Effect of 9-Hydroxy-10-oxo-12(Z), 15(Z)-octadecadienoic Acid (KODA) on Endodormancy Breaking in Flower Buds of Japanese Pear

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Abstract. The effect of 9-hydroxy-10-oxo-12(Z), 15(Z)-octadecadienoic acid (KODA) on endodormancy breaking was studied in flower buds of Japanese pear (*Pyrus pyrifolia* Nakai). The optimal concentration of KODA (100 μ M) for endodormancy breaking was established over a 2-year period during the endodormancy stage of 2006 and 2007 in Japanese pear cultivars Kosui, Natsushizuku, and Hosui. The effect of KODA on endodormancy breaking in flower buds was similar between 'Natsushizuku' and 'Kosui' but somewhat lower in 'Hosui'. These results indicate that KODA can be an effective agent for promoting endodormancy breaking of flower buds in 'Natsushizuku' and 'Kosui'. Although not as effective as hydrogen cyanamide, KODA may be preferable at late endodormancy stages because it has no apparent phytotoxicity.

In woody plants, the ability of a plant or plant tissue to enter the quiescent physiological state known as endodormancy is an important adaptive strategy for surviving severe winter freezes (Lang, 1987). However, recent winter temperature aberrations, which have been associated with global warming conditions, have given new impetus to the study of endodormancy breaking and particularly to dysfunctional breaking, which may be the result of changes in climate. Low temperature requirements for the development of complete endodormancy in Japanese pear are now often not met in southwestern regions of Japan, especially in regions where forcing culture under heated plastic-house conditions is used. Thus, dysfunctional endodormancy breaking has become an obstacle to stable economic production of Japanese pear. In these regions, artificial cooling to compensate for insufficient

natural chilling (Erez, 1995; Shulman et al., 1983) is now required to maintain commercial Japanese pear production.

A number of chemical and physical treatments can be used to overcome endodormancy, including mineral oils (Chandler et al., 1937), calcium cyanamide (Iwasaki, 1980; Kuroi et al., 1963; Shulman et al., 1983), hydrogen cyanamide (HC) (Nir and Lavee, 1993; Shulman et al., 1983; Siller-Cepeda et al., 1992), thiourea (Shulman et al., 1983), diallyl sulfides in garlic (Kubota et al., 1999), high temperatures (Orffer and Goussard, 1980; Tamura et al., 1993), freezing (Olmsted, 1951; Sparks et al., 1976), mechanical injury (Iwasaki, 1980; Paiva and Robitaille, 1978; Shulman et al., 1983), electric current (Kurooka et al., 1990), anaerobic conditions (Erez et al., 1980; Tamura et al., 1993), and hydrogen peroxide (Kuroda et al., 2005). However, some treatments such as mineral oils or cyanamide can sometimes cause negative effects not only to fruit trees, but also to humans. Other chemicals, which may have some endodormancy breaking effect, are not potent enough to compensate for the lack of chilling. Therefore, the identification of effective alternative chemicals or treatments that have robust and reproducible effects on endodormancy breaking with less toxicity for plants and mammals would be a great benefit to the fruit industry.

α -Ketol linolenic acid [KODA; 9-hydroxy-10-oxo-12(Z), 15(Z)-octadecadienoic acid] is a signal compound expressed in *Lemna paucicostata* (Duckweed) after exposure to drought, heat, or osmotic stresses (Yamaguchi et al., 2001; Yokoyama et al., 2000). KODA is an oxylipin, a common compound in green plants (Vick and Zimmerman, 1987). Oxylipins are bioactive lipids derived by oxygenation of polyunsaturated fatty acids (Lee et al., 2008). KODA is synthesized from linolenic acid (C18:3) by a 9-specific lipoxygenase (Howe and Schillmiller, 2002) (Fig. 1). In addition, applications of 10 or 100 μ M KODA during paradormancy promote bud breaking in strawberry flower buds (*Fragaria $\times$ ananassa* Duchesne) (Yokoyama, personal communication), suggesting that KODA may also break endodormancy in deciduous fruit trees, including Japanese pear.

Materials and Methods

Plant materials. Mature Japanese pear trees grown at the National Institute of Fruit Tree Science (Tsukuba, Japan), located at long. 36°3' N and lat. 140°8' E, were used for all experiments. We selected three to five current 1-year-old shoots with six to nine flower buds on shoots between 60 and 80 cm in length per treatment. All mature Japanese pear trees were managed according to the ordinary cultural practices used in Tsukuba (Ibaraki Prefecture).

Effect of KODA on endodormancy breaking in 'Kosui' flower buds. Current shoots with flower buds in the endodormant stage (Dec. 2006) were cut from mature Japanese pear trees (*Pyrus pyrifolia* Nakai, 'Kosui') at the National Institute of Fruit Tree Science (Tsukuba, Japan). Branches were sprayed to runoff with distilled water (control), 10, 100, or 1000 μ M KODA, which was provided by Shiseido Co., Yokohama, Japan. After treatment, cut ends of the branches were placed in distilled water in a phytotron at 25 $\pm$ 1.0 °C. Water in the 500-mL vials was changed at 2- to 3-d intervals. The percentage of flower bud breaking, which is indicative of endodormancy breaking, was determined at 2- to 4-d intervals. Flower bud breaking was defined as the stage at which green tissue is visible. The bud breaking percentage was first calculated on each branch with six to nine flower buds. Then, summed up percentage from each branch divided by the number of branch (three to five branches) gave the average bud breaking percentage. Seasonal effects on endodormancy breaking were determined by treating mid to late endodormancy. Branches were cut and sprayed on 7 Dec. 2006, 15 Oct., 30 Oct., 15 Nov., 22 Nov., 29 Nov., and 6 Dec., 2007, and treated as stated previously, but without a 1000- μ M dose.

Comparison of KODA on three Japanese pear cultivars. Current shoots from 'Hosui' and 'Natsushizuku' mature trees were cut and treated as stated previously with distilled water or 100 μ M KODA on 29 Oct., 11 Nov., 26 Nov., and 3 Dec. 2008 during endodormancy. 'Kosui', which was sampled on the

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Fig. 1. Proposed biosynthesis pathways for KODA and jasmonic acid.

same date, served as a longitudinal control to confirm the reproducibility of the experiments conducted in 2006 and 2007.

Comparison of KODA and hydrogen cyanamide on endodormancy breaking of flower buds. KODA was compared with HC, which is registered in Japan as a dormancy breaking agent of Japanese pear. Current shoots from 'Kosui' trees were cut on 29 Oct. and 27 Nov. 2009 and treated as stated previously with distilled water (control), 100 μ M KODA, or 1% (w/v) (0.24 M) HC (registered as CX-10; Nippon Carbide Industries Co., Inc., Minato-ku, Japan).

Statistical analysis. Data in 2006 were done with the Kruskal-Wallis test and Scheffé's test. Data in 2007, 2008, and 2009 were analyzed for significant differences by analysis of variance and least significant difference values were calculated for comparison of means.

Results and Discussion

Generally, endodormancy of flower buds of Japanese pear is deepest around the middle to end of October under natural conditions, gradually releasing as manifest by an increase in bud breaking (Fig. 2). We investigated the effect of KODA on endodormancy breaking in 'Kosui' in 2006 and 2007. In 2006, 100 and 1000 μ M of KODA promoted significant endodormancy breaking. KODA treatment at 10 μ M also tended to promote breaking (Table 1). Based on 2006 results, in 2007, we evaluated treatments at different intensities of endodormancy to determine the most effective timing for KODA application.

Fig. 2. Seasonal changes of endodormancy status in flower buds of 'Kosui' Japanese pear in 2007 to 2008 (A) and 2008 to 2009 (B). Vertical bars are the SE (n = 3–5).

Endodormancy breaking occurred earlier in flower buds treated with 100 μ M KODA on 15 Oct., 30 Oct., 15 Nov., and 22 Nov. than in controls (Fig. 3A–D). Toward the last stage of endodormancy (on 29 Nov. and 6 Dec.), breaking tended to occur earlier with 100 μ M KODA (Fig. 3E–F). Treatment with 10 μ M KODA on 15 Oct. and 30 Oct. resulted in breaking flower bud endodormancy before the controls (Fig. 3A–B). Treatment on 29 Nov. and 6 Dec. with 10 μ M KODA resulted in breaking on the same date as controls, but 100% of endodormancy breaking was attained earlier with KODA treatment (Fig. 3E–F). There was no observable difference in the final percentages of endodormancy breaking between 10 μ M KODA treatment and control

Table 1. Effect of KODA on endodormancy breaking in flower buds 'Kosui' Japanese pear (2006).

Treatment	Bud breaking (%) ^z
KODA 10 μ M	78.9 $\pm$ 10.0 ab ^y
KODA 100 μ M	98.0 $\pm$ 2.0 a
KODA 1000 μ M	100.0 a
Control	30.4 $\pm$ 18.1 b

^zPercentage of flower bud breaking 21d posttreatment.

^yThe data are presented as mean $\pm$ SE (n = 3–5). Different letters within a column indicate significant differences by the Scheffé's test.

with branches cut and treated on 15 Nov. or 22 Nov. (Fig. 3C–D), but the reason for this is not yet known. Collectively, 100 μ M was a more effective dose than 10 μ M KODA for

Fig. 3. Effect of concentration of KODA on endodormancy breaking in flower buds of 'Kosui' Japanese pear (2007). Treatment on 15 Oct. (A), 30 Oct. (B), 15 Nov. (C), 22 Nov. (D), 29 Nov. (E), and 6 Dec. (F). Control ($\diamond$), KODA 10 μ M (Δ), KODA 100 μ M ($\blacksquare$). Values are means $\pm$ SE ($n = 3$). Vertical bar indicate least significant difference ($P = 0.05$). NS, *, **Non-significant or significant at $P < 0.05$ or 0.01, respectively, by analysis of variance.

breaking endodormancy of flower buds among the concentrations tested.

One effect of seasonal timing on endodormancy breaking of 100 μ M KODA-treated flower buds was that breaking of treated buds preceded breaking of the controls on 29 Oct., 11 Nov., and 26 Nov. (Fig. 4A–C) with the percentage of treated buds increasing more rapidly than the control, including the treatment on 3 Dec. (Fig. 4). This result was in agreement with the results from 2007. 'Natsushizuku' breaking tended to be the same as 'Kosui' (Fig. 5A–C), but 'Hosui' breaking was not advanced with 100 μ M KODA on any date (Fig. 6). However, the final percentage in 100 μ M KODA-treated flower buds was higher than in controls (Fig. 6A–B and D), except for the treatment on 26 Nov. (Fig. 6C). Thus, the effect of KODA, at

least with the timing used in these experiments, was lower in 'Hosui' compared with 'Kosui' and 'Natsushizuku' (Fig. 6). Asano and Okuno (1990) reported that the period required for endodormancy breaking corresponds to the chilling requirements, which was shorter in 'Hosui' than in 'Kosui' after cold treatment. 'Kosui' and 'Natsushizuku' have about the same breaking period (Sudo et al., 2009). 'Hosui' thus requires the least time at low temperature to break endodormancy among the three cultivars. This trait is likely to be related to the diminished effect of KODA on endodormancy breaking in 'Hosui'. No phytotoxicity symptoms were observed during the course of these experiments.

HC is one of the most effective endodormancy breaking agents (Nir and Lavee, 1993; Shulman et al., 1983; Siller-Cepeda et al.,

1992). KODA and HC-treated flower buds broke endodormancy before the control treatment on both 29 Oct. and 27 Nov. (Fig. 7A–B), but KODA was generally inferior to HC for both timing and extent of bud break. However, the differences between HC and KODA decreased with the approach of normal endodormancy breaking (Fig. 7). In addition to the effect of advanced bud breaking, Bound and Jones (2004) reported that the flowering period from pink bud to full bloom in 'Fuji' apple was compressed by the HC application 40 d before the estimated bud breaking, whereas HC application did not always result in the compression of the flowering period, especially under higher temperature conditions. In this study, although both treatments advanced bud breaking, no apparent compression of flowering period (from bud breaking to full bloom) was observed by both KODA and HC treatments (data not shown), which may be the result of to high temperature (25 ± 1.0 °C) condition under phytotron as suggested by Bound and Jones (2004). The phytotoxicity of HC is well known and is dependent on concentration and application date (Kuroda et al., 2002; Siller-Cepeda et al., 1992). Therefore, with refinement of application schedules and doses, KODA could be the preferable treatment for endodormancy breaking for Japanese pear flower buds at the late endodormancy stage. However, it should be noted that this work was done on branches cut from the trees in the orchard; therefore, it could be required to examine the effect of KODA on bud breaking using the potted trees.

It remains to be demonstrated how KODA breaks endodormancy in Japanese pear. Here, it is worthy to note that KODA is synthesized from linolenic acid by a 9-specific lipoxygenase as opposed to the jasmonic acid pathway (Fig. 1). Therefore, when we considered the mechanism underlying the potent ability for endodormancy breaking by KODA in terms of lipid metabolism, that is, major changes in the phospholipid contents, a large decrease in linoleic acid (C18:2) concomitant with an increase in linolenic acid in 'Delicious' apple buds was observed when the chilling requirement is satisfied (Wang and Faust, 1990). Gemma et al. (1993) also reported that lipid metabolism in the buds of Japanese pear 'Kosui' was greatly accelerated during dormancy, resulting in an increase in phospholipids, especially linolenic acid. These reports suggest that the accumulation of linolenic acid is an important factor in breaking endodormancy. Indeed, linolenic acid itself may have promoted endodormancy breaking in our preliminary experiment (data not shown). Also, Taniguchi et al. (2003) reported that the breaking of dormancy in apical and lateral buds in horse chestnut (*Aesculus turbinata*) seedlings was promoted by treatment with jasmonic acid. Taking into consideration these results, accumulation of either linolenic acid or its oxylipin metabolites, including KODA and jasmonic acid, may be involved in endodormancy breaking. Further studies will be necessary to clarify the detailed physiological functions of linolenic acid, its

Fig. 4. Effect of KODA on endodormancy breaking in flower buds of 'Kosui' Japanese pear (2008). Treatment on 29 Oct. (A), 11 Nov. (B), 26 Nov. (C), and 3 Dec. (D). Control ($\diamond$), KODA 100 μ M ($\blacksquare$). Values are means $\pm$ SE (n = 3–5). Vertical bar indicate least significant difference ($P = 0.05$). ns, *, **Nonsignificant or significant at $P < 0.05$ or 0.01, respectively, by analysis of variance.

Fig. 5. Effect of KODA on endodormancy breaking in flower buds of 'Natususizuku' Japanese pear (2008). Treatment on 29 Oct. (A), 11 Nov. (B), 26 Nov. (C), and 3 Dec. (D). Control ($\diamond$), KODA 100 μ M ($\blacksquare$). Values are means $\pm$ SE (n = 3–5). Vertical bar indicate least significant difference ($P = 0.05$). ns, *, **Nonsignificant or significant at $P < 0.05$ or 0.01, respectively, by analysis of variance.

metabolites, or related compounds, like jasmonic acid for endodormancy breaking in Japanese pear.

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Fig. 6. Effect of KODA on endodormancy breaking in flower buds of 'Hosui' Japanese pear (2008). Treatment on 29 Oct. (A), 11 Nov. (B), 26 Nov. (C), and 3 Dec. (D). Control ($\diamond$), KODA 100 μ M ($\blacksquare$). Values are means $\pm$ SE ($n = 3-5$). Vertical bar indicate least significant difference ($P = 0.05$). NS, *, **Nonsignificant or significant at $P < 0.05$ or 0.01 , respectively, by analysis of variance.

Fig. 7. Effect of KODA or hydrogen cyanamide (HC) on endodormancy breaking in flower buds of 'Kosui' Japanese pear (2009). Treatment on 29 Oct. (A) and 27 Nov. (B). Control ($\diamond$), KODA 100 μ M ($\blacksquare$), HC 1% (w/v) ($\triangle$). Values are means $\pm$ SE ($n = 5$). Vertical bar indicate least significant difference ($P = 0.05$). NS, *, **Nonsignificant or significant at $P < 0.05$ or 0.01 , respectively, by analysis of variance.

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