Effects of GA\textsubscript{3+4} and GA\textsubscript{4+7} Application Either Alone or Combined with Prohexadione-Ca on Fruit Development of Japanese Pear ‘Kosui’

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In order to promote increases in the size of ‘Kosui’ Japanese pear [\textit{Pyrus pyrifolia} (Burm.) Nakai] fruit by plant growth regulators, we applied gibberellin (GA)\textsubscript{3+4} paste [2.7% (w/w), A\textsubscript{3}:\textsubscript{A_{4}} = 90:10] in combination with prohexadione-calcium [1%, PCa; BAS-125 (3-oxido-4-propionyl-5-oxo-3-cyclohexene-carboxylate)], an inhibitor of GA 2\beta-hydroxylation that catabolizes active GA into an inactive form, to fruit pedicels at approximately 30 days after full bloom. GA\textsubscript{3+4}+PCa treatment advanced fruit growth only in the early stages, but fruit weight did not show any significant differences between the untreated control and GA\textsubscript{3+4}+PCa-treated fruits at harvest. In contrast, when GA\textsubscript{4+7} [2.7% (w/w), A\textsubscript{4}:\textsubscript{A_{7}} = 66:34] was applied, the fruit weight at harvest was greater than that of untreated fruit. Moreover, GA\textsubscript{4+7} treatment in combination with PCa resulted in an even higher fruit weight at harvest. The GA\textsubscript{4} concentration in fruit flesh was not affected by GA\textsubscript{3+4} application at 1 week after the treatment (WAT) either with or without PCa, but GA\textsubscript{4} levels increased with GA\textsubscript{4+7}+PCa treatment, resulting in a significant increase in fruit weight at harvest. A single GA\textsubscript{4+7} application almost doubled the GA\textsubscript{4} concentration compared with the untreated control, but the difference was not significant. These results indicate that fruit weight at harvest was greater when the GA\textsubscript{4} concentration was higher in the fruit flesh at 1 WAT. The higher concentration of GA\textsubscript{4} in the GA\textsubscript{4+7}+PCa-treated fruit compared with the GA\textsubscript{4+7} treatment alone may be attributed to the function of PCa that acts to prevent the inactivation of GA\textsubscript{4} to GA\textsubscript{3+4} by inhibiting 2\beta-hydroxylation.

Key Words: bioactive GA, GA biosynthesis, gibberellin, fruit maturation, \textit{Pyrus pyrifolia} (Burm.) Nakai.

Introduction

In Japan, earlier harvest times and larger Japanese pears can result in higher prices in fresh fruit markets. Approximately 75% of harvested Japanese pears are marketed in August and September due to the maturation time for the two major early cultivars, ‘Kosui’ and ‘Hosui’ (Kajiura, 1996). To accelerate maturation and harvest time as much as possible, some Japanese pear growers in the southern pear-growing area have developed methods to force the culture of the early-ripening cultivar, ‘Kosui’.

To increase fruit size, pears are usually subjected to hand-thinning before six weeks after pollination, and subsequently only one fruit per three to five clusters is left to mature. In addition, to improve fruit size and maturation, growers apply a GA paste (GA\textsubscript{3+4}, containing both GA\textsubscript{3} and GA\textsubscript{4}) to the pear fruit pedicels at approximately 30 days after full bloom (DAF). GA paste is registered as an agricultural chemical in Japan, and is used commercially to enhance fruit size, and promote Japanese pear maturation, although the effect varies among cultivars and growing areas. Prohexadione-calcium [PCa; BAS-125 (3-oxido-4-propionyl-5-oxo-3-cyclohexene-carboxylate)], a GA biosynthesis inhibitor (Owens and Stover, 1999), is registered as a shoot
growth retardant for rice, turf grass, and other crops in Japan. PCa is known to reduce the vegetative shoot growth of stone fruits, citrus and pome fruits (Rademacher and Kober, 2003). The main target of PCa is 3β-hydroxylase, an enzyme that catalyzes primarily the conversion of inactive GA20/GA9 into highly active GA3/GA4 in either the early 13-hydroxylated pathway or the early non-13-hydroxylation pathway, respectively (Fig. 1). To a lesser extent, PCa also inhibits the 2β-hydroxylation that converts GA4/GA5 into their inactive forms of GA8/GA34, respectively. Therefore, conversion of exogenously applied bioactive GAs (e.g., GA1 or GA4) to inactive forms by 2β-hydroxylation can be inhibited by simultaneously treatment with PCa that also increases GA activity.

We have already reported that a combination treatment of GA paste (GA3+4) and PCa can increase the fruit size of Japanese pear ‘Hosui’ compared with applying GA paste alone (Itai and Honda, 2008; Itai et al., 2008), and is accompanied with a higher level of GA4 (Itai et al., 2008), the bioactive form of GA in Japanese pears (Ito et al., 2000; Nakagawa et al., 1979; Zhang et al., 2007, 2008), in the treated fruit. However, in practice, ‘Hosui’ growers may experience some difficulty in using GA paste for fruit enlargement because ‘Hosui’ is a cultivar susceptible to “water core”, a disorder known to be aggravated by GA application (Chun et al., 2003; Sakuma et al., 1995). In contrast, water core symptoms are seldom found in ‘Kosui’ fruits and, thus, growers can safely apply GA paste to ‘Kosui’. Therefore, it would be of great benefit to pear growers if ‘Kosui’ fruits could be enlarged by GA paste and PCa application because the harvest time for ‘Kosui’ is earlier than ‘Hosui’ by approximately two weeks. As a result, much higher profits would be realized by larger fruit in combination with an early harvest/market. However, the effect of GA paste and PCa has not yet been examined in this cultivar.

In this study, we first examined the effect of GA paste in combination with PCa to develop a practical technique for enlarging ‘Kosui’. Commercial GA paste in Japan contains 2.7% (w/w) GA3 + GA4 at a ratio of 90:10. Unexpectedly, we found that GA paste treatment in combination with PCa did not result in any additional increase in ‘Kosui’ fruit size compared with a single GA paste application, unlike our experience with ‘Hosui’. We hypothesized that this unexpected result with GA paste may be due to differences in the GA response between ‘Hosui’ and ‘Kosui’. Here, we show the effect of GA paste (GA3+4) and PCa on ‘Kosui’ fruit development, especially with regard to fruit size. We subsequently also examined the effect of GA4+7, instead of GA3+4, in combination with PCa.

**Materials and Methods**

**Plant materials**

Experiments were conducted in 2007–2009. Mature Japanese pear trees (32-year-old in 2007), Pyrus pyrifolia ‘Kosui’, growing in an experimental orchard at the NARO Institute of Fruit Tree Science (Ibaraki, Japan) were used. Trees were grown in a flat-pergola system (trellis height = 180 cm), and managed according to the methods employed at the Institute. Fruits were hand-thinned before the treatments according to commercial practice where fruit number was reduced to one fruit per four or five retained clusters.

**Plant growth regulators**

GA3+4 paste [2.7% (w/w), A3 + A4 mixture, 90:10] was commercially purchased (Kyowa Hakko Bio Co. Ltd., Tokyo, Japan). Prohexadione-Ca paste [1% (w/w)] was a gift from Kumiai Chemical Industry Co. Ltd. (Tokyo, Japan). GA4+7 (66:34 mixture, purity > 90%) was purchased from Lemandou Enterprise Ltd. (Chijiazhuang, China). GA4+7 was mixed with lanolin to a final concentration of 2.7% (w/w) or 0.27% (w/w). We did not discriminate between GA4 and GA7 when applied in a mixture (GA4+7) because these two

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![Fig. 1. The gibberellic acid (GA) biosynthetic pathway from the GA12-aldehyde as modified from Hedden and Kamiya (1997). Underlined GAs indicate those measured in this experiment.](image-url)
molecules have equal effects on fruit enlargement in Japanese pears (Zhang et al., 2008).

GA$_{3+4}$ and GA$_{4+7}$ were applied to fruit pedicels alone or in combination with PCa. When GA$_{3+4}$ and GA$_{4+7}$ were used individually, 30–40 mg of paste containing GA$_{3+4}$ or GA$_{4+7}$ was applied to each fruit. For treatments where GAs were combined with PCa, an equal weight of the GA mixture and PCa paste were mixed well, and then 60–80 mg of the mixture was applied to each fruit pedicel.

Treatments

To elucidate the combined effects of GA$_{3+4}$ and PCa application on fruit size and quality of ‘Kosui’, the following treatments were conducted in 2007 and 2008: (1) no treatment (control), (2) GA$_{3+4}$ application (GA$_{3+4}$), and (3) GA$_{3+4}$ in combination with a PCa application (GA$_{3+4}$+PCa). For each treatment, four and three adult trees were used in 2007 and 2008, respectively.

Similarly, the following treatments were conducted in 2008 and 2009 with GA$_{4+7}$: (1) no treatment (control), (2) 2.7% GA$_{4+7}$ application (GA$_{4+7}$), and (3) 2.7% GA$_{4+7}$ in combination with a PCa application (GA$_{4+7}$+PCa). Four adult ‘Kosui’ trees were used for each treatment both in 2008 and 2009.

In addition, in order to apply only early non-13-hydroxylated bioactive GAs (GA$_3$ and GA$_4$) at concentrations similar to those in GA$_{3+4}$ paste, we also made two additional applications in 2009 to ‘Kosui’: (4) 0.27% GA$_{4+7}$ application (1/10 GA$_{4+7}$) and (5) 0.27% GA$_{4+7}$ in combination with a PCa application (1/10 GA$_{4+7}$+PCa).

These plant growth regulators were applied at approximately 30 DAF. Uniformly growing fruits were randomly selected from these trees before the treatments, and the plant growth regulators were applied to the middle portion of the pedicels. At least 100 fruits per treatment were used for each treatment, except for the treatments of GA$_{4+7}$ and GA$_{4+7}$+PCa in 2008, where 15 fruits per treatment were used.

Fruit diameter was periodically measured every two weeks after treatment for 13–15 fruits, though we did not measure fruits treated with 1/10 GA$_4$ alone or in combination with PCa in 2009. The fruits were collected at harvest to assess fruit quality. The timing of fruit harvest was determined according to fruit color. In 2008, for the analysis of GA contents in ‘Kosui’ fruit flesh, six fruits × three biological replications per treatment were collected at 1 and 4 WAT.

GA analysis

The frozen fruit flesh (approx. 10 g per extraction) was collected, and analyzed for GA$_1$, GA$_3$, GA$_4$, and GA$_{34}$ concentrations by gas chromatography-mass spectrometry-selected ion monitoring (GC-MS-SIM) after purification by high performance liquid chromatography (HPLC). Hormone extraction was performed as described by Ito et al. (1999), and HPLC and GC-MS-SIM analyses were conducted as described by Phuoc et al. (2008) with slight modifications. Briefly, samples were homogenized, and extracted three times in a 4-fold volume (v/w) of 80% (v/v) aqueous methanol. After addition of internal standards (50 ng each of [H$_3$]GA$_1$, [H$_3$]GA$_3$, [H$_3$]GA$_4$, and [H$_3$]GA$_{34}$), the extract was concentrated in vacuo, and the aqueous residue was subjected to solvent partitioning to obtain an acidic ethyl acetate-soluble fraction. The resulting residue was pre-purified using a Sep-Pak C18 cartridge (Waters, Milford, MA, USA) followed by a Bondesil DEA (5 g) column. The eluate was subjected to HPLC on an ODS column (ODS-5, 150 mm × 10 mm i.d.; Nomura Chemical, Aichi, Japan), and eluted with a 60-min program of two linear gradients of methanol: water containing 0.1% (v/v) acetic acid [45% to 50% (v/v) methanol for 25 min and then 50% to 80% (v/v) methanol for 25 min, after which methanol was kept at 80% (v/v) for 10 min] at a flow rate of 2 mL per min at 40°C. HPLC fractions were collected every 2 min. The GAs-like activity of the HPLC fractions was confirmed with retention times and bioassays using dwarf rice (Oryza sativa L.) (Nishijima and Katsura, 1989). The fractions with retention times of 6 to 10 min for GA$_1$ and GA$_3$, 34 to 40 min for GA$_{3+4}$, and 40 to 46 min for GA$_4$ were, respectively, combined, followed by further chromatography by HPLC on a Senshu Pak N(CH$_3$)$_2$-4151-N column (150 mm × 10 mm i.d.; Senshu Scientific, Tokyo, Japan) that was eluted with methanol containing 0.1% (v/v) acetic acid at a flow rate of 2 mL·min$^{-1}$ at 40°C. Four mL fractions were collected, and bioassayed as already described. The fractions with retention times of 26 to 30 min for GA$_1$, 34 to 38 min for GA$_3$, 18 to 26 min for GA$_4$, and 26 to 34 min for GA$_{34}$ were, respectively, dissolved in MeOH (20 μL), and methylated with ethereal CH$_3$N$_2$ (100 μL) at room temperature. The samples were then dried, and trimethylsilylated in glass tubes with N-methyl-N-trimethylsilyl trifluoroacetamide (MSTFA, 20 μL) at 70°C. The derivatives were analyzed using an Agilent 6890N gas chromatograph coupled to an Agilent 5973N Mass Selective Detector (Agilent Technologies, Wilmington, DE, USA). The GC was equipped with a splitless injector and a DB-1 capillary column (30 m length, 0.25 mm i.d., 0.25 μm film thickness). The oven temperature program started at 120°C, and after 2 min was increased by 16°C·min$^{-1}$ to 216°C, was kept at 216°C for 5 min, then increased by 8°C·min$^{-1}$ to 280°C, and finally kept at 280°C for 10 min. Quantities of GAs were analyzed in selected ion monitoring mode, and were calculated from the ion peak area ratios of non-labeled GAs against $^3$H-labeled GAs.
Results

Effect of GA\textsubscript{3+4} and PCa on fruit development of ‘Kosui’

GA\textsubscript{3+4} applications resulted in larger fruit diameters compared with the control at the early stages of growth (ca. 60 to 110 DAF; i.e., 30 to 80 days after treatment) in 2007 and 2008 regardless of whether the fruits were also treated with PCa (Fig. 2). However, the increase in fruit diameter by GA\textsubscript{3+4} decreased in the later stages of fruit development, resulting in fruit diameters at harvest that were not significantly different (2007) or whose fruit diameter differences were very small (2008) compared with the untreated control. GA\textsubscript{3+4} promoted fruit maturation; therefore, the harvest date was advanced by three (2007) and five (2008) days compared with the control (Table 1). There were no significant differences between GA\textsubscript{3+4} and GA\textsubscript{3+4}+PCa for the mature fruit diameter or the harvest date.

Fruit size and quality at harvest are shown in Table 1. Fruit weight was slightly increased by GA\textsubscript{3+4} in 2008, although the GA\textsubscript{3+4}+PCa treatment in 2008 and GA\textsubscript{3+4} treatments with or without PCa in 2007 resulted in no significant effects on fruit weight. Fruit length and diameter were also positively affected by GA\textsubscript{3+4} treatments. Fruit Brix in 2008 was increased by the GA\textsubscript{3+4} treatment irrespective of PCa treatment, but was not affected in 2007. There were no significant differences among the treatments for fruit color, firmness, or pH in either year of the study.

Effect of GA\textsubscript{4+7} and PCa on fruit development of ‘Kosui’

GA\textsubscript{4+7} treatment with or without PCa enhanced fruit diameter compared with the control with significant levels at several time points starting as early as 14 days after the treatment and thereafter (45 DAF for GA\textsubscript{4+7}+PCa in 2008, and 44 DAF for GA\textsubscript{4+7}+PCa in 2009) or 28 days (59 DAF for GA\textsubscript{4+7} in 2008, and 58 DAF GA\textsubscript{4+7} in 2009) (Fig. 3). The fruit diameter was larger in the fruits receiving the GA\textsubscript{4+7}+PCa treatment compared with GA\textsubscript{4+7}-treated fruits in the early stages of fruit growth, but the difference between these two

Table 1. Effect of GA\textsubscript{3+4} application, either alone or in combination with a PCa treatment, on the quality of ‘Kosui’ Japanese pear fruit.

<table>
<thead>
<tr>
<th>Year</th>
<th>Treatment</th>
<th>Weight (g)</th>
<th>Length (mm)</th>
<th>Diameter (mm)</th>
<th>Ground color</th>
<th>Firmness (lb)</th>
<th>Brix</th>
<th>pH</th>
<th>Harvest date</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>Control</td>
<td>402.3 NS</td>
<td>76.6 b</td>
<td>94.1 NS</td>
<td>2.5 NS</td>
<td>5.7 NS</td>
<td>11.6 NS</td>
<td>5.1 NS</td>
<td>20 Aug</td>
</tr>
<tr>
<td></td>
<td>GA\textsubscript{3+4}</td>
<td>415.9</td>
<td>78.2 a</td>
<td>94.1</td>
<td>2.6</td>
<td>5.7</td>
<td>11.5</td>
<td>5.0</td>
<td>17 Aug</td>
</tr>
<tr>
<td></td>
<td>GA\textsubscript{3+4}+PCa</td>
<td>429.4</td>
<td>76.7 b</td>
<td>96.2</td>
<td>2.5</td>
<td>5.8</td>
<td>11.7</td>
<td>5.0</td>
<td>17 Aug</td>
</tr>
<tr>
<td>2008</td>
<td>Control</td>
<td>383.1 b</td>
<td>76.2 b</td>
<td>93.0 b</td>
<td>2.7 NS</td>
<td>5.6 NS</td>
<td>11.5 b</td>
<td>5.0 NS</td>
<td>28 Aug</td>
</tr>
<tr>
<td></td>
<td>GA\textsubscript{3+4}</td>
<td>415.6 a</td>
<td>79.0 a</td>
<td>97.0 a</td>
<td>2.8</td>
<td>5.6</td>
<td>11.8 a</td>
<td>5.0</td>
<td>23 Aug</td>
</tr>
<tr>
<td></td>
<td>GA\textsubscript{3+4}+PCa</td>
<td>411.8 ab</td>
<td>77.5 ab</td>
<td>96.0 ab</td>
<td>2.6</td>
<td>5.5</td>
<td>11.9 a</td>
<td>5.0</td>
<td>23 Aug</td>
</tr>
</tbody>
</table>

\(n \geq 100\).
Letters denote significant differences between treatments determined using Tukey’s LSD test. NS: not significant.
treatments became smaller at later stages and was still significant at harvest. GA\textsubscript{4+7} also promoted fruit maturation; thus, the harvest date advanced by eight (2008) and six (2009) days compared with the control (Table 2). There was no significant difference in harvest date between the GA\textsubscript{4+7} and GA\textsubscript{4+7}+PCa treatments.

Fruit size and quality at harvest are shown in Table 2. Fruit weight increased by 18% after GA\textsubscript{4+7} treatments in 2009 compared with the control, although GA\textsubscript{4+7} in 2008 did not affect fruit weight significantly. In contrast, the GA\textsubscript{4+7}+PCa treatment had a larger impact on fruit weight than a single GA\textsubscript{4+7} treatment, and significantly increased fruit weight in both years (27% and 30% compared with the control in 2008 and 2009, respectively). Fruit length and diameter were also positively affected by GA\textsubscript{4+7} treatments. The statistical discrepancy of fruit diameters at harvest shown in Figure 3 and Table 2 may result from differences in the number of intact fruits used for measuring diameter: 13 fruit and 15 fruit were used in 2008 and 2009, respectively (Fig. 3), while fruit quality at harvest used 14–16 fruit in 2008 and 133–139 fruit in 2009 (Table 2).

**Effect of 1/10 GA\textsubscript{4+7} and PCa on fruit size of ‘Kosui’**

Considering that the bioactive form of GA in Japanese pears is GA\textsubscript{4} (Zhang et al., 2008), we hypothesized that GA\textsubscript{4} at a concentration of ≈0.27% in the GA\textsubscript{3+4} treatment (i.e., 1/10 of 2.7% GA\textsubscript{3+4} paste) may be insufficient to enlarge ‘Kosui’ fruit, but sufficient for ‘Hosui’, whereas GA\textsubscript{4+7} at a concentration of 2.7% may be necessary for ‘Kosui’ enlargement. To re-confirm the effective GA\textsubscript{4+7} concentration in ‘Kosui’, we applied 1/10 GA\textsubscript{4+7} either alone or in combination with PCa in 2009. Application of 1/10 (0.27%) GA\textsubscript{4+7} did not affect ‘Kosui’ fruit size, but contrary to our expectations 1/10 GA\textsubscript{4+7} treatment in combination with PCa significantly increased fruit weight by 13% in comparison with the control (Table 2). There were no significant differences among treatments for fruit color, firmness, Brix, or pH.

**Effects of GA\textsubscript{3+4} and GA\textsubscript{4+7} on GA concentrations in fruit flesh**

The amount of GAs measured here was the sum of endogenously synthesized GAs and the exogenously applied GAs, although we did not discriminate between endogenous and exogenous GAs. We postulated that the applied GAs were similarly transferred, and retained within the fruits that received the same plant growth regulator(s), as the appearance and size of these treated fruits were not significantly different within each treatment.

The most abundant GA in pear fruit flesh was GA\textsubscript{4}, and comparable concentrations of GA\textsubscript{3} were also detected in Japanese pear fruit flesh obtained at 1 and 4 WAT, as shown in Table 3. GA\textsubscript{1} was also detected, although at a much lower level than for GA\textsubscript{4} and GA\textsubscript{3}. GA\textsubscript{34}, an inactivated form of GA\textsubscript{4}, was barely detectable. The levels of all GA forms measured at 1 WAT were lower at 4 WAT.

GA\textsubscript{4+7} treatment temporarily increased the GA\textsubscript{4} concentration in fruit flesh at 1 WAT; however, the GA\textsubscript{4} concentration decreased at 4 WAT, and was lower than the control. In contrast, GA\textsubscript{4+7}+PCa treatment increased the GA\textsubscript{4} concentration in fruit flesh compared with the control at both times after treatment, and resulted in higher GA\textsubscript{4} levels in fruit than from treatment with GA\textsubscript{4+7} alone.

GA\textsubscript{34}, an inactive metabolite of GA\textsubscript{4}, was found only...
in the fruits treated with GA_{4+7} regardless of whether a PCa treatment was also applied, but this form disappeared at 4 WAT in all of the treatments. The GA_{34} concentration in GA_{34}+PCa-treated fruits was smaller than that in fruits receiving the GA_{3+4} treatment. GA_{3+4} treatment did not affect the GA_{3} and GA_{34} concentrations, irrespective of whether the GA_{3+4} treatment was in combination with PCa or not.

The effect of GA_{3+4} or GA_{4+7} applications on GA_{3} concentrations in fruit flesh was obscure at 1 WAT because of the large variation between the replicates; however, the GA_{3} concentration in fruit flesh at 4 WAT increased after GA_{3+4} and GA_{3+4}+PCa treatments compared with the control. GA_{3+4} either alone or in combination with PCa treatment did not have a significant effect on the GA_{3} concentration in fruit flesh.

GA_{1} was also found in the fruit flesh of Japanese pears, although the level was very low compared with the other bioactive GAs, GA_{3}, and GA_{4}. GA_{3+4} treatment significantly increased the GA_{1} level at 1 WAT, and combining the treatment by also treating with PCa emphasized this effect.

### Discussion

In Japan, registered GA is only available in the form of a GA_{3+4} mixture. GA_{4} and GA_{3}, as well as their analogs GA_{1} and GA_{34}, are the main biologically active GAs in plants, but the relative roles of the various forms are unclear (Eriksson et al., 2006; Yamaguchi, 2008). In this experiment, treatments with both GA_{3+4} and GA_{4+7} provided at a concentration of 2.7% accelerated fruit maturation. The effect on fruit maturation was greater after GA_{3+4} treatment than after GA_{4+7} treatment, but fruit size enhancement in ‘Kosui’ occurred only after GA_{4+7} treatment (Tables 1 and 2). Fruit weight was slightly (8%) increased by GA_{3+4} in 2008, but GA_{3+4} treatments in 2007 resulted in no significant effects (Table 1). Moreover, the GA_{3+4} treatment in combination with PCa did not provide any additional increase in fruit size for ‘Kosui’ compared with the GA_{3+4} treatment. In contrast, GA_{4+7} treatment alone increased fruit size 5% and 18% in 2008 and 2009, respectively (Table 2), whereas GA_{4+7} in combination with PCa resulted in an additional increase in fruit size of 18% and 30% in 2008 and 2009, respectively (Table 2). GA_{4+7} application to ‘Kosui’ fruit may be economically beneficial to pear growers in Japan, whereas GA_{3+4} application may, at least in some cases depending on the cultivar, be insufficiently useful to increase fruit size and accelerate fruit maturation. The reason why the effects of GA_{3+4} on fruit size differed between 2007 and 2008 is obscure; however, the final fruit size of the control was larger in 2007 than in 2008. We hypothesize that the more favorable fruit growth may have overridden the applied GA effects in 2007.

In Japanese pears, the bioactive forms of GA are

### Table 2. Effect of GA_{4+7} application, either alone or in combination with a PCa treatment, on the quality of ‘Kosui’ Japanese pear fruit.

<table>
<thead>
<tr>
<th>Year</th>
<th>Treatment</th>
<th>Weight (g)</th>
<th>Length (mm)</th>
<th>Diameter (mm)</th>
<th>Ground color</th>
<th>Firmness (lb)</th>
<th>Brix</th>
<th>pH</th>
<th>Harvest date</th>
</tr>
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<tbody>
<tr>
<td>2008</td>
<td>Control</td>
<td>376.2 b</td>
<td>76.3 b</td>
<td>92.7 b</td>
<td>2.6 NS</td>
<td>5.8 NS</td>
<td>11.6 NS</td>
<td>5.0 NS</td>
<td>26 Aug</td>
</tr>
<tr>
<td></td>
<td>GA_{3+4}</td>
<td>393.4 ab</td>
<td>77.4 ab</td>
<td>95.1 ab</td>
<td>2.6</td>
<td>6.2</td>
<td>12.4</td>
<td>5.1</td>
<td>18 Aug</td>
</tr>
<tr>
<td></td>
<td>GA_{3+4}+PCa</td>
<td>476.0 a</td>
<td>83.9 a</td>
<td>100.5 a</td>
<td>2.8</td>
<td>5.8</td>
<td>12.0</td>
<td>5.1</td>
<td>17 Aug</td>
</tr>
<tr>
<td>2009</td>
<td>Control</td>
<td>410.5 d</td>
<td>78.6 d</td>
<td>95.4 d</td>
<td>2.6 NS</td>
<td>5.1 NS</td>
<td>11.4 NS</td>
<td>5.0 NS</td>
<td>20 Aug</td>
</tr>
<tr>
<td></td>
<td>GA_{3+4}</td>
<td>485.9 b</td>
<td>84.1 b</td>
<td>101.4 b</td>
<td>2.7</td>
<td>5.2</td>
<td>11.4</td>
<td>5.0</td>
<td>14 Aug</td>
</tr>
<tr>
<td></td>
<td>GA_{3+4}+PCa</td>
<td>531.7 a</td>
<td>86.2 a</td>
<td>105.1 a</td>
<td>2.6</td>
<td>5.1</td>
<td>11.5</td>
<td>5.0</td>
<td>13 Aug</td>
</tr>
<tr>
<td></td>
<td>1/10 GA_{3+4}</td>
<td>429.7 cd</td>
<td>80.4 cd</td>
<td>97.2 cd</td>
<td>2.6</td>
<td>5.1</td>
<td>11.3</td>
<td>5.0</td>
<td>16 Aug</td>
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<tr>
<td></td>
<td>1/10 GA_{3+4}+PCa</td>
<td>462.5 bc</td>
<td>83.1 bc</td>
<td>99.5 bc</td>
<td>2.6</td>
<td>5.2</td>
<td>11.5</td>
<td>5.0</td>
<td>17 Aug</td>
</tr>
</tbody>
</table>

### Table 3. Effects of GA_{3+4} and GA_{4+7} applications, either alone or in combination with a PCa treatment, on the GA concentrations (ng·g\(^{-1}\) FW) in the fruit flesh of ‘Kosui’ Japanese pear at 1 and 4 weeks after treatment (WAT).

<table>
<thead>
<tr>
<th>GA</th>
<th>GA_{34} (ng·g(^{-1}) FW)</th>
<th>GA_{34} (ng·g(^{-1}) FW)</th>
<th>GA_{3} (ng·g(^{-1}) FW)</th>
<th>GA_{1} (ng·g(^{-1}) FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 WAT</td>
<td>4 WAT</td>
<td>1 WAT</td>
<td>4 WAT</td>
<td>1 WAT</td>
</tr>
<tr>
<td>Control</td>
<td>4.99 b(^+)</td>
<td>3.25 b(^+)</td>
<td>nd(^\text{o})</td>
<td>nd(^\text{o})</td>
</tr>
<tr>
<td>GA_{3+4}</td>
<td>4.75 b</td>
<td>2.35 bc</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>GA_{3+4}+PCa</td>
<td>5.49 b</td>
<td>2.26 bc</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>GA_{4+7}</td>
<td>8.88 ab</td>
<td>1.81 c</td>
<td>0.43 a</td>
<td>nd</td>
</tr>
<tr>
<td>GA_{4+7}+PCa</td>
<td>11.21 a</td>
<td>4.61 a</td>
<td>0.13 b</td>
<td>nd</td>
</tr>
</tbody>
</table>

\(^a\) Letters denote significant differences between treatments determined using Tukey’s LSD test. NS: not significant.

\(^\text{o}\) nd: not detected.
early non-13-hydroxylated GAs such as GA₄ and GA₇ (Ito et al., 1999), whereas other bioactive forms of early 13-hydroxylated GA₃/GA₄ have no effect on fruit size (Nakagawa et al., 1979; Zhang et al., 2008), a finding that is in accordance with our result. Zhang et al. (2008) applied GA₅, GA₇, GA₈, and GA₁₀ to Japanese pear fruit, and reported that their effectiveness for fruit enlargement is GA₅ ≈ GA₇ > GA₈ > GA₁₀. Nakagawa et al. (1979) showed that GA₄ is more abundant than GA₂ in pear fruit; therefore, we hypothesized in this study that the positive effect of GA₃+₇ treatment on fruit enlargement (if any) would be due to GA₄ and not GA₂. In addition, GA₂ has a double bond at the 1, 2 position (Hedden and Kamiya, 1997); thus, GA₃ is not inactivated (i.e., not 2β-hydroxylated) by GA2-oxidase (Appleford et al., 2007; Sakamoto et al., 2001). As a result, PCₐ has no role in inhibiting the inactivation of GA₃. That is, GA₃ did not affect fruit enlargement, and the GA₃ level was similar to that found in fruit after treatments with either GA₃+₄ or GA₃+₄ with PCₐ. Contrary to this result, GA₄ had an effect on fruit size, and its effect was accelerated by the combination treatment with PCₐ. Taken together, these results suggest that GA₄, not GA₃, has a primary role in enlarging fruit size, and that the additive effects of PCₐ on fruit size may be attributed to the inhibition of GA₄ conversion to GA₃+₄, the inactive form.

A single treatment of GA₃+₄ transiently increased the GA₄ concentration in fruit flesh at 1 WAT due to exogenously applied GA₄, but by 4 WAT the GA₄ level had decreased to a value lower than the untreated control (Table 3). On the contrary, GA₃+₄ treatment in combination with PCₐ maintained a higher concentration of GA₄ at least for 4 weeks. The higher GA₄ concentration for a longer period in fruit treated with GA₃+₄+PCₐ may be caused by the activity of PCₐ that inhibits the conversion of applied bioactive GA₄ into inactive GA₃+₄. This long-lasting activity of PCₐ may explain this larger effect on mature fruit size.

In addition, our data may also demonstrate that reduction of GA₄ by 2β-hydroxylase in fruit flesh could happen when GA₃+₄ is present in flesh at a certain amount or more without PCₐ because i) GA₄ was detected after GA₃+₄ application, albeit only transiently (1 WAT), and ii) the GA₃+₄ concentration was higher in GA₃+₄ treated fruit than in GA₃+₄+PCₐ treated fruit where the GA₄ concentration was greater in the latter fruits than the former ones.

On the other hand, when GA₃+₄ was applied, the GA₄ concentration in fruit flesh did not statistically change, but the GA₁ and GA₃ concentrations increased. The commercial GA₃+₄ paste contains very small amounts of GA₁ (and GA₃) as sub-products of GA₃ and GA₄ (Kyowa Hakko Bio Co. Ltd, personal communication), and thus these increases in GA₁ and GA₃ originated from the applied commercial GA₃+₄ paste. The contrast in GA₁ (increased level) and GA₄ (constant level) after GA₃+₄ application shows that 2β-hydroxylation (GA degradation) may not be the sole pathway for maintaining GAs homeostasis because i) 2β-hydroxylase can equally convert both GA₁ and GA₄ into their respective inactivated forms, GA₅ and GA₃+₄, and ii) the GA₄ concentration in fruit flesh did not statistically change either with or without PCₐ treatment despite GA₃+₄ application. Thus, it is possible that GA₃+₄ application may retard GA₄ biosynthesis, possibly in the step for 3β-hydroxylation or an earlier point in the pathway, by which the GA₄ concentration was lowered. Indeed, previous reports indicated that exogenous application of bioactive GA down-regulates the expression of genes encoding GA biosynthetic enzymes concomitant with up-regulation of genes encoding GA deactivation enzymes (Ayele et al., 2006; Itai et al., 2008; Thomas et al., 1999), observations that also support our idea. We assume that homeostasis of GA may be more strongly driven by bioactive GA₄ than inactive GA₁ and GA₃, but this hypothesis should be investigated in the future.

In contrast to the effect on ‘Kosui’, the GA₃+₄ mixture is effective for enlarging the fruit of another Japanese pear cultivar, ‘Hosui’, and treatments with a combination of GA₃+₄ with PCₐ had additional positive effects (Itai et al., 2008). As mentioned above, the functional (active) GAs for enlarging Japanese pear fruit are GA₄ and GA₅, whereas GA₃ is much less effective. Therefore, the positive effect of GA₃+₄ paste on fruit enlargement, if any, is attributable to the minor component GA₄ (approx. 1/10 in 2.7% GA₃+₄), but not to the major component GA₅ (9/10 of 2.7% GA₃+₄). First, we hypothesized that 0.27% GA₄ in 2.7% GA₃+₄ paste (A₅:A₄ = 90:10) was insufficient to promote increases in ‘Kosui’ fruit weight, but was sufficient for ‘Hosui’.

To re-confirm the effective GA₄ concentration promoting fruit size, we investigated whether a similar concentration of GA₃+₄ in 2.7% GA₃+₄ paste, i.e., 0.27% GA₃+₄, would have a positive effect on fruit size in combination with PCₐ for fruit enlargement of ‘Kosui’. However, contrary to our expectation, treatments using 0.27% GA₃+₄ with PCₐ significantly increased ‘Kosui’ fruit size (Table 2). This result shows that the GA₄ concentration in GA₃+₄ paste (= 0.27%) is, when applied with PCₐ, sufficient to enlarge the fruit of ‘Kosui’. The promotive effects of the GA₃+₄ mixture were accompanied by an increase in the GA₄ concentration in ‘Hosui’ fruit (2008), but not in ‘Kosui’ flesh in this experiment. These results re-confirmed that the GA₄ concentration retained in fruit is positively correlated with fruit size at harvest both in ‘Kosui’ and ‘Hosui’. We hypothesize that ‘Kosui’ may control the GA₄ concentration more finely than ‘Hosui’; thereby, excess GA₄ in ‘Kosui’ fruit flesh is quickly degraded, whereas the same amount of GA₄ in ‘Hosui’ promotes fruit growth without reducing the GA₄ amount in fruit. In addition, ‘Kosui’ responded to 0.27% GA₃+₄+PCₐ treatment with an increased fruit size, but did not respond to the same
amount of GA$_4$ in GA$_{3+4}$ paste. The reason(s) for this different response is not yet known; however, components may coexist in GA$_{3+4}$ that might influence the effectiveness of applied GA$_{4+7}$.

In conclusion, GA$_{4+7}$ application enhanced fruit size and maturation in the ‘Kosui’ Japanese pear, and the combination of GA$_{4+7}$ with PCa yielded an additional improvement in fruit size; however, the GA$_{3+4}$ mixture which is registered as an agricultural chemical in Japan did not affect fruit size or maturation either when applied alone or in combination with PCa in ‘Kosui’. GA$_{4+7}$ application increased the GA$_4$ concentration by 1.8 times that of the control at 1 WAT in fruit flesh, although the increase was transient, and the effect of GA$_{4+7}$ in combination with PCa increased the GA$_4$ concentration by 2.2 times that of the control at 1 WAT and for a longer period. GA$_{3+4}$ treatment did not affect the GA$_4$ concentration in fruit flesh. A positive relationship was found between fruit size at harvest and GA$_4$ concentration in the fruit flesh at 1 WAT. ‘Kosui’ may more strictly regulate the GA concentration in fruit flesh, and the exogenously applied excess amount of GA may be more quickly removed. GA$_{4+7}$ may retain the GA$_4$ concentration in fruit flesh more efficiently in applications combined with PCa, thereby supporting the effect of GA$_4$ in enhancing fruit size and maturation.

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Literature Cited


