Inhibitory Factors That Affect the Ripening of Pear Fruit on the Tree

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Pear (Pyrus communis L.) fruit are not usually allowed to ripen on the tree. Indeed, they are harvested at the mature green stage and ripened off the tree. In this study, we examined inhibitory factors that affect the ripening of pear fruit on the tree. The relationship between ethylene and fruit abscission was investigated in ‘Bartlett’ pears. Fruit at the mature green stage produced little ethylene, but most fruit rapidly produced ethylene 2–3 days before dropping. Fruit drop was stimulated by ethylene treatment of fruit on the tree, but was delayed by treatment with 1-methylcyclopropene. We also investigated how fruit ripening on the tree was affected by a continuous supply of assimilates to the fruit via phloem transport. To suppress phloem transport, a girdling treatment was applied to the branch above the abscission zone. Fruit ripening on the tree was promoted by this girdling treatment, but the fruit dropped before softening to an edible firmness. 1-Naphthaleneacetic acid (NAA) was highly effective in suppressing fruit drop. NAA treatment alone did not induce full ripening on the tree, but fruit did ripen on the tree when the NAA and girdling treatments were combined. Two factors may explain why pear fruit do not fully ripen on the tree: the production of ethylene by the fruit during ripening on the tree does not occur until after the optimal harvest time and the continuous supply of assimilates to the fruit via phloem transport may delay ripening.

Key Words: ethylene, fruit softening, girdling, Pyrus communis.

Introduction

Fruit can be divided into two groups based on whether ripening occurs on the tree or after harvest. Fruit that usually ripen on the tree include apple, tomato, grape, and strawberry. Fruit that do not usually ripen on the tree need to ripen for several days to a few weeks after harvest. Pear, avocado, kiwifruit, and mango belong to this group. Both types of ripening are found among the climacteric fruit. Climacteric fruit such as apple and tomato can ripen on the tree or vine, but others such as pear and kiwifruit need a postharvest period to ripen. These characteristics indicate the absence of a relationship between climacteric and non-climacteric status and the ability to ripen on the tree. No non-climacteric fruit require ripening after harvest.

Pear (Pyrus communis L.) fruit are usually harvested at the mature green stage. Fruit maturity is judged using various indices, including starch content, flesh firmness, or the number of days after full bloom (Kingston, 1992). Fruit ripening off the tree is initiated under room temperature conditions. Biological, chemical, and physical changes occur during ripening. The biological changes include ethylene production and a transient increase in respiration called a climacteric peak. The chemical changes include degradation of starch to sugars, degradation of chlorophyll, decomposition of cell wall constituents, and the biosynthesis of volatile compounds. Fruit softening and the development of a juicy and buttery texture are among the physical changes that occur during pear ripening. These changes during ripening off the tree are required for pear fruit to be of good quality.

Pear fruit are not usually allowed to ripen on the tree. We investigated fruit ripening on the tree after the optimal harvest time in ‘Marguerite Marillat’ and ‘La France’ pears (Murayama et al., 1998). Both cultivars softened gradually on the tree, but neither softened to an edible firmness. Sugar and Einhorn (2011) also investigated fruit firmness on a weekly basis for 5 weeks after initial maturity was reached in ‘d’Anjou’ pears. Firmness decreased with each week of delay, but the fruit never reached ripeness with a buttery and juicy texture. In addition, average fruit firmness at commer-

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cial harvest time was 66.4 N and decreased only to 55.2 N after a 1-month harvest delay in ‘d’Anjou’ pears (Bai et al., 2009). Why do pear fruit not ripen on the tree? We observed that ‘Marguerite Marillat’ and ‘La France’ pears produced ethylene after the optimal harvest time, even on the tree (Murayama et al., 1998). Therefore, the inability of pear fruit to produce ethylene on the tree does not prevent the completion of ripening.

Pear fruit increase in size and accumulate sugars, even after the optimal harvest time, although the rate of increase in fruit size slows compared with the rate during maturation. These characteristics led us to propose an alternative hypothesis involving inhibitory factors that affect the ripening of pear fruit on the tree. The supply of assimilates to the fruit via phloem transport is necessary to increase fruit size and sugar content. Assimilates are also thought to be substrates for the synthesis of polysaccharides, such as pectin, hemicellulose, and cellulose. Therefore, the termination of assimilate import may act as a signal for cell wall decomposition and fruit softening. We applied a girdling treatment to the fruit branch above the abscission zone on a ‘Bartlett’ pear tree to inhibit the supply of assimilates and/or other substances to the fruit (Murayama et al., 2006). With this girdling treatment, fruit ripening on the tree was stimulated. However, all fruit dropped before they reached an edible phase with good quality.

Fruit drop is caused by endogenous ethylene, which is generally considered to be the main factor controlling fruit abscission (Kende, 1993). In apples, chemicals that inhibit ethylene biosynthesis and the ethylene signal cascade have been shown to reduce preharvest drop efficiently (Dal Cin et al., 2008; Yuan and Carbaugh, 2007). In contrast, pear fruit are harvested at the mature green stage, when ethylene production is low (Murayama et al., 1998), and preharvest drop is not a significant problem under actual cultivation conditions. Little research on fruit drop has been conducted, although many studies have examined fruitlet abscission for thinning (Wertheim, 2000).

We investigated the relationship between ethylene and fruit abscission in ‘Bartlett’ pears and examined the effect of the continuous supply of assimilates to the fruit on ripening on the tree. To suppress phloem transport, a girdling treatment was applied to a fruit branch of a ‘Bartlett’ pear tree above the abscission zone. We also treated the abscission zone with lanolin paste containing 1-naphthaleneacetic acid (NAA) to suppress fruit drop. We showed that pear fruit have the ability to ripen fully even on the tree. We also discuss the inhibitory factors that affect the ripening of pear fruit on the tree.

Materials and Methods

Plant material and treatments with ethylene and 1-methylocyclopropene (1-MCP)

We used three pear trees (24 years old, Pyrus communis L. ‘Bartlett’ on ‘Yamanashi’, Pyrus pyrifolia Nakai, rootstocks) grown in an orchard at Yamagata University, Japan. ‘Bartlett’ pears are usually harvested 110–120 days after full bloom in Yamagata, Japan. In this experiment, 120 days after full bloom was regarded as the optimum harvest day, when fruit reached commercial maturity. We tagged 20 fruit at the optimal harvest time. A cylindrical propylene tube of 40 mm in length and 14 mm in diameter was made from a centrifuge tube (Asahi Glass Co., Tokyo, Japan) (Fig. 1). It was attached to the fruit using adhesive (Dow Corning Co., Midland, MI, USA) that was harmless to the plant. Each tube had a screw cap with a septum. Ethylene production by the fruit was measured every day until the fruit dropped. One hour after the tube was capped, a
500-μL gas sample was withdrawn using a syringe and injected into a gas chromatograph (Model GC-8A; Shimadzu Co., Kyoto, Japan) fitted with an activated alumina column and a flame ionization detector. Ethylene production was measured as the rate per surface area.

To investigate the relationship between ethylene and fruit drop, fruit were covered with polyethylene film (150 × 170 mm, 0.8-mm thickness) at the optimal harvest time. Ethylene or 1-methylcyclopropene (1-MCP) was added to yield a final concentration of about 500 ppm or 1 ppm, respectively. After 24 h, the film was removed. The number of fruit that dropped every day was counted.

Girdling above the abscission zone and application of NAA to the abscission zone

A 20-mm-wide girdling treatment was applied about 50 mm above the abscission zone using a sharp knife on the optimal harvest date, when fruit reached commercial maturity (Murayama et al., 2006). The optimum shoots that occurred between the abscission zone and girdle were removed. This girdling treatment was applied to 32 fruit per tree. The abscission zones of half of the fruit were treated with lanolin paste containing 3% NAA (w/w). Ninety-six fruit were left on the tree without girdling and half of them were treated with NAA. Another 48 fruit were harvested on the initial day of girdling and ripened off the tree at 20°C. NAA was dissolved in lanolin at 60°C and added to a silicon tube (2-mm i.d.). After the lanolin in the tube cooled and solidified, it was cut into 20-mm lengths. Each 20-mm lanolin paste strip was used for NAA treatment of a single fruit.

Defoliation treatment

Defoliation treatment was applied to a 4-year-old branch of a tree. All leaves were removed at the optimal harvest time and the base portion of the 4-year-old branch was girdled to inhibit phloem transport from other branches. The abscission zone of fruit was treated with lanolin paste containing 3% NAA to suppress fruit drop during ripening on the tree.

Measurements

Five fruit from each group were sampled every 4 days after the optimal harvest time and ethylene production rates were immediately measured as follows. Individual fruit were placed in 1.5-L glass desiccators that were flushed with air and then sealed for 1 h. A 1-mL gas sample was withdrawn using a syringe and injected into a gas chromatograph.

Flesh firmness was determined on opposite sides of each pear fruit using a rheometer (Sun Scientific, Tokyo, Japan) with an 8-mm plunger. Results are expressed in Newtons (N). Color was recorded with a colorimeter (Model NF333; Nippon Denshoku Industries Co., Tokyo, Japan). Hue angle was calculated as the arctangent (b*/a*) and is expressed in degrees.

All fruit remaining on the tree were counted every day. The cumulative percentage of fruit drop on each date was calculated relative to the number of fruit (n = 16) that were tagged on day 0.

Results

Relationship between ethylene and fruit abscission

We tagged 20 fruit at the optimal harvest time and measured ethylene production of the fruit every day until fruit abscission. The timing of the increase in ethylene production differed significantly among individual fruit. However, most fruit rapidly produced ethylene 2–3 days before dropping (Fig. 1). Only one fruit (#14) produced little ethylene, even just before fruit abscission.

We investigated the effect of ethylene on fruit abscission. Fruit drop increased after day 16 on the tree without treatment, but was stimulated on the tree treated with ethylene (Fig. 2). Fruit drop rapidly increased after day 6. In contrast, fruit drop was suppressed by 1-MCP treatment. Fruit drop did not increase until after day 20 and the cumulative fruit drop after day 28 was the same with or without 1-MCP treatment.

Limitation of assimilate availability to the fruit

No fruit dropped from the untreated tree for the first 13 days (Fig. 3). Fruit drop then increased linearly and the cumulative fruit drop was more than 80% by day 30. NAA treatment on the abscission zone dramatically reduced fruit drop. Cumulative fruit drop was less than 5% even 30 days after the optimal harvest time. Girdling treatment stimulated fruit drop with a rapid increase beginning on day 10, which reached more than 90% by day 13. Fruit drop in fruit treated with girdling...
was also suppressed by NAA treatment, but was greater after day 20 that for fruit treated only with NAA.

Ethylene production in fruit off the tree increased after day 4, and reached a peak on day 16 (Fig. 4A). The ethylene production in fruit on the tree with no treatment showed little change throughout the experimental period. Fruit that received a single NAA treatment produced little ethylene, but showed a slight increase in ethylene production on day 20. Ethylene production in fruit receiving a single girdling treatment did not change for the first 8 days and then increased slightly on day 12. However, the majority of fruit dropped, preventing us from continuing the investigation. Fruit treated with girdling and NAA produced ethylene after day 4, similar to fruit that were harvested from the tree.

Fruit flesh firmness off the tree decreased after day 4, and then softened to an edible firmness (< 20 N; Fig. 4B). The firmness of fruit on the tree with no treatment or with a single NAA treatment showed little change for the first 16 days. Firmness then decreased in fruit treated with NAA, but did not reach an edible level. In contrast, the firmness of fruit on the tree with the girdling treatment decreased from day 4 to day 8 regardless of NAA treatment. Fruit treated with NAA softened to an edible firmness, whereas those without NAA treatment did not soften and dropped before reaching full ripeness. In fruit treated with both girdling and NAA, flesh firmness decreased after day 4 and then reached an edible level on day 16, similar to fruit that had been harvested from the tree.

The changes in the hue angle of fruit epidermis corresponded to the changes in flesh firmness (Fig. 4C). In ‘Bartlett’ pears, fruit color changed from greenish-yellow to yellow during ripening. In the absence of treatment and with NAA or girdling treatment, hue angle showed little change throughout the experimental period in fruit that did not fully ripen on the tree. In fruit that fully ripened off the tree or those on the tree that received girdling and NAA treatments, hue angle decreased after day 4 and reached 90°, corresponding to the index for yellow color.

The results of girdling treatment showed that limiting the assimilate availability to the fruit stimulated ripening on the tree. We confirmed this finding with a defoliation treatment that also limited assimilate availability to the fruit. In this case, the abscission zone was treated with NAA and the defoliation treatment stimulated fruit softening (Fig. 5). With the defoliation treatment, fruit flesh firmness decreased after day 4 and reached edible firmness by day 16. The final firmness was slightly higher than for the fruit ripened off the tree.

Discussion

Fruit drop

In a previous study, we investigated the effect of girdling on the ripening of fruit on the tree (Murayama et al., 2006). We showed that ethylene stimulated fruit
findings suggest that endogenous ethylene also plays an important role in controlling fruit drop in pears and that which was produced by the fruit itself during ripening on the tree (Murayama et al., 1998). These findings confirmed that pear fruit produced little ethylene at the optimal harvest time, even when still on the tree (Murayama et al., 1998). These findings suggest that fruit drop might be promoted by ethylene, even on the tree after the optimal harvest time. We showed that ethylene treatment stimulated fruit drop, whereas 1-MCP delayed it (Fig. 2). The firmness of fruit on the tree with girdling treatment decreased from day 4 to day 8, whereas fruit on the untreated tree showed little change for the first 16 days. One of the best known effects of girdling is the accumulation of assimilates above the girdle (Goren et al., 2003). Conversely, in our study, assimilate availability was limited in fruit treated with girdling treatment because the treatment was carried out near the abscission layer and all leaves between the girdle and the fruit were removed. The suppression of assimilate availability may change the physiological status of fruit. Génard et al. (2003) investigated the effects of assimilate supply, metabolism, and dilution on sugar concentration in the mesocarp of peach fruit during fruit development, and concluded that sorbitol was the most important carbohydrate in fruit metabolism. In pear fruit, sorbitol is also an important form of transport for assimilates. The suppression of assimilate availability might induce the utilization of polysaccharides as an energy source, including starch or cell wall materials that are related to fruit ripening.

Fruit softened to edible firmness with girdling above the fruit abscission zone and with NAA application to the abscission zone. Fruit color also changed from yellowish-green to yellow, as was seen for fruit off the tree. This finding indicates that pear fruit can fully ripen on the tree even before being fully ripened by ethylene.

Preharvest drop is common in apples and causes economic loss. Chemicals resembling indoleacetic acid, such as 2,4,5-trichlorophenoxypropionic acid (2,4,5-TP), NAA, and daminozide, or methods that inhibit ethylene biosynthesis and action have been used to control fruit drop (Dal Cin et al., 2005; Schupp and Greene, 2004). Application of NAA or other synthetic auxins delayed fruit drop, even though fruit ethylene production and fruit softening were increased (Yuan and Carbaugh, 2007). We used NAA in a lanolin paste to suppress fruit drop. NAA treatment on the abscission zone resulted in a dramatic decrease in fruit drop (Fig. 3).

**Limitation of assimilate availability to the fruit**

In a previous study, we investigated the effect of girdling above the fruit abscission zone on ‘Bartlett’ pear fruit ripening on the tree (Murayama et al., 2006). Fruit ripening on the tree was stimulated by girdling treatment. We confirmed this finding in the current study. The firmness of fruit on the tree with girdling treatment decreased from day 4 to day 8, whereas fruit on the untreated tree showed little change for the first 16 days. One of the best known effects of girdling is the accumulation of assimilates above the girdle (Goren et al., 2004). Conversely, in our study, assimilate availability was limited in fruit treated with girdling because the treatment was carried out near the abscission layer and all leaves between the girdle and the fruit were removed. The suppression of assimilate availability may change the physiological status of fruit. Génard et al. (2003) investigated the effects of assimilate supply, metabolism, and dilution on sugar concentration in the mesocarp of peach fruit during fruit development, and concluded that sorbitol was the most important carbohydrate in fruit metabolism. In pear fruit, sorbitol is also an important form of transport for assimilates. The suppression of assimilate availability might induce the utilization of polysaccharides as an energy source, including starch or cell wall materials that are related to fruit ripening.

Ethylene plays a major role in organ abscission in a wide range of plant species (Dal Cin et al., 2005, 2008; Reid, 1985). We showed that ethylene treatment stimulated fruit drop, whereas 1-MCP delayed it (Fig. 2). This finding is in agreement with the results of studies in apples. The application of ethephon, an ethylene-release compound, effectively promoted mature fruit abscission and ripening in apples (Edgerton and Blanpied, 1970), whereas aminooxyacetic acid (AVG), an inhibitor of ethylene biosynthesis, or 1-MCP delayed mature fruit abscission (Yuan and Carbaugh, 2007). In ‘Bartlett’ pears, 1-MCP also reduced the incidence of premature fruit drop in comparison with that of untreated fruit (Villalobos-Acuna et al., 2010). These findings suggest that endogenous ethylene also plays an important role in controlling fruit drop in pears and that the fruit do not fully ripen on the tree because they drop before being fully ripened by ethylene.
after storage for 3 or more months. In this study, fruit developed a melting texture, even on the tree, with girdling and NAA treatments. These differences might be caused by differences in the availability of assimilates used for cell wall synthesis.

Kondo and Takano (2000) demonstrated that the application of 2,4-DP solutions to whole ‘La France’ pear trees produced good-quality fruit that ripened on the tree. This synthetic auxin is used on apples in Japan to inhibit preharvest fruit drop. 2,4-DP may suppress the formation of an abscission layer in pear fruit. We cannot explain the mechanism underlying the completion of ripening on the tree with only synthetic auxin treatment in ‘La France’ pears. In this study, ‘Bartlett’ pears treated only with auxin did not reach the edible phase. The method of auxin application differed between studies. Kondo and Takano (2000) sprayed solutions onto whole trees, whereas we applied lanolin paste only to the fruit abscission zone. 2,4-DP may have stimulated fruit ripening because ethylene biosynthesis was initiated by auxin (Abeles et al., 1992; Hansen and Grossmann, 2000). The difference may also be due to the difference in cultivars. ‘Bartlett’ is an early-ripening cultivar that is usually harvested at the end of August in Japan. ‘La France’ is a middle-late-ripening cultivar that reaches full ripeness at the end of October, when the photosynthetic capacity of leaves is thought to be very low. The availability of assimilates during ripening on the tree may thus differ between these cultivars.

Differences in ethylene production, flesh firmness, and hue angle between control and NAA-treated fruit were not observed until 20 days after harvest. At that point, NAA tended to stimulate fruit ripening. Auxin treatment is known to affect fruit ripening. The application of NAA or other synthetic auxins delayed apple fruit abscission, despite increased fruit ethylene production and fruit softening (Yuan and Carbaugh, 2007). Moreover, early application of the synthetic auxin 2,4-dichlorophenoxy-propionic acid (2,4-DP) enhanced the red coloration of ‘Cripp’s Pink’ apples (Stern et al., 2010). On the other hand, ripening was delayed by a pre-veraison NAA treatment in grape berries (Bottcher et al., 2011; Ziliotto et al., 2012). We used NAA treatment to suppress the fruit drop in this study. The effect of such a treatment on fruit ripening needs to be investigated further.

In conclusion, we showed that pear fruit have the ability to ripen fully, even on the tree. Two factors may explain why fruit generally do not ripen fully on the tree (Fig. 6). One is ethylene, which is not produced by fruit on the tree until after the optimal harvest time, and this ethylene induces fruit abscission before fruit reach the full ripening stage. The other is the continuous supply of assimilates to fruit via phloem transport, which might delay the ripening of fruit.

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