EVALUATION OF TECHNIQUES USED IN DETERMINING FROST TOLERANCE OF FOREST PLANTING STOCK: A REVIEW

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ABSTRACT

This paper reviews the equipment available for evaluating frost hardiness of forest tree planting stock. The procedures and precautions necessary in undertaking such work are also outlined. The most common and accessible method of evaluating frost hardiness is to raise plants at one or more field sites where plants grow, harden and freeze naturally. However, the frequency and severity of frosts in the field are unpredictable and uncontrollable. To overcome these problems various commercial cold rooms and cabinets, often modified to provide controlled rates of freezing and thawing, have been used. In some cases these cabinets are portable to allow field plants to be frosted **in situ.** Freezing bars have also been developed which allow samples of plants to be frosted over a range of temperatures at the same time. Recently, specialised radiation and advective frost rooms have been designed which, although costly to construct and operate, provide very precise, controlled and reproducible "natural" frosts.

Most techniques can distinguish differences in frost tolerance between plants, but only those providing control of all phases of the frost treatment will allow the results to be extrapolated confidently from laboratory to the field. Access to more than one frost temperature is essential if differences in frost tolerance are to be quantified. The final test of the frost tolerance of forest planting stock, however, must remain its performance in the field.

INTRODUCTION

The nursery and early establishment phases of forest production are generally recognised as being the most vulnerable to frost damage. For example, frost was a major factor responsible for the failure of several thousand hectares of Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) planted at Kaingaroa Forest from 1930-1933 (Kirkland, 1969). Such losses are normally assessed on the basis of numbers of seedlings killed, and little account is taken of losses in production from slower growth of

surviving seedlings, or of the increased silvicultural costs and poorer timber quality arising from uneven growth of the remaining crop. When losses of this extent are liable to occur even one year in ten, it is not surprising that foresters often select more frost-hardy and usually less productive species. Thus Kirkland (1969) notes that after 1936 these potential Douglas fir sites in Kaingaroa Forest were planted with *Pinus contorta* Dougl., *P. nigra* Arnold and *P. ponderosa* Laws.

The ability of stock to survive frost undamaged is one attribute of planting stock quality and is often one of the prime requirements in many forest production areas. However, around the world actual levels of frost, and frost conditions, differ considerably. Whereas subfreezing temperatures in the tropics are extremely rare (Richards, 1952), in much of North America and Northern Europe freeze conditions last continuously for weeks or months. In New Zealand, frosts normally last for only a few hours and by the following afternoon air temperatures can be around 15 °C. In many forest areas of New Zealand killing frosts can occur at any season of the year (Washbourn, 1978), whereas in continental areas damaging frosts are generally confined to the period from late autumn to early spring. These features of field frost conditions should be borne in mind when considering the validity of any particular artificial frost technique or programme used for predicting the performance of planting stock in the field. Such studies should not only identify the most hardy types of planting stock, but also the levels of low temperature that can be tolerated in order to match the most appropriate plants to sites with high potential for freezing stress.

Ideally a forester would like to assess the likely frost tolerance of planting stock visually or carry out a quick, simple test *in situ* to indicate the frost temperatures that planting stock would tolerate. In the past, various attempts have been made to use morphological or biochemical characteristics to correlate with plant cold hardiness, but none have been reliable (Alden and Hermann, 1971; Steponkus, 1978). It is generally accepted that a seedling in its spring flush of height growth is less frost hardy than a dormant seedling. However, our experience with radiata pine (*Pinus radiata* D.Don.) seedlings suggests that frost tolerance levels are often grossly misjudged when they are assessed on seedling appearance only. It is also difficult to gauge the degree of frost tolerance of stock during autumn and spring when hardening and dehardening are occurring. The idea that a biochemical compound might be used to indicate cold hardiness has been intensively examined in the past (Alden and Hermann, 1971), with the objective that a sample of the plant could be analysed quickly to assess the concentration of an indicator compound. Compounds which have been associated with cold hardiness include carbohydrates (Aronsson *et al.,* 1976), the sugar raffinose (Parker, 1963), hormones (Kacperska-Palacz, 1978), proteins (Brown, 1978), and phospholipids (Steponkus, 1978). As Steponkus (1978) observes there are numerous biochemical and physiological changes associated with cold hardening. Once a more complete understanding is achieved it may be possible to rely on an indicator compound, or a change in metabolism, to gauge a plant's cold hardiness. This will only be possible through a better understanding of the processes involved, which will require access to reliable frosting techniques.

Comprehensive reviews of the literature on frost hardiness from several perspectives, including work with forest seedling material, are available (Mazur, 1969; Weiser, 1970; Alden and Hermann, 1971; Levitt, 1972; Burke *et al.,* 1976; Glerum, 1976; Timmis,

1976; Li and Sakai, 1978; Steponkus, 1978). However, the equipment available for determining frost hardiness does not appear to have been reviewed. In this paper the utilisation of (a) Field Conditions, (b) Controlled Enclosures, and (c) Freezing Bars for assessing frost tolerance of forest tree planting stock is evaluated. The procedures and precautions that should be taken in such studies are also outlined.

A: FIELD STUDIES

The most common and accessible method of evaluating plants for response to low temperature is to grow them at one or several field sites. With this method the plants can grow, harden and freeze in response to natural conditions. Damage can be assessed in the field soon after frosting, or in the spring/early summer when bud development and new growth occurs, or by taking plants into a glasshouse at frequent intervals after natural frost. Although scientists and foresters may feel that field tests afford the only real measure of winter hardiness, these tests have major limitations. However, it should be realised that field tests must remain the real test of cold hardiness of planting stock and provide a yardstick to evaluate the results of any artificial frost test.

A significant difficulty with field tests for cold hardiness is the unpredictability of the field conditions. In some years plants with differing frost tolerances may all be killed by an abnormally severe frost, while in other years all the plants may come through the winter totally undamaged. In neither case is information on differences in frost tolerance obtained and the time and money spent in raising plants, preparing the site, and running a research programme is lost. The frequency and severity of frosts must be close to the long-term average if tests for a given locality are to be reliable. This often means that trials need to be run for a number of years. Autumn and early winter weather should also reflect the long-term conditions as these can affect the extent and rate of hardening and dehardening. These processes go through an annual cycle and it is only through the interaction of environmental factors (such as daylength and temperature), and the genetic potential of a plant, that a change in cold hardiness occurs. In an attempt to overcome the problem of unpredictability at any one site, multiple location testing sites have been used (Dexter, 1956; Glerum, 1976).

Another problem with field tests of frost tolerance is to know precisely what frost conditions the plants have experienced. It is possible to measure and record the frost conditions but this itself can be a demanding operation. In field tests it can also be difficult to find large uniform sites, as even slight microsite variation can produce large differences in natural frost conditions (Horiuchi and Sakai, 1978). Although the effect of variation resulting from non-uniform sites can be partially overcome by increased replication, this increases the size and complexity of a research programme. Thus the precision of the study will often depend on recognising any significant microsite differences. The effects of frost in the field are also often confounded with the effects of other environmental factors (e.g., drying winds, weed competition) and field tests can provide misleading results on frost tolerance *per se,* unless the role of these other environmental factors is understood.

B: CONTROLLED ENCLOSURE STUDIES

The difficulty of obtaining predictable frosts in the field has encouraged scientists over the past fifty years to develop a range of devices for carrying out controlled freezing

studies. These devices range from fairly standard commercial cold rooms and freezing cabinets to very specialised and precise frost units which can produce specific types of frost. All of this equipment provides a means of controlling the occurrence and severity of frost. Some of it is portable allowing tests to be run *in situ* on field-grown material.

1. *Cold Rooms and Freezer Cabinets*

(a) *Laboratory Units:* Low temperature studies on plants under controlled conditions closely followed the development of mechanical refrigerators (Dexter, 1956). Although some workers continue to use facilities such as cool-store rooms, the temporal and spatial variation in temperature (e.g., 4% °C in the room used by Eagles *et al.,* 1975), dry atmospheres, variable wind speeds and absence of programming control are undesirable features which make precise and repeatable work difficult to achieve. To overcome these problems modified programmable freezer chests and refrigerator cabinets have been developed where rates of freezing and thawing and the duration of a low temperature period can be controlled. Voisey (1963) and Voisey and Moulton (1969) published early descriptions of modifications to domestic freezer units. These modifications reduced the maximum temporal variations from ± 3.0 to ± 0.1 °C, and spatial variations (not including measurements within 5 cm of the walls or floor) from 6.0 to 0.8 °C. The freezer operated satisfactorily at temperatures ranging from — 20 °C to + 5.0 °C.

Other workers have used a range of techniques to resolve the problems of temperature variation. For example, Gusta *et al.* (1978) incorporated a large aluminium shelf in a freezer to dampen temperature fluctuation. Samples were placed in aluminium pans on the shelf to further reduce variation. Their system could control air temperature between — 85 °C and +50 °C with a spatial uniformity of ± 0.5 °C. Others have used liquid baths (e.g., ethanol/water) into which leaf, stem or whole plant samples in plastic bags or glass flasks were immersed (e.g., Carrier, 1951; Wessel and Hermann, 1969; Fowler *et al.,* 1973; Pomeroy and Fowler, 1973; Christersson, 1978; Larsen, 1978). Irving and Lanphear (1967a) placed 16 cm stem tissue samples into styrofoam boxes in freezers also presumably to improve temperature stability.

A number of workers have used wide-mouthed large vacuum bottles (Dewar flasks) within the freezer to hold severed plant part (e.g., leaf or stem) samples (van Huystee *et al.,* 1967; McLeester *et al.,* 1969; Howell and Weiser, 1970; Fuchigami *et al.,* 1971; Graham and Mullin, 1976; Harrison *et al.,* 1978). The vacuum bottles are effective in minimising temperature variations and in slowing down the rates of freezing and thawing. For example, Graham and Mullin (1976) found that the cooling rate was reduced from 180-240 °C/hr to 15-20 °C/hr when using vacuum bottles in a freezer operating at — 65 °C. Vacuum bottles can also be used to avoid problems of supercooling. Plant tissue supercools if the temperature of the cell solution falls below freezing point without the formation of ice. Supercooling may protect plants incapable of hardening much below their freezing point. However, when ice does form in supercooled tissues, crystals form very rapidly and are more damaging to tissue than ice that forms slowly in association with little or no supercooling (Levitt, 1972). Inoculating plants with ice overcomes appreciable supercooling during artificial freezing tests. To achieve this, plant samples in freezers have been wrapped in damp cheesecloth

in vacuum bottles (McKenzie and Weiser, 1975), placed in wet sand (Gusta *et al.,* 1978) or seeded with ice crystals (Timmis and Worrall, 1975; Christersson, 1978).

In some cold rooms and freezer cabinets the rates of freezing and thawing can be controlled by using either cam or electronic controllers (Greenham and Daday, 1960; Lapins, 1962a, b, 1965; Glerum, 1973; Andrews *et al.,* 1974; Tanaka and Timmis, 1974; Timmis and Worrall, 1975; van den Driessche, 1976; Mexal *et al.,* 1979). Freezing and thawing rates used are typically 1-5°C/hr and 2-20°C/hr respectively. In other cases (e.g., Irving and Lanphear, 1967a) control over freezing rate is achieved by transferring samples between freezers set, for example, at 5°C differentials. Although some workers appear to control the duration of freezing, a large number of those using freezers and low temperature rooms appear to remove samples at the treatment temperature once that temperature is reached in the monitored sample (e.g., Irving and Lanphear, 1967a, b; Gusta and Fowler, 1977). In many cases the samples are removed from the freezer while still at the minimum temperature, allowing them to thaw uncontrolled at laboratory or refrigerator temperatures.

The amount of damage that occurs in a freezing cycle is dependent on the low temperature level attained, on the rate of freezing and rate of thawing, and on the duration of the low temperature period. In many instances the separate effects are not distinguishable as, for example, in freezers without rate control where it takes samples longer to reach lower minimum temperatures. Equally, in instances where samples are moved from freezers to refrigerators or laboratory benches for thawing, the rate of thawing will be greatest with the lowest minimum temperature used. In general it is recognised that rapid rates of freezing and thawing and long periods of freezing increase the amount of damage in comparison with slower rates and shorter durations. Hence Aronsson and Eliasson (1970) observed greater damage with Scots pine frozen at rapid rates (> 150°C/hr) than at slow rates (5°C/hr). Weaver and Jackson (1969) found that a slow rate of freeze (4.5°C/hr) to —20°C caused substantially less damage to dormant peach buds than a more rapid freeze of 8.1°C/hr. Ashton (1958) obtained up to 27% less damage when *Eucalyptus regnans* F.Muell. seedlings were cooled slowly over a period of 14hr to —2°C than when rapid freezing was used. In both treatments an 8hr minimum temperature period was used, so although tissue would have been frozen for longer under the slow rate of freeze conditions, less damage was sustained. Sprague (1955) and Kilpatrick *et al.* (1966) demonstrated that cold-acclimated stolons of red and white clovers ,and roots and crowns of alfalfa, were injured less when cooled slowly (1.7°C/hr) than when cooled rapidly (2.2, 2.8 or 5.6°C/hr) to a freezing temperature of —9.4°C maintained for 18hr. Tissues cooled slowly withstood lower temperatures for a longer period of time than tissues cooled rapidly.

Several other investigators have shown that injury increases with the length of exposure to cold, particularly as the lethal temperature is approached (Greenham and Daday, 1960; Hudson and Brustkern, 1965; Thomas and Lazenby, 1968; Lorenzetti *et al.,* 1971; Rammelt, 1972; Pomeroy *et al.,* 1975; Gusta and Fowler, 1977). For example, Gusta and Fowler (1977) found with winter cereals that if the duration of frost was increased from 1 to 24 hours the LT_{50} temperature increased by 4°C. Similarly damage to radiata pine (Warrington, unpubl.) increased as the length of the freezing period increased. The rate of thawing can also influence the degree of injury. Gusta and 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/hr).

Cold rooms and freezer chambers have been used in laboratory studies with forest tree species in a number of different ways. For example, they have been effective in studying the influence of environmental variables, such as day or night temperatures or photoperiod, on the physiology and biochemistry of cold hardiness development (e.g., McGuire and Flint, 1962; Irving and Lanphear, 1967a; Glerum, 1973; Aronsson, 1975; Timmis and Worrall, 1975; Christersson, 1978; Harrison *et al.*, 1978; McCreary *et al.*, 1978). A further use of freezers is provided by Sakai and Wardle (1978) who showed a good agreement between cold tolerance and the geographical and ecological distribution of New Zealand trees and shrubs. Differences between conifers and dicotyledonous species were also readily identified. Ashton (1958) and Eldridge (1968) used a cold room to define a gradation in frost resistance between provenances of *Eucalyptus regnans* collected along altitudinal gradients. Similar work was carried out on *Eucalyptus viminalis* Labill by Paton (1972). Ashton (1958) stated, however, that differences obtained were relative and absolute frost resistance, assessed from field studies, was underestimated using the cold room. Whereas the emphasis in all of these studies was on defining relative differences in cold tolerance, measurement of absolute differences should be possible if the precautions outlined earlier in this review are carefully noted.

(b) *Field units:* Freezer chambers have also been modified for field use. Reid *et al.* (1976) describe a simple, inexpensive portable freezing cabinet with a programmable controller which could give linear cooling rates of 1 to 10°C/hr. It could maintain constant or cyclical temperatures and the programmes could be quickly changed. A range of temperatures down to —25°C was obtainable. Air temperatures inside the cabinet were spatially uniform within 1°C and followed a linear rate of change program within 2°C. At a constant low temperature the cabinet kept within 1.1°C. The chamber size of 0.82 m^3 was ideally suited for small plot and plant work so it could be effectively used in forest nursery beds to assess planting stock hardiness. Wiltbank and Rouse (1973) describe a small portable freezer designed for leaf freezing point determination on citrus.

Larger portable systems have been developed by Cooper and Gorton (1954) and by Scott and Spangelo (1964). The latter constructed a 3.2 × 4.7 × 2.8 m freezing enclosure which they could manually place over young apple trees. Refrigerated air supply was via flexible insulated air ducts from a separate equipment trailer. Using the programming cam developed by Voisey (1963) temperatures as low as —50°C could be achieved within 3-4 hr from an ambient of —7°C. Temperature variation within the test enclosure was ± 0.6°C and variation about the control point when operating at a steady test temperature was also ± 0.6°C.

(c) *Liquid nitrogen-based systems:* Chambers cooled using the latent heat of vapourisation of liquid nitrogen have also been constructed. Systems for laboratory use are described by Voisey and Andrews (1970), Quamme *et al.* (1972) and Weaver and Jackson (1969). Liquid nitrogen was used either directly for cooling biological samples or indirectly by cooling air (in a heat exchanger) that was then passed through the sample chamber. Heating could be included to accelerate warming. The working range

of the control systems described by Quamme *et al.* (1972) was from + 50°C to — 100°C. In Weaver and Jackson's design the rate of temperature decrease could be controlled with accuracy to ± 2°C/hr with a maximum spatial variation of ± 1°C. Temperatures down to — 30°C were tested. Voisey and Andrews (1970) claimed a better spatial and temporal variation across any shelf level in the cabinet (± 0.7°C) and also claimed a lower minimum (— 85°C). Control configurations suitable for chambers large enough to hold whole plants or small enough to fit on a microscope stage have been developed (Quamme *et al.,* 1972). Such equipment has been used, for example, to study the relationship between the occurrence of low temperature exotherms and the distribution of woody plant species in North America (George *et al.,* 1974).

Scott (1966) described a portable chamber (3.2 × 4.7 × 2.8 m) with an overhead spray-nozzle grid which distributed the liquid nitrogen uniformly across the chamber. Temperature was controlled within ± 1.5°C under all conditions (via an electronic controller) and was uniform throughout the chamber within ± 0.3°C. Initial reduction of temperature was rapid (for example, 0 to — 18°C in 5 min) after which the temperature was reduced at a constant rate of 11.1°C/min. The chamber was tested down to — 68°C. The advantages of such a design include low construction, maintenance and operating costs, as well as ease of portability compared with a mechanical refrigeration system (e.g., Scott and Spangelo, 1964). Although the effect of a nitrogen atmosphere on dormant fruit trees must be considered, Voisey and Andrews (1970) avoided the problem by placing samples in polyethylene bags.

2. *Controlled Environment Rooms for Providing Specific Types of Frost*

Whereas some research workers have used commercial cold rooms and freezer cabinets incorporating the various modifications outlined above, others have used commercial controlled environment chambers in an attempt to get reliable, reproducible frost conditions (e.g., Cochran and Berntsen, 1973). The refrigeration systems used in most controlled environment chambers, however, do not allow precise temperature studies below 4-5°C and few automatic defrost systems operate without causing a substantial rise in temperature during every defrost cycle (Downs, 1975). This latter problem can be overcome, in part, by installing two or more evaporators and improved defrosting devices. Other problems encountered when using standard controlled environment chambers below 0°C include sensor protection and effectiveness, lack of humidity control, and a limited range of temperature control. This has led to the construction of special low-temperature frosting rooms to meet the requirements of a low-temperature research programme.

Controlled environment rooms designed to produce specific kinds of frost are of two main types, viz., radiation frost rooms and advective frost rooms. Both frost types occur naturally. In radiation frosts, heat is radiated from the surface of the earth and its vegetation during still, clear nights, while advective frosts are caused by the mass movement of subfreezing air.

(i) *Radiation Frost Chambers*

In a radiation frost room the leaves and branches of experimental plants radiate to an absorber surface set at an appropriate night-time sky temperature. Details of room designs and use have been presented by Aston and Paton (1973), Ludlow and

Taylor (1974) and by Ivory and Whiteman (1978). Aston and Paton (1973) used a large, 3.04 × 3.04 × 2.44 m high, commercially prefabricated cold room with insulated walls and floor. The ceiling comprised a 20 cm deep enclosed tank of thick galvanised iron with a black painted underside that formed the absorber surface. An intermittent spray of liquid nitrogen from a perforated supply pipe inside the tank provided cooling. The temperature sensors used were platinum-in-glass thermistors and placement of these close to the leaf surface, together with short on-off cycling of a solenoid valve on the liquid nitrogen supply line, allowed close control of leaf temperature.

In the radiation frost room used by Ludlow and Taylor (1974) convective air movement, caused by cold air moving downwards from the absorber surface, was prevented by a polyethylene film, transparent to long-wave radiation, positioned between the surface and the plants. They claimed negligible air movement around the plants which were therefore cooled by radiative heat exchange. Aston and Paton (1973) used a similar technique in initial work, but as frosting of the film was considerable, they doubted whether its use was justified.

In Aston and Paton's room, temperatures at least as low as —70°C were achieved with a spatial and temporal variation of less than ± 0.13°C. The rate of cooling could be as high as 4°C/min. or as low as 0.1°C/hr. Although some workers have relied on manual controlling (e.g., Ludlow and Taylor, 1974), automatic controllers can be incorporated which allow control over the rates of cooling and thawing and the duration of freezing. Care must be taken in radiation frost rooms to ensure that ice crystals form on the leaf surface at freezing temperatures in order to reduce the tendency of tissue supercooling. Plants are usually sprayed with water at the start of the freezing cycle to ensure ice crystal formation.

With their radiation frost room, Aston and Paton (1973) were able to confirm results from an earlier study (Paton, 1972), that high levels of within-provenance and within-family variability occurred in *Eucalyptus pauciflora* Sieb. ex Spreng. and *E. viminalis*. Both types of variability were characterised by a low frequency of plants with intermediate injury which could have been due to chance supercooling under convective cooling in the cold room used earlier. Moreover, they suggested that because radiation values in the radiation frost room were similar to those in the field, frost damage values could be expected to be comparable.

Gill and Waister (1976) describe a similar system for simulating natural frosts on strawberry plants growing in the field. They used a portable open-bottomed chamber with an overhead plate over which liquid nitrogen was sprayed. The freezing rate was controlled in a similar way to the method described by Voisey (1963). In field use the variation of temperature with time in a zone adjacent to the plants was ± 1°C at any maintained temperature. The greatest air temperature gradient was within 10 cm of the cooling plate, spatial variation below this being less than ± 0.5°C.

Gill and Waister's data illustrate the variation that can occur in rates of heat loss from different parts of plants during a radiation frost. Temperatures at the top of the plant that were exposed directly to the night sky (or cold chamber ceiling) were 4-5°C cooler than those at the plant base (i.e., the strawberry crown). Hence, under radiation frost conditions, for differing parts within a plant, a family of cooling curves existed. The part of the plant that is important in the survival and subsequent growth of the plant is therefore of primary concern. Leaf damage may not be a useful indication of

the plant's ability to survive if buds are unaffected and survive low temperature conditions. Care must be taken therefore with the interpretation of results from various controlled freezing techniques and there is a constant need to compare controlled freezing test results with field observations to ensure that the controlled test results are relevant.

Simple portable enclosures which rely on the sublimation of solid carbon dioxide (dry ice) to provide cooling on the ceiling of an enclosure around plants growing *in situ* have been described by Paulsell and Lawrence (1963) and Glerum and Farrar (1966). Injury symptoms similar to those occurring under natural radiation frost conditions were obtained, but temperature fluctuations within these enclosures were great (Glerum *et al.,* 1966).

Radiation frost chambers are simple in concept and are straightforward to operate except for the sophistication of monitoring and control equipment. Special skills are required in their construction, maintenance, and operation in comparison with conventional cold room and freezer cabinet units. A ready supply of liquid nitrogen has also to be available and this can be expensive where intensive low temperature work is being carried out.

(ii) *Advective Frost Enclosures*

Robotham *et al.* (1978) describe a large 2.70 × 2.70 × 3.25 m high walk-in controlled environment room capable of reproducing precisely advective frost conditions. Air temperature can be controlled to ± 0.5 °C from + 15 °C maximum to — 20 °C minimum. Design features incorporate sequential defrosting of multiple evaporator coils, and automatic defrosting of control and monitor sensors, which enable low temperature conditions to be maintained for several hours without control loss. Maximum to minimum and minimum to maximum temperature changeovers are independently programmed and electronically controlled and rates of temperature change can be varied between 1° and 20 °C/hr. Air relative humidity is controlled; white frosts are produced using saturated low-temperature air, and black frosts are formed with cold air of low moisture content. High relative humidity conditions, which should minimise supercooling, have been shown to induce greater damage than low relative humidity conditions at the same temperature. However, varieties being screened were ranked fairly similarly when frosted at the two different humidities (Hacker *et al.,* 1974).

The advective frost rooms, unlike radiation frost chambers, allow the use of photosynthetic irradiance levels of up to 150-170 Wm^{-2} during frost conditions. In the field plants may be under daylight conditions for at least part of the low temperature period, and, in most cases, for the thawing period. As a consequence plants may absorb energy and thaw out more rapidly than would be indicated by screen temperature records. Light may also have a direct effect on physiological or biochemical processes through a direct interaction with low temperature (Rowley and Taylor, 1972).

Consistent with many controlled freezing studies, Robotham *et al.* (1978) provided a means of root temperature control by using soil heating cables at the base of insulation-covered pots to maintain soil temperatures above 0 °C if required. Other workers have used a range of techniques; Ashton (1958), Aston and Paton (1973), and Tanaka and Timmis (1974) packed pots in vermiculite, sawdust, or perlite to insulate them from cold air conditions; Timmis and Worrall (1975) and Clements and Ludlow (1977)

placed the roots in an insulated box; Schmid and Hackel (1977) grew fruit trees in a divided chamber where roots and shoots could be grown in temperatures differing by as much as 60°C; and Christersson (1978) maintained root temperatures at 20°C with the pot in air while the shoot was maintained at the required temperature while enclosed in a flask in a freezing bath. In situations where soil does become frozen, winter hardiness is as much dependent on root survival as on shoot survival, so root temperature control is considered essential in any frosting work.

A disadvantage of using advective frost conditions is that temperature gradients which develop in plant canopies (e.g., grass swards, dense nursery beds) under radiation frost conditions cannot be simulated. However, this may not be a significant constraint where individual, spaced plants are being studied. Their cost of construction and maintenance is high and both require skilled personnel. In this respect they are even more demanding than radiation frost rooms. Therefore they are likely to remain a specialised facility available only in a few centres which will require the experimental plants to be transported to them or to be raised in close proximity.

The advective frost rooms have been used in several studies with *Pinus radiata* and *Eucalyptus* spp. For example, the seasonal variation in frost tolerance of radiata pine has been examined by subjecting young field grown plants to controlled frosts at different times throughout the year (Rook *et al.,* 1974; Menzies, 1976; Green and Warrington, 1978). Radiata pine withstood —4°C frosts in summer with no damage and developed natural hardiness in the autumn with a sharp rise in frost tolerance to —7°C in May. Thereafter hardiness increased to a peak in late August when plants were only moderately damaged at —12.5°C. Loss of winter tolerance occurred rapidly during September (—10°C), and tolerance to —7°C frosts tapered off by November to the summer minimum. Seedlings raised under colder conditions at higher altitudes were able to tolerate frosts some 3°C colder throughout the year than seedlings raised in warmer, lower altitude nurseries (Menzies, unpubl.). The nature of damage and seasonal change in tolerance were consistent with field observations recorded over a number of years and sites.

Rook *et al.* (in press) compared the results obtained from screening 38 provenances of *Eucalyptus regnans* for frost tolerance in the field and in the advective frost rooms. Close agreement between seedling frost damage data from the field and the artificial frost room was found with estimated correlation coefficients of 0.8. Whereas the field study enabled the different provenances to be ranked according to their relative frost tolerance, results from the controlled environment rooms provided not only a similar relative ranking but also, by being able to provide three known levels of frost, allowed the absolute differences in frost tolerance between provenances to be quantified.

C: FREEZING BARS

A major problem with deep-freeze units or low-temperature controlled-environment rooms is that either a large number of units, or several repeat runs at different temperatures, are required if a range of low temperatures are to be examined. Setting the units at fixed temperature intervals can somtimes lead to insensitivity in the test if there are only small species differences in freezing sensitivity. Several workers have attempted to overcome these problems by designing low-temperature gradient bars

which allow study of freezing tolerance under stable, constant conditions covering a continuous temperature range.

Rowley *et al.* (1975) used a temperature gradient bar (107 cm long) made of insulated aluminium. This large (17,800 cm^3 volume), well-insulated mass enabled stability and linearity of the temperature gradient to be maintained during operation. The bar was designed to operate with a variable temperature differential of 3° to 15°C anywhere within the temperature range of 0° to —20°C. Thermocouples set into the bar at regular intervals allowed temperatures at treatment points to be monitored. Temperature control was maintained with a fixed cooling load at one end of the bar and with proportional heating at both ends. Because of their mass, temperature gradient bars require several hours to reach equilibrium and are usually operated overnight before treatments are commenced. Similar devices are described by Hodges *et al.* (1970) and Timbers and Hocking (1971).

Freezing bars allow a wide range of temperatures to be set up in one treatment run; the units are compact, compatible with a laboratory operation, and are inexpensive to operate and maintain. The disadvantage is that only a small part of a plant (e.g., foliage) is used. Several studies have shown that some parts of a plant are more tolerant to cold stress than other parts or to the intact plant as a whole (e.g., Horiuchi and Sakai, 1978). The dynamics of the frosting cycles used are also unnatural. For example, the tissue is abruptly placed at the treatment temperature at the start of a cycle, and at the end of a cycle is allowed to thaw out very rapidly to allow removal for injury assessment (usually by leachate electroconductivity methods). Normally samples are left on the bar for 18-24hr but shorter freezing periods of 2-4hr should be possible. Although these are major disadvantages they do not completely negate the use of freezing bars. Rather caution is required to ensure that evaluations on freezing bars are correctly interpreted in respect to field or low temperature frost-room observations. Rowley *et al.* (1975) found that the ranking of freezing sensitivity in leaves of C4 grasses as measured with the freezing bar agreed with visual observations of frost damage in the field during late autumn and early winter. The technique, of course, only assessed the freezing sensitivity of leaf tissue and the results could not be used to assess whole plant overwintering ability or growth at low temperatures.

DISCUSSION

The purpose of carrying out low temperature work differs between various disciplines. In some tree breeding programmes, the breeder merely wishes to separate the most tolerant cultivars or provenances from the least hardy and use the hardy ones in further breeding work. In tree establishment work it may be necessary not only to identify the most hardy plants but also to know and understand what levels of low temperature can be tolerated in order to match the most appropriate plants to sites with potentially high levels of cold temperature stress. The physiologist may be chiefly interested in the changes in hardening and dehardening that occur seasonally in many plants, and in correlating these changes with physiological, developmental, and biochemical alterations. In each of these cases there are important advantages of being able to carry out multiple temperature screening of plants for cold hardiness (i.e., have access to more than one stress temperature at any one time). Such screening

requires more than one freezing unit, or successive treatments through a single unit, or more than one field site. However, it does allow comparisons of plant types to be made with more confidence by identifying even small differences in plant hardiness. Exposing plants to a range of freezing temperatures can also allow differences in frost tolerance to be quantified. Single temperature screening, whether in the field or in a single freezing unit, can provide misleading results due to genotype/environment interactions unless large differences in frost hardiness are expected (Fowler *et al.,* 1973).

In most of the work carried out using artificial frost techniques plants are exposed only once to the frost treatment. Artificial frost techniques which allow repeated low-temperature cycles to be programmed, allow plants to be exposed to more than one frost cycle, which more closely follows a normal sequence of events in the field. Repeated freezing and thawing generally results in increased injury. For example, Green and Warrington (1978) showed that repeating damaging frosts several times increased the amount of damage incurred to radiata pine seedlings. This result is in agreement with work by Cochran and Berntsen (1973) on both lodgepole and ponderosa pine. Gusta and Fowler (1977) found that injury to winter cereals occurred at a higher temperature with each successive freeze but the most pronounced effect occurred after the first cycle.

Following the frosting of intact plants, or of plant samples, damage can be assessed by a number of different methods and related to frost temperature levels in various ways. Timmis (1976) summarises and reviews some of the physiological, chemical, electrical, and physical methods that have been used to assess damage and viability following a freezing stress. Visual injury assessment methods have also been extensively used, particularly where whole plants or seedlings have been stressed. For example, Menzies (1977) developed a system of visual damage assessment for radiata pine seedlings. The seedlings are assessed one month after frosting and scored on a 0 to 5 scale as follows: 0 = no damage, 1 = buds undamaged, needles reddening, 2 = buds may be damaged, 10 to 30% needles killed, 3 = 50% of needles killed, buds moderately damaged, 4 = 90% of needles killed, buds heavily damaged, 5 = seedling dead. Field tests have shown using this method that a seedling will recover from a damage rating of 2 or less, but consecutive winter frosts will kill a seedling that has a rating of 3 or above. Similar systems have been developed by Ashton (1958) with *Eucalyptus regnans,* Lapins (1962a, 1965) with apple and sweet cherry, and by Harrison *et al.* (1978) with red-osier dogwood (*Cornus stolonifera* Michx.).

A number of definitions have been used to classify the response of plants to a range of low temperature conditions. The minimum temperature at which 50% of a test population is killed is called the median lethal temperature (LT_{50}). Although the concept is useful, especially when dealing with relatively homogeneous material, such as needles or small seedlings, and has been used by a number of workers in forest tree studies (e.g., Siminovitch, 1963; McLeester *et al.,* 1968; Weaver and Jackson, 1969; Pomeroy *et al.,* 1970; Tanaka and Timmis, 1974; Timmis and Worrall, 1975; van den Driessche, 1976; Mexal *et al.,* 1979) it has the limitation of not distinguishing between populations that may have the same LT_{50} values but different temperature-versus-survival responses (Steponkus, 1978). In addition the LT_{50} may not reflect the survival potential of the material under study and a different LT value may have to be used to account for this. The temperature at which 100% damage or death occurs has been used by some workers (e.g., Irving and Lanphear, 1967a; Howell and Weiser,

1970; Chen and Li, 1976) to define the changes that occur in plants growing under hardening conditions. This has the same shortcoming as LT_{50} estimation where a precise test for tissue or plant death is required. This concept may be useful in some physiological work but the forester has little interest in severely damaged and weakened seedlings.

The lowest temperature to which plants can be exposed without being damaged has been termed the "frost hardiness temperature" (or "lowest survival temperature", Fuchigami *et al.,* 1971). This concept has been used by Glerum (1976) to define the frost-hardiness changes in white pine (*Pinus strobus* L.) and American larch (*Larix laricina* (Du Roi)Koch) in response to changing seasonal conditions. Rook and co-workers (unpublished) have also used this concept to define seasonal hardiness changes in radiata pine from different forest nursery sites around New Zealand.

CONCLUSION

It is clear that frost tolerance of forest planting stock can be accurately evaluated by a range of field and laboratory frosting techniques. All of the techniques described here have been successfully used in plant survival studies. No obvious standardisation of treatment procedures appears to have been adopted between different laboratories. Although most techniques appear to be useful for distinguishing differences in frost tolerance of plants, only those laboratory techniques which have full control of all aspects of the frost treatment will allow results to be extrapolated with confidence to the field. Where information on absolute differences in frost tolerance, rather than a relative ranking is required, the techniques must be able to provide, in addition to full control of all features, a range of known frost temperatures. Whereas the final test of cold hardiness of planting stock will remain its performance in the field, artificial frosting techniques are essential in providing known, reproducible low temperatures for improving our understanding of the mechanisms of cold hardiness and for quantifying differences in frost tolerances of forest tree planting stock.

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