INJURY TO RADIATA PINE AS INFLUENCED BY FREEZING AND THAWING RATE, AND LOW TEMPERATURE DURATION

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Seedlings of **Pinus radiata** D. Don. were subjected to a series of different diurnal frost temperature programmes in a low temperature controlled environment room. Four rate of freeze, 4 rate of thaw, and 4 low temperature duration treatments were examined over 3 seasons and at 2 minimum temperatures. Increasing the rate of freezing from 2.3° to 10°C/h and increasing the freeze duration from 2 to 8 h both resulted in increased seedling damage. The response to the rate of thaw treatments was consistent with the duration response probably because plants were frozen longer when slow (2.5°C/h) rather than fast (10°C/h) thaw rates were used. Frost damage caused during thawing in darkness was closely similar to that caused when thawing occurred under light conditions potentially capable of sustaining photosynthesis. Within all treatments, decreasing the minimum temperature used from —8° to —10°C resulted in increased damage but did not alter the overall responses obtained. The responses were also similar over 3 different seasons when plants were at different levels of cold hardiness development.

INTRODUCTION

Artificial freezing techniques are used to study cold hardiness in various plant species. Such studies may be designed to investigate the mechanisms involved with cold hardiness processes or to define those environmental factors which control cold hardening and dehardening. Other studies may use a range of low temperature treatments to discriminate between frost-hardy and non-hardy individuals in a population or to identify differences in frost hardiness among genotypes. In these, and in other related studies, the low temperature programmes used are generally selected to have features, such as rates of freezing or the duration of frost, programmed to be consistent with natural field conditions or within reasonable limits from physiological considerations. Accordingly, many of the artificial freezing devices developed in recent years have included at least some control over air temperature cooling rate, the duration of the low temperature period, and sometimes over thawing rate (Warrington & Rook 1980). Experimenters using these devices must, therefore, choose a diurnal programme consisting of a combination of these components and consider, among other factors, the need to use root temperature control, the need to control relative humidity and the need to provide artificial lighting during all or part of the low temperature cycle.

Previously published work has shown that increasing the rate of freezing or extending the duration of frost both increase the level of plant damage that occurs in a freezing test (Mazur 1969; Steponkus 1978). However, little work on these aspects has been previously carried out with conifers. This study examined the effects of several rates of freezing, rates of thawing, and different low temperature durations on damage to radiata pine seedlings. The effect of light during the thawing period was also examined since in many other facilities the freezing test is carried out entirely in darkness.

MATERIALS AND METHODS

Seedlings of *Pinus radiata* D. Don. (1½/0) from the New Zealand Forest Service nursery at Bulls or the Forest Research Institute nursery at Rotorua, were transplanted into 1.2 *l* pots containing fertilised soil : peat : pumice (40 : 40 : 20 v/v) growing medium and were grown outside at Palmerston North. The Bulls stock was transplanted in April and the Rotorua stock in June. All pots were well watered and no transplanting shock was apparent with either stock. Under these growing-on conditions the plants cold hardened or dehardened naturally before the artificial freezing treatments.

The experiments were carried out in the low temperature controlled environment rooms previously described by Robotham *et al.* (1978). In these rooms the freezing and thawing rates can be independently selected and are determined by the difference between the maximum and minimum temperatures used and by the duration of the maximum/minimum and minimum/maximum temperature changeover periods. The programmes used, and the consequent rates of temperature change and times that the plants spent below 0°C, are shown in Table 1. The rate of freeze, rate of thaw, and duration treatments chosen, each consisted of 4 frost programmes run at 2 minimum temperatures (—8° and —10°C). Within a particular treatment series (e.g., freeze rate) only the component relevant to the treatment was changed (i.e., maximum/minimum changeover duration) while the other two programme components (i.e., minimum temperature duration and minimum/maximum temperature changeover duration) were held constant.

In all treatments, seedlings were placed in the low temperature controlled environment rooms on trolleys where root temperatures were maintained around 5°C during each low temperature programme (Robotham *et al.* 1978). The "day" or maximum temperature used was 10 ± 0.3°C and the corresponding relative humidity approximately 40%. The relative humidity was maintained at approximately 100% during the minimum temperature period and the diurnal changes for humidity were closely similar to those for temperature. Ten new randomly selected plants were used in each frost programme run.

Two low temperature rooms were used in the study and wherever possible the 4 programmes within a treatment series were run sequentially within one room with the 2 minimum temperatures being run on alternative days. The rate of freeze treatments preceded the duration treatments in one room, and the rate of thaw treatments were run concurrently in the other room. During the running of each programme, the performance of each room was continuously monitored with 0.2 mm diameter copper : constantan thermocouples. Plants were discarded and a frost cycle repeated if any programme or mechanical malfunctions were recorded. Between-room differences were assumed to be small.

TABLE 1—Rate of freeze, rate of thaw, and duration programmes and resulting freeze and thaw rates, and times below 0°C for each —8°C and —10°C low temperature treatment

Treatment		Frost programme*	Freeze rate (°C/h)		Thaw rate (°C/h)		Time (h) below 0°C (approx.)	
		h-h-h	—8	—10	—8	—10	—8	—10
Freeze rate	1	2-6-8	9.0	10.0	2.3	2.5	10.5	11.0
	2	4-6-8	4.5	5.0	2.3	2.5	11.3	12.0
	3	6-6-8	3.0	3.3	2.3	2.5	12.2	13.0
	4	8-6-8	2.3	2.5	2.3	2.5	13.1	14.0
Thaw rate	1	8-6-2	2.3	2.5	9.0	10.0	10.5	11.0
	2	8-6-4	2.3	2.5	4.5	5.0	11.3	12.0
	3	8-6-6	2.3	2.5	3.0	3.3	12.2	13.0
	4	8-6-8	2.3	2.5	2.3	2.5	13.1	14.0
Duration	1	8-2-8	2.3	2.5	2.3	2.5	9.1	10.0
	2	8-4-8	2.3	2.5	2.3	2.5	11.1	12.0
	3	8-6-8	2.3	2.5	2.3	2.5	13.1	14.0
	4	8-8-8	2.3	2.5	2.3	2.5	15.1	16.0

* Coded sequence for: time (h) from maximum temperature (10°C) to minimum temperature – duration of minimum temperature – time from minimum temperature to maximum temperature.

Each treatment series was repeated 3 times during 1979; once in May–June (autumn) with Bulls nursery stock and once each in July–August (winter) and September (spring) with Rotorua nursery stock. The duration treatment series in spring was omitted. The treatments were imposed at these 3 times of the year to examine possible changes in response with changes in plant cold hardiness. Within a season each treatment series was completed in as short a time as practicable to minimise the effect of any changes in natural plant cold hardiness. In practice, the 4 programmes within a treatment, each run at the 2 minimum temperatures, were completed within 10–12 days and the entire treatment series was completed within 30 days.

Each of the above programmes was run with the plants in darkness throughout. In addition, in winter and spring, treatments were imposed to study the effects of thawing plants under light. In the 8–6–2, 8–6–4, and 8–6–6 thaw treatment programmes plants were irradiated during the thaw period with light with a photosynthetic energy flux density of 155 W/m² (equivalent to a photosynthetic photon flux density of 630 μE/m²/sec). Thaw treatments with either lights on or off were run on alternate days.

The 8–6–8 programme, which was common to each of the 3 treatment series, was run only twice each season; once in the rate of freeze and once in the duration treatment series. The 8–6–8 rate of thaw programme values were taken from either of these depending on which sequence coincided closest with the rate of thaw sequence. Consequently, the rate of freeze 8–6–8 values were used in autumn and spring and the duration values were used in winter. This compromise allowed the thaw treatment series, run with and without lights and concurrently with the rate of freeze and duration treatments, to be completed within a similar time period.

After each treatment the plants were returned to the outside site and after 1 month they were individually assessed for damage on a 0–5 scale; 0, no damage; 1, buds undamaged, some needle reddening; 2, 10–30% needles killed; 3, approximately 50% needles killed; 4, upper stem and foliage dead, approximately 90% remaining needles dead; 5, seedling completely dead.

The data were analysed using analysis of variance techniques and where interaction terms were not statistically signficant the analyses were re-run to test main treatment effects only.

RESULTS

An increase in freezing rate markedly increased the amount of plant damage at both of the autumn minimum temperatures (—8° and —10°C) and at the —8°C spring temperature conditions (Fig. 1). Complete plant death occurred at all freezing rates at —10°C in spring; very little damage was caused by all rates at —8°C in winter. Under the —10°C winter temperature conditions, only the highest freezing rate caused significant plant damage. In autumn, a decrease in temperature resulted in an increase in plant damage but there was no significant interaction of freezing temperature with freezing rate.

An increase in the duration of the minimum temperature period and a decrease in the minimum freezing temperature both significantly increased plant damage (Fig. 2). There were no marked differences in the response to duration between the 2 seasons tested (i.e., between different stages of cold hardiness development) or marked inter-action between minimum temperature and duration within either season.

Within the thaw treatments the greatest amount of plant damage occurred with the slowest rates of thawing and the least with the fastest rates (Fig. 3). Decreasing the minimum temperature produced the same overall effects but increased the amount of plant damage obtained. The same trends and degree of damage occurred irrespective of whether thawing occurred in light or in darkness.
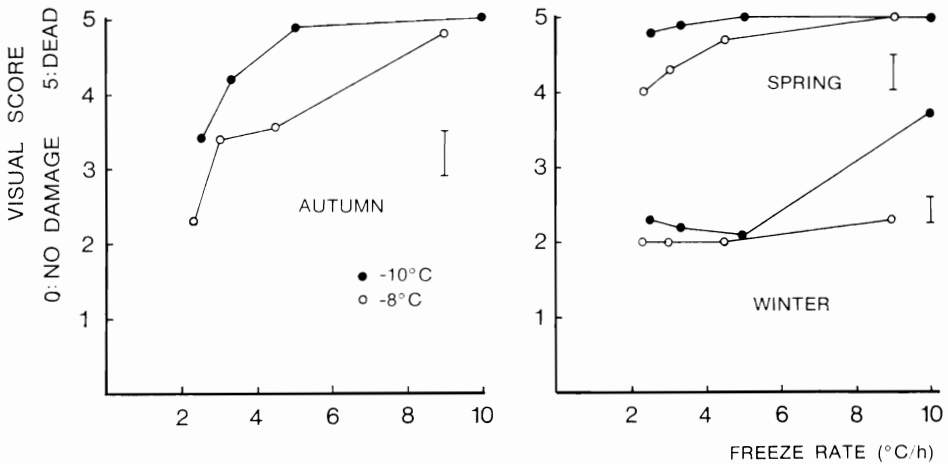

FIG. 1—Change in radiata pine seedling damage in response to freeze rate. Least significance difference values (5% probability level) for comparisons among rates or between temperatures are shown as vertical bars.
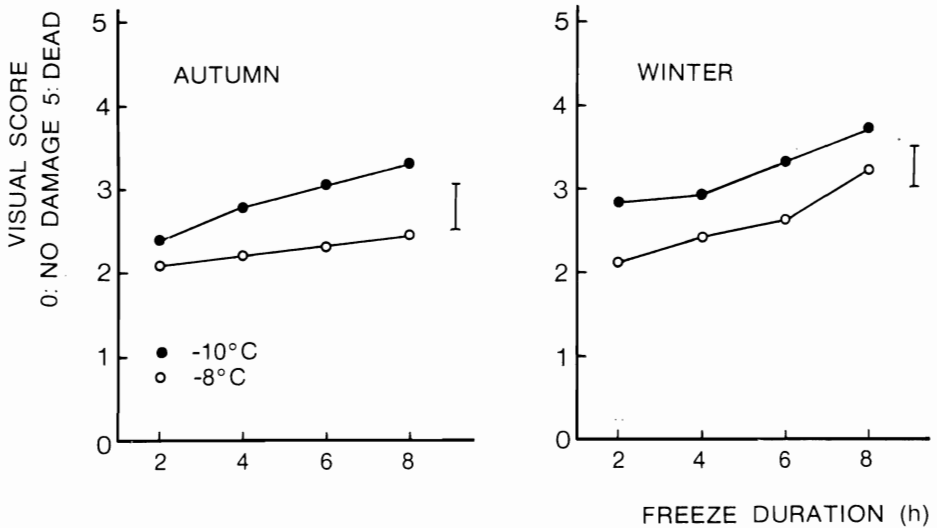
FIG. 2—Change in radiata pine seedling damage in response to change in the duration of the minimum temperature period.
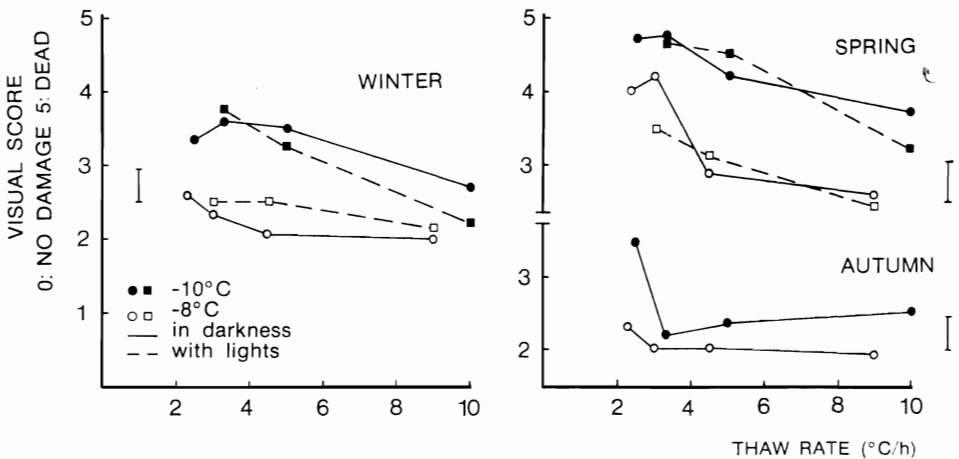

FIG. 3—Change in radiata pine seedling damage in response to thaw rate where the main lighting system was either on or off during the thaw period.

There was no obvious difference in damage resulting from the 8–6–8 treatment at the beginning and end of the autumn and spring series. However, plants were more extensively damaged at the end compared with the beginning of the winter series indicating that some loss of hardiness had occurred during the 30-day period between mid July and mid August. Accordingly, this should be considered if comparisons between rate of freeze, rate of thaw, and duration treatments in winter are made.

DISCUSSION

The enhancement of freezing damage by rapid rather than slow freezing observed in these studies is consistent with those results reported previously for a range of species including Scots pine (Aronsson & Eliasson 1970), peach (Weaver & Jackson 1969), *Eucalyptus regnans* (Ashton 1958), and pasture legumes (Sprague 1955; Kilpatrick *et al.* 1966). However, whereas many of these studies used only 2 contrasting rates and detected 2 contrasting levels of damage, one of which was often lethal, the present study has shown that a gradual change in the amount of plant damage sustained can take place when a range of rates is used. The likelihood of intracellular freezing, which is usually regarded as being lethal, increases with higher rates of freezing. Higher freezing rates can also increase the rate of cellular dehydration when extra-cellular ice formation occurs, and result in more plant damage through an increase in intracellular disruption (Mazur 1969).

Our observation with pine seedlings that the extent of freezing damage increased with an increase in the period of the freezing temperature is similar to previous observations made predominantly with herbaceous species (Greenham & Daday 1960; Hudson & Brustkern 1965; Thomas & Lazenby 1968; Lorenzetti *et al.* 1971; Rammelt 1972; Hacker *et al.* 1974; Pomeroy *et al.* 1975; Gusta & Fowler 1977; Ivory & Whiteman 1978). In contrast, there are few studies which have examined the influence of thawing rate on plant damage and in some instances the thawing rates reported were abnormally high in relation to those possible in field situations. Hence Gusta & Fowler (1977) found that more damage was caused to winter wheat crowns when they were thawed very rapidly (2–4°C/min) than when they were thawed slowly (0.5–2°C/h). However, in a study with tropical grass species, where thawing rates similar to those occurring in natural environments were used, generally more damage was sustained where thawing rates were controlled (5°C/h) than where an immediate transfer from the frost to the maximum temperature was used (Ivory & Whiteman 1978). A consequence of a slow thawing rate, particularly where a similar freezing rate, minimum temperature, and freeze duration are used, is that plants are exposed to critical freezing temperatures longer than where fast thawing rates are used. In this current study there was a close similarity between the patterns of response obtained under the freeze duration and rate of thaw treatments. In both instances increased damage would be expected from prolonged freezing through an increase in cellular dessication and a consequent increase in stresses that follow (Steponkus 1978).

Most low temperature frost studies using whole plants are carried out in the dark usually because the facilities used are not provided with artificial lighting systems (Warrington & Rook 1980). In chilling sensitive species, such as maize and sorghum, the damage sustained under chilling conditions ($< 10°C$) when the plants are under medium photosynthetic irradiance levels is much greater than when the plants are cold-stressed in darkness. The damage under light is due to destruction of the photosynthetic apparatus including major disruption of chloroplast integrity (Taylor & Rowley 1971). In the present study, the damage sustained by the radiata pine seedlings was very similar under either light or dark conditions indicating that light was not important in frost tolerance studies on this chilling-insensitive species. Therefore, artificial freezing tests can probably be carried out completely in darkness and yield results applicable to natural field conditions.

In conclusion, this study has shown that each of the components of a diurnal frost temperature sequence can influence the amount of plant damage that can occur. More damage occurred with high freezing rates and long freeze durations than with slow rates and short durations. Where relative differences between species are sought (i.e., ranking for frost tolerance) the temperature sequence chosen is probably not critical provided high freezing rates are avoided. However, some preliminary screening under a range of different programmes may be required to select the diurnal sequence which maximises the separation of the genotypes under study. Rook *et al.* (1980) used a 5–3–4 programme to screen 38 provenances of *Eucalyptus regnans* for frost tolerance. The ranking under the artificial conditions closely agreed with multiple field site data for the same provenances and the choice of the 5–3–4 sequence allowed the completion of 2 complete diurnal sequences each 24 h. If, however, data are sought which are to have relevance to a particular field situation, the most applicable results are likely to come from plants exposed to a diurnal temperature sequence which closely matches the characteristics of the field site. Menzies (1976) used a combination of 8–8–4, 6–6–4, and 4–4–2 programmes with different temperature minima to examine seasonal hardiness patterns of radiata pine. The programme sequences, determined from field meteorological data, which resulted in rates of freezing from 2.7° to 3.9°C/h provided quantitative results which agreed closely with field observations on the seasonality of frost suscepti-bility in this species and produced damage symptoms consistent with those occurring after the incidence of natural frosts.

ACKNOWLEDGMENTS

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