Use of ^1H -NMR to Determine Grape Bud Water State during the Photoperiodic Induction of Dormancy

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Abstract. Nuclear magnetic resonance longitudinal (T_1) and transverse (T_2) times were used to monitor changes in bud water state during the photoperiodic induction of dormancy in grape (*Vitis riparia* Michx.). Short day (SD) treatments were used to promote a rapid onset of bud dormancy, and long day (LD) treatments were used to prevent the onset of dormancy. Water content (WC) and the state of bud water were monitored after 2, 4, and 6 weeks of LD or SD treatment in three bud developmental stages. There was no difference in WC in the SD and LD treatments after 2 weeks. WC decreased in LD and SD buds of all stages during the 6 weeks of treatments, but there was a greater decrease in WC in SD treatments. The state of bud water changed during the SD treatments, shown in changes in T_1 and T_2 relaxation times. The SD T_1 relaxation times were shorter than the LD T_1 values at all measurement times. The SD T_2 times were shorter than the LD T_2 values in the 4- and 6-week treatment only. Tissue age was an element in lowering the T_1 and T_2 times but not the primary factor. A comparison between the bud dormancy response in the SD and LD treatments and the relaxation times showed that the shorter relaxation times indicate a restriction of motional freedom. The short relaxation times of the SD treatment correlated with the onset of dormancy. When dormancy is fully induced, T_2 times correlated better with dormancy than T_1 times.

The transition from active growth to dormancy [rest or endodormancy (Lang, 1987)] is a winter survival mechanism used by temperate woody plants in the northern latitudes. In grape, endodormancy develops in response to a decreasing photoperiod (Fennell and Hoover, 1991). Whole-plant studies of a northern *Vitis riparia* clone show that changes in plant water status (leaf water potential, stomatal resistance, and root hydraulic conductivity) are promoted by a decreasing photoperiod (Fennell et al., 1986). However, bud dormancy in *V. riparia* precedes the decrease in root hydraulic conductivity and decreased leaf water potential.

In contrast, changes in the state of water in buds have been correlated with various stages of bud dormancy or freezing tolerance in several woody perennials (Burke et al., 1974; Faust et al., 1991; Gardea et al., 1994; Kaku, 1993; Kaku et al., 1984; Rowland et al., 1992). The state of water commonly refers to its motional freedom, which is affected by the degree of association of water to macromolecules or concentration of paramagnetic ions in the tissue (Walter et al., 1992). Proton nuclear magnetic resonance (^1H -NMR) has been used to monitor the state of water in a variety of plant tissues (Burke et al., 1974; Chen et al., 1978; Coliere et al., 1988; Gusta et al., 1979; Kaku et al., 1984; Walter et al., 1992). NMR allows rapid nondestructive determination of changes in the ordered state of the water by measuring changes in proton relax-

ation times [longitudinal (T_1) spin-lattice or transverse (T_2) spin-spin relaxation times] (Burke et al., 1974; Chen et al., 1978; Gusta et al., 1979; Walter et al., 1992). Recent studies using ^1H -NMR or nuclear magnetic resonance imaging (MRI) have correlated the levels of water associated with macromolecules vs. free water (bound vs. free water) with the transition from endodormancy to ecodormancy (Faust et al., 1991; Gardea et al., 1994). When terminal buds were removed or thiadiazuron was applied to paradormant apple buds, MRI studies showed an increase in free water and a decrease in bound water that correlated with bud growth potential in lateral buds (Liu et al., 1993). In apple, blueberry, and wine grape buds, there also was more ordered or bound water in endodormant vs. ecodormant buds (Gardea et al., 1994; Faust et al., 1991; Rowland et al., 1992). In overwintering apple and blueberry buds, the presence of free water correlated with the end of endodormancy. The absence of free water in buds before fulfilling the chilling requirement, therefore, is criterion for endodormancy (Faust et al., 1991; Rowland et al., 1992).

The apple, blueberry, and wine grape studies were focused on the fulfillment of the chilling requirement and transition to ecodormancy rather than the initiation of endodormancy. The results from the apple and blueberry studies suggest that there could be an opposite change in the state of bud water (from free to bound or long T_2 times to short T_2 times) during the transition from paradormancy to endodormancy at the end of a growing season. Our objective was to further test the hypothesis that the state of water can be used to identify the stage of bud dormancy. In these studies, traditional methods of monitoring tissue water content and dormancy also were compared with nondestructive ^1H -NMR assessment of tissue water state during the induction of dormancy. These experiments were designed to determine whether changes in bud water state are correlated with dormancy and whether this nondestructive method can be used as a reliable tool to measure the onset of dormancy.

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Materials and Methods

Fig. 1. Stage of grape bud development. Buds were sampled from three developmental stages. A = basal (nodes 3 to 7), B = middle (nodes 8 to 12), and C = apical (nodes 13 to 17). Starting at the base of the plant, nodes 3, 8, and 13 (representing stage A, B, and C, respectively) were used for the NMR and water content measurements.

Table 1. Water content analysis of variance.

Source	df	Mean square	Significance
Main effects			
S ^z	2	7.5	NS
W ^y	2	60.2	***
PP ^x	1	20.9	***
Interaction			
S × W	4	0.8	NS
S × PP	2	0.6	NS
W × PP	2	5.7	NS
S × W × PP	4	0.1	NS
Error	126	0.4	NS

^zStage (S) = developmental stage: A, B, or C.

^yWeek (W) = week 2, 4, 6 of photoperiod treatment.

^xPhotoperiod (PP) = long-day and short-day treatments.

NS, *** Nonsignificant at $P = 0.05$ or significant at $P = 0.001$, respectively.

Plant materials. Two-year-old spur-pruned vines of *V. riparia* (clone origin: Manitoba, Canada, 52 °N) were used for these studies. Ecodormant bare root plants were placed in 15-L containers with media consisting of a 1 soil : 2 peat : 2 perlite (by volume) mixture. Three to four shoots (one on each spur) were trained vertically on each plant (Fig. 1), and all flower clusters were removed. The plants were grown under a long photoperiod (PP) (15 h) at 25 to 30 °C in unshaded glasshouses in Brookings, S.D., with 600 to 1400 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ photosynthetic photon flux density. All plants were watered daily and fertilized weekly with a complete nutrient solution (Lorenz and Maynard, 1980). After 45 d of growth, uniform plants were randomly assigned to either a short-day (SD) (8-h PP) or long-day (LD) (15-h PP) treatment at 25 to 30 °C. These treatments represent a dormancy-inducing or non-inducing treatment, respectively. Development of endodormancy and changes in bud water content and the state of the bud water were monitored at 2, 4, and 6 weeks of PP treatment. Three developmental stages were sampled during each time period (Fig. 1). The two basal nodes of each cane were left on each of three spurs on each plant after sampling for dormancy determination.

Bud water state. The state of the bud water was monitored using ¹H-NMR to determine changes in free vs. bound water in the bud during the transition from paradormancy to endodormancy. Node sections 3, 8, and 13 (counting from the base), representing the three stages of maturity (A to C) (Fig. 1), were harvested from the cane. Each stage of maturity was measured separately. One compound bud was cut from the shoot, wrapped in teflon tape, placed in a teflon holder, and inserted into a 7-mm zirconia rotor. The vertical axis of the bud was always oriented parallel to the pulse field to maximize signal resolution and to minimize anisotropic effects between samples (Burke et al., 1974; Gardea et al., 1994; McCain and Markley, 1985). In preliminary experiments, T₁ and T₂ data also were acquired with the sample spinning, yielding results similar to those acquired from stationary samples. It was difficult to maintain constant spinning speeds; therefore, all data presented were taken with the samples stationary and parallel to the pulse field.

Pulsed measurements of T₁ (the spin-lattice relaxation time) and T₂ (spin-spin relaxation time) and estimates of water content were made on the same samples. Eight single-bud replications from the three developmental stages were measured for each PP treatment. T₁ values were determined by the inversion recovery method (Farrar and Becker, 1971), and T₂ measurements were determined by the Carr-Purcell-Meiboom-Gill method (Farrar and Becker, 1971). T₁ and T₂ calculations were performed using integrated peak areas due to variations in the line shapes and line widths. All NMR measurements were made at 9.4 Tesla, corresponding to a proton NMR frequency of 400-MHz, using a NMR spectrometer (model ASX400; Bruker, Billerica, Mass.). A complete phase cycle of eight scans was used. The delay between scans was always greater than five times T₁.

Bud water content. Bud water content was estimated using ¹H-NMR by comparing the integrated signal intensity to that of a 10- μL reference sample of water. A one pulse sequence, with a 90° pulse of 3.5 μs , was used for estimation. Actual bud water content was determined immediately after NMR measurements. The fresh mass (FM) was measured, the buds were dried at 70 °C for 1 week, and bud dry mass (DM) was determined. Bud water content (WC) was expressed as $\text{WC} = \text{g}\cdot\text{g}^{-1}\text{ DM}$.

Bud dormancy. The pruned plants were returned to LD growing conditions after sampling to determine depth of dormancy. Dor-

mancy was determined by examining 48 buds, two buds on each of three spurs on eight plants. The relative depth of bud dormancy induced by the SD is expressed as the difference between the mean number of days to budbreak for the SD plants and the mean number of days to budbreak for LD plants.

Data analysis. An analysis of variance was used to determine significant treatment effects (Tables 1–3). A Student–Newman–Kuels test at $P \leq 0.05$ was used for mean separation of significant effects.

Results

Bud dormancy. Two weeks of SD treatment initiated bud dormancy. However, the dormancy process was still reversible. After 2 weeks of SD, budbreak was delayed by an average of 6 d. After ≥ 4 weeks, SD treatment the plants were endodormant. Only three buds on two plants, of 48 buds on eight plants, showed any growth after 4 weeks of SD. The growth of these three buds was stunted and leaves were a bronze color. These two plants never resumed normal leaf color or shoot growth, indicating dormancy was advancing. There was no growth after 6 weeks of SD, confirming that the dormancy process was well advanced and chilling was required for budbreak to occur.

Bud WC. There was no difference in bud WC between PP treatments or stage of development in the 2-week treatment (Fig. 2, Table 1). In the 4- and 6-week samples, PP and stage of development effects were significant. In the 4- and 6-week treatments, bud WC was always greatest for stage C > stage B > stage A. Bud WC decreased with tissue age as can be seen by the decrease in WC in the LD buds over the 6 weeks of treatment. SD promoted a more pronounced decrease in WC, from 80% to 60% of the FM, whereas the LD plants decreased from 80% to 70% after 6 weeks.

Bud WC, estimated by $^1\text{H-NMR}$, was linearly related with WC determined by drying the samples (Fig. 3). Regression analysis indicated that the $^1\text{H-NMR}$ estimation of the WC was about two-times that of the actual bud WC ($r^2 = 0.68$, $Y = 2.1X$). There were no significant differences in regression between treatments.

Bud water state. The proton relaxation times decreased in the LD and SD plants over time (Fig. 4). Within each PP treatment and measurement time, the T_1 times were not different for the three stages of development (Table 2). Over the 6 weeks, however, the T_1 times decreased from 300–400 ms at 2 weeks to ≈ 250 ms in the 6-week LD buds. In the SD buds, T_1 times decreased from 270–180 ms. The T_1 times were much shorter in the SD treatments than the LD treatments at 2, 4, and 6 weeks of treatment.

T_2 relaxation times were the same (≈ 25 ms) for all stages of development in LD and SD treatments at 2 weeks of treatment (Fig. 5, Table 3). After 4 weeks of treatment, SD plants had shorter relaxation times than LD plants. There were no differences in T_2 times among the three stages of buds within a PP treatment at 4 weeks. By 6 weeks of treatment, the difference between SD and LD was much greater, and the most mature segment had the lowest T_2 time.

T_1 and T_2 relaxation times were not strongly correlated with bud WC; coefficients of correlation were $r = 0.50$ and 0.58 , respectively.

Discussion

Dormancy was initiated within 2 weeks of SD treatments, and the grape plants were endodormant after 4 weeks of SD. This strong dormancy induction response allowed us to compare other measured characteristics with the dormancy response and assess their relatedness.

Table 2. Longitudinal time (T_1) analysis of variance.

Source	df	Mean square	Significance
Main effects			
S^z	2	3,930	NS
W^y	2	115,146	***
PP^x	1	156,877	***
Interaction			
$S \times W$	4	4,803	NS
$S \times PP$	2	657	NS
$W \times PP$	2	1,190	NS
$S \times W \times PP$	4	810	NS
Error	126	2,703	NS

^zStage (S) = developmental stage: A, B, or C.

^yWeek (W) = week 2, 4, 6 of photoperiod treatment.

^xPhotoperiod (PP) = long-day and short-day treatments.

NS, ***, *** Nonsignificant at $P = 0.05$ or significant at $P = 0.001$, respectively.

Table 3. Transverse time (T_2) analysis of variance.

Source	df	Mean square	Significance
Main effects			
S^z	2	113.5	**
W^y	2	645.7	***
PP^x	1	706.7	***
Interaction			
$S \times W$	4	58.6	*
$S \times PP$	2	5.2	NS
$W \times PP$	2	135.5	**
$S \times W \times PP$	4	8.9	NS
Error	126	20.2	

^zStage (S) = developmental stage: A, B, or C.

^yWeek (W) = week 2, 4, 6 of photoperiod treatment.

^xPhotoperiod (PP) = long-day and short-day treatments.

NS, *, **, *** Nonsignificant at $P = 0.05$ or significant at $P = 0.05, 0.01, 0.001$, respectively.

Fig. 2. Bud water content for the three bud stages (A, B, and C) after 2, 4, and 6 weeks of long or short days (LD and SD, respectively). LD are indicated by solid bars and SD by hatched bars. Values for 2, 4, and 6 weeks of treatment are expressed as the mean of eight replications $\pm$ SE.

As has been reported in other studies, decreased bud WC was related to increasing tissue age over time (weeks) in the experimental treatment (Wolpert and Howell, 1985). WC was highest in the 2-week treatment, lower in the 4-week treatment, and lowest in the 6-week treatment. There was a superimposed effect of SD on the decrease of water content. After 4 weeks of treatment, the effect of tissue age on WC became apparent. The buds from the oldest

Fig. 3. Total bud water content (grams) vs. water content estimated using $^1\text{H-NMR}$. Regression equation: $Y = 2.1X$, $r^2 = 0.68$.

Fig. 4. Spin-lattice relaxation time (T_1) for the three bud stages (A, B, and C) after 2, 4, and 6 weeks of long and short days (LD or SD, respectively). LD are indicated by solid bars and SD by hatched bars. Values are expressed as the mean of eight replications $\pm$ SE.

segments (A) of the plants had lower WC than the respectively younger segments (B and C) in SD and LD, with SD always lower. The change in WC was not directly related to induction of dormancy, as dormancy was initiated after 2 weeks of SD, whereas WC in SD buds was the same as that of LD buds. In addition, after 4 weeks, WC was lowered with age (A to C), even in the LD plants that never developed dormancy.

Comparison of actual tissue WC with WC estimated by $^1\text{H-NMR}$ indicated that the $^1\text{H-NMR}$ measurements can provide a nondestructive alternative for determining bud WC as has been shown for leaves (Coliere et al., 1988; Gusta et al., 1979). The relationship is linear; however, the NMR method detects all protons that contribute to the relatively broad line near 4.5 ppm, not just those associated with water. Therefore, a direct relationship cannot be expected between values determined from protons and actual WC. Measurements over a wider range of bud hydration levels would need to be made and a water vs. proton concentration curve developed before using NMR to routinely determine bud WCs.

Many investigators using $^1\text{H-NMR}$ determine either T_1 or T_2 relaxation time because they feel that either one expresses the state of water adequately. Because dormancy is a complicated process, we felt it important to determine both relaxation times to see if either fit the induction of dormancy process better. While relaxation times have been decreased with decreased WC, it is not a simple relationship. It is the interaction of water with macromolecules rather than the total amount of water in a tissue that leads to different relaxation times (Walter et al., 1992).

Decreasing T_1 times had a strong correlation with tissue age as expressed by weeks of treatment in SD and LD plants. It is apparent that the SD treatment superimposed an effect on T_1 values that was greater than the effect of tissue age. In the SD treatment, the decrease in T_1 times occurred after 2 weeks of treatment before there was a change in WC. A similar decrease in T_1 before a change in WC was noted in water-stressed barley leaves (Coliere et al., 1988). During the first 4 weeks of this study, the oldest grape buds had the longest T_1 time but lowest WC. The decrease of T_2 relaxation times with weeks of treatment was much less pronounced than the decrease exhibited with T_1 times. There was no reduction of T_2 times in LD plants until the sixth week of the experiment. There was no definite pattern in T_2 times with developmental stage of bud tissues (A to C), even after 4 weeks. After 6 weeks, the pattern is the same as with T_1 times, namely the youngest tissue showing the longest T_2 time. T_2 times appear to correlate more with the induction of dormancy than T_1 times. SD did not induce an appreciable change in T_2 times when dormancy was still clearly reversible after 2 weeks of treatment. After 4 weeks of SD, T_2 times were lower by $\approx 20\%$, and dormancy was imposed with the exception of two plants in which dormancy was advanced but not completed. After 6 weeks of SD, T_2 times were quite low ($\approx 50\%$ of original values) and dormancy was irrevocably imposed. A cold treatment was necessary to terminate dormancy after the 6-week SD treatment. From previous experiments (Fennell and Hoover, 1991), we know that after 6 weeks of SD, buds are dormant regardless of their shoot developmental stages. Yet in this study, buds showed a differential T_2 time, similar to the pattern in T_1 times, contingent on their shoot developmental stage. Thus, we must conclude that in addition to dormancy, there is an additional factor that influences T_2 times that is related to the age of the tissue.

Although T_2 times seem to correlate with dormancy, we must not discount the direct effect of WC on T_2 times. Relaxation times are strongly influenced by the availability of the water and the presence of macromolecules to which water molecules can be

Fig. 5. Spin-spin relaxation time (T_2) for the three bud stages (A, B, and C) after 2, 4, and 6 weeks of long or short day (LD and SD, respectively). LD are indicated by solid bars and SD by hatched bars. Values are expressed as the mean of eight replications $\pm$ SE.

“bound.” There is a strong correlation between reduction in T_2 and decreased WC in several plant tissues (Burke et al., 1974; Gusta et al., 1979); however, our results suggest that other factors may be contributing to the motional restriction of water that results in the shortened relaxation times. A number of cellular changes, such as the accumulation of hydrophilic solutes, changes in cellular pH, or other factors specific to dormancy induction, could contribute to a more ordered state of water, thereby resulting in decreased relaxation time (Coliere et al., 1988; Faust et al. 1991, Gusta et al., 1979; Kaku, 1993; Kaku et al., 1984; 1992). From our data, it is obvious that WC and other factors must be changing. Studies of changes in bud cellular composition must be made before we can reach definite conclusions on the factors influencing relaxation times in grape.

NMR does provide a nondestructive method of monitoring changes in bud water state. The results of these experiments indicate that there are changes in the bud water state and that these changes occur shortly after exposure to SD PP. The correlation between the short relaxation times and induction of dormancy supports the hypothesis that the presence of motionally restricted water is an indicator of endodormancy. Currently, it appears that T_2 is a better indicator of dormancy than T_1 .

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