Effects of pre-chilling potassium nitrate and post-chilling hydrogen cyanamide application on carbohydrate and water dynamics of 'Housui' Japanese pear spur buds during dormancy under mild winter conditions

[1]Humberto Mitio Horikoshi, [2]Yoshihiko Sekozawa, [3]Sumiko Sugaya

[1]Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba 305-8572, Japan, humberto_hmh@hotmail.com
[2]Faculty of Life and Environmental Sciences, University of Tsukuba, Tsukuba 305-8572, Japan, sekozawa.yoshihik.ga@u.tsukuba.ac.jp
[3]Faculty of Life and Environmental Sciences, University of Tsukuba, Tsukuba 305-8572, Japan, sugaya.sumiko.fw@u.tsukuba.ac.jp

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Abstract - Japanese pear (*Pyrus pyrifolia* Nakai) trees were treated with potassium nitrate (KNO_3) before the onset of chilling and hydrogen cyanamide (CH_2N_2) after the trees had accumulated 600 chilling hours (CH) to determine the effects on bud dormancy release, carbohydrate dynamics, and water content. For the carbohydrate and water content analyses, Japanese pear spur buds were collected after 0, 300, and 600 CH, and after the accumulation of 2000, 4000, 6000, and 8000 growing degree hours (GDH) during the dormancy period. Bud burst and flowering were hastened by both KNO_3 and CH_2N_2 application, and both chemicals affected carbohydrate and water dynamics during dormancy. Sucrose concentration tended to increase during the chilling period, and to decrease under forcing conditions. Hexose concentrations increased in buds treated with KNO_3, CH_2N_2, or both, and were associated with advances in bud burst. Sorbitol concentration decreased early in buds treated with CH_2N_2, suggesting rapid sorbitol catabolism. Water content gradually increased under forcing conditions in all buds, although those treated with both KNO_3 and CH_2N_2 exhibited the highest values. Accordingly, we recommend the use of KNO_3 and CH_2N_2, before and after chilling, respectively, to promote the release of bud dormancy and increase bud burst rate in 'Housui' Japanese pear grown in regions with mild winter conditions.

Keywords – Endodormancy. Water content. Sugar. Bud break.

Resumo - As árvores de pêra japonesa (*Pyrus pyrifolia* Nakai) foram tratadas com nitrato de potássio (KNO_3) antes do início do frio e cianamida hidrogenada (CH_2N_2) após terem acumulado 600 horas de frio (CH-chilling hours) para determinar seus efeitos na quebra de dormência de gemas, dinâmica de carboidratos e no conteúdo de água. Para as análises de carboidratos e conteúdo de água, foram coletadas gemas (esporões) de pereira japonesa após 0, 300 e 600 CH, e após o acúmulo da soma térmica de 2000, 4000, 6000 e 8000 (GDH-growing degree hour) durante o período de dormência. A brotação e a floração foram aceleradas pela aplicação de KNO_3 e CH_2N_2, e ambos os produtos químicos afetaram a dinâmica de carboidratos e água durante a dormência. A concentração de sacarose tendeu-se a aumentar durante o período de frio e a diminuir sob condições de forçamento. As concentrações de hexose aumentaram em gemas tratadas com KNO_3, CH_2N_2, ou ambos, e foram associadas com os avanços das brotações. A concentração de sorbitol diminuiu antes em gemas tratadas com CH_2N_2, sugerindo uma rápida degradação do sorbitol. O conteúdo de água aumentou gradualmente sob condições de forçamento em todos os tratamentos, embora aqueles tratados com KNO_3 e CH_2N_2 exibiram os valores mais elevados. Sendo assim, recomenda-se o uso de KNO_3 e CH_2N_2, antes e depois do frio, respectivamente, para promover a quebra de dormência das gemas e aumentar a taxa de brotação em pereira japonesa cv. Housui cultivadas em regiões sob condições de inverno ameno.

Palavras-chave – **E**ndodormência. Conteúdo de água. Açúcar. Quebra de dormência.

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1 INTRODUCTION

Bud dormancy is an adaptive mechanism that allows woody plants to survive harsh winter conditions (FAUST *et al.*, 1997). Several problems related to dormancy occur in temperate fruits trees such as kiwifruit (WALTON; CLARK; BOLDINGH, 1991), grapes (DOOKOOZLIAN *et al.*, 1995; BEN MOHAMED *et al.*, 2012), peaches, and nectarines (DOZIER *et al.*, 1990) when they are grown in regions where winter conditions are not as cold as in their region of origin. In the Japanese pear (*Pyrus pyrifolia* Nakai), bud dormancy release is negatively affected in regions with mild winters, such as New Zealand (KINGSTON; KLINAC; EPENHUIJSEN, 1990) and Brazil (PETRI; HERTER, 2002).

Deciduous fruit trees need a certain amount of chilling time to overcome dormancy (LANG, 1996), and in some cases only a portion of this requirement can be satisfied via chilling substitutes (FAUST *et al.*, 1997). Several chemicals have been reported to be effective in this regard, such as mineral oils (PETRI; STUKER, 1995), thiourea (KÜDEN; SON, 1997), calcium cyanamide (IWASAKI, 1980), hydrogen cyanamide (DOOKOOZLIAN *et al.*, 1995; KUROKI *et al.,* 2013) and potassium nitrate (EREZ; LAVEE; SAMISH, 1971; GEORGE; NISSEN, 1993; AKSOY *et al.,* 1995). However, the trees' responses to these chemicals are variable, and depend on the chemical concentration and the time of application.

Hydrogen cyanamide (CH_2N_2) has been reported as one of the most effective dormancy-breaking agents for many deciduous plant species (FUCHIGAMI; NEE, 1987), improving bud break in grapevines (DOOKOOZLIAN *et al.*, 1995), peaches and nectarines (DOZIER *et al.*, 1990), and Japanese pears (KUROKI *et al.,* 2013). Effects of CH_2N_2 on the carbohydrate metabolism have been reported for grapes (BEN MOHAMED *et al.,* 2012), kiwifruit (RICHARDSON *et al.*, 2010), and apples (CUTTING *et al.,* 1991). However, few studies have been conducted on Japanese pears.

Potassium nitrate (KNO_3) has similarly been used as an alternative dormancy-breaking agent to promote bud break in peaches (EREZ; LAVEE; SAMISH, 1971; GEORGE; NISSEN, 1993), grapes (HASSAN, 2004), and apricots (AKSOY *et al.,* 1995). Furthermore, KNO_3 promotes changes in total free amino acid concentration in dormant grapevine buds (SUZUKI *et al.,* 1997). In addition, this compound provides potassium, which is an essential element for plant growth that activates more than 60 enzymes, and is important in enabling plants to tolerate stresses such as high and low temperatures (MALVI, 2011)

Soluble sugars are important signaling molecules that are involved in various processes in a plant's lifecycle (SMEEKENS, 2000). In addition to their essential role as osmotic agents (SAKAI, 1960), protective aids for proteins and membranes (STEPONKUS *et al.,* 1977), and in conferring tolerance for freezing (YOSHIOKA *et al.,* 1988), carbohydrates are also the main source of energy for metabolic activities during dormancy, spring sprouting, and blooming (SHERSON *et al.,* 2003). Dormancy in trees is also related to changes in water movement (WELLING; PALVA, 2006). Furthermore, water dynamics are essential for carbohydrates transport between source and sink during dormancy phase (MARAFON *et al.,* 2011).

The effects of KNO_3 and CH_2N_2 application on carbohydrate and water content during bud dormancy in the Japanese pear under mild winter conditions are still poorly understood. Thus, the main objective of this study was to determine the effects of these compounds on bud break and on carbohydrate and water dynamics during bud dormancy in the 'Housui' Japanese pear under mild winter conditions.

2 MATERIAL AND METHODS

2.1 Plant material

The experiment was conducted in the boreal winter of 2012–2013 at the Agricultural and Forestry Research Center of University of Tsukuba, Tsukuba, Japan (36°N, 140°E), using 'Housui' Japanese pear trees grown in pots. The experimental design was completely randomized, with four treatments (water [control], KNO_3, CH_2N_2, and a combination of KNO_3 and CH_2N_2) in three replicates (three trees per replicate). Spur buds

were treated with 4% KNO_3, using a hand sprayer, before the onset of chilling (22 October 2012). The trees were maintained under field conditions until they had accumulated 600 chilling hours (CH), after which 1% CH_2N_2 was applied with a hand sprayer until the drip point. The trees were then subjected to forcing temperatures (minimum of 13 °C) in a greenhouse. CH were classified as the number of hours below 7.2 °C under field conditions. The number of growing degree hours (GDH) was calculated by subtracting 4.5 °C from each hourly temperature, and then summing the values (RICHARDSON *et al.,* 1975). Spur buds were collected at 0, 300, or 600 CH, and at 2000, 4000, 6000, or 8000 GDH, frozen in liquid nitrogen, and kept in an ultra-freezer (–80 °C) for further analysis.

2.2 Determination of bud burst, flowering and number of flowers per cluster

Trees were examined for bud burst and flowering every two days after these were subjected to forcing conditions in the greenhouse. Bud burst was identified as the point when buds reached the phenological stage C3 (COUTANCEAU, 1971; CALVET; GUIRBAL, 1979), and trees were recorded as flowering when they had at least one completely open flower. The number of flowers per cluster was counted once the petals had one completely opened flower.

2.3 Water content determination

Water content was measured by drying 500 mg of fresh spur bud samples at 80 °C in an oven for 24 hours. Dried samples were weighed and water content was calculated according to the formula: Water content = (FW – DW) / FW × 100, where FW is fresh weight and DW is dry weight, both expressed in grams.

2.4 Determination of sugar concentration

To determine the sugar content, approximately 300 mg of frozen spur buds were weighed accurately, ground using liquid nitrogen in a mill (ForceMill; Osaka Chemical, Osaka, Japan), and extracted twice with 6 mL of 80% ethanol. After the addition of 1 mL of pentaerythritol (1%) as an internal standard, the extract was evaporated in a vacuum at 40 °C. To remove phenolic compounds, three milligrams of polyvinylpolypyrrolidone (PVPP) was added into 1 mL aliquots of the extract, then centrifuged at 14.000 rpm for 10 min and filtered through a Millipore filter (0.45 µm) attached to a syringe. 20-µL samples of extract solution were then subjected to high-performance liquid chromatography (HPLC) using an auto-sampler with a pre-column (Shin-pack SPR-Ca; Shimadzu, Japan), a packed column (SC 1011, SHODEX; Showa Denko K.K., Tokyo, Japan), and a refractive index detector (RI-101, SHODEX; Showa Denko K.K.). Ultra-pure water (18 mΩ) was used for the mobile phase, and the equipment was set to a flow rate of 1.0 mL min^{-1} and a column temperature of 80 °C. Carbohydrate content was quantified based on calculations of peak area obtained from regression curves of standards.

2.5 Determination of starch concentration

Starch was quantified using a total starch kit (AOAC Official Method 996.11; Megazyme International Ireland, Bray, Ireland). The pellet remaining after the ethanol extraction for sugar determination was oven-dried overnight at 60 °C. The dried samples were stirred in a vortex mixer with 0.2 mL of 80% (v/v) ethanol. Immediately, 3 mL of thermostable α-amylase diluted in 50 mM MOPS buffer (pH 7.0) was added, and the samples were incubated for 12 min in boiling water. The tubes were placed in a water bath at 50 °C and 4 mL of 200 mM sodium acetate buffer (pH 4.5) was added, followed by 0.1 mL of amyloglucosidase, and incubated for 30 min. The samples were then adjusted to 10 mL with deionized water, followed by centrifugation at 3000 rpm. Duplicated 0.2-mL aliquots of the diluted solution were transferred to a glass tube (16 × 100 mm) and 3 mL of glucose oxidase/peroxidase (GOPOD) reagent was added, followed by incubation for 20 min at 50 °C. D-glucose was used as the standard, and distilled water was used as the blank. Absorbance at 510 nm was determined using a UV/VIS spectrophotometer (V-550; Jasco, Tokyo, Japan).

2.6 Statistical analysis

Means and standard errors were calculated from the replicates. The data were analyzed using one-way analysis of variance (ANOVA), and the means were compared using the Tukey-Kramer test at 5% of probability in JMP 5.0.1. All percentage data were arc-sine transformed before analysis. The graphics were produced using Graphpad Prism 5.0 (GraphPad Software).

3 RESULTS AND DISCUSSION

To replicate mild winter conditions, trees were exposed to 600 CH that accounts to 80% of the 750 CH (≤ 7.2 °C) necessary to release endodormancy in Japanese pear (NISHIMOTO; KISAKI; FUJISAKI, 1995). Knowing that potassium provides plants tolerance against stress, such as high and low temperatures (MALVI, 2011), we tried applying KNO_3 before the chilling accumulation period to serve not only as a bud break agent, but also as an aid for buds in tolerating low temperatures.

The application of KNO_3 before and of CH_2N_2 after chilling hastened bud burst relative to the control trees (Fig. 1A). Bud burst under forcing conditions started 16 days after spraying of CH_2N_2 and the combined treatment, whereas it took 22 days in the KNO_3 treatment and 24 days in the control. In all trees, except those in the control treatment, 100% of buds burst; bud burst in the control was 93%. Application of KNO_3 and CH_2N_2 advanced the onset of flowering in spur buds relative to the control (Fig. 1B). Flowering began first in the CH_2N_2 and combined treatments, followed by the KNO_3 and control treatments. Treatment of 'Housui' Japanese pear trees with KNO_3 and/or CH_2N_2 resulted in earlier bud burst and flowering than in the control. Similar results had been reported by George and Nissen (1993) and Aksoy *et al.* (1995), who found an advance in bud break as a result of using KNO_3 in a low-chill peach cultivar and in apricots, respectively. In another study, treating buds with KNO_3 resulted in a bud break percentage similar to that following CH_2N_2 treatment in 'Perlette 'grapes (HASSAN, 2004). In our results with Japanese pear, KNO_3 applied before the chilling period, in combination with CH_2N_2 sprayed after 600 CH had accumulated, resulted in earlier bud burst than KNO_3 treatment alone, which is similar to the findings of another study, in which bud break was advanced when KNO_3 was combined with other chemicals (EREZ; LAVEE; SAMISH, 1971). The effect of CH_2N_2 on bud break in Japanese pear flower buds depends on its concentration, accumulated chilling, and the cultivar (KUROKI *et al.*, 2013). A similar tendency has been observed in other studies, where CH_2N_2 advanced bud break in grapevines (DOOKOOZLIAN *et al.,* 1995) and in peaches and nectarines (DOZIER *et al.,* 1990).

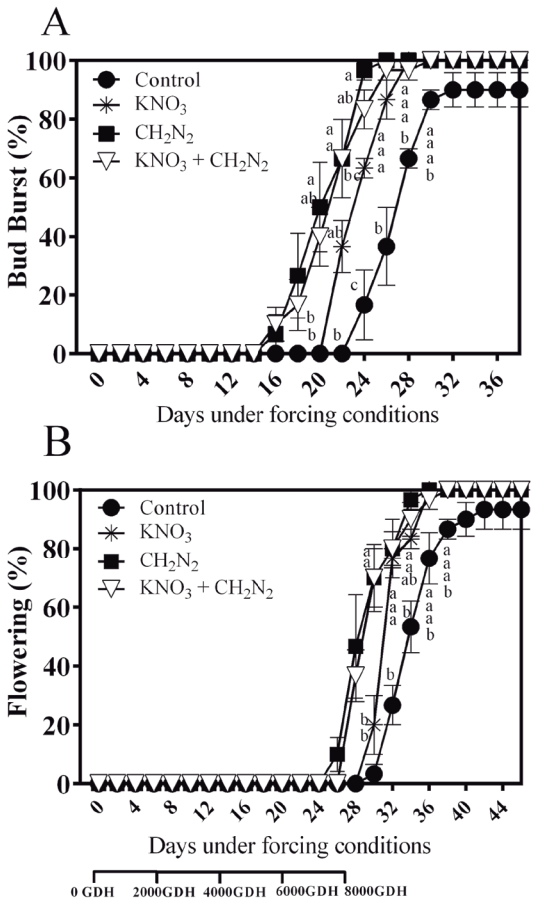

Figure 1. Bud burst (A) and flowering (B) in Japanese pear spur buds treated with pure water (control, closed circles), KNO_3 before chilling (stars), CH_2N_2 after chilling (closed squares), or both KNO_3 and CH_2N_2 (open triangles) under mild winter conditions. Bud burst was classified as the point when buds reached phenological stage C3, and flowering was recorded as the point when a tree had at least one completely open flower. Different letters indicate significant differences among treatments at each sampling time. Means compared by Tukey-Kramer test at 5% probability, ± SE (n=3).

The number of flowers per cluster (approx. four) was similar across treatments (Figure 2). The number of flowers per cluster was not affected by the treatment. In contrast, it has been reported that the application of CH_2N_2 to kiwifruit increased the total number of flowers (MCPHERSON *et al.,* 2001).

The water content of the buds remained low during the chilling period, and then gradually increased under forcing conditions (Figure 3). Also, it was observed that under forcing conditions, the water content of the buds at 8000 GDH in the CH_2N_2, KNO_3, and combined treatments was higher than that of the control buds. Simões *et al.* (2014) observed similar results, where water content increased in 'Housui' Japanese pear buds throughout dormancy. It has been reported that CH_2N_2 might increase the influx of water into buds, and this accumulation of water may assist in advancing the release of bud dormancy (YOOYONGWECH *et al.*,

2012). Thus, the application of KNO_3 and CH_2N_2 may have changed the water dynamics, leading to the earlier bud burst in this study.

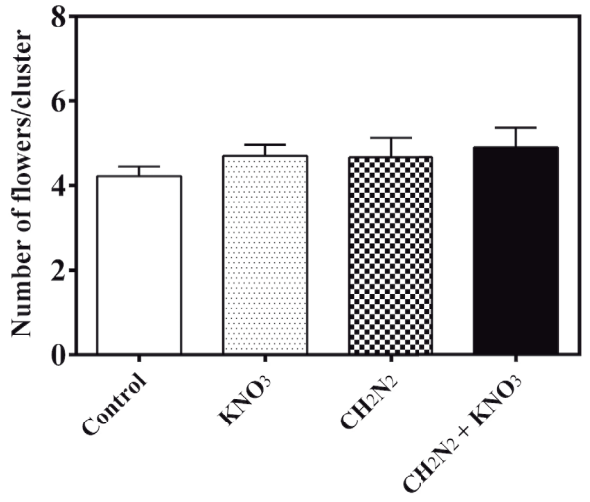

Figure 2. Number of flowers per cluster in Japanese pear spur buds treated with pure water (control, white), KNO_3 before chilling (stipples), CH_2N_2 after chilling (checks), or both KNO_3 and CH_2N_2 (black) under mild winter conditions. Different letters indicate significant differences between treatments at each sampling time. Means compared by Tukey-Kramer test at 5% probability, ± SE (n=3).

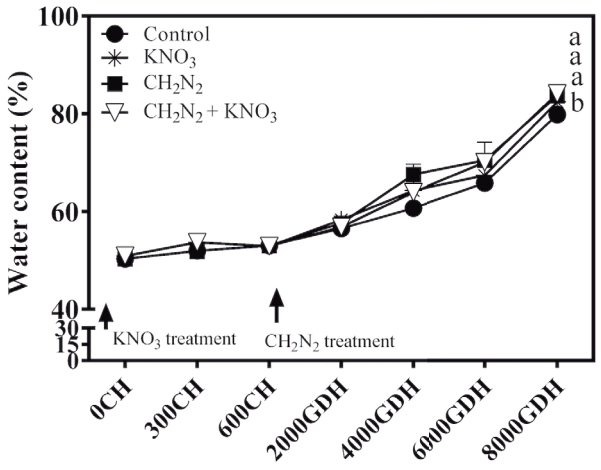

Figure 3. Water content of Japanese pear spur buds treated with pure water (control, closed circles), KNO_3 before chilling (stars), CH_2N_2 after chilling (closed squares), or both KNO_3 and CH_2N_2 (open triangles) under mild winter conditions. CH (chilling hours): accumulated hours of chilling ≤ 7.2 °C; GDH (growing degree hours): accumulated temperature (°C) of warming. Different letters indicate significant differences between treatments at each sampling time. Means compared by Tukey-Kramer test at 5% probability, ± SE (n=3).

The soluble carbohydrate content of the buds fluctuated between the different phases of dormancy, tending to increase during the chilling period and decrease under forcing conditions. Application of KNO_3 negatively affected sucrose accumulation at 600 CH (Figure 4). Under forcing conditions, the control buds had the highest sucrose content after 4000 GDH, which then gradually decreased. The KNO_3-treated buds had higher sucrose concentrations than the combined treatment at 6000 GDH, and the highest concentration of all four treatments at 8000 GDH. Sucrose concentration increased during the endodormancy phase under chilling. According to Yoshioka *et al.* (1988), starch is degraded by amylase and metabolized into sucrose under the influence of cold temperatures, and that soluble carbohydrates play an important role in increasing freezing tolerance during winter and in providing energy for growth in spring.

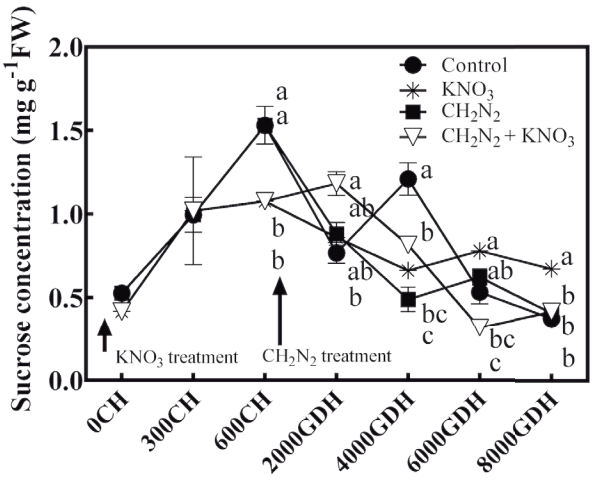

Figure 4. Sucrose concentration (mg.g^{-1} fresh weight) in Japanese pear spur buds treated with pure water (control, closed circles), KNO_3 before chilling (stars), CH_2N_2 after chilling (closed squares), or both KNO_3 and CH_2N_2 (open triangles) after accumulation of chilling hours under mild winter conditions. CH (chilling hours): accumulated hours of chilling ≤ 7.2 °C; GDH (growing degree hours) accumulated temperature (°C) of warming. Different letters indicate significant differences among treatments at each sampling time. Means compared by Tukey-Kramer test at 5% probability, ± SE (n=3).

Fructose concentration remained stable and similar across the treatments until forcing conditions began (Figure 5). The KNO_3, CH_2N_2, and combined treatments resulted in a gradual increase in fructose concentration from 4000 GDH, while in control buds the increase was only found at 8000 GDH. Glucose concentration remained low during chilling accumulation under field conditions, but increased under forcing conditions (Fig. 6). From 6000 GDH onwards, the control buds exhibited the lowest glucose concentration. In our experiment, glucose and fructose tended to increase, and sucrose to decline, as bud burst began under forcing conditions. A rapid decrease in sucrose and a simultaneous increase in hexose concentrations during spring are the earliest signs of high metabolic activity and resumption of growth (RICHARDSON *et al.,* 2010). In addition, bud break is followed by the rapid consumption of sucrose, glucose and fructose (BEN MOHAMED *et al.*, 2012). The delayed bud burst in our control trees was correlated with smaller increases in hexose

concentrations during the forcing period. Thus, the movement and availability of soluble carbohydrates appears to be essential for the resumption of growth, and the application of both CH_2N_2 and KNO_3 may enhance these dynamics, leading to early bud burst (and high rates of bud burst) in the Japanese pear.

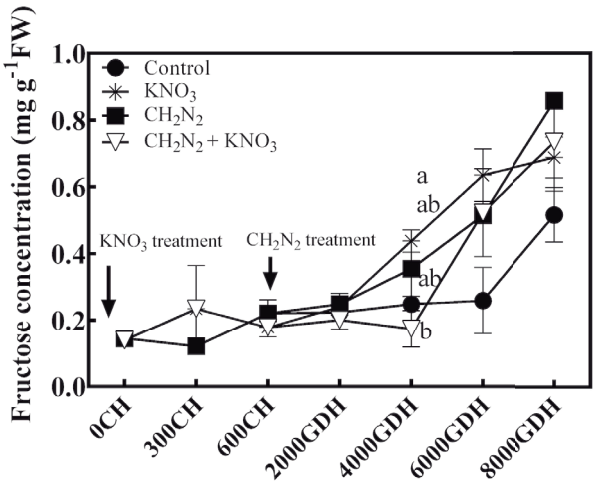

Figure 5. Fructose concentration (mg.g^{-1} fresh weight) in Japanese pear spur buds treated with pure water (control, closed circles), KNO_3 before chilling (stars), CH_2N_2 after chilling (closed squares), or both KNO_3 and CH_2N_2 (open triangles) after accumulation of chilling hours under mild winter conditions. CH (chilling hours): accumulated hours of chilling ≤ 7.2 °C; GDH (growing degree hours) accumulated temperature (°C) of warming. Different letters indicate significant differences among treatments at each sampling time. Means compared by Tukey-Kramer test at 5% probability, ± SE (n=3).

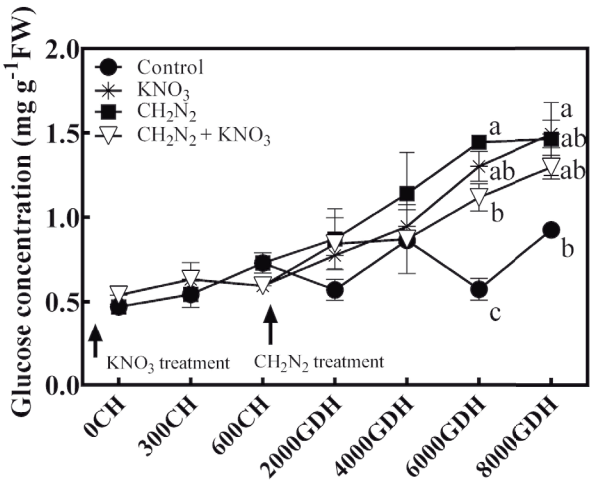

Figure 6. Glucose concentration (mg.g^{-1} fresh weight) in Japanese pear spur buds treated with pure water (control, closed circles), KNO_3 before chilling (stars), CH_2N_2 after chilling (closed squares), or both KNO_3 and CH_2N_2 (open triangles) after accumulation of chilling hours under mild winter conditions. CH (chilling hours): accumulated hours of chilling ≤ 7.2 °C; GDH (growing degree hours) accumulated temperature (°C) of warming. Different letters indicate significant differences among treatments at each sampling time. Means compared by Tukey-Kramer test at 5% probability, ± SE (n=3).

Sorbitol concentration in buds increased during chilling accumulation, and gradually decreased under forcing conditions (Figure 7). In the CH_2N_2 and combined treatments, the sorbitol concentration increased until the onset of forcing conditions, decreased rapidly after 2000 GDH, and remained low until the end of the trial at 8000 GDH, whereas in the buds in the control and KNO_3 treatments it decreased more slowly. The application of CH_2N_2 to buds after the accumulation of 600 CH caused the sorbitol concentration to decrease more rapid than in other buds. Similarly, CH_2N_2 application to apple trees promoted earlier bud break and a marked decrease in sorbitol concentration (CUTTING *et al.,* 1991). This suggests that CH_2N_2 application leads to rapid sorbitol catabolism, which provides energy and thereby advances bud break. This hypothesis is supported by our results. Sorbitol and sucrose concentration in the buds increased during the endodormancy period, i.e. under chilling; as noted above, these concentrations may be strongly correlated with the development of freezing tolerance (YOSHIOKA *et al.,* 1988; ITO; SAKAMOTO; MORIGUCHI, 2013). Sorbitol is often the primary carbohydrate that is translocated in fruit trees in the Rosaceae family (LOESCHER; EVERARD, 1996), and carbohydrate is transported from source to sink in this form in the Japanese pear during endodormancy (ITO; SAKAMOTO; MORIGUCHI, 2012). The sucrose and sorbitol accumulated during the endodormancy period is converted into glucose and fructose for the resumption of growth and flower development. Although it has been reported that just a small quantity of sorbitol is sufficient to satisfy the high demand for carbon and energy during the early stages of bud break in peaches (MAUREL *et al.,* 2004), the lack of capacity to metabolize sucrose and sorbitol to provide the required hexoses under cold deprivation conditions is associated with floral necrosis (BONHOMME *et al.,* 2005). We therefore suggest that the delay in bud burst and the occurrence of bud abortion in the control trees were caused by low rates of increase in hexose concentrations.

The starch concentration in the buds remained stable during the entire study period (Figure 8). During the chilling period, buds not treated with KNO_3 showed a decrease in starch concentration at 400 CH, followed by an increase at 600 CH. After the chilling period, CH_2N_2 application caused a slight decrease in the starch concentration at the onset of forcing conditions, after which starch content remained unchanged. Buds in the combined treatment exhibited the highest starch concentration at the beginning of forcing conditions, and then tended to decrease. Although we did not observe a

clear trend in this study, it has been reported that the completion of endodormancy is associated with increasing starch and decreasing sugar concentrations in the Japanese pear (HONJO *et al.,* 2002). In contrast, high starch and low soluble sugar concentrations have been observed during the onset of endodormancy in grapevines (BEN MOHAMED *et al.,* 2010). Furthermore, accumulated starch is used for the resumption of Japanese pear growth in spring (GEMMA, 1995), and an increase in α-amylase activity is associated with the release of bud dormancy in grapevines (BEN MOHAMED *et al.,* 2012). The effects of KNO_3 on sugar metabolism remain unclear, although it can be hypothesized that the involvement of potassium is important because of its function in activating more than 60 enzymes, including starch synthase (MALVI, 2011). Hence, carbohydrate dynamics play an important role during bud endodormancy and dormancy release, and further research is needed to clarify the effects of CH_2N_2 and KNO_3 on the physiological aspects during bud dormancy in the Japanese pear under mild winter conditions.

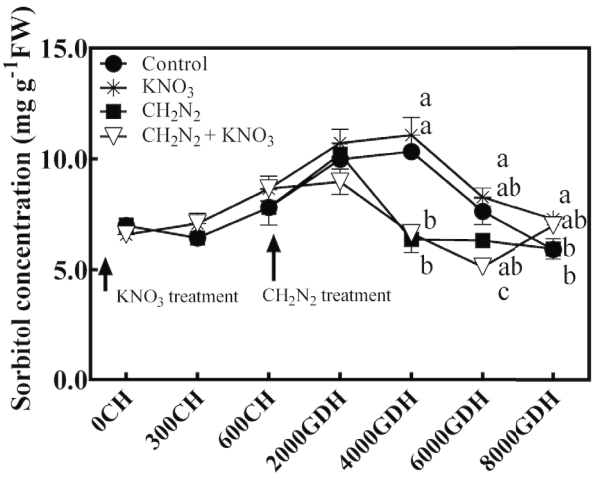

Figure 7. Sorbitol concentration (mg.g ^{1} fresh weight) in Japanese pear spur buds treated with pure water (control, closed circles), KNO_3 before chilling (stars), CH_2N_2 after chilling (closed squares), or both KNO_3 and CH_2N_2 (open triangles) after accumulation of chilling hours under mild winter conditions. CH (chilling hours): accumulated hours of chilling ≤ 7.2 °C; GDH (growing degree hours) accumulated temperature (°C) of warming. Different letters indicate significant differences among treatments at each sampling time. Means compared by Tukey-Kramer test at 5% probability, ± SE (n=3).

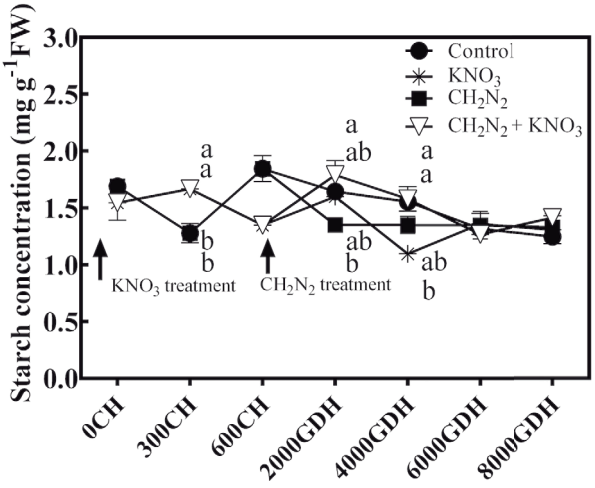

Figure 8. Starch concentration (mg.g^{-1} fresh weight) in Japanese pear spur buds treated with pure water (control, closed circles), KNO_3 before chilling (stars), CH_2N_2 after chilling (closed squares), or both KNO_3 and CH_2N_2 (open triangles) after accumulation of chilling hours under mild winter conditions. CH (chilling hours): accumulated hours of chilling ≤ 7.2 °C; GDH (growing degree hours) accumulated temperature (°C) of warming. Different letters indicate significant differences among treatments at each sampling time. Means compared by Tukey-Kramer test at 5% probability, ± SE (n=3).

4 CONCLUSIONS

Application of KNO_3 before the onset of chilling and of CH_2N_2 after the accumulation of 600 CH to Japanese pear spur buds hastened bud break. CH_2N_2 and KNO_3 increased water content and hexose concentrations, which resulted in improvements in bud burst under forcing conditions. The application of CH_2N_2 led to a rapid sorbitol catabolism under forcing conditions. We, therefore recommend using both KNO_3 as an alternative dormancy-breaking agent before the onset of chilling and employing CH_2N_2 after chilling accumulation to increase bud break rates in Japanese pear trees grown under mild winter conditions.

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