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Thermochimica Acta 457 (2007) 109–112
thermochimica acta
www.elsevier.com/locate/tca

# Respiratory response of apple buds treated with budbreaking agents

E. Carvajal-Millán*, A.A. Gardea, A. Rascón-Chu, J.A. Orozco, N. Ponce de León, J.A. Márquez-Escalante, A. Romo Chacón, V.M. Guerrero

*Centro de Investigación en Alimentación y Desarrollo, Unidad Cuauhtémoc, Apdo. Postal 781, Cuauhtémoc, Chihuahua 31570, Mexico*

Received 25 October 2006; received in revised form 22 February 2007; accepted 5 March 2007
Available online 12 March 2007

**Abstract**

Isothermal calorimetry was used to evaluate the effect of budbreaking agents (BBA) on metabolic rates $R_q$ and $R_{CO_2}$, metabolic efficiency ($R_q/R_{CO_2}$) and specific growth rate ($R_{SG}\cdot\Delta H_B$) in apple flower buds during late winter. Three BBA were tested (hydrogen cyanamide, HC at 1.0% (v/v); thidiazuron (1-phenyl-3-(1,2,3-thiadiazol-5-yl)urea), TDZ at 0.3% (v/v) and mineral oil, MO at 1% (v/v)). Water was used as a control. BBA application was performed at two $R_q$ rates (4.8 and 5.3 $\mu W\,mg^{-1}$ dw, corresponding to 30 and 15 days before budbreak, approximately). $R_q$, $R_{CO_2}$, $R_q/R_{CO_2}$ and $R_{SG}\cdot\Delta H_B$ were measured during 18 days at 3 day intervals under forcing conditions. Budbreak percentage was scored at the end of the experiment. Significant differences in the calorimetric parameters ($p \leq 0.05$) were found between BBA treatment and BBA application date. The highest $R_q$ (11.4 $\mu W\,mg^{-1}$ dw), $R_{CO_2}$ (32.2 mmol $CO_2\,mg^{-1}$ dw), $R_{SG}\cdot\Delta H_B$ (2.8 $\mu W\,mg^{-1}$ dw) and budbreak percentage (95%) values were found for HC treated buds when application was performed 15 days before budbreak. These samples showed the lowest $R_q/R_{CO_2}$ value (368 $kJ\,mol^{-1}$) confirming an increase in bud metabolic efficiency. These results indicate that HC is an effective BBA to overcome blooming problems associated with insufficient winter chilling on apples.


*Keywords:* Calorimetry; Metabolism; Apple buds; Budbreaking agents

## 1. Introduction

In northern Mexico, apple (*Malus domestica* Borkh) is produced under climatically marginal conditions, especially in terms of winter chilling. This region provides more than half of the national apple production, the most important cultivar being Golden Delicious. Golden Delicious satisfies its chilling requirements with 800–1000 chilling units (CU) defined as 1 h at 7.2 °C [1]. After this period starch will be converted back to sugars that provide buds with energy for development of flowers and leaves. Following ecodormancy, bloom stages are: silver point, green tip, half-inch green, tight cluster, first pink, king bloom, petal fall and fruit set [2].

Chemical budbreaking agents (BBA) have been used with success on apples to ensure a greater uniformity in budbreak. It is important to determine the metabolic responses of apple buds, while optimizing BBA application to overcome problems associated with insufficient winter chilling. A common difficulty in diagnosing the effect of BBA on apple budbreak is that available methods are time consuming and recommended actions derived from such information may be out of a time frame. Therefore, fast alternatives to optimize BBA application on apple buds are needed [3,4].

Calorimetric methods permit determination of the metabolic activity (heat rate, $R_q$ and $CO_2$ rate, $R_{CO_2}$) of plant tissue as functions of temperature, which allows a mathematical description of tissue growth rate ($R_{SG}\cdot\Delta H_B$) and efficiency for carbon substrate conversion ($R_q/R_{CO_2}$) [5–8]. Isothermal calorimetry provides a relatively rapid measurement. Chilling requirements of Golden Delicious apple leaf buds, the developmental stages of Golden Delicious and Red Delicious flower buds and the effect of BBA on dormant vegetative apple buds have been determined by isothermal calorimetry [3,4,9]. The effect of BBA application at different flower bud stages has not

* Corresponding author. Tel.: +52 158 12921; fax: +52 158 12921.
*E-mail address:* ecarvajal@cascabel.ciad.mx (E. Carvajal-Millán).

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doi:10.1016/j.tca.2007.03.004

previously been reported. The objective of this study was to evaluate the metabolic response of Golden Delicious flower buds to different BBA at two metabolic activity levels by isothermal calorimetry.

## 2. Experimental

Golden Delicious shoots (10–15 cm) with apical flower buds, were collected from a commercial orchard at two metabolic activity levels at midwinter (4.8 and 5.3 $\mu W\,mg^{-1}$ dw, which corresponds approximately to 30 and 15 days before budbreak), when the transition to the ecodormant stage is normally occurring. Shoots were wrapped in plastic bags containing wet paper to prevent dehydration during transportation to the laboratory. Golden Delicious shoots were sprayed with different BBA: (a) hydrogen cyanamide, HC at 1.0% (v/v), (b) thidiazuron (1-phenyl-3-(1,2,3-thiadiazol-5-yl)urea), TDZ at 0.3% v/v, c) mineral oil, MO at 1% (v/v) and water as a control. Shoots were transferred to an environmental chamber ($25 \pm 3\,^{\circ}C$) and 16 h photoperiod. At 3-day intervals, flower buds were randomly sampled and $R_q$ and $R_{CO_2}$ measurements made during 18 days. Budbreak was scored at the end of the experiment. A bud was considered as broken when the half-inch green stage was achieved.

Metabolic heat measurements were done with a CSC 4100 MC-DSC (Calorimetry Science Corporation, Lindon, UT) provided with four 1 $cm^3$ hastelloy ampoules.

From the shoots treated with BBA, nine buds were collected at sampling day. Three buds per ampoule were used, since they were enough to provide close to *a* 100 μW signal. Both $R_q$ and $R_{CO_2}$ measurements were done at 25 °C according to methods discussed elsewhere [6,7]. Isothermal data were baseline corrected and adjusted to sample dry weight. The experimental design was a completely randomized with six replicates. Data were analyzed with ANOVA and $R_q$, $R_{CO_2}$, $R_q/R_{CO_2}$, and $R_{SG}\cdot\Delta H_B$ means separation was according to Tukey ($p \leq 0.05$) (SAS 6.08 version) [10].

## 3. Results and discussion

### 3.1. Chilling units

For the commercial orchard used in this study, total CU record in 2006 was 750 (Utah model). On the other side, CU record at the two metabolic activity levels used in this study (4.8 and 5.3 $\mu W\,mg^{-1}$ dw) corresponded to 683 and 747. In this regard, Golden Delicious chilling requirements were not satisfied as needed from 800 to 1000 CU [11]. The latter confirms the requirement of BBA application to overcome blooming problems associated with insufficient winter chilling on apples in Northern Mexico.

### 3.2. Isothermal calorimetry

$R_q$ and $R_{CO_2}$ values showed a consistent positive response as a function of increased forcing time. A similar behavior was found for the different BBA treatments and the two BBA application dates (Figs. 1 and 2). In general, an increase in $R_q$ and $R_{CO_2}$ values was registered when BBA application was performed 15 days before budbreak. However, HC treated buds showed the highest $R_q$ and $R_{CO_2}$ values on both BBA application dates. Golden Delicious flower buds change from dormant stage to tight cluster (18 days forcing). These results followed a pattern

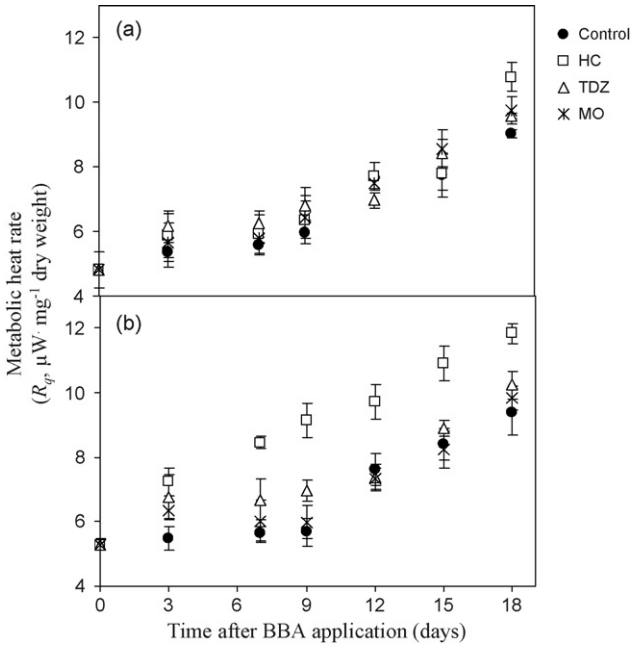


Fig. 1. Metabolic activity in flower buds of Golden Delicious apples treated with different BBA applied at 30 days (a) and 15 days (b) before budbreak and forced to grow at 25 °C for 18 days.

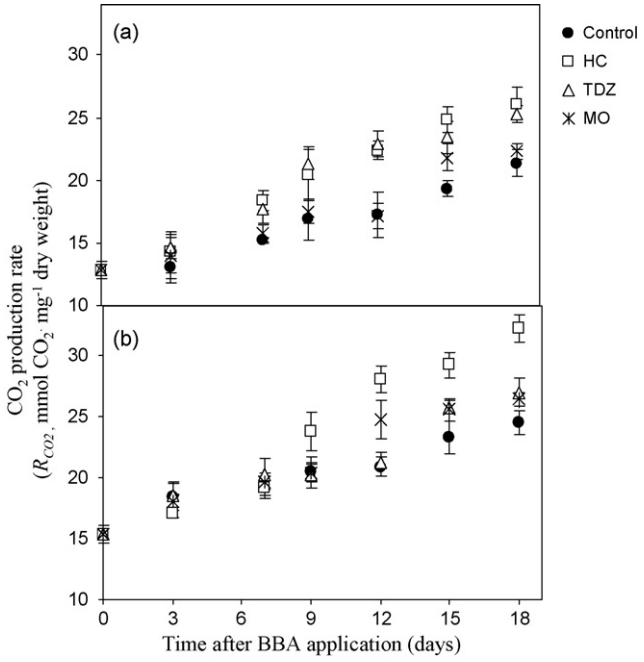


Fig. 2. $CO_2$ production rate in flower buds of Golden Delicious apples treated with different BBA applied at 30 days (a) and 15 days (b) before budbreak and forced to grow at 25 °C for 18 days.

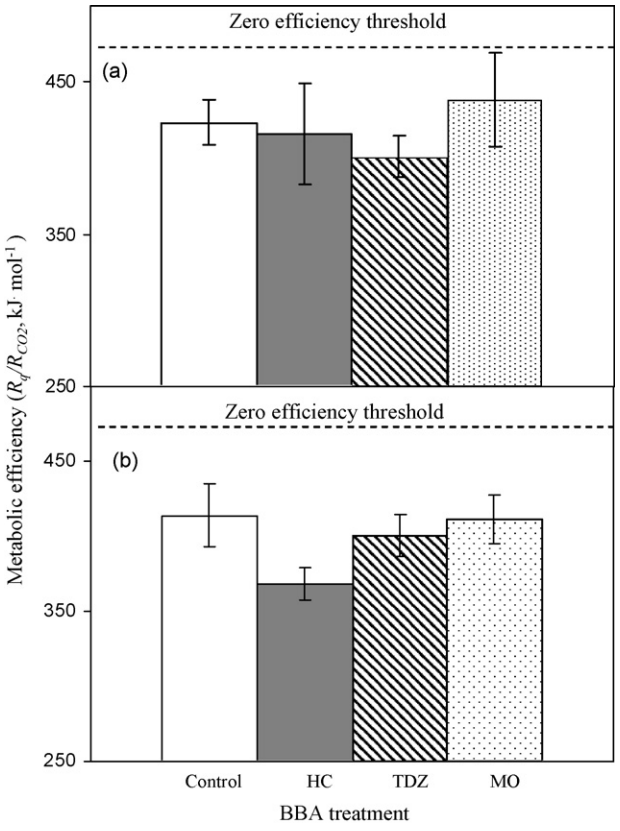


Fig. 3. Metabolic efficiency in flower buds of Golden Delicious apples treated with different BBA applied at 30 days (a) and 15 days (b) before budbreak and after 18 days at 25 °C.

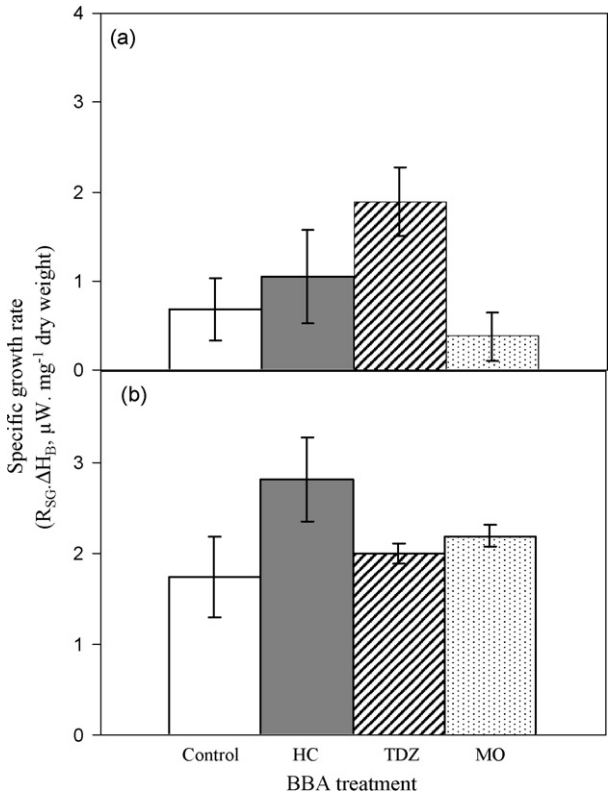


Fig. 4. Specific growth rate in flower buds of Golden Delicious apples treated with different BBA applied at 30 days (a) and 15 days (b) before budbreak and after 18 days at 25 °C.

similar to that reported elsewhere in Golden Delicious vegetative buds [3].

Although $R_q$ and $R_{CO_2}$ patterns look similar they show different aspects; their ratio is an indicator of substrate carbon conversion efficiency [6]. The higher the heat released the higher the ratio; therefore, metabolic efficiency decreases since less catabolic energy is used for tissue growth. In this study $R_q/R_{CO_2}$ did not show any consistent pattern during the forcing period (data not shown). However, after forcing conditions (18 days at 25 °C), no significant efficiency increase was registered for buds collected 30 days before budbreak (Fig. 3a). Conversely, buds collected 15 days before budbreak show a significant efficiency increase for HC treatment (Fig. 3b). HC treatment showed the lowest $R_q/R_{CO_2}$ ratio. The value representing zero efficiency of plant tissue is 455 kJ mol$^{-1}$ [5]. In this study all $R_q/R_{CO_2}$ values were under this efficiency threshold.

Plant tissue growth rate ($R_{SG}\cdot\Delta H_B$) results from calculations based on $R_q$, $R_{CO_2}$ and $R_q/R_{CO_2}$ [5]. Fig. 4 shows the plant tissue growth rate for all BBA treatments and both BBA application dates. In agreement with bud metabolic efficiency results discussed above, no consistent pattern on $R_{SG}\cdot\Delta H_B$ was found during bud forcing (data not shown). Nevertheless, $R_{SG}\cdot\Delta H_B$ values at the end of the experiment (after 18 days at 25 °C) showed a significant increase for HC treated buds (2.8 µW mg$^{-1}$ dw) when application was performed 15 days before budbreak (Fig. 4b).

The mechanism by which HC exerts its dormancy-breaking effect is not clear, but it has been shown to inactivate catalase in grape buds shortly after its application [12]. This decrease in catalase activity causes an increase in the level of $H_2O_2$ in bud tissue. The increased level of $H_2O_2$ might cause the activation of the pentose-phosphate pathway, and thus lead to dormancy termination, bud burst and rapid growth [13]. TDZ is a substituted phenylurea which has been found to mimic cytokinin-like activity releasing buds from dormancy of a wide variety of plant species [14]. The precise mechanism of action of TDZ is yet unknown; however, there are two hypotheses in this regard. It is possible that TDZ directly promotes growth due to its own biological activity in a fashion similar to that of *N*6-substituted cytokinins, or it may induce the synthesis and (or) accumulation of endogenous cytokinins [15]. Mineral oil also called dormant oil, is a highly refined petroleum oil that kills insects and their eggs by suffocating them. It is also applied in late winter or very early spring to improve bud breaking in several cultures [16]. MO increases the respiration rate of dormant buds of deciduous trees promoting dormancy release [17]. These differences in bud dormancy release mechanism for the three BBA tested in this study were reflected in the metabolic response of treated apple buds. The marked effect of HC treatment on apple buds could be explained by activation of bud respiration metabolism leading to a faster dormancy release.

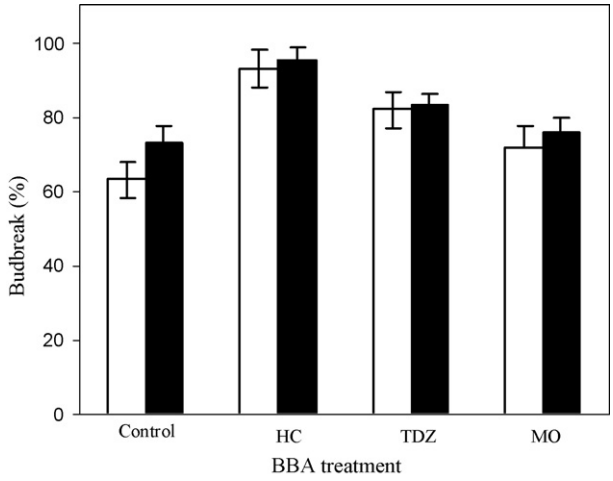


Fig. 5. Budbreak of flower buds of Golden Delicious apples treated with different BBA applied at 30 days (□) and 15 days (■) before budbreak and after 18 days at 25 °C.

### 3.3. Budbreak

In laboratory conditions, a significant increase in budbreak percentage was promoted by HC treatment on both BBA application dates. After 18 days forcing time HC treated buds reached values of 93 and 95 % when applied at 30 and 15 days before budbreak, respectively (Fig. 5). The latter values were 30 and 24% higher than controls, respectively. In these samples, bud phenophase was mostly tight cluster while for the others BBA treatments bud phenophase was uneven where half-inch green phenophase was predominant. Similar results were reported in Golden Delicious vegetative buds [3,4].

## Acknowledgements

This research was funded by Fundación Produce Chihuauha, UNIFRUT and CIAD.

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