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Plant Tissue Impedance and Cold Acclimation: A Re-analysis

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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 birdsfood 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, C_5 and R_4 represent the vacuole. The advantage of using model B for analysing impedance data is that protoplasm (R_2 in model A) is subdivided into cytoplasm (R_2 in model B) and vacuole (R_4 in model B). Similarly, membranes (C in model A) are subdivided into plasma membrane (C_3) and tonoplast (C_5) 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-

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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 1 110 000 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

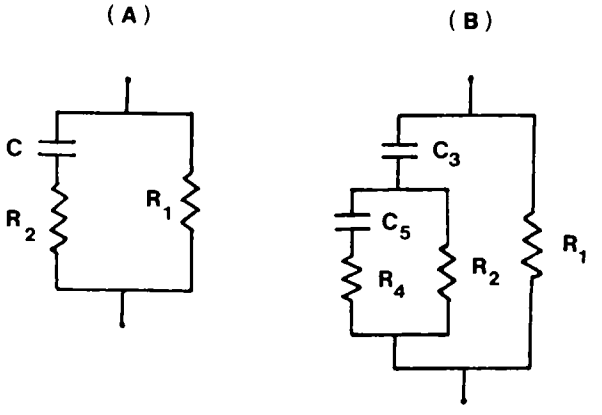

FIG. 1. (A) Model A. Note that the membrane resistance (R_3) in the original model from which this is derived is omitted here due to its very high resistance (Hayden *et al.*, 1969). R_1, the extracellular resistance; R_2, the intracellular resistance; C, the membrane capacitance. (B) Model B, the five-element model (Zhang *et al.*, 1990; Zhang and Willison, 1991). R_1, the extracellular resistance; R_2, the cytoplasmic resistance; R_4, the resistance of vacuole interior; C_3, plasma membrane capacitance; and C_5, the tonoplast capacitance.

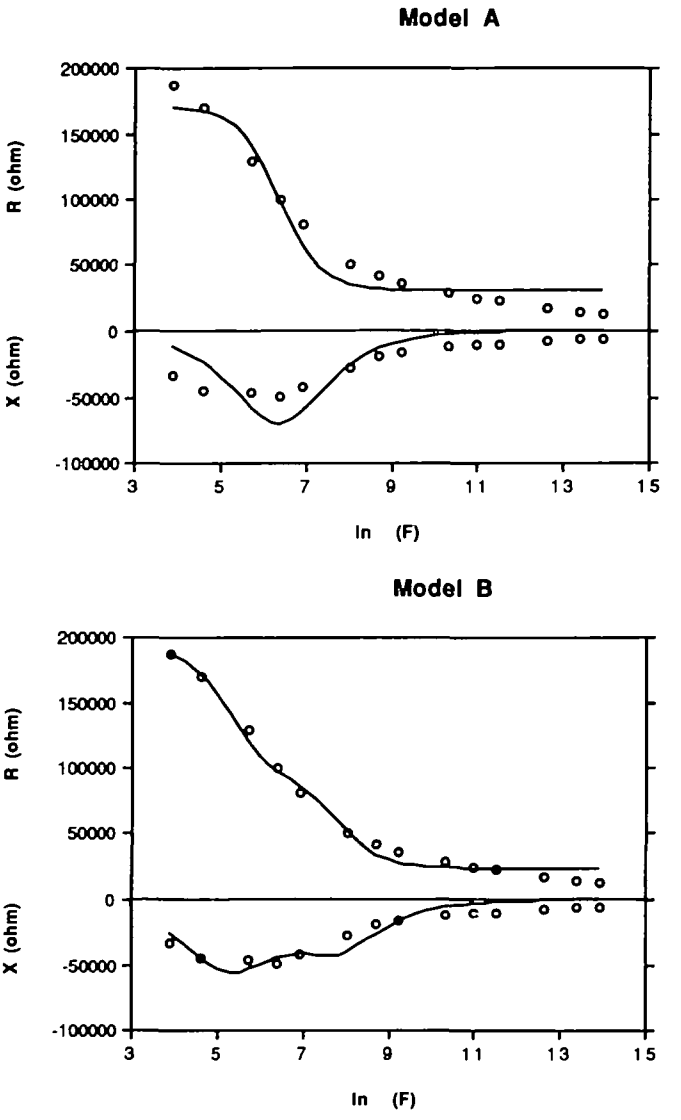

FIG. 2. An example of an impedance spectrum measured in birdsfoot trefoil and the best-fit theoretical spectra for models A and B. The data points are measured values and the continuous curves show the best-fit theoretical values. R—resistance, X—reactance, ln (F)—ln (frequency). (A) Model A. The best-fit parameter values are shown in Table 1, sample 3. (B) Model B. The best-fit parameter values are shown in Table 1, sample 3.

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 $P < 0.05$),

TABLE 1. *F-tests of the fitness of the 'best fit' for a theoretical model (A or B) to the measured impedance spectra (resistance and reactance) in birdsfoot trefoil*

Resistors (R) and capacitors (C) in the models are evident in Fig. 1. Original data, Stout *et al.* (1987). All resistors are in kilo-ohm and all capacitors are in nF.

Sample #	Model A	Model B	H[a]
1	$R_1 = 181$	$R_1 = 199$	
(ACCL[b] Leo)	$R_2 = 77{\cdot}8$	$R_2 = 224$	
	$C = 0{\cdot}155$	$R_4 = 52{\cdot}1$	
		$C_3 = 0{\cdot}401$	33·88
		$C_5 = 0{\cdot}027$	
2	$R_1 = 193$	$R_1 = 206$	
(ACCL Leo)	$R_2 = 80{\cdot}7$	$R_2 = 161$	
	$C = 0{\cdot}733$	$R_4 = 51{\cdot}8$	
		$C_3 = 1{\cdot}01$	38·65
		$C_5 = 0{\cdot}033$	
3	$R_1 = 178$	$R_1 = 193$	
(NA[c] Leo)	$R_2 = 36{\cdot}2$	$R_2 = 141$	
	$C = 1{\cdot}32$	$R_4 = 33{\cdot}4$	
		$C_3 = 2{\cdot}38$	15·49
		$C_5 = 0{\cdot}059$	
4	$R_1 = 134$	$R_1 = 143$	
(NA Leo)	$R_2 = 32{\cdot}6$	$R_2 = 64{\cdot}0$	
	$C = 2{\cdot}67$	$R_4 = 23{\cdot}1$	
		$C_3 = 3{\cdot}28$	13·56
		$C_5 = 0{\cdot}162$	
5	$R_1 = 163$	$R_1 = 172$	
(ACCL Vik[d])	$R_2 = 82{\cdot}0$	$R_2 = 142$	
	$C = 0{\cdot}746$	$R_4 = 52{\cdot}3$	
		$C_3 = 0{\cdot}967$	28·72
		$C_5 = 0{\cdot}020$	
6	$R_1 = 133$	$R_1 = 142$	
(ACCL Vik)	$R_2 = 61{\cdot}8$	$R_2 = 119$	
	$C = 0{\cdot}790$	$R_4 = 37{\cdot}9$	
		$C_3 = 1{\cdot}14$	25·18
		$C_5 = 0{\cdot}031$	
7	$R_1 = 112$	$R_1 = 118$	
(NA Vik)	$R_2 = 29{\cdot}9$	$R_2 = 63{\cdot}6$	
	$C = 1{\cdot}075$	$R_4 = 18{\cdot}1$	
		$C_3 = 1{\cdot}32$	22·27
		$C_5 = 0{\cdot}088$	
8	$R_1 = 141$	$R_1 = 146$	
(NA Vik)	$R_2 = 30{\cdot}3$	$R_2 = 64{\cdot}0$	
	$C = 0{\cdot}719$	$R_4 = 16{\cdot}5$	
		$C_3 = 0{\cdot}847$	17·06
		$C_5 = 0{\cdot}075$	

[a] The *F*-value, with degree of freedom (2, 11), at $P = 0{\cdot}005$, is 8·91 (Ott, 1988), thus model B is always a significantly better fit than model A because *H* is always bigger than *F*.
[b] ACCL—acclimated.
[c] NA—non-acclimated.
[d] Vik—Viking.

TABLE 2. *Effect of cultivar and cold acclimation on electrical properties of birdsfoot trefoil stems*

Original data, same source as Table 3 in Stout *et al.* (1987).

Cultivar	Acclimation	Resistance (ohm m)			Capacitance (μF m^{-1})	
		R_1	R_2	R_4	C_3	C_5
Leo	No	15·2	9·9	0·85	33·7	3·7
	Yes	14·4	14·9	1·15	10·7	0·5
Viking	No	14·8	8·2	0·72	10·6	0·9
	Yes	13·6	12·4	1·32	13·0	0·3
s.e. ($n = 3$)		2·2	1·8	0·11	3·6	0·5

Values are means for $n = 3$.

TABLE 3. *Effect of forage species and cold acclimation on electrical properties of plant stems*

Original data is data source for Fig. 8 in Stout (1988).

Parameter	Species	Acclimation		s.e. (*n*)
		No	Yes	
R_1 (ohm m)	Both[a]	13·74	17·05	0·93(18)
R_2 (ohm m)	Alfalfa	12·68	16·90	1·05(9)
	Birdsfoot trefoil	6·23	13·48	1·05(9)
R_4 (ohm m)	Both	3·12	4·58	0·76(18)
C_3 (μF m^{-1})	Both	21·79	11·42	2·05(18)
C_5 (μF m^{-1})	Both	2·10	0·68	0·33(18)

Values are means for $n = 9$ for cold acclimated and $n = 12$ for non-acclimated; when both species are combined, *n* is doubled.
[a] Data from both species were combined when the analysis of variance indicated no significant species by acclimation interaction.

and R_4 (significant at $P < 0{\cdot}01$) of the two birdsfoot trefoil cultivars (Table 2). Both C_3 and C_5 in Leo were reduced by cold acclimation, but neither C_3 nor C_5 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 R_1 ($P < 0{\cdot}01$) and R_4 ($P < 0{\cdot}05$), and decreased C_3 ($P < 0{\cdot}01$) and C_5 ($P < 0{\cdot}05$) in both alfalfa and birdsfoot trefoil (Table 3). Although cold acclimation increased R_2 of both species, it increased R_2 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 R_1 ($P < 0{\cdot}05$) and R_2 ($P < 0{\cdot}01$), and a larger C_5 ($P < 0{\cdot}05$) than those of alfalfa (Table 4). The two species also differed in C_3, but this difference depended on growth time. For alfalfa, C_3 decreased with growth time; for birdsfoot trefoil, C_3 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 ($P < 0{\cdot}005$) 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,

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).

Parameter	Acclimation time (d)	Birdsfoot trefoil	Alfalfa	s.e. (*n*)
R_1 (ohm m)	All[a]	13·63	16·70	0·86(21)
R_2 (ohm m)	All	9·34	14·49	0·66(21)
R_4 (ohm m)	All	2·85	4·65	0·71(21)
C_3 (μF m^{-1})	0	17·66	34·10	
	16	26·00	10·58	5·18(3)
	35	11·65	14·50	3·67(6)
	44	17·86	14·98	
	Mean	18·38	16·32	
C_5 (μF m^{-1})	All	1·94	1·04	0·31(21)

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

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

stems are differentiated and composed of cells of many different sizes.

Since model B provides more information with greater reliability than model A (Fig. 2; Table 1), the interpretation of the impedance data needs to be re-examined. The results of the birdsfoot trefoil cultivar trial (Table 2) and the legume species trial (Table 3) are quite consistent with each other. In both trials, cold acclimation increased R_2 and R_4. The earlier analysis concluded that intracellular resistance was increased by cold acclimation (Stout *et al.*, 1987; Stout, 1988), but this conclusion was largely based on the use of high frequency impedance, 1·11 MHz, rather than on estimates derived from model fitting. The present re-analysis by model fitting shows that the increase in intracellular resistance was a result of increases in both cytoplasmic (R_2) and vacuolar (R_4) resistance. The increased resistance of cytoplasm and vacuole could be a result of either decreased ion concentration or increased viscosity due to increased sugar concentration. As earlier studies have indicated that sugar content is increased during cold acclimation (Levitt, 1980), the increased resistances are more likely to reflect an increase in viscosity in both cytoplasm and vacuole during acclimation.

Lotus and *Medicago* stem tissue capacitances were not derived in the earlier analysis but have been derived in the re-analysis. In both trials C_3 and C_5 decreased during cold acclimation, which may indicate a general reduction in cell size. As more cells are aligned in a series, the total capacitance of these cells will be proportionally smaller (see Zhang and Willison, 1991 for detail). It is also possible that decreases in C_3 and C_5 indicate an increase in the proportion of xylem, because xylem is more resistant and less reactive than other tissues (Glerum and Krenciglowa, 1970).

The effect of cold acclimation on R_1 was inconsistent between trials: R_1 increased in the species trial but decreased insignificantly during the cultivar trial. In the original analysis, R_1 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 R_1 responds to cold acclimation (see R_1 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.

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