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Metabolic activity of low chilling grapevine buds forced to break

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ABSTRACT

This research documented the metabolic changes associated with the use of budbreak promoter sprays in vineyards. The objective was to evaluate metabolic heat production (R_q) and respiratory (R_{CO_2}) rates and water content of *Vitis vinifera* L. cv. 'Flame Seedless' buds during dormancy, and after spraying with the budbreak promoter, hydrogen cyanamide (H_2CN_2), assessing its effect on the developmental response of buds through several phenological phases in the vineyard. The study was conducted in the winter from November 24, 2005 to February 8, 2006 using isothermal calorimetry at 25 °C. Phenological stages monitored were dormant bud, swollen bud, half-inch green, first-flat leaf and second-flat leaf. On December 23, when 97 chilling units had accumulated, H_2CN_2 (0.82 M divided into two applications of 0.47 and 0.35 M) was applied and compared with untreated controls. Metabolic heat rates significantly ($p \leq 0.05$) increased from endodormant buds to the half-inch green stage, from 2 to 33 $\mu W\,mg^{-1}$ dry weight (dw). This occurred from November 24 to January 15. Bud respiration rate showed a similar pattern, increasing from 3.7 to 346 nmoles $CO_2\,s^{-1}\,mg^{-1}$ dw, and bud water content increased from 2.2 to 5.6 $mg\,mg^{-1}$ dw. In a 27-day period after spraying, treated vines reached 50% budbreak, while controls reached only 7%. Phenological development was heterogeneous due to insufficient chilling, as well as to low temperatures following H_2CN_2 application. Changes in R_q and R_{CO_2} were detected 12 days in advance compared with changes in bud hydration.

1. Introduction

In deciduous species, the dormant phase of development allows winter survival; its induction and fulfillment are genetically controlled and are affected by diverse factors, including temperature and photoperiod [1,2]. Traditionally, accumulation of chilling units (CU) has been used as an indicator of dormancy fulfillment to estimate dates of budbreak and to determine the need for spraying chemical budbreak promoters. Grapevines require a chilling exposure, depending on genotype, ranging between 50 and 400 h at temperatures ≤7 °C for normal bud development [3,4]. Inadequate exposure to low temperatures can cause erratic and/or heterogeneous budding or a limited number of buds and clusters in different stages of development [3], therefore spraying budbreak promoters becomes a must. The problem turns even more complex in those locations where winters are warm, as in the case of Sonora, Mexico, since productivity is strongly conditioned by the level of budbreak and, unfortunately, chilling accumulation in each crop cycle is irregular and sometimes even negligible. Deciding whether budbreak promoters should be applied to dormant vines can be a problem since their efficiency and phytotoxicity depend on the stage and intensity of bud dormancy [5–7].

Until now the method used to estimate budbreak, chilling accumulation, is based on an environmental variable and does not consider the intensity of bud dormancy [8,9]. Chilling unit accumulation has been used in large part because of the absence of visible changes and the lack of endogenous markers that allow determination of bud dormancy stage [9]. However, even though no changes are visible, dormancy is a state physiologically and biochemically active, during which changes occur in bud water content, growth regulators levels and other compounds, although respiration rate is slow [10,11]. Indeed, the events that occur in the bud during this phase and the mechanisms controlling its progress and causing its termination continue to elicit questions [1,12]. It is necessary to find other methods to estimate bud metabolic activity in a complementary fashion, so as to determine the most appropriate moment for the application of growth promoters to improve budbreak and, therefore, productivity.

Calorimetry can measure the metabolic response of plant tissues as a function of temperature. This technique makes it possible

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to quantify metabolic heat (R_q) and CO_2 production (R_{CO_2}) rates [13,14]. Therefore, if respiration parameters can be modeled during dormancy, practical applications of calorimetry can be used to monitor its evolution, estimate cold requirements for different germplasms, and evaluate the effect of cultural practices on bud development [15]. The use of this methodology could set other bases for determining grape bud metabolic activity pattern in real time, which in turn, would serve as the basis for anticipating the activities necessary for induction of homogeneous bud development. Thus, the objective of our study was to evaluate the metabolic heat and respiration rates of 'Flame Seedless' grape buds during dormancy by isothermal calorimetry and to evaluate bud metabolic and phenological responses to application of the budbreak promoter hydrogen cyanamide (H_2CN_2).

2. Experiment

The plant material used was 5-year-old 'Flame Seedless' table-grape vines from a commercial vineyard in Pesqueira, Sonora (altitude 376 m, 29°23′NL, 110°56′WL). In the first part of the study, bud calorimetric characterization and water content were determined. In the second part, the effect of H_2CN_2 on budbreaking, budbreak kinetics and phenological phases of the buds was monitored.

2.1. Calorimetric characterization

Samples were taken every 4 days as of November 24, 2005, until January 15, 2006. The samples were protected with moist absorbent paper and cold gels to prevent dehydration and transferred immediately to the laboratory in an ice chest.

R_q and R_{CO_2} were determined by isothermal calorimetry with a differential multicell scanning calorimeter, model CSC 4100 (Calorimetry Science Corporation, Pleasant Grove, Utah), equipped with four 1-cm^3 metal cells. The fourth cell was left empty during measurements, as a reference. Once bud scales were removed, to prevent dehydration the buds were placed with the cut base in contact with the bottom of the cells where 50 μL of sterile water had been previously added [15]. In each cell, 9 buds (weighing a total average of 80 mg) were placed at random. Both R_q and R_{CO_2} measurements were made at 25 °C for 3500 s, data were baseline adjusted and recalculated on a dry weight basis [16]. Fresh and dry weights of buds without scales were used to calculate water content, expressed in mg H_2O mg^{-1} dry weight (dw). The latter was obtained after dehydration in a drying oven at 65 °C for 48 h.

2.2. Effect of H_2CN_2 on budbreak, budbreak dynamics and bud phenophases

Application date and dosage of hydrogen cyanamide (H_2CN_2) followed commercial practices: application on December 23 at a total dosage of 0.82 M, divided into two applications of 0.47 and 0.35 M with a difference of 6 h between applications. At application date only 97 CU had accumulated. An absolute control without H_2CN_2 was used.

For the budbreak kinetics study considering five phenological stages, vines were evaluated every 4 days as of December 26, 2005, until February 8, 2006. Monitoring was done visually, comparing bud development as reported elsewhere [17] as dormant bud, swollen bud, half-inch green, first-flat leaf and second-flat leaf. The experimental design was a completely randomized and an analysis of variance by sampling date was performed. When percentages values were used, the arcsine transformation was used. Means were compared by the Tukey test ($p \leq 0.05$). The statistical software SAS version 8.01 [18] was used. Mean standard errors were calculated to construct graphs.

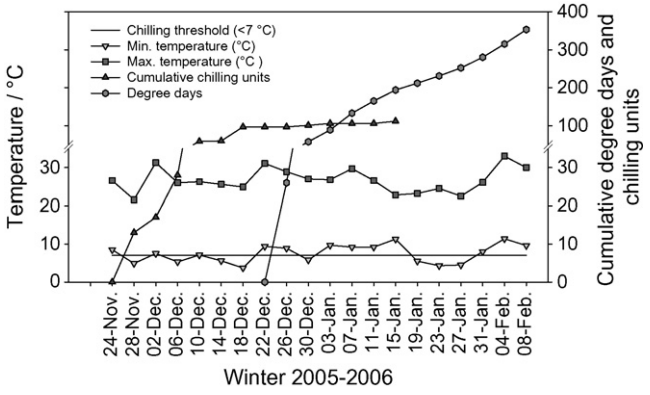

Fig. 1. Temperature regime during the evaluation period in Pesqueira, Mexico.

3. Results and discussion

3.1. Thermal regimen

Fig. 1 shows the prevailing climatic conditions during the period of this study. Low temperatures supplementing the chilling required to fulfill bud dormancy occurred from November 24 to December 6 and resulting in only 28 CU, in the following 4 days a cold front accounted for 57 CU. By December 22, before the H_2CN_2 application, a total of 98 CU were accumulated, no significant increases occurred afterward. These results show that chilling accumulation by buds was below the 200 CU reported for acceptable levels of budding [19]. After December 23, when H_2CN_2 was applied, minimum temperatures did not add anymore chilling, although by the end of January temperatures returned to below the physiological threshold. Those low temperatures, however, had an effect in delaying bud development, since growth is dictated by warm temperatures. They may have caused the heterogeneous budbreak observed. Nor did the thermal regimen caused sufficient heat accumulation needed to stimulate later development of the buds, once the chemical was used. Thus, daily average heat accumulation after H_2CN_2 application and up to the half-inch green stage was only 9 degree days (DD) for a total of 194 DD in this period. Nevertheless, reports on 'Pinot Noir' winegrapes found that a base temperature of 4 °C was required to induce budbreak and 7 °C for leaves to appear; concluding that the base temperature increases for successive phenological phases [20]; thus, the heat reported in our study is referenced only to the base temperature of 7 °C [3,4].

3.2. Metabolic heat (R_q)

Grape buds R_q pattern is presented in Table 1, with values ranging from 1.9 to 3.0 μW mg^{-1} dw from November 24 to December 26; by December 30 a significant increase to 3.4 μW mg^{-1} dw was recorded. Further increases peaked at 33.2 μW mg^{-1} dw on January 15, when the half-inch green stage was achieved, having accumulated 194 DD after H_2CN_2 spray. This progressive increase in the R_q pattern is similar to those reported in both 'Pinot Noir' grapevines and 'Golden Delicious' apple trees [21–23]. It was also observed that right after H_2CN_2 spray, on December 26, a slight, but temporary, significant decrease in R_q was recorded, with a value of 2.4 μW mg^{-1} dw. Since temperatures did not fall notably during the same period, it is likely that this was a response to the application itself. However, by December 30 bud R_q showed a significant

Table 1
Seasonal behavior of metabolic heat, respiration rate, water content and phenological development of 'Flame Seedless' grapevine buds.

Sampling date 2005–2006	Days after H_2CN_2	Metabolic heat rate, R_q ($\mu W\,mg^{-1}$ dw)	Respiration rate, R_{CO_2} (nmoles $CO_2\,s^{-1}\,mg^{-1}$ dw)	Water content (mg $H_2O\,mg^{-1}$ dw)	Predominant phenophase
24-November	–	2.0 gh	3.7 ef	2.15 cde	Dormant
28-November	–	1.9 gh	2.0 g	1.93 e	Dormant
2-December	–	2.3 fg	4.1 dfg	2.19 cde	Dormant
6-December	–	2.1 gh	2.1 g	2.14 cde	Dormant
10-December	–	1.7 h	6.0 d	2.20 cde	Dormant
14-December	–	2.7 ef	3.6 ef	2.25 cd	Dormant
18-December	–	3.0 de	5.3 de	2.34 cd	Dormant
22-December	–	3.0 de	2.9 fg	2.04 de	Dormant
26-December	3	2.4 fg	4.3 def	2.12 cdef	Dormant
30-December	7	3.4 d	6.0 d	2.15 cde	Dormant
3-January	11	4.7 c	10.0 c	2.37 c	Initial swelling
7-January	15	5.2 c	13.3 c	2.39 c	Swollen bud
11-January	19	20.7 b	43.7 b	4.16 b	Swollen/half-inch green
15-January	23	33.2 a	345.8 a	5.61 a	Half-inch green

Within columns, values followed by the same letters are statistically the same (Tukey ≤ 0.05). H_2CN_2 was sprayed at dosages of 0.47 + 0.35 M on December 23, 2005.

increase to 3.4 $\mu W\,mg^{-1}$ dw and kept increasing until reaching the maximum value mentioned above.

Even though no visible changes in buds were noticed during the first sampling dates, it was possible to detect increases in metabolic activity by calorimetry. The observed bud R_q is likely due to energy reserves consumption. At the beginning of the study, R_q rates were typically low since during endodormancy respiration rates are equally low and energy consumption is very low as well [24].

3.3. Respiration rate (R_{CO_2})

The R_{CO_2} pattern was very similar and concomitant to that of R_q. Table 1 shows that bud R_{CO_2} initially accounted for 3.7 nmoles $CO_2\,s^{-1}\,mg^{-1}$, and increased until reaching a peak at 345.8 mmoles $CO_2\,s^{-1}\,mg^{-1}$. Likewise, a decrease occurs after the H_2CN_2 application, but later increased significantly until reaching the half-inch green stage. From December 26 on, each date recorded a significant increase.

3.4. Bud water content

No significant differences were found in bud water content during the dormant stage, with values oscillating between 1.93 and 2.34 mg $H_2O\,mg^{-1}$ dw (Table 1). However, as the buds began to swell on January 3, hydration increased continuously from 2.37 up to 5.61 mg $H_2O\,mg^{-1}$ dw; this occurred from bud initial swelling to the half-inch green stage. The significant changes in hydration occurred 12 days after the changes in R_q and R_{CO_2}. Studies conducted on 'Pinot Noir' winegrapes found a highly significant increase in total water content in most of the phenological phases, reporting very similar values [25]. Considering that the first significant increase in hydration was detected on January 11, it is highly probable that the vascular connection between buds and the stem vascular system [26] may have occurred between the stages bud swelling and half-inch green. This is very relevant since it signals a period in which water demand is greatest.

3.5. Budbreak kinetics

Phenological bud development is presented in Fig. 2. Only 41% of the sampled buds did not develop normally; since 3% did not burst and 38% remained in the bud swelling stage; nevertheless 59% did develop normally. Considering January 23 as the date in which most of the treated buds opened, phenological evolution occurred in the following manner: 4.9% dormant buds, 41.4% swollen buds, 42.3% half-inch green, 8.2% in first-flat leaf, and 3.2% in second-flat leaf. This overlapping of stages is common [27]. Under normal development this may be an advantage when a freezing episode occurs, since buds lose cold hardiness as they develop. Therefore, it is feasible that late buds survive, since they are hardier. However, in this case, the large number of non-developing buds could point out that the H_2CN_2 stimulus was insufficient [28].

3.6. Dormancy interruption by effect of hydrogen cyanamide

H_2CN_2 induced a budbreak initiation 4 days earlier than controls (Fig. 3). H_2CN_2 caused significant differences in bud burst. Twenty-seven days after application, and 212 DD, treated buds achieved 50% budbreak, while controls reached only 7%. However, 47 days after application, and 353 DD, bud opening achieved 59% in treated buds, while only 8% in controls. The latter is a clear indication that the amount of chilling was clearly insufficient to satisfy this cultivar dormancy requirements. However, the budbreak percentage promoted by H_2CN_2 could have been affected by low temperatures occurring after application, reducing its effectiveness [29]. The regression analysis shows that in the rapid response phase,

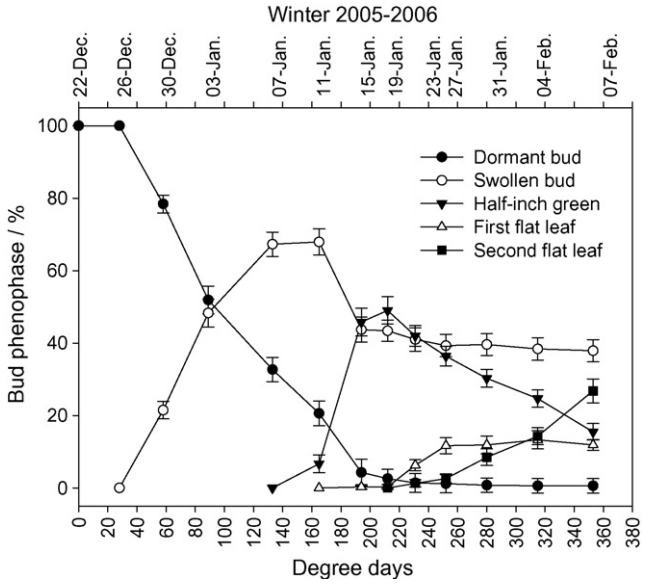

Fig. 2. Budbreak dynamic of 'Flame Seedless' grapevines in five phenological stages of development, shown as percentage of the total population. Each mean represents the average of 30 replications ± standard error.

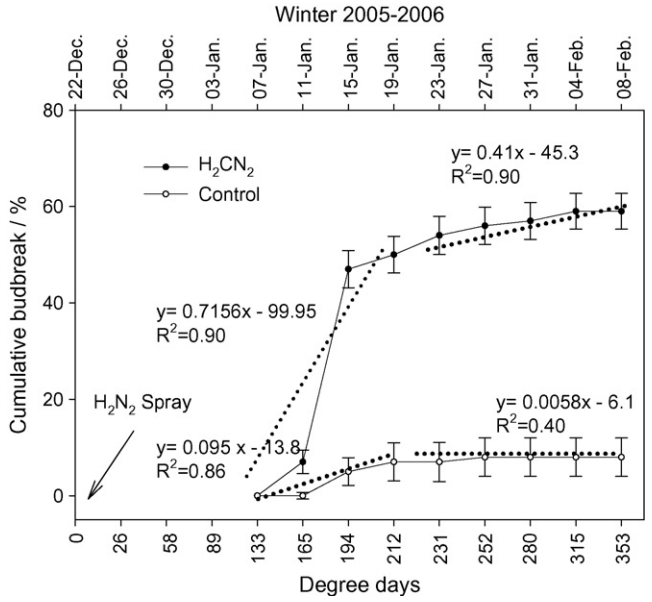

Fig. 3. Budbreak response to hydrogen cyanamide (H_2CN_2) by sprayed and unsprayed 'Flame Seedless' grapevines. Two regression analyses were performed on each data set, showing their parameters and corresponding to their fast and slow opening rates. Each mean represents the average of 20 replications ± standard errors.

treated buds opened at a rate over seven times faster than control buds (β values of 0.716 vs. 0.095). Nonetheless, after 212 DD the response changed, while treated buds opening slowed down to a rate of 0.41 buds/DD, control buds hardly opened at all. As to the effect of H_2CN_2 on earlier and more abundant budbreak, the results are similar to those reported by other authors [30,31]. However, in our work the number of open buds was below the desired minimum of 75% [4]. For example in studies conducted on 'Perlette' grapevines, H_2CN_2 application at concentrations of 0.25 and 1.25 M increased budbreak by 72 and 83%, respectively [32].

4. Conclusions

Metabolic heat in 'Flame Seedless' grape buds increased gradually during budbreak. Changes found in the interval between the stages of bud swelling and half inch green were 2–33 $\mu W\,mg^{-1}$ dw in R_q. While R_{CO_2} increased from 3.7 to 346 nmoles $CO_2\,s^{-1}\,mg^{-1}$ dw, concomitantly water content increased from 2.2 to 2.6 $mg\,mg^{-1}$. These changes were favored by H_2CN_2 sprays. Under field conditions cyanamide treated buds opened 4 days earlier than controls; treated buds also reached 50% budbreak 23 days after application, while budbreak in controls was only 7% for the same period. However, total budbreak was lower then the desired minimum of 75% because buds were in uneven stages when H_2CN_2 was applied. Development of the phenological phases was heterogeneous due to insufficient chilling before application and to low temperatures occurring once budbreak started.

Calorimetric techniques detected buds responses to H_2CN_2 application and provided a tool for prediction of the date of initial budbreak, once the buds were stimulated. Changes in R_q and R_{CO_2} were detected 12 days before changes in bud hydration. This is an added tool in budbreak management decision-making.

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References

[1] M.A. Faust, A. Erez, L.J. Rowland, S.Y. Wang, H.A. Norman, HortScience 32 (1997) 623–629.
[2] F.J. Pérez, W. Lira, J. Plant Physiol. 162 (2005) 301–308.
[3] G.F. Gil-Salaya, Fruticultura: El Potencial Productivo, Segunda Edición, Alfaomega, México, 1999.
[4] N.K. Dokoozlian, HortScience 34 (1999) 1054–1056.
[5] A. Erez, S. Lavee, J. Am. Soc. Hort. Sci. 96 (1971) 711–714.
[6] J.H. Siller-Cepeda, L.H. Fuchigami, T.H.H. Chen, Plant Cell Physiol. 33 (1992) 867–872.
[7] B.W. Wood, J. Am. Soc. Hort. Sci. 118 (1993) 690–693.
[8] L.H. Fuchigami, M. Wisniewski, HortScience 32 (1997) 618–622.
[9] R.L. Arora, L.J. Rowland, K. Tanino, HortScience 38 (2003) 911–921.
[10] L.C.T. Young, J.T. Winneberger, J.P. Benet, J. Am. Soc. Hort. Sci. 99 (1973) 146–149.
[11] L.E. Powell, HortScience 22 (1987) 845–850.
[12] L.D. Nooden, J.A. Weber, Dormancy and Developmental Arrest, Academic Press Inc., New York, 1978.
[13] L.D. Hansen, D.K. Taylor, B.N. Smith, R.S. Criddle, Russ. J. Plant Physiol. 43 (1996) 691–697.
[14] R.S. Criddle, R.W. Breidenbach, D.R. Rank, M.S. Hopkin, L.D. Hansen, Thermochim. Acta 172 (1990) 213–221.
[15] A.A. Gardea, E. Carvajal-Millán, J.A. Orozco, V.M. Guerrero, J. Llamas, Thermochim. Acta 349 (2000) 89–94.
[16] R.S. Criddle, A.J. Fontana, D.R. Rank, D. Paige, L.D. Hansen, R.W. Breidenbach, Anal. Biochem. 194 (1991) 413–417.
[17] M.G. Mullins, A. Bouquet, L.E. Williams, Biology of the Grapevine, Cambridge University Press, 1992.
[18] SAS, Version 8.01, SAS Institute Inc., 1999.
[19] N.K. Dokoozlian, L.E. William, R.A. Neja, HortScience 30 (1995) 1244–1247.
[20] M.W. Moncur, K. Rattigan, D.H. Mackenzie, G.N. Mclintry, Am. J. Enol. Vitic. 40 (1989) 21–26.
[21] A.A. Gardea, Y.M. Moreno, A.N. Azarenko, P.B. Lombard, L.S. Daley, R.S. Criddle, J. Am. Soc. Hort. Sci. 119 (1994) 756–760.
[22] E. Carvajal-Millán, F. Goycoolea-Valencia, V. Guerrero-Prieto, J. Llamas-Llamas, A. Rascón-Chu, J.A. Orozco-Avitia, C. Rivera-Figueroa, A.A. Gardea, Agrociencia 34 (2000) 543–551.
[23] J. Llamas-Llamas, E. Carvajal-Millán, A. Orozco-Avitia, A. Rascón-Chu, A. Romo-Chacón, V. Guerrero-Prieto, V.A. González-Hernández, A.A. Gardea-Béjar, Rev. Fitotec. Mex. 25 (2002) 411–418.
[24] W. Lira, M.SC. Tesis, Universidad de Chile, 2006.
[25] A.A. Gardea, Ph.D. Thesis, Oregon State University, 1992.
[26] K. Esau, Anatomy of Seed Plants, 2nd ed., John Wiley and Sons, New York, 1977.
[27] A.A. Gardea, M.S. Thesis, Oregon State University, 1987.
[28] A. Erez, Acta Horticulturae 385 (1995) 81–95.
[29] A. Erez, HortScience 22 (1987) 1240–1243.
[30] M. Angulo, M.E. Wisniewski, L.J. Rowland, Rev. Fitotec. Mex. 3 (1992) 75–86.
[31] E.J. Pires, M.M. Terra, C.V. Pommer, R.S. Passos, V. Nagai, G.M.B. Ambrosano, Acta Horticulturae 395 (1995) 169–176.
[32] Y. Shulman, G. Nir, L. Fanbersetin, S. Lavee, Scientia Horticulturae 19 (1983) 97–104.