Cherry Fruit Abscission: A Role for Ethylene in Mechanically Induced Abscission of Immature Fruits

V. A. Wittenbach and M. J. Bukovac
Michigan State University, East Lansing

Abstract. Mechanical injury to the seed of immature sour cherry (Prunus cerasus L., cv. Montmorency) fruit caused an immediate and marked increase in ethylene evolution followed by abscission of the fruit at the peduncle:pedicel zone. Ethylene evolution was induced when the seed, but not the pericarp, was injured. The magnitude of ethylene evolution was related to stage of fruit development at time of injury, and was most pronounced when the nucellus was the dominant tissue in the seed. Ethephon (500 and 1000 ppm) also caused immature fruits to abscise and abscission was preceded by embryo abortion. The site of ethephon-induced fruit separation differed depending on stage of fruit development at time of treatment. Fruit separation occurred at the peduncle:pedicel abscission zone when immature fruits were treated, and at the pedicel fruit zone when mature fruits were treated. The role of ethylene in the abscission of immature sour cherry fruit is discussed.

The role of the seed in the abscission of immature fruit remains unclear. Tukey (19) showed that mechanical destruction of the seed in developing sour cherry and peach fruit during Stage II of growth resulted in an abrupt cessation of fruit growth followed by abscission. A similar effect was demonstrated by maleic hydrizide-induced seed abortion in apricot fruit prior to pit hardening (5). However, as seed destruction was successively delayed, a higher percentage of fruits persisted and developed normally (5, 19). Such studies suggested that the seed plays a dominant role in abscission during the early stages of fruit development.

Previously, we observed that ethephon, an ethylene-releasing chemical, caused seed abortion and abscission of immature fruit at the peduncle:pedicel zone when applied to sweet cherries during Stage II (3). By contrast, treatment early in Stage III resulted in an acceleration of maturity and hastening of abscission at the pedicel:fruit abscission zone. Ethephon also caused abscission of immature peach fruit (2, 6, 16); however, separation occurred primarily at the fruit:receptacle juncture, the same site as mature fruit abscission (6).

Questions remain as to the role of the seed and ethylene in immature fruit abscission. Does seed abortion induce abscission by eliminating the need of auxin or some other juvenility factor to the zone (11, 18), or does abortion of the seed result in a production of ethylene which in turn promotes abscission (7, 17)? We summarize in this paper a series of experiments designed to further our understanding of the role of the seed and of ethylene in mechanically-induced abscission of immature sour cherry fruit.

Materials and Methods

General methods. Sour cherry fruit were selected as a test system because of the marked dependency of fruit growth on the seed and the well-defined stages of fruit and seed development (13, 20). Furthermore, separation of immature fruit occurs at the peduncle:pedicel zone while mature fruit abscise at the pedicel:fruit zone, thereby allowing abscission at the 2 zones to be studied independently.

Fruit development was monitored by determining fresh wt twice weekly on a sample of 20 fruit. Seed development was recorded by photography of fruits cut in half longitudinally through the suture.

Abscission at the peduncle:pedicel zone was quantitatively determined by measuring the fruit removal force (FRF) with a Hunter Mechanical Force Gauge (Hunter Spring, Hatfield, PA.) fitted with a claw. FRF was obtained by holding the spur in the claw at its point of attachment to the branch and pulling the pedicel from the spur in line with the long axis. FRF at the pedicel:fruit zone was determined as previously described (3).

Injury-induced ethylene evolution and abscission. To determine if there was a relationship between tissue injury, ethylene evolution and abscission, different tissues of the fruit and seed were injured mechanically, and ethylene evolution and abscission were recorded. Injury was induced by drilling (high speed hobby drill, 0.79 mm diam.) into the mesocarp, endocarp, or embryo through the micropylar and into the nucellus at the chalazal end (Fig. 1). Each treatment, consisting of approximately 100 fruits, was replicated 3 times.

Ethylene evolution was determined by enclosing a sample of 10 fruit collected from each treatment immediately after injury in a 25 to 50 ml flask, depending on stage of fruit development. A filter paper wick saturated with 10% KOH was sealed in each flask to absorb CO₂. The flasks, including appropriate controls (lacking only fruit), were held in a water bath at 25 ± 1°C for 2 hr. Ethylene evolution was determined by assaying 1.0 ml of the gas phase by gas chromatography (15). Abscission was recorded by counting the number of fruits which abscised after 2 wk.

Injury of the seed at different stages of development. To further elucidate the role of the seed in abscission of fruit at various stages of development, the seed was injured by drilling either into the micropylar or chalazal end (Fig. 1). These treatments were repeated weekly from late Stage I until early Stage III of fruit development (corresponding to periods A-E of Figs. 3 and 5-1, insert). The effect of injury on subsequent development of the seed and on ethylene

Fig. 1. Diagram of the sour cherry fruit in Stage II of development showing four positions of drilling to induce injury to the fruit and seed (e, endocarp; n, nucellus; i, integuments; en, endosperm; em, embryo; m, mesocarp). Position A, drilled into the nucellus at the chalazal end of the seed; B, into the endocarp; C, into the mesocarp; D, into the embryo through the micropylar end of the seed.

Results

Fruit and seed development. Development of the ‘Montmorency’ sour cherry fruit occurs in 3 distinct stages (Fig. 2). The FRF at the peduncle:pedicel zone for persisting fruit remains relatively constant from late Stage I through Stage II, thereafter increasing slightly with maturity (Fig. 2). No decline in FRF at this zone was observed in healthy fruit prior to or during “June drop”.

Seed development is closely related to fruit growth. During Stage I the nucellus and integuments reach their maximum size (Fig. 3A). With the onset of Stage II the endosperm and embryo begin a period of rapid enlargement (Fig. 3 B-D). As the endosperm and embryo develop, the nucellar tissue is consumed. By early Stage III the embryo is completely developed and occupies almost the entire seed compartment (Fig. 3E). Only remnants of the endosperm can be seen at the outer edges of the embryo.

Injury-induced ethylene evolution and abscission. Ethylene evolution as a result of injury was related to the fruit tissue damaged (Table 1). There was no significant increase in ethylene evolved when injury was limited to the ovary wall tissues (mesocarp and endocarp). In contrast, 22 to 43 times more ethylene was evolved following damage to the seed as compared to the control (not damaged) or when only the ovary wall was injured. Fruit abscission was induced only following injury to the seed, the same treatment that caused a marked increase in ethylene evolution (Table 1).

Injury of the seed at various stages of development. Damaging the seed at either the micropylar or chalazal end during Stage I resulted in degradation of the nucellar tissue and shriveling of the integuments (Fig. 4, A and D). In addition, ethylene evolution shortly after injury was markedly increased in comparison with uninjured control fruit (Fig. 5-I), and the FRF at the peduncle:pedicel zone was greatly reduced after 10 days. All injured fruits abscised (Fig. 5-II) within 2 wk.

Similar results were obtained for the early and mid-Stage II treatments (Fig. 5-I and II, B and C). However, neither the endosperm tissue nor embryo appeared to undergo degradation similar to the nucellar tissue (Fig. 4, B and E). The endosperm appeared to shrivel later, while the embryo showed only the scar of injury from the micropyly end and retarded growth corresponding to

Table 1. Effect of mechanical injury to various fruit tissues on ethylene evolution and abscission early in Stage II of fruit growth.

<table>
<thead>
<tr>
<th>Tissue injured</th>
<th>( \text{C}_2\text{H}_4 ) (( \mu \text{L kg}^{-1} \text{hr}^{-1} ))</th>
<th>% Abscission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (non injured)</td>
<td>0.24b(^a)</td>
<td>4b</td>
</tr>
<tr>
<td>Mesocarp</td>
<td>0.46b</td>
<td>5b</td>
</tr>
<tr>
<td>Mesocarp + endocarp</td>
<td>0.36b</td>
<td>4b</td>
</tr>
<tr>
<td>Seed (micropyly end)</td>
<td>10.33a</td>
<td>88a</td>
</tr>
</tbody>
</table>

\(^a\)Mean separation (in columns) by Tukey’s \( \alpha \) test, \( P = 0.01 \).
the time of treatment. Furthermore, the rate of shriveling of the integuments appeared to be dependent on the degradation of the underlying tissue, i.e., whether it was the endosperm or nucellus.

In late Stage II of development, injury-induced ethylene evolution was greatly reduced (Fig. 5-1, D). Associated with this lower rate of induced ethylene evolution was a slight decrease in FRF at the peduncle: pedicel zone, and the number of fruits which abscised due to injury was considerably less than when injury was induced early in Stage II (Fig. 5-II). With the onset of Stage III of fruit growth, damage to the seed resulted in no appreciable ethylene evolution (Fig. 5-I, E). Moreover, the fruit did not abscise (Fig. 5-II, E), but persisted with no visible effects other than the scar left by the drill (Fig. 4, C and F). Injury to the seed at the end of Stage II or early Stage III resulted in no measurable acceleration of maturity.

Although the abscising fruit separated at the peduncle: pedicel zone, there was a significant reduction in FRF at the pedicel: fruit zone in response to seed injury (Fig. 5-III). Furthermore, the magnitude of reduction in FRF increased with subsequent treatments until late in Stage II (Fig. 5-III, D) when the injury induced ethylene was greatly reduced. Thus, in the last 2 treatments (Fig. 5-III, D and E) the FRF at the pedicel: fruit zone of the injured fruit was only slightly lower than that of the control fruit.

Influence of ethylene. Ethylene caused a marked increase in the rate of ethylene evolution shortly after treatment at all stages (Fig. 6-I). Fruit treated with either 500 or 1000 ppm at the end of Stage I abscised at the peduncle: pedicel zone (Fig. 6-II, A). Furthermore, at this early date both concn caused seed abortion (Fig. 7A). However, only the higher concn induced seed abortion and abscission during early and mid-Stage II (Fig. 6-II and 7B), even though both concn caused a significant increase in ethylene evolution (Fig. 6-I). By the onset of Stage III, neither concn induced seed abortion or abscission (Fig. 6-II), although both markedly enhanced ethylene evolution (Fig. 6-I). Ethylene in late Stage II slightly accelerated fruit coloring.

Ethylene also caused a reduction in FRF at the pedicel: fruit zone (Fig. 6-III). The lower concn was effective during Stages I and III, but only the higher concn caused a significant reduction in FRF during Stage II.

Treatment of detached fruiting branches with 10 μl/l ethylene caused a significant reduction in FRF at the upper zone after 80 hr (Table 2). However, no apparent damage to the seed was observed at the end of the experiment other than a slight brown discoloration at the chalazal end.

Ethylene applied to the fruits during mid-Stage II induced an enhanced rate of ethylene evolution (Table 3). But, if ethylene was applied later in development no increase in ethylene evolution was observed.

Influence of injury-induced ethylene. Injuring the seeds, at the micropylar end of fruits on detached, defoliated branches late in Stage I failed to cause a reduction in FRF after 80 hr (Table 2). Similar results were obtained with a second experiment run for 200 hr (unpublished data). Ethylene at either 10 μl/l or 50 μl/l enhanced abscission. Reducing the level of injury-induced ethylene to one-fifth had no significant effect on abscission (Table 2). Injury to the seeds did, however, result in seed abortion.

**Discussion**

Mechanical injury to the seed of immature sour cherry fruit caused an immediate and marked increase in ethylene evolution, followed by degradation of the nucellus and integuments and abscission of the fruit. Ethylene evolution and fruit abscission occurred only when the
TIME OF TREATMENT

Fig. 6. Ethylene evolution (I) and fruit removal force (FRF) at the peduncle:pedicel (II) and pedicel:fruit (III) abscission zones of immature sour cherry fruit. Ethylene determinations were made during the first 4 hr and FRF 10 days after treatment. Control (0 ppm) and ethephon (500 and 1000 ppm) treated fruits. See insert Fig. 5-1 for description of time of treatment. Vertical brackets indicate standard deviations. +, injured fruits abscised; ±, approximately one-half of the injured fruits abscised.

Table 2. Effect of exogenous and injury-induced ethylene late in Stage I of fruit growth on fruit removal force (FRF) at the peduncle:pedicel abscission zone and on seed abortion.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>FRF (g)</th>
<th>Seed abortion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air, at 760 mm</td>
<td>768a</td>
<td>-x</td>
</tr>
<tr>
<td>10 μl/1 C₂H₄ in air, at 760 mm</td>
<td>358b</td>
<td>*</td>
</tr>
<tr>
<td>50 μl/1 C₂H₄ in O₂, at 150 mm</td>
<td>372b</td>
<td>*</td>
</tr>
<tr>
<td>Injured seed in air, at 760 mm</td>
<td>734a</td>
<td>+</td>
</tr>
<tr>
<td>Injured seed in O₂, at 150 mm</td>
<td>717a</td>
<td>+</td>
</tr>
</tbody>
</table>

*Branches with treated fruit were held in desiccators at the indicated pressure and gas phase conditions for 80 hr.
*Mean separation by Tukey's ω test, P = 0.01.
*Seed healthy (-), seed aborted (+), seed healthy except for a small brown discoloration at chalazal end of nucellus (*).

Table 3. Effect of ethylene pretreatment on the subsequent evolution of ethylene by detached sour cherry fruit at various stages of development.

<table>
<thead>
<tr>
<th>Date</th>
<th>Stage of development</th>
<th>Air</th>
<th>10 μl C₂H₄⁻¹ hr⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>June 9</td>
<td>Mid-Stage II</td>
<td>0.15b</td>
<td>1.83a</td>
</tr>
<tr>
<td>June 18</td>
<td>Late Stage II</td>
<td>0.11a</td>
<td>0.20a</td>
</tr>
<tr>
<td>July 3</td>
<td>Mid-Stage III</td>
<td>0.04a</td>
<td>0.05a</td>
</tr>
</tbody>
</table>

*12-hr pretreatment followed by 2 hr in ethylene-free air prior to enclosure and sampling.
*Mean separation (in rows) by Tukey's ω test, P = 0.01.

seed was damaged. Injury to the mesocarp or endocarp did not induce ethylene production or fruit abscission (Table 1).

The magnitude of ethylene evolution and fruit abscission was related to the stage of fruit development at time of injury. Fruit in Stage I to mid-Stage II of growth showed a marked increase in ethylene production and abscised as a result of injury to the seed, regardless of the site of injury (Fig. 5-1 and II). Damaging the seed in Stage III, however, failed to induce ethylene evolution or abscission.

By relating injury-induced ethylene evolution to seed development at the time of injury, it appears that the nucellus may be the primary tissue responsible for the ethylene evolved (Fig. 5-1 vs. Fig. 4). That the nucellar tissue may be important is further supported by data showing that ethylene treatment can induce ethylene production in the fruit during Stage II, but not later in development (Table 3). Although these data are not conclusive, they do show that ethylene or injury-induced ethylene was greatest when the nucellus was a dominant tissue in the seed. The nucellus may have a much higher capacity to produce ethylene than other seed tissues and, if this is the case, any treatment which will trigger this mechanism, e.g. mechanical, chemical or environmental injury to the seed, will result in a high rate of ethylene production that may lead to abscission of the fruit. It remains to be established if naturally aborting embryos are sites of ethylene production and play a role in fruit abscission in this way.

The integuments are probably of little significance since little or no ethylene was evolved when the seed was damaged in Stage III (Fig. 5-1). Similarly, the endosperm and embryo are apparently not involved, since neither begins to enlarge until the onset of Stage II, and yet a high rate of ethylene evolution was observed when the seed was injured in Stage I. Furthermore, injuring the fully developed embryo in Stage III did not significantly increase ethylene evolution or cause fruit abscission (Fig. 5-1, II vs. Fig. 4).

The differential response of the 2 abscission zones with fruit development is of interest. Even though a significant increase in FRF occurred at the pedicel:fruit zone, all fruits abscised at the peduncle:pedicel zone when the seed was damaged during late Stage I and early and mid-Stage II. Application of ethephon resulted in a marked

Fig. 7. Photomicrographs depicting ethephon-induced seed abortion. A, 6 days after treatment with 500 ppm in late Stage I; B, 8 days after treatment with 1000 ppm in early Stage II.
reduction in FRF at the peduncle:pedicel zone early in fruit development, but not in the latter stages of fruit growth. In contrast, the FRF of the pedicel:fruit zone was markedly depressed by ethephon in Stage III but not earlier. Much higher concn of ethephon were needed to effect separation at the peduncle:pedicel zone with fruit development. These data suggest that the nature of abscission at the 2 zones is similar but that they change in sensitivity to ethylene with fruit development, i.e. early in the growth of the fruit ethylene causes separation at the peduncle:pedicel zone and late in fruit development at the pedicel:fruit zone. The basis for this change is not known, but offers an interesting experimental system for the study of fruit abscission.

There appears to be a relationship between ethephon-induced seed abortion and fruit abscission. However, fruit abscission was induced on detached branches at the peduncle:pedicel zone late in Stage I with ethylene and without evidence of seed abortion (Table 2). This suggests that ethylene can induce abscission at the peduncle:pedicel zone provided it can be supplied to the zone at an effective concn. Perhaps ethylene produced in the nucellus during seed abortion can directly influence abscission of immature fruit, since the vascular system may provide a direct path of low diffusion resistance from the seed to the peduncle:pedicel zone. However, it is also possible that ethylene influences abscission indirectly by reducing auxin transport (1) or movement of some other juvenility factor to the zone.

The reason for failure of fruits on detached branches to abscise in response to seed injury is not clear (Table 2). These results suggest that injury-induced ethylene may not be the sole factor controlling abscission at the peduncle:pedicel zone. Crane and Nelson (5) showed that apricot fruits with maleic hydrazide-induced aborted seeds persisted as healthy fruit so long as competition between their growth and vegetative growth was reduced to a minimum. Removal of leaves in our study, rather than having the intended effect of enhancing the rate of abscission, may have reduced competition with the fruit.

Ethylene may play still another role in abscission of immature fruit. Growth hormones present in the seed may function to mobilize and direct substrates to the fruit (4). Auxins, gibberellins, and cytokinins cause movement of inorganic and organic compounds as well as other growth hormones to the site of application (8, 9, 14). The levels of these hormones are known to be high in the sour cherry (10, 12). On the other hand, ethylene has been shown to have a possible role in auxin destruction (12). Hence, ethylene production induced by injury to the seed prior to completion of growth of the embryo may result in the destruction of the metabolic gradient established by the hormones of the seed. As a result, the transport of hormones through the peduncle:pedicel zone toward the fruit might be inhibited and abscission induced.

Literature Cited
