Genetics and Molecular Research

Pre-harvest foliar application of ethephon strengthens gibberellins-induced fruit expansion in *Pyrus pyrifolia*

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ABSTRACT. To identify the roles of ethylene in fruit development in Japanese pear *Pyrus pyrifolia* 'Niitaka', one of the non-climacteric genotypes, source-sink strength and fruit development during fruit expansion were investigated when ethephon was applied after a conventional gibberellic acid (GA) lanolin paste treatment on the pedicel. The results demonstrate that the conventional GA treatment during the early stage of fruit expansion resulted in larger fruit size and advanced fruit maturation, but pre-harvest foliar application of ethephon only advanced fruit maturation. However, pre-harvest foliar application of ethephon with a preceding conventional GA treatment during the early stage of fruit expansion dramatically improved fruit size and advanced fruit maturation over GA or ethephon alone. Moreover, the early foliar

application of ethephon showed a better efficacy in increasing fruit size than the late spraying. A further study revealed that when ethephon was applied after the conventional GA treatment, it improved source-sink strength associated with leaf photosynthesis and the specific rate of [^{13}C] accumulation in fruit, and also strengthened cell expansion more than did GA or ethephon alone.

Key words: *Pyrus pyrifolia*; Source-sink strength; Cell expansion; Fruit development; Gibberellin; Ethylene

INTRODUCTION

To facilitate harvest scheduling of fruit, ethephon (2-chloroethylphosphonic acid) has been used as an important growth regulator for fruit crops, including Japanese pear (*Pyrus pyrifolia*) (Smith and Whiting, 2010). Ethephon's mode of action in regulating growth can be attributed to the release of ethylene in plant tissue (Abeles et al., 1992). Ethylene has long been recognized as a growth inhibitor; for example, it reduces the production and availability of leaf assimilates by increasing photorespiration through its effect on chlorophyll degradation (Choe and Whang, 1986). Ethylene also inhibits leaf expansion as well as hypocotyl and stem elongation (Choi, 2007; Jackson, 2008). There is, however, growing evidence indicating that growth promotion is a common feature in ethylene responses, including hypocotyl growth in the light (Smalle et al., 1997), stem growth and shade avoidance response (Pierik et al., 2004), and submergence-induced shoot elongation (Jackson, 2008).

Cell expansion is an important determinant of quality in fruit crops and is involved in the fruit ripening process and regulation of fruit size. To satisfy the rising demand for high quality pear fruit, many practical techniques are being developed and applied for fruit production, including cropload management, premier varieties, and plant growth regulators (PGRs) (Zhang et al., 2005a,b, 2009). In fruit crops, research in the roles of ethylene has focused on the processes of fruit ripening, maturation, and postharvest (Pech et al., 2008; Lin et al., 2009); however, little information related to growth stimulation, including cell expansion, is available in the literature. In the past decade, the information showing that ethylene promotes cell expansion and cell elongation has been largely obtained from model plants of rice, *Arabidopsis*, and *Rumex palustris* (Pierik et al., 2006, 2007; Choi, 2007; Jackson, 2008; Dugardeyn and Dominique, 2008), but not from fruit crops. Ethephon has also been reported to stimulate and advance ripening, improve fruit color, and reduce the storage potential of both climacteric fruits, such as apple (Whale et al., 2008) and blueberry (Ban et al., 2007), and non-climacteric fruits, including citrus (Zhou et al., 2010) and strawberry (Yildiz and Yilmaz, 2002), but it does not affect fruit size at harvest. However, in sweet cherry, ethephon-treated fruit were significantly heavier and darker than non-treated fruit (Smith and Whiting, 2010).

In *P. pyrifolia*, the application of gibberellic acid (GA) lanolin paste to the fruit pedicel during the early stage of fruit expansion has been widely used for fruit production because of its remarkable efficacy in increasing fruit size (Zhang et al., 2005a, 2009). Recently, the mechanism of GA-improved fruit size has been partly clarified and we concluded that GA increases fruit size by stimulating the activity of enzymes related to fruit sink activity to increase sink demand, and also by increasing the partitioning of assimilates to fruit and cell expansion (Zhang et al., 2005a). Interestingly, we also found that pre-harvest foliar application

of ethephon with a preceding conventional GA treatment on fruit during fruit expansion dramatically increased fruit size and advanced fruit maturation over GA treatment alone in *P. pyrifolia*. Therefore, the current work attempts to unravel the physiological roles of ethylene in its synergistic effects on fruit size with GA by investigating source-sink strength and fruit development in *P. pyrifolia*.

MATERIAL AND METHODS

Plant materials

Twenty-year-old *P. pyrifolia* trees 'Niitaka' growing in the orchard of Tottori University, Japan, were used in this experiment. The trees were trained as a flat-canopied pergolar system and with uniform cropload as described by Zhang et al. (2005b). Flowers were hand-pollinated with pollen of 'Chojuro' at anthesis and fruit were hand-thinned to one per spur at 30 DAA according to commercial practice.

GA and ethephon application

The experiment included eight treatments as follows: a) the control (pure lanolin); b) the conventional GA treatment was conducted at 40 days after anthesis (DAA) according to commercial practice and the pedicel portion was treated with 20-30 mg GA paste per fruit (concentration of GA_{3+4}: 2.7%, W/W); c) ethephon applied as a foliar application by hand sprayer at 78 DAA at 6.25, 12.5, and 25 mg/L until run-off; d) the combination of pre-harvest foliar application of ethephon (6.25, 12.5, and 25 mg/L) at 78 DAA with the conventional GA treatment. e) Ethephon at 25 mg/L was applied at 130 DAA in a separate trial with or without a preceding conventional GA treatment. Only four treatments, including the control, GA, ethephon (25 mg/L), and GA plus ethephon (25 mg/L), were selected for investigation of fruit ethylene evolution, leaf photosynthesis, and [^{13}C] partitioning in fruiting spur. The experiment had a randomized complete block design with a single tree representing treatment and was replicated three times.

Fruit growth and quality analysis

Ten fruit per treatment were selected for measurement of fruit length and width immediately after the conventional GA treatment and/or pre-harvest foliar application of ethephon with digital caliper at intervals of 7 to 14 days until commercial harvest. At harvest, all fruit were individually weighed for mean fruit fresh weight and 10 fruit were selected for measurement of color, flesh firmness, soluble solid content (°Brix), and juice pH. Fruit color was measured on both sun- and shade-sides on fruit surface around the equator by using a handheld color meter (MR-3000, Nippon Denshoku Co., Tokyo, Japan) as L^*, a^* and b^*. The data were expressed as Commission International de L'eclairage (CIE) lightness (L^*), chroma (C^*), and hue angle ($h°$). Flesh firmness was measured from two peeled sides of each fruit using a Rheometer (RT-3010D, Rheotech, Japan) fitted with a 0.8 mm diameter flat-tipped probe. The crosshead speed of Rheometer was 100 mm/min, and driving depths were 10 mm. Soluble solid content was determined by measuring the refractive index of juice using an infrared digital refractometer. Juice pH was measured with a digital pH meter. The occurrence of watercore in fruit was also recorded according to the method described by Zhang et al. (2009).

Determination of fruit ethylene evolution

Fruit ethylene evolution at harvest and 2 weeks after storage at 4°C was measured according to the method described by Zhang et al. (2008). Intact fruit were placed in 1.5 L sealed jars for 2 h at 20°C for ethylene measurement. Two-milliliter headspace gas samples were then drawn from each jar through a syringe. The ethylene concentration in the sample was measured with a gas chromatograph with a flame ionization detector and 60/80-mesh activated alumina column (Model 163, Hitachi, Tokyo, Japan).

Measurement cell number and cell length of mesocarp

After measurement of fruit ethylene evolution at harvest, ten fruit were cut along the equatorial region, then the diameter of the core was measured, and mesocarp width was calculated from the difference between the longest width of the transverse section of fruit and core according to the method described by Zhang et al. (2005a). Ten observation zones per section were measured. Cell number of the mesocarp along the equatorial region was then calculated by dividing the mesocarp width by average cell length, and this was taken as an indicator of total cell number per fruit.

Gas exchange measurement

Prior to measuring gas exchange, the relative chlorophyll content of leaves was measured with a chlorophyll meter (SPAD-502, Konica Minolta Sensing Inc., Sakai, Japan). Photosynthetic rate (P_N), stomatal conductance (g_s), and leaf internal CO_2 concentration (C_i) were investigated for both the control and PGR (GA and/or ethephon) treatments using open flow systems LI-6400 (LI-COR, Lincoln, Nebraska, USA). Response of leaf photosynthesis to intercepted photosynthetic photon flux density (PPFD) was measured for individual mature spur leaves using the 6400-02 LED light source (LI-COR, Lincoln, Nebraska, USA). Six fully expanded mature fruiting-spur leaves per treatment were selected and the measurements were repeated. Leaf photosynthesis was measured at 6 days after PGRs treatments for both dates of pre-harvest foliar ethephon application (78 and 130 DAA).

Specific rate of [^{13}C] accumulation in fruit

The [^{13}C] labeling experiment was conducted according to Zhang et al. (2005a,b) and was modified for the purposes of this experiment. Healthy, uniform 2-year-old fruiting spurs without bourse shoots on lateral branch were selected for [^{13}C] labeling at 86 and 138 DAA after ethephon treatment, respectively. The fruit were covered with aluminum foil to prevent them from fixing carbon dioxide. The [^{13}C]O_2 was generated by injecting 3 mL 70% lactic acid into 1.6 g Ba[^{13}C]O_3 with an abundance of 99% [^{13}C]. To ensure uniform labeling among the spurs, 1.5 h after the start of ^{13}C labeling, unlabelled CO_2 was produced by injecting lactic acid into another vial containing 1.5 g of $BaCO_3$ in the polyethylene bag. Four spurs in each treatment were immediately harvested 2 h after [^{13}C] labeling for evaluation of the specific rate of [^{13}C] accumulation in fruit during 2 h [^{13}C] labeling. Harvested spurs were separated into leaves, current shoots, old wood, and fruit, then stored on ice and brought to the laboratory. The fruit were freeze-dried and then weighed. Current shoots, old wood, and leaves were

oven-dried at 65°C for 10 d to determine dry weight. [^{13}C] abundance and carbon content were determined using an infrared [^{13}C]O_2 analyzer according to Zhang et al. (2005a,b). The absolute amounts (mg) of labeled [^{13}C] recovered in each organ were calculated as total carbon in each organ x[^{13}C] atom %.

RESULTS

Fruit growth and quality

As shown in Figure 1 and Table 1, the conventional GA application at 40 DAA significantly increased fruit size (length and width) and fresh weight compared to the control in *P. pyrifolia* 'Niitaka'. Interestingly, pre-harvest foliar application of ethephon alone at all three levels at 78 DAA did not increase both fruit size and fresh weight, but these features were significantly improved when ethephon was applied after conventional GA treatment (Figures 1 and 2). The early single application of ethephon at 78 DAA with a preceding conventional GA treatment is more effective in increasing fruit size than the late single application of ethephon with GA (Figure 2). No significant difference in the relationship between ethephon levels and fruit weight or size was observed in the current study in both treatments of ethephon alone or in combination with GA. The histological studies revealed that the application of PGRs in all treatments did not increase core diameter (size) and cell number of mesocarp along the equatorial region at harvest (Table 2). Treatments both of ethephon and GA alone increased cell size and it appears that cell size was dependent on ethephon concentration. When ethephon was applied after the conventional GA treatment, it strengthened cell expansion more than did GA alone.

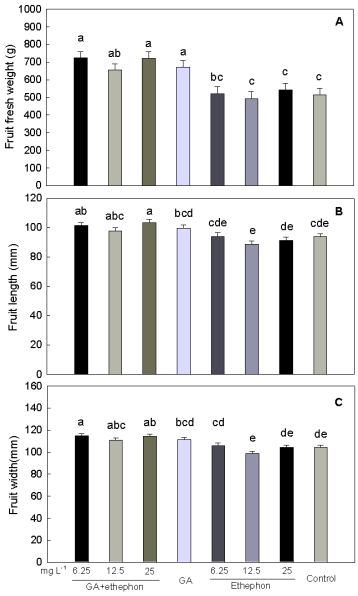

Figure 1. Effects of pre-harvest foliar application of ethephon alone or combination with a preceding conventional GA treatment during the early stage of fruit expansion on fruit size in *P. pyrifolia* 'Niitaka': fruit fresh weight (**A**), fruit length (**B**), and fruit width (**C**). GA was applied to pedicel with lanolin paste at 40 days after anthesis (DAA), and ethephon was applied at 78 DAA. P = 0.05, N = 10.

Table 1. Effects of pre-harvest foliar application of ethephon with or without a preceding conventional GA treatment on fruit fresh weight in *P. pyrifolia* 'Niitaka'.

Treatment	Fruit fresh weight (g)		P^y
	78 DAA[z]	130 DAA	
Control	542.9c[x]	542.9c	-
Ethephon 25.0	543.1c	561.5c	ns
GA	654.9b	654.9b	-
GA + ethephon 25.0	753.2a	702.6a	*

[z]Ethephon was applied at 78 or 130 days after anthesis (DAA). GA was applied to pedicel with lanolin paste at 40 DAA. [y]Significance test was conducted for date trial of ethephon application at $P < 0.05$ (*). ns = not significant. [x]Any two means within a column followed by different letters are significantly different at $P < 0.05$. N = 10.

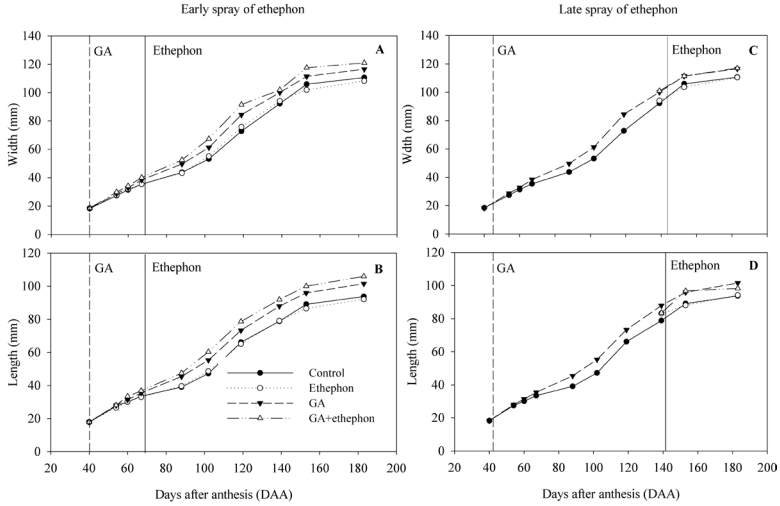

Figure 2. Fruit growth patterns after pre-harvest application of GA and/or ethephon in *P. pyrifolia* 'Niitaka'. GA was applied at 40 days after anthesis (DAA), and ethephon (25 mg l-1) was applied at 78 DAA (early) and 130 (late) DAA, respectively. A, C: fruit width; B, D: fruit length. The dates of GA and ethephon application are indicated by dotted and solid lines, respectively. N = 10.

Table 2. Effects of pre-harvest foliar application of ethephon with or without a preceding conventional GA treatment on core size (diameter), cell number and cell size of the mesocarp along the equatorial region at harvest in *P. pyrifolia* 'Niitaka'.

Treatment	Cell number (No.)	Cell size (μm)	Core diameter (mm)
Control	394.4ab[z]	157.6[e]	32.3[bc]
Ethephon 6.25[y]	402.5[ab]	177.5[d]	31.9[c]
Ethephon 12.5	352.3[bc]	196.3[bc]	33.4[abc]
Ethephon 25.0	338.8[c]	204.1[abc]	32.8[bc]
GA[x]	418.2[a]	190.3[cd]	35.4[a]
GA + ethephon 6.25	385.8[abc]	216.3[a]	33.7[abc]
GA + ethephon 12.5	370.4[abc]	215.8[a]	34.4[ab]
GA + ethephon 25.0	384.5[abc]	212.0[a]	34.5[ab]

[z]Any two means within a column followed by different letters are significantly different at $P < 0.001$. [y]Ethephon was applied at 78 days after anthesis (DAA). Numbers listed after ethephon show the concentrations were 6.25, 12.5, and 25.0 mg l^{-1}, respectively. [x]GA was applied to pedicel with lanolin paste at 40 DAA and followed by foliar application of ethephon in combination treatments.

The changes in fruit firmness, total soluble solids (TSS) (°Brix) content and juice pH were clearly detected after ethephon application (Figure 3), suggesting that pre-harvest foliar ethephon advanced fruit ripening. Both GA and ethephon alone reduced fruit firmness and no significant difference was observed between them. Fruit firmness was further reduced with a conventional GA treatment followed by a foliar application of ethephon at 78 DAA. The firmness data showed decreased flesh firmness at higher ethephon concentrations, and the GA treatment appears to have generally reduced firmness. These observations suggest that both GA and ethephon may have advanced fruit maturation.

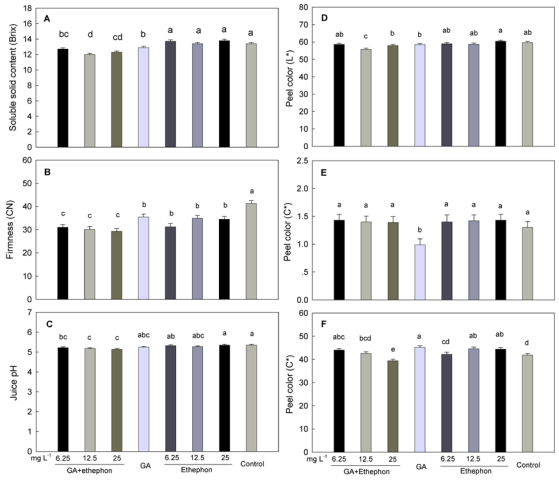

Figure 3. Effects of pre-harvest foliar application of ethephon alone or combination with a preceding conventional GA treatment during the early stage of fruit expansion on fruit quality in *P. pyrifolia* 'Niitaka': soluble solid content (**A**), firmness (**B**), juice pH (**C**) and peel color (L*, C*, h) (**D-F**). P = 0.05, N = 10.

Pre-harvest foliar application of ethephon alone did not influence total soluble solids and the occurrence of watercore (data not shown), but ethephon applied after the conventional GA treatment resulted in a significantly lower TSS compared to the control, as well as to the GA treatment. Similarly, ethephon alone did not significantly reduce juice pH. GA alone tended to reduce juice pH, but with no significant difference relative to the control. However, ethephon applied after the conventional GA treatment significantly reduced juice pH.

Both ethephon alone and a combination of ethephon and GA did not affect lightness and hue angle, but increased chroma (color saturation) compared to the control and conventional GA treatments (Figure 3). Either GA or ethephon treatments advanced fruit maturation and exhibited improved chroma when compared to the control.

Ethylene evolution

All treatments, including the control, showed an extremely low ethylene evolution at harvest and two weeks after storage at 4°C (data not shown). Although the earlier application of ethephon alone or in combination with GA exhibited higher fruit ethylene evolution than the late ethephon application, however, no statistically significant difference was observed (data not shown).

Leaf photosynthesis and [^{13}C] accumulation in fruit

Single applications of ethephon on both dates or the conventional GA treatment did not affect relative chlorophyll content, but the combination of ethephon and GA on both dates significantly increased relative chlorophyll content (Table 3). Both GA and/or ethephon at two application dates (78 and 130 DAA) showed an increased P_N when compared with the control, (Figures 4 and 5). Single ethephon application at 78 DAA exhibited significantly higher P_N and C_i than that at 130 DAA, and the increased P_N and C_i under the conventional GA treatment at 78 DAA was not observed at 130 DAA. However, only the combination of GA and ethephon treatment at 130 DAA still remained a higher P_N, about 19.6 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$, while P_N in the other three treatments was at 12.7 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$. The application of PGRs slightly increased leaf stomatal conductance, and both ethephon alone and the combination of ethephon and GA increased g_s on two spray dates (Figure 6).

Table 3. Effects of the foliar application of ethephon with or without a preceding conventional GA treatment on leaf relative chlorophyll content in *P. pyrifolia* 'Niitaka'.

Treatment	Relative chlorophyll content (SPAD value)		P^y
	78 DAA^z	130 DAA	
Control	46.4^{abx}	46.7^b	ns
Ethephon 25.0	44.5^b	48.7^{ab}	*
GA	46.3^{ab}	47.9^{ab}	*
GA + ethephon 25.0	48.1^a	50.9^a	ns

^{z}Ethephon was applied at 78 or 130 days after anthesis (DAA). GA was applied to pedicel with lanolin paste at 40 DAA. ^{y}Significance test was conducted for date trial of ethephon application at $P < 0.05$ (*). ns, not significant. ^{x}Any two means within a column followed by different letters are significantly different at $P < 0.05$. N = 8

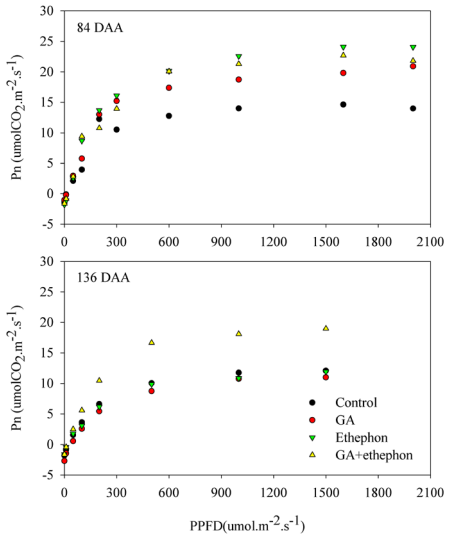

Figure 4. Effects of pre-harvest application of GA and/or ethephon on light response curves of leaf net photosynthesis in *P. pyrifolia* 'Niitaka'. GA was applied to pedicel with lanolin paste at 40 days after anthesis (DAA), and ethephon (25 mg l-1) was applied at 78 (early) or 130 (late) DAA. Leaf photosynthesis was measured at 84 DAA (upper part) and 136 DAA after (lower part), respectively. N = 6.

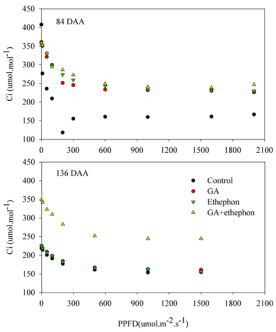

Figure 5. Effects of pre-harvest application of GA and/or ethephon on light response curves of internal CO2 concentration of leaves in *P. pyrifolia* 'Niitaka'. The intercellular CO2 concentration was measured at 84 DAA (upper part) and 136 DAA after (lower part), respectively. N = 6.

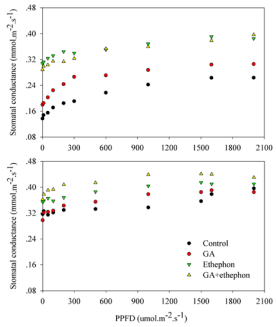

Figure 6. Effects of pre-harvest application of GA and/or ethephon on light response curves of leaf stomatal conductance in *P. pyrifolia* 'Niitaka'. The leaf stomatal conductance was measured at 84 DAA (upper part) and 136 DAA after (lower part), respectively. N = 6.

[^{13}C]-labeling study showed that source strength increased after PGR application (Figure 7), and the treatments of ethephon with or without a preceding conventional GA treatment increased sink strength associated with the specific rate of [^{13}C] accumulation in fruit at 78 DAA (Figure 8), but there was no significant difference between the control and the treatment of ethephon alone at 130 DAA.

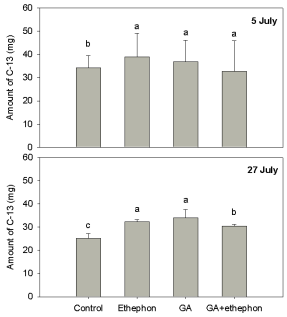

Figure 7. Effect of GA and pre-harvest foliar application of ethephon (25 mg/L) on C-assimilation of spur leaves (source strength) at 86 (8 days after ethephon spray) or 138 DAA (8 days after ethephon spray) in *P. pyrifolia* 'Niitaka'. P = 0.05, N = 6.

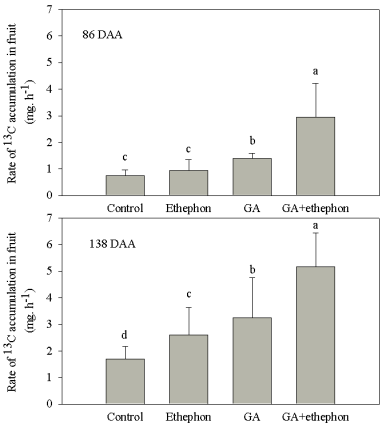

Figure 8. Effects of pre-harvest application of GA and/or ethephon on specific rate of [^{13}C] accumulation in fruit at 86 and 138 days after anthesis (DAA), respectively, in *P. pyrifolia* 'Niitaka'. N = 6.

DISCUSSION

Ethylene as a stimulator in cell expansion

The role of ethylene in plant growth regulation has been a controversial topic (Pierik et al. 2006). Ethylene is generally considered a growth inhibitor and its role as such has been well documented (Abeles et al., 1992; Pierik et al., 2006; Dugardeyn and Dominique, 2008). There is, however, growing evidence indicating that growth promotion is a common feature in ethylene responses. Ethylene stimulates hypocotyl growth in the light (Smalle et al. 1997), induces root hair formation, stimulates stem growth, shade avoidance responses (Pierik et al., 2004), and submergence-induced shoot elongation (Choi, 2007; Jackson, 2008). These growth-stimulatory effects of ethylene were species-, time-, position-, and condition-dependent (Pierik et al., 2006; Dugardeyn and Dominique, 2008; Jackson, 2008).

Most ethylene-mediated growth responses occur at the cell expansion level, although ethylene can affect cell division as well (Dan et al. 2003). In fruit crops, ethylene modulates fruit growth dynamics and the ripening process (Abeles et al., 1992). In this study, pre-harvest foliar application of ethephon with a preceding conventional GA treatment exhibited a better efficacy in increasing fruit size by promoting cell expansion than GA or ethephon alone (Figure 1 and Table 2). Moreover, the earlier application of ethephon is much better than the late spray of ethephon in strengthening GA-induced cell expansion (Table 1 and Figure 2). Cell expansion requires the synergistic action of cell wall loosening, deposition of cell wall materials, and water absorption. In most fruit crops, including *P. pyrifolia*, fruit expansion and softening are widely recognized as changes in cell walls that accompany fruit maturation (Zhang et al., 2007). A number of wall-loosening agents have been identified, and most of them act primarily on the cellulose:hemicellulose network. Xyloglucan endotransglycosylase/hydrolase (*XTH*) and various aquaporins have been postulated to play important roles in ethylene-induced berry expansion in grape (Chervin et al., 2008). In pear, ethylene-regulated expansins are involved in fruit ripening (Hiwasa et al., 2003). Cell wall extensibility is considered a major regulatory point in cell growth. Ethylene has been shown to induce apoplastic acidification, which would help to increase cell wall extensibility because expansins are active at low pH. Apoplastic

acidification resulting from H^+ extrusion probably sets the optimal acidic environment for the action of cell wall loosening proteins. Reduced fruit firmness (Table 1) by GA or ethephon alone indicated advanced fruit maturation and cell wall extensibility. Both juice pH and fruit firmness were further reduced when ethephon was applied after the conventional GA treatment, which was associated with increased fruit expansion (Figures 1 and 2). Therefore, to elucidate the regulatory routes of ethylene in cell expansion, much will depend on crosstalk with other hormones, particularly GA.

In this study, pear fruits were bagged with double-layered water-resistant bags to prevent fruit from experiencing direct contamination by ethephon. Compared with the conventional GA treatment, the intracellular ethylene in fruit, which was released from ethephon translocated from leaves, might be responsible for the changes of fruit size and quality (Figures 1 and 2) when ethephon was applied after the conventional GA treatment. This synergistic effect of GAs and ethylene also can be observed in light-grown *Arabidopsis* seedlings (Weiss and Ori, 2007).

Ethylene as a regulator in source-sink strength

Ethylene has long been recognized as a modulator of photosynthesis in plants (Pierik et al., 2006; Woodrow et al., 1989), but conflicting results on its effects on net photosynthetic rate have been reported (Pallas and Kays 1982; Ma et al., 2015; Tholen et al., 2008). Studies of whole plants or attached whole leaf gas exchange have indicated that net carbon uptake is reduced in some cultivars surveyed (Choe and Whang, 1986; Woodrow et al., 1989). It has been demonstrated that the ethylene transduction pathway is involved in the regulation of photosynthesis in mustard (Khan, 2004) and the absence of a functional ethylene receptor leads to a reduction in Rubisco content and photosynthetic capacity in tobacco (Tholen et al., 2008).

In present study, ethephon application significantly increased relative chlorophyll content in leaves when combined with a conventional GA treatment (Table 3), which is consistent with previous reports in *Quercus ilex* (Munné-Bosch et al., 2004). Ethephon application at both spray dates after the conventional GA treatment increased leaf photosynthesis rate, relative chlorophyll content and source strength (Table 3, Figures 4, 7). Growing evidence shows that ethylene can have a stimulating effect on photosynthesis by affecting carboxylation capacity and the diffusion rate of CO_2 from the atmosphere to the intercellular cavities (Tholen et al., 2008). In this study, the increased photosynthesis associated with source strength can be explained by the possible effect of ethylene on stomatal conductance (Figure 6), which increases the intercellular CO_2 concentration (Figure 5). The observed large differences in photosynthesis (Figure 4) were coupled with relatively small changes in intercellular CO_2 concentration (Figure 5). These results suggest that the effect of ethylene on stomatal conductance is concentration-dependent. Therefore, ethylene is not necessarily a growth-inhibiting hormone under all circumstances, but can also act as a stimulant for photosynthesis and growth (Pierik et al., 2006).

Sink strength is established and regulated by plant growth regulators, which stimulate nutrient transport and increase phloem unloading, or act upon metabolism and compartmentalization of sucrose and sorbitol (Kuiper, 1993). Our previous reports showed that GA is proposed for serving as a mobilizer of assimilates to fruit and as a modulator for carbon partitioning in fruit of *P. pyrifolia* (Zhang et al., 2005a, 2007). In this study, ethephon applied after the conventional GA treatment significantly increased the specific rate of [^{13}C] accumulation in fruit on both spray dates compared to the conventional GA treatment.

Chalmers (Chalmers et al., 1976) also reported that ethylene participated in the control of sink strength of peach. The enhanced sink activity and sink size (Figure 1) can be attributed to an increase in the specific rate of [^{13}C] accumulation in fruit (Figure 8). Therefore, reduced photosynthesis in both treatments of ethephon or GA alone at late spray date (Figure 5) might result from the difference in fruit strength or fruit size relative to the combination of GA and ethephon treatment.

Ethylene and fruit quality in *P. pyrifolia*

Fruit ripening of *Pyrus* spp, including *P. pyrifolia*, has been classified as climacteric. However, evidence suggests that *P. pyrifolia* comprises climacteric and non-climacteric genotypes (Itai et al., 1999). 'Niitaka' is considered as non-climacteric as it produces very little ethylene (<0.1 μL·kg·h) and has a non-climacteric respiratory pattern. The role of ethylene in the ripening process of climacteric fruit has been studied extensively for many years (Lin et al., 2009), and very little is known about the role of ethylene in the ripening of non-climacteric fruit. In climacteric fruit, ripening events such as fruit softening, chlorophyll degradation, coloring, and sugar accumulation are considered to be induced by ethylene (Pech et al., 2008). Ripening control in non-climacteric fruit was originally thought to be independent of ethylene, but ethylene can affect non-climacteric fruit (Abeles et al., 1992). In the current study, fruit ethylene production in all treatments was not enhanced by GA treatment, even when GA and ethephon were both used (Figure 3). However, our results showed that ethylene is capable of affecting the physiological processes during fruit maturation in non-climacteric 'Niitaka.' Ethephon advanced fruit maturation according to reduced fruit firmness and improved fruit skin color at harvest (Figure 2). Sugar accumulation and acidity (juice pH) likely were ethylene-independent, and fruit firmness and chlorophyll degradation in fruit were ethylene-dependent (Figure 2).

In conclusion, pre-harvest foliar application of ethephon with a preceding conventional GA treatment resulted in synergistic effects in fruit size through enhancing source-sink strength and GA responsiveness in cell expansion in *P. pyrifolia*.

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