Application of Livestock Waste Compost as a Source of Nitrogen Supplementation during the Fall-winter Season Causes Dead Flower Buds in Japanese Pear ‘Kosui’

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Dead flower buds were frequently observed in the Japanese pear cultivar ‘Kosui’ (Pyrus pyrifolia) when trees were cultured under open field conditions in the southwestern regions of Japan. To elucidate the causes of dead flower buds, we studied the effect of nitrogen (N) supplements using two experimental designs that included trees grown in pots and in the field. First, we investigated the effect of applying chemical N fertilizer during the winter using potted trees (controlled temperature experiment). We also investigated the effect of applying livestock waste compost during the fall-winter months on flower bud freezing tolerance and on the N and sugar contents of flower buds from trees cultivated in the field. In controlled temperature conditions, the percentage of dead flower buds significantly increased when N fertilizer was applied in December and January. In field conditions, compost application in the fall-winter months significantly reduced the freezing tolerance of flower buds concomitant with a significant increase in the percentage of dead flower buds compared to compost application in the spring. Application of compost in the fall-winter months resulted in a significantly higher N content compared to that in spring. In contrast, the relationship between the sugar content and freezing tolerance of flower buds is unclear. This potential connection remains to be elucidated in the near future. Collectively, these results suggest that compost application during the fall-winter season can adversely affect freezing tolerance through an increase in the N content, thus promoting dead flower buds.

Key Words: freezing injury, Japanese pear, N content.

Introduction

The Japanese pear is widely cultivated from the northern (Hokkaido region) to the southern (Kyushu region) regions of Japan. Japanese pear ‘Kosui’ trees in the southwestern regions of Japan have recently been observed to have dead flower buds in the spring. Application of nitrogen (N) fertilizers in the late fall season reduces cold hardiness and enhances the frequency of freezing injury in apples (Kuroda et al., 1984). In addition, livestock waste compost (compost) as a N supplement reduces the freezing tolerance of chestnut trees (Sakamoto et al., 2015b). Nevertheless, it is customary for pear farmers to N-fertilize their orchards during the fall/winter season with compost in addition to chemical fertilizers (Ishizuka, 1984). Before 1999, compost was traditionally stored in the open air, leading to losses in the N content through leaching; however, in 1999 a Japanese law that prohibited the open-air storage of compost was enacted. This new law led to an increase in the total N content of compost of more than 1% compared with that before the law was enacted (Fujita, 2014). The total N content of the compost used in this study was approximately 2.0%. Given that this phenomenon tends to happen in years with a warmer fall-winter season and that excessive N application in the fall reduces the freezing tolerance of pears (Matsumoto et al., 2010), we hypothesized that the occurrence of dead flower buds was related to the timing of N

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application using compost. In this study, we fertilized Japanese pear (‘Kosui’) trees with compost in October or December and evaluated the freezing tolerance, N, and sugar contents of trees to determine if these parameters influenced the incidence of dead flower buds.

**Materials and Methods**

**Plant materials**

For the controlled temperature experiment, 12 three or four-year-old potted trees (25 L in 2012/2013 and 20 L in 2013/2014) of the Japanese pear cultivar ‘Kosui’, growing in an experimental orchard at the NARO Institute of Fruit Tree Science (Tsukuba, Japan), located at 36°3′N and 140°8′E, were used. The experiment was conducted during the winter seasons of 2012/2013 and 2013/2014. In both growing seasons, the warm fall and winter field conditions (from mid-November to mid-March) similar to those recorded in Satsumasendai, Kagoshima, where the incidence of dead flower buds was observed in 2008/2009, were simulated using greenhouses equipped with heat pump systems (SPW-AGP180E; E’s Inc., Tokyo, Japan). Specifically, maximum air temperatures were set to 15°C (from 0400 to 1600), and the average air temperature was set to 13°C (from 1600 to 1800) and 7°C (from 1800 to 0400). Air temperatures calculated from a 5-day moving average during the experiments in 2012/2013 and 2013/2014 simulated temperatures in Satsumasendai in 2008/2009 (Fig. 1).

For the field experiment, 9 mature Japanese pear ‘Kosui’ trees, growing in the experimental orchard at the Kagoshima Prefectural Institute for Agricultural Development, Hokusatsu Branch (Satsumasendai, Japan), located at 31°5′N and 130°2′E, were used. The experiment was conducted during the three fall-winter seasons of 2012/2013, 2013/2014, and 2014/2015. To analyze changes in freezing tolerance and the N and sugar contents of flower buds, we sampled three uniform, current season’s shoots (CSSs, 60–120 cm in length) with 5–10 flower buds per each CSS from October to March at about 2–4 week intervals, and then used the flower buds to analyze the parameters indicated below. As for fertilizer management before the experiments (2011), mature trees were managed according to the ordinary cultural practices used in Japanese pear orchards in Satsumasendai. Briefly, mixed organic (40%)-inorganic (60%) fertilizer (mikan-aki4gou; JA Butsuryu, Kagoshima, Japan) was applied in September (20%, 3.6 kgN/10a), October (50%, 9 kgN/10a), and November (30%, 5.4 kgN/10a) and compost (2 t /10a) was applied in December.

**Fertilizer treatment**

For the controlled temperature experiment, we used three potted trees per treatment, in which 2 g of readily available chemical fertilizer (ammonium sulfate; Mitsubishi Chemical Co., Tokyo, Japan) was applied to each pot at three different times in mid-December, mid-January, and mid-February. One set of pots was used as a control and did not receive any fertilizer. The potted trees were first kept in an open field and then transferred to a greenhouse equipped with a heat pump system (SPW-AGP180E; E’s Inc.) on 13 November (2012) and were returned to an open field on 8 March (2013). Similarly, for the 2013/2014 season, the pots were transferred to a greenhouse on 20 November (2013) and were returned to an open field on 10 March (2014).

For the field experiment, we used nine mature trees (three mature trees per treatment) and applied 18 kgN/10a of mixed organic-inorganic fertilizer and 2 t/10a of compost that was purchased from Japan Agricultural Cooperatives (JA Kitasatsuma) in Satsuma-cho, Kagoshima every year according to the following experimental design that is summarized in Table 1. Mixed organic-inorganic fertilizer (18 kgN/10a) was applied in March in all experiments. In 2012/2013 and 2014/2015, compost was applied in March (“Compost in spring”) or December (“Compost in winter”). In 2013/2014, compost was applied in March (“Compost in spring”) or October (“Compost in winter”).
The total N concentration of compost used in this study was approximately 2.0%.

**Status of flower buds at flowering**

The number of dead flower buds in the controlled temperature experiment was also investigated. During flowering, 6–33 flower buds (apical flower buds of the spur) on each of the three potted pear trees were examined. Similarly, 72–208 flower buds from each of the three trees in each treatment were randomly chosen during flowering (three biological replications each having 72–208 flower buds), and the number of dead apical flower buds on spurs and axillary flower buds of the CSSs were counted separately. The data were transformed using an arcsine transformation and then analyzed by a two-way ANOVA.

**Freezing tolerance of flower buds in the field experiment**

Freezing tolerance experiments using CSSs were conducted from November to March at about 4-week intervals. Fifteen CSSs from three mature trees for each treatment were randomly chosen and used to evaluate the freezing tolerance. Freezing tolerance was evaluated using an environmental test chamber (SU-642; ESPEC Corp., Osaka, Japan). Three CSSs with 15–26 flower buds were collected from each treatment, wrapped in polyethylene bags and precooled for 3 h at 0°C. After leaving the CSSs at selected temperatures, i.e., −5, −8, −12, −16, or −20°C, for 16 h, the samples were transferred to 0°C for 3 h and then 5°C for 5 h. To evaluate the viability of flower buds after treatment, CSSs were placed in plastic containers with rockwool cubes submerged in distilled water in a chamber (MLR-351; SANYO Electric Co. Ltd., Osaka, Japan) set to 20°C under cycling conditions, 12 h/12 h (light/dark), for two weeks. Thereafter, injury due to freezing was evaluated visually by monitoring the browning of floral primordia and the number of buds that failed to sprout. Freezing tolerance was defined as the temperature at which half of the flower buds were dead and was expressed as the lethal temperature 50 ($LT_{50}$) using the Spearman-Kärber method that was previously used to evaluate blueberry flower buds and Japanese pear (Bittenbender and Howell, 1974; Honjo and Omura, 1987). Calculation of the $LT_{50}$ for a treatment is 1 replication. Data were analyzed $t$-test.

**Flower bud total N and sugar contents in the field experiment**

Total N and sugar contents were analyzed in the flower buds. Five to ten flower buds per tree were randomly chosen from three mature trees and were collected at about 2- or 3-week intervals from late-November. The flower buds including scales were dried (24 h at 80°C) using a constant temperature oven (DK600; Yamato Scientific Co. Ltd., Tokyo, Japan), and the total N content was analyzed using an NC analyzer (SUMIGRAPH; NC-220F, Sumika Chemical Analysis Service Ltd., Tokyo, Japan). Dried flower buds from each tree were also used for extraction and analyses of soluble sugars (sorbitol, sucrose, fructose, and glucose) as described by Ito et al. (2012). Data were analyzed by $t$-test.
Results

Status of flower buds at flowering

In the controlled temperature experiment (Table 2), the percentage of dead flower buds was significantly higher in the December or January treatments compared with the no fertilizer control. There were no significant differences in the percentage of dead flower buds between the February treatment and the no fertilizer application control, but the February treatments had higher numbers of dead flowers than the no fertilizer control in both years studied. In the field experiment (Table 3), the average percentage of dead flower buds increased with compost application in the fall and winter (“Compost in winter” and “Compost in fall”) compared with compost application in the spring (“Compost in spring”) for all types of flower buds (apical flower buds of spurs and axillary flower buds of the CSSs). Furthermore, when these data were analyzed by a two-way ANOVA, compost application in the fall and winter significantly increased the percentage of dead flower buds in all three experiments compared with the “Compost in spring” treatment. In addition, the percentage of dead flower buds was significantly higher in axillary flower buds of the CSSs than in apical flower buds of spurs in 2013 and 2015, but there were no significant differences in 2014 (Table 3).

Freezing tolerance of flower buds in the field experiment

The effect of the timing for compost application on freezing tolerance is shown in Table 4 and in Figure 2. Freezing tolerance was assessed by measuring the LT$_{50}$, defined as the temperature at which half of the flower buds were dead. The “Compost in winter” treatment had a significantly higher LT$_{50}$ than that in the “Compost in spring” treatment during the experimental periods (Table 4; Fig. 2). Similarly, the “Compost in spring” treatment had a significantly higher freezing tolerance than the “Compost in fall” treatment during the experimental periods (Table 4; Fig. 2). Freezing tolerance of flower buds in the “Compost in winter” treatment was lower than that of the “Compost in spring” treatment in mid-December and mid-January. Similar

### Table 2. Effect of the timing of nitrogen fertilizer application on the occurrence of dead flower buds (%) in the controlled temperature experiment.

| Year          | Treatment (Abbreviation)                      | Dead flower bud (%) |
|---------------|----------------------------------------------|---------------------|
| 2012–2013     | No fertilizer application (Cont.)            | 24.7 by             |
|               | Chemical fertilizer applied in mid-December  | 86.7 a              |
|               | Chemical fertilizer in mid-January (January) | 86.2 a              |
|               | Chemical fertilizer in mid-February (February)| 43.7 ab             |
| 2013–2014     | No fertilizer application (Cont.)            | 27.4 b              |
|               | Chemical fertilizer applied in mid-December  | 100 a               |
|               | Chemical fertilizer applied in mid-January   | 100 a               |
|               | Chemical fertilizer applied in mid-February  | 78.9 ab             |

* The status of the buds was investigated at flowering.
** Different letters within a column indicate significant differences by the Tukey-Kramer Test.

### Table 3. Effect of N supplementation from livestock waste compost during fall-winter on the occurrence of dead flower buds in the field experiment.

| Year          | Treatment (Abbreviation)                      | Dead flower bud (%) for each type of flower buds | Significance |
|---------------|----------------------------------------------|--------------------------------------------------|--------------|
|               |                                              | Apical flower buds on spurs | Axillary flower buds of the CSSs | Treatment | Types of flower buds | Interaction |
| 2012–2013     | Fertilizer (0.8 kg in Mar.) and compost in spring | 0.0 | 2.1 | * | ** | NS |
|               | Fertilizer (0.8 kg in Mar.) and compost in winter | 2.9 | 14.1 |     |     |             |
| 2014–2015     | Fertilizer (0.8 kg in Mar.) and compost in spring | 0.6 | 3.5 | * | ** | NS |
|               | Fertilizer (0.8 kg in Mar.) and compost in winter | 2.8 | 4.3 |     |     |             |
| 2013–2014     | Fertilizer (0.8 kg in Mar.) and compost in spring | 0.3 | 2.1 | ** |   | NS |
|               | Fertilizer (0.8 kg in Mar.) and compost in fall  | 8.1 | 6.6 |   |     |             |

* 72–208 flower buds from each tree were randomly chosen during flowering, and the status of the buds was investigated at flowering.
** NS, *, and ** indicate not significant, or significant differences at $P<0.05$, and 0.01, by a two-way ANOVA (n=3).
x Mixed organic-inorganic fertilizer.
trends were observed in the “Compost in fall” treatment, in which the freezing tolerance was lower than that of the “Compost in spring” treatment in mid-February (Fig. 2).

**Flower bud total N and sugar content in the field experiment**

The effect of the timing for compost application on the total N content of flower buds is shown in Table 4. Total N was significantly higher for the compost treatments in fall and winter over all three seasons. In addition, differences in the freezing tolerance (°C) between the “Compost in spring” and “Compost in winter” treatments or between the “Compost in spring” and “Compost in fall” treatments per each sampling date in three years were significantly and positively correlated with the differences in the N concentration (%) between the “Compost in spring” and “Compost in winter” treatments or between the “Compost in spring” and “Compost in fall” treatments per month for each sample (Fig. 3).

There were significant differences in the total sugar content between the “Compost in spring” treatment and the “Compost in fall” treatment, but there were no significant differences between the “Compost in spring” treatment and the “Compost in winter” treatment (Table 4).

**Discussion**

In controlled temperature conditions, the percentages of dead flower buds significantly increased when fertilizer was applied in winter (December and January) (Table 2). In field conditions, application of compost in the fall-winter months reduced the flower buds’ freezing tolerance concomitant with a significant increase in the percentage of dead flower buds (Tables 3 and 4). Thus, we obtained positive correlations between i) an increase in the number of dead flower buds and low freezing tolerance and ii) low freezing tolerance and N application in the fall-winter period.

Orimoto and Ishitsuka (1989) reported that excessive N chemical fertilizer application caused an increase in

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**Table 4.** Effect of the N supplementation from livestock waste compost during fall-winter months on the average freezing tolerance, N concentration, and total sugar content of flower buds during the fall-winter months in the field experiment.

| Year     | Treatment Description          | Abbreviation       | Freezing tolerance (°C) | Total N concentration (mg·g⁻¹ DW) | Total sugar content (mg·g⁻¹ DW) |
|----------|--------------------------------|--------------------|-------------------------|-------------------------------|-------------------------------|
| 2012–2013 | Fertilizer (0.8 kg in Mar.) and compost in spring | Compost in spring | −10.2 | *         | 8.8 | * | 50.3 | NS |
|          | Fertilizer (0.8 kg in Mar.) and compost in winter | Compost in winter | −7.9 | x         | 9.0 | x | 49.4 | NS |
| 2014–2015 | Fertilizer (0.8 kg in Mar.) and compost in spring | Compost in spring | −9.3 | **        | 9.7 | * | 37.8 | NS |
|          | Fertilizer (0.8 kg in Mar.) and compost in winter | Compost in winter | −6.8 | **        | 9.9 | * | 38.2 | NS |
| 2013–2014 | Fertilizer (0.8 kg in Mar.) and compost in spring | Compost in spring | −10.1 | **        | 8.8 | * | 49.7 | * |
|          | Fertilizer (0.8 kg in Mar.) and compost in fall | Compost in fall | −8.5 | **        | 9.0 | * | 47.6 | * |

* The average freezing tolerance of flower buds shows the mean value from mid-November to mid-March, the average N concentration and total sugar content of flower buds shows the mean value from late-November to mid-March.

* Mixed organic-inorganic fertilizer.

* NS, *, and ** indicate not significant, or significant differences at P<0.05, and 0.01 as determined by a t-test.

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**Fig. 2.** The effect of the timing of livestock waste compost application on the freezing tolerance of ‘Kosui’ flower buds in 2012/2013 (A), 2013/2014 (B), and 2014/2015 (C). Compost in spring, mixed organic-inorganic fertilizer and compost in spring (×); Compost in winter, mixed organic-inorganic fertilizer in spring and compost in winter (△); Compost in fall, mixed organic-inorganic fertilizer in spring and compost in fall (◇). Freezing tolerance was defined as the temperature at which half of the flower buds were dead and was expressed as the LT₅₀.
dead buds of Japanese pear cultivars ‘Kosui’ and ‘Hosui’ in the following spring. In addition, application of N chemical fertilizers in the late-fall reduced cold hardiness by stimulating root activity and inversely increased the potential for freezing injury in apple buds (Kuroda et al., 1984). In our study, the N content of flower buds significantly increased after the fall-winter compost applications compared with compost application in the spring (Table 4). There is a possibility that excess N from compost application activated tree nutrient uptake in the winter, unlike the bud-sprouting period (March) that usually increases the level of nutrient uptake when trees experience warm winter conditions. Sakamoto et al. (2015b) reported that the depth of endodormancy was shallow in chestnut trees after excessive N from compost application activated tree nutrient uptake in the winter, as is the case for pear (Ito et al., 2013), apple (Kuroda et al., 1985) and chestnut trees (Sakamoto et al., 2015a). Thus, it is clear that there is a close relationship between sugar content and freezing tolerance. The basis for this observation remains to be elucidated in the near future.

Collectively, these results suggest that compost application during the fall-winter months can adversely affect freezing tolerance through an increase in the N content, thereby promoting dead flower buds.

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