Plant Tissue Impedance and Cold Acclimation: A Re-analysis

M. I. N. ZHANG¹,³, D. G. STOUT² and J. H. M. WILLISON¹

¹ Department of Biology, Dalhousie University, Halifax, N.S. Canada B3H 4J1
² Agriculture Canada, Range Research Station, Kamloops, B.C. Canada V2B 8A9

Received 22 July 1991; Accepted 4 September 1991

ABSTRACT

A new five-element electrical model was proposed recently (Zhang, Stout, and Willison, 1990; Zhang and Willison, 1991) to represent plant tissues. In previous studies on the relationship between electrical impedance and cold-hardiness, one of us had analysed data in relation to a simpler three-element electrical model. Here, we have re-analysed these data in relation to the more complex model. F-tests showed that the new model always fitted measured impedance spectra significantly better (P < 0.005) than the earlier model. The previously reported increase in intracellular resistance during cold acclimation was found to be related to increased resistance of both the cytoplasm and vacuole.

In the species trial of birdsfoot trefoil and alfalfa, cold acclimation was accompanied by an increase in extracellular resistance and a decrease in capacitances of both the plasma membrane and tonoplast. In the cultivar trial of birdsfoot trefoil, cold acclimation did not affect plasma membrane capacitance in Viking and extracellular resistance in both Leo and Viking. In the species and growth time trial, cold acclimation was accompanied by a decrease in plasma membrane capacitance in alfalfa but not in birdsfoot trefoil.

Key words: Electrical impedance, cold acclimation, equivalent electrical circuit.

INTRODUCTION

By measuring electrical impedance at two or more alternating-current frequencies, electrical parameters of plant tissues may be estimated without serious injury to the tissue. Using this method, measurements of extracellular resistance, intracellular resistance and membrane capacitance have been made (Hayden, Moyse, Calder, Crawford, and Fensom, 1969; Stout, Hall, and McLaughlin, 1987) and the approach has provided some useful insights into the physiology of cold acclimation and freeze-thaw stress (Hayden et al., 1969; Hayden, Dionne, and Fensom, 1972; Stout et al., 1987; Stout, 1988).

An equivalent electrical model whose components can be related to specific plant structures is required for useful analysis of impedance data. In the past, the model of choice has usually been a three-element model that was developed by Hayden and co-workers (1969), see model A (Fig. 1A). Recently, Zhang and co-workers (Zhang, Stout, and Willison, 1990; Zhang and Willison, 1991) showed that this Hayden model was inadequate because of differences between measured impedance spectra and theoretical impedance spectra. The inadequacy of the Hayden model was also demonstrated by results obtained by Stout and co-workers (see Fig. 11 in Stout et al., 1987).

Zhang and co-workers (1990) proposed the use of a five-element electrical model (see model B, Fig. 1B) for plant tissue which included elements representing the vacuole that were missing in the Hayden model. In electrical model B, C₅ and R₄ represent the vacuole. The advantage of using model B for analysing impedance data is that protoplasm (R₂ in model A) is subdivided into cytoplasm (R₂ in model B) and vacuole (R₄ in model B). Similarly, membranes (C in model A) are subdivided into plasma membrane (C₃) and tonoplast (C₅) in model B. Recently, Zhang and Willison (1991) have shown that the 5-element 'double shell' model fits impedance data for several plant tissues better than the simpler 3-element model. F-testing showed that the improved fitness is not due simply to the addition of more degrees of freedom, but to better modelling (Zhang and Willison, 1991).

Here, we have re-analysed previously reported imped-
ance data from the stems of *Lotus corniculatus* and *Medicago sativa* at various levels of cold-hardiness acclimation (Stout et al., 1987; Stout, 1988) in order to test whether or not the measured impedance spectra are better represented by the new 5-element model. We also consider the implications of the new results to the physiology of cold acclimation.

**MATERIALS AND METHODS**

The procedures used to measure total impedance and to eliminate electrode impedance have been described previously (Stout et al., 1987; Stout, 1988). Resistance and reactance were measured at 14 frequencies ranging from 49 to 1110000 Hz. The procedure for simultaneous fitting of theoretical spectra to both the measured resistance and reactance was based on the Marquardt algorithm (Grant, Sheppard, and South, 1978; Tsai and Whitmore, 1982; Zhang and Willison, 1991). An *F*-test which was used to compare the relative fit of the two models followed the procedure of Grant et al. (1978) (also see Zhang and Willison, 1991).

In a cultivar trial, stem electrical impedance was measured for birdsfoot trefoil (*Lotus corniculatus* L.) plants which were either non-acclimated or cold-acclimated for 6 weeks (Stout et al., 1987). In a species trial, stem impedance was measured for birdsfoot trefoil and alfalfa (*Medicago sativa* L.) plants following either 0, 16, 35, or 44 d of cold acclimation (Stout, 1988). In both trials, 3 replicates per treatment were used. Data were subjected to analysis of variance using ‘Proc GLM’ of SAS (SAS Institute Inc., 1985).

**RESULTS**

*A comparison of the two models*

Examples of the best-fit theoretical spectra for models A and B (Fig. 1) to measured impedance spectra are shown in Figs 2A and 2B, respectively. The best-fit values for the parameters of each of the components of the two models, for each of 8 stems, are listed in Table 1. Also given in the same table are *H*-values (for the calculation of *H* values, see Zhang and Willison, 1991) used for *F*-tests of the quality of fit between the two models and the measured data. In each case, model B fitted the measured data significantly (*P* < 0.005) better than model A.

**Application of model B to the analysis of impedance data in relation to cold acclimation**

I. The effect of cultivar and cold acclimation on electrical properties of birdsfoot trefoil stems. (Data source: the original data for Table 3 in Stout et al., 1987.)

Cold acclimation did not significantly affect *R*\(_1\) at the *P* < 0.05 level, but it increased *R*\(_2\) (significant at *P* < 0.05),
and R₄ (significant at *P* < 0.01) of the two birdsfoot trefoil cultivars (Table 2). Both C₁ and C₃ in Leo were reduced by cold acclimation, but neither C₁ nor C₃ in Viking were affected by cold acclimation.

II. The effect of forage species and cold acclimation on electrical properties of plant stems. (Data source: the original data for Fig. 7 in Stout, 1988.)

Cold acclimation increased R₁ (*P* < 0.01) and R₄ (*P* < 0.05), and decreased C₁ (*P* < 0.01) and C₃ (*P* < 0.05) in both alfalfa and birdsfoot trefoil (Table 3). Although cold acclimation increased R₁ of both species, it increased R₂ in birdsfoot trefoil more than in alfalfa.

III. The effect of forage species and growth time on electrical properties of plant stems. (Data source: the original data for Fig. 7 in Stout, 1988.)

Birdsfoot trefoil stems had a smaller R₁ (*P* < 0.05) and R₂ (*P* < 0.01), and a larger C₃ (*P* < 0.05) than those of alfalfa (Table 4). The two species also differed in C₃, but this difference depended on growth time. For alfalfa, C₃ decreased with growth time; for birdsfoot trefoil, C₃ varied with time but showed no consistent trend.

### DISCUSSION

Similar to the findings of a previous study using potato tubers and carrot roots (Zhang and Willison, 1991), model B always fitted better than model A (*P* < 0.005) for all the impedance data used in this re-analysis. By contrast with the results of Zhang and Willison (1991), however, even the best-fit model, B, did not fit the present impedance data very satisfactorily (see the difference between the theoretical curve and the experimental data in Fig. 2b). This unsatisfactory fit of model B to the impedance data probably results from the heterogeneous nature of the stem tissues used in this study. Unlike the relatively homogeneous potato tuber or carrot root cortical tissues,
properties of plant stems

TABLE 4. Effect of forage species and growth time on electrical properties of plant stems

Original data, same source as for Fig. 7 in Stout (1988).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Acclimation time (d)</th>
<th>Birdsfoot trefoil</th>
<th>Alfalfa</th>
<th>s.e. (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1 (ohm m)</td>
<td>All*</td>
<td>13-63</td>
<td>16-70</td>
<td>0.86(21)</td>
</tr>
<tr>
<td>R2 (ohm m)</td>
<td>All</td>
<td>9-34</td>
<td>14-49</td>
<td>0.66(21)</td>
</tr>
<tr>
<td>R4 (ohm m)</td>
<td>All</td>
<td>2-85</td>
<td>4-65</td>
<td>0.71(21)</td>
</tr>
<tr>
<td>C3 (μF m⁻¹⁻)</td>
<td>0</td>
<td>17-66</td>
<td>34-10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>26-00</td>
<td>10-58</td>
<td>5-18(3)</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>11-65</td>
<td>14-50</td>
<td>3-67(6)</td>
</tr>
<tr>
<td></td>
<td>44</td>
<td>17-86</td>
<td>14-98</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>18-38</td>
<td>16-32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C4 (μF m⁻¹⁻)</td>
<td>All</td>
<td>1-94</td>
<td>1-04</td>
<td>0.31(21)</td>
</tr>
</tbody>
</table>

Values are means. n=3 for 0 time and n=6 for all other times. Means for all have n=21.

* Data from all acclimation times were combined when the analysis of variance indicated no significant acclimation time by species interaction.

The effect of cold acclimation on R1 was inconsistent between trials: R1 increased in the species trial but decreased insignificantly during the cultivar trial. In the original analysis, R1 was estimated by using the resistance measured at the lowest frequency used, 49 Hz, but no effect of cold acclimation was detected. It therefore remains unclear whether R1 responds to cold acclimation (see R2 in Tables 2 and 3) and better data will be required if this question is to be answered.

To conclude, re-analysis of previously reported data using an improved electrical model indicates that the previously reported increase in intracellular resistance during cold acclimation was related to increased resistance of both the cytoplasm and vacuole. It is suggested that a decrease in the capacitance of both the plasma membrane and the tonoplast during cold acclimation indicates either or both, a reduction in cell size and an increase in the proportion of xylem.

LITERATURE CITED


