AN ABSTRACT OF THE THESIS OF

Alfonso A. Gardea for the degree of Doctor of Philosophy in Horticulture presented on March 20, 1992.

Title: Water Partitioning and Respiration Activity of Dormant Grape Buds

Abstract approved: _____

Porter B. Lombard

Dormancy of 'Pinot Noir' grapevines was characterized. Primary buds required ca 300 cumulative chilling hours (CCH) to initiate the transition from endodormancy to ecodormancy, which occurred in mid November. Additional chilling, up to 1000 CCH, improved budbreak. Changes in bud water content were estimated by gravimetric and spectroscopic methods. Total, intra-, and extracellular water were measured gravimetrically. The total water and the intracellular fraction increased during the ecodormant period.

A method to evaluate bud water status by proton-nuclear magnetic resonance was developed. Grape buds present anisotropic behavior. Using this method, water content was partitioned into free and bound fractions. Free water was always larger than bound water. Furthermore, bound water reached its peak in January when ecodormant plants were exposed to colder temperatures. These results support former evidence that bound water is composed of one population with restricted mobility, and another in a transient state.

Respiration pattern of overwintering buds was investigated using

differential scanning calorimetry. Endodormant buds decreased their respiration. In February, an increase in respiration, typical of the period preceding budbreak, was observed in ecodormant buds. Ecodormant and breaking buds were classified into four developmental stages. Those stages were: ecodormant, initial swelling, fully swollen, and broken buds. Isothermal experiments at 25°C were done to evaluate metabolic heat rate, CO_2 evolution rate, and metabolic efficiency. Also, buds were scanned from 1 to 60°C (7°C/hr) to estimate activation energy and Q_{10} values. Metabolic heat rates were 5, 17, 28, and 29 μW/mg dw, respectively, for the four bud developmental stages listed above. In the same order, CO_2 evolution increased from 2 to 3, 6, and 7 μW/mg dw. The efficiency of metabolism, however, decreased during the initial swelling stage. High apparent activation energy values for ecodormant and initial swelling buds showed that metabolism at those stages is slow, mostly because it is energetically expensive. As the buds developed, activation energy decreased, indicating a more favorable condition from a thermodynamic standpoint. Q_{10} values demonstrated that grapevines are highly responsive to temperature. Water content and respiration activity are useful indicators of metabolic activity.

WATER PARTITIONING AND RESPIRATION ACTIVITY OF DORMANT GRAPE BUDS

By

Alfonso A. Gardea

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Completed March 20, 1992

Commencement June 1992

APPROVED:

Professor of Horticulture in charge of major

Head of the Department of Horticulture

Dean of the Graduate School

Date thesis is presented March 20, 1992.

Typed by Alfonso A. Gardea and Georgina Vargas

Dedicated to Georgina Vargas and Porter B. Lombard.

Also to Alfonso J. and Manuel Fco. because I love them so much.

ACKNOWLEDGMENTS

In a chronological order my gratitude is expressed to many persons, who contributed in my research program along the way. Dr. Leslie Fuchigami helped me to start my program. My association with Dr. Larry Daley resulted in a fruitful experience. "If a door gets close for you, the Lord shall open two." In the most difficult moments, the support and friendship of my major professor Dr. Porter B. Lombard was crucial, and he was a cornerstone in the completion of this program, I owe him so much! I am lucky that I crossed paths with Dr. Richard Criddle, for he is a scientist of intense creativity, who really cares for his neighbor.

In my committee I am very thankful to Dr. Larry W. Moore for his unconditional support and for the values he shares. Working with Dr. Anita N. Azarenko was a delighting experience, full of support and challenge. Dr. Arnold Appleby found the time in his busy schedule to review the manuscript and suggest critical inputs. I am in debt with Dr. Shawn Mehlenbacher for his critical observations of my research project.

Many hours were spent in critical discussions with my fellow graduate students, and I express my gratitud to Jorge H. Siller, Sanliang Gu, Enrique Sánchez, Ursula Shuch, Yerko Moreno, Glen Creasy and Suozhan Cheng.

My children Alfonso and Manuel were a continuous source of inspiration and strength, while Georgina was a major support in the completion of my program.

The financial support from the Department of Horticulture is appreciated.

Note: This thesis is presented in manuscript format as three publications. Chapter 3 is written in the format of the Journal of Spectroscopy, and Chapters 4 and 5 in the format of the Journal of the American Society of Horticultural Science.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDIX FIGURES

LIST OF APPENDIX TABLES

WATER PARTITIONING AND RESPIRATION ACTIVITY OF DORMANT GRAPE BUDS

Chapter 1

INTRODUCTION

The dormancy of grapevines, associated metabolic changes, have been the objective of intense research. Still, most of the mechanisms remain unknown. Martin (1984) summarized the present knowledge of this topic in the introduction to the international symposium on bud dormancy in grapevines. This meeting focused mostly on the effect of cyanamide, a dormancy breaking agent; however, little is known about the dormancy requirements of grapes. Some workers reported dormancy requirements of grapevines in a general way (Winkler et al. 1974; Westwood, 1988), while others found requirements for specific cultivars (Iwasaki and Weaver, 1977). The objective of this dissertation was to characterize dormancy of 'Pinot Noir' grapevines in the Willamette Valley of Oregon. To achieve such an objective, as an initial step, the dormancy requirements of this cultivar were evaluated for two consecutive dormant periods. Forcing budbreak under controlled conditions is the most common method to assess dormancy (Fuchigami et al., 1982). Still, this is a time-consuming operation, and the results are not readily available; shorter-term methods should be explored. Siller (1991) studied the changes in the oxidized and reduced forms of glutathione, as a biochemical marker. Although this method proved useful in several woody species, it did not work in grapes. Further research was directed toward evaluation of physical and biochemical variables

associated with dormancy development. Particularly those related to changes in water content and respiration activity of the primary buds.

The water content of grape buds and its relationship with their cold hardiness status was reported in 'Concord', a labrusca grape which is considerably hardier than the vinifera grapes (Wolpert and Howell, 1986; Howell,1988a). Furthermore, even though the gross water content of the buds was inversely related with its cold hardiness, no specific differences in cold hardiness were correlated with the bud water content (Wolpert and Howell, 1984). Those reports were based on gravimetric determinations of the water content. The limitation of such a method is the lack of determining water allocation, and the most attainable information is the partitioning of the water content in its extracellular and intracellular fractions.

Early work by Burke et al. (1974) reported the advantages of proton nuclear magnetic resonance spectroscopy to study water in plant tissues, since it is a non-invasive technique allowing the visualization of water in situ. Rajashekar and Burke (1986) described this spectroscopic method to study the freezing process in plants. Recently, Faust et al. (1991) used magnetic resonance imaging techniques to visualize water in apple bud tissues. They proposed that determinations of the bound and free fractions of the water content could be used to identify endodormant buds.

Thus, proton nuclear magnetic resonance spectroscopy is an adequate method to evaluate changes in tissue water content, but these techniques are

complicated by the effect of sample geometry (Burke et al., 1974) and anisotropic behavior (McCain et al., 1984). Consequently, the immediate goal of this research was to develop a spectroscopic method appropriate for bud evaluations. Once this was done, the efforts were focused on the partitioning of the water content into the bound and free fractions during the dormant period, so that the hypothesis of Faust et al. (1991a) could be tested.

Ecodormant buds increase their water content before breaking (Bittenbender and Howell, 1975; Gardea, 1987; Colombo,1990); however, most of this gain in water is through the vascular connection between stem xylem and buds, which was reported to be interrupted by undifferentiated parenchyma (Esau, 1965). Thus, vascular connection determination is of paramount importance, and may explain, at least partially, some of the results found by Faust et al. (1991a).

Improvement in design allows the use of modern differential scanning calorimeters to measure heat, in the order of thousandths of a calorie. Since heat evolution is an inevitable byproduct of metabolism (Criddle et al., 1991), it is theoretically possible to evaluate plant respiration during the dormant stage, when metabolism is at its lowest (Westwood, 1988).

In 1991, the Department of Horticulture acquired a microcalorimeter, and after appropriate calibrations were made, repeated measurements were conducted after early experiments in Dr. Criddle's laboratory at Davis, California. Isothermal and scanning experiments were performed to evaluate several variables related to metabolic activity. Hard copies of the instrument outputs are included in the

appendix section for illustration.

The results of this research point out that gravimetric measurements of the bud water content are associated with the development of the ecodormant period of grapevines, while proton-nuclear magnetic resonance were useful in separating the bound and free water fractions. Until the actual molecular mechanism (s) controlling the onset, transition, and termination of dormancy are identified, microcalorimetry is a suitable technique allowing instant determinations of metabolic activity.

Chapter 2

LITERATURE REVIEW

BUD DORMANCY.

Definitions and Terminology.

An extensive and sometimes confusing terminology has been used in dormancy reports. In an early review, Doorenbos (1953) indicated that plants go through periods of growth by elongation, alternating with periods in which no such growth occurs. This state of inactivity was called 'dormancy' and he avoided the use of the term 'rest'. That review proposed the use of three terms; 'imposed dormancy' when the environment imposed growth inactivity, 'summer dormancy', when terminal buds inhibited lateral buds, and 'winter dormancy' when the cause of dormancy is not systemic, but lies within the tissue itself.

Almost simultaneously, in an independent review, Samish (1954) also described this phenomenon. He defined 'dormancy' as the temporary suspension of any visible growth, especially of buds and seeds, despite its cause. When external conditions stopped growth he called it 'quiescence'. In cases where internal factors caused the dormant state even under favorable conditions, he called it 'rest'. Martin (1984) used these two terms to clarify terminology in an International Symposium on Grape Bud Dormancy.

In an attempt to unify terms used in dormancy research, Lang et al. (1987) proposed "a descriptive system of physiological terminology, based on processes and inputs that can be easily understood, translated, and expanded as complexities

are revealed." They coined three terms to classify dormancy. 'Endodormancy' implies that the initial reaction leading to growth control is a specific perception of an environmental or endogenous signal within the affected structure alone. 'Paradormancy' involves a specific biochemical signal originating in a structure other than the affected structure as an initial reaction. 'Ecodormancy' includes all cases of dormancy resulting from one or more unsuitable environmental factors, which are generally non-specific in their effect on general metabolism.

Genetic Control of Dormancy.

The genetic aspects of dormancy have been the focus of continuous research. Tuan and Bonner (1964), reported that the genetic material of dormant potato buds was largely in a repressed state. The breaking of dormancy was accompanied by derepression of the genetic material. Following the metabolic changes in cherry flower buds, Wang et al. (1985), reported that a sharp increase in DNA and RNA characterized the transition from dormancy to active growth on late and early-blooming cultivars.

Large variations in chilling requirements have been reported to be a function of the genotype. Faust et al. (1976) reported that phenological differences between crosses of early and late blooming pear species resulted from the genetic transmission of bloom date. They suggested that discovery of late-blooming germplasm was essential for the development of late-blooming pears. Differences in the onset, intensity, and dissipation of rest in several pecan cultivars were reported by Amling and Amling (1980).

Dennis (1986) concluded that the genetic control of the chilling requirement for flowering and breaking of dormancy varies greatly within and among species. Sometimes this is controlled by a single gene, but multigenic control seems to be the rule.

Effects of Chilling.

To overcome dormancy, woody plants must be exposed to a certain amount of chilling (Westwood, 1988). Martin (1984) reported that grapevines respond to accumulation of chilling between 0 to 7°C, and chilling requirements vary greatly among genotypes (Westwood, 1988). Martin (1984) noted that grapevines require about 200 hrs of winter chilling at the above temperatures, although, 'Zinfandel' vines did not break dormancy before 330 chilling hours were accumulated, with a budbreak of 60 %, after 670 hrs, budbreak rose to 100%, and no changes were found after 1340 hrs. (Iwasaki and Weaver, 1977). Chilling requirements also were documented for grape seeds. 'Almeria' requires a stratification period of 4 to 6 weeks at 33 to 40°F (Harmon and Weinberger, 1959). Kachru et al. (1969) needed to stratify grape seeds for 3 months at 5°C, to find increasing synthesis of growth-promoting substances.

Lack of chilling in grapevines resulted in low percent budbreak, lack of uniformity in shoot growth, and very few clusters (Hatch and Ruiz, 1987). It also resulted in erratic shoot growth, poor cluster development, and irregular berry set (Martin, 1984). Insufficient chilling in peaches also was the cause of prolonged dormancy (Weinberger, 1954).

Winter chilling is crucial for temperate woody plants to synchronize their seasonal development, and insure survivability (Martin, 1984). In tropical areas, however, successful culture of temperate zone fruit species depends on inducing successive growth cycles throughout the year. These prevent the onset of dormancy and thus avoid the requirement for chilling (Edwards, 1984). Under such a scenario, cultivars with high chilling requirements are potentially as well suited as low chilling cultivars. In those conditions, water management seems to be the main factor in avoiding dormancy (Buttrose, 1974).

The temperature ranges at which chilling is accumulated have been the object of intensive research. Weinberger (1954) reported that brief high temperature periods during the breaking of dormancy of peach buds delayed blossoming and foliation and reduced set. Later, Erez and Lavee (1971) found that 6 °C is optimum as the base temperature for bud release from dormancy in peaches. However, when continuous chilling was compared with alternate treatments of high and low temperatures, Erez et al. (1979) reported that 15 hrs at low temperature, followed by 6 hrs of high temperature enhanced lateral burst of peach leaf buds.

Under field conditions, continuous temperatures do not occur. In an early report, Weinberger (1967) described that in the San Joaquin valley in California, adequate chilling, to break rest of most peach cultivars, was assisted by uniform temperatures below 7 °C during long foggy periods. But warm maximum temperatures in November and December partially counteract chilling, becoming

the primary determinants of the rate at which rest breaking occurred. Gianfagna and Mehlenbacher (1985) evaluated the heat requirements for budbreak and time of flowering in late- and early-blooming apple cultivars. Their results suggest that late flowering in apples result from high heat and high minimum temperature requirements for bud growth, as well as high chilling requirements. A mathematical model relating environmental temperatures to rest completion for 'Elberta' and 'Redhaven' peaches was proposed by Richardson et al. (1974). This model equates temperature to effective chill units, such that rest completion can be predicted with a high degree of accuracy. It considers the nullifying effect of temperatures above 16°C, and that no chilling is accumulated below 1.5°C. Erez et al. (1979) subsequently showed that only the chilling accumulated 20 to 40 hrs before to the onset of the high temperature was susceptible to this negative effect. The Utah model became popular, although it lacked accuracy when tested under mild conditions (Buchanan et al., 1977). Gilreath and Buchanan (1981) improved upon the model by incorporating the response to chilling and non-chilling temperatures in 'Sungold', a low chilling nectarine.

Different attempts have been made to quantify dormancy. Timmis et al. (1981) measured and interpreted the attenuation of electrical square waves in woody plants. Yet, difficulties in interpreting and reproducing quantitative data showed this technique to be of little value. A degree growth stage model was described by Fuchigami et al. (1982). In this model, the end of rest is determined by placing dormant plants or plant parts in a warm environment and recording the

time for budbreak. End of rest is estimated by extrapolation of two regression lines. One line indicates the rapid loss of rest in response to increasing chilling, and the other shows a slow change in development of quiescent buds.

Bud Vascular Differentiation.

Vacuolated parenchyma separates dormant axillary buds and the vascular cylinder of the stem. When the bud begins to grow, a direct vascular connection is established with the nearest vascular strands in the center cylinder through the vacuolated parenchyma (Esau, 1977). The timing and direction of differentiation vary in relation to the character of the bud. In axillary buds close to the apical meristem, the distance between the bud and the vascular region of the shoot is so short that it is difficult to recognize the direction in which differentiation happens. Although it is believed to occur acropetally in many plants; while buds distant from the shoot tip differentiate basipetally (Esau, 1965). In studying the development of xylem in the gynoecium of apple blossoms, MacKenzie and Costa (1991) found that the lignification of the xylem within the capillary bundles spread acropetally, simultaneously another wave of lignification spread basipetally from a point below the stigma.

This cambial growth is associated with metabolic changes, which occur during the dormant and quiescent stages before any other anatomical changes become visible (Savidge, 1989). Auxins seem the major agent-signals involved in the control of all aspects of vascular differentiation, but the control mechanisms require further investigation (Aloni, 1987).

Metabolic Changes During Dormancy.

Endogenous hormones. During dormancy development, ABA commonly increases in buds and seeds, however, there has been no proof that ABA is responsible for dormancy. Although it is generally diminished during winter, contrasting evidence suggests that chilling temperatures have little to do with this diminution (Powell, 1987). There is evidence that gibberellins (GA) influence dormancy. In hazelnut seeds, several inhibitors that interfered with germination, also interfered with GA production during chilling (Ross and Bradbeer, 1971). Bianco et al. (1984) found large quantities of GA in dormant embryos of 'Golden Delicious' apples extracted with Tris buffer, in contrast to trace amounts with conventional ethanolic solvents. This underlines the importance of the extraction method. Furthermore, they suggested that chilling temperatures may affect GA through membrane permeability. Generally, researchers agree that more work is needed to establish if gibberellins and dormancy have a cause-effect relationship (Powell, 1987).

Also, in contrast, there are reports on the effect of cytokinins in releasing dormancy. Rest-breaking treatments caused a rapid increase in xylem sap cytokinin concentration of apple trees, peaking just before or at budbreak (Cutting et al., 1991). The same response was found in extracts of breaking buds from sugar maple (Taylor and Dumbroff, 1975). Nonetheless, Powell (1987) citing several studies with little or no change in cytokinins during bud development, concluded that additional research with cytokinins would be profitable.

There is little evidence that auxins play an important role in regulating rest in buds or chill-requiring seeds (Powell, 1987).

Enzymes. The efficiency of utilizing the respiratory enzyme system remains higher in organs approaching growth, than in deep dormancy. Since the same occurs in embryonic axis of cherry seeds, Pollock (1960) suggests that it may be a general phenomenon. When the temperature decreases in winter, it is followed by a decrease in catalase activity. The intensity of dormancy in grapevines seems directly related to the activity of catalase. Nir and Shulman (1984) found that dormancy breaking agents caused a decrease in catalase activity, and they suggested that an increased level of hydrogen peroxide in the bud tissue might activate the pentose phosphate pathway (PPP), leading to the termination of dormancy. The involvement of glycolysis and PPP in controlling the dormancy of wild oat seed also was proposed by Kovacs and Simpson (1976), after finding increasing activities of glucose-6-phosphate dehydrogenase, isocitrate dehydrogenase, glutamate-oxaloacetate transaminase, and acid phosphatase during steeping. But Hu and Couvillon (1990) strongly questioned the role of PPP and catalase activity in the breaking of dormancy. They measured the activities of catalase, glucose-6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase in nectarine seeds, and could not correlate enzymatic activity with seed germination. Apparently, many enzymatic interactions remain to be determined (Powell, 1987).

Lipids. From winter dormancy to spring budbreak, the degree of saturation

of the fatty acids of membrane lipids increase in apple buds. They also change the composition of their polar headgroups, increasing membrane phospholipid content, and changing sterol levels and composition (Wang and Faust, 1990). However, it is possible that these changes are be related to bud cold hardiness instead of dormancy. Yoshida (1984) found a significant increase in phospholipids and fatty acid unsaturation during cold acclimation of mulberry bark cells. He also reported changes in the electrophoretic pattern during fall and winter, while glycoproteins levels were coincident both with changes in growth stages and with cold hardiness development. Later (1986), he found reversed changes during deacclimation in the spring.

Methods for **Breaking Dormancy.**

Reasons given to control plant dormancy status were summarized by Martin (1984). The primary objectives are: a) induce uniform bud growth, b) induce early bud growth where frost is not a hazard, c) delay bud growth, d) delay dormancy, e) advance dormancy, and e) satisfy winter chilling in warm years.

Cultural methods. Pruning and irrigation are two cultural practices outstanding as managerial tools to control dormancy in grapevines. Hatch and Ruiz (1987) recommended pruning in December to increase early and uniform, budbreak in 'Perlette', in areas with insufficient chilling. On the other hand, delayed pruning delays budbreak and avoids frost (Howell, 1988b). Still, pruning of peach trees after rest completion reduced flower bud hardiness by decreasing rehardening after a thaw, becoming more susceptible to subsequent freezes

(Durner, 1990).

Williams et al. (1991) reported that suspending irrigation of 'Perlette' vines by 15 September in the Coachella valley resulted in earlier budbreak the following spring. In Israel, evaporative cooling also induced early uniform budbreak in grapevines by decreasing the bud temperature exposed to direct sunlight from 30 to 16 °C, and unexposed buds from 25 to 13 °C (Nir et al., 1988).

Defoliation is another cultural practice used to manage dormancy. Early defoliation the reduces depth of dormancy throughout the winter in low-chill peaches (Lloyd and Firth, 1990). Early defoliation is commonly done in Taiwan in combination with pruning and chemical treatments to avoid dormancy and produce two crops of table grapes per year (Lin, 1984).

Chemical methods. Various chemicals have been used to break dormancy. The following is an example of their diversity. Hosoki et al. (1986) isolated volatile compounds from garlic and horseradish capable of breaking dormancy of corms and ornamental trees. The compounds were identified as allyl sulfide, methyl disulfide, and n-propyl disulfide in garlic, and allyl disulfide, allyl isothiocyanate, and n-propyl sulfide in horseradish.

Working with detached apple shoots, Paiva and Robitaille (1978) did not find evidence for a role of ethylene in breaking bud dormancy, either directly or indirectly, in DNOC-stimulated budbreak. Erez et al. (1971) used a combination of KNO_3 and kinetin to break dormancy of peach flower buds, while leaf buds were more responsive to thiourea sprays. The same compounds proved more

effective than cytokinins and gibberellins, when sprayed on apple flower buds (Shaltout and Unrath, 1983), while 6-benzylamino purine (6-BA) and GA_{4+7} affected both flower and leaf buds. Broome and Zimmerman (1976) broke bud dormancy in tea crabapple with 6-BA, while DMSO enhanced the effect of the cytokinin. In another study, Nauer and Boswell (1981) stimulated growth of quiescent citrus buds with 6-BA dissolved with DMSO and mixed with Tween 20. Since 6-BA alone did not work, and the solvent and the spreader were not used by themselves, it is difficult to conclude if their action was merely synergistic or if they have their own effect. GA was used to shorten the production time of azaleas by reducing the chilling required to break dormancy (Black et al., 1990).

Cyanamide sprays. Currently the use of cyanamide is the most popular method to break dormancy in grapevines and many reports have documented its effectiveness (Wicks et al., 1984; Williams and Smith, 1984; Lavee et al., 1984; and Bracho et al. 1984). Nonetheless, a report from Whitins and Coombe (1984) summarizes the problems associated with the use of this compound. Timing and concentration are the main factors defining cyanamide performance. They reported that cyanamide (0.83M) sprayed 30 days before budbreak caused bud mortality of 'Cabernet Sauvignon' vines. Siller (1991), in a time course study, found that the mortality of peach buds depended upon concentration and timing of cyanamide concentration. Generally, the more advanced the bud development, the more sensitive it is to a given cyanamide concentration.

^{14}C-labelled cyanamide applied through the cut end of grape cuttings was

rapidly metabolized. A significant amount was transformed to urea and subsequently hydrolyzed by urease (Goldbach et al., 1988). These results also indicated that cyanamide decomposition in plant material is partly of pure chemical nature. In a complementary work, Vilsmeier and Amberger (1988) fed rooted grapevine cuttings with ^{15}N-labelled cyanamide, and found an increase in arginine concentrations. They suggested that cyanamide reacted with ornithin to yield arginine.

Cyanamide causes a decrease in catalase activity in dogwood and grapes (Fuchigami and Nee, 1987; Nir and Shulman, 1984; and Amberger, 1984). This decrease is hypothesized to cause an increase in the level of hydrogen-peroxides, leading to a shift from EMP cycle to PPP pathway. This increased production of reduced nucleotides is typical of intensified metabolism and synthesis, conditions similar to those occurring prior to budbreak. However, Amberger (1984) postulated that the resulting accumulation of peroxides, also leads to a higher concentration of glutathione, which in turn can control the metabolism of dormancy.

Cyanamide has other less conventional uses including: delaying budbreak of 'Thompson Seedless' grapevines when applied later in the season (Williams, 1987), development of latent buds on old bare cordon trained grapevines (Lavee et al., 1984), and defoliation of nursery plants without phytotoxic side effects (Erez, 1985). Substitution of chilling with cyanamide during stratification of grape seeds resulted in shorter breeding cycles (Spiegel-Roy and Shulman, 1987). Also,

cyanamide induced budbreak of insufficiently chilled peach cultivars, when applied six weeks after normal budbreak period (Dozier et al., 1990).

Budbreak.

Development of ecodormant buds begins after exposure to warm temperatures, which lead the buds to break. Budbreak is evaluated in different ways depending on the sophistication required. Field observations usually consider the appearance of green shoot tissue (Williams and Smith, 1984; Hatch and Ruíz, 1987). For comparative purposes budbreak is usually recorded as the moment when 50% of the buds burst (Fuchigami et al., 1982). Still, the dynamics of different phenological stages of breaking buds may yield more qualitative and quantitative information (Gardea, 1987).

Swanepoel et al. (1990) considered the prediction of budbreak date has many practical applications including timing of pruning. Treatments against diseases and pests could be more accurately timed. Uniform and increased budbreak could also be induced more precisely.

Becker (1988) stated that the duration of development until budbreak was statistically dependent on soil and temperature. A close correlation was found between the summation of hourly temperatures above 10°C, and the period from March to full bloom. Traditionally, a mean temperature of 10°C has been used as a reference point to evaluate grapevine development (Winkler et al., 1974). However, Moncur et al. (1989) found no experimental evidence in the literature for the appropriateness of 10°C as a base temperature for heat accumulation.

Instead, they presented evidence that a base temperature of 4°C was required for budbreak of 'Pinot Noir' vines, and 7°C for leaf appearance, concluding that the base temperature increases for successive stages in the annual cycle. These stages were defined by Lin (1984) as: dormant, swollen, budburst, one-three and five leaf opening. Other authors used slightly different nomenclature, but essentially the same phenological stages (Proebsting, 1978; Flaherty et al., 1982; Gardea 1987).

During budbreak, grapevines are very responsive to periodic temperature fluctuations, which coincide with a window of susceptibility to spring freeze damage (Howell, 1988b). The phenological stages of bud development are closely related with critical temperatures (Gardea, 1987; Howell, 1988a), and germplasm influences damage via genetic influence on both, bud phenology and hardiness at same phenological stage (Howell, 1988a). This was later illustrated by Wolf and Cook (1991), who observed that 'Cabernet Franc' dormant buds were 1 or 2°C hardier than 'Cabernet Sauvignon', but, because they deacclimated faster, by spring 'Cabernet Sauvignon' was comparatively hardier.

WATER STATUS OF OVERWINTERING BUDS.

The Grape Primary Bud.

Grape buds are usually classified depending on the structures they contains (leaf or fruit bud), or their position along the shoot (basal or axillary). The bud consists of three partially developed shoots with rudimentary leaves, or with both rudimentary leaves and flower clusters (Winkler et al, 1974). The primary,

secondary, and tertiary buds remain dormant, and in winter form a large compound bud, the whole bud is covered with suberin-impregnated scales to protect against mechanical injury, and the scales are lined with hairs to avoid dehydration of the inner parts (Winkler et al, 1974).

The primary bud is more likely to contain differentiated clusters, and is also the first to emerge in the spring. The primary bud is the least hardy of the three lateral growing points contained in the compound bud (Slater et al, 1991). Primary buds are also less hardy than stem tissues (Wolpert and Howell, 1984).

Water Content of Grape Buds.

Seeds at maturity have a moisture content of less than 20% (Berrie, 1985). Unlike seeds, buds are partially well hydrated structures (Dumbroff et al, 1979; Colombo, 1990). However, many analogies can be found between the two structures. Germinating apple seeds showed an increasing water uptake during the transition from germination sensu stricto to growth (Perino and Côme, 1991). In the same way, sugar maple buds have a winter moisture content of 40%, which rises to 70% during budbreak (Dumbroff et al, 1979). 'Pinot Noir' grapevines have a water content of 54% (v/w) during the winter, increasing to 85% when they are breaking in the spring (Gardea, 1987).

Many physiological changes are associated with the amount of water in the buds. Colombo (1990) found a strong correlation between the dormancy status of black spruce, and their cold hardiness and shoot moisture content. In grapevines, the gross water content of primary buds was inversely related with cold hardiness

in a general way. Specific hardiness differences, however, could not be correlated with changes in water content (Wolpert and Howell, 1984). The same authors in a subsequent report (1986) found that basal cane and primary bud tissues acclimated earlier than apical tissues. An inverse relationship between hardiness and water content was also noted. Although those changes in bud hydration are accompanied by changes in cold hardiness, it is still not clear whether it is a cause-effect relationship, or if they are only events occurring simultaneously.

Terminology Used in Water Reports.

Different terms are used to describe water status in biological systems. Water is called symplastic or apoplastic according to the path used for water molecules to move through the plant. *Symplastic water* is a continuous system of communicating cytoplasm created by intercellular connections (Nobel, 1983). Some authors, however, exclude the large central vacuoles. The connections are made through plasmodesmata in the cell walls (Salisbury and Ross, 1985). *Apoplastic water* moves in the cell walls (Nobel, 1983) or in the hollow lumens of xylem vessels, the dead part of the cell (Salisbury and Ross, 1985).

In relation to the degree of binding of water molecules to themselves, and to other structures, water is called either *bound* or *free*. The bound fraction is held with great force to hydrophilic surfaces (Salisbury and Ross, 1985), remains liquid at temperatures as low as -50°F (Toledo et al., 1968), has restricted motion (Burke et al., 1974), and is associated with macromolecules (Vertucci, 1990; Faust et al., 1991a). For the most part, free water has characteristics unlike those

described above. Some controversy on the extent of the region of restricted water mobility was reported by Burke et al. (1974). Kerhoas and Dumas (1986) found several water compartments in pollen grains, each with different physiological effects. Furthermore, Vertucci (1990) proposed that two populations of bound water exist around a hydrated macromolecule, one that has severely restricted mobility, and one with properties similar to free water.

Depending on the location of the water molecules in the cell, water can be either *extracellular* or *intracellular* (T.H.H. Chen, personal communication).

Methods to Estimate Water Content.

Several ways have been devised to estimate the amount of water in plant tissues. Some of those techniques include gravimetric, calorimetric, and spectroscopic methods.

Gravimetric methods measure the total amount of water by recording the difference in tissue weight before and after dehydration (Wolpert and Howell, 1986). The extracellular fraction can be estimated by centrifuging plant parts, and comparing the weights before and after centrifugation. Intracellular water is determined by dehydrating the tissue after being centrifugated, and subtracting the dry weight from the weight after centrifugation (Gusta et al. 1979, 1983; and L.V. Gusta, personal communication).

Calorimetric methods. Vertucci (1990) used differential scanning calorimetry to study the thermodynamic properties of water in seeds with different water content, based on their freezing and melting transitions. This method

depends on knowing the heat of fusion of cellular water, which is not necessarily identical with the value of pure water (Burke et al., 1974).

Spectroscopic methods are based on the fact that the spectroscopic properties of water change as a function of both temperature (Burke et al., 1974), and magnetic fields surrounding the sample (McCain, 1986). A specific technique is described below.

Proton-Nuclear Magnetic Resonance Spectroscopy (^{1}H-NMR). Certain atomic nuclei (including protons) possess energy levels that diverge in a magnetic field. ^{1}H-NMR allows the measure of those energy labels (McCain, 1986). Nobel laureate Richard Ernest as quoted by Amato (1991) said, "Compared with other analytical tools, NMR gives information about the local structure of molecules, allowing chemists to determine a molecule's overall structure." Infrared spectroscopy only describes the presence of chemical groups, but not how those groups arrange themselves in a specific molecular structure (Amato, 1991).

In a typical ^{1}H-NMR experiment, a sample containing protons in a variety of chemical environments is exposed to a constant, uniform magnetic field. Instead of a single peak, the resulting spectrum often displays several peaks at different resonance frequencies, which demonstrates that nuclei are exposed to different magnetic field strengths. This provides information about the magnetic environment at each nucleus, which is used for chemical identification (McCain, 1986).

Used by physicists for 45 years, NMR was adopted by chemists, and it is being used extensively in the biological areas because of parallel improvements in instrumentation and methods (Levy and Craik, 1982).

^{1}H-NMR is a particularly convenient tool for the study of water in plants because it is non-destructive, and rich in structural, and dynamical information (Burke et al., 1974). Since water is the predominant component of plants, their ^{1}H-NMR spectra is essentially that of liquid water because protons from other compounds in solution are too dilute to make a significant contribution to the spectra, while protons in the solid state or in membranes have peaks too wide to be detected (Burke et al., 1974; Kerhoas and Dumas, 1986; McCain, 1986).

^{1}H-NMR Parameters.

Four parameters can be measured from NMR spectra and can be used in quantitative and/or qualitative analysis. Those parameters are: chemical shifts, spin-spin constants, relaxation times, and integration. *Chemical shift* is referred to different resonance signals found for various protons in a molecule because they reside in different chemical environments (Günter, 1985). Those resonance signals depend on the shielding effect of the nucleus. The number of peaks in the ^{1}H-NMR spectrum defines the number of positions occupied by protons (Ando and Webb, 1983).

The second parameter is the *spin-spin constant* resulting from the magnetic interaction of one nucleus with another. Since not all spectral lines are simple (singlets), characteristic splitting patterns form triplets or quartets (Günter, 1985). The spin-spin constant also depends on chemical environments, and is obtained

directly from spectral values (Ando and Webb, 1983).

The third parameter is the *relaxation times*, representing the time needed for the excited proton molecules to dissipate the excess energy and return to the ground state (Ando and Webb, 1983). They affect widths and spectra intensities. Two kinds of relaxation times can be obtained.

1) Spin-lattice relaxation time (T_1) which measures the time it takes to dissipate energy from the excited state of nuclei to their surroundings (Faust et al., 1991b).

2) Spin-spin relaxation time (T_2) which measures the time required to transfer energy from the excited nuclei, not to their surroundings, but to adjacent nuclei at ground level energy. Consequently, T_2 must be less than or equal to T_1 (Ando and Webb, 1983; Williams, 1986; and Faust et al., 1991b). T_2 values are particularly important since they are related to the degree of binding of water molecules. T_2 times in free water are much longer than in bound water. It is desirable to measure T_2 in milliseconds to get quantitative data (Faust et al., 1991b). Toledo et al. (1968) established that T_2 can represent the amount of bound water, only if all the free water is frozen. Consequently it should be measured at temperatures insuring this condition (usually -50°F).

There is an inverse relationship between the spectrum peak at half its height, and its corresponding T_2 value (Burke et al., 1974; Gusta et al., 1979; Brevard and Granger, 1981; and Williams, 1986). This is given by the equation:

$$\Delta\nu_{\frac{1}{2}} = 1/\pi T_2$$

$$T_2 = 1/\pi \Delta \nu_{\frac{1}{2}}$$

Where, $\Delta \nu_{\frac{1}{2}}$ is the peak width at half its height, π is a constant, and T_2 is the spin-spin relaxation time. While estimation of T_1 requires specific manipulation of the sample, T_2 can be estimated directly from the spectrum. This is an important consideration, since this is an expensive technique.

The fourth parameter is *integration.* It measures the area under a resonance signal and is proportional to the number of protons giving rise to that signal (Günter, 1985). So, if two or more resonances are found and their areas are measured, then it is easy to determine the relative number of spin-active nuclei for each resonance (Williams, 1986).

Further refinement of the parameters discussed above can be done by second derivative analysis, which enhance information on position, width and integration (Holler et al., 1989).

Uses of ^{1}H-NMR in the Plant Sciences.

Once a technique exclusive to physicists and chemists, NMR has become increasingly popular in different disciplines of the plant sciences. In a pioneer work in stress physiology, Burke et al. (1974) studied the state of unfrozen water at low temperatures. They concluded that the ability of dogwood to survive low temperatures depended on its ability to tolerate diminished quantities of liquid water. Gusta et al. (1979) evaluated varietal responses of acclimating cereals and found a negative correlation between $\Delta \nu_{\frac{1}{2}}$ and crown water content, which in turn determined their levels of hardiness. McCain (1986) also analyzed the changes in

liquid water of hawthorn leaf discs at low temperatures, and the effects on their ^{1}H-NMR spectra. Thermal injury to chloroplast envelope membranes in vivo, at temperatures between 53 and 57 °C was detected by McCain et al. (1989). The injury was associated with a sudden loss of water from the chloroplast.

In plant anatomy studies, Brown et al. (1988) used H-NMR microscopy, as a non-destructive tool, for in vivo studies of tissue structures of Pelargonium hortorum, with sufficient resolution to discern individual cells.

Plant-water relation experiments have also been performed by H-NMR. McCain and Markley, (1985) evaluated in vivo the water permeability of chloroplast envelope membranes. Bottomley et al. (1986) followed water distribution and transport in plant root systems in situ. While changes in root water content during periods of rapid transpiration were followed by Brown et al. (1986). Kerhoas and Dumas (1986) used gravimetric methods in combination with ^{1}H-NMR to determine pollen quality, and changes in viability associated with water content. Johnson et al. (1987) distinguished different stem tissues based on their resonance properties given by differences in water content after periods of rapid transpiration. Finally, McCain et al. (1988) found that water is allocated differently to chloroplasts in exposed and shaded hawthorn leaves, with the former having 1.7x more water/unit area than shaded leaves.

McCain et al. (1984) were among the first to report anisotropic behavior in plant leaves, defined as the changes in spectral characteristics depending on sample orientation in the magnetic field. They analyzed more than 50 species, and

found this characteristic in one fourth of the surveyed plants.

^{1}H-NMR imaging, or magnetic resonance imaging (MRI) describes a class of experiments in which NMR signals are used to obtain information having spatial significance. Although the instrumentation may be different than conventional spectrometers, they operate on the same principles (Levy and Craik, 1982). In 1979, Hinshaw et al. obtained images of several fruit specimens, demonstrating the integrity and resolution of this new method. Aguayo et al. (1986) used a modified instrument to get the first NMR images of a single cell, forecasting the advent of ^{1}H-NMR imaging microscopy. In a recent publication, Faust et al. (1991a) used MRI to image dormant apple buds. They concluded that the lack of images of unchilled buds was because most of the water was in the bound form. After chilling, however, the water became free and the images of the buds were possible to visualize. They proposed that this technique can differentiate endodormant buds from eco- and paradormant buds.

PLANT RESPIRATION.

Definition and Stoichiometry.

Respiration is an ATP-generating process, in which an inorganic compound (such as O_2) serves as the ultimate electron transport (Stryer, 1988). The stoichiometry of respiration is described as:

$$C_6H_{12}O_6+6O_2=6H_2O+6CO_2+686 \text{ kcal}$$

Oxygen is needed to metabolize sugars, the result is a liberation of energy

and a concomitant formation of water and evolution of CO_2. A total of 36 ATPs is formed from the oxidation of a glucose molecule, assuming that the glycerol-phosphate shuttle is used to transfer electrons from the cytosol to the respiratory chain. This represents an investment of 263 kcal, out of the 686 liberated (Stryer, 1988). The other 423 kcal are dissipated as metabolic heat.

Consequently, cellular metabolic activities are accompanied by characteristic heat effects (Loike et al., 1981), and heat production is an inevitable byproduct of all growth processes (Criddle et al., 1991). Measurements of respiratory CO_2 or O_2 exchange in photosynthetic tissues in the light are complicated by concomitant reverse fluxes due to photosynthesis (Amthor, 1989).

Plant metabolic rates can be determined by three major methods (Criddle et al., 1988). One is to determine biomass production over a given time. The second method is to use some biochemical measure such as the rate of CO_2 release, O_2 uptake, or the incorporation of some labelled nutrient. A third method of evaluating metabolism is to measure the heat evolution rates, which are interpreted as a measurement of overall metabolic rate. This will be discussed in detail later in the text.

Seasonal Fluctuations in Plant Respiration.

As in all plant metabolism, respiration is a seasonally dependent activity. The general pattern is a decrease before the plants go dormant, low respiratory levels during the dormant phase, and an increasing activity as budbreak approaches (Westwood, 1988). The root system of Atriplex confertifolia

undergoes seasonal adjustment as well (Holthausen and Caldwell, 1980). Its respiration diminishes by late summer, and rises to peak capacity early in the spring. At greater depths in the soil profile, the timing of minimum and peak capacities of respiration is progressively delayed.

The clearest statement that can be made about respiratory metabolism during dormancy is that those treatments which break dormancy give enhanced respiratory activity (Ross, 1984). Wrzesniewski (1985) reported very low respiration rates of cherry seeds stratified at 3°C. However, thermal induction of secondary dormancy (25°C for 2 weeks) caused a temporary rise in respiration, but after a week, respiration rates dropped. When the low temperature was restored, the seeds respired at low levels again.

Cole et al. (1982) compared the respiration of flower buds of Pyrus communis (late blooming) and Pyrus calleryana (early blooming) during dormancy. At 5°C, P. calleryana respired at twice the rate of P. communis, but no difference was observed at 25°C. This indicated that early blooming buds have a mechanism that provided an advantage at low temperature conditions.

After chilling is satisfied, ecodormant buds and seeds begin to respire more actively. Pollock (1960) reported that respiration rates of buds from chilled maple trees rose as a result of low-temperature exposure, which broke rest. While respiration of unchilled buds dropped during the same period. He concluded that the efficiency of the utilization of the respiratory enzyme system always remained higher in organs approaching growth than in those in which the rest period was

not broken. Changes during the transition from endo- to ecodormancy in after-ripening cherry seeds were also marked by an increasing respiration rate at 25°C (Pollock and Olney, 1959). However, those differences are not always as noticeable. Chen and Varner (1970) found that the respiration rate of water imbibed dormant oat seeds was only 20% less than imbibed non-dormant seeds in the period before actual germination. Butler and Landsberg (1981) followed the seasonal changes in respiration of apple trees. They found that at the same temperature, respiration rates were low during the endodormant phase, rose to a peak in spring, and declined steadily through the season. However, the ecodormant phase of fully chilled apple trees can be delayed by reducing the oxygen concentration to 3%. Young and Blankenship (1991), working under greenhouse conditions at 6°C, were able to suppress growth development for as long as 35 weeks. Once the oxygen concentration was restored, budbreak and shoot growth were normal.

Respiration rates also differ among different organs in the same tissue. When Zimmerman et al. (1970) estimated winter respiration in pear buds, they reported that meristems respired 9 times more than brown scales, twice as much as green scales, and 5 times more than the bud as a whole. Pollock and Olney (1959) had reported previously that the embryonic axis of chilled cherry seeds respired more than the whole seed. Wrzesniewski (1985) found no difference in respiration of cherry embryos after removal of stone and testa, during the stratification period.

Major Biochemical Pathways Involved in Respiration.

Glycolysis and Pentose Phosphate Pathway. Glycolysis, as well as PPP, occurs in the cytosol and use common substrates. Glucose 6-phosphate isomerase and glyceraldehyde 3-phosphate dehydrogenase are two important enzymes interconnecting these two pathways (Amthor, 1989).

PPP was reported to be a major route of glucose catabolism in several higher plants (Humpreys and Dugger, 1957). La Croix and Jaswal (1967) reported an increasing activity of PPP, based on the C-6/C-1 ratio, during the seventh week of stratification of cherry seeds, parallel to a transition from endo- to ecodormancy. Zimmerman and Faust (1969) concluded that overwintering pear buds respired mostly through PPP. But during bud swelling, glucose metabolism changes with a decrease in PPP activity, and a concomitant increase in the glycolytic pathway. A similar finding was reported by Bogatek and Lewak (1988) in apple seeds during stratification. Recent work (Perino and Côme, 1991) described two phases during germination of apple embryos, the transition from germination sensu stricto to growth was marked by changes in the respiratory activity. Substances that inhibit respiration or that stimulated PPP, also stimulated germination sensu stricto.

The alternative oxidases. The mitochondrial electron transport system is the final acceptor of electrons from NADH and $FADH_2$. Water is formed by the transfer of these free electrons to oxygen, and the energy release from this transfer drives the oxidative phosphorylation (Amthor, 1989).

However, in the presence of cyanide, an alternative pathway bypasses cytochrome c, resulting in little phosphorylation. This alternative pathway, which is not sensitive to cyanide, is important in heat production and may act as an overflow mechanism oxidizing excess carbon substrate (Amthor, 1989). The presence of this alternative pathway has been reported in many plants. For example, during the first 12 hrs of germination of chickpea seeds, the respiration occurred through the normal cytochrome c pathway, but after this time there was a shift to a cyanide resistant (alternate) pathway, which reached maximal resistance between 72 to 96 hrs of germination. A similar finding was reported in soybean seeds (Yentur and Leopold 1976), except that the alternative oxidase was functional only between the 4th and 8th hrs of germination. After that, respiration experienced a transition to the cyanide sensitive cytochrome c pathway.

Hydroxamic acids (R-CONHOH) are specific inhibitors of the cyanide resistant alternate oxidase described above. The nature of the R group in these compounds affects the concentration at which they are effective (Schonbaum et al, 1971). Salicyl-hydroxamic acid (SHAM) is an inhibitor commonly used in investigations of the capacity and engagement of the alternative pathway. It is used in combinations with cyanide to measure electron flux through the alternative and cytochrome oxidases (Siedow and Girvin, 1980; and Amthor, 1989).

However, even in the presence of cyanide and SHAM, O_2 uptake is not always totally inhibited. Assuming that the concentration of both compounds

worked at peak capacity, it was suggested that the presence of an active oxidase other than the alternative and cytochrome oxidases (Amthor, 1989). This component of O_2 uptake has been called residual respiration. It is not associated with mitochondrial respiration, and only appears in intact tissues, but not in isolated mitochondria. Whether it occurs in the absence of SHAM and cyanide is unknown (Amthor, 1989). Cole et al. (1982) studied the respiration of pear dormant flower buds at 5°C. Their results indicated that up to 70% of oxygen flow was through the alternative oxidase. The presence of both SHAM and cyanide, inhibited respiration only partially. This residual respiration, whose nature was unknown, was higher in P. calleryana than in P. communis.

Differential Scanning Calorimetry (DSC).

As mentioned earlier in this section, measurement of heat evolution from plant tissue is a sensitive, non-destructive way to estimate metabolic rates. Nonetheless, until recently this was limited by available instrumentation (Criddle et al., 1988). Calorimetric methods are based on the fact that "whenever a material undergoes a change in physical state, or whenever it reacts chemically, heat is either absorbed or liberated" (McNaughton and Mortimer, 1975). They described that the differences between differential thermal analysis and DSC is that in the former, both sample and reference are heated by a single heat source. In DSC, sample and reference are each provided with individual heaters, making possible the use of the "null-balance" principle by two control loops. One controls the temperature of sample and reference according to a predetermined scanning

rate, while the other insures that no temperature differential is developed between the sample and the reference by adjusting the power input (for details see McNaughton and Mortimer, 1975).

Modern DSC instruments work in two basic modes. In an isothermal mode, the instrument is held at a given temperature, and the heat differential between sample and reference represent a measurement of its heat of metabolism. In the scanning mode, the temperature of sample and reference changes at a programmed rate; and the heat difference between sample and reference changes at each temperature (Criddle, personal communication). Thus, DSC provides a non-invasive method for studying heat evolution in living tissues (Loike et al., 1981).

Applications of DSC in Plant Respiration.

This section describes relevant publications on each of the two basic modes of modern DSC.

Isothermal heat conduction calorimetry was used to evaluate the effect of temperature and oxygen depletion on metabolic rates of tomato and carrot cell cultures and cuttings by Criddle et al. (1988). They reported that accurate prediction of growth properties of intact plants was possible in this two species, and that it represented a practical tool for germplasm screening and selection. Criddle et al. (1988) studied the effect of NaCl on metabolic heat production by barley roots. Two levels of inhibition by increasing salt were found. Up to 150mM of salt, the inhibition was similar for the three cultivars. However, the

50% inhibition -before 150mM was reached- was germplasm dependent. At higher salt concentrations, metabolism was further decreased and no differences among the cultivars were noted. Iversen et al. (1989) examined cut fresh pineapple metabolism, and defined suitable storage conditions to avoid heat-producing spoilage related with microflora growth. Based on the metabolic rate of larch shoot tips, Hansen et al. (1989) predicted long term growth of several clones. Hansen and Criddle (1989) devised a batch injection attachment for calorimeters, which permitted environment modifications in the ampules without affecting the DSC performance. Calorimetric studies do not allow the stirring of cell cultures, and oxygen diffusion becomes a limiting factor. This problem was solved by Fontana et al. (1990) by floating the cells on high density media allowing long time studies. The effect of high and low temperature stresses on the metabolic activity of plant tissues and cell cultures were determined by calorimetric procedures by Breidenbach et al. (1990). Their data show that metabolism inactivation was a complex function of both temperature, and time of exposure to the stress. Simultaneous measurements of heat rate, CO_2 production and O_2 consumption were possible when Criddle et al (1991) devised a system compatible with commercial calorimeters.

Differential scanning calorimetry is a tool well suited for studing sample responses over a temperature range, at a predeterminated temperature rate. DSC of pea and soybean cotyledons allowed Vertucci (1990) to study their thermodynamic properties of water. She identified five states of water, and those

results concluded that two populations of bound water exist around a hydrated macromolecule. Rank et al. (1991) measured decreasing metabolism of cultured tomato cells as a result of exposures to high and low temperatures. Models were used to describe thermal inactivation and prediction of activity loss, following a stress.

At the end of the 19th century, Arrhenius proposed the following equation to describe the rate of a chemical reaction (Montgomery and Swenson, 1976; Nobel, 1983)

$$k = Ae^{-EA/RT}$$

where k is the rate constant, A is a constant, -Ea is activation energy, R is the gas constant, and T is absolute temperature in Kelvin. Data collected from scanning experiments can be analyzed this way. After several assumptions were met, and appropriate substitutions were done, Criddle et al. (submitted) modified the original equation revised by Johnson et al. (1974) to:

$$q = Ce^{-\mu/T}$$

Where q is the heat rate, C is a constant, and $\mu = {}^{Ea}/_{R}$. Considering the natural log of both sides of the equation.

$$\ln q = \ln C - \mu/T$$

This represents an Arrhenius plot, where the natural log of the heat rate is plotted against the inverse of the temperature in Kelvin. ln C is the intercept of the regression line and $-\mu$ represents the slope, also known as the activation energy. In the same work they proposed that activation energy is a useful

criterion to explain spatial distribution of temperate plant species. Activation energy also was determined in dormant apple buds, demonstrating that it decreased as carbon metabolism diminished (Young, 1990).

Chapter 3

VARIATIONS IN ^{1}H-NMR SPECTRAL COMPOSITION OF GRAPE BUDS.

ABSTRACT

This study was done to develop a method to characterize changes in water by ^{1}H-NMR spectroscopy of grape buds. The influence of sample position in the magnetic field, and the effect of extracellular water depletion, were determined. Primary buds are the most hydrated organ in a compound grape bud. Thereby making them more suitable for water studies. Bud spectra were done in a Bruker AM 400 nuclear magnetic resonance spectrometer. The spectra were recorded at 298°K, and second derivative analyses were done for each spectra. First, the spectra of buds with their longitudinal axes parallel to the direction of the magnetic field were recorded. Then, the buds were rotated 90°, such that their longitudinal axes were perpendicular to the direction of the magnetic field, and their spectra were recorded again. The results confirm that anisotropy exists, although it appears to be more of a quantitative than a qualitative response, possibly affected by sample morphology. Extracellular water depletion caused a decrease in signal size.

INTRODUCTION

Biological material yields complex spectral signals; however, modern spectroscopy and signal processing permits resolution of these complex signals into defined and consistent entities. These entities permit rational interpretation of biological phenomenon, otherwise not directly accessible.

Nuclear magnetic resonance spectroscopy has become an important technique in plant physiology studies. ^{13}C NMR was used to demonstrate that potato cell wall suberin is a polyester with the phenylpropanoid groups characteristic of lignin (Garbow et al., 1989). The nitrogen metabolism of white spruce buds was studied with ^{14}N and ^{15}N NMR, concluding that NH_4^+ and NO_3^- were both required for the induction of nitrate- and nitrite reductase (Thorpe et al., 1989). For the first time, the energy status and the intracellular pH associated with sodium uptake by excised corn roots was monitored with ^{23}Na and ^{31}P NMR (Gerasimowicz et al., 1986). ^{31}P NMR was instrumental in studies involving ATP measurements, like the intracellular pH of cotton embryos and seed coats (Hendrix et al., 1987), the in vivo effects of DNP in barley roots (Jackson et al., 1986), and phosphate concentrations on elicitor-treated bean cell cultures on phytoallexin formation (Ojalvo et al., 1987). ^{19}F and ^{1}H NMR were used to follow the uptake of trichloroacetic acid by tomato stems and leaves, demonstrating the movement and location of a xenobiotic in vivo (Rollins et al., 1989).

^{1}H-NMR is a particularly convenient tool for the study of water in plants,

because it is non-destructive and rich in structural, as well as dynamical, information (Burke et al., 1974). This method was used to study the freezing process and heat injury mechanisms in plants (Rajashekar and Burke, 1986; Abbas and Rajashekar, 1991). ^{1}H NMR was used to determine water allocation and transfer in several plant species (Bottomley et al., 1986; Brown et al., 1986; Brown et al., 1988; Johnson et al.,1987; Kerhoas and Dumas, 1986; McCain and Markley, 1985; and McCain et al., 1988). It was also used in cold acclimation studies of ornamentals (Burke et al., 1974) and cereals (Gusta et al., 1979). Proton magnetic resonance imaging (^{1}H-MRI) was used to image water in single cells (Aguayo et al., 1986), fruits (Hinsaw et al., 1979), and buds (Faust et al., 1991a). McCain et al. (1984) demonstrated that sample position in the magnetic field affected the spectral composition. This anisotropic behavior of plant tissue, should be taken in consideration when developing an spectroscopic method.

A grape bud consists of three partially developed shoots, with rudimentary leaves or with both rudimentary leaves and flower clusters (Winkler et al., 1974). The primary, secondary, and tertiary buds remain dormant, forming a large compound bud in winter (Pool et al, 1978). The whole bud is covered with suberin-impregnated scales to protect against mechanical injury, and the scales are lined with hairs to avoid dehydration of inner parts (Winkler et al., 1974). Primary buds are more likely to contain differentiated clusters, and they are also the first to emerge in the spring (Winkler et al., 1974). Primary buds are the least hardy of the three laterals and also less hardy than stem

tissues (Slater et al., 1991; Wolpert and Howell, 1984).

'Pinot Noir' grape primary buds have a water content of 54%(v/w) during the winter, when they are endodormant, and in the early ecodormant stage. However, water content increases to 85% during the late ecodormant period, when they are breaking in the spring (Gardea, 1987). Many physiological changes are associated with this increase in water content, such as a reduction in cold hardiness (Wolpert and Howell, 1984) and an increasing metabolic activity (Chapter 5). Changes in water content were determined by gravimetric methods, but those techniques do not allow in situ observations of water.

The objectives were to develop a spectroscopic method to characterize changes in water in grape buds. This was done first by determining if grape buds present an anisotropic behavior. Secondly, by evaluating changes in their spectroscopic properties, such as second derivative composition (Holler et al., 1989), as affected by water extraction.

MATERIALS AND METHODS

Instrumentation. Bud spectra were done in a Bruker AM 400 nuclear magnetic resonance spectrometer (USA Bruker Instruments Inc., San Jose, CA 95134-9977). All spectra were acquired at 400.136 MHz, with a synthesized frequency of 133.09 MHz. Transients of 4096 data points were recorded using a spectral width of 16,129 Hz. The number of points used was 512, and the resolution was 7.876 Hz/data point. The spectra were recorded at 298°K. Second derivative analyses were done for each spectra. When required, extracellular water extraction was achieved with an Eppendorf 5415 microcentrifuge (Brinkman Instruments Inc., Westbury, N.J. 11590-0204). Bud weights were recorded using a Metler AE-200 analytical balance (Metler Instruments Co., Highstown, N.J. 08520).

Plant material. Grapevine cuttings Vitis vinifera L. cv. 'Pinot noir' were rooted, potted and grown outdoors for one season. Current season shoots were sampled, and defoliated when required. In a preliminary study, we partition the compound bud biomass into scales, primary, secondary, and tertiary buds. The information was analyzed as a completely randomized design with ten replications. Based on results, which will be discussed later, we decided to direct our efforts to the primary buds. Primary buds were excised, fresh weights recorded, and the buds were kept in a humid container until the experiment began. During handling, further dehydration was avoided by keeping the buds in a Petri dish with wet Whatman No. 1 filter paper. Teflon tube, 3mm diameter,

was cut in 5 cm pieces, and each piece was cut lengthwise. Nine buds were inserted on each tube piece and wrapped with teflon tape. Care was taken to place the buds in an aligned position along the bud filled tube section (tubing). Once the spectra were obtained, the buds were dehydrated for a week at 75°C. Dry weights were recorded, and the total water content was estimated.

Since sample orientation may affect the spectral pattern (McCain et al., 1884), we started by defining the possible anisotropic behavior of grape buds.

Experiment 1. Effect of sample orientation in the magnetic field on ^{1}H-NMR spectra. Samples were prepared as described above. First, the spectra of buds with their longitudinal axis parallel to the direction of the magnetic field were recorded (McCain et al., 1984). Next, keeping the buds in the same order along the tubing, the buds were rotated 90°, such that their longitudinal axis were perpendicular to the direction of the magnetic field, and their spectra were recorded again. For each test, three tubings were fit in a standard 10mm NMR probe, on every sampling date. This study was done in 8 Sep., 12, 23, 28, and 30 Oct., and 1 Nov. 1989. The treatments were bud position in the magnetic field, either parallel, or perpendicular. Nine replications were used for each treatment. The changes in signal shape were assigned peak positions using the second derivative minima (Daley, 1990).

Experiment 2. Effect of extracellular water depletion on signal size. Here spectra were recorded only for buds parallel to the magnetic field. This procedure was done, first with intact buds. Using the same set of buds, the

extracellular water was extracted by centrifuging the buds at 4°C for 30 min at 8160 *g* (for details see Chapter 4). The buds were replaced in the 'tubings' in their original order. Then, the spectra of extracellular water-depleted samples were recorded.

RESULTS AND DISCUSSION

In a compound grape bud (Fig. 1), the scales are relatively dehydrated structures protecting the bud. The biomass increases from the tertiary to the secondary and primary buds. Primary buds are the ones with differentiated clusters, and are responsible for the season crop (Winkler et al., 1974). Primary buds are not only the largest component on a fresh and dry basis, but also the most hydrated, which is an advantage making them more suitable for water studies.

Experiment 1. Effect of sample orientation in the magnetic field on ^{1}H-NMR spectra. ^{1}H-NMR undifferentiated spectra of grape buds was not as conspicuous and orientation dependent as described in Crataegus leaf discs by McCain et al. (1984). Table 3.1 shows the location of those bands along the resonance frequency of the ^{1}H-NMR spectra for buds in both, parallel and perpendicular positions in the magnetic field. There are at least 10 reproducible bands in the second derivative analysis. These bands exist in both orientations. However, quantitatively, they are much stronger in the parallel position (Fig. 3.2a). The simplest explanation is that anisotropy exists, but the structure of the buds is sufficiently irregular, than even in the perpendicular orientation, the response is detected (Fig. 3.2b). Therefore, such bands appears a quantitative, rather than an absolute qualitative response.

Experiment 2. Effect of extracellular water depletion on signal size. When extracellular water was extracted, a decrease in signal size was observed

fewer protons were available for the instrument to read, which resulted in a reduced signal.

CONCLUSIONS

Grape buds presented an anisotropic behavior, although changes in the signal size are not visible. Furthermore, second derivative analysis showed that spectral composition changes quantitatively, but not qualitatively. Further studies should consider this condition. ^{1}H-NMR spectroscopy was a sensitive method able to detect extracellular water depletion from the buds.

Table 3.1. Description of assigned bands from the second derivative analysis of ^{1}H-NMR spectra of grape buds, as affected by sample orientation in the magnetic field.

Assigned Band	Band Frequency (ppm) Sample Position	
	Parallel	Perpendicular
1	-1.6830 a	-
2	-1.4280 ab	-1.3005 b
3	-1.0054 c	-1.0200 c
4	-0.7140 d	-0.7140 d
5	-0.3431 e	-0.3287 e
6	0.0306 f	0.0073 f
7	0.3124 g	0.2921 g
8	0.6184 h	0.6290 h
9	0.8568 i	0.8670 i
10	1.1424 j	1.1560 j
11	1.7340 k	1.6524 k
12	2.1420 l	-

Assigned bands were peaks found in the signal of the second derivative analysis of ^{1}H-NMR spectra of grape buds.

Band frequency was the location of these peaks along the horizontal axis of the spectra. Units are referred as the resonance frequency in ppm of magnetic strength.

Sample position is the orientation of the longitudinal axis of the buds, with respect to the direction of the magnetic field.

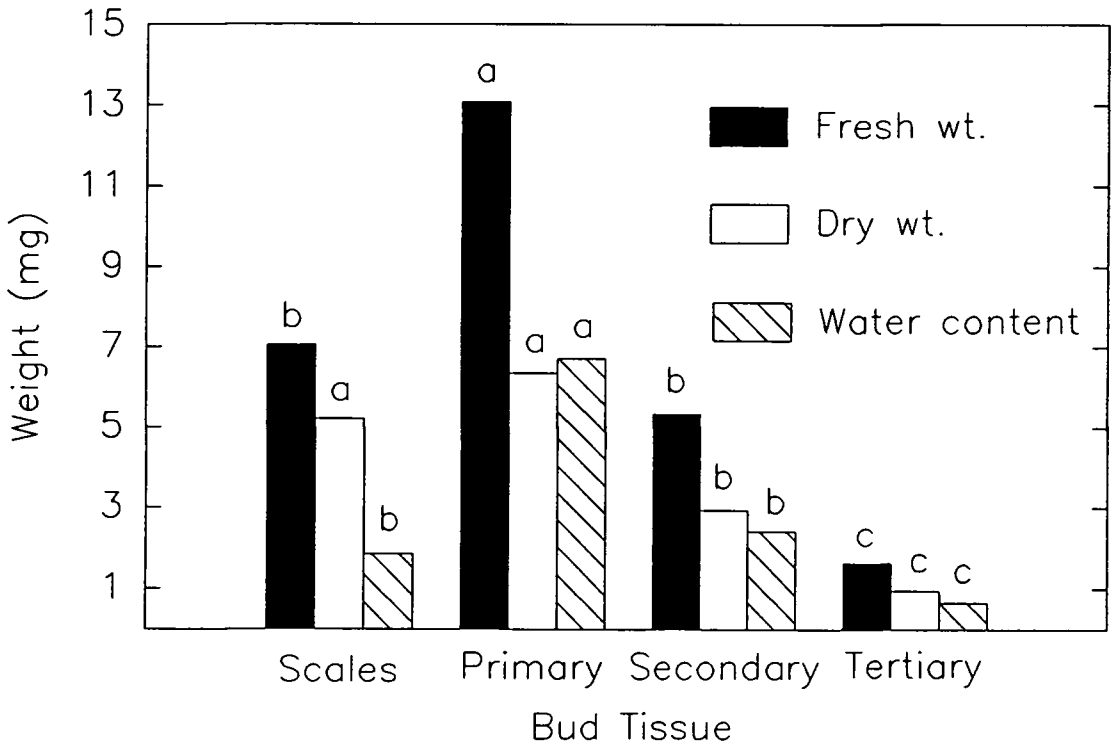


Figure 3.1 Partitioning of the compound grape biomass into the fresh and dry weights of each component, as well as their water content. Mean separation by Fisher's protected LSD at p=0.01

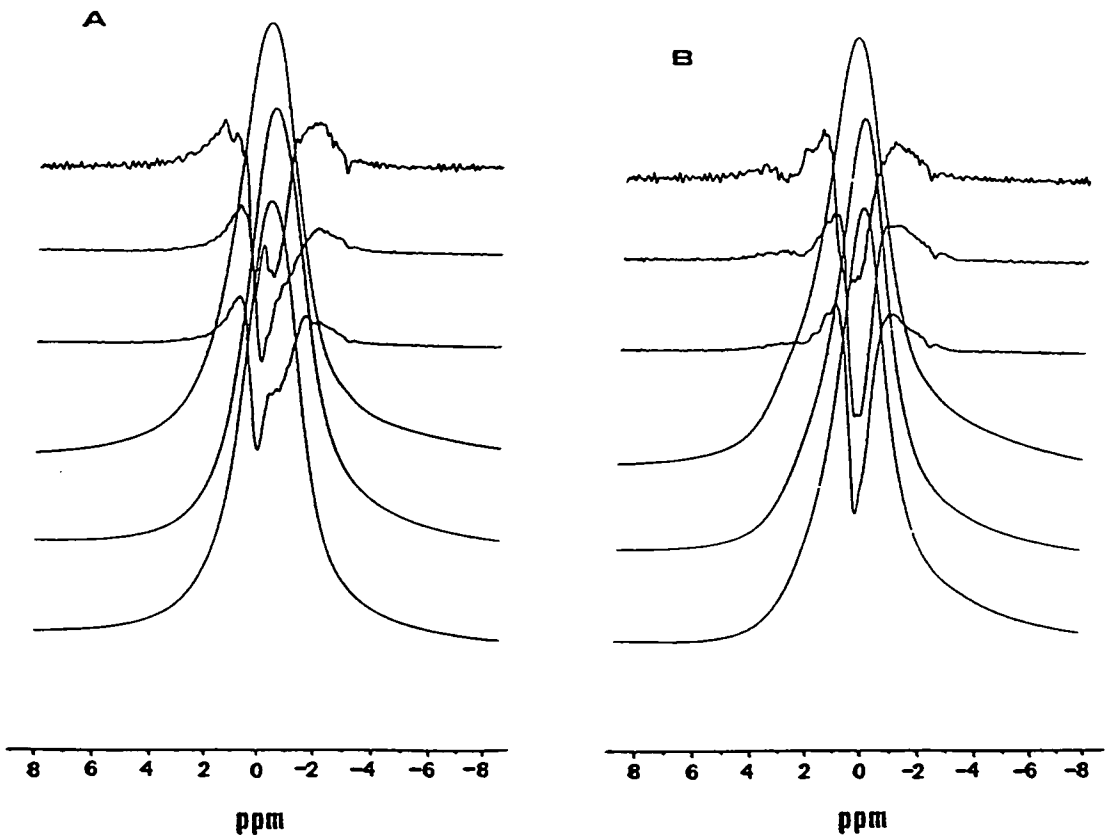


Figure 3.2. ^{1}H-NMR spectra of 'Pinot Noir' primary buds showing the anisotropic behavior of three sets of buds in the position parallel to the magnetic field (a), and the same sets in the perpendicular position (B). Second derivative analyses show the changes in the spectral complexity.

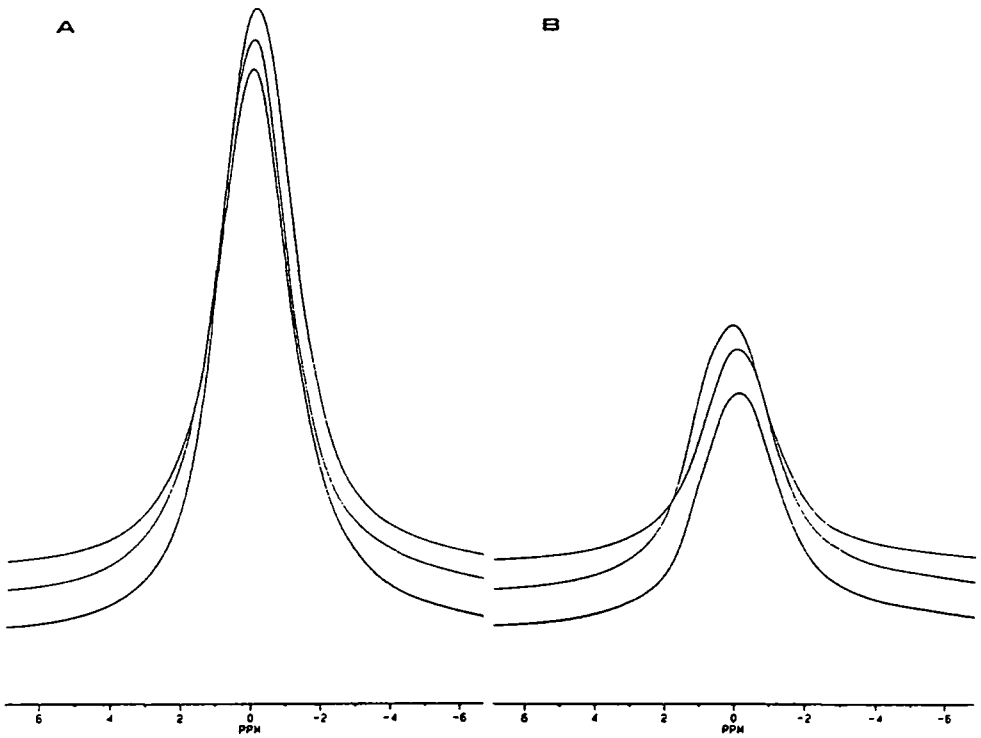


Figure 3.3. Effect of extracellular water depletion by centrifugation on the ^{1}H-NMR signal response of buds before centrifugation (A), and after being centrifuged (B). Scale in horizontal axis is parts per million (ppm) of the magnetic strength.

LITERATURE CITED

Abass, M., and C.B. Rajashaker. Characterization of heat injury in grapes using ^{1}H nuclear magnetic resonance methods. Plant Physiol. 96:957-961 (1991).

Aguayo, J.B., S.J. Blackband, J. Schoniger, M.A. Mattingly, and M. Hintermann. Nuclear magnetic resonance imaging of a single cell. Nature 322:190-191 (1986).

Bottomley, P.A. H.H. Rogers, and T.H. Foster. NMR imaging shows water distribution and transport in plant root systems in situ. Proc. Natl. Acad. Sci. U.S.A. Botany. 83:87-89 (1986).

Brown, J.M., G.A. Johnson, and P.J. Kramer. In vivo magnetic resonance microscopy of changing water content in Pelargonium hortorum roots. Plant Physiol. 82:1158-1160 (1986).

Brown, J.M., J.F. Thomas, G.P. Cofer, and G.A. Johnson. Magnetic resonance microscopy of stem tissues of Pelargonium hortorum. Bot. Gaz. 149(3):253-259 (1988).

Burke, M.J., R.G. Bryant, and C.J. Weiser. Nuclear magnetic resonance of water in cold acclimating red osier dogwood stem. Plant Physiol. 54:392-398 (1974).

Colombo, S.J. Bud dormancy, frost hardiness, shoot moisture content, and readiness of black spruce container seedlings for frozen storage. J Amer. Soc. Hort. Sci. 115(2):302-307 (1990).

Daley, L.S. French's nomenclature use: in vivo chlorophyll complexes. Plant Physiol. Biochem. 28(2):271-282 (1990).

Faust, M., D. Liu, M.M. Millard, and G.W. Stutte. Bound versus free water in dormant apple buds. A theory for endodormancy. Hortscience 26(7):887-890 (1991).

Garbow, J.R., L.M. Ferrantello, and R.E. Stark. ^{13}C Nuclear magnetic resonance study of suberized potato cell wall. Plant Physiol 90:783-787 (1989).

Gardea, A.A. Damage to Pinot noir (Vitis vinifera L.) as affected by bud development, INA-bacteria and a bacterial inhibitor. Thesis. Oregon

State University (1987).

Gerasimowicz W.V., S.I. Tu, and P.E. Pfeffer. Energy facilitated Na^+ uptake in excised corn roots via ^{31}P and ^{23}Na NMR. Plant Physiol. 81:925-928 (1986).

Gusta, L.W., D.B. Fowler, and P. Chen. A nuclear magnetic resonance study of water in cold acclimating cereals. Plant Physiol 63:627-634 (1979).

Hendrix, D.L., J.W. Radin, and R.A. Nieman. Intracellular pH of cotton embryos and seed coats during fruit development determined by ^{31}P nuclear magnetic resonance. Plant Physiol. 85:588-591 (1987).

Hinshaw, W.S., P.A. Bottomley, and G.N. Holland. A demonstration of the resolution of NMR imaging in biological systems. Experientia 35:1268-1269 (1979).

Holler, F., D.H. Burns, and J.B. Callis. Direct use of second derivatives in curve fitting procedures. Applied Spectroscopy 43(5):877-883 (1989).

Johnson, G.A., J. Brown, and P.J. Kramer. Magnetic resonance microscopy of changes in water content in stems of transpiring plants. Proc. Natl. Acad. Sci. U.S.A. Botany 84:2752-2755 (1987).

Jackson, P.C., P.E. Pfeffer, and W.V. Gerasimowicz. Use of ^{31}P NMR to assess effects of DNP on ATP levels in vivo in barley roots. Plant Physiol. 81:1130-1133 (1986).

Kerhoas, C., and C. Dumas. Nuclear magnetic resonance and pollen quality. In: Nuclear Magnetic Resonance. H.F. Linskens and J.F. Jackson (Eds.). pp 169-190. Springer-Verlag, New York (1986).

McCain, D.C., J. Croxdale, and J.L. Markley. Water is allocated differently to chloroplasts in sun and shade leaves. Plant Physiol. 86:16-18 (1988).

McCain, D.C., J. Croxdale, and J.L. Markley. Thermal damage to chloroplast envelope membrane. Plant Physiol. 90:606-609 (1989).

McCain, D.C., and J.L. Markley. Water permeability of chloroplast envelope membranes. In vivo measurement by saturation-transfer NMR. FEBS Letters 183(2):353-358 (1985).

McCain, D.C., T.C. Selig, Govindjee, and J.L. Markley. Some plants have orientation-dependent EPR and NMR spectra. Proc. Natl. Acad. Sci. U.S.A. 81:748-752 (1984).

Ojalvo, I., J.S. Rokem, G. Navon, and I. Goldberg. ^{31}P NMR study of elicitor treated Phaseolus vulgaris cell suspension cultures. Plant Physiol. 85:716-719 (1987).

Pool, R.M., C. Pratt, and H.D. Hubbard. Structure of base buds in relation to yield of grapes. Am. J. Enol. Vitic. 29:36-41 (1978).

Rollins, A., J. Barber, R. Elliott, and B. Wood. Xenobiotic monitoring in plants by ^{19}F and ^{1}H nuclear magnetic resonance imaging and spectroscopy. Plant Physiol. 91:1243-1246 (1989).

Slater, J.V., M.R. Warmund, M.F. George, and M.R. Ellersieck. Deacclimation of winter hardy Seyval blanc grape tissue after exposure to 16°C. Scientia Horticulturae 45:273-285 (1991).

Thorpe, T.A., K. Bagh, A.J. Cutler, D.I. Dunstan, D.D. McIntyre, and H.J. Vogel. A ^{14}N and ^{15}N nuclear magnetic resonance study of nitrogen metabolism in shoot-forming cultures of white spruce (Picea glauca) buds. Plant Physiol. 91:193-202 (1989).

Winkler, A.J., J.A. Cook, W.M. Kliewer, and L.A. Lider. General viticulture. Second edition. 710 pp. University of California Press. Berkeley. (1974).

Wolpert, J.A. and G.S. Howell. Effects of cane length and dormant season pruning on cold hardening and water content of Concord bud and cane tissues. Am. J. Enol. Vitic. 35(4):237-241 (1984).

Chapter 4

CHARACTERIZATION OF WATER CONTENT IN GRAPE PRIMARY BUDS DURING DORMANCY.

ABSTRACT

Bud dormancy status of 'Pinot Noir' was evaluated by forcing budbreak at 20°C. Primary buds required 300 cumulative chilling hours (CCH), to initiate the transition from the endodormant to the ecodormant state, which occurred in mid November. Further chilling to 1000 CCH improved the budbreak percentage. Changes in water content of dormant primary buds were determined by gravimetric and proton-nuclear magnetic resonance(^{1}H-NMR) methods in 1989-1990 and 1990-1991. Measurements of total, intra- and extracellular water fractions of primary buds were made gravimetrically. Extracellular water was extracted by centrifuging the buds at 8160 *g* for 30 min, followed by dehydration to estimate intracellular water. During the ecodormant phase prior to budbreak, the intracellular fraction rose from 0.51 to 4.40 mg/mg dry weight for mid-January and early April, respectively. This increase in intracellular water was highly correlated to the increase in total water content (R^2=0.9984). ^{1}H-NMR detected the amount of liquid water in the buds. At freezing temperatures, signal size and spin-spin relaxation time (T_2) decreased. T_2 at -30°C represented the bound fraction. During the dormant season, the free fraction was always larger than the bound one; however, changes in bound water indicated that a transient population was present.

INTRODUCTION

Grape buds make a transition from an endodormant to an ecodormant state (Lang et al., 1990) during the winter, after being exposed to at least 200 (CCH) (Martin, 1984). Concurrent changes in bud and cane moisture content have been reported (Wolpert and Howell, 1984). During the ecodormant period before budbreak, a strong hydration takes place in buds of blueberry (Bittenbender and Howell, 1975), black spruce (Colombo, 1990), and 'Pinot Noir' grapes (Gardea, 1987). These reports, however, are based on total water content, and little is known about water partitioning. Centrifugation has been used to extract the extracellular water fraction in cold acclimating wheat crowns (Gusta et al., 1979) and the sap content in xylem parenchyma cells (Gusta et al., 1983).

Although gravimetric methods partition the water into extracellular and intracellular fractions, bound and free water cannot be directly differentiated in this way. ^{1}H-NMR techniques were used to quantify bound water in colloidal systems (Toledo et al., 1968). The amounts of bound and free water change depending on the dormancy status of apple buds (Faust et al., 1991) and seed tissues (Vertucci, 1990). Faust et al. (1991) proposed that bud endodormancy is characterized by water in the bound form, while ecodormant buds contain free water.

^{1}H-NMR detects and characterizes tissue water non-destructively. Thus,

it has been used in many areas of research, including in vivo plant anatomy studies (Brown et al., 1988), plant-water relations studies to determine the water transport in roots under normal conditions and during rapid transpiration (Bottomley et al., 1986; Brown et al., 1986), and to quantify water content of transpiring stems (Johnson et al., 1987); in cold hardiness studies to evaluate varietal responses of acclimating cereals (Gusta et al., 1979), to understand freezing mechanisms in dogwood stems (Burke et al., 1974), and to characterize freezing of cultured cells (Chen et al., 1984); in chloroplast studies to determine water allocation (McCain et al., 1988), and thermal injury to membranes (McCain et al., 1989). H-NMR has also been used in imaging single cells (Aguayo et al., 1986), fruits (Hinsaw et al., 1979), and lately for free water imaging of apple buds depending on their dormancy status (Faust et al., 1991).

The objective of this study was to evaluate changes in the water status of grape primary buds during dormancy. This was done through evaluation of intracellular and extracellular water by gravimetric methods and to quantification of seasonal changes in the free and bound fractions by H-NMR spectroscopy.

MATERIALS AND METHODS

Dormancy determination. One-year old 'Pinot Noir' grape plants (Vitis vinifera L.) were used during the 1989-1990 and 1990-1991 dormant seasons. Vines were grown at the Lewis-Brown farm (O.S.U.) near Corvallis, Ore. Beginning in October, and at weekly intervals, five plants were collected, pruned to 10 buds, potted, and transferred to a greenhouse. Environmental conditions were 12 h photoperiod with temperature 20±3°C, and the pots were watered weekly. Variables evaluated included time elapsed to reach 10 and 50% budbreak, and the total number of broken buds. Chilling between 0 and 7°C (Martin, 1984) was calculated based on daily temperature records from October to March and expressed as cumulative chilling hours (CCH). An attempt to establish the occurrence of vascular connection between bud and stem was done in 1990. Small 4-node cuttings were prepared weekly from the prunings obtained from the plants of the dormancy study described above. The basal ends of the cuttings were submerged in a 5% azosulfamide solution. After one week, longitudinal sections of buds and stems were cut and examined for the absence or presence of the dye in the buds.

Gravimetric analysis. In an initial experiment the optimum centrifuge conditions to extract extracellular water (ECW) from the buds were determined. A modified method, previously described by Gusta et al. (1979, 1983) was used. Centrifuge vials (1 ml) were loaded with tissue paper, and a disc of Whatman

filter paper No. 1 was placed on top. Vials were then weighed individually. Primary buds were excised and immediately transferred to a humid environment. Ten randomized sets of three buds each were weighed and placed vertically in a vial with the excised side in contact with the paper. The vials were centrifuged at 82, 325, 1310, 2940, 5220, and 8160 *g* for 5 min each, in an Eppendorf 5415 microcentrifuge (Brinkman Instruments Inc., Westbury, NJ 11590-0204). The buds were removed from the vials, weighed again, and then oven dried to a constant weight at 75C for one week. Calculations were made to determine the ECW, intracellular water (ICW) and total water content (TWC) of the grape primary buds. These calculations were ECW = fresh weight - centrifuged weight, ICW = centrifuged weight - dry weight, and TWC = fresh weight - dry weight. Bud weight did not change after centrifugation at 8160 *g* (Fig. 4.1). Thus, we concluded that centrifuging the samples for 30 min at 8160 *g* was sufficient to attain constant centrifuged weight. Therefore, this centrifuge rate was used in subsequent experiments to determine ECW and ICW.

Water content partitioning was done every other week from 3 Nov. 1989 to 30 Mar. 1990, and from 17 Jan. to 9 Apr. 1991. TWC was also estimated from 3 Oct. 1990 to 9 Apr. 1991. TWC, ICW, and ECW were expressed as mg H_2O/mg dry weight. The experimental design was completely randomized with dates as treatments, and 27 replications for the water content partitioning on both seasons, and 5 replications for TWC in 1990-1991.

Spectroscopic analysis. Parallel to the gravimetric analysis, nine buds

were excised, weighed, and inserted into a 5 mm NMR probe. Spectral patterns of the sample water protons were obtained in a Bruker AM 400 Nuclear Magnetic Resonance spectrometer (USA Bruker Instruments Inc., San Jose, CA 95134-9977). Initial bud water spectra were obtained at 25°C. While in the spectrometer, the sample was subjected to a stream of liquid nitrogen to lower the temperature to -10, -20, and -30°C, at a rate of 1°C/min. Spectra were recorded for 15 min at each temperature. Signal size, as area under the peak, was determined by 10 independent measurements using a Li-Cor area meter Model 3100 (Licor Inst. Corp., Lincoln, NE 68504). Signal size at 25°C represented the initial amount of liquid water present in the sample (Kerhoas, 1986), and liquid water at freezing temperatures was estimated from the signal size at 25°C. Variables quantified were amount of liquid water, and $\Delta\nu_{1/2}$, the line width at half peak height, which is inversely correlated with the spin-spin relaxation time (T_2) (Burke et al., 1974; Brevard and Granger, 1981). The experimental design was completely randomized with three replications of nine buds and four scanning temperatures (25, -10, -20, and -30°C) for four sampling dates: 22 Jan., 28 Feb., 8 Apr. and 5 Nov. 1991. The above experiment was repeated using ECW-depleted buds after centrifugation.

RESULTS AND DISCUSSION

Dormancy determination. Data from 1989-1990 showed increasing budbreak as the winter season progressed (Fig. 4.2A). In October, only one bud per plant broke, while in January more than nine did. Some of these vines did not attain 100% budbreak because some buds were physically damaged during handling. The time required for the plants to break at least one bud (10%) is shown in Fig. 4.2B. Apparently, three stages can be distinguished. During the first stage, we observed a decrease in the time required for buds to break. When approximately 300 CCH were accumulated, budbreak occurred in only 30 days, yet less than 50% of the buds broke. The second stage is characterized by a relatively rapid budbreak, with at least 50% of the buds breaking. This is a more conventional reference for budbreak (Fuchigami et al., 1982). During the final stage, maximum budbreak is attained and the time to budbreak is shortened. Still, chilling in excess of 1000 CCH did not affect the final budbreak percentage. These results agree with a previous report (Iwasaki and Weaver, 1977), and were confirmed in the following season when comparable trends were observed. Some differences were noted: sub-freezing temperatures in late December injured plants in the field, and were responsible for the decrease in broken buds on the corresponding sampling dates (Fig. 4.2C). Afterward, the effect of the freeze was noted in a reduction in the final budbreak to about 70%. At such low temperatures, no chilling was accumulated, and therefore, the rate

of chilling was decreased. The steep decrease in the time required to reach 10% budbreak changed to a slower rate after 300 CCH (Fig. 4.2D), except the two dates in late December when the freeze occurred.

Based on these results, we concluded that 'Pinot Noir' required at least 300 CCH to begin the transition from the endodormant to the ecodormant stage. Chilling up to 1000 CCH improves the final number of buds that break.

Gravimetric analysis. Water content dynamics and bud weights during the dormant season are presented in Fig. 4.3. The water content partitioning for the 1989-1990 season (Fig. 4.3A) showed constant values for ECW, ICW, and TWC up to late December. Then on 16 Jan., a significant increase in ECW was recorded, marking the beginning of the hydration process before budbreak. This has been also reported for black spruce (Colombo, 1990), blueberry (Bittenbender and Howell, 1975), and 'Concord' grapevines (Wolper and Howell, 1984). These findings were confirmed in the 1991 season, although ECW increase was observed a week later (Fig. 4.3B). In both seasons, ECW increase was paralleled with a decrease in ICW. Given the number of replications and the small standard errors, it was unlikely that this represented an experimental procedure error. Rather, it was related with a change in water status that allowed a larger fraction to be extracted by centrifugation. This was supported by the fact that, up to 16 Jan., no xylem mobile dye was evident in the bud (Appendix 1). This implied that the bud was a closed system until then, because of the lack of xylem connection and diffusion per se being a slow process

(Nobel, 1983). Furthermore, bud fresh and dry weights were constant, indicating that biomass production, and water uptake had not begun (Figs. 4.3C and D). Little change occurred in bud dry weight until early March. Prior to March, the increase in fresh weight observed was primarily due to intracellular hydration. This was followed by increases in both dry and fresh weights, as previously reported (Bittenbender and Howell, 1975; Gardea,1987; Wolpert and Howell, 1984).

The relationship between TWC and ICW for two consecutive seasons, as determined by simple linear regression analysis, was high with R^2 values of 0.99 (Table 4.1). Therefore, TWC is an estimate of water content sufficient for field studies. The regression of TWC and ECW was not significant and is not shown. ECW measurements were more prone to error than TWC and ICW, from bud variation, bud dehydration during sample preparation, or a lack of sensitivity in the weighing method.

Spectroscopic analysis. Seasonal bud hydration, determined gravimetrically, was confirmed by ^{1}H-NMR. The effect of low temperature on signal size was dependent upon the sampling time (Fig. 4.4). ^{1}H-NMR spectra of acclimating dogwood stems (Burke et al., 1974) and hawthorn leaf discs (McCain, 1986) had a similar decrease in signal size as free water froze. Analyses of T_2 values in milliseconds, showed a highly significant interaction between sampling date and scanning temperature. As the season progressed, the spectra became narrower, having smaller $\Delta\nu_{\frac{1}{2}}$ values, and consequently

increasing T_2. Intact buds, before being frozen, had T_2 values of 0.139, 0.195, and 0.266 msec for 23 Jan., 28 Feb., and 4 Apr. respectively (Fig. 4.5A). Therefore, T_2 values increased as buds became more hydrated. At -30°C on the same dates, the values were 0.096, 0.082, and 0.070 msec.

Toledo et al. (1968) found in a complex colloidal system, that the T_2 values were an estimate of the bound water fraction. However, this was only so, when the signal was recorded at temperatures cold enough to freeze all available free water. Measurements of T_2 at room temperature include the resonance properties of both free and bound water. ECW extraction by centrifugation had no effect on the bound water fraction, since the T_2 values were similar to the intact buds at -30°C (Fig. 4.5B).

Changes in TWC of intact primary buds determined by ^{1}H-NMR are shown in Fig 4.5C. Water content rose from 0.935 to 1.222 and then to 3.617 mg H_2O/mg dw on 23 Jan. 1991, 28 Feb., and 4 Apr., respectively. Therefore, the decreased signal size is most logically attributed to increasing water freezing at later dates. At -10°C, the liquid portion was 0.881, 0.932, and 0.648 mg H_2O/mg dw, for 23 Jan., 28 Feb., and 4 Apr., respectively. Ratios to signal size show this represents 6, 24, and 82% of the total water content. An increasing amount of water froze at -20 and -30°C. Water remaining in liquid form at -30°C represents the bound fraction, and at this temperature differences among sampling dates were smaller.

When the ECW was depleted after centrifugation, the remaining ICW

had a similar response (Fig. 4.5D). Slight negative water content values at -30°C in the April sample are attributed to small and random variations in the model. Since intact and ECW-depleted buds behave in the same manner. Thus, this response pattern strongly suggests that the amount of unfrozen water at -30°C (bound water) is independent of the presence of the ECW fraction. These data also provide evidence that the ECW is in the free state, and centrifugation does not affect the bound fraction. The regression equations, coefficients of determination, and the significance probabilities of plots in Fig. 4.5 are shown in Table 4.2.

Therefore, we have considered that liquid water at -30°C represents the bound fraction. Changes in the partitioning of water into free and bound fractions are shown in Fig. 4.6. Total water followed the same pattern described before, but the bound fraction of endodormant buds was 0.23 mg H_2O/mg dw on 6 Nov., and increased to 0.32 in ecodormant buds on 22 Jan. Then, bound water decreased to 0.26 and 0.20 on 28 Feb. and 8 Apr., respectively. The free fraction decreased in January, corresponding to a significant increase in the bound fraction. Since the total water content remained constant, and we observed no movement of water soluble dyes between the bud and the stem xylem by 16 Jan., we concluded that free water decreased because some of it became bound. After January, the free water content rose because of increasing vascular connection and also the release of some bound water. Since bound water decreased as the season progressed, it is reasonable to conclude that

transient water exists, as the bound water becomes free. This agrees with calorimetric studies of the state of water in pea and soybean cotyledons (Vertucci, 1990). These studies concluded that at least two populations of water exist around a macromolecule, one having severely restricted mobility and the other with properties similar to bulk water. Although the actual mechanism remains unclear, Vertucci postulated that this occurred because of changes in water structure, or in the macromolecules associated with the water.

Changes in bud water content from the bound to the free form were proposed to distinguish endodormant from ecodormant buds (Faust et al., 1991). Our results demonstrated that free water was always more abundant than the bound fraction in grape buds. Also, the bound water in the early endodormant period on 6 Nov. was lower than two of the three sampling dates of ecodormant buds. The bound water pattern observed most closely correlates to cold hardiness of grape buds than dormancy status (Wolpert and Howell, 1984; Slater et al., 1991).

CONCLUSIONS

This study provides evidence that, in the Willamette Valley, Oregon 'Pinot Noir' primary buds require ca. 300 CCH to begin to make the transition from the endodormant to the ecodormant phase. Chilling up to 1000 CCH increases budbreak percentage. The hydration process, preceding budbreak, was started by a change of ICW to ECW, although no vascular connection between bud and stem xylem was observed at the time. Bud hydration continued via an increase in ICW, while ECW remained constant. Increases in bud weight were clearly seen in early March. Bound water was assessed by ^{1}H-NMR at -30°C. The results agreed with the concept published by Vertucci (1990) that the bound fraction may be composed of two populations, one with very restricted mobility, and the other in a transient state. Although the free fraction was always larger than the bound one, small changes in the latter may implicate major changes in the bud physiology, perhaps more related with its cold hardiness than the dormancy status.

Table 4.1. Parameters estimated for the regression analyses between total water content and intracellular water of 'Pinot Noir' primary grape buds for two consecutive dormant seasons.

Season	Parameter			
	a	b	R^2	p
1989-1990	0.2167	1.3081	0.9935	0.0001
1991	0.1919	1.0131	0.9952	0.0001

Table 4.2. Regression equations, coefficients of determination (R^2), and model significance ($P>F$) of T_2 and water content for intact and ECW-depleted 'Pinot Noir' grape buds, scanned at 0, -10, -20, and -30°C.

Dependent variable	Equation	R^2	P>F
Intact buds:			
T_2 (msec)	$Z=0.28+41.3E\text{-}4(X)+12E\text{-}8(X^2Y^2)\text{-}57.7E\text{-}7(Y^2X)+36.8E\text{-}7(X^2Y)$	0.8875	0.0001
Water content (mg H_2O/mg dw)	$Z=1.68\text{-}5.4E\text{-}3(X)\text{-}2E\text{-}3(Y^2)+8E\text{-}4(X^2)\text{-}2E\text{-}3(XY)+10.5E\text{-}7(X^2Y^2)+63E\text{-}6(X^2Y)$	0.9149	0.0001
ECW-depleted buds			
T_2 (msec)	$Z=0.28+42.3E\text{-}6(X^2)\text{-}45.3E\text{-}5(XY)+27E\text{-}8(X^2Y^2)\text{-}18.3E\text{-}6(Y^2X)\text{-}88E\text{-}7(X^2Y)$	0.8836	0.0001
Water content (mg H_2O/mg dw)	$Z=1.76\text{-}8.6E\text{-}2(X)+0.19(Y)+47.2E\text{-}4(Y^2)+12.4E\text{-}4(X^2)\text{-}12.8E\text{-}3(XY)+42.1E\text{-}7(X^2Y^2)\text{-}33.6E\text{-}5(Y^2X)+16.6E\text{-}5(X^2Y)$	0.9553	0.0001

X= Sampling date (days). Y= Scanning temperature (°C)

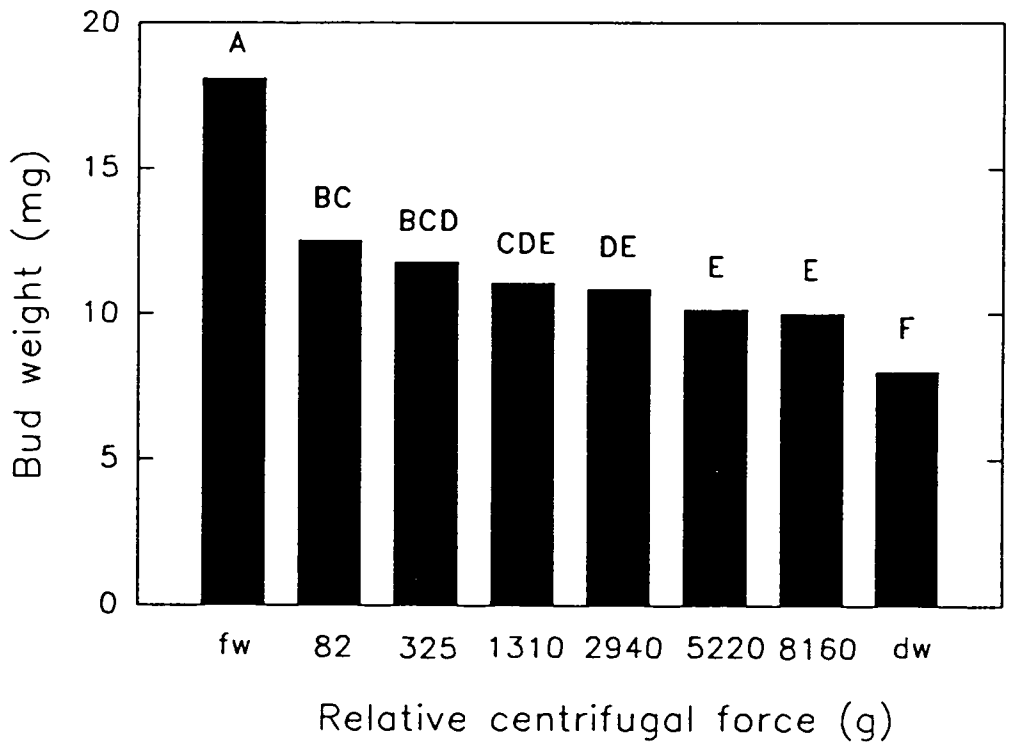


Figure 4.1. Changes in the grape primary bud weights from water depletion after centrifugation, as compared to fresh (FW), and dry (DW) weights. Means separation were by Fisher's Protected LSD at 0.01 level.

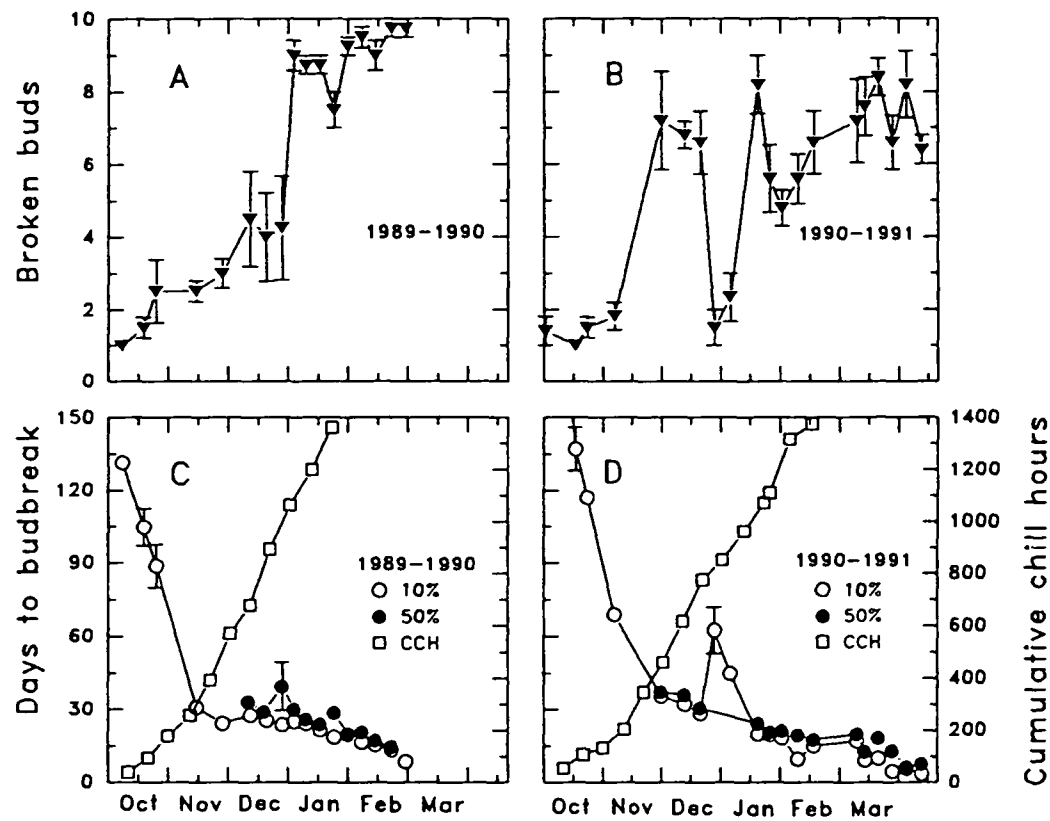


Figure 4.2. Number of broken buds (A,B), and number of days to reach 10 and 50% budbreak, and cumulative chilling hours (C,D), during dormancy of 'Pinot Noir' grapevines in the Willamette Valley, Oregon for the 1989-1990 and 1990-1991 seasons, respectively. Vertical bars represent ± S.E at p=0.05.

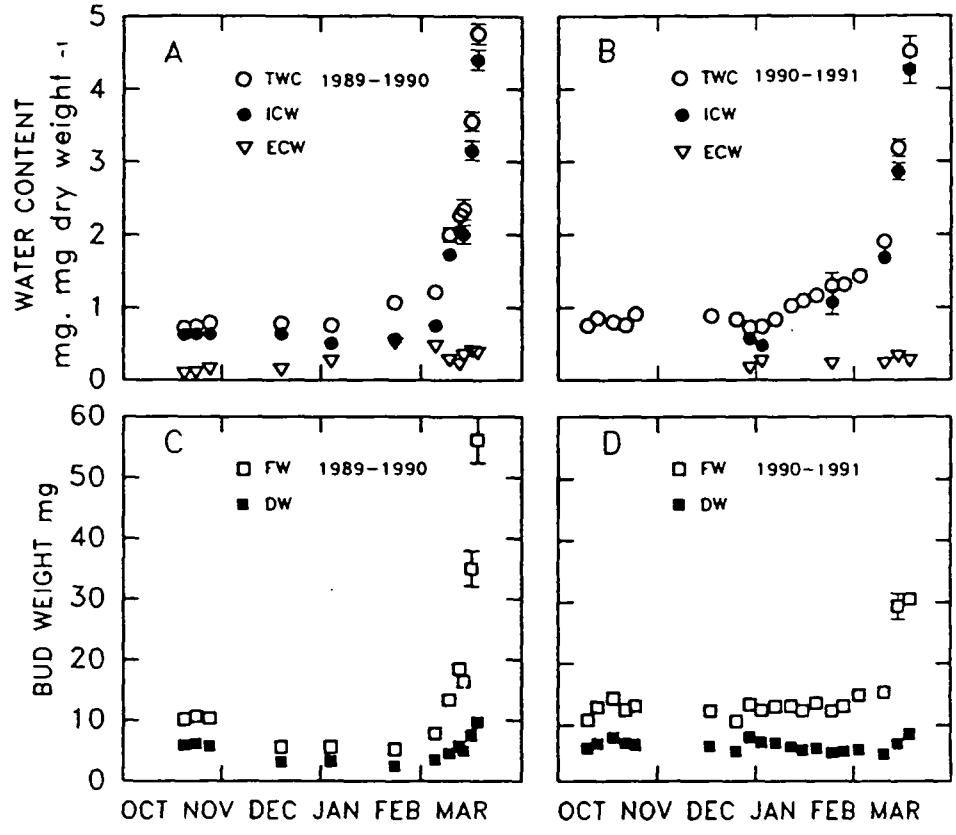


Figure 4.3. Changes in total water content (TWC), intracellular water (ICW), and extracellular water (ECW) (A,B), and fresh (FW) and dry (DW) weights of 'Pinot Noir' grape primary buds (C,D), during two dormant seasons in the Willamette Valley, Oregon. Vertical bars represent ± S.E. at p=0.05.

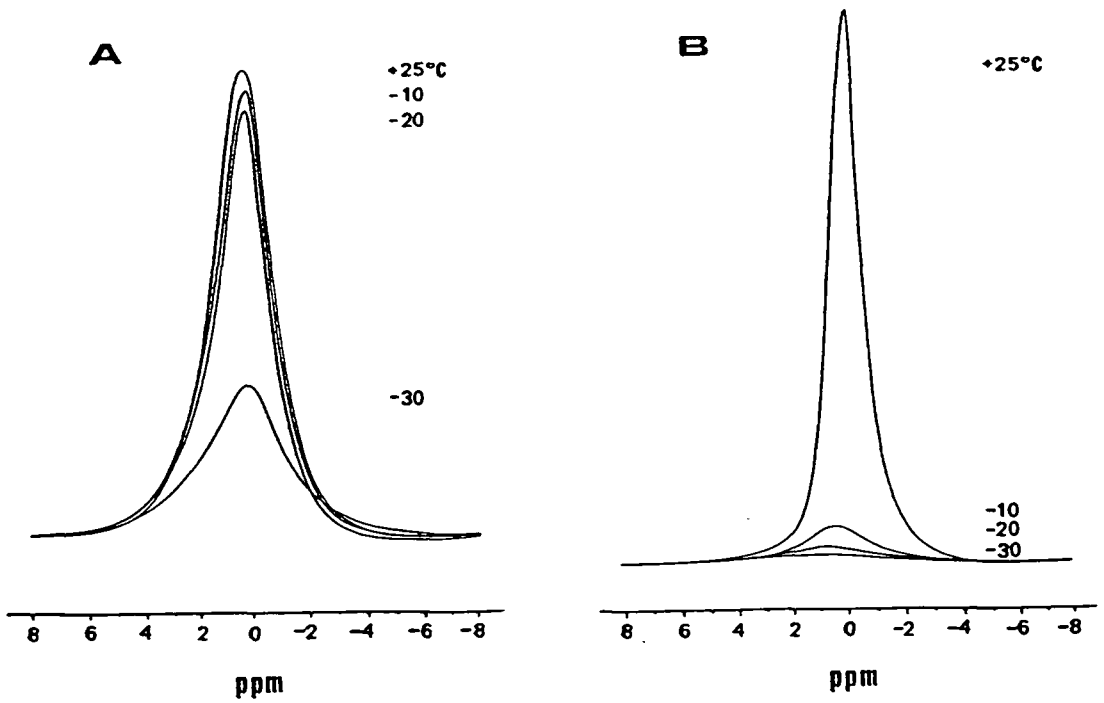


Figure 4.4. Effect of scanning temperature on the ^{1}H-NMR spectra of 'Pinot Noir' grape primary buds sampled on 22 Jan. (A) and 4 Apr. (B). Peaks represent liquid water at any given temperature. Signal size is comparable only within sampling dates.

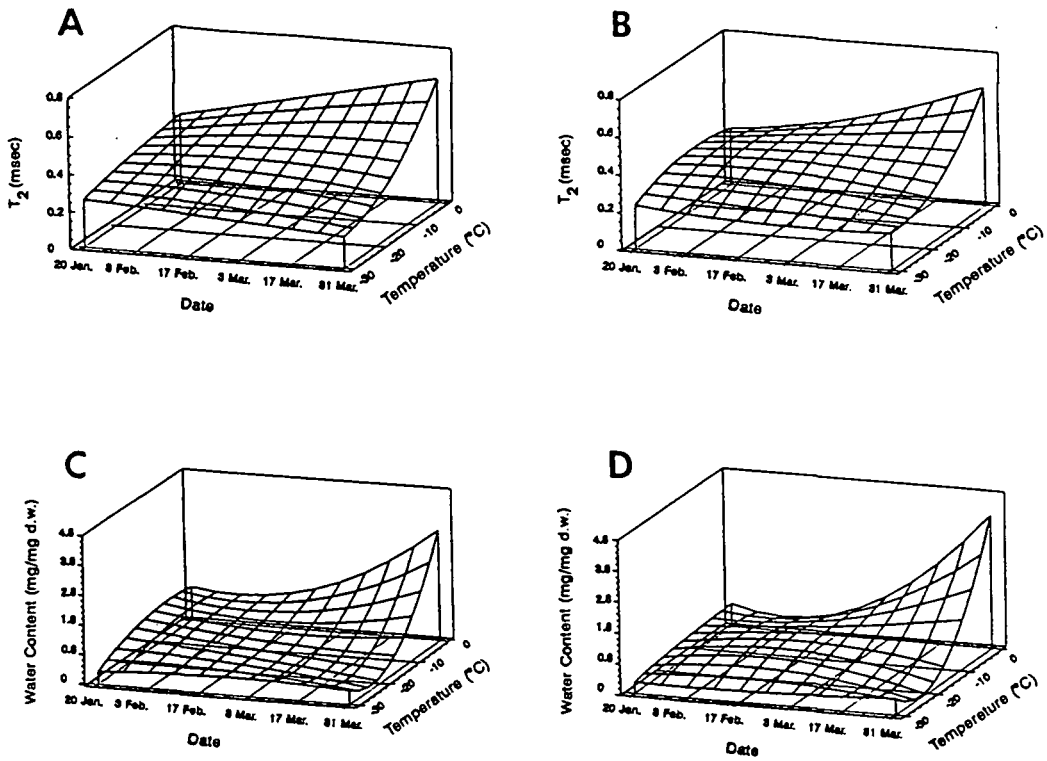


Figure 4.5. Effect of sampling date and ^{1}H-NMR scanning temperature of grape primary buds on spin-spin relaxation time (T_2) of intact buds (A), and extracellular water-depleted buds (B). As well as total water content of intact buds (C), and extracellular water-depleted buds (D). A highly significant interaction between sampling date and scanning temperature was determined for both, total water content and T_2. Bud total water content was estimated gravimetrically, and represented by the signal peak area recorded at room temperature, this value was extrapolated to the corresponding decreases in signal size at each temperature. T_2 was calculated from the $\Delta\nu_{\frac{1}{2}}$ value of the resonance signals.

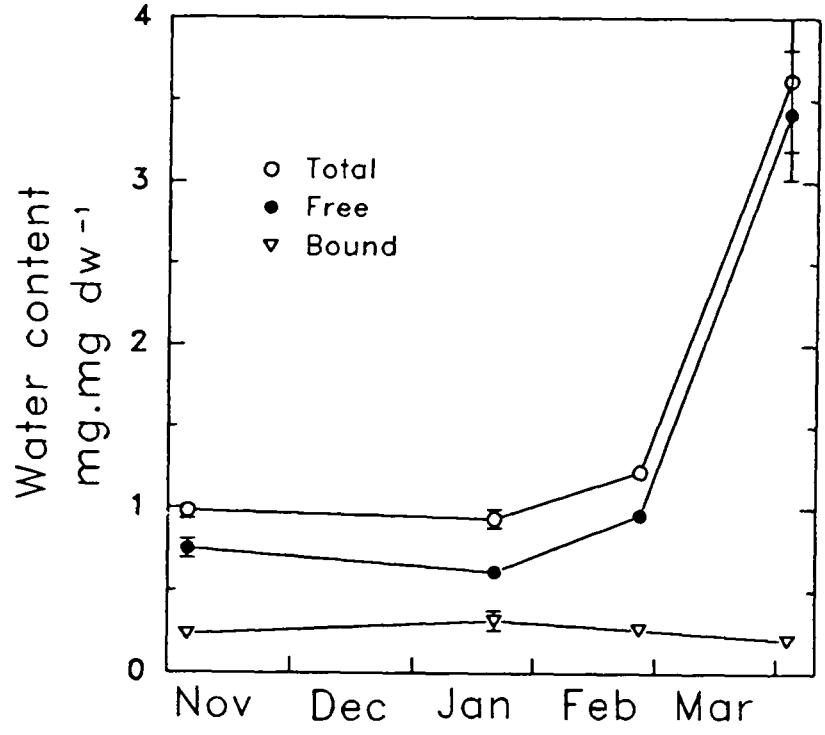


Figure 4.6. Partitioning of water content of 'Pinot Noir' primary buds into the free and bound fractions. Total water was determined by weight, bound water was estimated by ^{1}H-NMR at -30°C, and the free fraction was calculated by substraction. Vertical bars represent ± S.E. at p=0.05.

LITERATURE CITED

Aguayo, J.B., S.J. Blackband, J. Schoniger, M.A. Mattingly, and M. Hintermann. Nuclear magnetic resonance imaging of a single cell. Nature 322:190-191 (1986).

Bittenbender, H.C., and G.S. Howell. Interaction of temperature and moisture content on spring deacclimation of buds of highbush blueberry. Can. J. Plant Sci. 55:447-455 (1975).

Bottomley, P.A. H.H. Rogers, and T.H. Foster. NMR imaging shows water distribution and transport in plant root systems in situ. Proc. Natl. Acad. Sci. U.S.A. Botany. 83:87-89 (1986).

Brevard,C., and P. Granger. Handbook of high resolution multinuclear NMR. pp 229. Wiley-Interscience Publication. New York (1981).

Brown, J.M., G.A. Johnson, and P.J. Kramer. In vivo magnetic resonance microscopy of changing water content in Pelargonium hortorum roots. Plant Physiol. 82:1158-1160 (1986).

Brown, J.M., J.F. Thomas, G.P. Cofer, and G.A. Johnson. Magnetic resonance microscopy of stem tissues of Pelargonium hortorum. Bot. Gaz. 149:253-259 (1988).

Burke, M.J., R.G. Bryant, and C.J. Weiser. Nuclear magnetic resonance of water in cold acclimating red osier dogwood stem. Plant Physiol. 54:392-398 (1974).

Chen, T. H. H., K. K. Kartha, F. Constabel, and L.V. Gusta. 1984. Freezing characteristics of cultured Catharanthus roseus (L.) G. Don cells treated with dimethylsulfoxide and sorbitol in relation to cryopreservation. Plant Physiol. 75:720-725.

Colombo, S.J. Bud dormancy, frost hardiness, shoot moisture content, and readiness of black spruce container seedlings for frozen storage. J. Amer. Soc. Hort. Sci. 115:302-307 (1990).

Faust, M., D. Liu, M.M. Millard, and G.W. Stutte. Bound versus free water in dormant apple buds. A theory for endodormancy. Hortscience 26:887-890 (1991).

Fuchigami, L.H., C.J. Weiser, K. Kobayashi, R. Timmis, and L.V. Gusta. A degree growth stage (°GS) model and cold acclimation in temperate

woody plants. In: Plant cold hardiness. P.H. Li (Ed.) pp. 93-116. Academic Press, N.Y. (1982).

Gardea, A.A. Damage to Pinot noir (Vitis vinifera L.) as affected by bud development, INA-bacteria, and a bacterial inhibitor. M.S. Thesis. Oregon State University (1987).

Gusta, L.W., D.B. Fowler, and P. Chen. A nuclear magnetic resonance study of water in cold acclimating cereals. Plant Physiol 63:627-634 (1979).

Gusta, L.W., N.J. Tyler, and T.H.H. Chen. Deep supercooling in woody taxa growing north of the -40°C isotherm. Plant Physiol. 72:122-128 (1983).

Hinshaw, W.S., P.A. Bottomley, and G.N. Holland. A demonstration of the resolution of NMR imaging in biological systems. Experientia 35:1268-1269 (1979).

Iwasaki, K., and R.J. Weaver. Effects of chilling, calcium cyanamide, and bud scale removal on budbreak, rooting, and inhibitor content of Zinfandel grape (Vitis vinifera L.). J. Amer. Soc. Hort. Sci. 102:584-587 (1977).

Johnson, G.A., J. Brown, and P.J. Kramer. Magnetic resonance microscopy of changes in water content in stems of transpiring plants. Proc. Natl. Acad. Sci. U.S.A. Botany 84:2752-2755 (1987).

Kerhoas, C., and C. Dumas. Nuclear magnetic resonance and pollen quality. In: Nuclear Magnetic Resonance. H.F. Linskens and J.F. Jackson (Eds.). pp 169-190. Springer-Verlag, New York (1986).

Lang, G., J.D. Early, G.C. Martin, and R.L. Darnell. Endo-, para-, and ecodormancy: Physiological terminology and classification for dormancy research. Hortscience 22:371-377 (1987).

Martin, G.C. An introduction to bud dormancy. In: Proceedings of bud dormancy in grapevines. R.J. Weaver, J.O. Johnson, and A.S. Wicks (Eds.). pp. 1-4. University of California Press, Davis (1984)

McCain, D.C. Orientation of chloroplasts in leaves by H NMR spectroscopy. In: Nuclear Magnetic Resonance. H.F. Linskens and J.F. Jackson (Eds.). pp 127-147. Springer-Verlag, New York (1986).

McCain, D.C., J. Croxdale, and J.L. Markley. Water is allocated differently to chloroplasts in sun and shade leaves. Plant Physiol. 86:16-18 (1988).

McCain, D.C., J. Croxdale, and J.L. Markley. Thermal damage to chloroplast envelope membrane. Plant Physiol. 90:606-609 (1989).

Nobel, Park S. Biophysical plant physiology and ecology. pp. 10-20. W.H. Freeman and Co., New York (1983).

Slater, J.V., M.R. Warmund, M.F. George, and M.R. Ellersieck. Deacclimation of winter hardy Seyval blanc grape tissue after exposure to 16°C. Scientia Horticulturae 45:273-285 (1991).

Toledo, R., M.P. Steinberg, and A.I. Nelson. Quantitative determination of bound water by NMR. Journal of Food Science 33:315-317 (1968).

Vertucci, C.W. Calorimetric studies of the state of water in seed tissues. Biophys. J. 58:1463-1471 (1990).

Wolpert, J.A. and G.S. Howell. Effects of cane length and dormant season pruning on cold hardening and water content of Concord bud and cane tissues. Am. J. Enol. Vitic. 35:237-241 (1984).

Chapter 5

METABOLIC HEAT RATES OF DORMANT GRAPE BUDS.

ABSTRACT

A respiratory profile of dormant grape primary buds cv. 'Pinot Noir' was developed using differential scanning calorimetry. Metabolic heat rates decreased during endodormancy. Respiration of ecodormant buds was dependent upon the season, reaching minimum levels (ca. 1μW/mg dw) in January, but in February a steady increase in respiration began. Forcing ecodormant buds to break under controlled environmental conditions simulated the increase in respiration observed in field samples. Buds were classified into four developmental stages: ecodormant, initial swelling, fully swollen, and broken buds. Typical respiration profiles showed an increasing metabolic heat rate of 5, 17, 28, and 29 μW/mg dw as the buds developed through the four stages. CO_2 evolution from respiring buds also was related to stage of bud development and increased from 2, to 3, 6, and 7 μW/mg dw at advancing stages. Metabolic efficiency was higher in ecodormant buds (R-1=0.33), then decreased to 0.15 at initial swelling, and rose to .22 and .26 for fully swollen and broken buds. The activation energy decreased during the forcing period, which means that, metabolism is more energy expensive in ecodormant buds, but decreased as bud development advanced.

INTRODUCTION

Respiration of temperate plants follows a seasonal pattern, decreasing during the fall and increasing by the end of winter (Westwood, 1988). Such behavior was described in apple and pear buds (Butler and Landberg, 1981; Cole et al., 1982) and roots of Atriplex confertifolia (Holthausen and Caldwell, 1980). An increase in respiration rate was also reported during the transition from the dormant to non-dormant stage of cherry seed embryonic axis (La Croix and Jaswal, 1967). Chilling was required for maple buds to raise their respiration levels (Pollock, 1960), and Young (1990) demonstrated that during dormancy, chilling affected respiratory response of apple trees to different forcing temperatures. Ross (1984) concluded that, in general, termination of dormancy resulted in enhanced respiratory activity.

Several methods have been used to measure respiration. Among them are: Cartesian diver balances (Pollock and Olney, 1959; Pollock, 1960), Gilson respirometers (Chen and Varner, 1970; Zimmerman et al., 1970), infrared gas analyzer (Butler and Landsberg, 1981), manometric techniques (Wrzesniewski, 1985), and gas chromatography (Young, 1990). Criddle et al. (1991) considered that heat production is an inevitable byproduct of all metabolic processes, but until recently, measurements of heat evolution were limited by available instrumentation (Criddle et al., 1988). Differential scanning calorimeter (DSC) provides a non-invasive method for studying heat evolution in living cells (Loike et al., 1981). This technique has been used in different plant physiology studies:

predicting long-term growth rates of larch clones (Hansen et al. 1989); defining suitable storage conditions to avoid spoilage of fresh cut pineapple (Iversen et al. 1989); evaluating the state of water in pea and soybean cotyledons (Vertucci, 1990); measuring aerobic cell metabolism in unstirred cell cultures (Fontana et al. 1990); studying the responses of tomato cells to thermal extremes (Breidenbach et al. 1990); and determining high and low temperature inactivation of tomato cells (Rank et al. 1991). Criddle et al. (1991) devised a method to measure simultaneously metabolic heat rate, CO_2 production, and oxygen consumption in corn tissue. These works demonstrate that metabolic heat measurements are a reliable method to assess respiration.

Simultaneous measurements of the rates of metabolic heat production, and CO_2 evolution were proposed by Criddle et al. (1990) to estimate the unitless ratio 'R-1', which is inversely related to the calorespirometric ratio (Elliot and Davison, 1975). 'R-1' provides a useful method to determine metabolic efficiency and to quantify changes in metabolic pathways active in the tissues (Criddle et al., 1990).

Theoretical basis for the use of apparent activation energies (Ea) were discussed elsewhere (Johnson et al., 1974; Montgomery and Swenson, 1976). Ea was defined as the energy required to move a reaction or pathway forward (Young, 1990); the higher its value, the more energy is required. Ea decreased in apple trees approaching growth, once chilling was satisfied (Young, 1990), and Criddle et al. (submitted) proposed recently, that Ea is a useful criterion to

explain spatial distribution of temperate plant species in response to environmental conditions. Johnson et al. (1974) found no rational basis for the use of temperature coefficients of respiration (Q_{10}). However, Amthor (1989) suggested that this information is useful to compare results with those found in the literature.

The purpose of this study was to characterize the changes in grape bud respiration during endo- and ecodormancy (Lang et al., 1987) by calorimetric methods and by calculating metabolic heat production, CO_2 evolution, respiration efficiency, apparent activation energies, and Q_{10} values at several temperatures in a relevant physiological range.

MATERIALS AND METHODS

Plant material. For seasonal changes in bud respiration, buds were sampled, either from one-year old nursery 'Pinot Noir' (Vitis vinifera L.) vines, or a mature vineyard at Lewis-Brown Research Farm near Corvallis, Ore. from September to November 1990, and from January to March 1991. For budbreaking studies, cuttings were collected from the vineyard in late February 1990 and 1991. The cuttings were kept ecodormant in a 4°C room, and covered with wet sawdust until used.

Forcing conditions. Five-node cuttings were forced to break at two-day intervals in a 20°C greenhouse, with the basal ends of the cuttings immersed in water, which was changed every other day. The same procedure was repeated in 31 May 1991, except that a growth chamber was used, and the environmental conditions were 20°C and 18 hr photoperiod.

Respiration measurements. Bud heat of metabolism was measured in a Hart 4207 differential scanning calorimeter (Hart Scientific, Pleasant Grove, Utah 84062). The instrument has a base line noise of ±1μWatt, and a working range of -30 to 110°C. Temperature around the DSC chamber was kept at 15°C with a Fisher 9500 refrigerated circulating bath (Fisher Scientific, Pittsburgh, PA. 15230) using 30% (v/v) ethylene glycol as refrigerant. A flux of argon was used to prevent moisture condensation inside the DSC, in experiments running below room temperature. Data acquisition was done in the DSC embedded computer, while data processing was done in a 16 MHz 80386

computer system (Microsolutions, Corvallis, Ore. 97330). For system set up see Appendix 2. Samples were held in three-1.0 ml hasteloy ampules with removable lids, a fourth ampule with a copper weight was used as a reference.

Isothermal experiments were run at 25°C for 3000 sec, data were collected at 20 sec intervals. The last 20 observations were averaged, base-line corrected, and expressed on a dry weight basis. Measurements of CO_2 evolution heat rate, and 'R-1' calculations were done according to Criddle et al. (1990). See Appendix 3.

Scanning experiments were done in the temperature range of 1 to 60°C, at a rate of 7°C/hr, with data acquisition at 20 sec intervals. The descriptive program is included in Appendix 4, while raw data are displayed in Appendix 5. Heat measurements were base-line corrected, and expressed on a fresh weight basis (Appendix 6). Heat data were transformed to their natural logarithmic values, and corresponding scanning temperatures were expressed in absolute terms (1000/T°K). Arrhenius plots were developed using this information (Nobel, 1983; Fontana et al., 1990). An example of these plots is shown in Appendix 7. Ea was calculated from the slope of these plots in the temperature interval 15 to 35°C (Johnson et al., 1974; Montgomery and Swenson, 1976; Young, 1990; Criddle et al., submitted). Q_{10} values for the intervals 10 to 20, 20 to 30, 30 to 40, and 40 to 50°C were calculated directly from heat readings (Young, 1990).

Sample preparation. Primary buds were excised from the cuttings, and

fresh weights recorded. The excised buds were placed on moist filter paper in a closed petri dish, before proceeding with the experiment. During the dormant season, as well as the ecodormant stage in the budbreak study, the sample size was 5 buds per ampule. Since increasing bud development resulted in increasing metabolic heat rates, in the later stages of the budbreak study, the sample size was adjusted accordingly, so that the total heat output per ampule was ca 100 μW. To avoid dehydration, the buds were placed on a thin layer of 50 μl of sterile-double distilled water on the bottom of the ampule. Since scanning experiments lasted an average of 11 hr, anaerobic conditions were avoided by substituting air with oxygen. Dry weights were recorded once the runs were completed.

Experiment 1. *Seasonal changes in bud respiration.* In this study canes were sampled directly from the field, during the endodormant and ecodormant periods specified above. Buds were excised, followed by immediate determination of heat of metabolism by isotherms at 25°C.

Experiment 2. *Heat of metabolism of forced buds.* Ecodormant cuttings were forced to break, as described before. Heat of metabolism was measured isothermally by sampling buds at two-day intervals for ten days. A preliminary study was done on January 26, 1990, and repeated on 20 April 1990 and 15 May 1991. The responses were similar, therefore only data from the April experiment are presented.

Experiment 3. *Metabolic efficiency determination.* This and the following

experiment were started on 31 May, 1991, and repeated on 25 August, 1991. The results from the last date are reported here. Sampling was done according to four bud phenological stages to diminish variability, those stages were; ecodormant, initial swelling, fully swollen, and broken buds. Metabolic and CO_2 evolution heat rates were assessed isothermally. Using these values, the ratio, 'R-1' was calculated according to Criddle et al. (1990).

Experiment 4. *Scanning study.* Using the same sampling criterion as in experiment 3, scanning experiments were done to calculate energy of activation and Q_{10} values as described in the respiration measurements section.

Statistical procedures. Seasonal changes in bud respiration (Exp. 1) were analyzed as a completely randomized design with sampling dates as treatments, and six replications per treatment. 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. In experiment 2, metabolic heat rate was assessed on five replications in a completely randomized design for 0, 2, 4, 6, 8, and 10 days under forcing conditions as treatments. Information corresponding to experiments 3 and 4, regarding the measurement of metabolic and CO_2 evolution heat rates, 'R-1', Ea, and Q_{10} was analyzed as a completely randomized design with nine replications per treatment. Treatments were the four bud developmental stages described above.

RESULTS AND DISCUSSION

Experiment 1. *Seasonal changes in bud respiration.* The transition of 'Pinot Noir' buds from the endodormant to the ecodormant stage was achieved by mid November 1990 (Chapter 4), and the pattern of bud heat of metabolism during that period is presented in Fig. 5.1. A decrease in metabolic heat of endodormant buds was observed in the fall. The higher heat of metabolism of young nursery vines was caused by an active growth in late fall. Ecodormant buds showed a steady increase in respiration in February 1991. Since in the Willamette Valley of Oregon, 'Pinot Noir' buds, on the average, break during the 2nd week in April, the increase in respiration began well in advance, and long before any morphological change was visible.

Experiment 2. *Heat of metabolism of forced buds.* The results on ecodormant buds above were confirmed with ecodormant buds kept at 4 °C, and forced to break in 20 April, 1990 (Fig. 5.2). These buds respired at rates similar to those observed in the field during January, and a lag period of four days was noted under forcing conditions. After that, an exponential increase in metabolic heat was recorded, and breaking buds respired at a rate of 31 μW/mg dw, and after 10 days under forcing conditions, growing shoot tips respired at 49 μW/mg dw.

Experiment 3. *Metabolic efficiency determination.* The increase in respiration of ecodormant buds was also confirmed in the growth chamber experiment, where the metabolic heat rates of ecodormant, initial swelling, fully

swollen, and breaking bud stages were 5, 18, 28, and 29 μW/mg dw (Fig. 5.3). CO_2 evolution, as measured by its exothermic reaction with 0.4M NaOH, increased from 1.7 to 2.4, 6.3, and 7.4 μW/mg dw for the four consecutive developmental stages. The increase in metabolic heat and CO_2 evolution rates was not parallel. In the transition from ecodormant to initial swelling, CO_2 evolution increased only 41%, while metabolic heat rose 226%. Criddle et al. (1990) calculated a hypothetical 'R-1' value of 0.24, which was confirmed with tomato leaflets in the range of 12 to 30°C, where 'R-1' of 0.26 was calculated. Metabolism of ecodormant buds is a slow, but efficient process as confirmed by a 'R-1' value of 0.33. When buds change to the initial swelling stage a decrease in metabolic efficiency was recorded, and R-1 value decreased to 0.15. We hypothesized that this decrease in efficiency may be related with engagement of another pathway (Criddle et al., 1990), or change in substrate (Criddle, personal communication). As bud development proceeded, the 'R-1' ratio increased to 0.22, and 0.26 for fully swollen and broken buds.

Experiment 4. *Scanning study.* Calculation of apparent activation energies confirmed the results above (Fig. 5.4). During the four successive stages, Ea decreased from 54.11 to 52.48, 42.15, and 41.46 Joules/mole/°K. This implied that ecodormant bud metabolism, although efficient, proceeded at the slowest rate, because it was energetically expensive, as demonstrated by a high Ea. Even though, Ea decreased steadily during bud development, the biggest drop occurred from the initial swelling to the fully swollen stage.

Afterwards, it appeared that the metabolism continued at a less expensive rate. In the same way, Young (1990) concluded that Ea was lower during active growth than in dormancy.

Changes in Q_{10} values were dependent on the temperature range as well as bud developmental stage (Fig. 5.5). The Q_{10} of respiration is often about 2.0 in the physiologically relevant temperature range (Amthor, 1989). In the interval 10 to 20°C, the Q_{10} values were 2.81 to 3.88, 3.19, and 3.63 for the four consecutive stages (Fig. 5.5A). These high values are not uncommon (Young, 1990), and explain the quick response of grapevines to temperature (Iwasaki and Weaver, 1977; Moncur et al., 1989). However, in the 20 to 30°C interval (Fig. 5.5B), Q_{10} values decreased, as the buds developed, to 2.09, 1.88, 1.70, and 1.68. This suggests that, although metabolic heat increases, its rate actually decreased in the higher temperature regime. In the upper 30°C, characteristic peaks in the Arrhenius plots were observed, denoting bud stress at any stage, and lower Q_{10} values were found (Fig. 5.5C). In the 40 to 50°C interval, respiration rates decreased quickly, and buds died despite developmental stage, which was demonstrated by Q_{10} values smaller than 1.00 (Fig. 5.5D). These progressive decreases in Q_{10} with rise in temperature were also reported by Johnson et al. (1974).

CONCLUSIONS

Seasonal variation in bud respiration of grapevines follows the same pattern reported for other species. It decreases during the endodormant stage, and rises again during the ecodormant stage. Ecodormant buds respired at the lowest rate, as demonstrated by their low metabolic heat and CO_2 evolution rates. This low rate is explained by a high Ea; yet, based on the 'R-1' value, metabolism was highly efficient. The initial swelling stage was characterized by increasing respiratory activity, but Ea remained high, however, metabolic efficiency dropped, based on metabolic heat and CO_2 evolution rates. This may explain the longer period observed in the field for this developmental stage. Respiration increased up to the broken bud stage, and so did metabolic efficiency, while Ea decreased to a minimum. This means that once growth is initiated, there is not only a recovery in metabolic efficiency, but also less energy is required to keep the system going on. During budbreak, grapevines were highly responsive to temperature in the range from 10 to 20°C, but temperatures up to 30°C, caused a reduction in respiration. Above this temperature, buds were stressed and died.

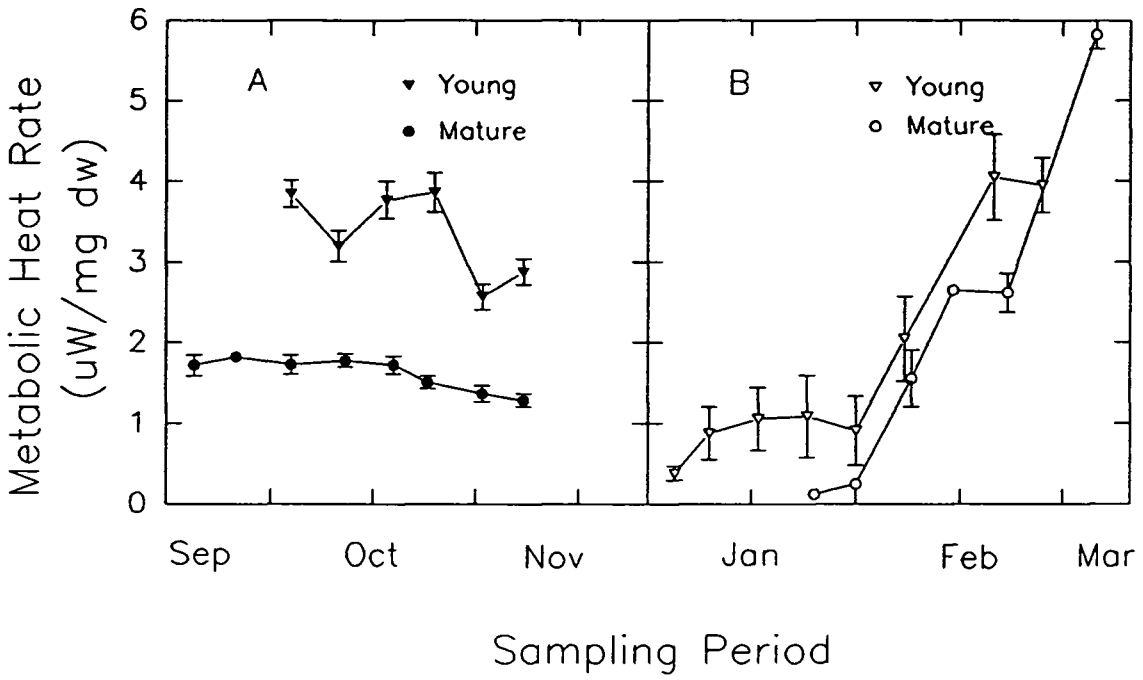


Figure 5.1. Seasonal variation in metabolic heat rate of grape primary buds sampled from young and mature vines, during the endodormant period (September to November) in 1990 (A), and the ecodormant period (January to March) in 1991 (B). Mean separation by FPLSD at p=0.5.

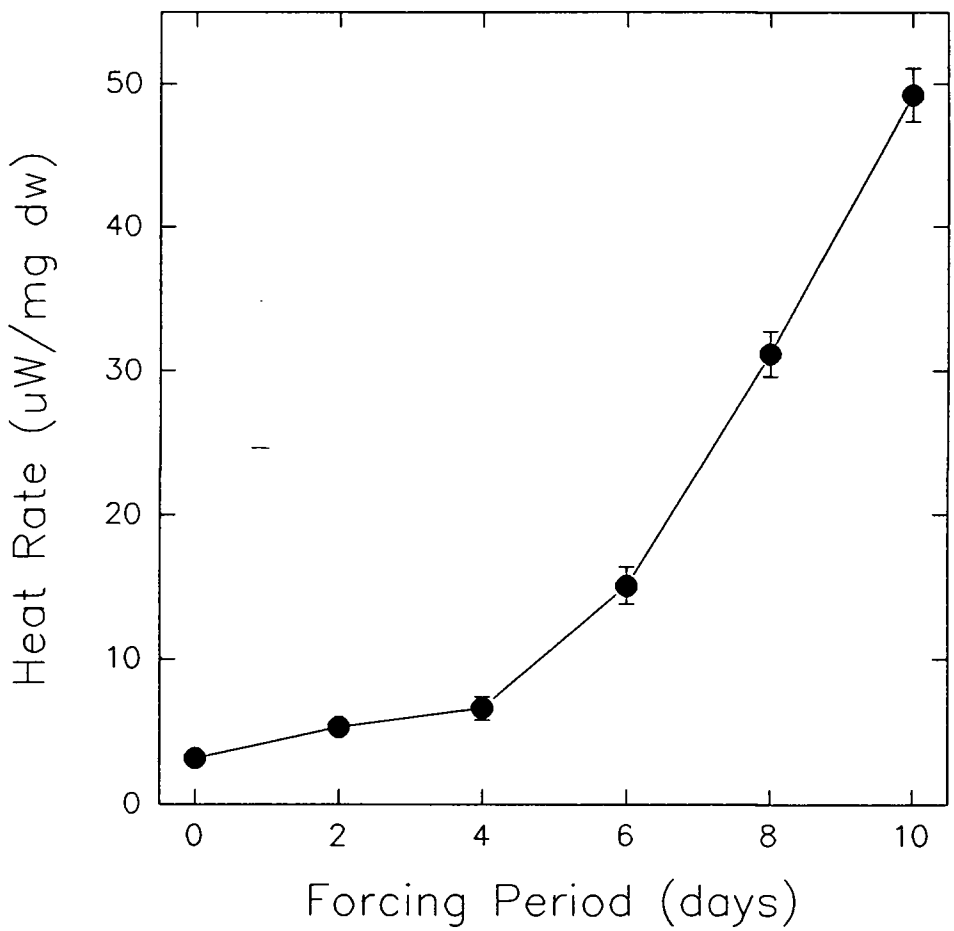


Figure 5.2. Increase in metabolic heat rate of ecodormant grape primary buds, forced to break under controlled conditions. Mean separation by FPLSD at p=0.05.

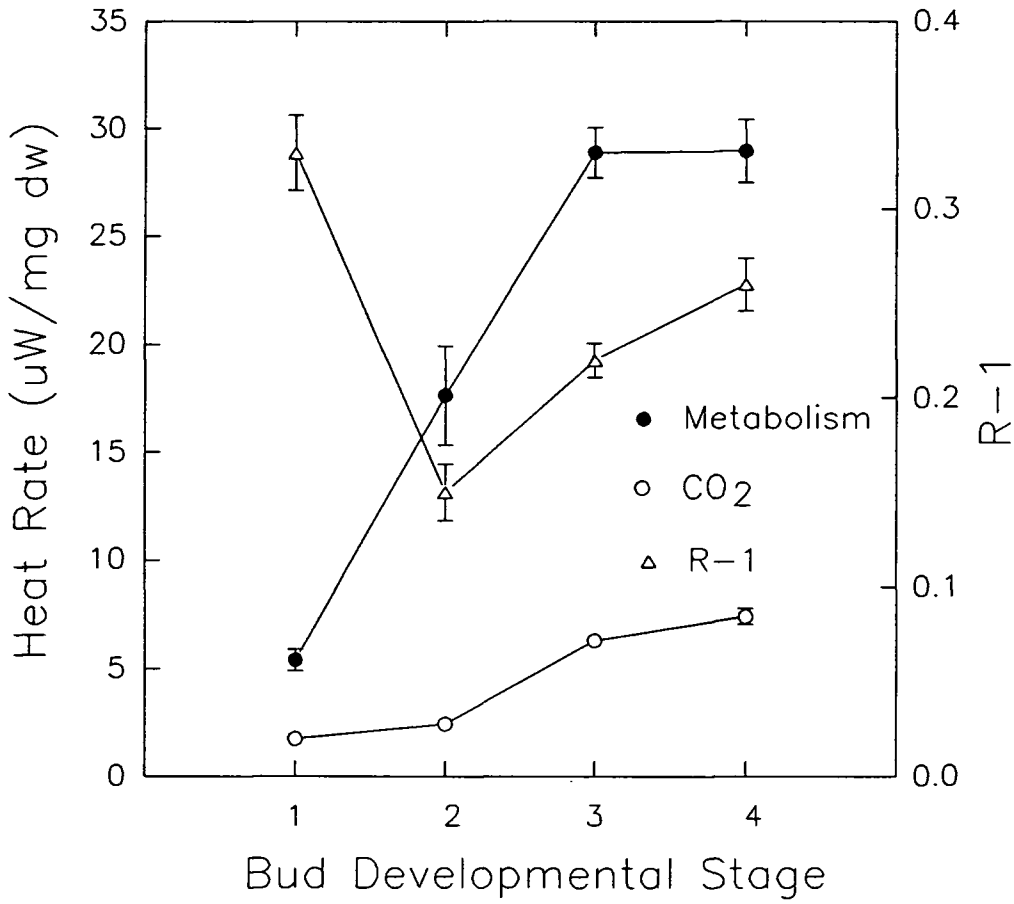


Figure 5.3. Changes in metabolic and CO_2 evolution heat rates, and variations in metabolic efficiency (R-1) of grape primary buds forced to break. Bud developmental stages were ecodormant (1), initial swelling (2), fully swollen (3), and broken buds (4). Mean separation by FPLSD at p=0.05.

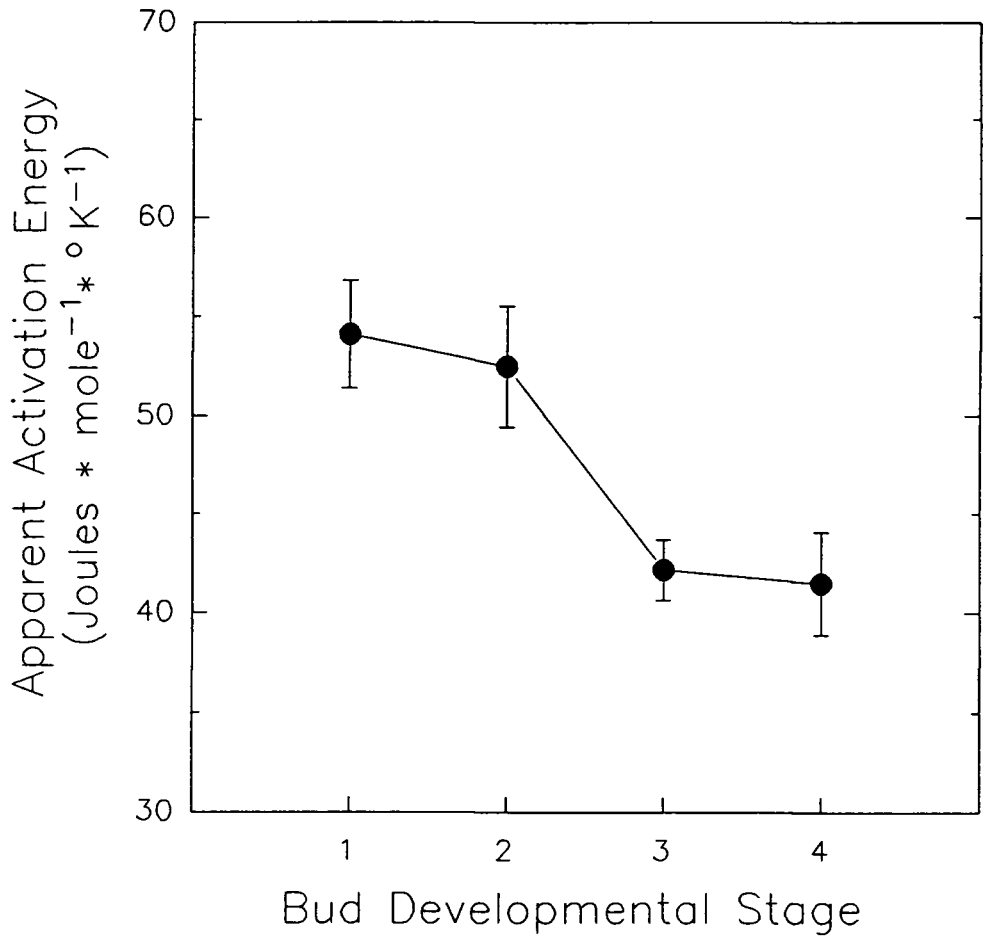


Figure 5.4. Apparent activation energy of grape primary buds at four developmental stages during budbreak forcing. Activation energy was calculated from the slope of Arrhenius plots between 1000/T (K) and natural log of heat rate. The temperature interval was 15 to 35°C. Mean separation by FPLSD at p=0.05.

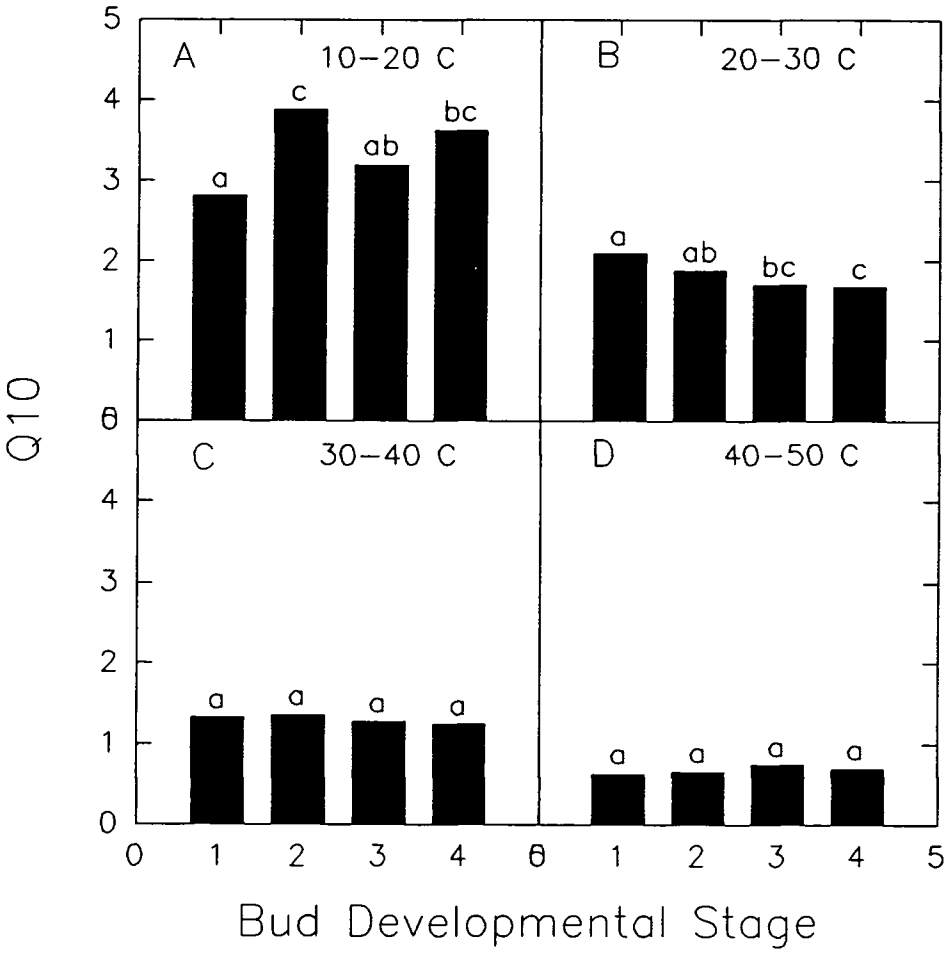


Figure 5.5. Temperature coefficient of respiration (Q10) of grape primary buds at four intervals, 10 to 20°C (A), 20 to 30°C (B), 30 to 40°C (C), and 40 to 50°C (D). Bud developmental stages are the same as described before. Mean separation by FPLSD at p=0.05.

LITERATURE CITED

Amthor, J.S. Respiration and crop productivity. 215 pp. Springer-Verlag. New York (1989).

Breidenbach, R.W., D.R. Rank, A.J. Fontana, L.D. Hansen, and R.S. Criddle. Calorimetric determination of tissue responses to thermal extremes as a function of time and temperature. Thermochim. Acta 172:179-186 (1990).

Butler, D.R., and J.J. Landsberg. Respiration rates of apple trees estimated by CO_2-efflux measurements. Plant Cell and Environment 4:153-159 (1981).

Chen, S.S.C., and J.E. Varner. Respiration and protein synthesis in dormant and after ripened seeds of Avena fatua. Plant Physiol. 46:109-112 (1970).

Cole, M.E., T. Solomos, and M. Faust. Growth and respiration of dormant flower buds of Pyrus communis and Pyrus calleryana. J. Amer. Soc. Hort. Sci. 107(2):226-231 (1982).

Criddle, R.S., R.W. Breidenbach, E.A. Lewis, D.J. Eatough, and L.D. Hansen. Effects of temperature and oxygen depletion on metabolic rates of tomato and carrot cell cultures and cuttings measured by calorimetry. Plant, Cell and Environment 11:695-701 (1988).

Criddle, R.S., R.W. Breidenbach, D.R. Rank, M.S. Hopkin, and L.D. Hansen. Simultaneous calorimetric and respirometric measurements on plant tissues. Thermochim. Acta. 172:213-221 (1990).

Criddle, R.S., A.J. Fontana, D.R. Rank, D. Paige, L.D. Hansen, and R.W. Breidenbach. Simultaneous measurements of metabolic heat rate, CO_2 production, and O_2 consumption by microcalorimetry. Anals Biochem. 194:413-417 (1991).

Criddle, R.S., M. Hopkin, E.D. McArthur, and L.D. Hansen. The relationship between temperature coefficient of respiration and plant distribution. American Naturalist (submitted, 1992).

Elliot, J.M., and W. Davison. Energy equivalents of oxygen consumption in animal energetics. Oecologia 19:195-201 (1975).

Fontana, A.J., L.D. Hansen, R.W. Breidenbach, and R.S. Criddle. Microcalorimetric measurements of aerobic cell metabolism in unstirred cell cultures. Thermochim. Acta 172:105-113 (1990).

Iwasaki, K., and R.J. Weaver. Effects of chilling, calcium cyanamide, and bud scale removal on bud break, rooting and inhibitor content of buds of Zinfandel grape (Vitis vinifera L.). J. Amer. Soc. Hort. Sci. 102(5):584-587 (1977).

Hansen, L.D., E.A. Lewis, D.J. Eatough, D.P. Fowler, and R.S. Criddle. Prediction of long term growth rates of larch clones by calorimetric measurements of metabolic heat rates. Can. J. For. Res. 19:606-611 (1989).

Holthausen, R.S., and M.M. Caldwell. Seasonal dynamics of root system respiration in Atriplex confertifolia Plant and Soil 55:307-317 (1980).

Iversen, E., E. Wilhelmsen, and R.S. Criddle. Calorimetric examination of cut fresh pineapple metabolism. J. Food Sci. 54(5):1246-1249 (1989).

Johnson, F.H., H. Eyring, and B. Jones. The theory of rate processes in biology and medicine. pp. 550. John Wiley and Sons. New York (1974).

La Croix, L.J.,and A.S. Jaswal. Metabolic changes in after-ripening seed of Prunus cerasus. Plant Physiol. 42:479-480 (1967).

Lang, G, J.D. Early, G.C. Martin, and R.L. Darnell. Endo-, Para-, and Ecodormancy: Physiological terminology and classification for dormancy research. Hortscience 22(3):371-377 (1987).

Loike, J.D., S.C. Silverstein, and J.M. Sturtevant. Application of differential scanning microcalorimetry to the study of cellular processes: Heat production and glucose oxidation of murine macrophages. Proc. Natl. Acad. Sci. Biochemistry U.S.A. 78(10):5958-5962 (1981).

Moncur, M.W., K. Rattigan, D.H. MacKenzie, and G.N. McIntyre. Base temperature for budbreak and leaf appearance of grapevines. Am. J. Enol. Vitic. 40(1):21-26 (1989).

Montgomery, R., and C.A. Swenson. Quantitative problems in the biochemical sciences (2nd edition) pp. 370. W.H. Freeman and Co. New York (1976).

Nobel, P.S. Biophysical plant physiology and ecology. (3rd. Ed.) 608 pp. W.H. Freeman and Co. New York (1983).

Pollock, B.M. Studies of the rest period. III Respiratory changes in leaf primordia of maple buds during chilling. Plant Physiol. 35(6):975-977

(1960).

Pollock, B.M., and H.O. Olney. Studies of the rest period. I Growth, translocati on, and respiratory changes in the embryonic organs of the after-ripening cherry seed. Plant Physiol. 34:131-142 (1959).

Rank, D.R., R.W. Breidenbach, A.J. Fontana, L.D. Hansen, and R.S. Criddle. High and low temperature inactivation of tomato. Planta 185:576-582 (1991).

Ross, J.D. Metabolic aspects of dormancy. In: Seed physiology and reserve mobilization. D.R. Murray (Ed.). pp 63-75. Academic Associated Press. Orlando, FL (1985).

Vertucci, C.W. Calorimetric studies of the state of water in seed tissues. Biophys. J. 58:1463-1471 (1990).

Wrzesniewski, W. Respiration of Mazzard cherry (Prunus avium L.) seeds during cold stratification interrupted several times with a dormancy inducing warm temperature. Arboretum Kónickie 30:351-366 (1985).

Westwood, M.N. Temperate-zone pomology. Revised edition 428 pp. Timber Press. Portland, Oregon (1988).

Young, E. Changes in respiration rate and energy of activation after chilling and forcing dormant apple trees. J. Amer. soc. Hort. Sci. 115(5):809-814 (1990).

Zimmerman, R.H., M. Faust, and A.W. Sherve. Glucose metabolism of various tissues of pear buds. Plant Physiol. 76:839-841 (1970).

BIBLIOGRAPHY

Abass, M., and C.B. Rajashekar. Characterization of heat injury in grapes using ^{1}H nuclear magnetic resonance methods. Plant Physiol. 96:957-961 (1991).

Aguayo, J.B., S.J. Blackband, J. Schoniger, M.A. Mattingly, and M. Hintermann. Nuclear magnetic resonance imaging of a single cell. Nature 322:190-191 (1986).

Aloni, R. Differentiation of vascular tissues. Ann. Rev. Plant Physiol. 38:170-204 (1987).

Amato, I. Chemistry: a certain resonance. Science Vol. 254:518-519 (1991).

Amberger, A. Uptake and metabolism of hydrogen cyanamide in plants. In: Proceedings of bud dormancy in grapevines. R.J. Weaver, J.O. Johnson, and A.S. Wicks (Eds.). pp 5-7. University of California Press, Davis (1984).

Amling, H.J., and K.A. Amling. Onset, intensity, and dissipation of rest in several pecan cultivars. J. Amer. Soc. Hort. Sci. 105(4):536-540 (1980).

Amthor, J.S. Respiration and crop productivity. 218 pp. Springer-Verlag, New York (1989).

Ando, I., and G.A. Webb. Theory of nuclear magnetic resonance parameters. pp 1-7. A.P.I. Ltd. London (1983).

Becker, N. Site climate effects on development, fruit maturation and harvest quality. In: Proceedings of the Second International Symposium for the Cool Climate Viticulture and Enology. R.E. Smart, R.J. Thornton, S.B. Rodriguez, and J.E. Young (Eds.). pp 13-18. New Zealand Soc. for Vitic. and Oenol. Auckland, New Zealand (1988).

Berrie, A.M.M. Germination and dormancy. In: Advanced plant physiology. M.B. Wilkins (Ed.). pp 440-468. Pitman Publishing Inc. (1985).

Bianco, J., S. Lasserchere, and C. Bulard. Gibberellins in dormant embryos of Pyrus malus L. cv. Golden Delicious. Plant Physiol. 116:185-188 (1984).

Bittenbender, H.C., and G.S. Howell. Interaction of temperature and moisture content on spring deacclimation of buds of highbush blueberry. Can. J. Plant Sci. 55:447-455 (1975).

Black, L.A., T.A. Nell, and J.E. Barrett. Dormancy- breaking method effects in azalea longevity. Hortscience 25(7):810 (1990).

Bogatek, R. and S. Lewak. Effects of cyanamide and cold treatment on sugar catabolism in apple seeds during dormancy removal. Physiologia Plantarum 73:406-411 (1988).

Bottomley, P.A., H.H. Rogers, and T.H. Foster. NMR imaging shows water distribution and transport in plant root systems in situ. Proc. Natl. Acad. Sci. U.S.A. Botany. 83:87-89 (1986).

Bracho, E., J.O. Johnson, A.S. Wicks, L.A. Lider, and R.J. Weaver. Using hydrogen cyanamide to promote uniform budbreak in Cabernet Sauvignon in California. Perliminary results. In: Proceedings of bud dormancy in grapevines. R.J. Weaver, J.O. Johnson, and A.S. Wicks (Eds.). pp 11-13. University of California Press, Davis (1984).

Breidenbach, R.W., D.R. Rank, A.J. Fontana, L.D. Hansen, and R.S. Criddle. Calorimetric determination of tissue responses to thermal extremes as a function of time and temperature. Thermochim. Acta 172:179-186 (1990).

Brevard, C., and P. Granger. Handbook of high resolution multinuclear NMR. pp. 9, 67-71, 72-75. Wiley-Interscience Publication, New York (1981).

Broome, O.C., and R.H. Zimmerman. Breaking bud dormancy in tea crabapple [Malus hupehensis (Pamp.) Rehd.] with cytokinins. J. Amer Soc. Hort. Sci. 101(1):28-30 (1976).

Brown, J.M., G.A. Johnson, and P.J. Kramer. In vivo magnetic resonance microscopy of changing water content in Pelargonium hortorum roots. Plant Physiol. 82:1158-1160 (1986).

Brown, J.M., J.F. Thomas, G.P. Cofer, and G.A. Johnson. Magnetic resonance microscopy of stem tissues of Pelargonium hortorum. Bot. Gaz. 149(3):253-259 (1988).

Buchanan, D.W., J.F. Bastholic, and R.H. Biggs. Manipulation of bloom and ripening dates of three Florida grown peach and nectarine cultivars through sprinkle and shade. J. Amer. Soc. Hort. Sci. 102:466-470 (1977).

Burke, M.J., R.G. Bryant, and C.J. Weiser. Nuclear magnetic resonance of water in cold acclimating red osier dogwood stem. Plant Physiol. 54:392-398 (1974).

Butler, D.R., and J.J. Landsberg. Respiration rates of apple trees estimted by CO_2-efflux measurements. Plant Cell and Environment 4:153-159 (1981).

Buttrose, M.S. Climatic factors and fruitfulness in grapevines. Hort. Abstr. 44:319-326 (1974).

Chen, S.S.C., and J.E. Varner. Respiration and protein synthesis in dormant and after ripened seeds of Avena fatua. Plant Physiol. 46:109-112 (1970).

Chen, T.H.H., K.K. Kartha, F. Constabel, and L.V. Gusta. Freezing characteristics of cultured Catharanthus roseus (L). G. Don cells treated with dimethylsulfoxide and sorbitol in relation to cryopreservation. Plant Physiol. 75:720-725 (1984).

Cole, M.E., T. Solomos, and M. Faust. Growth and respiration of dormant flower buds of Pyrus communis and Pyrus calleryana. J. Amer. Soc. Hort. Sci. 107(2):226-231 (1982).

Colombo, S.J. Bud dormancy, frost hardiness, shoot moisture content, and readiness of black spruce container seedlings for frozen storage. J. Amer. Soc. Hort. Sci. 115(2):302-307 (1990).

Criddle, R.S., R.W. Braidenbach, E.A. Lewis, D.J. Eatough, and L.D. Hansen. Effects of temperature and oxygen depletion on metabolic rates of tomato and carrot cell cultures and cuttings measured by calorimetry. Plant, Cell and Environment 11:695-701 (1988).

Criddle, R.S., R.W. Braidenbach, D.R. Rank, M.S. Hopkin, and L.D. Hansen. Simultaneous calorimetric and respirometric measurements on plant tissues. Thermochim. Acta. 172:213-221 (1990).

Criddle, R.S., A.J. Fontana, D.R. Rank, D. Paige, L.D. Hansen, and R.W. Braidenbach. Simultaneous measurements of metabolic heat rate, CO_2 production, and O_2 consumption by microcalorimetry. Anals Biochem. 194:413-417 (1991).

Criddle, R.S., L.D. Hansen, R.W. Braidenbach, M.R. Ward, and R.C. Huffaker. Effects of NaCl on metabolic heat evolution rates by barley roots. Plant Physiol. 90:53-58 (1990).

Criddle, R.S., M. Hopkin, E.D. McArthur, and L.D. Hansen. The relationship between temperature coefficient of respiration and plant distribution. American Naturalist (submitted, 1992).

Cutting, J.G.M., D.K. Strydom, G. Jacobs, D.U. Bellstedt, K.J. Van Der Merwe, and E.W. Weiler. Changes in xylem constituents in response to rest-breaking agents applied to apple before budbreak. J. Amer. Soc. Hort. Sci. 116(4):680-683 (1991).

De la Fuente-Burguillo, P., and G. Nicolas. Appearance of an alternate pathway cyanide-resistant during germination of seeds of Cicer ariethorum. Plant Physiol. 60:524-527 (1977).

Dennis, Jr., F.G. Two methods of studying rest: Temperature alternation and genetic analysis. Hortscience 22(5):820-824 (1987).

Doorenbos, J. Review of the literature on dormancy in buds of woody plants. Meded. Landbouwhogeschool, Wageningen 53:1-24 (1953).

Dozier Jr., W.A., A.A. Powell, A.W. Caylor, N.R. McDaniel, E.L. Carden, and J.A. McGuire. Hydrogen cyanamide induces budbreak of peaches and nectarines following inadequate chilling. Hortscience 25(12):1573-1575 (1990).

Dumbroff, E.B., D.B. Cohen, and D.R. Webb. Seasonal levels of abscisic acid in buds and stems of Acer saccharum. Physiol. Plant. 45:211-214 (1979).

Durner, E.F. Dormant pruning reduces rehardening of peach pistils after a winter thaw. Hortscience 25(8):980 (1990).

Edwards, G.R. Producing temperate-zone fruit at low latitudes: Avoiding rest and chilling requirement. Hortscience 22(6):1236-1239 (1987).

Elliot, J.M., and W. Davison. Energy equivalents of oxygen consumption in animal energetics. Oecologia 19:195-201 (1975).

Erez, A. Defoliation of deciduous fruit trees with magnesium chlorate and cyanamide. Hortscience 20(3):452-453 (1985).

Erez, A., G.A. Couvillon, and C.H. Hendershott. Quantitative chilling enhancement and negation in peach buds by high temperatures in a daily cycle. J. Amer. Soc. Hort. Sci. 104(4):536-540 (1979).

Erez, A., G.A. Couvillon, and C.H. Hendershott. The effect of cycle length on chilling negation by high temperature in dormant peach leaf buds. J. Amer. Soc. Hort. Sci. 104(4):573-576 (1979).

Erez, A., and S. Lavee. The effect of climatic conditions on dormancy development of peach buds. I. Temperature. J. Amer. Soc. Hort. Sci. 96(6):711-714 (1971).

Erez, A., S. Lavee, and R.M. Samish. Improved methods for breaking rest in peach and other deciduous fruit species. J. Amer. Soc. Hort. Sci. 96(4):519-522 (1971).

Esau, K. Vascular differentiation in plants. Biology studies. 160 pp. Holt, Rinehart, and Winston, Inc. New York (1965).

Esau, K. Anatomy of seed plants. (2nd ed.). 551 pp. John Wiley and Sons, New York (1977).

Faust, M., D. Liu, M.M. Millard, and G.W. Stutte. Bound versus free water in dormant apple buds. A theory for endodormancy. Hortscience 26(7):887-890 (1991a).

Faust, M., G.W. Sutte, D. Liu, M.M. Millard, and M.J. Cline. Magnetic resonance imaging: A non-destructive analytical tool for developmental physiology. Hortscience 26(7):818,935 (1991b).

Faust, M., R. Zimmerman, and T. van der Zwet. Genetic transmission of bloom date in pears. Hortscience 11(1):59-60 (1976).

Flaherty, D.L., F.L. Jensen, A.N. Kasimatis, H. Kido, and W. Moller. Grape pest management. pp 12-15. Publication 1981. University of California Press. Davis (1982).

Fontana, A.J., L.D. Hansen, R.W. Braidenbach, and R.S. Criddle. Microcalorimetric measurements of aerobic cell metabolism in unstirred cell cultures. Thermochim. Acta 172:105-113 (1990).

Fuchigami, L.H., and C.C. Nee. Degree growth stage model and rest-breaking mechanisms in temperate woody perennials. Hortscience 22(5):836-845 (1987).

Fuchigami, L.H., C.J. Weiser, K. Kobayashi, R. Timmis, and L.V. Gusta. A degree growth stage (°GS) model and cold acclimation in temperate woody plants. In: Plant cold hardiness and freezing stress. P. Li and A. Sakai (Eds.). Academic Press Inc. New York. pp. 93-114 (1982).

Garbow, J.R., L.M. Ferrantello, and R.E. Stark. ^{13}C Nuclear magnetic resonance study of suberized potato cell wall. Plant Physiol. 90:783-787 (1989).

Gardea, A.A. Damage to Pinot noir (Vitis vinifera L.) as affected by bud development, INA-bacteria and a bacterial inhibitor. M.S. Thesis. Oregon State University (1987).

Gerasimowicz, W.V., S.I. Tu, and P.E. Pfeffer. Energy facilitated Na^+ uptake in excised corn roots via ^{31}Pnand ^{23}Na NMR. Plant Physiol. 81:925-928 (1986).

Gianfagna, T.J., and S.A. Mehlenbacher. Importance of heat requirement for budbreak and time of flowering in apple. Hortscience 20(5):909-911 (1985).

Gilreath, P.R., and D.W. Buchanan. Rest prediction for low-chilling Sungold nectarine. J. Amer. Soc. Hort. Sci. 106(4):426-429 (1981).

Goldbach, H., Ch. Thaler, A. Wünsch, and A. Amberger. Decomposition of ^{14}C-labelled cyanamide in Vitis vinifera cuttings. Plant Physiol. 133:299-203 (1988).

Günter, H. NMR spectroscopy: An introduction. Translation by R.W. Gleason. pp 9-15. John Wiley and Sons. New York (1985).

Gusta, L.V., D.B. Fowler, and P. Chen. A nuclear magnetic resonance study of water in cold acclimating cereals. Plant Physiol 63:627-634 (1979).

Gusta, L.V., N.J. Tyler, and T.H.H. Chen. Deep supercooling in woody taxa growing north of the -40°C isotherm. Plant Physiol. 72:122-128 (1983).

Hansen, L.D., and R.S. Criddle. Batch-injection attachment for the Hart differential scanning calorimeter. Thermochim. Acta 154:81-88 (1989).

Hansen, L.D., E.A. Lewis, D.J. Eatough, D.P. Fowler, and R.S. Criddle. Prediction of long term growth rates of larch clones by calorimetric measurements of metabolic heat rates. Can. J. For. Res. 19:606-611 (1989).

Harmon, F.N., J.H. Weinberger. Effects of storage and stratification on germination of vinifera grape seeds. J. Amer. Soc. Hort. Sci. 73:147-150 (1959).

Hatch, R.L., and M. Ruíz. Influence of pruning date on budbreak of desert table grapes. Amer. J. Enol. Vitic. 38(4):326-328 (1987).

Hendrix, D.L., J.W. Radin, and R.A. Nieman. Intracellular pH of cotton embryos and seed coats during fruit development determined by ^{31}P nuclear magnetic resonance spectroscopy. Plant Physiol. 85:588-591 (1987).

Hinshaw, W.S., P.A. Bottomley, and G.N. Holland. A demonstration of the resolution of NMR imaging in biological systems. Experientia 35:1268-1269 (1979).

Holler, F., D.H. Burns, and J.B. Callis. Direct use of second derivatives in curve fitting procedures. Applied Spectroscopy 43(5):877-883 (1989).

Holthausen, R.S., and M.M. Caldwell. Seasonal dynamics of root system respiration in Atriplex confertifolia Plant and Soil 55:307-317 (1980).

Hosoki, T., Y. Sakai, M. Hamada, and K. Taketami. Breaking bud dormancy in corms and trees with sulfide compounds in garlic and horseradish. Hortscience 21(1):114-116 (1986).

Howell, G.S. Cultural manipulation of vine hardiness. In: Proceedings of the Second International, Symposium for Cool Climate Viticulture and Enology. R.E. Smart, R.J. Thornton, S.B. Rodriguez, and J.E. Young (Eds.). pp 98-102. New Zealand Soc. for Vitic. and Oenol. Auckland, New Zealand (1988a).

Howell, G.S. Factors affecting vine survival in winter. Grapegrowing. November issue pp 35-40 (1988b).

Hu, H., G.A. Couvillon. Activities of catalase and pentosa phosphate pathway dehydrogenases during dormancy release in nectarine seed. J. Amer. Soc. Hort. Sci. 115(6):987-990 (1990).

Humpreys, T.E., and W.M. Dugger, Jr. The effect of 2,4-dichlorophenoxyacetic acid on plant pathways of glucose catabolism in higher plants. Plant Physiol. 32:136-140 (1957).

Iversen, E., E. Wilhelmsen, and R.S. Criddle. Calorimetric examination of cut fresh pineapple metabolism. J. Food Sci. 54(5):1246-1249 (1989).

Iwasaki, K., and R.J. Weaver. Effects of chilling, calcium cyanamide, and bud scale removal on bud break, rooting, and inhibitor content of buds of Zinfandel grape (Vitis vinifera L.). J. Amer. Soc. Hort. Sci. 102(5):584-587 (1977).

Jackson, P.C., P.E. Pfeffer, and W. V. Gerasimowicz. Use of ^{31}P NMR to assess effects of DNP pn ATP levels in vivo in barley roots. Plant Physiol. 81:1130-1133 (1986).

Johnson, F.H., H. Eyring, and B. Jones. The theory of rate processes in biology and medicine. pp. 550. John Wiley and Sons. New York (1974).

Johnson, G.A., J. Brown, and P.J. Kramer. Magnetic resonance microscopy of changes in water content in stems of transpiring plants. Proc. Natl. Acad. Sci. U.S.A. Botany 84:2752-2755 (1987).

Kachru, R.B., E.K. Chacko, and R.N. Singh. Physiological studies of dormancy in grape seeds (Vitis vinifera L.). I. On the naturally occurring growth substances in grape seeds and their changes during low temperature storage. Vitis 8:12-18 (1969).

Kerhoas, C., and C. Dumas. Nuclear magnetic resonance and pollen quality. In: Nuclear Magnetic Resonance. H.F. Linskens and J.F. Jackson (Eds.). pp 169-190. Springer-Verlag, New York (1986).

Kovacs, M.I.P., and G.M. Simpson. Dormancy and enzyme levels in seeds of wild oats. Phytochemistry 15:455-458 (1976).

La Croix, L.J., and A.S. Jaswal. Metabolic changes in after-ripening seed of Prunus cerasus. Plant Physiol. 42:479-480 (1967).

Lang, G., J.D. Early, G.C. Martin, and R.L. Darnell. Endo-, Para-, and Ecodormancy: Physiological terminology and classification for dormancy research. Hortscience 22(3):371-377 (1987).

Lavee, S., Y. Shulman, and G. Nir. The effects of cyanamide on budbreak of grapevines (Vitis vinifera L.). In: Proceedings of bud dormancy in grapevines. R.J. Weaver, J.O. Johnson, and A.S. Wicks (Eds.). pp 17-29. University of California Press, Davis (1984).

Levy, G.C., and D.J. Craik. Introduction. In: NMR spectroscopy: New methods and applications. G.S. Levy (Ed.) pp 1-5. ACS symposium series 191. American Chemical Society. Washington, D.C. (1982).

Lin, C.H. Viticulture in Taiwan and experiments in dormancy. In: Proceedings of bud dormancy in grapevines. R.J. Weaver, J.O. Johnson, and A.S. Wicks (Eds.). pp 30-35. University of California Press, Davis (1984).

Lloyd, J., and D. Firth. Effect of defoliation time on depth of dormancy and bloom time for low-chill peaches. Hortscience 25(12):1575-1578 (1990).

Loike, J.D., S.C. Silverstein, and J.M. Sturtevant. Application of differential scanning microcalorimetry to the study of cellular processes: Heat production and glucose oxidation of murine macrophages. Proc. Natl. Acad. Sci. Biochemistry U.S.A. 78(10):5958-5962 (1981).

MacKenzie, K.A.D., and J. Costa Tura. Xylem development in the gynoecium of the apple (Malus pumila L.) cv. Cox's Orange Pippin. Annals of Botany 67:383-398 (1991).

Martin, G.C. An introduction to bud dormancy. In: Proceedings of bud dormancy in grapevines. R.J. Weaver, J.O. Johnson, and A.S. Wicks (Eds.). pp 1-4. University of California Press, Davis (1984).

McCain, D.C. Orientation of chloroplasts in leaves by H-NMR spectroscopy. In: Nuclear Magnetic Resonance. H.F. Linskens and J.F. Jackson (Eds.). pp 127-147. Springer-Verlag, New York (1986).

McCain, D.C., J. Croxdale, and J.L. Markley. Water is allocated differently to chloroplasts in sun and shade leaves. Plant Physiol. 86:16-18 (1988).

McCain, D.C., J. Croxdale, and J.L. Markley. Thermal damage to chloroplast envelope membrane. Plant Physiol. 90:606-609 (1989).

McCain, D.C., and J.L. Markley. Water permeability of chloroplast envelope membranes. In vivo measurement by saturation-transfer NMR. FEBS Letters 183(2):353-358 (1985).

McCain, D.C., T.C. Selig, Govindjee, and J.L. Markley. Some plants have orientation-dependent EPR and NMR spectra. Proc. Natl. Acad. Sci. U.S.A. 81:748-752 (1984).

McNaughton, J.L., and C.T. Mortimer. Differential scanning calorimetry. In: I.R.S.; Physical Chemistry Series 2. Vol. 10. pp 1-43. H.A. Skinner (Ed.). Butterworths, London (1975).

Moncur, M.W., K. Rattigan, D.H. MacKenzie, and G.N. McIntyre. Base temperature for budbreak and leaf appearance of grapevines. Am. J. Enol. Vitic. 40(1):21-26 (1989).

Montgomery, R., and C.A. Swenson. Quantitative problems in the biochemical sciences (2nd edition) pp. 370. W.H. Freeman and Co. New York (1976).

Nauer, E.M., and S.B. Boswell. Stimulating growth of quiescent citrus buds with 6-benzilaminopurine. Hortscience 16(2):162-163 (1981).

Nir, G.I., I. Klein, S. Lavee, G. Spieler, and U. Barak. Improving grapevine budbreak and yields by evaporative cooling. J. Amer. Soc. Hort. Sci. 113(4):512-517 (1988).

Nir, G., and Y. Shulman. The involvement of catalase in the dormancy of grapevine buds. In: Proceedings of bud dormancy in grapevines. R.J. Weaver, J.O. Johnson, and A.S. Wicks (Eds.). pp 40-43. University of California Press, Davis (1984).

Nobel, P.S. Biophysical plant physiology and ecology. (3rd. Ed.) 608 pp. W.H. Freeman and Co. New York (1983).

Ojalvo, I., J.S. Rokem, and I. Goldberg. ^{31}P NMR study of elicitor treated Phaseolus vulgaris cell suspension cultures. Plant Physiol. 85:716-719 (1987).

Paiva, E., and H.A. Robitaille. Breaking bud rest on detached apple shoots: Effects of wounding and ethylene. J. Amer. Soc. Hort. Sci. 103(1):101-104 (1978).

Perino, C., and D. Côme. Physiological and metabolical study of the germination phases in apple embryo. Seed Sci. & Technol. 19:1-14 (1991).

Pollock, B.M. Studies of the rest period. III Respiratory changes in leaf primordia of maple buds during chilling. Plant Physiol. 35(6):975-977 (1960).

Pollock, B.M., and H.O. Olney. Studies of the rest period. I Growth, translocation, and respiratory changes in the embryonic organs of the after-ripening cherry seed. Plant Physiol. 34:131-142 (1959).

Pool, R.M., C. Pratt, and H.D. Hubbard. Structure of base buds in relation to yield of grapes. Am. J. Enol. Vitic. 29:36-41 (1978).

Powell, P.E. Hormonal aspects of bud and seed dormancy in temperate-zone woody plants. Hortscience 22(5):845-850 (1987).

Proebsting, E., V. Brummund, and W.J. Clore. Critical temperatures for Concord grapes. Extension leaflet EM 4330. Washington State University (1978).

Rajashekar, C.B., and M.J. Burke. Methods to study the freezing process in plants. Methods Enzymol. 127:761-771 (1986).

Rank, D.R., R.W. Breidenbach, A.J. Fontana, L.D. Hansen, and R.S. Criddle. High and low temperature inactivation of tomato. Planta 185:576-582 (1991).

Richardson, E.A., S.D. Seeley, and D.R. Walker. A Model for estimating the completion of rest for Redhaven and Elberta peach trees. Hortscience 9(4):331-332 (1974).

Rollins A., J. Barber, R. Elliot., and B. Wood. Xenobiotic monitoring in plants by ^{19}F and ^{1}H nuclear magnetic resonance imaging and spectroscopy. Plant Physiol. 91-1243-1246 (1989).

Ross, J.D. Metabolic aspects of dormancy. In: Seed physiology and reserve mobilization. D.R. Murray (Ed.). pp 63-75. Academic Associated Press. Orlando, FL (1985).

Ross, J.D., and J.W. Bradbeer. Studies in seed dormancy: VI The effects of growth retardants on the gibberellin content and germination of chilled seeds of Corylus avellana L. Planta 100:303-308 (1971).

Salisbury, F.B., and C.W. Ross. Plant physiology (3rd ed.). 540 pp. Wadsworth Publishing Co. Belmont CA. (1985).

Samish, R.M. Dormancy in woody plants. Annu. Rev. Plant Physiol. 5:183-204 (1954).

Savidge, R.A. Phytohormonal regulation of cambial growth in trees. In: Fast growing trees and nitrogen fixing trees. D. Werner and P. Müller (Eds.). pp 142-151 Gustav Fisher Verlag. New York (1989).

Schonbaum, G.R., W.D. Bonner, Jr., B.T. Storey, and J.T. Bahr. Specific inhibition of the cyanide-insensitive respiratory pathway in plant mitochondria by hydroxamic acids. Plant Physiol. 47:124-128 (1971).

Shaltout, A.D., and C.R. Unrath. Effect of some growth regulators and nutritional compounds as substitutes for chilling of delicious apple leaf and flower buds. J. Amer. Soc. Hort. Sci. 108(6):898-901 (1983).

Siedow, J.N., and M.E. Girvin. Alternative respiratory pathway. Plant Physiol. 65:669-674 (1980).

Siller-Cepeda, J.H. The role of glutathione in the development and release of rest on 'Redhaven' peach. Ph.D. Thesis. Oregon State University (1991).

Slater, J.V., M.R. Warmund, M.F. George, and M.R. Ellersieck. Deacclimation of winter hardy Seyval blanc grape tissue after exposure to 16°C. Scientia Horticulturae 45:273-285 (1991).

Spiegel-Roy, P., Y. Shulman, I. Baron, and E, Ashbel. Effect of cyanamide in overcoming grape seed dormancy. Hortscience 22(2):208-219 (1987).

Stryer, L. Biochemistry. 3rd edition 1089 pp. W.H. Freeman and Co. New York (1988).

Swanepoel, J.L., F.S. de Villiers, and P. Pouget. Predicting the date of budburst in grapevines. S. Afr. J. Enol. Vitic. 11(1):46-49 (1990).

Taylor, R.J., and E.B. Dumbroff. Bud, root, and growth regulator activity in Acer saccharum during the dormant season. Can. J. Bot. 53:321-331 (1975).

Timmis K.A., L.H. Fuchigami, R. Timmis. Measuring dormancy: The rise and fall of square waves. Hortscience 16(2):200-202 (1981).

Toledo, R., M.P. Steinberg, and A.I. Nelson. Quantitative determination of bound water by NMR. Journal of Food Science 33:315-317 (1968).

Thorpe, T.A. K. Bagh, A.J. Cutler, D.I. Dunstan, D.D. McIntyre, and H.J. Vogel. A ^{14}N and ^{15}N nuclear magnetic resonance study of nitrogen metabolism in shoot-forming cultures of white spruce (Picea glauca) buds. Plant Physiol. 91:193-202 (1989).

Tuan, D.Y.H., and J. Bonner. Dormancy associated with repression of genetic activity. Plant Physiol. 39(5):768-772 (1964).

Vertucci, C.W. Calorimetric studies of the state of water in seed tissues. Biophys. J. 58:1463-1471 (1990).

Vilsmeier, K., and A. Amberger. Uptake and metabolism of ^{15}N labelled cyanamide by cuttings of grapevine. Vitis 27:2232-228 (1988).

Wang, S.Y., and M. Faust. Changes of membrane lipids in apple buds during dormancy and budbreak. J. Amer. Soc. Hort. Sci. 115(5):803-808 (1990).

Wang, S.Y., M. Faust, and G.L. Steffens. Metabolic changes in cherry flower buds associated with breaking of dormancy in early and late blooming cultivars. Physiol. Plant. 65:89-94 (1985).

Weinberger, J.N. Some temperature relations in natural breaking of the rest of peach flower buds in the San Joaquin Valley, California. J. Amer. Soc. Hort. Sci. 91:84-89 (1967).

Weinberger, J.N. Effects of high temperatures during the breaking of the rest of Sullivan Elberta peach buds. J. Amer. Soc. Hort. Sci. 63:157-162 (1954).

Westwood, M.N. Temperate-zone pomology. Revised edition 428 pp. Timber Press. Portland, Oregon (1988).

Whiting, J.R., and B.G. Coombe. Response of Sultana and Cabernet Sauvignon grapevines to cyanamide. In: Proceedings of bud dormancy in grapevines. R.J. Weaver, J.O. Johnson, and A.S. Wicks (Eds.). pp 44-47. University of California Press, Davis (1984).

Wicks, A.S., J.O. Johnson, F. Jensen, E. Bracho, R. Neja, L. Lider, and R.J. Weaver. Induction of early and more uniform budbreak in Vitis vinifera L. cvs. Perlette, Flame Sedless, and Thompson Seedless grapes with hydrogen cyanamide. In: Proceedings of bud dormancy in grapevines. R.J. Weaver, J.O. Johnson, and A.S. Wicks (Eds.). pp 48-55. University of California Press, Davis (1984).

Williams, D.A.R. Nuclear magnetic resonance spectroscopy. In: Analytical Chemistry by Open Learning Series. D.J. Mowthorpe (Ed.). pp 1-45. John Wiley and Sons. New York (1986).

Williams, L.E. The effect of cyanamide on budbreak and vine development of Thompson Seedless grapevines in the San Joaquin Valley of California. Vitis 26:107-113 (1987).

Williams, L.E., R.A. Neja, J.L. Meyer, L.A. Yates, and E.L. Walker. Postharvest irrigation influences budbreak of Perlette grapevines. Hortscience 26(8):1081 (1991).

Williams, L.E., and R.J. Smith. The effects of cyanamide application on the growth and development of Thompson Seedles grapevines used for raisin production. Preliminary results. In: Proceedings of bud dormancy in grapevines. R.J. Weaver, J.O. Johnson, and A.S. Wicks (Eds.). pp 56-58. University of California Press, Davis (1984).

Winkler, A.J., J.A. Cook, W.M. Kliewer, and L.A. Lider. General viticulture. Second edition. 710 pp. University of California Press. Berkeley. (1974).

Wolf, T.K., and M.K. Cook. Comparison of Cabernet Sauvignon and Cabernet Franc grapevine dormant bud cold hardiness. Fruit Varieties Journal 45(1):17-21 (1991).

Wolpert, J.A., and G.S. Howell. Effects of cane length and dormant season pruning on cold hardening and water content of Concord bud and cane tissues. Am. J. Enol. Vitic. 35(4):237-241 (1984).

Wolpert, J.A., and G.S. Howell. Cold acclimation of Concrod grapevines. III. Relationship betwwen cold hardiness, tissue water content, and shoot maturation. Vitis 25:151-159 (1986)

Wrzesniewski, W. Respiration of Mazzard cherry (Prunus avium L.) seeds during cold stratification interrupted several times with a dormancy inducing warm temperature. Arboretum Kónickie 30:351-366 (1985).

Yentur, S., and A.C. Leopold. Respiratory transition during seed germination. Plant Physiol. 57:274-276 (1976).

Yoshida, S. Chemical and biophysical changes in the plasma membrane during cold acclimation of mulberry bark cells (Morus bombycis Koidz cv. Goroji). Plant Physiol. 76:257-265 (1984).

Yoshida, S. Reverse changes in plasma membrane properties upon deacclimation of mulberry trees (Morus bombysis Koids). Plant Cell Physiol. 27(1):83-89 (1986).

Young, E. Changes in respiration rate and energy of activation after chilling and forcing dormant apple trees. J. Amer. soc. Hort. Sci. 115(5):809-814 (1990).

Young, E., and S.M. Blankenship. Low oxygen delays budbreak of apple trees in greenhouse and prolongs storage life. Hortscience 26(7):890-891 (1991).

Zimmerman, R.H., and M. Faust. Pear bud metabolism: Seasonal changes in glucose utilization. Plant Physiol. 44:1273-1276 (1969).

Zimmerman, R.H., M. Faust, and A.W. Sherve. Glucose metabolism of various tissues of pear buds. Plant Physiol. 76:839-841 (1970).

APPENDIX

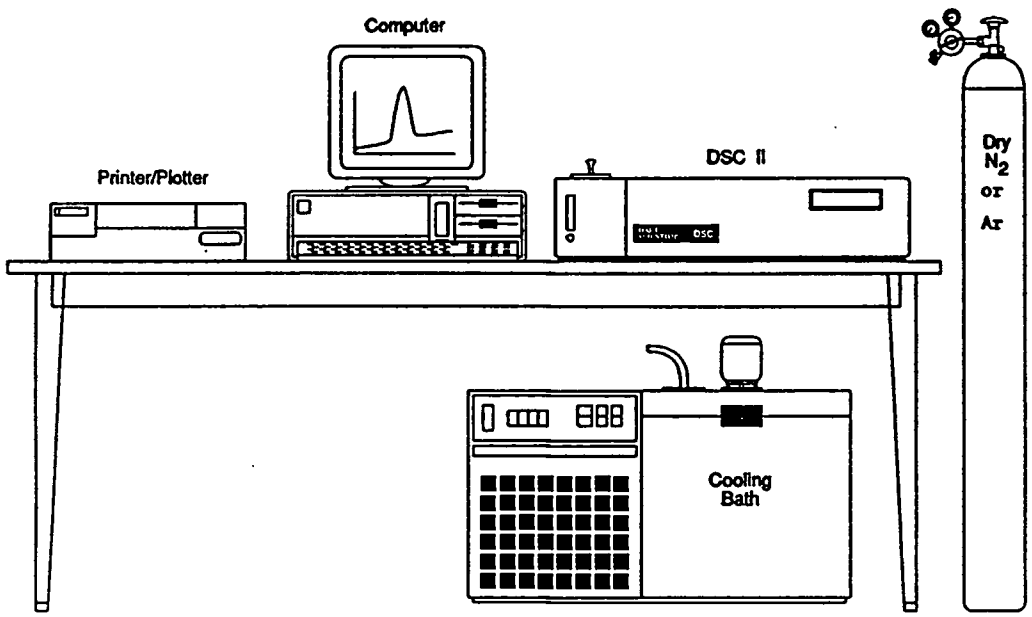
Appendix 1. Determination of vascular connection between bud and stem xylem followed by translocation of a 5% azosulfamide solution.

DATE	TMT	Number of excised buds showing vascular connection								TOTAL
		1a	1b	2a	2b	3a	3b	4a	4b	
12/18/89	12/10/89	0	0	0	0	0	0	0	0	0/32
12/20/89*	12/18/89	0	0	0	0	0	0	0	0	0/32
01/02/90	12/26/89	0	0	0	0	0	0	0	0	0/32
01/05/90	01/02/90	0	0	0	0	0	0	0	0	0/32
01/15/90	01/08/90	0	0	0	0	0	0	0	0	0/32
01/23/90	01/15/90	0	0	0	0	0	0	0	0	0/32

NOTE: On every date when plants are transferred to greenhouse, and pruned to check for dormancy status. The prunings (a + b) of each sample were checked for vascular connection. This material is the same as that for dormancy determination and can be correlated. A green layer can be observed just below the bud in a continuous line with surrounding tissue in the stem.

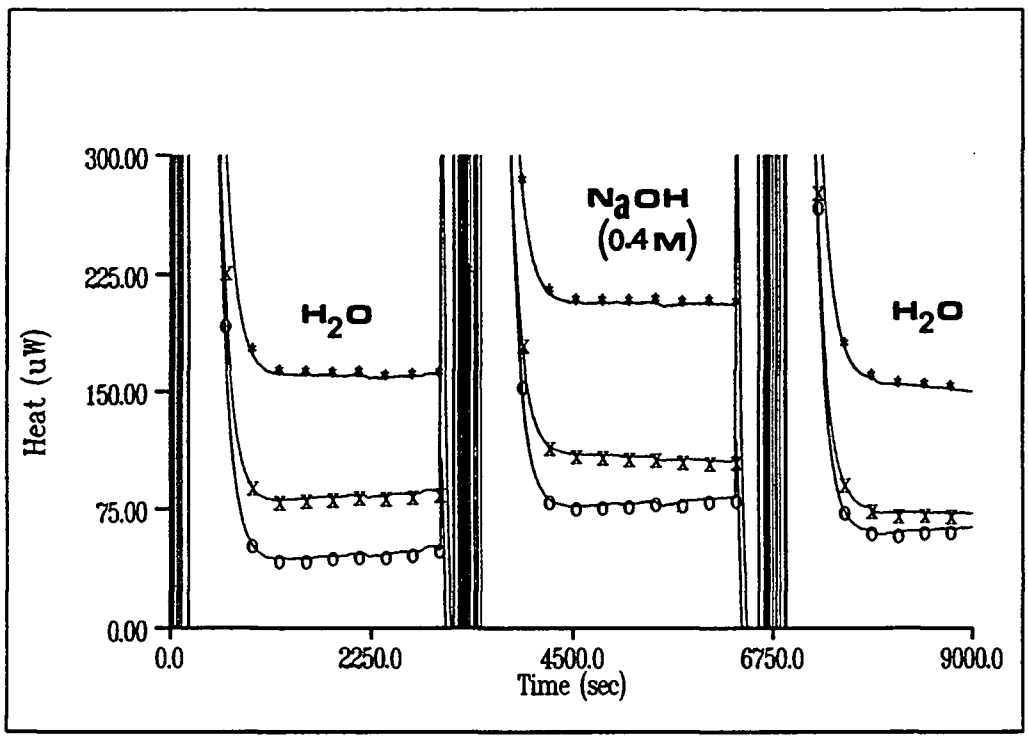
When dissecting the wood the dyed tissue can be easily noticed. Since the samples are one-year old shoots, just one-season xylem is observed. Making cross sections of the buds from the tip going down to the base (acropetally), the tissue looks green. Below the point where the secondary bud is attached to the axis of the primary bud, it is possible to observe the first traces of dyed tissue. This means that the non-differentiated parenchyma layer should lay below or at this point.

Appendix 2. Illustration of the differential scanning calorimeter system set up.



Reproduced from Hart differential scanning calorimeter manual. Hart Scientific, Pleasant Grove, Utah 84062-0435.

Appendix 3. Isothermal thermograph describing heat responses of the same set of buds in the presence of water, and a CO_2-trap system.



Three samples were loaded. Series of vertical lines at 3000 and 6750 sec represent the time when the instrument is opened. Once the chamber is open, it takes ca. 1500 sec for the DSC to equilibrate. In the first 3000 sec, a mini ampule containing 50 μl of sterile double-distilled water is included with the buds in the 1 ml-ampule, and the heat of metabolism is recorded. At 3000 sec, the ampules are removed from the DSC, and the water is replaced with 50μl of 0.4M NaOH. At 3300 sec, the ampules are replaced in the DSC, and the heat of metabolism plus

the heat of carbonation are recorded. At 6300 sec, the ampules are removed again, and the NaOH is replaced by sterile double-distilled water to check the metabolic heat. Metabolic heat is calculated by averaging the last 20 readings of each section. Heat of the carbonation reaction is estimated by subtracting the metabolic heat from the total heat output in the presence of the NaOH solution. Calculation of metabolic efficiency was done according to the algorithm:

$$R\text{-}1 = q_{CO2}/q_{met}$$

Where R-1 is metabolic efficiency, q_{CO2} is the heat produced by the carbonation reaction between the NaOH solution and the CO_2 of respiration, and q_{met} is the heat of metabolism. For theoretical considerations see Criddle et al. (1990). Later in 1992, a report will be published by Hansen et al., this publication will propose changes in the algorithm above, to determine an estimate of the efficiency of energy metabolism. For the time being, calculations were done as cited.

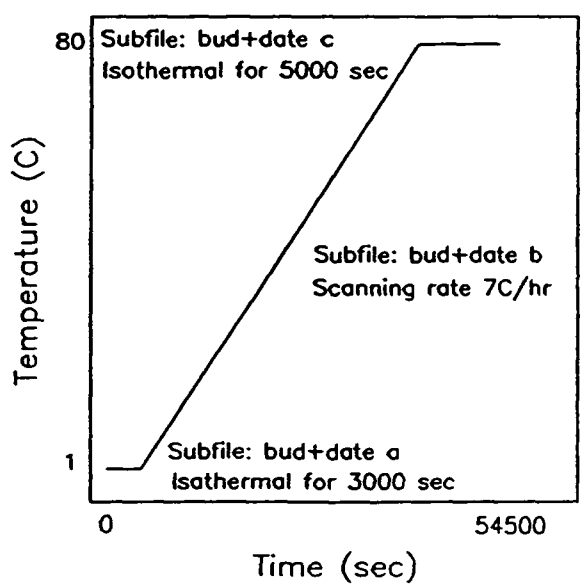
Appendix 4. Example of a descriptive file used to program an scanning experiment in the differential scanning calorimeter, and diagrammatic representation of the program.

```
Current file: bud.ptr
    1  heat2
    2  heat3
    3  time 20
    4  scanuprate 7
    5  scandownrate 7
    6  settemp to 1
    7  file bud+datea
    8  hold for 3000
    9  file bud+dateb
   10  scanup to 80
   11  file bud+datec
   12  hold for 5000
   13  quit
   14  # enter any comment #
   15  **********
   16  **********
   17  **********
   18  **********
   19  **********
   20  **********
   21  **********
   22  **********
```

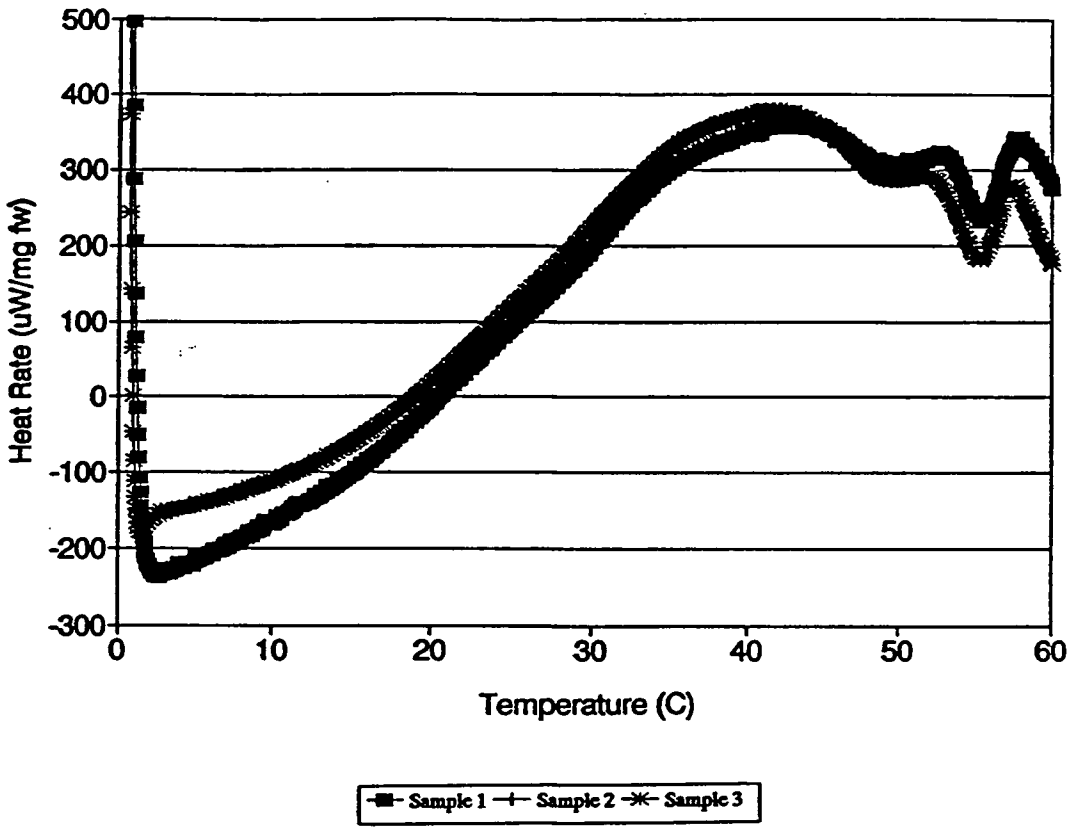
```
d scandown      h hold
D scandownrate  t time
u scanup        g getheat
U scanuprate    j heat2
s settime       k heat3
S settemp       c comment
n newfile       q quit
```

```
'R' removes current line
'I' inserts a line
'E' saves and exits
'F' loads a template file
'L' toggle options menu

Enter choice:
```

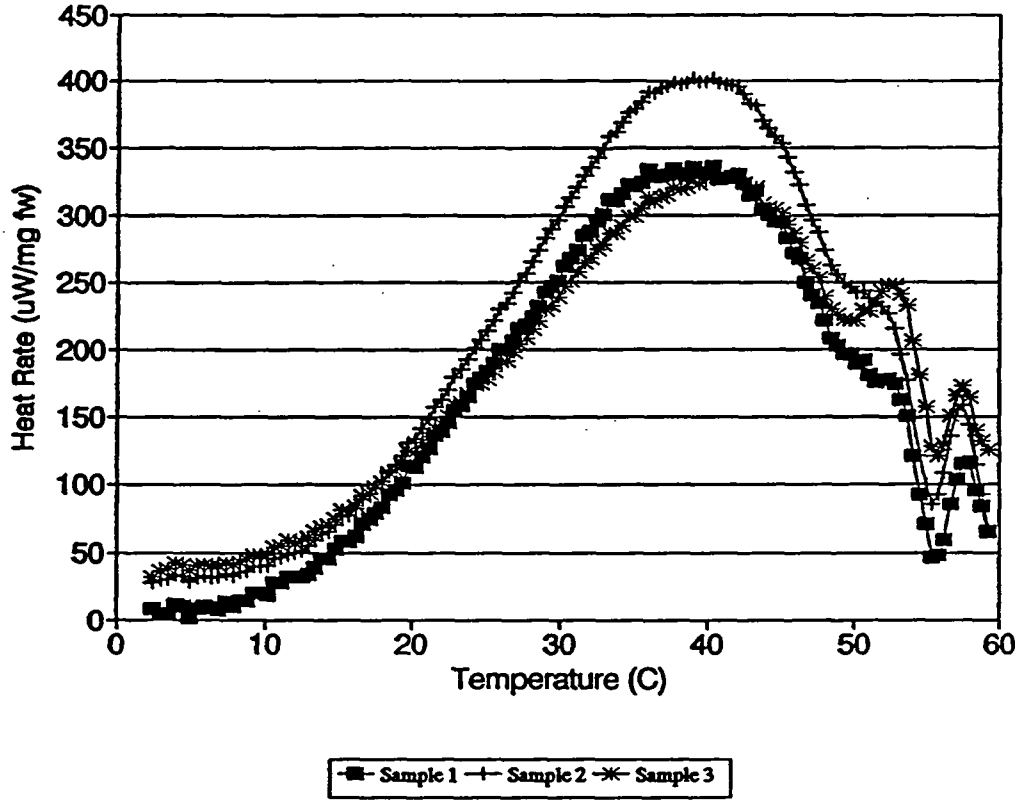


Appendix 5. Typical thermograph of a grape bud scanning showing raw data.



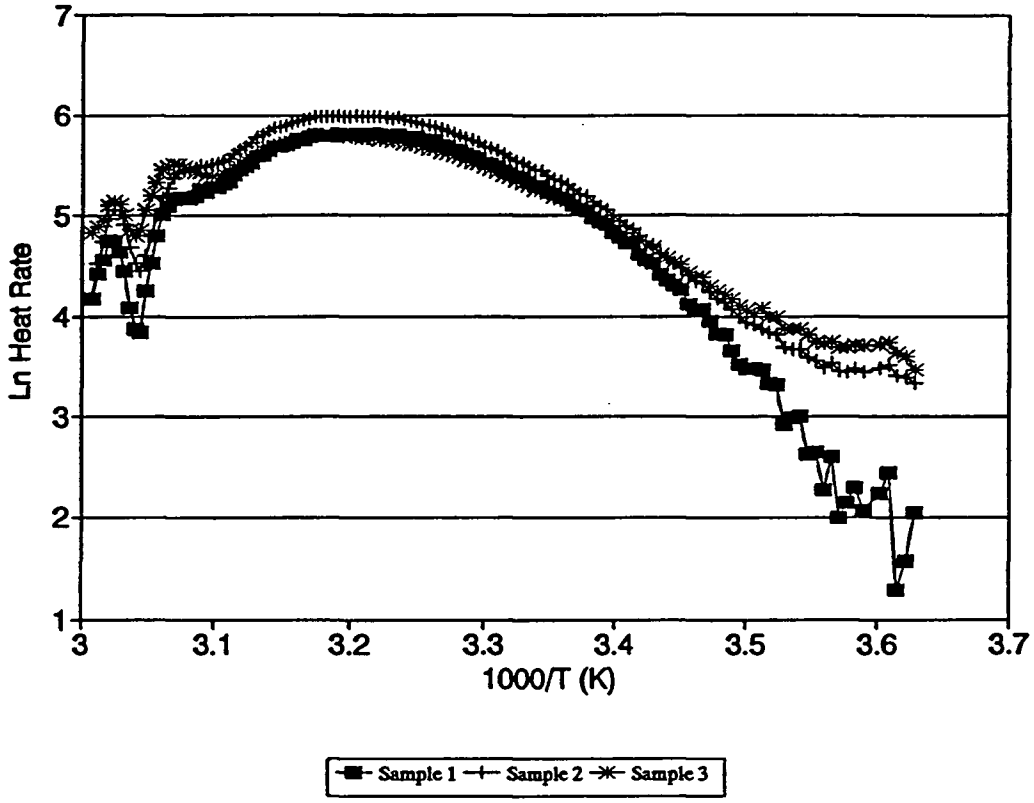
Data were collected at 20 sec intervals, must be base-line corrected, and expressed on a fresh weight basis.

Appendix 6. Scanning thermograph showing the same set of data as Appendix 5, but base-line corrected, and expressed on a fresh weight basis.



Heat measurements at 10°C intervals are used to calculate Q_{10} values. After attaining maximum respiration values, decreasing rates represent stress responses and tissue damage. Lesser peaks, in the 50 to 60°C, are believed to be related with degradation of tissue components. Only one tenth of the original data are plotted.

Appendix 7. Arrhenius plot developed from the data set shown in Appendix 6.



Information obtained from this plots include the apparent activation energy (Ea), calculated from the slope of the regression equation fitting the data in the 15 to 35 °C range (3.47 and 3.25 in the abscissa). Breaking points, associated with changes in metabolic activity, are identified by changes in the slope of the lines.