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Effect of chilling on calorimetric responses of dormant vegetative apple buds

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Abstract

Chilling exposure is important in apple buds to overcome dormancy. The objective of this work was to determine the effect of different chilling treatments on apple bud calorimetric responses. One-year-old Golden Delicious trees were kept in a controlled temperature chamber at 4°C for up to 1600 h. At 200 h intervals, five trees were transferred to forcing conditions at 25°C. Isothermal calorimetry was used to determine metabolic activity (q), respiration rate (R_{CO_2}), respiration efficiency (q/R_{CO_2}) and specific growth rate ($R_{SG}\,\Delta_{HB}$) in vegetative buds. DSC was used to estimate activation energy (E_a). Calorimetric assays were performed at 0, 15 and 30 days at forcing conditions and budbreak was recorded. No significant differences ($p \leq 0.05$) in the above respiration parameters were found between chilling treatments during cold storage and immediately when the trees were transferred to growing conditions (0 days at 25°C). Also, the lowest values registered for q (2.5 μW mg/dw) and R_{CO_2} (8 mmol CO_2 mg/dw) were found during the above two periods. However, after 15 and 30 days at forcing conditions, buds increased their metabolism at a slow, but significant pace, as a direct function of chilling exposure time. After 15 days under forcing treatments q increased from 2.5 to 4 μW mg^{-1} dw, R_{CO_2} from 9 to 13.5 mmol CO_2 mg^{-1} dw and $R_{SG}\,\Delta_{HB}$ from 1 to 2.5. After 30 days, these responses kept increasing, q from 2.5 to 5 μW mg^{-1} dw, R_{CO_2} rose from 10 to 17 mmol CO_2 mg^{-1} dw and $R_{SG}\,\Delta_{HB}$ from 2 to 3. On both periods (15 and 30 day), highest values were found after chilling exposures more than 1000 h at 4°C, which coincides with an increased budbreak of 12%. No consistent pattern was observed in q/R_{CO_2}, regardless of exposure to forcing conditions. Cumulative chilling resulted in decreasing E_a of 29.8 and 33.3 J mol^{-1} K after forcing for 15 and 30 days, respectively. In general, dormant vegetative apple buds increased their calorimetric responses after forcing for 15 and 30 days, but this was significant only when chilling equaled or exceeded 1000 h. Calorimetric parameters agree with information available elsewhere on chilling requirements for this variety

Keywords: Chilling; Calorimetry; Dormancy; Apple; Budbreaking

1. Introduction

Perennial plants from the temperate zone developed dormancy as a mechanism to overcome winter adverse conditions [1]. Dormancy can be described as a process that inhibits growth of several meristematic tissues. Dormancy allows plants to synchronize growth and development so that making it possible to survive under harsh environments [2]. Many apple (*Malus domestica* Borkh) varieties need exposures from 1200 to 1500 h under temperatures below 7°C to fulfill chilling requirement to overcome dormancy. Once

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such a condition is met during the spring, starch will be converted back to sugars that provide buds with energy for development of flowers and leaves [3].

Calorimetry is a quick and precise method allowing measurement of metabolic responses of different plant tissues as a function of temperature. This technique makes it possible to quantify metabolic heat rate (q), and CO_2 production rate (R_{CO_2}) [4,5]. Furthermore, these variables allow a biochemical description of tissue growth rate ($R_{SG}\,\Delta_{HB}$) and its efficiency for carbohydrate substrate (q/R_{CO_2}) conversion [6]. At present, a common difficulty in diagnosing plant dormancy status is that available methods are time consuming and faster alternatives are needed. Therefore, if respiration parameters can be patterned during dormancy, then practical applications may be developed, that is, a quick system to diagnose dormancy development, chilling requirements for different germplasm and effect of orchard cultural practices on bud development.

The objective of this study was to evaluate calorimetrically the response of vegetative dormant buds of apple kept at different cold exposures followed by conditions conducive to growth.

2. Experimental

Forty one-year-old Golden Delicious trees were carefully dug out from a commercial nursery. Their roots were wrapped in plastic bags containing wet sawdust to prevent dehydration during transportation to the laboratory and cold storage. The trees were pruned to ten nodes and stored from 0 to 1600 h in a 4°C growth chamber. At 200 h intervals, five trees were randomly sampled and planted in individual containers with a standard propagation medium. They were forced to grow for up to 40 days in a 25°C greenhouse. Metabolic heat measurements of vegetative buds were made after 0, 15 and 30 days at forcing conditions following exposure to every chilling interval. Budbreak was scored after 40 days at forcing conditions. A bud was considered as broken when the half-inch green stage was achieved.

Metabolic heat measurements were done using a CSC 4100 DSC (Calorimetry Science Corporation, Pleasant Grove, Utah) provided with four 1 cm^3 hastelloy ampoules with removable lids. The instrument has a ± 1 μW baseline sensitivity and a working range of −30–110°C. Temperature around the DSC chamber was maintained at 15°C with a refrigerated circulating bath (PolyScience, Niles, II). A 175 g cm^{-2} flux of dry nitrogen was used to prevent moisture condensation inside the instrument, when the test was carried out below room temperature.

From the five plants exposed to chilling treatments, nine buds were collected at every forcing period. Their fresh weight was recorded after blotting to eliminate excess moisture. Buds were placed in the ampoules with their basal cuts submerged in 50 μl of sterile water to avoid dehydration during the runs. Preliminary tests were done to determine amount of tissue to include, so that enough signal was recorded. For example, in isothermal studies, three buds per ampoule were used, since they were enough to provide close to a 100 μW signal. Both q and R_{CO_2} measurements, as well as q/R_{CO_2} and $R_{SG}\,\Delta_{HB}$ determinations were done at 25°C according to methods discussed elsewhere [4,7]. Scannings were run from 5–65°C at a rate of 10°C h^{-1}, and data were collected at 60 s intervals. Arrhenius plots were done from the scanning data. We found that in vegetative apple buds, the region of linear metabolic response was between 10 and 20°C (3.53 and 3.41 K^{-1}). Such data were used to calculate activation energy (E_a) [6]. Both, isothermal and scanning data were baseline corrected and adjusted to sample dry weight. The experimental design was a completely randomized with six replicates. Data were analyzed with an ANOVA and q, R_{CO_2}, q/R_{CO_2}, $R_{SG}\,\Delta_{HB}$ and E_a means separation was according to Tukey ($p \leq 0.05$) (SAS 6.08 version) [8].

3. Results and discussion

3.1. Isothermal calorimetry

Vegetative apple buds q values were not statistically different under 0 days at 25°C, following chilling, regardless of the exposure period. Right after chilling, q showed the lowest response (2.5 μW mg^{-1} dw), at this stage buds need to be exposed to warm temperatures to activate their metabolism and attain activity levels leading to growth [9]. Only after forcing at 25°C for 15 and 30 days, q showed a consistent positive response, as a function of increased exposure to chil-

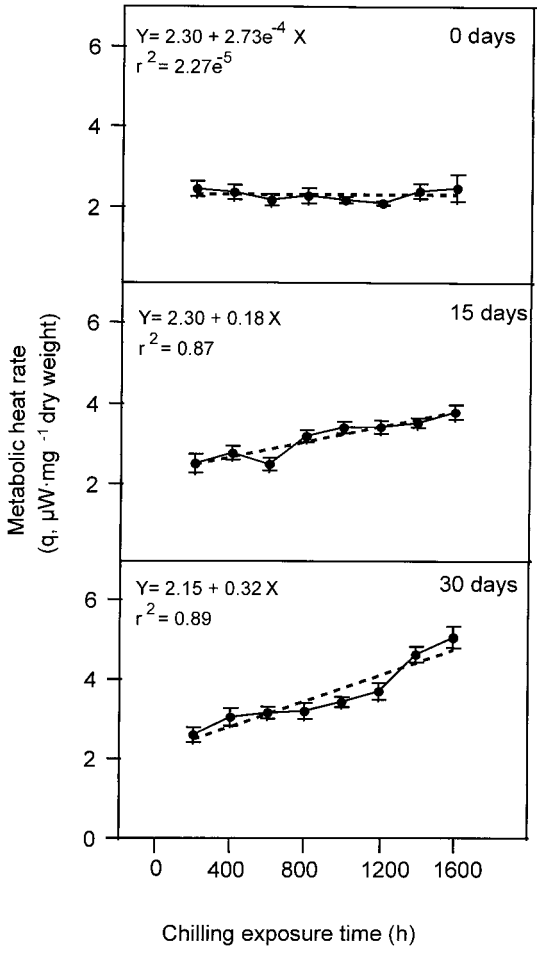

Fig. 1. Metabolic activity in vegetative buds of Golden Delicious apples exposed to different chilling and force to grow at 25°C for 0, 15 and 30 days.

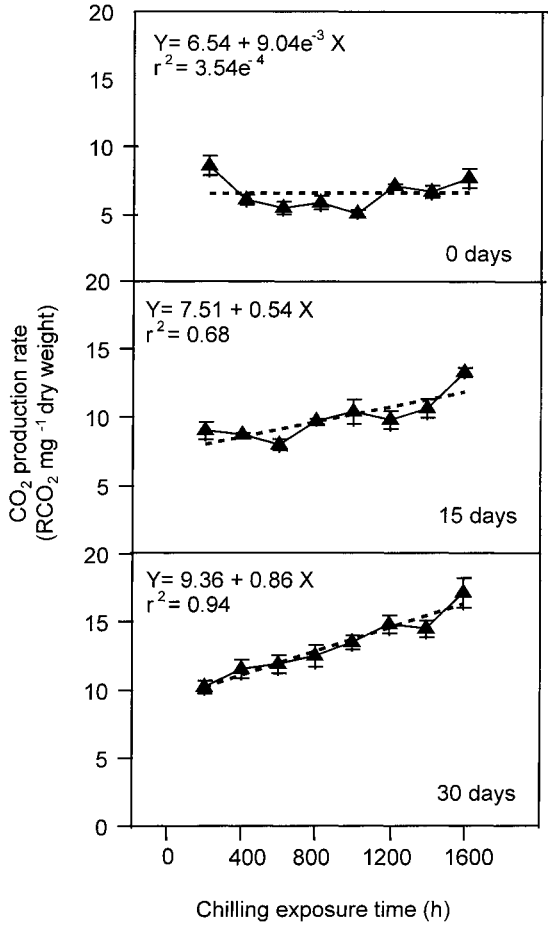

Fig. 2. CO_2 production rates in vegetative buds of Golden Delicious apples during different times of exposure to chilling and forcing conditions at 25°C.

ling time, as shown in Fig. 1. Following exposure to 200 and 1600 h of chilling, q increased from 2.4 to 4 and from 2.5 to 5 µW mg^{-1} dw, after 15 and 30 days in forcing conditions, respectively.

Metabolic heat as measured by calorimetry is a measurement of heat loss during dark respiration [10]. Therefore, q determination must be complemented by an analysis of CO_2 produced by tissues, which indicates respiration rate (R_{CO_2}) [5] or energy potentially available for growth. Fig. 2 shows results of the R_{CO_2} measurements. In all chilling treatments before exposure to 25°C (0 days), R_{CO_2} of buds remained low and without a definite pattern with overall values of around 8 mmol CO_2 mg^{-1} dw. However, after 15 days, values ranging from 9 to 13.5 mmol CO_2 mg^{-1} dw. By the time the buds reached 30 days at 25°C, R_{CO_2} increased in proportion to the length of chilling period the plants were previously exposed, increasing from 10 to 17 mmol CO_2 mg^{-1} dw (Fig. 2). Although q and R_{CO_2} patterns look somewhat similar they do show two different, but complimentary aspects; the first is heat released by metabolism, while the second indicates the amount of CO_2 produced during respiration. Both measurements are important, since their ratio is known as an indicator of plant tissue metabolic efficiency [6]. The higher the heat released the higher the ratio; therefore, metabolic inefficiency increases since less catabolic energy is used for tissue growth. In this

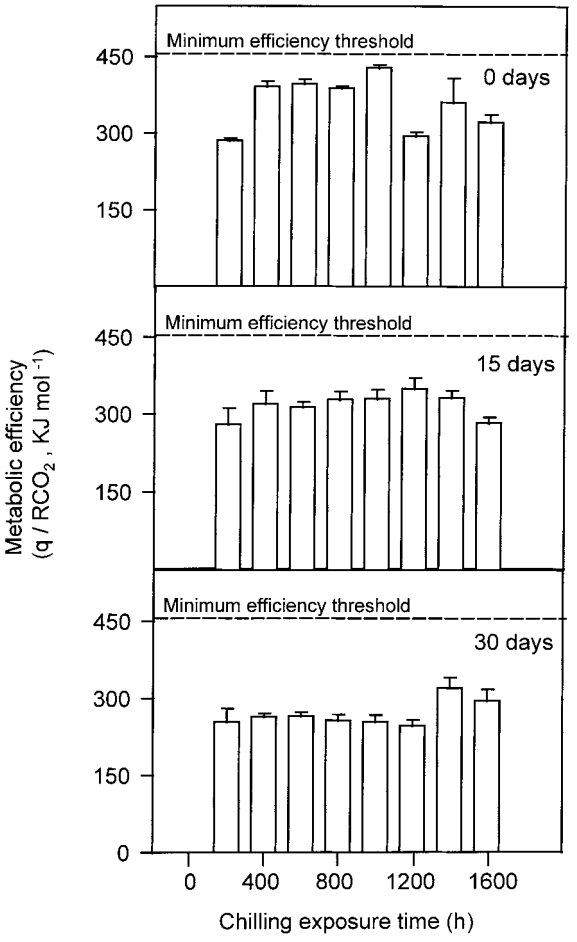

Fig. 3. Metabolic efficiency in vegetative buds in Golden Delicious apples at different chilling exposure and three forcing conditions at 25°C. Minimum efficiency threshold of 455 kJ mol^{-1} as reported in [6].

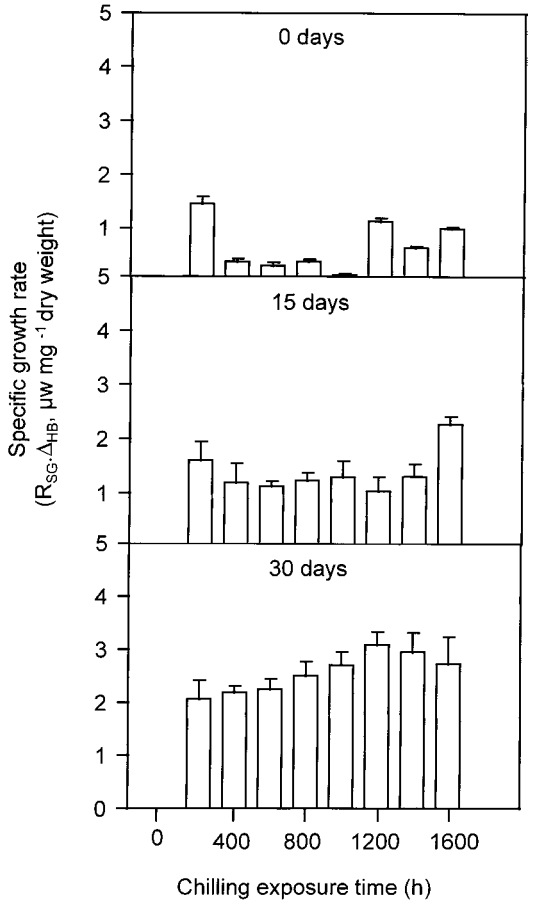

Fig. 4. Specific growth rate in vegetative buds of Golden Delicious apples at different chilling exposure and three forcing conditions at 25°C.

study, q/R_{CO_2} did not show any consistent pattern with the duration of chilling exposure, regardless the length of forcing period. However, the comparison among exposure to forcing conditions for every chilling treatment (0, 15 and 30 days at 25°C) did show that efficiency increases as bud metabolism increases (Fig. 3).

Plant tissue growth rate (R_{SG} Δ_{HB}) as shown in Fig. 4, results from calculations based on q, R_{CO_2} and q/R_{CO_2} [5]. The effect of chilling treatments on this variable is noticeable only after 30 days at 25°C, when average values increased from 2 to 3 µW mg^{-1} dw in a response pattern proportional to chilling duration.

3.2. Differential scanning calorimetry

As apple trees overcome dormancy, their metabolism becomes fast [2]. The scanning studies evaluated another aspect of metabolic response in vegetative buds of Golden Delicious, when exposed to different chilling periods. The isothermal calorimetry showed that the longer the exposure to forcing conditions, the higher the q and R_{CO_2} responses, especially after exposure to 30 days at 25°C. This forcing condition increases the rate of chemical reactions involved in different metabolic pathway by directly affecting E_a. Since plant metabolism represents a pool of closely related reactions, then estimations of E_a actually represents an apparent E_a [11]. A high E_a value

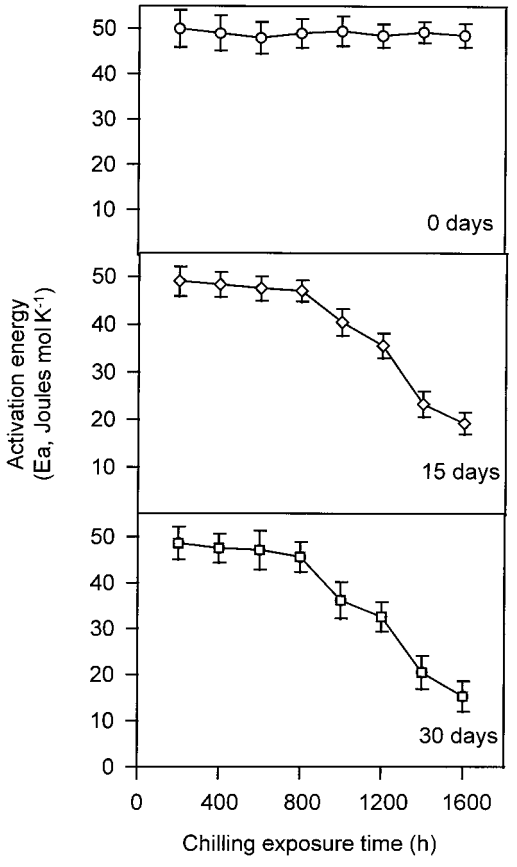

Fig. 5. Changes in the apparent activation energy of vegetative buds of Golden Delicious apples as a function of chilling exposure and length of forcing conditions.

indicates high energy requirements for reactions to take place [11,12]; therefore it implies slow metabolic processes, since reactions are more difficult to proceed forward [13].

Results shown in Fig. 5 demonstrate that at the beginning of forcing conditions, no significant differences were found among chilling treatments, with E_a values showing small deviations from 50 J mol^{-1} K. As trees were exposed to warm temperatures for longer periods, dramatic decreases in E_a values were observed, particularly in those treatments exceeding 1000 chilling hours. After 1000 h, E_a values decreased to 49.5, 40.5 and 36.2 J mol^{-1} K, after 0, 15 and 30 days at 25°C, respectively. In the same order, buds exposed to 1600 h decreased their E_a from 48.6 to 19.2 and to 15.3 J mol^{-1} K. Therefore, once chilling requirements are fulfilled, exposures to growing temperatures resulted in an increased metabolic rate, characterized by decreasing activation energy, which means that less energy is needed for reactions to take place.

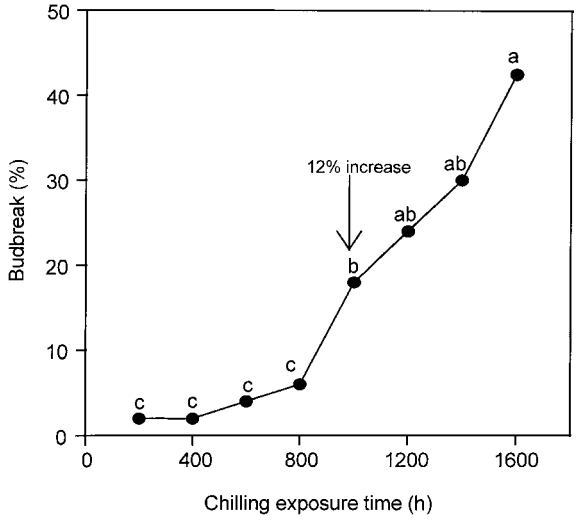

Fig. 6. Budbreak of vegetative buds of Golden Delicious apples as a function of chilling exposure and after 40 days at 25°C.

Budbreak responses in the greenhouse are shown in Fig. 6. After 1000 chilling hours, budbreak attained a significant increase of 12%. This sets a lower threshold of 1000 h at temperatures of 4°C for Golden Delicious vegetative buds to attain metabolic activity leading to a significant increase in budbreak. Below that threshold, buds may remain dormant as a result of insufficient chilling [14].

4. Conclusions

Metabolic activity of apple vegetative buds increase gradually as a function of cumulative chilling duration, followed by exposure to temperatures leading to growth. Metabolic heat rate values ranged from 2.5 μW mg^{-1} dw in buds without chilling exposure to 5 μW mg^{-1} dw in buds fully chilled and later exposed to warm conditions for 30 days.

CO_2 production rate of buds also doubled as a result of cumulative chilling and exposure to growing temperatures. Average values ranged from 8 to 17 mmol CO_2 mg^{-1} dw.

Within the same exposure periods to growing temperatures (i.e. 0, 15 or 30 days) no difference between chilling treatments was observed in metabolic efficiency. However, comparison of individual chilling treatments along the forcing period resulted in increased efficiency (i.e. decrease in q/R_{CO_2} values).

Specific growth rate ranged from 1.5 to 3.0 $\mu W\ mg^{-1}$ dw. It showed an increasing response, but only after buds were forced to 30 days at growing temperatures.

Cumulative chilling also resulted in decreasing activation energy of 29.8 and 33.3 $J\ mol^{-1}\ K$ after forcing for 15 and 30 days, respectively. In general, dormant vegetative apple buds increased their calorimetric responses after forcing for 15 and 30 days, but this was significant only when chilling equaled or exceeded 1000 h.

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