Water Status in Relation to Maintenance and Release from Dormancy in Blueberry Flower Buds

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ABSTRACT. Three blueberry (*Vaccinium* section *Cyanococcus*) genotypes, that have different chilling requirements and levels of cold hardiness, were studied. Depth of dormancy was evaluated and water status was determined, using nuclear magnetic resonance (NMR), throughout the accumulation of chilling that leads to release from dormancy. Among the two highbush cultivars studied, 'Bluecrop' (*Vaccinium corymbosum* L.) was the most dormant and 'Gulfcoast' (*Vaccinium corymbosum* L. $\times$ *Vaccinium darrowi* Camp) was the least dormant. The rabbiteye cultivar 'Tifblue' (*Vaccinium ashei* Reade) had an intermediate dormancy. It appeared that the cultivar with the deepest dormancy had also the highest chilling requirement (CR). The NMR results showed that 'Bluecrop' buds had the lowest relaxation times (T2), indicating that water was relatively more bound in 'Bluecrop' buds than in the buds of the two other cultivars. Whatever the cultivar, no significant variation of T2s and water content of the buds was noted throughout the accumulation of chilling, even after CRs were satisfied. Within 1 day of forcing (24 °C, long day), there was a shift towards freer water but no change in the water content. Forcing was ineffective in freeing water until the plants received enough chilling to satisfy their CRs.

Buds of woody plants enter dormancy and develop cold hardiness to survive winter conditions. Control of this type of winter dormancy resides in the bud itself and is called endodormancy, as opposed to paradormancy, which is controlled by a factor outside the bud (for example, apical dominance), and ecodormancy, which is imposed by environmental factors (Lang, 1987; Lang et al., 1987). For breaking endodormancy, exposure to low temperature is required. This requirement is referred to as the chilling requirement (CR) and is genotype dependent. According to the French school, endodormancy is characterized by the occurrence of a permeability barrier and an inability to increase the non-adenylic nucleotide pool (reviewed by Crabbé and Barnola, 1996). Other metabolic processes associated with release from para- and endodormancy include changes in lipid and sterol metabolisms (Liu et al., 1991; Wang and Faust, 1990), respiratory metabolism (Wang et al., 1991), polyamines (Wang and Faust, 1994a), and antioxidant systems (Wang and Faust, 1994b). Efforts to characterize changes in gene expression associated with changes in dormancy status have resulted in the identification of three major dehydrins in blueberry floral buds, whose levels increase with chill unit (CU) accumulation and decrease with the resumption of growth (Muthalif and Rowland, 1994). However, more recent work suggests that levels of these proteins are more closely associated with cold hardiness levels than with dormancy status (Arora et al., 1997).

In the search for the mechanism of dormancy control in buds, some researchers have focused on studying the changes in water status, using nuclear magnetic resonance (NMR) or magnetic resonance imaging (MRI) techniques (Erez et al., 1998; Faust et al., 1991; Liu et al., 1993; Rowland et al., 1992). These techniques detect T1 or T2 proton relaxation times of water. A short relaxation time is interpreted as an indication of bound water (water associated with macromolecules) and a longer relaxation time is indicative of freer water. With MRI, images are created that allow the visualization of states of water associated with different tissues. Because the biological

activity of tissues depends on the physiological state of water, changes in the mobility of water are expected to accompany or to precede the physiological changes that occur in the buds of woody plants during dormancy induction and release. Faust et al. (1991) with apple buds and Rowland et al. (1992) with blueberry flower buds reported that the state of water significantly changes with development of endodormancy. Bound water is associated with the dormant stage while water less restricted in its motion is associated with the satisfaction of the CR. More recently, Erez et al. (1998) reported that in peach flower buds water is freed with resumption of growth rather than by satisfaction of the CR.

Here, we report the results from experiments designed to better distinguish if changes in the water status of blueberry flower buds are correlated with the transition from endo- to ecodormancy, i.e., with the satisfaction of CR, or with the resumption of growth.

Materials and Methods

PLANT MATERIAL. Potted blueberry (*Vaccinium* section *Cyanococcus*) plants of rabbiteye cultivar 'Tifblue' (*Vaccinium ashei* Reade) and of highbush cultivars 'Bluecrop' (*Vaccinium corymbosum* L.) and 'Gulfcoast' (*Vaccinium corymbosum* L. $\times$ *Vaccinium darrowi* Camp) were kept in a heated greenhouse (minimum temperature of 18.3 °C) until November 1995 or 1996. By that time, they were dormant as judged by the inability to force budbreak but had not received any chilling. Seven plants of each cultivar were moved to a constant temperature cold room and incubated at 4.4 $\pm$ 0.5 °C under a 10-h photoperiod. Each hour at 4.4 °C was considered as 1 CU according to the Utah model modified by Norvell and Moore (1982) for highbush blueberries. Shoots with floral terminal buds were periodically collected for determination of dormancy and for NMR analysis to determine the water state and content.

DORMANCY DETERMINATION. To evaluate the dormancy status of the floral buds, 10 shoots 5 to 6 cm long with one terminal floral bud were randomly cut from seven plants of each cultivar at each sampling time (every 150 CU or 300 CU for 'Tifblue', every 300 CU for 'Bluecrop', and every 100 to 150 CU for 'Gulfcoast') and placed in jars of water in a growth chamber maintained at 24 °C with a 14-h photoperiod. Budbreak was recorded three times a week for 2 months. Budbreak was estimated according to Spiers (1978). Buds

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were considered as broken when the bud scales had separated and the apices of the flowers were visible. Results were expressed as percentage budbreak and as average time of budbreak (Champagnat, 1992). The average time of budbreak (T_b) was given by $T_b = n / \sum t_i^{-1}$ where t_i = the individual times of budbreak and n = the number of buds. By its formulation, T_b allowed us to consider the buds that did not break after a certain time ($t_i = \text{infinity}$, $t_i^{-1} = 0$).

The CR of each cultivar was defined as the number of hours at 4.4 °C necessary for >50% budbreak after 3 weeks of forcing at 24 °C.

NMR ANALYSIS. NMR analysis of protons was used to determine the status of water in blueberry buds during 1) CU accumulation and 2) release from dormancy. To examine the effect of CU accumulation on water status, shoots of 'Tifblue', 'Bluecrop', and 'Gulfcoast' were collected every 150 or 300 CU, 300 CU, and 100 to 150 CU, respectively, and imaging of terminal floral buds was performed immediately after collection. To examine the effect of release from dormancy on water status, similar shoots of 'Gulfcoast' were also kept for 24 h in a growth chamber at 24 °C with a 14-h photoperiod before analysis. Three or five single-bud replications from the different cultivars were performed at each sampling time. The shoots were randomly cut from the seven plants of each cultivar placed in the cold chamber.

The shoots were cut ≈ 1 mm from the bottom of the terminal buds and dipped into melted wax to prevent dehydration during the measurements. Depending on the size of the buds, the samples were placed into one of two solenoidal coils, either 4.5 mm in diameter and 6 mm long or 5.5 mm in diameter and 8 mm long. The coils were placed in a Bruker imaging probe. Data were collected using a Bruker MSL 400 9.4T system (Bruker Instr. Co., Bellerica, Mass.).

Before imaging the samples, a 20-point Carr-Purcell-Gill-Meiboom spectroscopic measurement was made with echo times (t_e) of 1 to 20 milliseconds (ms) in 1 ms increments. This provided an estimate of the average T2 (spin-spin relaxation) time of the bud. The same experiment was repeated after imaging. A large decrease in the T2 values would indicate desiccation during the experiment. Before the experiments, spectroscopic T1 (spin-lattice relaxation) times were measured on several reference samples. This allowed selection of a repetition time (time between radiofrequency pulses), which minimized the effects of T1 relaxation on the acquired data (Morris, 1986).

For imaging, a three-dimensional spin-echo experiment was performed. Five separate acquisitions were run on each sample with echo times of 12.2, 16.2, 20.2, 24.2, and 32.2 ms. A repetition time of 1 s, over five T1s, was used. In total, 256 complex points were collected in the frequency encode direction perpendicular to the axis of the shoot. Sixty-four phase encode steps were used along the other perpendicular axis, and eight phase encode steps were used along the shoot axis. The field of view was $4 \times 4 \times 5$ mm respectively for the smaller coil and $5 \times 5 \times 8$ mm for the larger coil. A two step phase cycle was used giving a total acquisition time of ≈ 20 min and a total experiment time of ≈ 1 h, 40 min. The raw data was trapezoidal multiplied along the frequency encode direction, zero filled to a matrix size of $256 \times 256 \times 8$ and Fourier transformed. The magnitude images of the eight sections perpendicular to the bud axis were viewed and the images with areas showing bud tissue were selected for T2 estimation.

T2 estimation images were constructed by median filtering the spin-echo images, segmenting the images into background and shape, and then performing a nonlinear least squares fit to the equation $I(t_e) = sd \exp(-t_e/T_2) + b$, where $I(t_e)$ = the magnitude of the image with echo time t_e , sd = the intensity of an image at a t_e of 0 (spin density), and b = a baseline > 0 (Bevington, 1969). The estimate was

made using a simplex method with the program Amoeba and Amotry (Press et al., 1988). The T2 image was then median filtered.

T2 images were binned into three values: T2 estimates < 4 ms, 4 to 7 ms T2 estimates, and T2 estimates ≥ 7 ms. These levels were

Fig. 1. Percentage budbreak versus days of forcing at 24 °C, 14-h photoperiod, for terminal flower buds of (A) 'Tifblue' that received (●) 740 chill units (CU), (○) 884 CU, (■) 1220 CU, (□) 1531 CU, (▲) 1748 CU and (⊞) 1892 CU, flower buds of (B) 'Bluecrop' that received (●) 908 CU, (○) 1199 CU, (■) 1508 CU, (□) 1845 CU and (▲) 2010 CU and flower buds of (C) 'Gulfcoast' after accumulation of (●) 40 CU, (○) 136 CU, (■) 208 CU, (□) 400 CU, (▲) 808 CU, and (⊞) 1000 CU.

Fig. 2. Dormancy status of (A)(●) 'Tifblue', (○) 'Bluecrop' and (B)(■) 'Gulfcoast' flower buds expressed as the average time of budbreak (Tb) versus chill unit (CU). 'Tifblue': $y = 691,615 \exp(-0.0074x) + 373.5$, $r^2 = 0.99$; 'Bluecrop': $y = 256,495 \exp(-0.0042x) + 207.6$, $r^2 = 0.99$; 'Gulfcoast': $y = 632 \exp(-0.0083x) + 168.3$, $r^2 = 0.99$. Data points are the inverse transformed of means of the inverse transformed of 10 replications.

chosen arbitrarily, to illustrate the most contrast across all images, by viewing histograms of the T2 estimate images. Color-coded images were constructed by assigning purple, blue, and yellow, respectively, to these segmentation levels. The percentage of the image area with corresponding T2 values was computed from the ratio of points in a range to the total number of points in the image. The first spin-echo image was used to divide the area of the image into flower and bud scale sections. The area corresponding to flowers was determined visually.

The relative amount of water per unit volume was calculated by dividing the spin density (sd), which is proportional to the amount of water giving an NMR signal, by the volume of the bud. To estimate the volume of the bud, a photograph was taken along its axis and then scanned into a computer. The central axis of the bud was determined by hand dividing the image into a top and a bottom half. The volume was estimated from this image using the method of shells for each half of the bud (Purcell and Varberg, 1984). Five single-bud replications from five different plants of the different cultivars were performed at each sampling time.

In another experiment to examine the effect of release from dormancy on water state, one shoot of 'Tifblue' and one shoot of 'Bluecrop', which had received 2000 CU, were placed under forcing conditions at 24 °C with a 14-h photoperiod. The terminal buds were imaged every day for 9 d. Because of the duration of the experiment, no replications were performed. Samples were placed in a 6-mm-diameter saddle coil with the base of the stem remaining in water throughout the experiment. The stems were kept long enough for the water not to interfere with the measurements. In total, 256 frequency encode points were used in the axis perpendicular to the bud axis. Sixty-four phase encode steps were used in the other perpendicular axis, and sixteen phase encode steps were used in the parallel axis. A 1-s repetition time and a two-step phase encode were used to give an acquisition time of ≈ 35 min. Five images were collected with the same echo time as above to give a total experiment time of ≈ 3 h. T2 images were estimated as above.

STATISTICAL ANALYSIS. Mean comparisons were made by using a t test at the 0.1 probability level, after a significant effect had been determined by the analysis of variance procedure or the F test (SAS Institute, Cary, N.C.). The average time of budbreak values (Tb) were fitted to a one-phase asymptotic decay function for each cultivar, using PROC NLIN (SAS Institute).

Results

DORMANCY. To determine the dormancy status of blueberry floral buds, shoots were collected from three different cultivars after different lengths of chilling, held at 24 °C with a 14-h photoperiod, and examined for budbreak every 2 or 3 d. Floral buds of 'Tifblue' (*Vaccinium ashei*) and 'Bluecrop' (*Vaccinium corymbosum*) plants did not show signs of budbreak until they had received 740 and 908 CU, respectively (Fig. 1 A and B). Thereafter, as the length of chilling increased, percentage budbreak increased and the number of days to budbreak decreased. Fifty percent budbreak was attained after 1220 and 1508 CU for 'Tifblue' and 'Bluecrop', respectively. One hundred percent budbreak was attained after 1892 and 2012 CU for 'Tifblue' and 'Bluecrop', respectively. At that point, ≈ 10 to 15 d of forcing were necessary for budbreak. 'Gulfcoast' (*Vaccinium corymbosum* $\times$ *Vaccinium darrowi*) a low-chilling variety grown in the southern United States, responded very quickly to chilling (Fig. 1C). Forty CU were enough to induce budbreak and to obtain high

Fig. 3. Changes in the spin-spin relaxation time (spectroscopic T2) of (●) 'Tifblue' and (○) 'Bluecrop' flower buds with chill unit (CU) accumulation. Data points are the mean $\pm$ standard deviation of three replications.

Fig. 4. (top) Color-coded T2 images of 'Tifblue' and 'Bluecrop' buds after exposure to 0 chill units (CU), 642 CU, 1219 CU, 1891 CU, and 0 CU, 666 CU, 1193 CU, and 2010 CU, respectively. T2 < 4 ms = purple; 4 ms ≤ T2 < 7 ms = blue; T2 ≥ 7 ms = yellow. BS = bud scales; R = rachis; F = flower.

percentages of flowering (70% overall, 50% by 20 d of forcing). One hundred percent budbreak occurred after only 136 CU. After 304 CU, percentage budbreak was not enhanced by an increased number of CU. Plants reached 100% budbreak in <10 d.

Results were also expressed as the average time for budbreak (Tb), after different lengths of chilling (Fig. 2). Whatever the cultivar, Tb were fitted to regression curves of general equation $y = A \exp(-Bx) + C$ (Fig. 2). Up until 1500 CU, Tb for 'Bluecrop' was higher than Tb for 'Tifblue' (Fig. 2A). Thus, floral buds of 'Bluecrop' were in a deeper state of dormancy than those of 'Tifblue'. For 'Tifblue' and 'Bluecrop', when Tb first decreased, it decreased quickly, indicating a rapid release from dormancy. This phase was then followed by a slower decrease in Tb that lasted 300 CU for both cultivars. After 1500 CU were accumulated for 'Bluecrop' and 1200 CU were accumulated for 'Tifblue', Tb reached minimum thresholds of ≈300 h. Thus, at this stage, floral buds of the two cultivars had the same ability to resume growth. 'Gulfcoast' was the least dormant of the three cultivars (Fig. 2B). At the deepest point of dormancy, the Tb was only 600 h, ≈10 and 5 times lower than the Tb for 'Bluecrop' and 'Tifblue', respectively. Dormancy release occurred very quickly in 'Gulfcoast' as well. After 300 to 400 CU, a Tb threshold was reached, of ≈175 h, about half the value of the Tb threshold for 'Tifblue' and 'Bluecrop'. Amongst the three cultivars studied, 'Bluecrop' was the most dormant while 'Gulfcoast' was the least dormant.

Under our experimental conditions the CRs of 'Tifblue', 'Bluecrop', and 'Gulfcoast' were estimated to be ≈1200, 1500, and, 140 CU, respectively.

EFFECT OF CU ACCUMULATION ON WATER STATUS OF FLOWER BUDS. No significant change in average spectroscopic T2 times of whole buds was observed through 2000 CU for either 'Tifblue' or 'Bluecrop' (Fig. 3). The T2 times remained relatively low, <6 ms. 'Bluecrop' had a T2 time one unit shorter than 'Tifblue' (4.5 vs. 5.5 ms), indicating that water was present in a more motionally restricted form in 'Bluecrop' buds than in 'Tifblue' buds.

Fig. 4. (bottom) Color-coded T2 images of 'Tifblue' and 'Bluecrop' buds after exposure to 2000 chill units (CU) and to forcing conditions (24 °C, 14-h photoperiod) for 0 to 9 d. T2 < 4 ms = purple; 4 ms ≤ T2 < 7 ms = blue; T2 ≥ 7 ms = yellow.

Fig. 5. Water content of (●) 'Tifblue' and (○) 'Bluecrop' buds, estimated using $^1\text{H-NMR}$, versus chill unit (CU). Data points are the mean $\pm$ standard deviation of five replications.

Fig. 6. Changes in the average T2 times of the images of (●) 'Tifblue' and (○) 'Bluecrop' buds with days of forcing.

From transverse sections of buds, proton signals were detected from rachis, flowers and bud scales (Fig. 4 top). From the color-coded images, it was evident that water with the longest T2 times was within the flowers and water with the shortest T2 times was detected in the external bud scales. Corroborating the spectroscopic results, no significant change in T2 times was seen with CU accumulation using MRI. Although the lesser amount of yellow in the image for 'Bluecrop' at 2010 CU suggests a lower T2 value and thus more restricted water, the change in T2 was not statistically significant.

The water content of the buds, measured throughout the accumulation of chilling, did not change significantly for either 'Tifblue' or 'Bluecrop' (Fig. 5).

EFFECT OF FORCING ON WATER STATUS OF FLOWER BUDS. After 2000 h of chilling, one shoot from 'Tifblue' and one shoot from 'Bluecrop' were exposed to forcing conditions (24 °C, 14-h photoperiod) and the terminal bud from each was imaged daily for 9 d. On color-coded images of T2 times, without forcing (day 0), water appeared to have longer T2 times in the 'Tifblue' bud than

in the 'Bluecrop' bud (Fig. 4 bottom). However, a dramatic shift towards longer T2 times occurred in the 'Bluecrop' bud after only 1 d of exposure to high temperature, whereas little change in T2 times occurred in the 'Tifblue' bud until about the third day of forcing. This trend in 'Bluecrop' continued for the following days but to a lesser extent. By the last days of forcing, the 'Tifblue' bud had begun to break, resulting in the appearance of gaps between the flowers. The 'Bluecrop' bud was swollen, resulting in an increase in the size of the pictures.

Results from analysis of the images indicated that the average T2 of the image of 'Bluecrop' increased from 4.5 ms to 6.5 ms during the first 24 h of forcing (Fig. 6). Then, for the following 8 d, the T2 continued to slightly and steadily increase, reaching 7.6 ms. The major change occurred within 1 d of forcing. For 'Tifblue', at day 0, the T2 of the image was already two units higher than for 'Bluecrop' (Fig. 6). After a slight increase during the first 2 d, changes in the relaxation time were more irregular, with two peaks at day 3 and day 6. The overall increase after 9 d was from 6.5 ms to 8.0 ms. Changes in the average T2 times of the images were due to changes in the flower and the bud scale sections of the images (results not shown).

The percentage of the image area with each of the three T2 levels (T2 < 4 ms, 4 to 7 ms T2, and T2 $\geq$ 7 ms) was computed. For 'Tifblue', during the first 24 h of forcing, the proportion of water with T2 < 4 ms slightly decreased while the proportion of water with 4 to 7 ms T2 increased (Fig. 7A). The percentage of water

Fig. 7. Changes in the percentages of water with (●) T2 < 4 ms, (○) 4 ms $\leq$ T2 < 7 ms, and (□) T2 $\geq$ 7 ms for the images of (A) 'Tifblue' and (B) 'Bluecrop' with days of forcing.

Fig. 8. T2 relaxation time as affected by chill unit (CU) accumulation of 'Gulfcoast' buds when (●) analyzed immediately after sampling and when (○) kept 24 h under forcing conditions before analysis. Data points are the mean $\pm$ standard deviation of three replications.

with $T_2 \geq 7$ ms did not change. Between the first and the ninth day of forcing, opposite changes occurred in the high and intermediate T_2 percentages. When the percentage of water with $T_2 \geq 7$ ms increased, the percentage of water with 4 to 7 ms T_2 decreased. The percentage of water with the lowest T_2 times did not change and remained $<10\%$. After 2 d at 24°C , the water component with the longest T_2 times ($T_2 \geq 7$ ms) was the major one, comprising more than half the total image area. In 'Bluecrop', during the first 24 h of forcing, the proportion of water with $T_2 < 4$ ms decreased while the proportion of water with $T_2 \geq 7$ ms increased (Fig. 7B). The proportion of water with the shortest T_2 times dropped from 40% to 10% while the proportion of water with the highest T_2 times, only a few percent at day 0, reached 40%. During that time, the proportion with the intermediate T_2 times decreased slightly. After the first day, the percentage of water with $T_2 < 4$ ms did not change and remained at $\approx 10\%$. Between the first day and the third day of forcing, the proportions of water with 4 to 7 ms T_2 and $T_2 \geq 7$ ms remained at $\approx 45\%$. Then, the proportion of water with 4 to 7 ms T_2 steadily decreased and the proportion of water with $T_2 \geq 7$ ms increased to reach 30% and 60%, respectively. From day 3 onward, the proportion of water with $T_2 \geq 7$ ms was the most abundant component.

In a separate experiment, 10 shoots of 'Gulfcoast' plants were collected every 100 to 150 CU. At each time, terminal buds from half of the shoots were analyzed immediately (d0) and the other half of the shoots were kept for 24 h under forcing conditions before imaging of terminal buds (d1). Without forcing, the spectroscopic relaxation time did not change significantly with CU accumulation, ranging from 4.85 ms to 5.15 ms (Fig. 8). With forcing for 1 d at 24°C , T_2 remained constant until 300 CU. Between 300 and 400 CU, T_2 increased slightly by 0.3 to 0.4 ms, then remained more or less constant until 900 CU, and finally increased again during the last 100 CU. Before 300 CU, T_2 s of d0 and d1 did not differ significantly. After 300 CU, a difference was evident and gradually increased thereafter, to a final difference of about one unit (T_2 of d0 = 5.0 ms, T_2 of d1 = 6.0 ms). This 300 to 400 CU period coincided with the time at which the T_b reached its minimum threshold. The water content did not change during CU accumulation in 'Gulfcoast' for either d0 or d1 buds (Fig. 9).

Discussion

Three blueberry cultivars, 'Bluecrop', 'Tifblue', and 'Gulfcoast', were used to study the water status of flower buds during CU accumulation and release from dormancy. 'Bluecrop' and 'Tifblue' are the most widely planted highbush (*V. corymbosum* L.) and rabbiteye (*V. ashei* Reade) blueberries, respectively (Moore, 1993). 'Gulfcoast' is a low-chilling southern highbush cultivar that is a hybrid of *V. corymbosum* L. and *V. darrowi* Camp (Gupton et al., 1994).

Dormancy status of the cultivars was evaluated by forcing detached budsticks after exposing the plants to increasing periods of chilling. 'Tifblue' and 'Bluecrop' have a deep dormancy characterized by an inability to resume growth until they have received 740 and 900 CU, respectively. On the contrary, 'Gulfcoast' has a shallow dormancy as shown by the high percentage of flowering after only 40 CU. Whatever the cultivar, they enter the same dormancy phases as previously described for other woody plants like walnut (Mauget, 1977) and plane tree (Ricaud et al., 1995). First, buds exhibit a very low, indeed no, ability to resume growth. Then, with increasing chilling, there is a rapid decrease in the mean time required for bud burst, indicating a rapid release from dormancy. This is followed by a slow decrease in T_b which corresponds to a residual dormancy left after partial chilling, also called postdormancy (Champagnat, 1992; Crabbé, 1994; Vegis, 1964). The phase of rapid dormancy release is equivalent in 'Bluecrop' and 'Tifblue', i.e., T_b decreases at the same rate in 'Bluecrop' as in 'Tifblue'. On the other hand, during the postdormant phase T_b decreases more slowly in 'Tifblue' than 'Bluecrop' (2.14 vs. 4.55 h/CU), indicating that the residual dormancy is harder to overcome in 'Tifblue' than in 'Bluecrop'. For woody plants, the relationship between the intensity of bud dormancy and the timing of budbreak varies. Thus, grapevine varieties that break dormancy early have a weaker dormancy (Pouget, 1966). On the other hand, two walnut cultivars, one with a weak dormancy and one with a deeper dormancy, might resume growth at the same time (Mauget and Germain, 1980). In blueberry, like in grapevine, the cultivar with the shortest time to budbreak (also shortest CR), 'Gulfcoast', has the weakest dormancy and the cultivars with longest CRs, 'Tifblue' and 'Bluecrop', have the deepest dormancy. After 1200 CU for 'Tifblue', 1500 CU for 'Bluecrop', and 400 CU for

Fig. 9. Changes in water content with chill unit (CU) accumulation of 'Gulfcoast' buds when (●) analyzed immediately after sampling and when (○) kept 24 h under forcing conditions before analysis. Data points are the mean $\pm$ standard deviation of five replications.

'Gulfcoast', the capacity for buds to resume growth is not enhanced by an increase in chilling, i.e., Tb for the different cultivars reaches minimum thresholds. This marks the end of endodormancy since further chilling no longer hastens budbreak (Dennis, 1994). Buds have the capability to break but resumption of growth is limited by insufficient exposure to warm temperature. This period of quiescence, imposed by unfavorable environmental conditions, is described as ecodormancy (Lang, 1987; Lang et al., 1987).

The CR was estimated for each cultivar as the number of hours at 4.4 °C necessary for >50% budbreak upon exposure to conditions favorable for growth (14-h photoperiod, 24 °C). Under our conditions, the CR was 1200 CU for 'Tifblue', 1500 CU for 'Bluecrop' and 140 CU for 'Gulfcoast'. For 'Tifblue' and 'Bluecrop', CRs are higher than most previous reports. Spiers and Draper (1974) reported that 'Tifblue' plants require 600 h of constant chilling (6 to 7 °C) for normal flowering. Austin and Bondari (1987a) reported that 'Tifblue' requires 500 h of artificial chilling at 4.4 °C and between 400 and 500 h below 7.2 °C in field experiments (Austin and Bondari, 1987b). Muthalif and Rowland (1994) estimated the CR of field-grown plants of 'Tifblue' to be ≈600 and 1200 h for 'Bluecrop'. Unlike the two other cultivars, in this study 'Gulfcoast' has a lower CR than what is cited in the literature. Previous results from Rowland et al. (1992) indicated that the CR of 'Gulfcoast' is ≈400 h. In the present study, the same conditions seem to be very efficient in breaking bud dormancy in 'Gulfcoast' and in delaying the satisfaction of CR for 'Tifblue' and 'Bluecrop'. In fact, among these blueberry cultivars, there might be some variability in the range of temperatures and in the optimum temperatures effective in satisfying the CR. 'Tifblue' has been reported to have a narrower range of effective chilling temperature (-1.2 to 12.9 °C) than 'Woodard' and 'Aliceblue', and to have a lower optimum chilling temperature (6.7 °C) (Gilreath and Buchanan, 1981; Shine and Buchanan, 1982). For 'Bluecrop', Norvell and Moore (1982) showed that of the temperatures studied (1, 6, and 12 °C), 6 °C was the most effective in breaking dormancy. With their modified chilling model, each hour between 2.5 and 9.1 °C is equivalent in breaking bud dormancy and contributes 1 CU. A substantial amount of research has focused on the effect of the chilling regime (constant vs. fluctuating temperatures, cold room vs. outdoor experiments) on the accumulation of CU, and on the evaluation of the best model to estimate rest completion. Although Darnell and Davies (1990) noted that the cold chamber regime reduces both the possibility of temperatures that might negate some of the chilling and the possibility that a greater amount of chilling is received than is recorded, most authors have concluded that natural chilling is more effective than constant chilling, and that blueberry plants exposed to natural field chilling break bud dormancy more quickly than those exposed to artificial chilling (Arora et al., 1997; Austin and Bondari, 1987a; Norvell and Moore, 1982; Spiers, 1976; Spiers and Draper, 1974). Arora et al. (1997) showed with blueberry that, if the continuous chilling treatment is interrupted at 50% CR by 2 weeks at 15 °C (12 °C at night), there is a slightly higher percentage of budbreak as compared to the continuous treatment. Erez and Lavee (1971), with peach, have also shown that temperatures >7.2 °C, which are not counted for in field studies, have a restbreaking activity and that alternating moderate temperature (15 °C) with chilling in a daily cycle, results in chilling enhancement (Erez et al., 1979). The possibility also exists that the cultivars may vary in their response to the forcing temperature. Indeed, forcing at 24 °C could have negated some of the chilling for 'Tifblue' and 'Bluecrop' but not for 'Gulfcoast'.

NMR was used to determine the water status of blueberry floral buds during CU accumulation. Two parameters were studied. The first parameter was the T2 relaxation time which reflects the state of water. The state of water refers to the degree of association of water

to macromolecules in the tissues. Water may be free or may interact with biological macromolecules either by hydrogen bonds between polar groups or by hydrophobic bonds between nonpolar residues (Fullerton and Cameron, 1988). The second parameter studied was the spin density, which is proportional to the amount of water producing an NMR signal. It was used to calculate the water content of the buds.

The average spectroscopic T2 times of the buds, during CU accumulation, for 'Bluecrop', 'Tifblue', and 'Gulfcoast' are 4.5, 5.5, and 5.0 ms, respectively. For 'Gulfcoast' the maximum values reached after 1 d of forcing do not exceed 6.0 ms. With chilling accumulation, the average T2 times of the images are ≈6.0 ms for 'Bluecrop' and 'Tifblue'. These estimates of T2 times are lower than previously reported. Measured after 1 d at room temperature, T2 times of the images of chilled buds range from 8.14 to 14.65 ms for 'Gulfcoast', 'Georgiagem', 'Bluecrop', and 'Duke' cultivars studied previously (Rowland et al., 1992). These values are similar to those from apple leaf buds in which T2 times average 12.5 ms. For buds of 'Winblo' peach, the image T2 time averages 9.0 ms during CU accumulation (Erez et al., 1998). For buds of 'Redhaven' peach, the image T2 increases from 7.0 to 12.0 ms after 9 d of forcing at 24/18 °C (day/night) with a 16-h photoperiod (Erez et al., 1998).

T2 relaxation times as well as water content do not change significantly in blueberry during CU accumulation. Satisfying CR alone is not enough to free water. These conclusions differ from our original conclusions (Rowland et al., 1992) and those of Faust et al. (1991). However, in those studies, shoots were held at room temperature for 20 to 24 h before NMR analysis, or equivalent to 1 d of forcing in our current study. Therefore, based on NMR images of dormant and nondormant vegetative buds of apple, Faust et al. (1991) postulated that water is bound at the beginning of dormancy and freed after the CR has been satisfied. In the previous study (Rowland et al., 1992), blueberry flower buds appeared to behave similarly to apple leaf buds. Water appeared present in a motionally restricted form in dormant buds and in a freer form after enough CU accumulation to satisfy the CR. Sugiura et al. (1995) also suggested that the tightly bound water in the tissues of peach flower buds changes into free or loosely bound water during the rest period. However, Sugiura et al. (1995) did not specify how their experiments were conducted. Further work of Faust et al. (1995) indicated that the change in the state of water is incremental, i.e., that an increasing proportion of water changes from bound to free as chilling increases.

In our present study, if blueberry plants receive enough chilling to satisfy their CRs and if shoots are placed under forcing conditions (14-h photoperiod, 24 °C) before NMR analysis, the T2 times increase within 1 d of forcing, showing a shift towards freer water. Based on these results, it appears that water status does not change with CU accumulation, and that forcing is the main factor in freeing water after the CR is satisfied. Erez et al. (1998), in their study with peach, also suggested that forcing is the factor responsible for freeing water. Here, too, the NMR analyses were carried out with and without forcing. It appears then that NMR can be developed as a tool to distinguish endodormant buds from ecodormant buds. Indeed, T2 times of ecodormant buds increase after 1 d of forcing, whereas they do not change if buds are still endodormant. From our results with 'Gulfcoast', there is no change in water content within 1 d of forcing, therefore the increase in T2 that occurs after 1 d of forcing may be attributed to changes in the state of water (freer water) rather than to movement of water into the buds. However, when shoots are kept under forcing conditions for longer periods of time and buds start to show signs of budbreak, the greater proportion of water with longer T2s might be due partially to an uptake of water.

Among macromolecules able to bind water, dehydrin proteins,

due to their high hydrophilicity (Close et al., 1993), have been suggested as possible candidates (Erez et al., 1998; Faust et al., 1997). Although we know that dehydrins levels are extremely high in cold hardy blueberry flower buds (Muthalif and Rowland, 1994), the relationship of dehydrins to the state of water in buds of woody plants has not been fully explored. Some authors have raised the possibility that bound water might be correlated with the level of cold hardiness rather than with the level of endodormancy (Erez et al., 1998; Kaku et al., 1984). Whether dehydrins are involved in the binding of water warrants further investigation.

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