Effect of girdling above the abscission zone of fruit on ‘Bartlett’ pear ripening on the tree

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Abstract

Pear fruit usually soften and develop a melting texture when harvested at the mature green stage and ripened. The reason why the fruit does not fully ripen on the tree is unknown. To clarify this, our attention was directed to the continuous supply of assimilates and/or other substances into the fruit via phloem transport. To determine the effect of inhibiting phloem transport on fruit ripening on the tree, a girdling treatment was applied to the branch above the abscission zone of ‘Bartlett’ pear (Pyrus communis L.). Girdling significantly enhanced the ethylene production of fruit on day 12 compared with control fruit. Fruit softening was also stimulated by girdling. On day 8, flesh firmness was similar in treated fruit on the tree and in fruit off the tree, and was significantly lower than that of untreated fruit on the tree. The patterns of transcript accumulation for the ethylene biosynthetic [1-aminocyclopropane-1-carboxylate (ACC) synthase (PcACS) and ACC oxidase (PcACO)] and polygalacturonase (PcPG1 and PcPG3) genes showed good correspondence with ethylene production and fruit softening, respectively. Thus, fruit ripening on the tree was stimulated via ethylene by girdling on the branch above the abscission zone of fruit to interrupt phloem transport. Assimilates and/or other substances in phloem sap may prevent fruit ripening on the tree.

Key words: Ethylene, fruit softening, girdling, Pyrus communis, ripening.

Introduction

Pears, like avocados and kiwifruit, require several days or weeks after harvest to ripen and develop good quality. Starch and chlorophyll degradation, and the biosynthesis of volatile compounds characteristic of ripe pear (Jennings et al., 1964; Shiota, 1990) all occur during the ripening process. In addition, fruit that are harvested at an optimum time soften and develop a buttery and juicy texture. By contrast, pears never soften appreciably on the tree, although exposure to cool temperatures can cause premature ripening of summer pears, such as ‘Bartlett’ (Wang et al., 1971). Murayama et al. (1998) investigated the ripening of ‘Marguerite Marillat’ and ‘La France’ pears on the tree for 28 d after optimum harvest time, and reported that both cultivars softened gradually on the tree, but neither softened to an edible firmness. The reason why pear fruit does not fully ripen on the tree is unknown.

In most ripening fruit, softening is accompanied by the solubilization of polyuronides, i.e. a decrease in the amount of insoluble polyuronides and a concomitant increase in soluble polyuronides. Solubilization also occurs during the softening of pear fruit (Ben-Arie and Sonego, 1979). The amount of water-soluble polyuronides increases slightly in pear fruit on the tree, but the amount in fruit harvested 28 d after the optimum harvest time was less than one-third of...
that in fruit harvested at the optimum harvest time and ripened off the tree (Murayama et al., 1998). This suggests that the suppression of polyuronide solubilization is related to the inability of pear fruit to ripen on the tree. Hiwasa et al. (2003, 2004) reported that the ethylene analogue, propylene, stimulated the solubilization of pectic polysaccharides, and the accumulation of mRNA of the polygalacturonase (PG) genes PC-PG1 and PC-PG2 occurred in parallel with the pattern of softening in fruit treated with either propylene or 1-methylecyclopropene. Thus, ethylene is thought to be important in the normal ripening of pear fruit. Murayama et al. (1998) observed that ‘Marguerite Marillat’ and ‘La France’ pears produced ethylene after the optimum harvest time, even on the tree. Therefore, it is not the inability of pear fruit to produce ethylene on the tree that prevents the completion of ripening.

The fact that ethylene is produced normally on the tree led to the proposal of another hypothesis. Pear fruit increase in size and accumulate sugars, even after the optimum harvest time, although the rate of increase in size slows from that during maturation. The supply of assimilates to the fruit via phloem transport is necessary for such increases. Assimilates are also thought to be substrates for the synthesis of polysaccharides, such as pectin, hemicellulose, and cellulose. Therefore, it is possible that the termination of assimilate import is the signal for cell wall decomposition and fruit softening.

Girdling has been used for decades to improve fruit quality and yield (Goren et al., 2004), and is also a major tool in the physiological studies of translocation and source/sink relationships (Goren et al., 2004; Hoch, 2005). A girdling treatment was applied to the branch above the abscission zone of ‘Bartlett’ pear fruit to inhibit the supply of assimilates and/or other substances to the fruit via phloem transport. The effect of this girdling on the ripening of fruit on the tree was then investigated.

Materials and methods

Plant material and treatments

Three pear trees (Pyrus communis L. cv. Bartlett) grown in an orchard at Yamagata University, Japan were used. A girdling treatment 2 cm in width about 5 cm above the abscission zone was applied using a sharp knife on 1 September 2001, when fruit reached commercial maturity (Fig. 1). Shoots that occurred between the abscission zone and the girdle were removed. The girdling treatment was applied to 20 fruit per tree; 60 fruit were left on the tree without girdling; another 60 fruit were harvested on the initial day of girdling; and ripened outdoors under the same temperature conditions. Five fruit from each group were sampled every 4 d after girdling and the ethylene production rate was immediately measured as follows. Individual fruit were placed in 1.5 l glass desiccators, which 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 (model GC-8A; Shimadzu Co., Kyoto, Japan) fitted with an activated alumina column and a flame ionization detector. The experiment was repeated in the 2002 harvest season.

Fig. 1. Girdling treatment near the abscission zone.

Extraction of pectin and hemicellulose

Flesh firmness was determined on the opposite sides of each pear using a rheometer (Sun Scientific, Tokyo, Japan) with an 8 mm plunger. Each fruit was then peeled, and two wedge-shaped sectors were cut from the fruit and diced into approximately 1 cm³ pieces. The samples then were freeze-dried and stored at −20 ℃ for cell wall analysis and −80 ℃ for RNA analysis until use. Alcohol-insoluble residue (AIR) was prepared using the method of Murayama et al. (2002). For starch determination, an equatorial slice was sampled from each fruit. Five tissue discs, 10 mm in diameter and 20 mm thick, were excised from the central cortex of slices. The dried AIR was resuspended in 100 mM sodium acetate buffer (pH 5.0) and boiled for 30 min. After cooling, the gelatinized starch was digested with β-amylase (from porcine pancreas; Sigma-Aldrich, St Louis, MO, USA) in 50 mM sodium acetate buffer (pH 6.5) at 37 ℃ for 2 h, and β-amylase (from sweet potato; Sigma-Aldrich) and iso-amylase (Nacalai Tesque, Kyoto, Japan) in 100 mM sodium acetate buffer (pH 4.5) at 37 ℃ for 2 h. The released glucose was measured using the glucose oxidase–peroxidase method of Barham and Trinder (1972).

The AIR was extracted sequentially into various cell wall fractions using the procedure described in Murayama et al. (2002). Briefly, AIR was dispersed in distilled water, mechanically shaken overnight at 20 ℃, and vacuum-filtered through a GF/C filter. The residue was then suspended in distilled water, shaken for 1 h, and filtered again. The filtrates were combined and designated as water-soluble polyuronides (WSP). The residue was then extracted with 50 mM ethylenediaminetetraacetic acid (EDTA; pH 6.5) at 20 ℃ twice. The filtrates were combined and designated chelator-soluble polyuronides (CSP). The residue was further extracted with 50 mM Na2CO3 containing 20 mM NaBH4 at 20 ℃ twice. The filtrates were combined and designated as alkaline-soluble polyuronides (ASP). The uronic acid content in each fraction was measured using the m-hydroxydiphenyl method (Blumenkrantz and Asboe-Hansen, 1973).

Depectinated AIR was treated for 2 h with α-amylase in 50 mM sodium acetate buffer (pH 6.5). The residue was then used for the subsequent extraction of hemicellulose. The residue was extracted with 4 M KOH containing 20 mM NaBH4 for 24 h, mechanically shaken overnight at 20 ℃, and vacuum-filtered through a GF/C filter.
The residue was then suspended in KOH solution, shaken for 1 h, and filtered again. The filtrates were combined and designated as hemicellulose. The total sugar content in this fraction was measured using the phenol-sulphuric acid method (Dubois et al., 1956).

The residue was washed with diluted acetic acid and a mixture of ethanol:diethyl ether (1:1, v/v). After drying, the residue was designated as cellulose. This fraction was dissolved in 72% H₂SO₄ kept for 1 h at room temperature, diluted with distilled water, and heated for 1 h at 120 °C. The total sugar content of the solution was measured using the phenol-sulphuric acid method (Dubois et al., 1956).

Cell wall polysaccharides were only analysed for samples taken in 2001.

RNA extraction and estimation of mRNA levels in fruit

The total RNA of each sample was extracted using the hot borate method described by Wan and Wilkins (1994). Poly (A)⁺ RNA was purified from total RNA using the method described by Toyomasu et al. (1998). Single-strand cDNA was prepared from 1 μg of poly (A)⁺ RNA from each sample by reverse transcription (RT) using SuperScript II (Invitrogen, Carlsbad, CA, USA). After treatment with RNase H (Takara Bio, Shiga, Japan), the cDNA was used as templates for semiquantitative RT–polymerase chain reaction (PCR). Based on the sequences of pear ACC synthase (ACS; accession number x87112) and ACC oxidase (ACO; accession number x87097), primer sets were designed as follows: ACSF: TACCCATTCACTGCAACAGC and ACSR: GCCCAAACCATGTCGTT; ACOF: TCCAGGATGACAAAGGGC and ACOR: TCGAGTAGTTGCAACAAAGGGTC-GGAT.

The gene-specific primer sets used for the amplification of each cDNA for the PG1 and PG3 genes were the same as those used by Sekine et al. (2006). The reaction mixture (20 μl) was as described above, with the exception of using 0.2 μM of each primer and Taq DNA polymerase (Roche Diagnostics, Basel, Switzerland). An initial denaturation step of 2 min at 94 °C was followed by cycles of 30 s at 94 °C, 30 s at 58 °C, and 30 s or 1 min at 72 °C, with a final extension step of 7 min at 72 °C. The amount of first-strand cDNA and the number of cycles were selected to assure that the reactions were in the linear range of PCR amplification. The RT–PCR products were separated on 1.0% (w/v) agarose gels and stained with ethidium bromide; the intensities of the PCR products were estimated using Scion Image Beta 4.02 Win (Scion Corporation, Frederick, MD, USA). The expression level of each gene was calculated after normalization to the level of the actin gene PCR product from the same sample.

The estimation of mRNA levels in fruit harvested in 2002 was carried out using northern blot hybridization. Total RNA (10 μg lane⁻¹) extracted from fruit samples was denatured, electrophoresed in 1% (w/v) agarose/2.2 M formaldehyde gels, and transferred onto nylon membranes (Hybond-N⁺; Amersham Biosciences, Buckinghamshire, UK) using standard blotting techniques (Sambrook et al., 1989). The membranes were prehybridized for 3 h at 68 °C and hybridized with the gene-specific [³²P]-labelled cDNA fragments for 18 h at 68 °C in a rapid hybridization buffer (Amersham Biosciences). The membranes were washed at room temperature in 3 × SSC with 0.1% (w/v) SDS, and were then washed sequentially for 15 min each in 2×, 1×, and 0.2× SSC/0.1% SDS at 68 °C. The radioactivity was recorded on an imaging plate using a Bio-Imaging Analyser (Fujix BAS1500; Fujifilm, Tokyo, Japan).

Results

All results shown, excluding gene expression, were obtained from fruit harvested in 2001 because the results from 2001 and 2002 were similar. The patterns of gene expression are shown for fruit harvested in both years because different measurement methods were used (RT–PCR and northern blot hybridization, respectively).

Ethylene production, fruit firmness, and starch content

Ethylene production of fruit off the tree increased after 4 d (Fig. 2A). The ethylene production of fruit on the tree without girdling showed little change for the first 8 d. It then increased gradually, but the rate was low even on day 12. Ethylene production of fruit with girdling increased 12 d after the treatment. However, all fruit dropped, so the investigation could not be continued. Flesh firmness of fruit off the tree decreased after day 4, and then softened to an edible firmness (<20 N; Fig. 2B). The firmness of fruit on the tree without girdling showed little change throughout the experimental periods. By contrast, the firmness of fruit on the tree with girdling drastically decreased from day 4 to day 8.

The starch content of fruit off the tree immediately decreased after harvest (Fig. 2C). That of fruit on the tree with girdling also decreased immediately after harvest; however, the starch content in these fruit on day 12 was higher than in fruit off the tree. By contrast, the starch content in fruit without girdling did not decrease until 8 d after harvest.

Cell wall polysaccharides

The WSP in fruit off the tree increased linearly after harvest and reached 200 mg 100 g⁻¹ FW on day 12 (Fig. 3A). That in fruit on the tree with and without girdling also increased on day 4, but then remained constant. As a result, the WSP content in these fruit on day 12 was about one-third that in fruit off the tree.

Irrespective of girdling, the CSP content was lowest among the three pectin fractions, and its change after the optimum harvest time was small (Fig. 3B). The ASP in fruit off the tree decreased gradually after harvest, drastically after 8 d, and reached about 100 mg 100 g⁻¹ FW on day 12 (Fig. 3C). That in fruit on the tree with and without girdling also decreased slightly on day 4, but then remained constant.

The hemicellulose content in fruit off the tree decreased rapidly after harvest and reached about 200 mg 100 g⁻¹ FW on day 8 (Fig. 4A). That in fruit on the tree with girdling also decreased rapidly on day 4, and was similar to that in fruit off the tree; however, it did not change from day 4 to day 8. In fruit on the tree without girdling, hemicellulose content decreased from day 4 to day 8. No change in hemicellulose content was observed in any fruit from day 8 to day 12. The cellulose content in fruit on the tree without girdling increased on from day 0 to day 4, and then decreased gradually (Fig. 4B). In fruit off the tree, the cellulose content did not change significantly until day 12, when it dropped. Little change in cellulose content was
observed in fruit on the tree with girdling throughout the experimental periods.

Gene expression

The expression patterns of the \textit{PcACS} and \textit{PcACO} genes after optimum harvest time were similar (Fig. 5). Expression levels were low on day 0. Expression levels in fruit off the tree increased from day 0 to day 4, and reached the highest levels among the three fruit treatments on day 12. By contrast, an increase in \textit{PcACS} and \textit{PcACO} gene expression only occurred from day 8 to day 12 in fruit on the tree without girdling. In comparison, girdling advanced the expression of both genes in fruit on the tree.

The expression patterns of the \textit{PcPG1} and \textit{PcPG3} genes after optimum harvest time were also similar (Fig. 6). Expression levels were low on day 0. Expression levels in fruit off the tree increased from day 0 to day 4, and reached their highest levels on day 12. By contrast, an increase in \textit{PcPG1} and \textit{PcPG3} gene expression only occurred from day 8 to day 12 in fruit on the tree without girdling. Girdling advanced the expression of both genes in fruit on the tree.

The expression patterns of the \textit{PcACS}, \textit{PcACO}, \textit{PcPG1}, and \textit{PcPG3} genes in fruit harvested in 2002 and analysed using northern blot hybridization were similar to those in fruit harvested in 2001 (Fig. 7).

Discussion

Pear fruit is generally harvested at a mature green stage, and tissues soften and develop a buttery and juicy texture during ripening. By contrast, fruit never ripens on the tree, although the fruit gradually softens after the optimum harvest time (Murayama \textit{et al.}, 1998). Why pear fruit does not fully ripen on the tree is unknown. Attention was directed to phloem sap including assimilates and/or other substances because pear fruit normally produce ethylene, even while on the tree (Murayama \textit{et al.}, 1998). To study the effect of the inhibition of phloem transport on fruit ripening on the tree, a girdling treatment was applied to the branch above the abscission zone of ‘Bartlett’ pear fruit (Fig. 1).

Effect of girdling on ethylene biosynthesis

Girdling near the abscission zone of fruit significantly stimulated ethylene production in fruit from day 8 to day 12, compared with fruit on the tree without girdling (Fig. 2A). The expression patterns of the \textit{PcACS} and \textit{PcACO} genes (Fig. 5) corresponded with changes in ethylene production. Agusti \textit{et al.} (1998) also demonstrated that

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Open circles, fruit off the tree; solid circles, fruit on the tree without girdling; closed triangles, fruit on the tree with girdling near the abscission zone. The vertical bar represents the LSD at $P < 0.05$ for comparison of means within the figure.
peach fruit on girdled branches initiated the ethylene climacteric before fruit on untreated branches. Thus, ethylene biosynthesis in fruit was enhanced by the girdling treatment. Wounding induces ethylene biosynthesis in fruit (Starrett and Laties, 1993; Yokotani et al., 2004), and in tomatoes, some ACS genes are induced by wounding (Tatsuki and Mori, 1999; Yokotani et al., 2004). Thus, it is possible that the stimulation of ethylene biosynthesis in fruit by girdling was caused by wounding. In the present study, the expression of PcACS that corresponds to PcACS1 or pPPACS1 was investigated because it was shown to be important in the capacity of ‘Passe Crassane’ pear fruit to ripen (El-Sharkawy et al., 2004) or the production of high levels of ethylene in Japanese pear fruit (Ito et al., 1999), respectively. El-Sharkawy et al. (2004) studied the regulation of ACS genes in pear fruit using the seven isolated Pc-ACS cDNAs. Further work is required to elucidate the
regulation of ethylene production by girdling using ACS isogenes.

**Effect of girdling on fruit softening**

Girdling also stimulated fruit softening from day 4 to day 8 compared with fruit on the tree without girdling (Fig. 2B). Sekine et al. (2006) suggested that PcPG1 was related to fruit softening in ‘La France’ pear. In addition, Hiwasa et al. (2003) found that the accumulation of PC-PG1 mRNA during ripening in ‘La France’ pear fruit paralleled the pattern of fruit softening in fruit treated with either propylene or 1-methylcyclopropene. It was confirmed here that PcPG1 is involved in the softening of pear fruit because the expression pattern of the PcPG1 gene (Fig. 6) corresponded with fruit softening. The accumulation of PcPG3 mRNA also paralleled the pattern of fruit softening (Fig. 6). Sekine et al. (2006) reported that PcPG3 may be involved in the development of the melting texture, rather than fruit softening, in ‘La France’ pear fruit because some fruit softened without the development of a melting texture. By contrast, it was found that fruit softening and the development of a melting texture occurred simultaneously, and the accumulation of mRNA of both genes paralleled the pattern of fruit softening.

Fruit off the tree softened to <10 N; this softening was accompanied by an increase in water-soluble polyuronides and a decrease in alkali-soluble polyuronides (Figs 2B, 3). These changes in polyuronides during ripening support previous results in pear fruit (Ben-Arie and Sonego, 1979; Yoshioka et al., 1993; Murayama et al., 1998). In fruit with the girdling treatment, flesh firmness drastically decreased from day 4 to day 8. However, the changes in polyuronides observed in fruit off the tree did not occur, suggesting that the solubilization of pectin was involved in later fruit softening. In addition, the correspondence between the expression pattern of the PcPG1 gene and fruit softening (Figs 2B, 6), suggests that PcPG1 is involved in the decomposition, rather than the solubilization, of pectin.

**Fruit ripening on the tree**

‘Bartlett’ pear fruit off the tree soften after day 4 and ripen with good quality on day 12 (Fig. 2B). The firmness of fruit on the tree without girdling was significantly hard, even
Effect of girdling on pear ripening

The inhibition of assimilate transport into fruit affected fruit ripening directly or indirectly. Pear fruit continues to increase in size after the optimum harvest time, suggesting that cell wall synthesis may continue on the tree. Thus, it is likely that both cell wall synthesis and decomposition occur after the optimum harvest time. The starch content in fruit on the tree with girdling decreased more rapidly than that in fruit on the tree without girdling (Fig. 2C). It is possible that starch decomposition was induced by the interruption of assimilate transport into the fruit by girdling. However, the termination of the import of assimilates may act as a signal for cell wall decomposition and fruit softening, and thus affect fruit ripening indirectly. The interruption of phloem transport by girdling should also affect the transport of other substances, including water or plant hormones. Dann et al. (1985) found that indole-3-acetic acid (IAA) accumulated transiently above the girdle. In strawberries, it is suggested that the decline in the concentration of auxin in the achenes as fruit mature modulates the rate of fruit ripening (Given et al., 1988). Thus, changes in plant hormone contents may also be involved in the effects of girdling on pear fruit ripening. In addition, some reports mention that the flux of water into fruit via phloem can affect the ripening of fruit on the tree. For example, in 'Shiraz' grape berries, late-ripening shrinkage seems to be attributed to decreased phloem flow into the berry (Rogiers et al., 2006). The relationship between fruit ripening and the flux of water into fruit in the future needs to be clarified.

Fruit on the tree with girdling did not soften after 8 d, and dropped before it was fully ripe. In general, the application of ethylene or ethylene-releasing compounds, such as ethephon, promotes abscission, whereas ethylene biosynthesis or perception inhibitors prevent or delay abscission (Abeles et al., 1992; Brown, 1997). Although recent work on ethylene-insensitive and delayed-abscession mutants of Arabidopsis demonstrated that ethylene does not initiate abscission (Bleecker and Patterson, 1997; Patterson and Bleecker, 2004), ethylene plays an important role in accelerating leaf and fruit abscission once abscission is initiated (Brown, 1997; Taylor and Whitelaw, 2001; Roberts et al., 2002). It is possible that abscission had already been initiated in the pear fruit because it had reached the stage suitable for harvest. Thus, pear fruit abscission may be promoted by ethylene, which was produced by the fruit itself during ripening on the tree. Kondo and Takano (2000) demonstrated that 2,4-dichlorophenoxy propanoic acid treatment could be used to produce good quality fruit ripened on the tree. The 2,4-dichlorophenoxy propanoic acid may inhibit the formation of the abscission zone; it is used on apples in Japan as a retardant against preharvest fruit drop. Further work is required to elucidate the relationship between girdling near the abscission zone and fruit abscission.

In conclusion, it was found here that fruit ripening on the tree was stimulated by girdling above the abscission zone of fruit to interrupt phloem transport. Assimilates and/or other substances in the phloem sap may be involved in the inhibition of fruit ripening on the tree.

Fig. 7. Changes in the levels of transcripts hybridizing with the PcACS, PcACO, PcPG1, and PcPG3 genes during ripening of 'Bartlett' pear fruit at 20 °C in 2002. Signal intensities were determined using an imaging analyser.

on day 12, although the fruit softened slightly after day 4. Ethylene plays an important role in the ripening of climacteric fruit, including pear; therefore, ethylene may be related to the inability of fruit to ripen on the tree. However, ethylene production occurred in fruit on the tree, although the production rate was less than in fruit off the tree. Murayama et al. (1998) reported that ‘Marguerite Marillat’ and ‘La France’ pears also produced ethylene on the tree. Therefore, it is confirmed that ‘Bartlett’ pear fruit did not ripen despite ethylene production while on the tree.

Fruit softening was stimulated by the girdling treatment. On day 8, the flesh firmness of fruit off the tree and fruit on the tree with girdling was similar, and was significantly lower than that of fruit on the tree without girdling. Girdling enhances maturation in various crops, particularly in grape berries and peaches (Agusti et al., 1998; Carreno et al., 1998). One of the best-known effects of girdling is the accumulation of assimilates above the girdle (Goren et al., 2004). In most cases, girdling involves removing a strip of bark from the circumference of the trunk or a scaffold branch. However, assimilate availability to the fruit was limited because the girdling was performed near the abscission zone, and all leaves between the girdle and the fruit were removed. Thus, these results cannot easily be compared with those of previous studies.

It is possible that the inhibition of assimilate transport into fruit affected fruit ripening directly or indirectly. Pear fruit continues to increase in size after the optimum harvest time, suggesting that cell wall synthesis may continue on the tree. Thus, it is likely that both cell wall synthesis and decomposition occur after the optimum harvest time. The starch content in fruit on the tree with girdling decreased more rapidly than that in fruit on the tree without girdling (Fig. 2C). It is possible that starch decomposition was induced by the interruption of assimilate transport into the fruit by girdling. However, the termination of the import of assimilates may act as a signal for cell wall decomposition and fruit softening, and thus affect fruit ripening indirectly. The interruption of phloem transport by girdling should also affect the transport of other substances, including water or plant hormones. Dann et al. (1985) found that indole-3-acetic acid (IAA) accumulated transiently above the girdle. In strawberries, it is suggested that the decline in the concentration of auxin in the achenes as fruit mature modulates the rate of fruit ripening (Given et al., 1988). Thus, changes in plant hormone contents may also be involved in the effects of girdling on pear fruit ripening. In addition, some reports mention that the flux of water into fruit via phloem can affect the ripening of fruit on the tree. For example, in 'Shiraz' grape berries, late-ripening shrinkage seems to be attributed to decreased phloem flow into the berry (Rogiers et al., 2006). The relationship between fruit ripening and the flux of water into fruit in the future needs to be clarified.

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