ASSESSMENT OF FROST DAMAGE IN RADIATA PINE SEEDLINGS USING THE DIFFUSATE ELECTROCONDUCTIVITY TECHNIQUE

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ABSTRACT

The diffusate electroconductivity technique of evaluating cold injury was studied on radiata pine seedlings subjected, at different times throughout the year, to below-freezing temperatures in a controlled environment frost room. The seedlings developed natural hardiness in May which increased to a peak in July to August when experimental frosts of —9° and —12°C caused little damage. Thereafter the seedlings lost their winter hardiness through September to November and returned to the summer minimum of —3° to —6°C. Relative electroconductivity values determined on upper stem tissue, within three days of the low temperature treatments, were in close agreement with visual assessments made one month later when the damage symptoms had had time to develop. Relative electroconductivity values of 0.5 or greater indicated that the low temperature treatment had been severe and would lead to subsequent death of the seedling.

The technique was also used to examine the influence of the number of frosts at any particular low temperature on seedling damage. Where the treatment temperature caused little damage (i.e. slight burning of needle tips) there was no increase in damage in response to the number of frosts the seedlings received. However, where a single low temperature frost caused considerable damage (i.e. many needles and buds killed), the amount of damage increased in response to an increase in the number of frosts. Damage assessment by the electroconductivity method again closely agreed with visual assessments.

INTRODUCTION

The low-temperature controlled environment "frost" rooms at the DSIR Climate Laboratory (Robotham, Lloyd and Warrington, 1978) have been used for a number of studies investigating the freezing tolerance of radiata pine (Rook, Menzies and Robotham, 1974; Menzies, 1976; 1977). In all such frosting studies it is essential to have an accurate technique whereby freezing injury can be evaluated. The present technique has involved holding material after frosting for 1-2 months and visually evaluating the extent of the induced damage, particularly the death of needles and buds; damage is rated on a scale of 0 (no damage) to 5 (dead). The main disadvantage of such a technique is that considerable time elapses between the treatment and the evaluation. In the interim, large numbers of plants must be watered and protected from natural frosts. There is

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also little opportunity, particularly with seedlings with rapidly changing frost hardiness, to carry out exploratory treatments prior to the main experimental frosts to be sure that the latter are in the lethal to sublethal range.

It would be desirable therefore to develop or modify a technique to enable a more rapid and quantitative evaluation of frost damage to be carried out. Several methods based either on the inactivation of enzyme and metabolic functions, or on the loss of permeability or destruction of cell membranes, are available and have been reviewed by Timmis (1976). The advantages of one such technique, the diffusate electrical conductivity method, are outlined in this paper. This technique was developed by Dexter *et al.* (1930, 1932) and has been used subsequently by a number of workers on a range of woody species which include Scots pine (Aronsson and Eliasson, 1970), Douglas fir (van den Driessche, 1969) and apple (Wilner, 1960; 1961). These workers have reported good agreement between relative electroconductivity determined shortly after a freezing test and longer term development of visible injury symptoms.

To test the value of the method for radiata pine, seedlings were subjected to various low temperature treatments at different times of the year during which the plants were either hardening (autumn and early winter), exhibiting a high degree of low temperature tolerance (mid- and late winter) or losing hardiness (spring and early summer). The effect of a single frost compared with up to four successive frosts at each treatment time was also investigated.

MATERIALS AND METHODS

Plant Material

Pinus radiata D. Don. seedlings ($1\frac{1}{2}/0$) were raised in the nursery at the Forest Research Institute, Rotorua, lifted and air-freighted to Palmerston North. They were potted into 1.2 litre containers in soil : peat : pumice (40 : 40 : 20 v/v) growing medium and held in a sheltered outside growing site where they were watered regularly.

Frosting Treatments

Low temperature programme

In all of the low temperature treatments examined, the high or "day" temperature was + 10°C; the temperature decline occurred over 6 hours, the low temperature duration was 6 hours and the rise to day temperature occurred over 4 hours. The relative humidity of the air was approximately 100% (0 mb vapour pressure deficit) during the period of frosting and 40% at + 10°C (7 mb vapour pressure deficit); the duration of humidity change from day to night and from night to day were the same as those for temperature. The main lighting (approximately 160 $W.m^{-2}$, photosynthetically active radiation) switched off at the start of the temperature decline and switched on 1 h before the temperature was raised. Soil temperature was kept above 5°C throughout using a simple soil heating system. The frost rooms are described in detail by Robotham, Lloyd and Warrington (1978).

Seasonal Study

Each month, March to October inclusive, low temperature treatments of 0°, —3°, —6°, —9° and —12°C were carried out on groups of 15 randomly selected seedlings.

Treatments of —15°C in August and September and 0°, —3° and —6°C in November were also imposed. The seedlings were taken from the outside growing site, where hardening/dehardening processes would have occurred naturally, were subjected to one low temperature cycle, sampled for the electroconductivity assessment (5 seedlings) and then returned to the outside site for subsequent visual assessment (10 seedlings). Plants remaining outside served as controls.

Repeated frost study

To investigate the influence of successive frosts on radiata pine, replicates of 15 seedlings were each subjected to either 1, 2, 3 or 4 frost cycles at a particular low temperature. Two low temperatures were selected each month from May through to October; i.e. 120 randomly selected seedlings and 15 controls were used each month.

Diffusate Electrical Conductivity Technique

The procedure adopted was similar to that of Wilner (1960). After the low-temperature frosting treatment, 2 pieces of stem, 2.5 cm long, from five individual plants were cut from either close to the soil surface or from the upper section of the stem immediately below the apical bud. They were then placed into capped glass tubes containing 15 ml distilled water and held in a water bath at 25°C. After 24 h the tubes were shaken and conductivity measured at 50 Hz using a Philips conductivity meter (GM 4249) and a Philips conductivity electrode (PW 9510). The stem samples were then killed by placing the tubes, in an ethanol bath, inside a deep-freeze for 24 h at —15°C. This method of killing was found to be superior to boiling where evaporative losses led to inconsistent conductivity values. The tubes were then replaced in the 25°C water bath for 24 h after which a final conductivity measurement was made.

Relative conductivity, R_t was calculated as:

$$R_t = L_t/L_k$$

where: L_t = specific conductance of leachate from sample frozen at temperature (t) and L_k = specific conductance of leachate from sample frozen at temperature (t) and then killed.

The "index of injury", I_t (Flint *et al.*, 1967), was calculated as:

$$I_t = 100\ (R_t\text{-}R_o)/(1\text{-}R_o)$$

R_o is the relative conductance of the control seedlings, given by L_o/L_d, where L_o = specific conductance of leachate from control seedlings and L_d = specific conductance of leachate from controls killed as indicated above.

Conversion of R_t values to index of injury, I_t, values bases the expression of injury on a scale where the unfrozen control is given a value of zero and the killed sample a value of 100. Differences confounded by changes in unfrosted seedling R_t values are, therefore, eliminated.

Visual Evaluation

One month after frosting, the plants were individually assessed for damage on a 0-5 scale; 0, no damage; 1, buds undamaged, some needle reddening; 2, buds partly damaged, 10-30% needles killed; 3, buds killed, approximately 50% needles killed; 4, upper stem and foliage dead, approximately 90% remaining needles dead; 5, seedling completely dead.

RESULTS AND DISCUSSION

A summary of the monthly relative conductivity, R_t, and visual assessment values, for March to November inclusive, is presented in Fig. 1. Separate values for April are

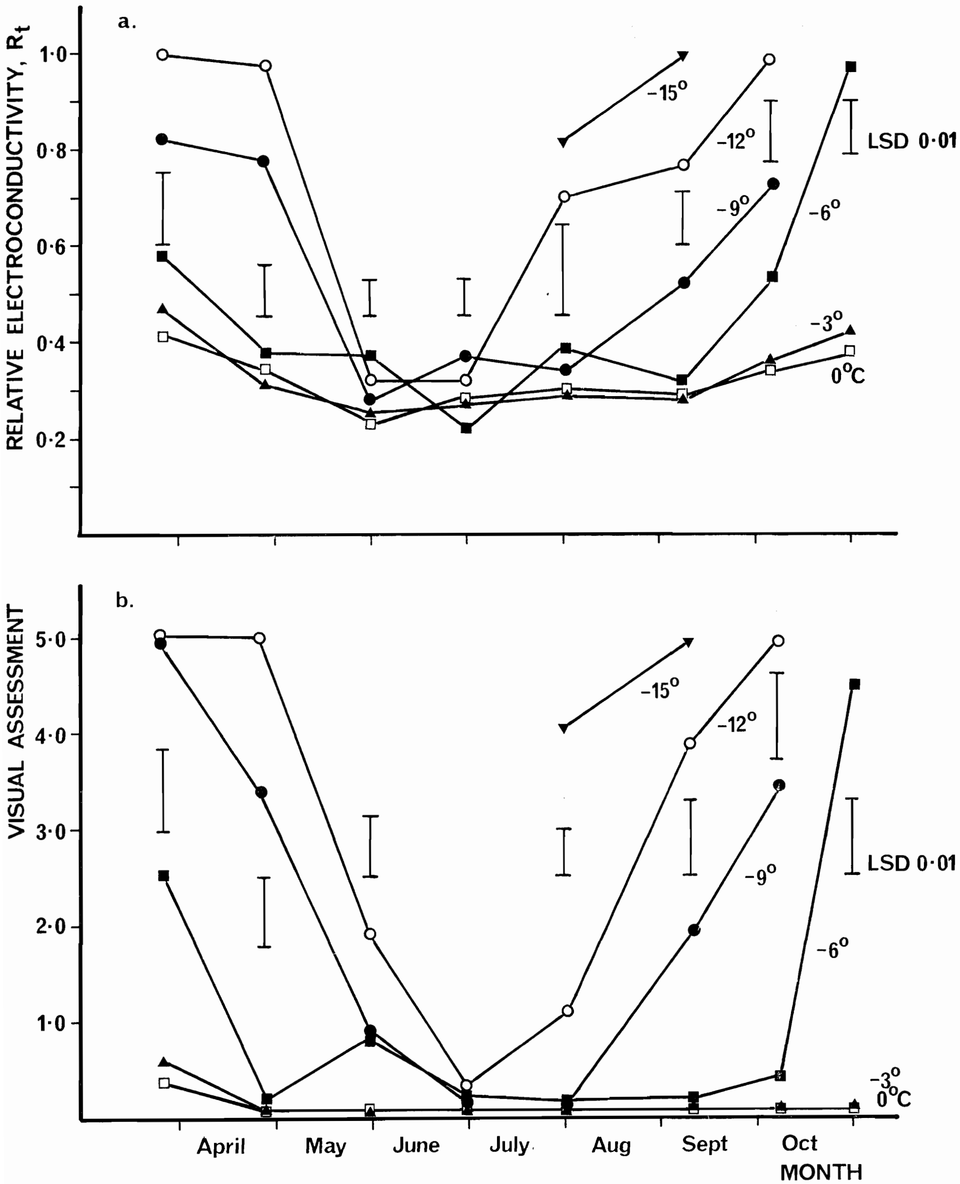

FIG. 1—(a) Changes in relative electroconductivity in upper stem sections from radiata pine seedlings subjected to 0 to —15°C treatments from March to November inclusive.

(b) Changes in visual damage assessed on radiata pine seedlings one month following each frosting treatment.

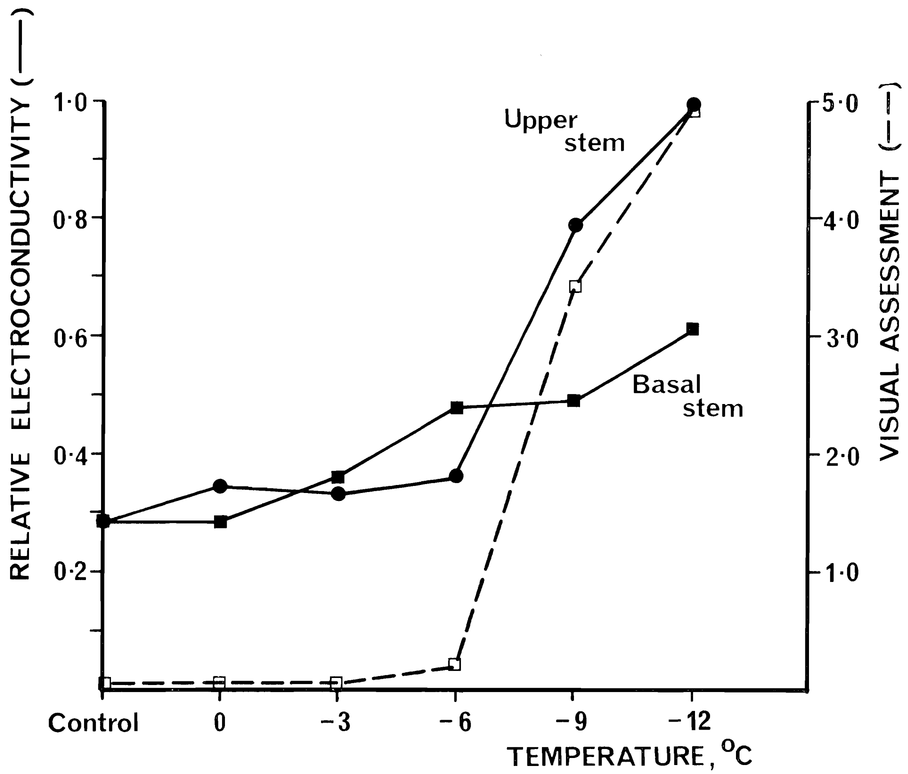

FIG. 2—Relative electroconductivity values recorded on upper or lower stem sections, and visual damage assessment values, for radiata pine seedlings subjected to each of the low temperature treatments in April.

shown in Fig. 2. The visual assessment values are consistent with previous results (Menzies, 1976) and show a development of natural hardiness in May which increased to a peak in July-August when frosts of —9° and —12°C caused little damage. Thereafter the seedlings lost their winter hardiness through September to November and returned to the summer minimum of —3° to —6°C.

In general, frost tolerance coincided with the growth pattern; maximum tolerance occurred when the seedlings were making negligible growth during the winter months but declined rapidly when growth recommenced in response to warmer growing conditions.

The R_t values from the upper portion of the stem followed the visual assessment values very closely (Fig. 1b) and consequently were of considerable value in assessing the low temperature damage of the experimental material under study. Field trial evaluations have shown previously that a visual damage assessment of 2 or greater will result in marked loss of vigour and often subsequent death of the frosted seedling; this generally corresponded to R_t values of 0.5 or greater.

Conversion of the electroconductivity results to the "index of injury" values, suggested by Flint *et al.* (1967), improved the relationship with visual assessment values even further ($r = 0.96$) and enhanced the differences between frosts at any treatment time.

R_t values obtained from basal stem samples were considerably more variable than upper stem sample values and were less sensitive to changes in low temperature damage (Fig. 2).

Unpublished work (Meeking, 1973) suggests that leaf tissue would be satisfactory test material and would have the advantage of not requiring the complete sacrifice of a treatment subsample to obtain the electroconductivity values. Leaf tissue was not used in the present study largely because of possible changes in that tissue between seasons and with needle age.

The results of the study examining the influence of successive frost numbers on seedling damage are presented in Table 1. Where the low temperature treatment caused little damage (i.e. —4.5° and —6°C in May, —6° and —9°C in June, —10°C in July, —9°C in September and —6°C in October) there was no marked increase in damage due to an increase in frost number. Where the low temperature treatment caused considerable damage (i.e. visual assessment greater than 2) after one frost cycle, increasing the number of frost cycles often resulted in increased damage (e.g. —12°C in July, August and September and —8°C in October). In all treatments the damage assessment using the relative electroconductivity method closely matched the visual assessment results.

In summary, the relative electroconductivity method was found to be a convenient, rapid and reliable method for assessing the degree of frost injury in radiata pine. Best results were obtained using upper stem sections. The technique could be used effectively

TABLE 1—Effect of frost number and frost temperature at different months of the year on stem section leachate electroconductivity of radiata pine seedlings

Month (day no.), Frost temperature	NUMBER OF FROSTS 0	1	2	3	4	
	RELATIVE ELECTROCONDUCTIVITY, R_t					
May (1-6)						
—4.5°C	0.30	0.32	0.38	0.36	0.36	ns
—6.0°C	0.30	0.40	0.36	0.38	0.41	ns
June (7-11)						
—6.0°C	0.24	0.28	0.29	0.34	0.27	ns
—9.0°C	0.24 a	0.32 bc	0.28 b	0.41 c	0.28 b	**
July (11-15)						
—10.0°C	0.29	0.36	0.33	0.35	0.38	ns
—12.0°C	0.29 a	0.36 a	0.36 a	0.33 a	0.47 b	**
August (22-26)						
—12.0°C	0.29 a	0.78 b	0.79 b	0.77 b	0.84 b	**
September (19-23)						
—9.0°C	0.33 a	0.52 b	0.62 bc	0.74 cd	0.83 d	**
—12.0°C	0.33 a	0.76 b	0.87 bc	0.84 bc	0.97 c	**
October (24-28)						
—6.0°C	0.36 a	0.66 b	0.81 b	0.72 b	0.75 b	**
—8.0°C	0.36 a	0.79 b	0.83 bc	0.92 bc	0.98 c	**

ns = not significant, ** = significant at 1% level, relative electroconductivity values with different subscripts are significantly different (1% level) within any given frost temperature treatment in studies examining the induction or loss of cold hardiness in radiata pine. It could also be used to assess damage in trials selecting for frost hardy families, or to discriminate against intolerant families, for growth in more rigorous, frost-prone growing sites.

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