulted in a 25% increase after 3 days, and a 50% increase after 6, 10, or 13 days. However, color differences could not be seen due to the increased coloration of control fruit.

There was no effect of ethephon on the respiration of berries held 1 day at 21°C following any harvest (Table 2). Neither was there any evidence of a respiratory rise in these samplings. All samples were actually kept for at least 5 days at 21°C and monitored daily for CO2 production, and in no sample was a respiratory rise similar to that recorded in the preceding 2 years detected. Figure 3 presents the data for the September 7 harvest, which corresponds in time with the harvests depicted in Fig. 1. Figure 3 is representative of all of the harvests except that of August 27, for which the values were more erratic. Nothing resembling a respiratory climacteric was recorded in this year.

Ethephon stimulated ethylene production by harvested cranberries (Fig. 4). Berries harvested 3 days after either application were producing about 10 times more ethylene than control fruit. Ethylene production declined sharply as time from application increased; berries harvested 14 days after the first application and 10 days after the second application were producing ethylene at essentially the same rate as the control fruit.

Ethylene production was monitored for at least 5 days at 21°C, and in Fig. 4 the direction of change during this time is indicated for each sample. Except for fruit from the first 2 harvests, production either remained constant or declined. The rate of evolution from the first-harvest berries during 18 days at 21°C indicated that for both control and treated fruit a gradual increase and subsequent decrease occurred (Fig. 5). For the
Table 2. CO₂ production from cranberries harvested at intervals following ethephon application. 1971.

<table>
<thead>
<tr>
<th>Date of application</th>
<th>Days from application to harvest</th>
<th>Respiration rate ( \text{Mg CO}_2/\text{kg-hr} )</th>
<th>( \text{Control} )</th>
<th>( \text{Treated} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>8/24</td>
<td>3</td>
<td>33.2 ± 3.6</td>
<td>29.8 ± 3.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>27.8 ± 0.2</td>
<td>27.4 ± 0.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>23.6 ± 0.4</td>
<td>24.1 ± 1.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>24.2 ± 1.4</td>
<td>22.7 ± 1.7</td>
<td></td>
</tr>
<tr>
<td>9/7</td>
<td>3</td>
<td>23.8 ± 1.2</td>
<td>25.1 ± 1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>26.4 ± 3.1</td>
<td>27.4 ± 2.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>27.9 ± 0.4</td>
<td>27.5 ± 0.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>29.4 ± 1.0</td>
<td>28.7 ± 3.1</td>
<td></td>
</tr>
</tbody>
</table>

\( ^2 \text{Rate of application, 0.92 kg/ha.} \)

\( ^\text{YDetermined after 1 day at 21°C following harvest.} \)

control fruit, at maximum, this was a 4-fold increase in production rate.

**Discussion**

We confirmed earlier reports (4, 9, 14) that preharvest application of ethephon increased anthocyanin content of harvested cranberries. Applications at various stages of maturity all resulted in increased pigment content. Percent increase tended to be less with late applications, but absolute increase of pigment remained at about 0.1 mg/g FW (Table 1 and Fig. 2). Pigment increased rapidly following application, reaching close to the maximum difference within 6 to 10 days (Fig. 2).

Increased pigmentation due to ethephon treatment improved the appearance of harvested berries only at the earlier harvest dates, since at later dates the controls were well colored. However, since a large percentage of the cranberry crop is processed and since total pigment content is extremely important in processing, the potential impact of this treatment on the cranberry industry is considerable. We employed the highest application rate used by Devlin and Demoranville (4); their results suggest that considerably lower rates should evoke similar responses.

The action of ethephon on plants is primarily through its capacity to generate ethylene (18, 19). On cranberries ethylene production was stimulated rapidly (Fig. 4) but the stimulation did not persist long. It appears that the ethylene was formed from the degradation of ethephon, and that an autocatalytic response was not evoked, even though the fruits are capable of producing relatively large quantities of ethylene (Fig. 5).

Since the ethephon stimulation of anthocyanin accumulation is presumably via ethylene, applications of ethylene should stimulate anthocyanin accumulation in cranberries. Fudge (12) reported that while ethylene applied at 100-2000 µl/liter caused chlorophyll breakdown of harvested ‘Howe’ cranberries, it did not stimulate anthocyanin accumulation and resulted in essentially colorless fruit. However, she kept the berries in the dark during and following treatment, and determined anthocyanin with an insensitive method. Craker (3) recently reported different findings. Application of 0.1-10.0 µl/liter of ethylene to ‘Early Black’ cranberries caused a marked increase in anthocyanin accumulation in the light and at room temperature for 4 days. Anthocyanin also increased in the dark, but to a lesser extent.

Whether or not ethephon or ethylene stimulates anthocyanin accumulation by accelerating ripening remains unclear. Our 1969 and 1970 results indicated that: 1) the cranberry was a climacteric-type fruit; 2) ethephon did not influence the time of the climacteric; and 3) ethephon was influencing anthocyanin without affecting ripening. However, our 1971 results challenged these conclusions. There was no evidence of a respiratory climacteric in this third year. Perhaps it occurred in the field either before or after the sampling period, but the range in appearance of the berries made this seem unlikely. It is possible that the earlier results were artifacts, in some way induced by the experimental procedures. Doubt about a typical respiratory climacteric response during cranberry ripening is also
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.

Literature Cited

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.

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

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