Involvement of Ethylene in the Pedicel in Preharvest Abscission of ‘Tsugaru’ Apple

Osamu Arakawa*, Aya Akagi, Takenori Asada and Yunosuke Shiozaki
Faculty of Agriculture and Life Science, Hirosaki University, Hirosaki, Aomori 036 – 8561

Summary

The involvement of ethylene in the pedicel in preharvest fruit drop and the relationship between ethylene in the pedicel and its production in the flesh and the seed were investigated in ‘Tsugaru’ apple. The induction of pedicel abscission was achieved by the injection of ethylene with a syringe, whereas it was inhibited by 2, 5-norbornadiene (NBD), an antagonist of ethylene action. The injection of ethephon into the fruit core, or the spray on the bourse resulted in increasing ethylene content in the pedicel and subsequent fruit abscission. The treatment with aminooxo-vinylglicine (AVG) of the fruit core, the inhibitor of ethylene synthesis, reduced the fruit abscission and ethylene content in the pedicel. Ethylene production of the seed and the flesh, and its content in the pedicel in ‘Tsugaru’ greatly increased before the rapid increase in fruit drop. Fruit of ‘Jonagold’ usually do not exhibit preharvest drop, the ethylene production and ethylene content are low; they increased little before harvest. Therefore, ethylene likely originates in the flesh and seeds and is translocated to the pedicel where it acts to induce fruit abscission.

Key Words: apple, ethephon, ethylene, fruit abscission, pedicel.

Introduction

Preharvest fruit drop is a serious problem in cultivars, such as ‘Tsugaru’ and ‘Delicious’, so that the use of chemicals, namely, 2, 4-dichlorophenoxypropionic (2, 4DCPP) acid has become virtually indispensable (Byun and Cuoi, 1988; Takishita et al., 1992). The mechanism of preharvest fruit drop problem remains to be satisfactorily elucidated.

Preharvest fruit drop has been studied in relation to the plant hormones auxin and ethylene (Brown, 1997; Edgeron, 1971; Leopold, 1971). A constant flow of auxin from the distal region of the leaf through the petiole or the fruit via the pedicel to the stem creates a gradient that prevents the formation of an abscission layer (Addicott et al., 1955; Luckwill, 1953). However, no difference in auxin balance of the bourse and the pedicel has been found between ‘Tsugaru’ and ‘Fuji’ which do not exhibit preharvest drop (Tsukahara, 1990).

The involvement of ethylene in preharvest drop has been demonstrated in previous studies as follows: the relationship between ethylene production of the fruit and abscission (Blanpied, 1972; Walsh, 1977); the induction of fruit drop by treatment with etephon (Tsukahara, 1990) and prevention of the abscission with aminooxoy-vinylglicine (AVG) which is the inhibitor of ethylene synthesis (Kondo and Hayata, 1995). Since the treatment of the fruit with etephon and AVG in previous studies affects the fruit ripening, the effect of ethylene on abscission is unclear (Kondo and Hayata, 1995; Tsukahara, 1990). Although Blanpied (1972) reported that the increase in ethylene concentration in the fruit and pedicel occurred simultaneously in the ‘McIntosh’ apple, it has not been reported that a relationship exists between ethylene content in the pedicel and ethylene production of the fruit in ‘Tsugaru’. Thus, direct evidence that the ethylene in the pedicel is involved in abscission is not yet available.

The purpose of this research is to: 1) to confirm the effect of ethylene in the pedicel on preharvest fruit abscission, 2) to examine the relationship between ethylene content in the pedicel and ethylene production of the fruit, and 3) to investigate the mechanism of preharvest fruit drop in ‘Tsugaru’.

Materials and Methods

Plant material

‘Tsugaru’ and ‘Jonagold’ apple trees (12-year old) on Malus prunifolia Borkh. var. ringo Asami rootstock planted and trained to an open center in an experimental orchard of the Teaching and Research Center for Bio-coexistence, Faculty of Agriculture and Life Science, Hirosaki University were used from 1999 to 2002.

Measurement of ethylene by gas chromatography

The ethylene concentration of the air was analyzed by gas–solid chromatography on a gas chromatograph (GC–8A, Shimadzu) fitted with a stainless column (3 mm × 3 m) packed with activated alumina and a flame ionization detector. The GC column was kept at 100°C and

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*Corresponding author (E-mail: oarakawa@cc.hirosaki-u.ac.jp).
eluted with nitrogen as a carrier gas at a rate of 100 kPa.

**Experiment 1. Effect of ethylene on the pedicel**

For the *in vivo* orchard experiment in 1999, the fruit was separated from the pedicel, leaving it attached to the spur, 123 days after full bloom (DAFB). A disposable plastic syringe (10 ml) containing 500 ppm ethylene was attached to the pedicel with a rubber tubing; the plunger was depressed gently twice; an air-filled (10 ml) syringe was used as a control. In the *in vitro* laboratory experiment, the syringe with 500 ppm ethylene or air was attached to the harvested pedicel on a bourse and spur and placed into a plastic bag lined with the moistened filter paper and kept at 20°C. For the 2, 5-norbornadiene (NBD) treatment, these pedicels were placed in a plastic box with the moistened filter paper, then liquid NBD (1 ml) was applied with a micropipette onto a sheet of filter paper in a small petri dish, which was immediately sealed with a cover. Liquid NBD readily volatilized at 20°C in a closed box (Blankenship and Sisler, 1989).

For measurement of the removal force of harvested pedicels in a laboratory, each pedicel, with bourse and spur, was fixed in a universal vise, then the attachment fastened to the pedicel was tightened and pulled by the force gauge.

**Experiment 2. Effect of AVG and ethephon on ethylene content in the pedicel**

In 2002, 100 ppm ethephon (1 ml) was applied on September 3 (125 DAFB) for ‘Tsugaru’ and on September 19 (141 DAFB) for ‘Jonagold’ using three methods: 1) injected into the fruit cores using a syringe; 2) sprayed on the fruit surfaces, or 3) applied to the bourse and pedicel. On September 3 (125 DAFB), 200 ppm AVG (1 ml) was injected into the cores of 10 fruit.

The ethylene content in the *in vivo* pedicel was measured by taking a 5-mm length, distal to the abscission zone and enclosing it in a barrel of a disposable plastic syringe (10 ml volume) in which the volume was fixed at 3 ml; the syringe was then sealed with a rubber stopper at the orchard. Within three hours after sampling, the syringe plunger was pulled to 10 ml to reduce the pressure and kept there for 1 min. One ml of interior atmosphere was withdrawn with a disposable plastic syringe through the rubber stopper, and the ethylene concentration was determined by gas chromatography.

The force required to separate the pedicel with the fruit from its bourse (spur) was measured with an attachment that held the fruit by applying a pull force gauge (Edgerton, 1971). The cross sectional area of the pedicel was measured and the removal force was expressed as kg·cm⁻².

**Experiment 3. The changes in ethylene production of the seed and the flesh, and ethylene content in the pedicel relating to fruit abscission**

In 2002, ten fruits were harvested randomly from three trees periodically; the ethylene content in the pedicel and ethylene production of the flesh and the seed were determined. The fruit from which the pedicel had been removed for the measurement of the ethylene content was cut at the equatorial diameter and the seeds removed. Half of the fruit was enclosed in a desiccators and the interior atmospheres pressure reduced by a vacuum pump to 133 hPa. The sample was placed in a box (1 liter) for 1 hr, then a 1-ml sample was withdrawn and analyzed for ethylene. Five seeds were enclosed in the barrel of a plastic disposable syringe (10 ml) in which the plunger was kept at 5 ml of volume. After the syringe was sealed with a rubber stopper for 2 hr, 1 ml of air was injected into the GC for ethylene analysis.

The cumulative rate of preharvest fruit abscission was determined daily by counting the number of fruits that dropped that day from each scaffold branch of three trees. The preharvest drop was recorded from the onset of the abscission to harvest. The percentage of abscised fruits was calculated by dividing the number that dropped by the total number of fruits harvested × 100.

**Experiment 4. Comparison of ethylene production and content of the abscised and not abscised fruit**

Branches of a ‘Tsugaru’ tree were shaken gently by hand; and the ethylene contents of the seed, pedicels, abscised and intact fruits collected from the tree were determined as above.

**Results**

**Experiment 1. Effect of ethylene treatment on the pedicel**

The ethylene content in the *in vivo* pedicel treated with 500 ppm ethylene was 6.7 ppm, whereas that of untreated control was less than 0.5 ppm. The higher ethylene content in the treated pedicel showed that ethylene was absorbed by the pedicel. The *in vitro* ethylene treatment of the pedicel after removing the fruit on a tree induced the pedicel to abscise; all treated pedicels dropped 9 days after treatment, while only 20% of the control pedicels dropped at day 9 (Table 1). Ethylene treatment of the harvested pedicels resulted in significant reduction in the removal force, which was significantly inhibited by NBD treatment (Table 2).

**Experiment 2. The effect of AVG and ethephon on ethylene content in the pedicel and fruit abscission**

The treatment with AVG significantly reduced the ethylene production of the seed and the flesh, and the ethylene content of the pedicel (Table 3). The cumulative abscission of the treated fruit was much less than
that of the untreated control fruit. The difference in the fruit removal force was not significant, although more was needed for AVG-treated fruit than for the untreated ones.

The effect of ethephon on the fruit abscission depended on the treatment. For ‘Tsurugai’, the injection of ethephon into the fruit core and bourse induced the fruit abscission three and four days after the treatment, respectively (Fig. 1), whereas surface application induced fruit abscission six days after the treatment. The ethylene content in the pedicel of the bourse treated with ethephon increased significantly two days after treatment. For ‘Jonagold’, fruit core ethephon treatment induced the rapid increase in ethylene concentration of the pedicel, followed by fruit abscission six days after treatment (Fig. 2).

**Experiment 3. The changes in ethylene production of the seed and the flesh, and ethylene content in the pedicel relating to fruit abscission**

Fruit abscission of ‘Tsurugai’ began 124 DAFB, and then sharply increased (Fig. 3). From 102 DAFB, the ethylene production of the seed preceded that of the flesh; ethylene in the pedicel was detected on the same day the seed started to produce ethylene. Ethylene production of the seed, pedicel and flesh clearly increased from 124 DAFB and increased thereafter. In ‘Jonagold’, no preharvest fruit drop occurred; ethylene production of the seed, flesh and fruit was very low, increasing slightly toward harvest maturity (Fig. 4).

**Table 1.** The effect of ethylene treatment on abscission of pedicel in ‘Tsurugai’ (%; n = 10).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Ethylene</td>
<td>30</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
</tr>
</tbody>
</table>

**Table 2.** The effect of ethylene and simultaneous treatment with ethylene and NBD on the pedicel removal force in ‘Tsurugai’ 7 days after treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Removal force (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.20 ± 0.25</td>
</tr>
<tr>
<td>Ethylene</td>
<td>1.34 ± 0.07</td>
</tr>
<tr>
<td>Ethylene+NBD</td>
<td>3.06 ± 0.32</td>
</tr>
</tbody>
</table>

'Mean ± SE (n=5).

**Fig. 1.** The effects of ethephon on cumulative fruit abscission and ethylene content in the pedicel in ‘Tsurugai’ apple. The fruit skin, the fruit core and the bourse were treated with ethephon. Vertical bars represent SE.

**Table 3.** Effects of AVG on fruit abscission, ethylene production of the seed and flesh, and ethylene content in the pedicel in ‘Tsurugai’.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ethylene production and content</th>
<th>Removal force (kg·cm⁻²)</th>
<th>Cumulative abscission (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Seed (m·g⁻¹·hr⁻¹)</td>
<td>Flesh (m·g⁻¹·hr⁻¹)</td>
<td>Pedicel (ppm)</td>
</tr>
<tr>
<td>Control</td>
<td>15.75</td>
<td>45.18</td>
<td>1.77</td>
</tr>
<tr>
<td>AVG</td>
<td>0.61</td>
<td>0.13</td>
<td>0.28</td>
</tr>
<tr>
<td>Significance</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
</tbody>
</table>

* AVG was applied on September 3, and all fruits were harvested and investigated on September 23.
* *** indicate significant at $P=0.001$ by paired $t$-test (n=5).
* Divided by cross sectional area of the pedicel.
Fig. 2. The effect of ethephon on cumulative fruit abscission and ethylene content in the pedicel in ‘Jonagold’ apple. The fruit skin, the fruit core and the bourse were treated with ethephon. Vertical bars represent SE.

Experiment 4. Comparison of ethylene production and content of the abscised and intact fruit

The ethylene production by the seed and the fruit and the ethylene content in the pedicel of the abscised fruit by shaking the branch were significantly higher than that of intact fruit 129 DAFB (Table 4).

Discussion

The involvement of ethylene in preharvest apple drop has been suggested in previous studies (Blanpied, 1972; Walsh, 1977; Kondo and Hayata, 1995); however, there has been no clear evidence of the involvement of ethylene in the pedicel during abscission. The finding that ethylene treatment to ‘Tsugaru’ pedicel promoted abscission while NBD inhibited it, demonstrated that ethylene participated in preharvest drop. The involvement of ethylene in the pedicel in the fruit abscission was also confirmed by the treatments with ethephon and AVG. That the injection of ethephon into the fruit core, or the spray on the bourse resulted in increasing ethylene content in the pedicel and subsequent fruit abscission, whereas the same treatment to the fruit was ineffective indicate that ethylene may be the causal factor in fruit abscission. In ‘Jonagold’, the same ethephon treatment
on the bourse was ineffective in increasing ethylene content in the pedicel and inducing fruit abscission for some unknown reason. The treatment with AVG, which prevents the fruit abscission by inhibiting ethylene production also indicate that ethylene in the fruit core, then in the pedicel induced the abscission. As the treatment with ethephon was effective in inducing abscission not only for 'Tsugaru', but also for 'Jonagold' which normally do not exhibit preharvest drop, points to ethylene as a factor for inducing abscission in apple fruit.

The induction or inhibition of the fruit drop by the treatment with ethephon or AVG has been reported previously (Kondo and Hayata, 1995; Tsukahara, 1990). The results here suggested that both treatments affected ethylene content in the pedicel so that ethylene in the fruit core could diffuse to the pedicel and then move to the abscission zone, as assumed by Blanpied (1972).

In 'Tsugaru', the increase in ethylene production of the seed and the flesh and ethylene content in the pedicel coincided with when fruit abscission started. Conversely, in 'Jonagold', theethylene content and the production was low and the fruit did not abscise. Likewise, fruits induced to abscise by shaking had a higher ethylene content in the pedicel and fruit parts than comparable parts of intact fruits.

That auxin is an important regulator of ethylene sensitivity and fruit abscission (Addicott et al., 1955; Brown, 1997) indicates that the responsiveness to ethylene increased with decreasing auxin level with fruit ripening. Although there has been no clear data about the change in auxin level before preharvest fruit drop, the results suggest that ethylene has a key role in the preharvest fruit drop in 'Tsugaru'.

Acknowledgments

We are thankful to Teaching and Research Center for Bio-coexistence, Faculty of Agriculture and Life Science, Hiroasaki University for providing the apple trees.

### Table 4. Ethylene production of the seed and flesh, and ethylene content in the pedicel in abscised or not abscised 'Tsugaru' fruit.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Seed (nl·g⁻¹·hr⁻¹)</th>
<th>Flesh (nl·g⁻¹·hr⁻¹)</th>
<th>Pedicel (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.10</td>
<td>10.35</td>
<td>0.56</td>
</tr>
<tr>
<td>Abscised fruit³</td>
<td>36.10</td>
<td>38.60</td>
<td>3.11</td>
</tr>
</tbody>
</table>

³ The fruits were dropped with shaking the branch.

⁴ *, **, *** indicate significance at P= 0.05, 0.01, and 0.001 by t-test, respectively (n=10).

### Literature Cited


リンゴ'つまる'の収穫前落果における果柄内エチレンの関与

荒川 修・赤城 文・浅田武典・塩崎雄之輔

弘前大学農学生命科学部 036-8561 弘前市文京町

摘 要

リンゴ'つまる'の収穫前落果における果柄内エチレンの関与について、果柄内のエチレン濃度と果肉および種子のエチレン生成との関係について検討した。果柄の誘発は注射器によるエチレンの注入によって引き起こされ、それはエチレンの作用阻害剤である2-5 ノルボルナジェンによって阻害された。果実部と果実へのエフェクト処理によって果柄内のエチレン濃度が増加し、果実が落果した。エチレン生成の阻害剤であるアミノエタキシビニルグリシンの果柄部への処理によって果柄内のエチレン濃度が減少し、果実の落果が抑制された。'つまる'の種子と果肉のエチレン生成および果柄内のエチレン濃度は果実の落果前に急激に増加した。収穫前に落果しない'ジョナゴールド'では種子と果肉のエチレン生成量が果柄のエチレン濃度が低く、その増加はわずかであった。これらのことから、果柄内のエチレンは果肉と種子から移動してくるものであり、それが果実の落果を誘導しているものと考えられた。