Plant Physiol. (1974) 54, 392–398

Nuclear Magnetic Resonance of Water in Cold Acclimating Red Osier Dogwood Stem[1]

Received for publication February 20, 1974 and in revised form April 19, 1974

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ABSTRACT

The pulsed and continuous-wave nuclear magnetic resonance of water in cold-acclimating red osier dogwood (*Cornus stolonifera* Michx) stem showed reduced relaxation times and increased line width. The reduction of relaxation times suggests an over-all restriction in the motional characteristics of the water. The increased line width is not related to a molecular property of the water, but is useful in estimating the initiation of cold acclimation. Biphasic relaxation characteristics may be related to partitioning of the water at the cellular level. The liquid water content of the stem was a weak function of temperature between −25 and −55 C, corresponding to approximately 0.15 gram of water per gram of dry stem. The quantity of unfrozen water at subfreezing temperatures was not strongly dependent on the degree of cold acclimation. It is concluded that the ability of dogwood to survive low temperatures depends on its ability to tolerate diminished quantities of liquid water.

Low temperature injury of plants is thought to result from intracellular ice formation or from freezing of extracellular water which may cause severe dehydration of the cells. Red osier dogwood (*Cornus stolonifera* Michx) has been studied previously and many details of the physiological alterations associated with acclimation to freezing stress and environmental factors which induce acclimation have been reported (10, 24). For example, changes in the quantities of water, lipids, carbohydrates, proteins, and nucleic acids usually occur (22, 34). Although water has not been studied extensively as part of this problem, the status of water almost certainly is a vital factor in determining the resistance of living organisms to freezing stress. Nuclear magnetic resonance provides a particularly convenient tool for the study of water in plants because it is nondestructive and rich in structural as well as dynamical information. NMR[2] is used in two ways in the present study: to study the state of the unfrozen water in dogwood stems at low temperatures and to determine the amount of unfrozen water at low temperatures.

NMR has been extensively used to study water in animal tissues such as muscle. In these samples, the NMR line is significantly broader than it is in pure water and the spin-spin relaxation time (T_2) is significantly shorter (2 to 6, 12, 13). These results are often interpreted to mean that cellular water consists of several populations of water molecule, including a bound fraction which experiences restricted motion. Some controversy has developed concerning the extent of the region of restricted water mobility and models range from a simplified two-phase model with a small fraction bound (2, 4, 5) to one consisting of all bound water (3, 6, 13). Regardless of the details of the interpretation of the NMR line shape, it is possible to measure the amount of water which is not solid because of the very large change in spectroscopic properties when water freezes. In particular, NMR line widths for solids are about 30,000 Hz or broader, line widths for ice fall in the 100,000 Hz range, while the NMR line widths for aqueous phases in tissues range from several Hz to about 8,000 Hz. Since these lines are narrow compared with the solid-state components of the spectrum, the narrow components are readily separated from the broad component. Although there are some problems associated with accurately integrating a very broad line, the integrated intensity of the observed NMR line in tissues or even frozen solutions is a reasonably accurate measure of the liquid content. Kuntz and co-workers (19–21) for example, have taken advantage of this property to study liquid water in frozen protein solutions. Toledo *et al.* (31), Sussman *et al.* (30), and Sussman and Chin (29) have used it to quantitatively measure the liquid water in frozen wheat-flour dough, and fish muscle. Several investigators have suggested that substantial fractions of liquid water in biological samples are not observable by NMR methods (6, 13–15). Subsequent investigations have not supported this hypothesis, although care must be exercised in the integration of the broad absorption lines which becomes more difficult as the width increases (1, 4, 7, 8, 11).

Other methods have been used to estimate liquid water content of plant tissues at various temperatures, but they are subject to considerable uncertainty. Calorimetric methods, for example,

[1] Scientific Journal Series Paper 8558 of the Minnesota Agricultural Experiment Station.

[2] Abbreviation: NMR: nuclear magnetic resonance.

depend on knowing the heat of fusion of cellular water, which is often assumed to be identical to the value for pure water (16–18, 33). This assumption is unwise because water which does not freeze at subfreezing temperatures is not pure water. The NMR evidence has suggested that some fraction of this water may be bound in some way to macromolecular structures. To freeze bound water, it is necessary to unbind it before freezing; therefore, the heat of fusion of the bound water will be the heat of fusion of pure water less the heat of binding. Since in many cellulose and protein systems, heats of binding may be as high as 300 cal/g, the heat of fusion for bound water can be negative, zero, or at least significantly different from that of pure water (28). There is therefore considerable uncertainty in calculations based on an assumed enthalpy of fusion for tissue water.

After a consideration of the various methods of measuring liquid water content, we conclude that NMR is at least as reliable as the other methods for determining the amount of liquid water in a tissue and has the advantage of relative speed and the general availability of equipment. The objective of this communication is to provide additional information on the state and freezing properties of water in plants acclimating to low temperature.

MATERIALS AND METHODS

Stem sections 0.1 to 3 cm long and up to 4 mm diameter were collected from the current season's growth of red osier dogwood (*Cornus stolonifera* Michx) plants of a single, vegetatively propagated, hardy clone native to Dickinson, N. D. Nonacclimated samples were obtained during the summer from plants growing in the field in St. Paul, Minn. and from plants growing under long days in a warm greenhouse at other times of the year. These nonacclimated, actively growing plants were characteristically killed at the moment of freezing (usually slightly below 0 C). Acclimated stem samples were obtained from plants in the field collected between December 15, 1972 and February 15, 1973. These stems from dormant plants were fully acclimated to below −196 C. Stem samples from plants which were acclimating to low temperature stress were obtained from plants in the field in the autumn or from plants acclimated under short days and low temperatures in a controlled environment chamber (10). For the most part these stems were from dormant plants which had just entered the initial phase of acclimation (34). Stems in this condition survived repeated freezing to −3 or −5 C, but were killed at relatively high temperatures (above −12 C). In the spring, stem samples were obtained from plants which were deacclimating. Partly deacclimated stems were also obtained from plants acclimated under short days and low temperatures in a controlled environment chamber and deacclimated under long days in a warm greenhouse. Excepting the fully acclimated plants, controlled freezing tests (24) were conducted at the time NMR measurements were made to determine hardiness. For samples used in freezing curve studies, the visual browning test (24) was used to estimate tissue injury. It was not possible to dissect stems into distinct tissues, but gross separations were made. The bark could be separated from the wood. The tissues of acclimated bark included living epidermis, cortex, and some phloem cells. Nonacclimated bark included all the phloem cells. The wood cylinder contained nonliving xylem cells and living xylem ray parenchyma cells. Pith cells in the center of the stem mature and die in the autumn.

The continuous-wave NMR measurements were made on a conventional Varian A60-D instrument operated at 60 MHz and on an XL-100 instrument operated at 100 MHz. Both spectrometers were equipped with standard variable temperature units. Sample temperature was monitored with a thermocouple which could be removed from the sample tube while spectra were recorded or with an alcohol thermometer which was imbedded in the sample. To assure good thermal contact between sample tube, sample, and thermocouple or thermometer, liquid perfluorobutyl-tetrahydrofuran (FC-80, Minnesota Mining and Manufacturing Company) was often added to the sample tube. Line widths were expressed as widths at one-half the maximum height of the absorption line. Complete freezing curves (plots of liquid water content *versus* temperature) were determined without moving the sample in the spectrometer. The amount of liquid water present at low temperature was calculated via equation 1.

$$L_T = L_{10}(A_T/A_{10})(T/283) \quad (1)$$

L_T is the liquid water content at temperature T(K), L_{10} is the total water content as determined by dry weight measurement in grams of water per gram dry weight, and A_T and A_{10} are the areas of the absorption lines at temperatures T and 283 K, respectively. The temperatures are included to make a first order correction to the change of spin population with temperature.

Samples were normally cooled in 10 C steps and time was allowed for the absorption line to reach an equilibrium (the longest time required was 3 hr when a sample was cooled from 0 to −10 C). The cooling and warming rates between 10° steps never exceeded 1° per minute. In some cases to guarantee tissue viability samples were cooled at −1 C/hr to −76 C. Samples held at this temperature were placed in a NMR spectrometer precooled to −76 C. Measurements were then made between −40 and −100 C. Samples were then slowly rewarmed (1 C/hr), incubated, and tested for viability (24).

Measurements of T_1 (the spin-lattice relaxation time) and T_2 (the spin-spin relaxation time) were made at 20 MHz on a Brucker M-20 pulsed spectrometer. T_1 values were determined after 180°, τ, 90° pulses from the slope of a semilog plot of $A_\infty - A_\tau$ *versus* τ (equation 2) (9).

$$\ln (A_\infty - A_\tau) = \ln 2A_\infty - \tau/T_1 \quad (2)$$

A_τ is the initial amplitude of the free induction decay following the 90° pulse which occurs at time τ after the 180° pulse. A_∞ is the limiting value of A_τ for a very long interval between the 180° and 90° pulses. T_2 values were determined by the Carr-Purcell-Meiboom-Gill method, from the slope of a semilog plot of A(echo at t) *versus* t (equation 3) (9).

$$\ln A(\text{echo at } t) = -t/T_2 - \gamma^2 G^2 D\tau^2 t/3 \quad (3)$$

Here A(echo at t) is the amplitude occurring at time t after the initial 90° pulse. The term including γ (magnetogyric ratio), G (spatial magnetic field gradient), D (diffusion coefficient), and τ (pulse separation) is negligible when τ is shorter than 2 msec. τ was generally chosen between 0.1 and 1 msec.

RESULTS AND DISCUSSION

A. CONTINUOUS AND PULSED NMR OF STEMS

Nonacclimated Stems Killed above −3 C. The NMR spectrum of nonacclimated stems in Figure 2 have a single continuous wave-absorption line and scanning 20,000 Hz either side of this resonance reveals no other lines using conventional high resolution methods. The line width of half maximum amplitude is always less than 20 Hz for stem sections collected from the first five apical internodes and it is usually between 1 and 10 Hz if the sample is spun. The line shape is neither Lorentzian nor Gaussian. Pulsed measurements of T_1 and T_2 made on the same sample are shown in Table I. The longer relaxation times T_2 (slow) and T_1 (slow) account for greater than 80% of the sample water, while the shorter relaxation times, T_2 (fast) and T_1 (fast) account for the remainder of the sample water. T_2 (slow) and T_2* calculated from equation 4 are in good agreement.

$$\Delta\nu_{1/2} = 1/\pi T_2^* \quad (4)$$

Table I. *Typical Pulsed and Continuous Wave NMR Results from a Nonacclimated Dogwood Stem which Was Killed at −3 C and an Acclimated Dogwood Stem which Was Killed at −196 C*

T_2* values are obtained from the NMR line widths as described in the text.

	$\Delta\nu_{1/2}$	T_2*	T_2[1]		T_1[1]	
			Fast	Slow	Fast	Slow
	Hz	*msec*				
Nonacclimated						
Apical internode	1.0[2]	320		240(100)		950(100)
Third internode	3.2[2]	106	10(20)	115 (80)	50(20)	520 (80)
Base internode	270	1.2	11(17)	97 (83)	50(13)	450 (87)
Acclimated						
Apical internode	250	1.3	4(37)	38 (63)	80(37)	370 (63)
Third internode	285	1.1	9(46)	44 (54)	100(36)	350 (64)
Base internode	265	1.2	5(35)	39 (65)	140(30)	400 (70)

[1] Numbers in parentheses refer to percentage of the fraction of water decaying at this relaxation time.

[2] Band width is measured with sample spinning. The band width was 5 Hz (apical internode) and 17 Hz (third internode) with the samples stationary.

Acclimated Stems not Killed at −196 C. Compared with nonacclimated stems, acclimated stems shown in Figure 1 have a broad and relatively complex continuous wave-absorption spectrum which is about 250 Hz wide when measured between 0 and 40 C at 60 MHz. The line width of acclimated stems is independent of the internode location of the stem sample and spinning generates significant changes in the spectrum. Relaxation times T_1 and T_2 for acclimated stems are shown in Table I. T_2 values for acclimated stems are substantially smaller than T_2 values obtained from nonacclimated stems and T_1 and T_2 relaxation is biphasic. The shorter relaxation times account for between 25 and 46% of the water, while the longer relaxation times account for the remainder. In the acclimated case, the T_2 values are considerably larger than T_2*, indicating that the magnetic field homogeneity within the sample is considerably lowered in the acclimated stem.

Cold-acclimating and Deacclimating Stems Killed between −3 and −18 C. For plants acclimating to low temperature in controlled environments, the transition from narrow to broad absorption line depends on the extent of hardiness and the part of the stem being examined. While we have not done a complete analysis correlating line width data with plant hardiness, some relationships were obvious. For example, line broadening at the first apical internode invariably occurred at hardiness level of between −10 and −18 C regardless of the source of the sample (field or controlled environment chamber). The same transition occurs at the second, third, fourth, and fifth internodes when the internodes were hardy to between −12 and −3 C. In studies of individual stems of current season growth, the line broadening proceeded from the proximal older internode tissues near the ground to the distal younger tissues nearer the apex. The change from broad to narrow absorption line usually occurred at a single node; however, sometimes an intermediate band width was observed in the transition. A similar, although less distinct, change occurred in T_2 (slow), which parallels the timing of the change in the absorption spectrum. The NMR spectra of stem samples taken from deacclimating plants in the spring were not related to the hardiness of the internode sample. Deacclimated stems had a broad absorption line regardless of the hardiness.

B. CONTINUOUS WAVE NMR OF STEM PARTS

As shown in Figure 2, the NMR spectra of stem parts differ significantly from the spectra of the whole stems; however, the NMR spectral differences are not reflected in the T_2 values for the same identical samples. Acclimated dogwood-bark cylinders and nonacclimated dogwood-bark cylinders when oriented perpendicular to the magnetic field have a broad absorption line consisting of two, or occasionally more, distinct maxima. The spectra shown in Figure 3, a and b, were made between 0 and 40 C at 60 MHz. The splitting between the two maxima is directly proportional to the magnetic field strength (350 Hz in a 14,092-gauss field and 600 Hz in a 24,500-gauss field). The general spectrum shape is not dependent on the stage of acclimation; however, as shown in Figure 3, c and d, the relative intensity of the two absorption maxima depends on the sample orientation in the magnetic field of the spectrometer. The over-all intensity of the absorption line, however, is independent of sample orientation.

For planar bark samples shown in Figure 3c only the upfield maximum is observed when the plane of the bark is parallel to the magnetic field and only the downfield maximum is observed when the plane of bark is perpendicular to the magnetic field. For cylindrical bark samples shown in Figure 3d, all absorption components are observed; however, only the upfield maximum is observed in cylinders with the cylinder axis parallel to the magnetic field (bark orientation parallel to the magnetic field). When observed alone, the single absorption lines are 60 ± 16 Hz wide, and the T_2* values (5.5 ± 1.5 msec) obtained from them are much smaller than the T_2 values obtained by pulsed methods which show that the major component T_2 (slow) for bark tissue is between 50 and 100 msec.

Acclimated stems with bark removed (wood) and whole acclimated stems have NMR spectra showing a sample orientation dependence similar to that observed with bark cylinders. In whole acclimated stems, as in bark cylinders, the separation between the two maxima is directly proportional to the magnetic field strength (250 Hz in a 14,092-gauss field and 440 Hz in a 24,500-gauss field). Therefore, the major difference between the whole acclimated stems and bark cylinders is that the separation between the two maxima is smaller in whole stems. The NMR spectra of nonacclimated stems and the wood cylinder from them have no detectable orientation dependence.

To summarize, there are two NMR absorption maxima for water in bark, acclimated wood, and acclimated whole stems, the relative maxima intensities are dependent on sample orientation, and the separation between the two maxima is dependent on the

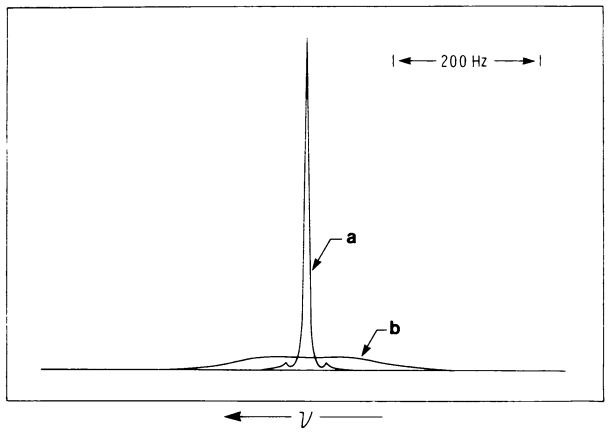

FIG. 1. NMR spectra at 60 MHz of dogwood stems oriented perpendicular to the external magnetic field. a: spectra from a nonacclimated stem with the sample spinning; b: spectra from an acclimated stem with the sample stationary. ν is the frequency in Hz. Spinning nonacclimated stems reduces the line width which is always less than 20 Hz wide with the sample stationary. Spinning acclimated stems does not change the line width but does change the line shape. This is dependent on the spinning rate.

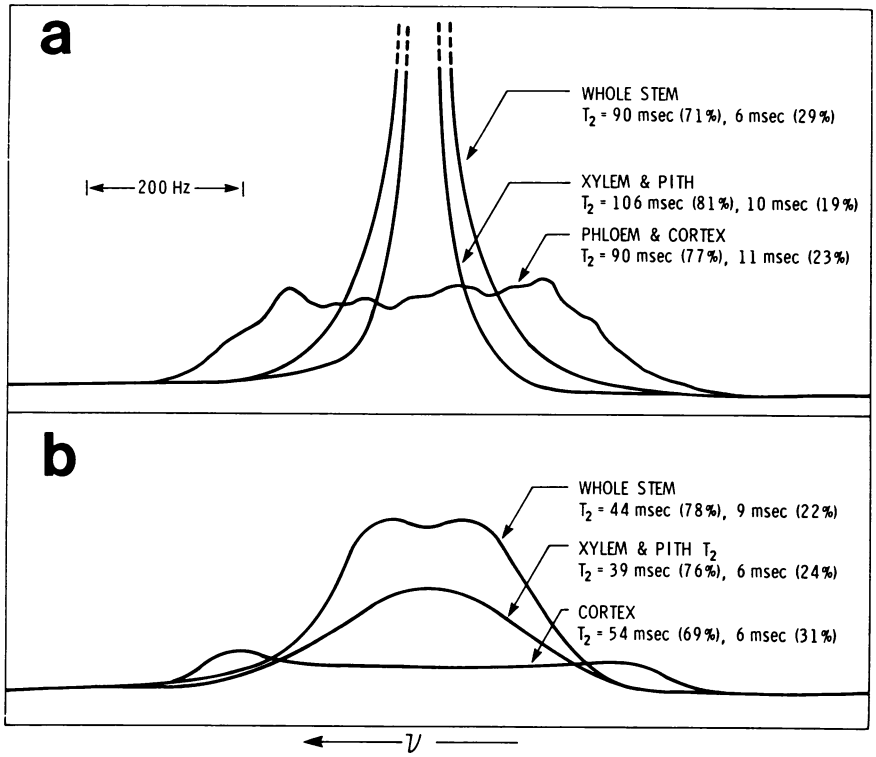

FIG. 2. NMR spectra at 60 MHz and spin-spin relaxation times (T_2) of whole dogwood stem and stem parts. a: spectra from a nonacclimated stem with the sample stationary; b: spectra from an acclimated stem with the sample stationary. All samples are cylindrical and are oriented perpendicular to the magnetic field. Two T_2 values are observed for each sample. The percentages in brackets following the relaxation times are the fractions of the total water decaying at the given relaxation rate.

external magnetic field strength. A common source of these effects is the variation of magnetic susceptibility within the sample, which has been discussed in detail by Zimmerman and Foster (36) for cylindrically symmetric samples. In the limit when the sample is a thin walled cylinder, as in Figure 3, perpendicular to the external magnetic field, the magnetic field H at any point in the sample will depend on the coordinate of that point θ according to equation 3 (36).

$$H = [1 - \tfrac{1}{2}(\mu_w - \mu_o) - \tfrac{1}{2}(\mu_w - \mu_o) \sin 2\theta]H_o \qquad (5)$$

H_o is the external magnetic field, μ_w and μ_o are the magnetic permeability of the sample and the sample surroundings, respectively, and θ is the angle between the applied field direction and the radius vector from the center of the cylinder to the point.

It can be seen from this relationship that the magnetic field within a sample will range between H_o and $(1 - (\mu_w - \mu_o))H_o$. Therefore the resonance intensity from a single resonance line H_r, the internal magnetic field strength necessary for resonance, will be spread and, in fact, will have two maxima, one each at H_r and $H_r/(1 - (\mu_w - \mu_o))$ (36). When a planar sample (i.e., a portion of a thin walled cylinder with infinite radius) is oriented parallel to the external field ($\theta = \pi/2$), resonance occurs at $H_r/(1 - (\mu_w - \mu_o))$ and when perpendicular to the external field ($\theta = 0$), resonance occurs at H_r. When the cylinder axis, shown in Figure 3e, is tilted away from the perpendicular position by an angle β the average magnetic field within the sample $\langle H \rangle_{avg}$ is given in equation 6.

$$\langle H \rangle_{avg} = [1 - \tfrac{1}{2}(\mu_w - \mu_o) - \tfrac{1}{2}(\mu_w - \mu_o) \sin^2 \beta]H_o \qquad (6)$$

It is clear from this relationship that when β approaches $\pi/2$, $\langle H \rangle_{avg}$ approaches the resonance frequency H_r. Such a model predicts precisely the observed results in Figure 3 for bark, acclimated stem, and wood tissues. In Figure 3, values of β and $(\mu_w - \mu_o)$ are given for each spectrum. The values of $(\mu_w - \mu_o)$ are chosen to account for the observed shifts.

These values are reasonable in sign and magnitude for a thin-walled cylinder of water, surrounded inside and out by organic materials with larger magnetic permeabilities. Most organic materials have larger magnetic permeabilities than water. Such a model suggests that the water is in the periphery of the samples. This model also predicts that the over-all resonance width is linearly dependent on H_o as is observed. These considerations suggest that the shape of the absorption spectra of bark, acclimated stems, and wood primarily reflect not a property of the water, but a geometric property of the sample. We have noticed such magnetic susceptibility effects in many woody tissues. This result, which in some circumstances is useful as noted in part A, does not enter direct measurements of relaxation times by pulsed NMR methods.

The spectra of whole stem sections do not appear to be the simple sum of the spectra of the stem parts in Figure 2. The absence of apparent simple additivity could suggest that the exchange of water between the bark and wood is rapid on the time scale of approximately 50 msec. Although exchange of water across the various portions of the sample may contribute to the apparent averaging of the spectrum, it is unlikely that this could explain all observed effects, because even pure liquid water does not diffuse rapidly enough to exchange between the bark and the wood in 50 msec. Other effects are therefore also important. Zimmerman and Foster (36) showed that the separation between the two maxima predicted by equation 5 depends on the difference in the permitivity of the two regions as well as the ratio of the inner to outer diameter of the observed region. The larger the annulus region, the smaller the splitting of the spectrum becomes. Therefore, adding dry or wet wood to the center of the bark cylinder may decrease the permitivity difference between the bark and the center and therefore decrease the splitting between the

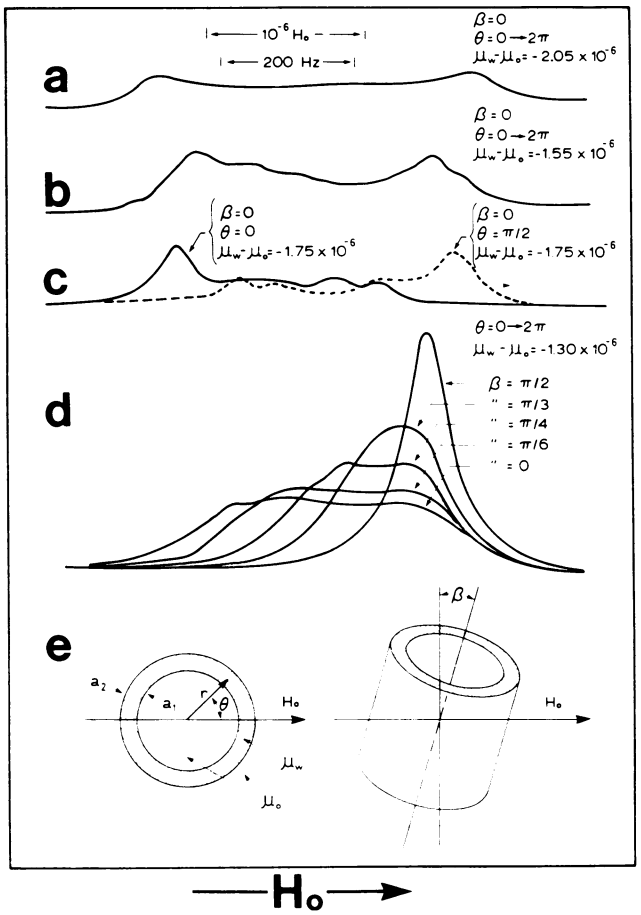

FIG. 3. NMR spectra at 60 MHz of dogwood bark. a: acclimated bark cylinder oriented perpendicular to magnetic field; b: nonacclimated bark cylinder oriented perpendicular to the magnetic field; c: a strip of nonacclimated bark oriented with its surface perpendicular (solid line) or parallel (broken line) to the magnetic field; d: nonacclimated bark cylinder having various orientations to the magnetic field; e: angles β and θ for a thin walled cylinder are defined. β is the angle in the y — z plane between the cylinder axis and the y axis. a_1 and a_2 are the internal and external radius, respectively. μ_o and μ_w are magnetic permeabilities. H_o is the external magnetic field direction.

observed maxima. This analysis suggests that the spectral changes associated with acclimation result in part from the loss of water from the central portion of the stem. This conclusion is consistent with the results of McKenzie *et al.* (24), who observed that the most obvious change during stem acclimation is loss of water from the wood. This analysis satisfactorily explains the observed spectral data without requiring significant changes in T_2.

C. LOW TEMPERATURE NMR OF STEMS

The intensity of the NMR signal in dogwood stem recorded by high resolution technqiues is shown in Figure 4. The intensity decreases as the temperature is lowered and eventually vanishes near −70 C, and a concomitant increase in the line width is observed. The integrated intensity A_T is related to the liquid water concentration and to temperature by equation 1. The liquid water concentrations, L_T of various cold-acclimating stem samples calculated by this means are plotted against temperature in Figure 5. As indicated earlier (24), the water concentration (ice and liquid) in dogwood stems depends on the stage of cold acclimation. The liquid water content in the temperature range between −15 and −30 C depended only on the tissue dry weight and it was independent of the stage of cold acclimation. There was less total water (liquid and ice) in cold-acclimated stems and because of this alone, the proportion of liquid water was higher in acclimated than nonacclimated stems at all subfreezing temperatures above −70 C. Figure 5 shows that the ability of dogwood to withstand low temperature is not related to maintenance of a substantial quantity of unfrozen water at low temperature. Indeed, these same NMR methods show that similar amounts of water remain unfrozen in paper down to −24 C (25) and −50 C (Burke, unpublished results), and proteins have more unfrozen water at these temperatures (19–21).

The integrated NMR intensity is shown in more detail for acclimated dogwood stem and cortex in Figure 6. Similar results were obtained with dead stems killed by plunging a sample at room temperature into liquid nitrogen. The liquid water content of the stem is a weak function of temperature between −25 and −55 C. However, beginning at −55 C a stronger temperature dependence is apparent which correlates with phase transitions reported by Rasmussen and MacKenzie (23, 26). The NMR line is not detected below −70 C by high resolution methods. The results obtained for acclimated bark show that there is less liquid water at low temperature compared with whole stem; however, the general behavior is similar to that in the whole stem. The approximate plateau in the freezing curve between −25 and −55 C may be taken as evidence for a bound water component corresponding to approximately 0.15 g water/g dry weight of stem. Although the quantity of liquid water is nearly constant over this temperature range, the line width at 100 MHz varies considerably from 560 Hz at −25 C to 3500 Hz at −55 C. Similar results have been reported for frozen protein solutions and in those systems the logarithm of the line width plotted against the reciprocal of the temperature gives a straight line (19). With stem samples, however, such Arrhenius plots deviate significantly from linearity. The deviation could be the result of a reduction in the liquid water component over the temperature range of the plot or could result

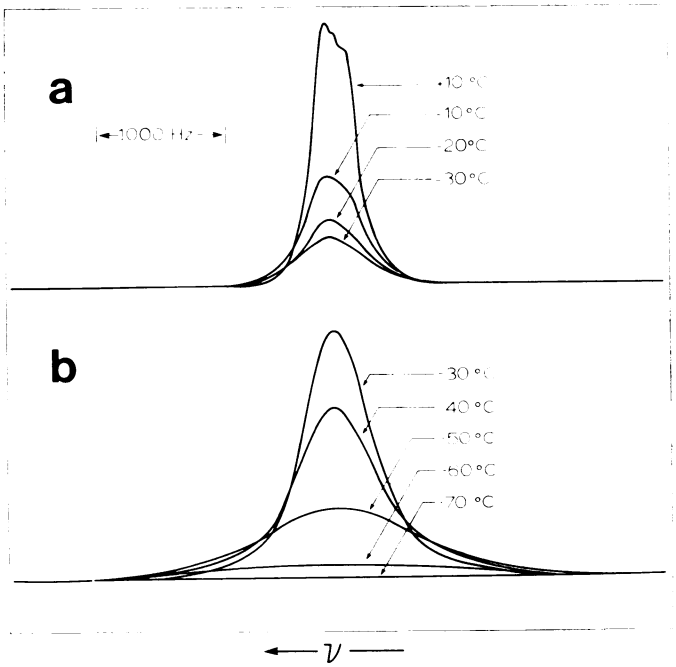

FIG. 4. NMR spectra at 100 MHz of an acclimated dogwood stem. a: spectra between +10 C and −30 C; b: spectra between −30 C and −70 C. In part b the radio frequency field strength was increased by approximately 13-fold which increased the sensitivity. The cylindrical sample was stationary and perpendicular to the external magnetic field.

from the existence of several activation enthalpies and entropies for unfrozen tissue water. The latter is supported by the earlier analysis of the hardy stem-line shape.

CONCLUSION

In the present study the NMR line shape is proportional to the sample geometry and is not related to the molecular properties of the water. This is shown by the analysis of the continuous wave spectra and the gross difference between the continuous wave-line width and the measured relaxation times.

Increased cold acclimation has been shown previously to result in lower water content in plants. This results in shorter values for T_2 and T_1. Shorter T_2 values in tissues with decreasing water contents are a general phenomenon in tissues and may be most easily explained by a model which includes at least two types of water in the sample. Such models have been proposed and discussed in detail elsewhere (35). One type of water is characterized by restricted motional characteristics and therefore has short T_2 values. This water has been called bound water. The other type of water has more normal characteristics and long T_2 values. If there is rapid chemical exchange of the water between these two environments, the qualitative changes in relaxation times observed in this study are predicted, provided there is a thermodynamic preference for the motionally less restricted component to be removed first.

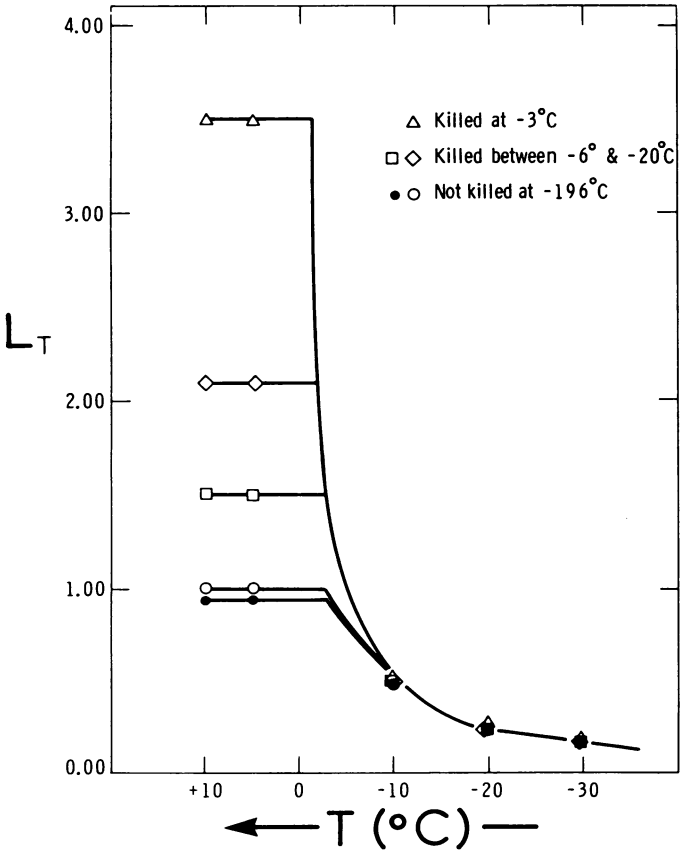

FIG. 5. Freezing curve of dogwood stems taken from plants different in hardiness. The data were obtained by integrating 1000 Hz of the continuous wave NMR spectra at 60 MHz. L_T is the liquid water content (equation 1) and is expressed in grams of liquid water per gram of dry sample. At and below −30 C the full band width sometimes exceeded 1000 Hz; therefore, the water content indicated at this temperature is an underestimate. At −20 C the full band width was always under 1000 Hz.

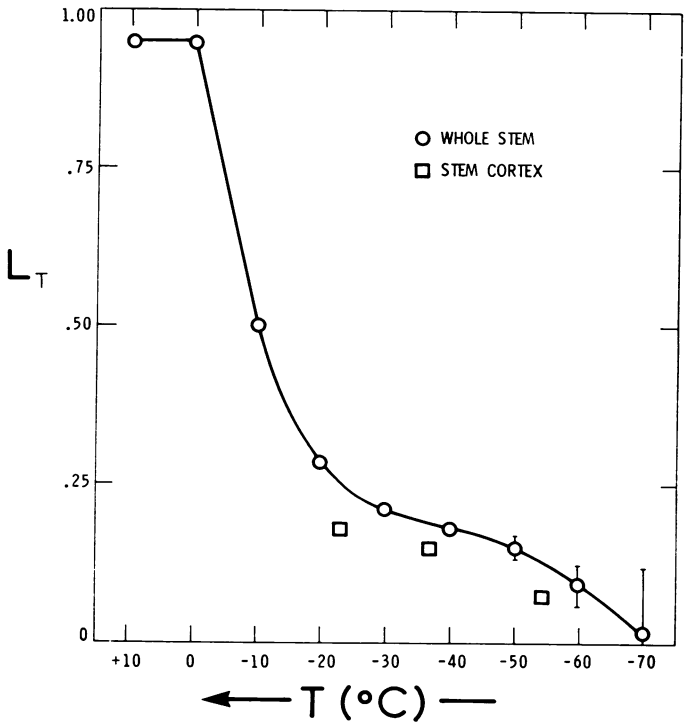

FIG. 6. Freezing curve of hardy dogwood stem and hardy dogwood bark. The data were obtained by integrating 40,000 Hz of the continuous wave NMR spectra at 100 MHz. L_T is defined in equation 1 and is expressed in grams of liquid water per gram of dry sample. The spectra used are those in Figure 4. The full band width never exceeded 8,000 Hz.

In the present experiments there is clear evidence for further partitioning of the water since both T_1 and T_2 are biphasic. The isolated tissues also had the biphasic relaxation characteristics, thereby reducing the possibility that the origin of the biphasic characteristics is two different stem tissues. The origin of biphasic behavior may be related to partitioning of water within the protoplasm (cytoplasm and vacuole) or between the protoplasm and extracellular spaces.

Cold injury in hardy plants is frequently attributed to dehydration of the protoplasm. This cellular dehydration is the result of the nucleation and growth of ice in the extracellular spaces at the expense of liquid water in the protoplasm. Various hypotheses have been suggested to explain the injurious result of the dehydration. Some of these suggest that a fraction of liquid water, sometimes called vital water, is associated with the living condition and death occurs when this fraction is withdrawn from the protoplasm or freezes (32, 34). Other approaches suggest that irreversible protein aggregation occurs as a result of close macromolecular contact in the condensed protoplasm (22). The present measurements make clear that the quantity of unfrozen water at subfreezing temperatures is not strongly dependent on the state, living or dead, or the degree of cold acclimation of the tissues, nor is there an ability of fully acclimated dogwood stems or stem cortical cells to maintain even as much liquid water as some frozen protein and cellulose solutions at subfreezing temperature. Therefore, it must be concluded that the ability of dogwood to survive low temperatures depends more on its ability to tolerate diminished quantities of liquid water and the resultant high protoplasmic concentrations. This idea is not new to the study of cold hardiness and it is not surprising in light of the major changes which occur in woody plant cells on cold acclimation, for example, protoplasmic augmentation (27).

It appears that NMR spectra may be useful for estimating the

time of initiation of acclimation to low temperature in the autumn. This could prove to be a useful tool in selecting early acclimating genotypes in a breeding program. Failure to acclimate early enough in the autumn leads to freezing in many fruit, ornamental, and forest species (34). Premature deacclimation in the spring is also a problem of practical significance in many woody crop species, but NMR, at least for dogwood, does not appear to be a good indicator for estimating hardiness loss.

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