ELECTRICAL IMPEDANCE RATIO TECHNIQUE FOR RAPID ASSESSMENT OF FROST DAMAGE IN PINUS RADIATA

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(Received for publication 23 December 1982; revision 31 March 1983)

ABSTRACT

A simple, low-frequency, impedance technique has been applied to frost damage evaluation in seedlings of **Pinus radiata** D. Don. Stem resistive impedance at 100 Hz was measured before and after a frost to calculate a pre- to post-frost impedance ratio (Z_R). By demonstrating a relationship between Z_R and the proportion of seedlings surviving a frost (with no more than 30% needle damage) a relationship between Z_R and expected survival was derived.

This relationship was used to predict the number of seedlings lightly or seriously damaged in each of two separate populations of seedlings subjected to a range of frosts. Frost damage, with up to one seedling in error out of each batch of six in one population and nine in the other, was correctly assessed in 78% and 90%, respectively, of the frosts of those populations. In frosts where more than 10% of the seedlings were incorrectly classified the impedance ratio tended to overpredict survival. Seedlings with a $Z_R < 0.9$ had a 100% chance of surviving, and this screening method could provide for rapid selection of material where no seedling loss is acceptable.

INTRODUCTION

Techniques to determine the extent of frost damage to seedlings of *Pinus radiata* in artificial freezing tests have been described by Menzies (1977) and Green & Warrington (1978). Menzies' technique was based on a visual evaluation of bud and needle damage 1 month after the frost; field tests have demonstrated that seedlings will succumb to subsequent frosts if more than 30% of the needles are damaged (Menzies & Chavasse 1982). Therefore this technique has been adopted for use in frost studies on *P. radiata* (Menzies *et al.* 1981; Warrington & Jackson 1981; Greer & Warrington 1982). The major disadvantage of the technique is the time lapse between the frost and subsequent damage assessment. A faster evaluation of frost-induced damage is frequently needed and desirable.

Green & Warrington (1978) have described a relatively rapid (72-h) diffusate electroconductivity technique that has been successfully used to assess the extent of frost damage in *P. radiata.* However, it is destructive, labour intensive, and requires considerable laboratory hardware when damage is to be evaluated in large numbers of seedlings.

New Zealand Journal of Forestry Science 13(1): 72–9 (1983)

A technique closely related to diffusate electroconductivity is that of electrical impedance. This non-destructive technique assesses frost damage by inserting small electrodes into stem tissue and measuring electrical impedance at one or more frequencies. The theory and application of impedance measurements to frost damage evaluation have been reviewed recently by Glerum (1980). In this paper the application of a simple, low-frequency, impedance technique to frost damage evaluation in seedlings of *P. radiata* is described.

MATERIALS AND METHODS

Plant Material

One-year-old *P. radiata* seedlings were raised in the nursery at the Forest Research Institute in Rotorua, lifted, air-freighted to Palmerston North, and potted into 1.2-*l* containers in Opiki peat loam : sand : pumice : peat (50 : 20 : 20 : 10 v/v). The slow-release fertiliser Magamp (W. R. Grace and Co., United States) (N 7%, P 14%, K 5%, Mg 13%) was incorporated into the growing medium at a rate of 3 kg/m^3. The seedlings were held in a sheltered outdoor site for at least 1 month before frosting and were watered frequently.

The advective frost rooms at the DSIR Climate Laboratory, Plant Physiology Division, were used to impose the artificial frost treatments (Robotham *et al.* 1978).

Impedance Measurement and Technique

The circuit used to measure resistive impedance was similar to that of Glerum & Zazula (1973) and the two-electrode probe was similar to that of Wilner *et al.* (1960). A wide range R-C oscillator (Marconi Inst., England) supplied a 100-Hz sine wave across the electrodes. The voltage was measured across a $1000 \pm 5\Omega$ standard resistor connected in series with one electrode. From this the current was calculated and the impedance of the stem between the electrodes given by $Z = V/I$ where Z in kΩ was impedance, V in volts the voltage across the electrodes, and I in amps the current of the circuit. Calibration of this system was carried out routinely by inserting a 33.0 ± 0.1 kΩ resistor in place of the stem.

The capacitance of the *P. radiata* seedlings was determined by a resistance/capacitance bridge (Marconi Inst., TF 2700, England) and found to be <1 pF at 100 Hz. Therefore all measurements with this circuit were considered to be the resistive component of impedance.

Immediately before each frost programme commenced, the impedance at the base, middle, and apical sections of the stem was measured on each seedling, providing the mean pre-frost impedance (Z_O) values. The differences in impedance between the stem sections were small (<2 kΩ) though impedance tended to be highest in the apical section. At the conclusion of each frost, the seedling mean post-frost impedance (Z_F) values were measured on the same tissue. All measurements were made at $22 \pm 1°C$. The impedance ratio (Z_R) was calculated for each seedling as follows:

$$Z_R = Z_O/Z_F$$

All seedlings were maintained for 1 month in outdoor shelter where foliage damage was then assessed visually on the scale of 0 (no damage) to 5 (dead) (Menzies 1977).

Establishing the impedance/frost-damage relationship

Paired impedance ratio and visual damage ratings were collected from a large number of seedlings (approximately 1000) which were conditioned to either natural or controlled environments and subsequently exposed to a range of artificial frosts. The seedlings used were:

(1) Those conditioned in the controlled environment treatments described by Greer & Warrington (1982) and exposed to frosts from –4° to –18°C;

(2) Those naturally conditioned which were exposed to frosts ranging from –3° to –15°C between August and October 1980;

(3) Those that were conditioned in glasshouses with natural (spring) or extended (14-h) daylengths and warm temperatures (25°/15°C day/night), or outside with natural temperatures and extended daylengths and exposed to frosts ranging from –3° to –15°C;

(4) Those which were grown outside between February and April 1981 and exposed to frosts ranging from –2.0° to –9.5°C.

Testing the impedance/frost-damage relationship

In a second experiment, paired impedance ratio and visual damage ratings were collected on 216 seedlings raised outside and frosted monthly between May and October 1981. Another 622 seedlings were conditioned for 50 days in controlled environments with two photoperiods (10 and 13.5 h) and two temperature regimes (12°/4°C and 16°/8°C), and frosted at regular intervals. Frosts for these seedlings ranged between –2.5° and –12.0°C.

RESULTS

Relationship Between Impedance Ratio and Frost Damage

All seedlings from the first set of experiments were grouped into two broad classes based on their visual damage rating – (a) little or no damage, i.e., ratings of 0 to 2 and no more than 30% needle damage, and (b) seriously damaged or dead, i.e., ratings of 3 to 5. The number of seedlings in each of the two classes at each impedance ratio is shown in Fig. 1.

The proportion of seedlings that survived at each impedance ratio was subsequently defined as the expected probability of survival (S_E) for each impedance ratio. A relationship between expected survival and impedance ratio was derived from these data by polynomial regression analysis (Fig. 2). The most significant ($p < 0.01$) relationship for these data between the Z_R limits of 0.9 and 3.5 was a cubic function where:

$$S_E = 1.01 + 0.25\,(Z_R) - 0.33\,(Z_R^2) + 0.05\,(Z_R^3)$$

with $Z_R < 0.9$ $S_E = 1$ and $Z_R > 3.5$ $S_E = 0$

Evaluation

The number of seedlings surviving each frost in the second experiment, and hence also those seriously damaged, was predicted by determining the impedance ratio and the expected probability of survival for each seedling in each frost, calculating the

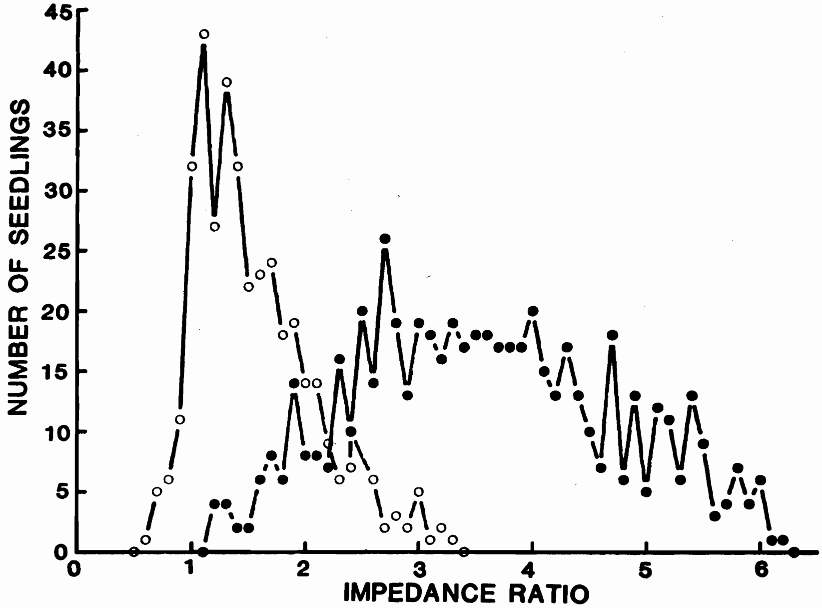

FIG. 1—Number of **P. radiata** seedlings at each impedance ratio in an undamaged class (○) and a damaged class (●) following artificial frosts after treatment in a wide range of conditions (approx. 1000 seedlings in all).

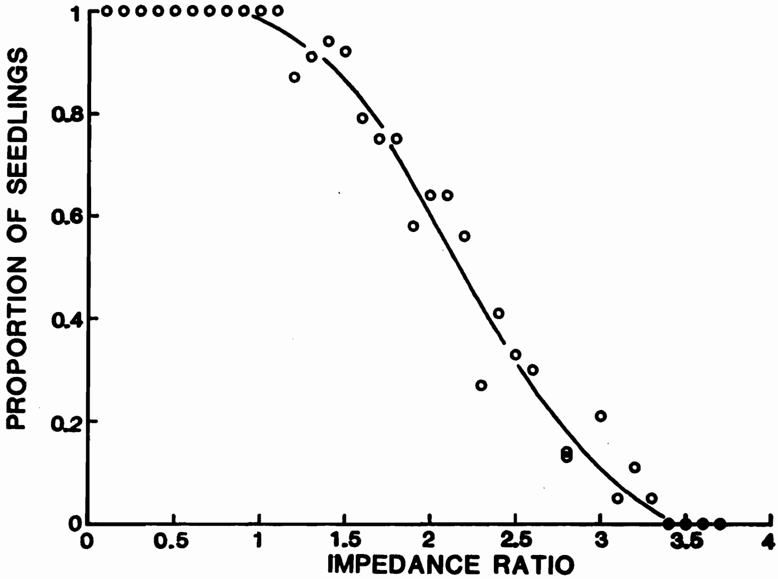

FIG. 2—Cubic regression between impedance ratio (Z_R) and the proportion of **P. radiata** seedlings expected to survive after exposure to an artificial frost.

mean expected probability of survival, and multiplying that by the number of seedlings in each frost. The expected number of seedlings surviving was subsequently compared with the actual number by regression analysis.

There were no significant differences between the slopes or intercepts of the regression lines of the two populations of seedlings. The over-all regression (Fig. 3), which accounted for 92% of the variation was,

$$S_A = 1.1 (S_E) - 0.05$$

where S_A was the actual survival and S_E the expected survival.

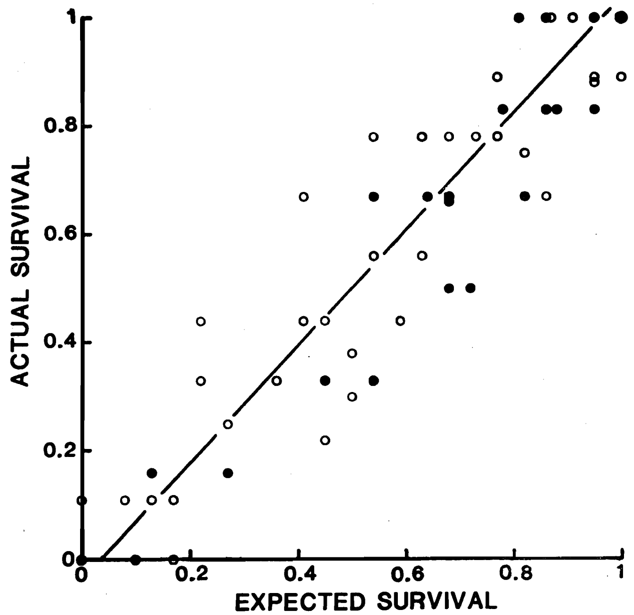

FIG. 3—Linear relationship between the expected survival and the actual survival of **P. radiata** seedlings after exposure to an artificial frost (● = seedlings conditioned outside between April and October, ○ = seedlings conditioned in controlled environments).

The number of frosts in which all seedlings, grown outdoors or in a controlled environment, were correctly assessed for frost damage is given in Table 1, together with the number of frosts in which between one and three seedlings per frost were incorrectly assessed.

DISCUSSION

Impedance ratio measurements have been used to measure frost damage and survival, with the kilohertz to megahertz ratio having been used most often (Greenham & Daday 1957; Glerum 1970; Van den Driessche 1973). The main advantage of this technique is that only post-frost measurements are required. However, at the high frequency the capacitive reactance component of impedance is important and must be accounted

TABLE 1—Number of frosts in which some seedlings per frost were incorrectly classified for frost damage, by the impedance ratio technique

	No. seedlings/frost incorrectly classified	No. frosts
(a) Conditioned outside April–Oct; 36 frosts, six seedlings/frost		
	0	16
	1	12
	2	6
	3	2
	4	0
(b) Conditioned in controlled environments; 68 frosts; nine seedlings/frost		
	0	31
	1	30
	2	6
	3	1
	4	0

for (Hayden *et al.* 1969), while reliable voltage measurements require high-quality voltmeters and screening problems become acute. Thus the measurement of impedance is made increasingly more complex. The simpler technique used here is to measure impedance at a single low (100-Hz) frequency before and after a frost. Both approaches have been used by Van den Driessche (1973) and Timmis (1976) to evaluate frost damage in *Pseudotsuga menziesii* (Mirb.) Franco and they appeared to favour the kilohertz-megahertz technique.

The present results indicate that *P. radiata* seedlings with a pre- to post-frost impedance ratio less than 0.9 have a 100% chance of survival whereas those with a ratio greater than 3.5 have no chance of survival. Hence a method for the rapid screening of material where no seedling loss is acceptable is now available. Similar impedance ratios for survival and death have been shown for seedlings of *Pseudotsuga menziesii,* though at a frequency of 1000 Hz (Van den Driessche 1973).

One feature of the technique described here that has apparently not yet been demonstrated for other impedance techniques is the close relationship between impedance ratio and expected survival. This has a useful consequence in that any level of seedling loss can be set and quickly screened for.

In the evaluation of the impedance ratio method, the significant and high correlation between the predicted and observed numbers of seedlings surviving each frost implied an accurate assessment of frost damage by this technique. However, frost damage of all seedlings was correctly assessed in only 45% of the frosts of both populations of *P. radiata.* With one seedling in error out of six for the outdoor-grown material and out of nine for that grown in a controlled environment, the number of frosts where seedling damage was correctly assessed increased to 78% and 90% respectively. In those frosts where several seedlings were incorrectly assessed there was some tendency for the impedance ratio to overpredict survival.

These data imply that the pre- to post-frost impedance ratio method could classify, to within 10%, the number of frost-damaged seedlings, in a sample as small as nine, at least 90% of the time. With smaller samples there would still be a high success rate but incorrect assessment of two or more seedlings would have greater impact on the sample. Timmis (1976) demonstrated that the kilohertz-megahertz impedance technique could correctly classify 87% of *Pseudotsuga menziesii* seedlings for frost damage where there were 15 seedlings in each frost.

The impedance ratio technique described here is a fast, non-destructive, and reliable method of assessing frost damage of *P. radiata* after artificial frosts. While it may be less effective than the kilohertz-megahertz method, the equipment and its operation are considerably cheaper and simpler and therefore frequently more useful for general application in frost studies.

ACKNOWLEDGMENTS

Ian Warrington of the Plant Physiology Division, DSIR, provided the impetus for this work and offered much valuable advice, and Michael Boyce (Plant Physiology Division, DSIR) provided technical assistance in setting up the measurement system. I owe thanks to Judi Galpin (Applied Mathematics Division, DSIR) and the Journal statisticians for helping with and improving the statistical analysis.

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