Changes in Metabolic Properties of Grape Buds during Development

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Abstract. The pattern of respiratory changes during dormancy and development of 'Pinot noir' primary grape (*Vitis vinifera* L.) buds through the broken-bud stage was determined using differential scanning calorimetry. Metabolic heat rates [$\mu\text{J}\cdot\text{s}^{-1}\cdot\text{mg}^{-1}$ dry weight (dw)] decreased during endodormancy and increased during ecodormancy and bud development. Respiration of ecodormant buds was at its minimum ($\approx 1 \mu\text{J}\cdot\text{s}^{-1}\cdot\text{mg}^{-1}$ dw) in January. Respiration steadily increased, beginning in February and continuing as buds passed through the four developmental stages: ecodormant, initial swelling, fully swollen, and broken bud. Heat of metabolism increased from 5 to 18, 28, and 29 $\mu\text{J}\cdot\text{s}^{-1}\cdot\text{mg}^{-1}$ dw as the buds developed from ecodormant to broken-bud stage. Carbon dioxide evolution from respiring buds increased from 15.9 to 22.2, 57.9, and $68.3 \times 10^{-6} \mu\text{mol}\cdot\text{s}^{-1}\cdot\text{mg}^{-1}$ dw as bud development advanced. Metabolic inefficiency, as quantified by heat (kJ) released per CO_2 (mol) produced, was lowest in ecodormant buds, then increased at initial bud swelling and declined again in fully swollen and broken buds. The temperature coefficient of metabolism (μ) [apparent activation energy (E_a)/gas constant (R)] was lower at low temperatures (5 to 15°C) in ecodormant buds than at the three more advanced stages. At higher temperatures (20 to 35°C), buds at the ecodormant and initial swelling stages had a higher μ than the fully swollen and broken buds. These results suggest there may be a major change in metabolic pathways between the ecodormant bud stage and bud swelling.

Respiration of buds from deciduous tree species follows seasonal patterns, decreasing during the fall and increasing again by the end of winter (Westwood, 1993). Ross (1985) concluded that, in general, termination of dormancy resulted in enhanced respiratory activity. Chilling is required to initiate the rise in respiration rate of maple buds and some other plants (Pollock, 1960). Young (1990) demonstrated that, during dormancy, chilling affected the respiratory response of apple trees to different forcing temperatures.

Criddle et al. (1991), recognizing that heat production is an inevitable by-product of all metabolic processes, used calorimetry to determine heat evolution as a general measure of metabolic activity of plants. Isothermal and differential scanning calorimetry (DSC) are noninvasive methods for studying heat evolution in living cells (Loike et al., 1981). These techniques have been used in plant physiology to determine the effects of temperature and O_2 depletion on tomato and carrot cell cultures (Criddle et al., 1988), measure the heat rate of dormant and developing buds and predict long-term growth rates of larch clones (Hansen et al., 1989), define suitable storage conditions to avoid spoilage of fresh-cut pineapple (Iversen et al., 1989), evaluate the physical state of water in pea and soybean cotyledons (Vertucci, 1990), measure aerobic cell metabolism in unstirred cell cultures (Fontana et al., 1990), study the responses of tomato cells to thermal extremes (Breidenbach et al., 1990), and determine high and low temperature metabolic inactivation of tomato cells (Rank et al., 1991).

Simultaneous measurements of metabolic heat production, O_2

consumption, and CO_2 evolution were proposed by Criddle et al. (1990) to determine the efficiency of plant metabolism. Heat rates per O_2 or CO_2 and measurements of metabolic efficiency are useful methods to quantify changes in metabolic pathways active in plant tissues (Criddle et al., 1990, 1991).

A theoretical basis for using apparent activation energy (E_a) in studies of biological systems has been discussed elsewhere (Johnson et al., 1974; Montgomery and Swenson, 1976). Apparent E_a is determined using the Arrhenius equation, the energy difference between reactants and the transition state. The higher its value, the more energy is required, and the greater the temperature dependence of the reaction. Apparent E_a values differ among plant species, among individual plants of the same species, and with season and growth stage of plants. Apparent E_a is, therefore, an additional parameter for monitoring physiological changes during growth. For example, apparent E_a decreased in apple trees approaching budbreak, once chilling was satisfied (Young, 1990).

Johnson et al. (1974) suggested using the temperature coefficient of metabolism of plants (μ) (i.e., apparent E_a/R , R = gas constant) when considering the effects of temperature on enzyme rates and catalytic efficiencies. These authors found the commonly used Q_{10} value much less useful. However, as pointed out by Amthor (1989) and others, this information is useful for comparing plant responses and is included here to compare results with those found in the literature. The temperature coefficient, μ , can be used to recalculate Q_{10} over any temperature range, as it generates in a single number the nonlinear relationship between each response and temperature. This nonlinear relationship of μ is a useful criterion to explain spatial distribution of temperate plant species in response to environmental conditions (Anekonda, 1992).

In this study, we characterize changes in 'Pinot noir' grape bud metabolic heat during endo- and ecodormancy (Lang et al., 1987) by a calorimetric method. Changes in metabolic heat production (q_{met}), CO_2 evolution, metabolic efficiency, μ , and Q_{10} s at several temperatures were determined for four stages of bud development: ecodormant, initial swelling, fully swollen, and broken bud.

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Materials and Methods

Plant material. To measure seasonal changes in the heat of bud metabolism, endodormant buds were sampled from mid-September to November 1990 and from January to March 1991 (ecodormant buds) from either 1-year-old nursery 'Pinot noir' grape vines or an 8-year-old mature vineyard at the Lewis-Brown Research Farm near Corvallis, Ore. For budbreak studies, cuttings were collected from the vineyard in late February 1991. The cuttings were covered with wet sawdust and kept ecodormant in a 4C room until used.

Forcing conditions. Five-node cuttings were forced to break at 2-day intervals in a 20C greenhouse, at times indicated later, with the basal ends of the cuttings immersed in water. This water was changed every other day. The same procedure was repeated on 31 May 1991, except that a growth chamber was used and the environmental conditions were 20C and an 18-h photoperiod.

Calorimetric and respiration (CO_2) measurements. Heat of metabolism was measured in a differential scanning calorimeter (model 4207; Hart Scientific, Pleasant Grove, Utah). The instrument has a baseline noise of $\pm 1 \mu\text{W}$ and a working range of -30 to 110C . Temperature around the DSC chamber was maintained at 15C with a refrigerated circulating bath (model 9500; Fisher Scientific, Pittsburgh) using 30% ethylene glycol as refrigerant. A flux of Ar was used to prevent moisture condensation in experiments below room temperature. Samples were measured in 1.0-ml hasteloy ampules with removable lids. Isothermal experiments measured heat rates at 25C for 3000 sec; data were collected at 20-sec intervals. Heat rates were expressed on a dry-weight basis. Carbon dioxide evolution and metabolic heat rate were measured and metabolic efficiency was calculated according to Criddle et al. (1990). Carbon dioxide evolution was quantified by measuring the exothermic reaction of carbonate formation with 0.4 M NaOH in the ampule and subtracting the heat generated by the bud in the absence of NaOH. The NaOH was placed in a small glass vessel to ensure that the bud did not contact the base. The inefficiency of energy metabolism was measured as the metabolic heat rate divided by the rate of CO_2 production.

Scanning experiments measured metabolic rates over the temperature range from 1 to 60C at a rate of 7C/h with data acquisition at 20-sec intervals (Hansen and Criddle, 1990). Metabolic heat rates were expressed on a fresh-weight basis. Arrhenius plots of the natural log of heat rates vs. the reciprocal of absolute (K) temperature ($\times 1000$) were used to determine apparent E_a and μ at 5C temperature intervals from 5 to 40C (Fontana et al., 1990; Johnson et al., 1974; Montgomery and Swenson, 1976; Nobel, 1991; Young, 1990). Q_{10} values for the intervals 10 to 20 , 20 to 30 , 30 to 40 , and 40 to 50C were calculated directly from heat readings (Young, 1990).

Sample preparation. Primary buds were excised from cuttings and fresh weights were recorded. The excised buds were stored briefly (≈ 5 min) on moist filter paper in a closed petri dish before the experiment. During the dormant season and the ecodormant stage in the budbreak study, the sample size was five buds per ampule. Since bud development resulted in increased metabolic heat rates, sample size was adjusted downward in the later stages of the budbreak study so that the total heat output per ampule remained $\approx 100 \mu\text{W}$. To avoid dehydration, $50 \mu\text{l}$ of sterile double-distilled water was added to the bottom of the ampule. Since scanning experiments lasted an average of 11 h, the ampules were flushed with O_2 before closing to avoid anaerobic conditions. After the scans, buds were dried and weighed.

To study the seasonal changes in bud respiration of endo- and

ecodormant buds, canes with their attached buds were harvested from the field and brought to the laboratory. Endodormant buds were sampled from September until November and ecodormant buds were sampled from January to March. Buds had not yet begun to swell when sampling ended in March. The buds were excised from the canes and heat of metabolism was measured at 25C .

For a preliminary bud development study, ecodormant cuttings were sampled on 26 Jan. 1991 and forced in a 20C greenhouse. Buds were excised at 2-day intervals for 10 days and heat of metabolism was measured isothermally at 25C . During the last week in February, when buds had received >1000 chilling hours, canes were collected from the field and stored in a 4C chamber. Cuttings were removed from storage on 20 Apr. and 15 May 1991 and forced as described above. Data were compared from the three dates and found to be similar; therefore, only data from 20 Apr. 1991 are reported here.

The four bud developmental stages were used later in isothermal determinations of the heat of metabolism (q_{met}) and CO_2 evolution rates and the calculation of metabolic efficiency ($q_{\text{met}}/\text{CO}_2$) (Criddle et al., 1990). Buds were excised at the appropriate stage from cuttings that had been removed from 4C storage on 31 May and 25 Aug. 1991 and forced. Another set of buds was scanned from 1 to 60C and μ and Q_{10} values were calculated. Only the data from 25 Aug. 1991 are reported.

Statistical procedures. Means of metabolic heat rates were calculated for each sampling date with six replications per date. During endodormant (September to November 1990) and ecodormant periods (January to March 1991), mature and young vines were sampled on six and eight dates, respectively. Metabolic heat rate was assessed on five randomized replications after 0, 2, 4, 6, 8, and 10 days under forcing conditions as treatments. Significance of metabolic heat rates, CO_2 evolution rates, and metabolic efficiency for the four bud developmental stages were determined by analysis of variance using a completely randomized design with nine replications. Mean separation was by Fisher's protected least significant difference. The μ and Q_{10} analyses and mean values were calculated from a minimum of three replications. Only statistically significant differences in mean values are discussed.

Results

The transition of 'Pinot noir' grape buds from the endodormant to the ecodormant stage was achieved by mid-November 1990 for young and mature plants when chilling requirements had been satisfied (Fig. 1) (Gardea, 1992). The pattern of bud heat of metabolism during that period is presented in Fig. 1A. A progressive decrease in metabolic heat of endodormant buds was observed in the fall. The active growth of young nursery vines in late fall is reflected in their higher heat of metabolism. The heat of metabolism of ecodormant buds (Fig. 1B) began to increase steadily starting in February 1991. Since budbreak began during the second week in April, the increased metabolism began well in advance of budbreak and long before any morphological change was visible.

The above results on ecodormant buds were confirmed with ecodormant buds stored at 4C then forced to break on 20 Apr. 1990 (Fig. 2). The buds stored at 4C respired at rates similar to those observed in field-grown samples in January. When forcing conditions were imposed, a 4-day lag period was followed by an exponential increase in metabolic heat rate. Broken buds respired at a rate of $3.1 \mu\text{J}\cdot\text{s}^{-1}\cdot\text{mg}^{-1} \text{ dw}$ and, after 10 days under forcing conditions, growing shoot tips respired at $49 \mu\text{J}\cdot\text{s}^{-1}\cdot\text{mg}^{-1} \text{ dw}$.

The increase in respiration of developing buds was similar

Fig. 1. Chilling hour accumulation and seasonal variation in metabolic heat rate of 'Pinot noir' primary grape buds sampled from young and mature vines during the endodormant period (September to November) in 1990 (A) and the ecodormant period (January to March) in 1991 (B). Bars represent $\pm$ SE ($n = 6$).

Fig. 2. Metabolic heat rate of ecodormant 'Pinot noir' primary grape buds forced to break at 20°C in greenhouse. Bars represent $\pm$ SE ($n = 5$).

when a growth chamber was used for forcing conditions (Fig. 3). The increases in metabolic heat and CO_2 evolution rates were not of the same magnitude. The metabolic heat rates of ecodormant, initial swelling, fully swollen, and broken-bud stages were 5, 18, 28, and 29 $\mu\text{J}\cdot\text{s}^{-1}\cdot\text{mg}^{-1}\text{ dw}$, respectively (Fig. 3). Carbon dioxide evolution also increased as buds developed. During the transition from ecodormant to initial swelling, there was a 40% increase in CO_2 evolution, while metabolic heat rose 260%. Thus, heat loss per CO_2 evolved increased significantly at the swelling stage. Metabolism of ecodormant buds was low and had a relatively low $q_{\text{met}}/\text{CO}_2$ value. As bud development proceeded, the $q_{\text{met}}/\text{CO}_2$ ratio increased to 810, then decreased thereafter.

In general, μ , calculated at 5C intervals, decreased progressively as temperature increased (Fig. 4). Ecodormant buds at low temperatures (5 to 15°C) had the lowest μ values. Between 10 and 20°C, initially swelling buds had the highest μ values, followed by fully swollen, broken buds, and ecodormant buds. As the temperature increased, the two most advanced bud stages had similarly low μ values, while ecodormant and initially swollen buds were higher. At the highest temperatures, >35°C, the μ value was the same for all four stages.

Fig. 3. Changes in metabolic heat rate, CO_2 evolution heat rates, and metabolic efficiency ($q_{\text{met}}/\text{CO}_2$) of 'Pinot noir' primary grape buds forced at 20°C in a growth chamber at four developmental stages. Mean separation by Fisher's protected LSD, $P \leq 0.05$. Each data point represents an average of nine replications.

Changes in Q_{10} values depended on the temperature range and bud developmental stage (Fig. 5). For grape buds at 10 to 20°C, the Q_{10} values were 2.81, 3.88, 3.19, and 3.63 for the four respective developmental stages (Fig. 5A). In the 20 to 30°C interval (Fig. 5B), Q_{10} values were lower than at 10 to 20°C and decreased as the buds developed, in agreement with scanning studies at 15 to 35°C. Scanning calorimetry showed that the maximum metabolic rate was reached at 30 to 40°C; however, the rate of increase with

Fig. 4. Temperature coefficient of metabolism (μ) of forced 'Pinot noir' primary grape buds calculated at 5°C intervals from 5 to 40°C during four developmental stages. Bars represent $\pm$ SE ($n = 3$).

temperature decreased and lower Q_{10} values were observed (Fig. 5C). These lower Q_{10} values seem to be a result of temperature stress. At 40 to 50°C, respiration rates decreased quickly and irreversibly. Buds were assumed dead irrespective of developmental stage because of Q_{10} values <1.00 (Fig. 5D).

Discussion

Seasonal variation in metabolic activity of grapevines follows a pattern similar to those reported for other species (Westwood, 1993). It decreases during the endodormant stage and increases during the ecodormant stage (Fig. 1). Ecodormant buds respire at a low rate, as shown by their low metabolic heat and CO_2 evolution rates (Figs. 2 and 3). Yet, based on the $q_{\text{met}}/\text{CO}_2$ value, metabolism seems highly efficient. The initial swelling stage was characterized by increasing metabolic heat and respiratory activity, a large increase in $q_{\text{met}}/\text{CO}_2$, and a decrease in metabolic efficiency. Respiration increased up to the broken bud stage. $q_{\text{met}}/\text{CO}_2$ decreased again at fully swollen and broken bud stages, indicating an increase in metabolic efficiency.

The metabolism of ecodormant buds depends less on temperature at the lower temperatures when compared to the more advanced developmental stages (Fig. 4). These data indicate that the metabolic pathways active in ecodormant buds at low temperatures are possibly different than initially swelling, fully swollen, and broken buds. At the intermediate temperatures (20 to 30°C), buds just beginning to swell and ecodormant buds had similarly low μ values, while μ was similar and higher for fully swollen and broken buds. These results signify comparable temperature dependence of metabolism between the two earlier and two later developmental stages. Also, these results partially agree with those of

Fig. 5. Temperature coefficient of respiration (Q_{10}) of forced primary grape buds at four developmental stages and four temperature intervals: 10 to 20°C (A), 20 to 30°C (B), 30 to 40°C (C), and 40 to 50°C (D). Mean separation by Fisher's protected LSD, $P \leq 0.05$. Each data point represents an average of at least three replications.

Young (1990), who concluded that apparent E_a was lower during active apple bud growth than in dormancy. One might hypothesize from these data that a transition in the metabolic pathways at lower temperatures is occurring between the ecodormant and the initial swelling stage. At the highest temperature ranges, all stages had similar μ values.

During budbreak, grapevines were highly responsive to temperatures of 10 to 20°C. Q_{10} values of respiration are often reported to be near 2.0 for plants in physiologically relevant temperature ranges (Amthor, 1989). Values >2 are common at low temperatures (Young, 1990) and may explain the sensitive response of grapevines to low temperatures. At temperatures $>20^\circ\text{C}$, vines were less responsive (Figs. 4 and 5), and $>40^\circ\text{C}$ buds began to die.

Two major causes of the large $q_{\text{met}}/\text{CO}_2$ increases between the ecodormant and initial bud swelling stage could be an inefficiency in energy coupling or a major change in metabolic pathways. It seems likely that both may occur during this period. For example, if a pathway such as the glyoxylate cycle is important in budbreak, as it is in germinating seeds (Goodwin and Mercer, 1983), O_2 is required to oxidize NADH and produce FADH_2 . However, these reactions do not generate CO_2 . Oxidative reactions are the major source of heat production in the cell. Thus, tissues in which the glyoxylate cycle pathway is very active may generate significant heat but produce little CO_2 , thus, $q_{\text{met}}/\text{CO}_2$ may be high. The change in $q_{\text{met}}/\text{CO}_2$ during bud development may reflect the change predominantly to carbohydrate metabolism through glycolysis and oxidative phosphorylation. The value of $424 \text{ kJ}\cdot\text{mol}^{-1} \text{ CO}_2$ obtained at the broken-bud stage is consistent with glycolytic metabolism at this stage. The Gibbs free energy released when glucose is oxidized to CO_2 and H_2O is $479 \text{ kJ}\cdot\text{mol}^{-1}$ of C (Nobel, 1991). We have shown in previous research that it is common for plant tissues to lose efficiency in response to nutrient or environmental stress factors (Breidenbach et al., 1990; Criddle et al., 1990). In such tissues, $q_{\text{met}}/\text{CO}_2$ and $q_{\text{met}}/\text{O}_2$ increase greatly. At the same time, growth rate decreases significantly. Thus, heat release per milligram of tissue biosynthesis becomes large, an obvious indication of energy-use inefficiency. This response may also be occurring in buds as they begin to break dormancy.

The metabolic efficiency data and the μ values calculated for low temperatures for the different grape-bud developmental stages suggest that there may be a major change in metabolic pathways between the ecodormant bud stage and bud swelling. Future research should focus on identifying the predominant metabolic pathways present during these distinct stages.

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