Relationship of Electrical Conductance at Two Frequencies to Cold Injury and Acclimation in *Cornus stolonifera* Michx.¹

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ABSTRACT

The ratio of electrical conductance measured at two frequencies can be used to predict the cold hardiness of stem sections of *Cornus stolonifera* Michx. during the first stage of cold acclimation. Electrical conductance at 50 hertz divided by electrical conductance at 100 kilohertz gave a better estimate of hardiness than measurements at either frequency alone. The observed increase in the electrical conductance ratio as hardiness increased is consistent with an increase in membrane permeability. After plants were exposed to non-lethal frost, hardness increased rapidly, and the relation between the conductance ratio and hardness changed. This change indicates that ice crystalization induces a significant physiological alteration in the plants. Contrary to expectations, stem sections exposed to lethal temperatures could not consistently be separated from sections exposed to nonlethal temperatures by electrical conductance ratio measurements made immediately after thawing.

Rapid techniques are needed for evaluating freezing injury and cold acclimation in plants. Most methods presently in use involve controlled freezing of plant materials at preselected test temperatures. Subsequent injury evaluation may take days, as in the case of electrolyte diffusion or tetrazolium chloride techniques (12, 16), or even weeks in the case of regrowth tests (11). Such delays present problems for physiological studies of rapidly acclimating plants unless large amounts of materials are available for frequent testing (8, 13). Freezing curve techniques (11) conserve plant material and provide a reasonably rapid evaluation of viability after controlled freezing, but the length of time required to run individual samples precludes the screening of large populations in hardness breeding programs.

Electrical measurements can be rapidly made and offer a possible method for measuring injury and predicting plant hardiness. In 1931 Luyet made detailed observations of the variation in electrical resistance of plant tissues over a wide range of frequencies after the tissues had been injured by heat, cold, or lipid solvents (10). Luyet attributed the decrease in electrical resistance observed at low frequencies to changes in the "cell surfaces" and stated that "The decrease in resistance at low frequencies seems to correspond to the degree of this destruction." Greenham and Daday (7) noted that in herbaceous plants, "The general decrease in low frequency resistance with increasing injury is interpreted as being due mainly to increasing damage to the plasmalemma and associated decrease in its impedance." The relation of cell permeability to cold acclimation was discussed by Levitt (9). He stated, "During hardening, the permeability of the cell to polar substances and therefore to water was found to increase. Only plants that actually increased in frost resistance on exposure to hardening temperatures showed this change."

Apparently cell membranes and particularly the plasmalemma are involved in injury and acclimation to cold, and the structural integrity of the plasmalemma influences low frequency electrical measurements more than high frequency measurements.

The electrical measurements noted above were made on herbaceous plants. Electrical measurements have also been made on woody plants (3, 5, 15, 18–20). Most of these studies were done at a single low frequency and without temperature control. Measurements at low frequencies are influenced by membrane changes and also changes in temperature (6), electrolyte concentration (10), stem diameter (18), and probably other variables. With the exception of the membrane effects, the same variables also influence the high frequency measurements. Thus a ratio of a low frequency measurement and high frequency measurement should increase the sensitivity of electrical measurements to changes in the cellular membranes. Because electrical measurements at two frequencies can be made rapidly (less than 2 min per sample) and can minimize many of the variables associated with single frequency measurements, they present an attractive possibility for evaluating cold hardiness.

Electrical measurements were made on sections cut from stems of red-osier dogwood (*Cornus stolonifera* Michx.) during cold acclimation and before and after lethal freezing to determine the suitability of electrical measurements for assessing cold acclamation and freezing injury.

MATERIALS AND METHODS

Electrical conductance of stems was studied for two clones of red-osier dogwood propagated from single plants, one from Dickinson, North Dakota, the other from Seattle, Washington. These clones were selected because previous studies had shown they differed markedly in the timing of cold acclimation in the field in Minnesota even though both clones survive a temperature of −196°C when fully acclimated (14). Softwood cuttings of the two clones were rooted in a mist bench and then grown in a warm greenhouse at a long (17-hr) photoperiod for about 9 months followed by natural photoperiods in the greenhouse.
from August 20 to December 4. On December 4 the plants in 15-cm pots were transferred to a controlled environment chamber which supplied a mixture of fluorescent and incandescent light at a total energy level of 80 kergs cm\(^{-2}\) sec\(^{-1}\) at pot height as measured by a YSI Kettering model 65 radiometer. For the first 4 days the day and night temperatures were 20 and 15 C, respectively; for the next 7 weeks 15 and 5 C; and for the next 2 weeks 5 and 2 C. Beginning February 5 (the 9th week) the night temperature was lowered to -1, -2, -5, -7, -8, -10, and -10 C on successive nights. The photoperiod was 10 hr throughout the 10-week hardening cycle.

At weekly intervals a plant from each clone was sacrificed for measurements. Electrical measurements were made on stem sections 1 cm long cut from the internodal regions of the terminal 20 cm of a stem from each clone. Beginning at the basal end of these 20-cm samples, three to five sections, cut and numbered to identify the section as well as the basal end, were used for electrical measurements.

The remaining portions of the 20-cm stem samples were used for moisture determination. Moisture content was determined from weight measurements before and after drying at 80 C for 1 week.

All of the remaining portions of each plant were cut into sections approximately 3 cm long, and cold hardiness was determined by the method of Fuchigami (4). Groups of six stem sections, three selected at random from each clone, were frozen to a range of temperatures 2 C apart, selected to include the killing temperature of each clone. After freezing, the samples were thawed and incubated in high humidity for 1 week at 30 C and then visually scored for injury (4).

Measurements made on living tissues show that these tissues have frequency-dependent electrical properties. At any frequency two electrical networks can be determined, each with properties identical to those of the sample. One network is a resistor and capacitor in parallel. Even though the electrical properties of the two networks are identical with those of the sample, all resistors and capacitors have different values. In this paper a parallel combination of a resistor and a capacitor is used to represent the sample, and conductance, the reciprocal of the parallel resistance, is used to indicate that the resistor and capacitor are in parallel rather than in series.

Electrical conductance and capacitance of each stem section was measured by a Wheatstone bridge over a frequency range of 50 hertz to 100 kilohertz (Fig. 1). The sample to be measured was moistened at each end with 0.01 M NaCl. Then a No. 27 stainless steel hypodermic needle, cut to a length of 0.8 mm, was inserted into the pith at the apical end of the sample, and the basal end was placed in a 0.01 M NaCl solution. Electrical contact at the two cut ends of the sample was made through the hypodermic needle and a constantan electrode in the NaCl solution. A 0.01 M NaCl solution was used because it gave minimal variation with time in preliminary measurements of electrical conductance and capacitance. A diagram of the electrode configuration with a sample in place is shown in Figure 2.

Electrical measurements were made on the same sample with the use of Ag/AgCl and metal electrodes with 0.01 M NaCl to investigate the effect of electrode polarization. These data indicate that the primary effect of polarization with metal electrodes is confined to frequencies below 200 hertz. The electrical impedance measured with the metal electrodes is within 10% of that measured with Ag/AgCl electrodes at all frequencies considered. Apparently the effect of electrode polarization is small in comparison with the impedance of the sample. Thus, from a practical standpoint, the difficulty in maintaining a uniform AgCl coating on the electrodes during use and the small variation between the two types of electrodes seem to justify the use of metal electrodes for field measurements. All electrical measurements on samples from acclimating plants were made with metal electrodes.

To measure the electrical conductance and capacitance of the samples at each frequency, the bridge was nullled with the sample in parallel with the reference resistor (R\(_r\)) and capacitor (C\(_s\)) (Fig. 1). The sample was then switched out of the circuit (S\(_l\)) and the bridge was renullled, again using the reference resistor and capacitor. This process was repeated at each frequency. No shift in the null point was observed for generator voltages less than approximately 2 v. For the 0.8 v normally used the sample current was less than 24 \(\mu\)A.

The capacitance of a sample is the difference between the two readings of the reference capacitor, and the conductance is...
the difference between the reciprocals of the two readings of the reference resistor.

Measurements of known resistors and capacitors indicated an over-all accuracy of 2% for the conductance and 2% or two picofarads, whichever was larger, for the capacitance. To achieve the 2% accuracy, it was necessary to calibrate the bridge. Calibration values over the entire frequency range for both capacitance and conductance were the differences between the measured and actual values of known metal film and carbon resistors and silver mica capacitors. Capacitances of samples were measured but are not included in this report. The calibration values and the raw resistor readings for each sample provided the inputs for a computer program which calculated the conductance of the sample at 50 hertz and 100 kilohertz (2).

After the electrical measurements had been made on stem sections, they were subjected to controlled freezing stress along with the sections used for hardness determination. Following freezing, the sections were rewarmed to room temperature and the electrical measurements were repeated. These sections were incubated and evaluated for injury with the samples used to determine hardness.

RESULTS

At the start of the experiment on December 4, freezing tests indicated that the Dickinson clone had already acclimated to some extent. This was probably due to exposure to natural short days in the greenhouse. Previous studies have shown that the Dickinson clone begins cold acclimation earlier in the fall than the Seattle clone even though the photoperiods in their native environments are the same (14). Plants from both clones acclimated during the nonfreezing temperature of the cold acclimation regime (Fig. 3).

During the final week of the experiment, after exposure to frost, both clones showed a rapid increase in cold hardness. Frost apparently induced the second stage of cold acclimation, as has been previously reported (18).

Moisture content varied from 97 to 138% on a dry weight basis and was not related to the cold acclimation of red-osier dogwood.

Figure 4 illustrates the variable relationship between hardiness and the electrical conductance at 50 hertz. When conductance at 100 kilohertz was plotted against hardness, similar results were obtained. These results illustrate the poor predictive value of single frequency measurements of the type widely reported in the literature.

Figure 5 shows the relationship between the level of cold acclimation and the ratio of the equivalent parallel electrical conductance at 50 hertz to the conductance at 100 kilohertz. Previous studies have shown that electrical conductance of a sample becomes independent of the physiological condition of the sample at high frequencies and that the integrity of the membranes has the most effect on conductance at low frequencies (1, 10). Therefore, a ratio of conductance at low frequency to conductance at high frequencies should be dependent on membrane integrity but not on stem diameter, temperature, or moisture content. In this study 100 kilohertz was not high enough for conductance to be independent of frequency, but conductance was nearly constant. Measurements at higher frequencies would probably have increased the sensitivity of these tests, but the equipment capabilities were limiting. The linear regression line was calculated, excluding the points at $-4 \, ^\circ C$ and $-6 \, ^\circ C$ because supercooling is sufficient to protect the plant material at this level; and the points at $-28 \, ^\circ C$ and $-32 \, ^\circ C$. 

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** FIG. 3.** Changes in cold hardness during the experiment. S: Seattle, Wash. clone; D: Dickinson, N. D. clone. Each point is the mean of three samples from a single plant.

** FIG. 4.** Cold hardness as a function of the electrical conductance at 50 hertz. S: Seattle, Wash. clone; D: Dickinson, N. D. clone. Each point is the mean for three samples from a single stem.
ductance measurements have been performed for a Seattle clone. The ratio of conductance freezing is expressed as the percentage increase relative to the conductance ratio before freezing to minimize the effect of the variation in the conductance ratio with hardness. The mean percentage increase for lethal and nonlethal freezing was compared for the two clones using an unequal sample $t$ test. Table I shows that the differences in the mean are not significant at the 0.05 probability level for the Dickinson clone but are significant for the Seattle clone. If only samples hardy to $-9^\circ$C or more are considered for the Seattle clone, the $t$ test value is 1.64 ($df = 6$), which is not significant at the 0.05 level. Thus electrical conductance measurements immediately after freezing apparently have limited ability to distinguish between lethal and nonlethal freezing. Table I also shows the percentage increase in the conductance ratio for the unfrozen controls. The samples used as controls were randomly selected after the initial electrical measurements were made. While the control stem sections were not frozen, they were otherwise treated the same as the frozen samples. The $t$ test values for the unfrozen controls indicate a mean increase in the conductance ratio significantly different from zero at the 0.05 probability level. The values were 2.70 ($df = 8$) and 2.45 ($df = 8$) for the Dickinson and Seattle clones, respectively. Apparently the storing of cut stem sections results in an increase in the conductance ratio.

Visual inspection of samples after incubation for 1 week following electrical measurements revealed no injury produced by the electrical measurements. In fact, stem sections used for electrical measurements generally appeared to have slightly more hardness in controlled freezing tests than sections used only for hardness measurements. While this general observation was not carefully examined, the possibility of an electrical treatment increasing hardness in less than an hour is intriguing and deserving of further study.

**DISCUSSION AND CONCLUSIONS**

Previous electrical studies (3, 15, 17, 19, 20) have examined changes in conductance at a single frequency during acclimation of woody plants. In general these studies revealed that conductance was lowest when hardness was greatest. The results, however, have been complicated by several factors. For example, stem diameter (17) and temperature (6) are known to influence electrical measurements. The influence of these factors on electrical conductance is illustrated by the scatter of the points in Figure 4 in which cold hardness is plotted against electrical conductance for a single frequency (50 hertz).

The decrease in electrical conductance as hardness increases, though generally observed for the Seattle clone in this study (Fig. 4), is inconsistent with the idea that permeability increases with increased hardness (3, 17, 20). The increase in electrical conductance at a single frequency during acclimation of woody plants is illustrated by the scatter of the points in Figure 4 in which cold hardness is plotted against electrical conductance for a single frequency (50 hertz).

**Table I. Changes in Electrical Conductance Ratio after Exposure to Lethal or Nonlethal Freezing Temperatures**

<table>
<thead>
<tr>
<th>Time</th>
<th>Dickinson Clone</th>
<th>Seattle Clone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lowest survival temperature</td>
<td>Treatment</td>
</tr>
<tr>
<td></td>
<td>Unfrozen control</td>
<td>Non-lethal freezing</td>
</tr>
<tr>
<td>weeks</td>
<td>$C$</td>
<td>% increase in conductance ratio</td>
</tr>
<tr>
<td>0</td>
<td>-12</td>
<td>14</td>
</tr>
<tr>
<td>1</td>
<td>-14</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>-12</td>
<td>-2</td>
</tr>
<tr>
<td>3</td>
<td>-16</td>
<td>14</td>
</tr>
<tr>
<td>4</td>
<td>-14</td>
<td>9</td>
</tr>
<tr>
<td>5</td>
<td>-18</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>-18</td>
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</tr>
<tr>
<td>7</td>
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<td>0</td>
</tr>
<tr>
<td>8</td>
<td>-20</td>
<td>0</td>
</tr>
<tr>
<td>Average increase</td>
<td>5.4</td>
<td>11.8</td>
</tr>
</tbody>
</table>

1. $t$ value for lethal versus nonlethal freezing ($df = 11$): 0.255 NS.

2. $t$ value for lethal versus nonlethal freezing ($df = 11$): 2.271 (means are significantly different at the 0.05 level).
crease in the conductance ratio (Fig. 5) is consistent with an increase in permeability of the membranes as hardiness develops. The decrease in electrical conductance measured at a single frequency as hardness increases may reflect lignification of the cell walls, changes in moisture content, or changes in electrolyte concentration rather than a change in membrane permeability.

The work of Luyet (10) and de Plater and Greenham (1) with herbaceous plants indicated the possibility of combining conductance measurements at two frequencies to reduce the variability of data and facilitate the prediction of hardiness. By combining measurements at 50 hertz and 100 kilohertz, it was possible to predict the hardness without freezing the material (Fig. 5). The correlation coefficient for the data in Figure 5 is 0.86 with 95% confidence limits of 0.63 and 0.92. The calculated regression line plotted in Figure 5 was based on the assumption that the conductance ratio was measured without error. The standard deviation of the conductance ratio for a single sample is 0.023. Hardiness was estimated to be 2 C for this study, and the calculated standard error of estimate using the conductance ratio and hardness data of Figure 5 is 2.46 C.

After plants were exposed to nonlethal frost, the hardiness of both clones increased rapidly. This hardiness increase seemed to be associated with a significant physiological change which altered the conductance values and changed the relationship between the conductance ratio and cold hardness (Fig. 5). Freezing injury of the plants as a result of the low night temperatures does not explain the changed relationship. Visual inspection of randomly selected control stem sections not frozen but otherwise treated the same as the samples used for electrical measurements and determining cold hardiness revealed no injury at the time of electrical measurements and after 1 week of incubation. Furthermore, freezing injury should cause an increase in the conductance ratio rather than the observed decrease. Additional studies will be needed to determine whether conductance measurements can be used for predicting cold hardiness following exposure to nonlethal frosts. The prediction of hardiness over the whole range of acclimation with a single regression line would have been convenient. Ultimately, however, the implication that frost does induce a substantial change in the plant which is associated with increasing hardiness may prove to be significant in attempts to resolve the nature of acclimation.

On the basis of other studies, it was anticipated that electrical measurements could also provide a basis for rapidly determining whether plants had been injured by freezing. In conifers injured by frost there was an immediate decrease in electrical resistance which was roughly proportional to the amount of injury (5). Studies on herbaceous plant tissues have indicated a similar rapid decrease in electrical resistance after freezing injury (1, 10). Other widely used viability tests based upon the integrity of the cellular membranes, such as electrolyte diffusion, indicate a loss of selective permeability after freezing injury (12, 16, 19).

Therefore, it would seem that conductance measurements before and after controlled freezing could be used to determine the killing temperature and to evaluate injury without the delays associated with other techniques.

Increases in the electrical conductance ratio after stem sections were frozen did not allow separation of lethal and nonlethal freezing in the Dickinson clone (Table 1). Previous studies have demonstrated a relationship between changes in the electrical properties and injury for herbaceous plants or woody plants of limited hardiness (to about —6 C) (1, 5, 10). The increase in the electrical conductance ratio did separate lethal and nonlethal freezing in the less hardy Seattle clone. Because freezing injury did not cause an increase in the conductance ratio over that observed for noninjured plants of the Dickinson clone, we hypothesize that the primary site of freezing injury changes as woody plants become hardy. The increases in electrical conductance following freezing stress in hardy plants reported by others (17, 19, 20) may be due to a general deterioration of cells and membranes during the interval between stress and electrical measurement.

If one accepts that membrane destruction would cause a sudden increase in conductance, it seems likely that freezing injury in tender tissue is due to irreversible membrane destruction, but that the plasmalemma is not the primary site of injury in acclimated woody tissues.

LITERATURE CITED