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Inhibition of carbohydrate metabolism by thermal fluctuations during endodormancy lead to negative impacts on bud burst and incidence of floral necrosis in 'Housui' Japanese pear flower buds

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ABSTRACT

Challenges related to bud dormancy release are important limiting factors to commercial fruit production of Japanese pear (*Pyrus pyrifolia* Nakai) in areas under mild winter conditions. This study aimed to elucidate the effects of thermal fluctuations that occur in areas with mild winters on endodormancy release of Japanese pear flower buds. 'Housui' Japanese pear trees exposed to thermal fluctuations were left to accumulate 600 chilling hours (CH) in order to simulate mild winter conditions and subsequently moved into a greenhouse to observe bud burst and number of flowers/cluster. During endodormancy progression, the concentration of sugars, starch and α-amylase activity were analyzed in lateral flower buds and stems collected at 0, 300 and 600 CH. In addition, necrosis of floral primordia was examined in buds collected over the same period. Thermal fluctuations during endodormancy caused floral primordia necrosis at 300 CH and 600 CH and tended to decrease bud burst percentages. Low concentrations of soluble sugars were observed in buds and stems subjected to thermal fluctuations during endodormancy. Lower percentage of bud burst and incidence of floral primordia necrosis may be a result of low sucrose observed in buds. Hexoses and sorbitol that are important for energy and carbon supply as well freeze tolerance during endodormancy were lower in buds and stem exposed to thermal fluctuations. Thermal fluctuations inhibited the activity of α-amylase in buds and retarded the degradation of starch, leading to low accumulation of soluble sugars during endodormancy. These results suggest that carbohydrate metabolism is inhibited by thermal fluctuations during endodormancy under mild winter conditions, and that low availability of sufficient carbohydrate during winter may lead to floral primordia necrosis and abnormal patterns of endodormancy release in Japanese pear.

1. Introduction

Japanese pear (*Pyrus pyrifolia* Nakai) buds enter into dormancy to survive harsh conditions during winter. Bud dormancy is defined as the inability to initiate growth combined with the capacity to resume growth under optimum conditions (Rohde and Bhalerao, 2007). Temperature has been considered as the most significant factor involved in the processes of dormancy initiation and termination in deciduous fruit trees (Perez and Lira, 2005). In deciduous fruit trees, endodormancy is released when chilling requirements under low temperatures are satisfied (Saure, 1985). This chilling requirement varies based on the species and cultivars levels, for example 'Housui' Japanese pear was estimated to have lower chilling requirement than 'Shinsetsu' Japanese pear (Tamura et al., 2001). In other studies, 750 h chilling requirement at the range of 0–6 °C is estimated to release flower bud endodormancy in 'Kousui' Japanese pear (Sugiura and Honjo, 1997). However, they also reported that some temperatures negatively affected dormancy release. In apples, temperatures of 25 °C over long periods partially cancelled chilling effects during dormancy (Anzanello et al., 2014).

Pear (*Pyrus* spp.) is an important fruit produced in many regions of the world, and Japanese pear is one of the important fruits produced in Japan. The commercial production of Japanese pear has also been extended to sub-tropical areas under mild winter conditions. such as Brazil (Faora and Nakasu, 2002) and New Zealand (White, 2002). However, Japanese pear trees grown in areas under mild winter conditions have shown adaptation problems, such as reduced number of flowers per bud and floral abortion (Petri et al., 2002; Petri and Herter, 2002). Moreover, areas under mild winter conditions face thermal fluctuations with large temperatures swings during the winter period. These thermal fluctuations are a negative factor involved in bud dormancy release (Anzanello et al., 2014). Furthermore, climate change has negatively affected winter temperatures (Baldocchi and Wong,

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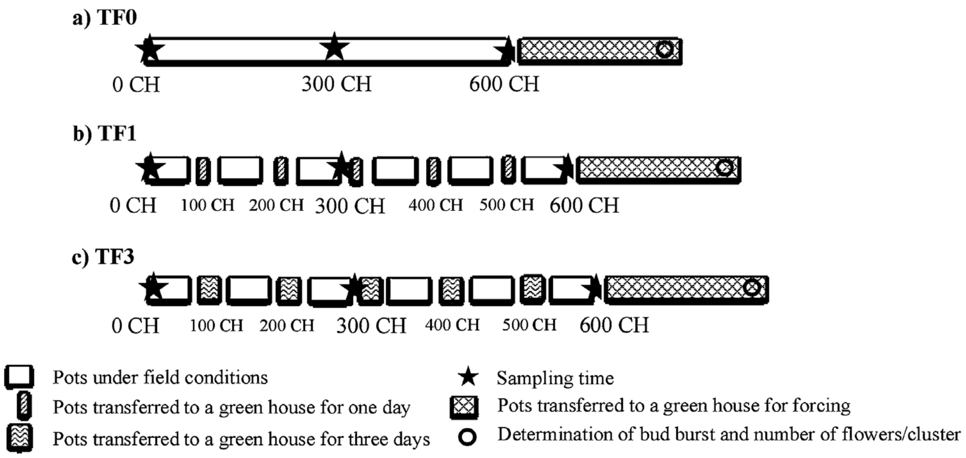

Fig. 1. Schematic model of the experimental treatments: (a) control trees that accumulated 600 chilling hours (CH) under field conditions without induced thermal fluctuations (TF0), (b) trees under field conditions that were transferred into a heated greenhouse to simulate thermal fluctuations for one day (TF1) or (c) three days (TF3) at every point when 100, 200, 300, 400 and 500 CH were progressively accumulated under field conditions.

2008). Global climate change has been greatly affecting fruit production areas due to insufficient chilling during winter period. Thus, it can be expected that in future climate change scenarios, the production of deciduous fruits, including Japanese pear, may be affected by higher temperatures and more thermal fluctuations during the winter period. Therefore, it is essential to study the effects of thermal fluctuations during the winter on endodormancy release, in order to minimize the problems related with dormancy under mild winter conditions.

Several studies have been conducted to elucidate the problems related with bud dormancy under mild winter conditions (Petri and Herter, 2002; Marafon et al., 2011; Yamamoto et al., 2010). However, only a few studies focused on the effects of thermal fluctuation on endodormancy. In apples, daily alternating temperature with high temperatures decreased bud break rates (Naor et al., 2003). In a more recent study, it has been reported that thermal fluctuation increased the chilling requirements for bud endodormancy release in apples (Anzanello et al., 2014). In Japanese pear, chilling interruption by high temperatures of 20 °C delayed bud break (Tamura et al., 1995). Gardin et al. (2002) suggested that climatic conditions of thermal fluctuations occurring in mild winters could be associated with a high incidence of floral abortion in Japanese pear. However, studies regarding the effects of thermal fluctuation in endodormancy release in Japanese pear are limited and this relationship remains poorly understood.

Carbohydrates are the main source of energy for metabolic activity during dormancy in winter, and for bud burst and blooming during spring (Flore and Layne, 1996). Furthermore, soluble sugars are important for their osmotic properties (Sakai, 1960), and in conferring freezing tolerance (Yoshioka et al., 1988), which is an essential mechanism that allows buds to survive winter conditions. During dormancy, starch is converted into soluble sugars due to increases in the activity of amylase in response to low temperatures (Elle and Sauter, 2000). Starch concentration has been found to decrease in Japanese pear flower buds during chilling accumulation (Hussain et al., 2015). Furthermore, starch is mobilized from the reserve tissues (stems) to the growth areas (meristems) of woody plants to increase the amount of soluble sugars available for use in cellular metabolism (Beck and Ziegler, 1989). Studies on cold deprivation in peaches indicated that sugar concentrations remained high during dormancy (Bonhomme et al., 2005). Marafon et al. (2011) suggested that reduced sucrose synthesis in wood tissues and low reducing sugar content in buds may lead to flower bud abortion under insufficiency of chilling. Although recent studies have been conducted to clarify the effects of temperature on carbohydrate metabolism during dormancy in Japanese pear (Marafon et al., 2011; Ito et al., 2013; Hussain et al., 2015), carbohydrate metabolism and its relationship with the dormancy release associated with thermal fluctuations under mild winter conditions remains unclear.

One hypothesis to explain floral primordia necrosis and irregular bud burst in areas under mild winters is that the insufficiency of chilling associated with thermal fluctuations may affect the physiological processes underlying dormancy (i.e. carbohydrate metabolism) in a more severe manner than that caused solely by lack of chilling. The objective of this study was therefore, to examine the effects of thermal fluctuations during endodormancy in Japanese pear under simulated mild winter conditions, and determine the consequences on bud dormancy release, floral primordia necrosis and carbohydrate metabolism in floral buds and stem.

2. Materials and methods

2.1. Plants and treatments

The study was conducted in the boreal winter of 2015–2016 at the Agricultural and Forestry Research Center of University of Tsukuba, Tsukuba, Japan (36°N, 140°E). Forty-five four-year-old 'Housui' Japanese pear trees grown in pots (15 L) were used in this experiment and were divided into three different treatment groups. Schematic model of experimental treatments are shown in Fig. 1. The treatments were as follows: (a) control trees that accumulated 600 chilling hours (CH) under field conditions without induced thermal fluctuations (TF0), (b) trees under field conditions that were transferred into a heated greenhouse to simulate thermal fluctuations for one day (TF1) or (c) three days (TF3) at every point when 100, 200, 300, 400 and 500 CH were progressively accumulated under field conditions. After all TF0, TF1 and TF3 trees had accumulated 600 CH, they were subsequently subjected to forcing temperatures (minimum of 15 °C) in the greenhouse. The CH were defined as the number of hours below 7.2 °C under field conditions. Chilling unit (CU) values were calculated using the Saitama method (Asano and Okuno, 1990). Lateral flower buds and the adjacent stem (1 cm of length) of each bud were collected at 0, 300 and 600 CH during endodormancy progression, frozen in liquid nitrogen, and kept in an ultra-low-temperature freezer (−80 °C) for further analysis.

2.2. Determination of bud burst and number of flower/cluster

The bud burst in potted trees of each treatment was calculated relative to the total number of lateral flower buds observed (fifteen buds per replicate). Observations were conducted two days under forcing conditions (minimum of 15 °C) in the greenhouse after trees had accumulated 600 CH. Bud burst was defined to be the point when buds reached phenological stage C3 or mouse-ear stage, when swelling of flower buds is apparent and green leaf tips are visible above the bud scales (Coutanceau, 1971; Calvet and Guirbal, 1979). The number of flowers per cluster was counted once the petals had completely opened in all flowers.