Relation between Early Drop of Apple Fruit and Ethylene Evolution under High Night-Temperature Conditions

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Summary

The present report aims to analyze the mechanism of fruit abscission under high night-temperature (HNT) conditions which promote the abscission.

The relationship between the rate of fruit abscission and ethylene evolution was investigated for the apple cultivars Tsugaru, Starking Delicious (SD) and Fuji. The rate of fruit abscission of ‘SD’ was the highest, followed by that of ‘Tsugaru’ and then ‘Fuji’. Ethylene evolution from these fruits also showed a similar trend.

Application of 100 ppm ethephon gradually increased the rate of seed abortion and fruit abscission with time after treatment. Aminoethoxy-vinylglycine (AVG) application prevented ethylene evolution and reduced the rate of seed abortion, fruit abscission and cellulase activity in the abscission zone.

Based on these results, it is concluded that the increase in endogenous ethylene levels of fruit prevented seed development and caused fruit abscission under HNT treatment, and that the most susceptible cultivars were the ones in which ethylene evolution was the highest.

Introduction

In a previous report (19) it was demonstrated that early fruit drop of apple was promoted by high night temperatures (HNT), and that the rate of fruit abscission was reduced by over 60% in comparison with the control by the inhibition of ethylene production in the fruit.

Walsh et al. (27) and Williams (30) showed that a high level of endogenous ethylene was associated with fruit abscission. In addition, studies have been conducted to determine whether ethephon (2-chloroethylphosphonic acid), an ethylene releasing chemical, could be utilized as a fruit thinning agent (16,17,24,27,32). Although a large number of reports (5, 6,7,10,31,34) have indicated that ethylene promotes fruit abscission, there have been only a few studies on how HNT affect the young fruit before abscission occurs.

The purpose of this report is to analyze the mechanism of fruit abscission under HNT conditions.

Materials and Methods

Experiment 1. Rate of fruit abscission and ethylene evolution in the apple cultivars Tsugaru, Starking Delicious and Fuji

Three 8- to 11-year-old trees of the apple cultivars Tsugaru, Starking Delicious (SD) and Fuji grafted on M.26 rootstocks were selected in 1983-1986, respectively. Flowers in the center of a cluster, which were hand-pollinated with the pollen of ‘Ohrin’ in full bloom, were used in the experiment. The 150 fruits which had grown uniformly were marked 10-15 days after full bloom (AFB), and the number of fruits remaining on the trees was recorded at intervals of 3-5 days until early July when fruit abscission ceased.

The fruits were collected for ethylene analysis at intervals of 3-5 days from May 28 (15 days AFB) to July 3 (51 days AFB) in 1985. Ethylene evolution from fruit was determined by gas-chromatography (FID) as described in the previous report (19).

Experiment 2. Effects of AVG and ethephon application on the formation of the abscission layer of the peduncle, cellulase activity in the abscission zone and seed degeneration

Four 9-year-old ‘SD’ apple trees grafted on M.26 rootstocks were selected in 1986. In the experiment on the effect of AVG application,
apple trees were enclosed in a pipe-frame greenhouse covered with a 0.1 mm clear polyvinyl chloride film and heated during the night (6:00 pm to 6:00 am) by a warm-air heating apparatus with a thermostat. The temperature treatments were continued as follows; (A): for 4 days from June 9 (23 days AFB), after 500 ppm AVG solution containing 0.01% wetting agent (Suntokten, Yamamoto Nohyaku Co., Ltd.) was hand-sprayed on only the fruits on June 7, (B): for 7 days from June 16 (30 days AFB), after 500 ppm AVG solution mixed with 0.01% wetting agent (Suntokten) was sprayed on only the fruits on June 12. Thereafter, the apple trees were sprayed with a 100 ppm ethylene solution (C) on June 8 (23 days AFB) or a 300 ppm solution (D) on June 18 (32 days AFB).

Fifty fruits, which had grown uniformly, were marked for each of the 4 treatments. The number of fruits remaining on the trees was recorded in the same way as in Exp. 1. In addition, the effects of these treatments on the rate of seed abortion and cellulase activity in the abscission zone were also examined. For these investigations, representative fruits which had grown uniformly in each treatment were collected, and the seeds with withered and browned inner parts were scored as having aborted.

Furthermore, the junction between the peduncle and bourse was fixed with F.A.A., dehydrated in an n-butyl alcohol series, embedded in paraffin and cut into 15 μm thick longitudinal sections prior to staining (hematoxylin solution). Cellulase activity was determined according to the method of Iwahori and Oohata (15). Hence, 2 mm sections including the abscission zone between the peduncle and bourse were cut, and 1 g of tissue homogenized in 5 ml of 67 mM phosphate buffer (pH 7) containing 100 mg polyclar AT and 5 mg sodium L-ascorbate, and then centrifuged. The procedures described above were carried out at 0–1°C. The reaction mixture, containing 2 ml of the supernatant (enzyme) solution and 4 ml of a 0.8% carboxy methylcellulose (CMC) solution, was placed into an Ostwald viscosity meter and then incubated for 20 hr at 30°C.

**Results**

**Exp. 1. Rate of fruit abscission and ethylene evolution in the apple cultivars Tsugaru, Starking Delicious and Fuji**

Fig. 1 shows the rate of abscission of the fruits of the 3 cultivars between 1983-1986. The rate of fruit abscission of ‘SD’ was the highest followed by that of ‘Tsugaru’, while that of ‘Fuji’ was the lowest throughout the 4 year period. In particular, ‘Fuji’ fruits did not

![Fig. 1. Rate of early fruit drop of apple cultivars Tsugaru, Starking Delicious and Fuji in 1983–1986.](#)

*Days after full bloom.*
Fig. 2. Ethylene evolution from young fruits of the apple cultivars Tsugaru, Starking Delicious and Fuji (1985). 

As in Fig. 1. Vertical bars indicate S.E.

Fig. 3. Effect of AVG application on ethylene evolution from fruit (1988).

As in Fig. 1. Vertical bars indicate S.E.

AVG solution (500 ppm) was applied on June 3.

Table 1. Mean temperature during the high night-temperature (HNT) treatment (1986).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>June 9</td>
<td>10</td>
</tr>
<tr>
<td>HNT</td>
<td>20.9</td>
<td>19.8</td>
</tr>
<tr>
<td>Outdoors</td>
<td>15.5</td>
<td>14.5</td>
</tr>
</tbody>
</table>

Fig. 4. Effect of AVG on ethylene evolution from fruit, fruit abscission and cellulase activity in the abscission zone. AVG application inhibited ethylene evolution from 50 to 5% for 25 days after the spray compared with the untreated control (Fig. 3).

In the HNT treatment applied on June 9 (23 days AFB), the mean temperature was 19.2–20.9 °C as shown in Table 1. The abscission rate of the fruits sprayed with AVG was only 8.0%, whereas that of untreated fruits was 50.0%. Cellulase activity in the abscission zone of fruits sprayed with AVG was lower than that of untreated fruits on both June 13 and 19 (6 and 12 days after the spray, respectively) (Fig. 4A). In the HNT treatment applied on June 16 (30 days AFB), the mean temperature was 20.2–23.7 °C (Table 1). The abscission rate of the fruits sprayed with AVG was 26.0%, and that of untreated fruits amounted to 90.0%. The cellulase activity in the abscission zone on June 23 (11 days after the spray) of the fruits sprayed with AVG was lower than that of untreated fruits (Fig. 4B).

Figs. 5 and 6 show the effect of ethephon application on the formation of the abscission layer of the peduncle, cellulase activity in the abscission zone and seed degeneration.
Fig. 4. Effect of AVG application on the rate of fruit abscission and cellulase activity in the abscission zone (1986).

As in Fig. 1. HNT: High night temperature.
The arrow (↓) indicates the day when the AVG solution was sprayed.

Cellulase activity (%) = \( \frac{a - b}{a - c} \times 100 \)

- \( a \): flow time at the start of incubation
- \( b \): flow time at the end of incubation
- \( c \): flow time of buffer solution

Fig. 5. Effect of ethephon application on the rate of fruit abscission and ethylene evolution from fruit (1986).

As in Fig. 1. Vertical bars indicate S. E.
The arrow (↓) indicates the day when the ethephon solution was sprayed.

Fig. 6. Effect of ethephon application on cellulase activity in the abscission zone (1986).

As in Fig. 1.
Ethephon was sprayed on June 8 (100 ppm) and June 18 (300 ppm), respectively.
application on ethylene evolution, fruit abscission and cellulase activity in the abscission zone. Ethephon application markedly increased ethylene evolution from the fruits. The fruits sprayed with 100 ppm ethphon on June 8 (22 days AFB) underwent gradual abscission from 9 days after application, and the final rate of fruit abscission amounted to 72.0% compared with 18.0% in the untreated control (Fig. 5A). The cellulase activity in the abscission zone of fruits treated with 100 ppm ethphon, which until June 16 (8 days after the spray) hardly differed from that in ethphon had been omitted, increased on and after June 18 (Fig. 6A). Over 80% of the fruits sprayed with 300 ppm ethphon on June 18 (32 days AFB) experienced abrupt fruit abscission 6-7 days after the application, and all fruits subsequently dropped (Fig. 5B). The cellulase activity in the abscission zone of the fruits treated with 300 ppm ethphon increased markedly on June 21 (3 days after the spray) (Fig. 6B). Thus, the effect of the concentration of the compound was conspicuous when sprayed on either date.

The cells in the abscission layer identified at the junction between the peduncle and bourse were smaller than the surrounding parenchyma cells. The thickness of the abscission layer was about 100 μm (Fig. 7).

Table 2 shows the effects of AVG and ethphon application on the rate of seed abortion. AVG application reduced the rate of seed abortion under both HNT treatments. The application of 100 ppm ethphon increased the rate of seed abortion with time after application, whereas the application of 300 ppm ethphon caused fruit abscission before seed development was influenced.

Discussion

Seed development is closely associated with early fruit drop at the young fruit stage (23). Weinbaum and Simons (28) also reported that the chemical thinning agents, NAA and Sevin, caused a reduction in seed size within 2 days of application while NAA caused a retardation in the development of all seed tissues. In Exp. 2 of this study, an application of 100 ppm ethphon also enhanced seed abortion with time after the spray. As fruits which had grown uniformly were used for observation of seeds,
it was considered that seed abortion occurred before fruit development had ceased. In contrast, it appears that the application of 300 ppm ethephon abruptly increased the cellulase activity in the abscission zone before seed development was affected and resulted in fruit abscission. It was shown that cellulase was localized in the separation layer of the abscission zone (1), its activity increased before and during abscission and that it was closely associated with fruit abscission (3, 12, 13, 14, 33, 36). On this point, our current results also support these reports.

It has been shown that ethylene is broken down to form ethylene by a simple base-catalyzed reaction at pH 3.5 and higher (3), and that it stimulates endogenous ethylene production (26, 34). Regarding the effects of ethylene application, Beyer (4, 5) demonstrated that the flow of auxin out of the leaf blade was reduced, Pallus and Kays (25) showed that net photosynthesis was inhibited, while Weinbaum and Murakoa (29) and Krewer et al. (20) reported that the translocation of photosynthates from the leaf into the fruit was retarded.

Ebert and Bangerth (11) reported that ethylene evolution was high at the younger stage of 'King of the Pippins' and 'Golden Delicious' apple fruits. The three cultivars in the current experiments also showed similar characteristics with ethylene evolution of 'SD' per fruit weight being the highest.

The abscission of fruit and leaf is affected by environmental factors (18, 22), and in a previous report (19) it was demonstrated that a high temperature of 25°C increased endogenous ethylene evolution from young fruits. Therefore, it is considered that the young fruits of 'SD', in which ethylene evolution was higher than in the 'Tsugaru' and 'Fuji' fruits, were affected significantly by the increased amounts of endogenous ethylene when the fruits were subjected to HNT treatment, as well as to the application of ethephon at low concentrations. Hence, the translocation of hormone-like substances to the fruits was inhibited (4, 5) and, due to the reduction in the amount of photosynthates translocated to the fruits (20, 25, 29), seed development was retarded. As a result, the amount of endogenous growth regulators (2, 21, 23) produced in the seed was reduced, leading to the formation of the abscission layer and eventually resulting in fruit abscission. This phenomenon may also account for the fact that fruit abscission of 'Fuji' was hardly observed under HNT conditions (18), and that the application of AVG, an inhibitor of ethylene biosynthesis (35), reduced the rate of seed abortion and fruit abscission due to the decreased levels of endogenous ethylene in the fruits. Although the effect of AVG application was investigated under HNT conditions which promoted fruit abscission, a reduction of fruit abscission by AVG application under natural environmental conditions has also been reported by Child and Williams (8) and Williams (30). In these cases where AVG was sprayed on the whole tree including leaves, branches and fruits, the reduction in fruit abscission was affected by the regulation of vegetative growth (9), as well as the reduction in ethylene evolution. However, in the current studies, the rate of fruit abscission could be reduced even when only fruits were sprayed with AVG. Thus, it was shown that fruit abscission under HNT conditions was due to the fact that ethylene evolution from fruit prevented seed development, while the inhibition of ethylene evolution led to a reduced rate of fruit abscission under such conditions. However, the inhibition of ethylene evolution hardly reduced the rate of fruit abscission under shading (19), and Nakagawa et al. (23) showed that in peaches the application of gibberellin was effective in promoting fruit set and development after the embryo had been destroyed. Therefore, it is assumed that other growth regulators such as auxins, gibberellins and cytokinins in the seeds play an important role in fruit development, as will be presented in a subsequent report.

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Literature Cited

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