

GENETIC VARIATION IN THE FROST HARDINESS
OF PINUS BANKSIANA LAMB. (JACK PINE)
IN NORTHWESTERN ONTARIO

Maria Davradou ©

A Graduate Thesis Submitted
In Partial Fulfillment of the Requirements
for the Degree of Master of Science in Forestry

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ABSTRACT

Davradou, Maria. 1991. Genetic variation in the frost hardiness of Pinus banksiana Lamb. (Jack pine) in northwestern Ontario. 102pp. Advisor : Dr. W.H. Parker

Keywords: frost hardiness; genetic variation; provenance variation; jack pine; symptoms of frost injury

To estimate the level and pattern of variation in frost hardiness, artificial freezing tests of 64 provenances of jack pine were conducted. The provenances originated from northern Ontario. Seedlings of the provenances were grown in a uniform environment in a shade house. Current-growth needles were collected in fall during three consecutive years, 1988 to 1990, and in mid-summer in 1990. Three test temperatures and a control were used for all freezing trials. Temperatures ranged from -19°C to -1°C and duration varied from three to one hours. Freezing injury was evaluated visually. Two way ANOVA indicated statistically significant provenance and provenance x temperature interactions. These results suggested that the tested jack pine provenances exhibited genetic variation in their development of frost hardiness and implied a certain risk in transferring seed from one environment to another. Differentiation among provenances could not be detected during early August 1990. Regression analyses examined the associations between various degrees of injury and climatic gradients. These analyses suggested that several selective forces, including precipitation and temperature, were partially responsible for differentiation among the tested provenances. Principal component analysis (PCA) of the data generated three significant principal components which accounted for approximately 60% of the total variation. Regression of PCA scores against climatic gradients also reflected adaptive variation. However, a number of provenances which originated from regions with low temperatures and very short frost-free periods showed no higher levels of frost hardiness than provenances from areas with longer frost-free periods and higher temperatures. Low levels of consistency were found among the different trials. Possible reasons for the observed inconsistencies were assumed to be i) weaknesses of the scoring technique, ii) the random effect of supercooling and, iii) the uneven distribution of temperature in the freezer.

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FORWARD

Every scientist is an agent of cultural change. He may not be a champion of change; he may even resist it, as scholars of the past resisted the new truths of historical geology, biological evolution, unitary chemistry, and non-Euclidean geometry. But to the extent that he is a true professional, the scientist is inescapably an agent of change. His tools are the instruments of change - skepticism, the challenge to established authority, criticism, rationality, and individuality.

Alexander Vucinich (bn. 1914)

Science in Russian Culture: A History to 1860. 1963 (Stanford, Calif: Stanford UP)

The most beautiful thing we can experience is the mysterious. It is the source of all true art and science.

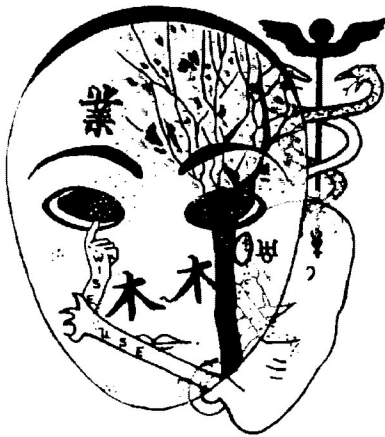
Albert Einstein (1879-1955)

What I Believe (1930)

You don't live in a world all alone. Your brothers are here too.

Albert Schweitzer (1875-1965)

On Receiving the Nobel Prize (1952)



Maria Davradou

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INTRODUCTION

Jack pine (*Pinus banksiana* Lamb.) is among the most widespread and most economically important species for planting and direct seeding in the Lake States and throughout much of the boreal forest of Canada (Yeatman, 1976; Rudolph and Yeatman, 1982). The species has a wide geographic distribution which includes broad ranges in latitude and climate (Yeatman, 1966). Although it occurs mainly in the boreal forest region of Canada (Rowe, 1959), it forms an important constituent of the Great Lakes-St. Lawrence forest region (Rudolf, 1958). Its natural range extends from 42° to 65° N latitude, from the Lake States of Wisconsin and Michigan to northcentral Quebec and northern Ontario, and 65° to 130° W longitude, from Nova Scotia to the Northwest Territories (Schantz-Hansen and Jensen, 1952; Mirov, 1967). Throughout its extensive range jack pine has been shown to possess considerable intraspecific variation (Giertych and Farrar, 1961; Mirov, 1967). A great number of investigations of such variability throughout the range of the species in Canada and the United States have been reported (Rudolph *et al.*, 1957; Giertych and Farrar, 1961; Schoenike, 1962; Rudolph, 1964; Maley, 1990).

According to Durzan and Chalupa (1968), climatic factors such as temperature, light, and moisture are crucial in determining seed production and plant distribution. Several provenance trials have shown that growth, phenology, susceptibility to diseases and survival of jack pine provenances are related to environmental parameters associated with latitude (photoperiod) and length and temperature of the growing season, and that the species shows clinal variation corresponding to these parameters (Yeatman, 1974; Yeatman, 1976; Skeates, 1978; Rudolph and Yeatman, 1982; Magnussen *et al.*, 1985).

Significant variation in potential growth among jack pine seed sources in Ontario has been shown by Skeates (1979). He emphasized the need to use good

quality, local seed to prevent damage due to frost and disease susceptibility. The same was shown by Holst and Yeatman (1959) who reported that jack pine provenances of Ontario origin varied significantly in height growth, and that a strong positive correlation existed between this variable and the length of the growing season of the area of origin.

Batzer (1961) showed significant differences in susceptibility to attack by the pest, white-pine weevil (Pissodes strobi (Peck)), existed among 17 jack pine seed sources from the Lake States. King and Nienstaedt(1965) observed differences in susceptibility to the fungus Hypodermella ampla Dearn., a parasite which causes defoliation to the species, among 29 seed sources from Minnesota, Wisconsin and Michigan.

Yeatman (1976) strongly recommended the use of the best regional seed sources especially for regions which use large quantities of seed, or at least, seed sources which are as good as that of the original stand so that the success of jack pine regeneration programs could be assured. Several other authors also stressed the importance of matching the suitable seed source with the site even within the natural range of jack pine in order to increase biomass production of the species (Batzer, 1961; Stevens and Wertz, 1971; Zavitkovski *et al.* ,1981; Strong and Grigal, 1987).

Schantz-Hansen and Jensen (1952; 1954) found variation among jack pine provenances in winter injury in a seed source test at Cloquet, Minnesota, after the severe 1947-48 winter. According to Yeatman (1976) frost hardiness is vital for the survival and growth of planted jack pine in climates where freezing occurs, such as the colder boreal climates of Canada.

Yeatman and Holst (1972) also pointed out the importance of cold hardiness by making the following statement "cold hardiness is the first criterion to be considered when selecting seed for reforestation in the boreal climates of Canada" (p.30). This is consistent with the conclusion drawn by Rudolph and Yeatman (1982)

that the greater the environmental differences between seed origin and planting site, the greater the risk of winter injury and susceptibility to disease.

The degree of cold hardiness of woody plants is strongly related to the minimum temperature over their distribution range (Flint, 1972; Sakai and Weiser, 1973). A clarification of the mechanism of frost damage and the relation between frost resistance and climate conditions of the origin area among ecotypes and climatic races of widely ranging species could be very useful in tree improvement programs (Sakai and Okada, 1971; Sakai and Weiser, 1973; Rehfeldt, 1980). Rehfeldt (1978; 1979) advanced a sound argument that the understanding of the ecological genetics of a species should be the base of any program for tree improvement. He added that in order to control the distribution of seed for reforestation, the ecological adaptations reflected by the differentiation of populations in cold hardiness should not be overlooked. Despite the demonstrated importance of frost hardiness on successful tree improvement programs and artificial regeneration, only a limited amount of information is available on the cold hardiness of jack pine.

Morgenstern (1979) pointed out that although seed zones in northern Ontario have not been adequately tested, there were indications of genetic variation within them. By referring especially to jack pine he indicated that a better distribution of seed stands across all seed zones is necessary in order to avoid a narrowing of gene pool of the species.

Climate of Northern Ontario

Northern Ontario is situated in central Canada. The central belt of Northern Ontario is a boreal forest region in which the major tree species are: black spruce (*Picea mariana* (Mill.) B.S.P.), balsam fir (*Abies balsamea* (L.) Mill.), tamarack (*Larix laricina* (Du Roi) K. Koch), aspen (*Populus tremuloides* Michx.), jack pine

(*Pinus banksiana* Lamb.) and white birch (*Betula papyrifera* Marsh.). The climate of this area is classified as a modified continental type climate (Chapman and Thomas, 1968). It is characterized by a long winter and a short summer as well as by sharp contrasts between the seasonal temperatures, day and night temperatures, and day to day temperatures (Hearn, 1981). This type of climate is due mainly to the proximity of the Great Lakes to the south, as well as, to a lesser degree, the presence of Hudson Bay on the north (Chapman and Thomas, 1968). The greatest differences in climate are found in winter minimum temperatures and lengths of the growing season (Yeatman and Morgenstern, 1979). Considerable local variations exist within the region when considering the occurrence of frosts. This is mainly the result of different types of weather, varied topography and type of soil, type of vegetation, and the existence of small lakes and clearings (Heggie, 1972). The different timing of the last frost in spring and the first frost in fall is another important characteristic of this region (Chapman and Thomas, 1968; Heggie, 1972).

Objective

The objective of the present study was to assess the level and pattern of variation in cold hardiness of jack pine from 64 provenances located in northern Ontario. This study forms part of a short-term program of jack pine provenance research whose goal is to show patterns of adaptive variation which are useful in establishing seed zones (Parker, 1992). The above program follows the experimental approach applied by Rehfeldt (1984). His research engages three different tests : short-term (3 to 5 years) growth and phenology field tests, a greenhouse phenological test, and a laboratory cold hardiness test conducted early in September. The level of genetic variation among provenances, estimated from statistics provided by analyses of variance and regression techniques against climatic gradients, is used to create seed transfer guidelines among the tested provenances. The results of this

study should increase the knowledge of geographic variation within jack pine and consequently aid the development of seed transfer guidelines in northern Ontario.

LITERATURE REVIEW

NATURE OF FROST HARDINESS

Frost hardiness of a seedling can generally be defined as the lowest temperature to which a seedling or a tree can be exposed without experiencing irreversible damage (Glerum, 1976; Glerum, 1985; Johnson and Gagnon, 1988). The process which determines the response of a tree to cold, either by increasing or by decreasing its resistance, is called the frost hardiness process (Levitt, 1980; Glerum, 1985). Numerous biochemical and physiological processes are associated with frost hardiness (Glerum, 1976; Steponkus, 1978) which make its understanding very difficult (Levitt, 1980). As the frost hardiness process enables trees to resist low temperatures, it is essential for the survival of trees growing in temperate and cold climates where freezing occurs (Sakai, 1970b; Glerum, 1976; Menzies *et al.*, 1987).

Two types of freezing are known to occur in plants, and they are distinguished by the location of ice formation in the plant tissues : intracellular and extracellular freezing. Intracellular freezing occurs when water freezes inside the cell and is considered to be lethal. Extracellular freezing occurs when water freezes outside the cells in the intercellular spaces and may or may not be lethal depending on the hardiness of the plant. Extracellular freezing is predominant in nature while intracellular freezing infrequently occurs under natural conditions (Mazur, 1969; Olien, 1967; Glerum, 1976; Glerum, 1985). Although ice formation occurs in both hardy and nonhardy tissues, only the former are able to survive (Glerum, 1985). Several authors agree that the fundamental cause of freezing injury is the dehydration of the cell which leads to membrane disruption (Lyons *et al.*, 1979; Steponkus, 1984; Glerum, 1985;).

Frost hardiness changes noticeably in relation to stages of development and in response to several environmental factors (Yeatman, 1966). Temperature and light (photoperiod) are the two main environmental factors which control the development of freezing tolerance in plants (McGuire and Flint, 1962; Scheumann and Bortitz, 1965; Jonsson *et al.*, 1980). Other factors, such as moisture and nutrients, must be adequate in order for the plant to harden maximally on exposure to hardening levels of temperature and light (Levitt, 1980). Fraser and Farrar (1957) concluded that a prolonged lack of water during summer has an adverse effect on hardiness. Although the effects of nutrients on frost hardiness are questionable (Glerum, 1985), several reports show that full hardening is not obtained in the presence of excess nitrogen or of insufficient potassium, phosphorus or calcium (Levitt, 1980).

According to Sakai (1970a) the freezing resistance in hardy plants shows a noticeable periodicity through the year. Levitt (1980) also pointed out the importance of seasonal changes by stating that, even in the most hardy species, the tolerance varies from a minimum value during spring growth to a maximum one in midwinter. Sakai (1974) noticed that the hardiest trees maintain this maximum hardiness throughout the winter and Tumanov *et al.* (1976) added that in less hardy trees the hardening capacity is reduced as the winter advances. Pomerleau and Ray (1957) stressed the importance of this seasonal change claiming that a light summer frost can cause damage in conifers which are considered to be among the most hardy of plants.

The frost hardiness process, under natural conditions, proceeds in two or three phases in woody plants native to temperate zones (Tumanov and Krasavtsev, 1959; Weiser, 1970; Glerum, 1973; Glerum, 1985). The first phase occurs in early fall and is associated with the cessation of growth and development (Glerum, 1985). This is in agreement with Weiser (1970) who believed that the first phase of

acclimation is induced by short days (decreased photoperiod). The second phase is induced by low temperatures below 0° C, and it is in this stage that large increases in frost hardiness occur (Weiser, 1970; Glerum, 1985). Rehfeldt (1979) reported that "autumn frost injuries occur during the first phase of cold acclimation when phenological events are not synchronized with the local climate; injuries occur during the second phase when dormant tissues have failed to harden sufficiently to withstand the minima of autumn and winter" (p.1). Sakai (1965) referred to extremely hardy species and proposed a third phase of hardening which allows plants to survive temperatures lower than usually found under natural conditions. Weiser (1970) considered this kind of hardiness as being lost in a limited time. A different third stage of hardening was described by Rehfeldt (1989). According to him, the third phase of the frost hardiness process occurs when the plants are becoming physiologically ready for spring.

FREEZING TESTS

Frost hardiness can be estimated either in field trials where plants are subjected to natural frosts or in controlled freezing tests (Sakai, 1970b; Hallam and Tibbits, 1988). Field trials have major limitations (Levitt, 1980; Warrington and Rook, 1980; Hallam and Tibbits, 1988). The unpredictability of the field conditions, the need for large, uniform sites and the difficulty of distinguishing the effects of frost from the other environmental factors are some of the problems which led scientists to the development of alternative, faster and more accurate methods of estimating the degree of frost hardiness of plants (Levitt, 1980; Warrington and Rook, 1980; Glerum, 1985). A concise and comprehensive summary of the various controlled freezing techniques has been provided by Warrington and Rook (1980).

Freezing chambers in which the hardiness of plants can be determined were first introduced by Harvey in 1918 (Levitt, 1980). This technique was highly

improved by Swedish investigators and artificial freezing tests have been used for many different kinds of plants since then (Levitt, 1980). According to Glerum (1985), uniformity of the temperature within the chamber should be obtained prior to any frost hardiness testing. Liquid baths (Wessel and Hermann, 1969; Christersson, 1978), wide-mouthed, large vacuum bottles known as Dewar flasks (van Huystee *et al.*, 1967; McLeester *et al.*, 1969; Howell and Weiser, 1970) and fans (Rehfeldt, 1980) have been used to resolve the problems of temperature fluctuation.

Cams or electronic controllers have been used in order to control the rates of freezing and thawing (Glerum, 1973; Tanaka and Timmis, 1974; Timmis and Worrall, 1975; van den Driessche, 1976). Glerum (1985) discussed the effects of the rate of freezing, the rate of thawing and the duration of the minimum temperature exposure. He noted that although rapid rates of freezing, more than 6° C/h, and long periods of freezing, more than 24 hours, considerably increase the amount of injury, the rate of thawing can be faster than the rate of freezing without causing any significant damage. These observations are consistent with the conclusion drawn by several other authors (Ashton, 1958; Aronsson and Eliasson, 1970; Gusta and Fowler, 1977). Timmis (1977) studied the susceptibility of male and female buds of Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) to low temperatures and used a rate of freezing of 5° C/h and a duration of the test temperature of 2 hours. Timmis and Worrall (1975) investigated cold acclimation in Douglas fir during germination, active growth and rest. They used cooling rates of 7° C/h and warming rates of 20° C/h. Recently Joyce (1987) also used a rate of freezing of 5° C/h when he studied cold hardiness of eastern larch (*Larix laricina* (Du Roi) K. Koch) originating from northern Ontario.

Levitt (1980) emphasized the value of artificial freezing tests observing that they have usually been found to give excellent agreement with winter survival in the field. Reports of significant correlations between field estimates and laboratory

estimates of frost hardiness in coniferous species have been made for : Pseudotsuga menziesii (Beissn.) Franco var. glauca (Rehfeldt, 1979), Pinus contorta Dougl. (Jonsson et al., 1980), Abies sachalinensis Mast. (Eiga and Sakai, 1984), and conifers in general (Sakai and Okada, 1971).

Liquid nitrogen-based techniques have also been used for frost hardiness assessment. Liquid nitrogen was used either directly for cooling samples or indirectly by cooling air which was then transferred to the freezing chamber (Warrington and Rook, 1980). Weaver and Jackson (1969) give a detailed description of a liquid nitrogen freezing chamber and its related equipment. They reported a rate of freezing of 2° C/h, with a maximum variation of $\pm 1^{\circ}\text{C}$. Their system was tested at temperatures down to -30°C . Other researchers have tested temperatures down to -85°C (Voisey and Andrews, 1970). Advantages and disadvantages of this method are discussed by Scott (1966). Several authors studied the degree of frost hardiness of conifers using the liquid nitrogen method (Sakai, 1960; 1983; Sakai and Otsuka, 1970; Sakai and Weiser, 1973).

FREEZING INJURY ASSESSMENT TECHNIQUES

Two methods are mainly used for assessing cold hardiness : visual evaluation and electrolytic methods (Ritchie, 1984; Glerum, 1985). Visual evaluation is a qualitative method which is used for the estimation of the frost injury on plant tissues such as buds, needles and cambium. Depending on the time of the year when the tissue is being tested for frost hardiness, a valid evaluation can be made from 3 to 10 days after freezing (Jonsson *et al.*, 1980; Glerum, 1985). Two characteristics of this method are considered weaknesses by Levitt (1980); i.e., the subjectivity of estimating injury and the considerable time which elapses between the test and the evaluation.

Browning, as a criterion for rating frost injury, has been successfully used with alpine plants (Sakai and Oztuka, 1970) and with several North American tree species (Sakai and Weiser, 1973). Sakai (1970b) investigated the degree of freezing resistance of several coniferous species and used the browning of various tissues, stems, leaves and twigs as a sign of frost damage. The same author (1983) studied the differences in frost hardiness among a number of coniferous families and genera originating in different parts of the world. He used the extent of browning of twigs as the criterion of measuring the degree of frost injury.

Wilner (1962) provided a concise and comprehensive description of the electrolytic methods for evaluating the frost hardiness of plants. The electrolytic conductivity method, originated by Dexter in 1932 (Levitt, 1980), is assumed to be a quantitative measure of the amount of cell membrane damage which has occurred in response to freezing (Glerum, 1985). This method is mainly used on shoot tips or needles and requires 3 days to complete (Colombo *et al.*, 1984). The conductivity measurements are usually expressed as either a ratio or 'index of injury' (Johnson and Gagnon, 1988). The 'index of injury', which has been introduced by Flint *et al.* in 1967 is a scale where a value of zero is given to the undamaged sample and a value of 100 to the completely damaged one; thus, the release of electrolytes is expressed as a percent (Glerum, 1985). According to Colombo *et al.* (1984) the advantages of the 'index of injury' are the independence of the statistic from sample volume and seasonal changes in the quality of free electrolytes released by unfrozen tissue. The electrolytic conductivity method has been used by a number of workers on a range of woody species which include *Pinus radiata* D. Don (Green and Warrington, 1978), *Eucalyptus delegatensis* R.T. Baker (Webb *et al.*, 1983), *Pinus silvestris* L. (Aronsson and Eliasson, 1970), *Pseudotsuga menziesii* (Mirb.) Franco (van den Driessche, 1969a;b).

Glerum (1980) reviewed the theory and application of the measurements of the electrical impedance of plant tissues to frost damage evaluation. This method has been strongly supported by Glerum (1973) and Greer (1983). Wilner (1962) stressed the importance of the electrolytic methods and noted that when these techniques are used correctly they can be as dependable as prolonged field survival tests. According to Green and Warrington (1978) a good agreement exists between relative electroconductivity measured shortly after a freezing test and longer term development of visible damage symptoms. Burke *et al.* (1976) discussed the probability of overestimating frost hardiness under certain conditions with testing tissue samples, as small samples of plant tissue tend to supercool more than whole plants.

FROST HARDINESS USED TO ESTIMATE GEOGRAPHIC AND ECOTYPIC VARIATION

Frost hardiness of different geographic provenances representative of a range of habitats has been examined in a large number of species. A common method of studying intraspecific variation is the uniform environment plot (Sakai and Larcher, 1987). There are several approaches to this methodology.

Flint (1972) studied the frost hardiness of twigs of young trees of Quercus rubra L. grown on a single site from seeds representing 38 different geographic origins. He found that variation among provenances was strongly related to latitude of the place of origin. Trees from colder provenances hardened more rapidly than those from warm regions. Frost hardiness was strongly related to the average annual minimum temperature of the place of origin. Average annual minimum temperature, extreme minimum temperature and length of the frost-free period were strongly intercorrelated and also highly correlated with the latitude of the provenance.

Alexander *et al.* (1984) examined the cold hardiness of stem sections of white ash (Fraxinus americana L.) from 10 geographic origins in eastern North America. They reported that variation in cold hardiness was related to latitude of the place of origin. More specifically, they found that northern provenances were more frost resistant than the southern ones in autumn and winter. In an experiment with Liquidambar styraciflua L. provenances from United States, Mexico and Central America, Williams and McMillan (1971) found photoperiod control of frost hardiness. It was demonstrated that the level of frost hardiness was greatest in northern origin provenances. According to Smithberg and Weiser (1968), photoperiod initiates a series of physiological changes involved in cold acclimation. In their study of red-osier dogwood (Cornus stolonifera Michx.) clones from 21 locations representing the natural range of the species, they observed that climatic races from northern sites were acclimatized to cold stress in the fall prior to any freezing temperature.

Mergen (1963) conducted provenance experiments on variation in eastern white pine (Pinus strobus L.). Frost hardiness was tested along with several morphological and physiological characteristics to determine the patterns of variation of the species. He concluded that northern sources were less sensitive to freezing than the southern ones. In an experiment with 100 provenances of black spruce (Picea mariana (Mill.) B.S.P.), Morgenstern (1978) also found that northern provenances were more frost resistant than southern provenances. Campbell and Sorensen (1973) found that frost hardiness in western American Douglas-fir (Pseudotsuga menziesii Mirb. Franco) provenances was correlated with latitude. Joyce (1987) studied the adaptive differentiation in frost hardiness of 66 populations of eastern larch (Larix laricina (du Roi) K. Koch) in northern Ontario. He found that the northern and western provenances were the most hardy; provenances from southwestern and eastern Ontario exhibited lower levels of hardiness.

The influence of the geographic origin on the frost hardiness of Pinus contorta Dougl. was determined by Jonsson *et al.* (1980) in a study of twelve populations with a distribution of 62° to 47° N and elevation 300m to 1000m. Both the northern provenances and those from high elevations were characterized by an early development of frost hardiness. They concluded that photoperiod was the main factor governing frost hardiness.

The relationship between cold hardiness of Pinus contorta Dougl. provenances and environmental gradients has been studied in a number of experiments conducted by Rehfeldt (Rehfeldt 1980; 1983a; 1985a;b;1986a;b). Freezing tests were conducted to study cold acclimation in 2-year-old seedlings representing 30 populations of P. contorta from the northern Rocky Mountains (Rehfeldt 1980). It was found that 78% of the variance in hardiness among provenances was attributable to elevation and geographic region of the seed origin. Freezing tests were also conducted to follow frost hardiness in 4-year-old seedlings representing 28 populations of P. contorta from northern Idaho (Rehfeldt 1983a). Adaptive variation was strongly related to elevation of the seed origin.

Rehfeldt (1985a) did similar experiments with P. contorta from the Wasatch and Uinta Mountains of Utah and found that 77% of the variance among the provenances was related to the elevation and geographic locations of the seed source. The same relationships were found by Rehfeldt (1985b) in P. contorta provenances from central Idaho. Similar clinal patterns of adaptive variation have been found by Rehfeldt (1986a) in 64 provenances of the same species from the same area. He reported that 61% of the variance among provenances was related to the elevation and geographic location of the seed source. In another experiment with 60 provenances of lodgepole pine (P. contorta var. latifolia Engelm.), Rehfeldt (1986b) again found adaptation to the elevation and geographic location of the place of

origin. Provenances from west-central regions, eastern Idaho, and western Wyoming exhibited the lowest frost hardiness.

Frost hardiness of Douglas-fir (*Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco) was also thoroughly examined in a series of studies conducted by Rehfeldt (Rehfeldt 1978; 1979; 1982a; 1983b). Rehfeldt (1978) examined growth potential, phenology and frost hardiness in 5-year-old seedlings representing 18 populations of Douglas-fir from the northern Rocky Mountains. He found a distinct differentiation of populations into 3 provinces. One included provenances from cool environments, regardless of geographic origin, while the other two included provenances from warmer environments. Variation in cold hardiness in 2-year-old seedlings from 51 provenances of Douglas-fir originating mainly from northern Idaho and eastern Washington was also studied by Rehfeldt (1979). He observed that the variation in cold hardiness was strongly related to geographic and ecologic parameters of the place of origin. He stated that provenances originating from high latitudes and high elevations exhibited the highest levels of cold hardiness.

Rehfeldt (1982a) did similar experiments with 1-year-old seedlings from 54 populations of the same species from Western Montana. He demonstrated that at a constant elevation, frost hardiness increases northward in the western zone and southward in the eastern one. Rehfeldt (1983b) did experiments with 3-year-old seedlings from 74 populations of Douglas-fir from central Idaho. By examining growth, phenology and cold hardiness he concluded that the genetic variation among populations was closely related to the elevation, geography, and climate of the seed place of origin.

MATERIALS AND METHODS

The seedlings

Sixty four populations of jack pine were selected from the area around Lake Nipigon. The locations of the sources studied are shown in Figure 1 and tabulated in Table 1. Interpolated climatic data of the seed sources are presented in Table 2. Detailed explanations of how each climatic record was interpolated are given by Maley (1990). Between late May and early August, 1987, ten trees were selected within each site. These trees were separated by at least 20m. Criteria for the selection of the study area, for the site selection, and for the selection of the individual trees are given in detail by Maley (1990).

The seedlings were grown from seeds bulked by provenance which were sown in small Ferdinand containers with a mixture of peat and vermiculite at the end of October 1987 by Maley.(1990) Between March 15 and March 25 of the following year the seedlings were transplanted to larger Ferdinand containers. On May 30th, 1988, the seedlings were placed in a shadehouse and finally they were transplanted to pots with the same soil mixture at the end of April 1989. A mixture of nitrogen, phosphorous and potassium (1:1:1) was applied to all seedlings on July 1989. The seedlings were grown outdoors where they naturally developed frost hardiness.

Freezing tests

Ten seedlings per provenance were sampled on 7 dates : 26 September 1988; 31 August, 7 September and 17 September 1989; 19 July, 3 August and 20 September 1990. At each date needles were removed from the current growth from seedlings representing each site. All the freezing tests consisted of removing

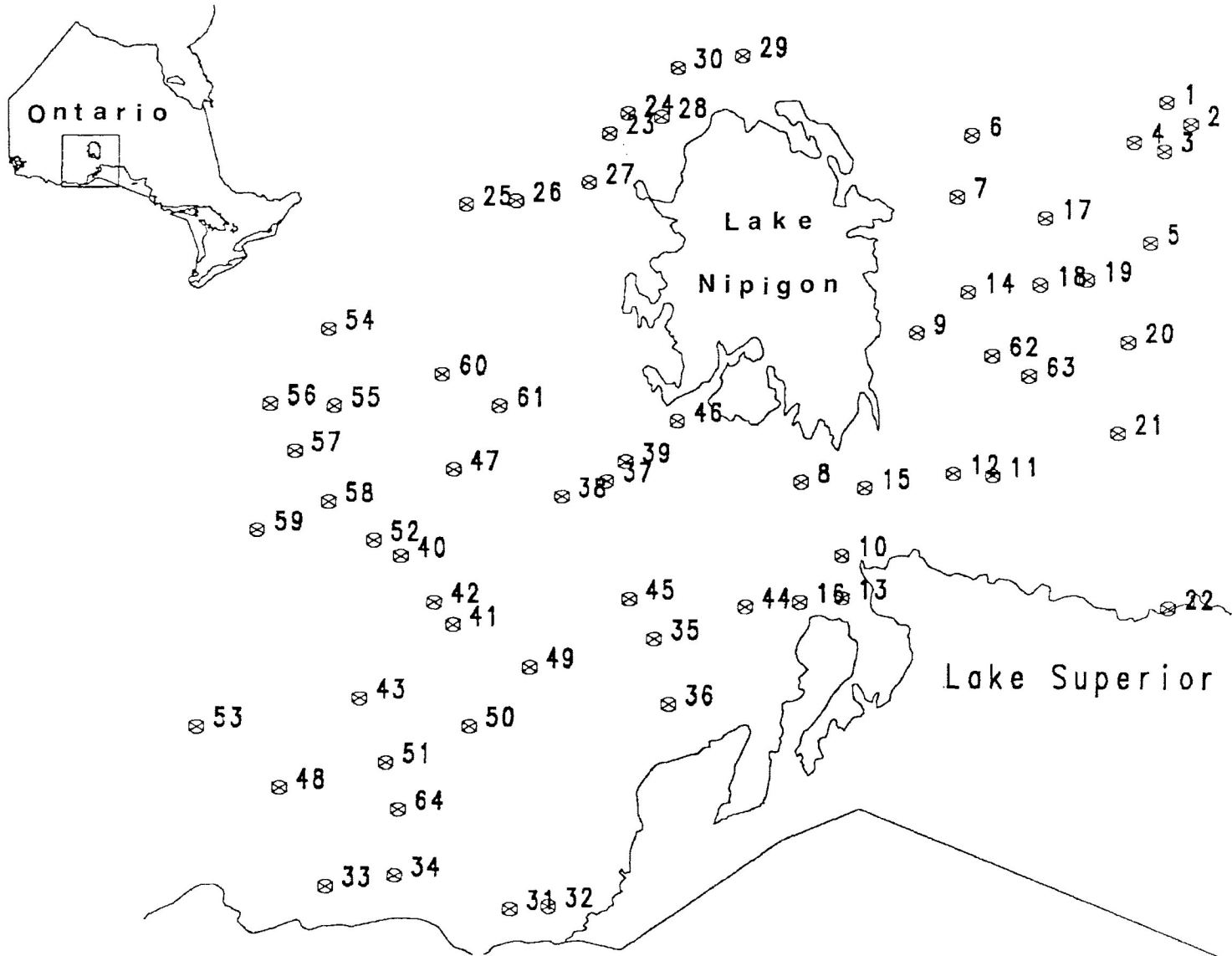


Figure 1. Study area and locations of jack pine provenances (after Parker 1992)

Table 1. Latitude, longitude and elevation for each *Pinus banksiana* collection site.

SITE NO	LATITUDE	LONGITUDE	ELEVATION (m)
1	50°12'	86°52'	1050
2	50°07'	86°47'	1080
3	50°03'	86°54'	1150
4	50°05'	87°01'	1100
5	49°48'	87°00'	1150
6	50°09'	87°39'	1050
7	49°59'	87°44'	1050
8	49°11'	88°25'	950
9	49°37'	87°57'	1050
10	49°01'	88°20'	950
11	49°12'	87°43'	1500
12	49°13'	87°52'	1400
13	48°54'	88°21'	650
14	49°43'	87°44'	1150
15	49°12'	88°13'	800
16	48°54'	88°31'	900
17	49°54'	87°24'	1100
18	49°43'	87°27'	1100
19	49°43'	87°16'	1100
20	49°33'	87°10'	1250
21	49°17'	87°13'	1300
22	48°47'	87°06'	900
23	50°16'	89°03'	1200
24	50°18'	89°01'	1150
25	50°04'	89°42'	1450
26	50°02'	89°29'	1350
27	50°07'	89°13'	1100
28	50°17'	88°53'	1050
29	50°26'	88°32'	1050
30	50°27'	88°42'	1050
31	48°05'	89°47'	1100
32	48°10'	89°37'	1250
33	48°14'	90°30'	1700
34	48°14'	90°11'	1600
35	48°50'	89°06'	1550
36	48°39'	89°04'	1500
37	49°17'	89°14'	1350
38	49°15'	89°25'	1450
39	49°20'	89°09'	1150
40	49°07'	90°03'	1550
41	48°55'	89°53'	1600
42	48°59'	89°57'	1450
43	48°44'	90°15'	1600

Table 1. (Continued).

SITE NO	LATITUDE	LONGITUDE	ELEVATION (m)
44	48°54'	88°44'	1100
45	48°57'	89°11'	1500
46	49°26'	88°56'	750
47	49°21'	89°50'	1500
48	48°30'	90°36'	1600
49	48°47'	89°36'	1500
50	48°38'	89°51'	1450
51	48°35'	90°09'	1500
52	49°18'	90°11'	1600
53	48°41'	90°54'	1600
54	49°46'	90°17'	1450
55	49°33'	90°17'	1550
56	49°34'	90°32'	1600
57	49°26'	90°26'	1550
58	49°17'	90°20'	1550
59	49°13'	90°37'	1550
60	49°37'	89°51'	1450
61	49°31'	89°38'	1500
62	49°32'	87°40'	1350
63	49°28'	87°32'	1450
64	48°25'	90°08'	1450

Table 2. Interpolated climatic records for each *Pinus banksiana* collection site.

SITE	JUMAX	JAMIN	MNDLY	EXMAX	EXMIN	PCIPSN	PCIPTL	DDH	DDG	FFDYS	FFS	FFA
1	22.9	-26.0	-0.6	35.1	-46.9	290.0	807.8	6819.1	1167.6	74.9	166.0	240.9
2	22.9	-26.0	-0.6	34.9	-46.8	291.4	809.3	6820.0	1166.4	74.4	166.2	240.6
3	23.0	-26.2	-0.6	34.8	-47.0	292.8	806.7	6815.1	1172.9	73.7	166.4	240.1
4	23.0	-26.3	-0.6	34.9	-47.2	294.7	804.1	6816.3	1173.5	74.3	166.2	240.5
5	23.5	-27.3	-0.2	34.0	-48.1	281.9	801.3	6679.7	1279.3	64.0	168.5	232.5
6	23.2	-27.0	-0.7	35.8	-48.8	291.8	780.9	6851.0	1168.1	67.2	169.6	236.8
7	23.4	-27.3	0.2	34.0	-48.3	335.7	784.0	6530.3	1273.5	80.0	163.0	243.0
8	23.9	-22.8	1.6	39.5	-46.8	245.9	792.2	6050.9	1379.3	98.8	155.1	253.7
9	23.4	-26.6	0.2	34.0	-48.3	335.7	784.0	6530.3	1273.5	80.0	163.0	243.0
10	23.2	-22.1	1.6	37.5	-44.4	208.6	768.2	6021.3	1317.4	98.9	155.3	254.4
11	22.4	-23.8	0.9	33.0	-43.4	365.7	798.8	6272.2	1234.0	94.2	155.9	251.0
12	22.8	-23.7	1.2	34.1	-44.4	268.1	797.2	6173.3	1281.1	95.2	155.9	251.8
13	22.7	-22.6	1.4	35.6	-43.0	180.2	716.3	6096.1	1258.5	88.6	159.9	248.7
14	23.4	-27.0	0.0	33.9	-48.7	338.9	785.3	6601.1	1263.9	77.1	164.5	241.6
15	23.6	-22.4	1.7	38.7	-46.6	243.7	801.7	6002.2	1359.4	103.1	153.8	257.0
16	23.0	-23.0	1.3	36.1	-43.4	108.7	703.5	6117.7	1277.5	85.7	161.2	246.8
17	23.2	-27.0	-0.4	34.2	-48.4	322.6	792.4	6732.3	1221.2	74.1	166.2	240.2
18	23.4	-27.1	0.0	33.8	-48.4	320.6	789.4	6617.6	1270.0	73.7	165.5	239.1
19	23.4	-27.2	-0.1	33.9	-49.0	303.1	792.6	6801.0	1281.7	69.7	166.6	236.3
20	23.5	-27.9	-0.5	35.7	-46.5	260.5	706.1	6801.0	1247.3	63.6	169.9	233.5
21	22.5	-26.0	-0.2	34.9	-46.5	236.9	716.0	6669.5	1184.1	75.8	164.2	240.4
22	19.0	-19.6	1.3	32.6	-42.1	214.4	861.7	6108.3	1090.3	112.4	144.7	256.9
23	23.6	-28.2	-1.1	38.4	-49.9	258.8	737.5	6985.6	1136.1	50.3	177.7	228.0
24	23.6	-28.2	-1.1	38.4	-50.0	259.7	737.1	6990.8	1130.8	49.9	178.1	228.0
25	23.6	-27.7	-0.9	37.8	-45.3	257.1	753.3	6909.9	1181.9	61.2	172.1	233.3
26	23.6	-27.9	-0.9	38.0	-46.7	256.9	746.8	6928.9	1165.5	56.1	174.6	230.7
27	23.6	-28.0	-1.0	38.1	-48.5	259.0	741.4	6946.4	1149.4	52.1	176.6	228.7
28	23.6	-28.2	-1.1	38.3	-50.6	262.3	738.6	6990.3	1130.2	50.0	178.0	228.0
29	23.5	-28.2	-1.1	38.2	-51.7	266.5	742.4	6990.8	1119.9	50.8	178.0	228.8
30	23.6	-28.2	-1.1	36.6	-51.7	262.2	737.2	6990.8	1119.3	49.2	178.8	227.9
31	23.5	-20.0	2.7	36.5	-39.9	217.3	692.5	5653.8	1449.0	125.6	141.4	268.9
32	23.4	-19.7	2.8	36.5	-39.2	209.4	696.8	5612.9	1448.8	121.8	142.6	264.3

JUMAX = maximum June temperature; JAMIN = minimum January temperature; MNDLY = mean daily temperature; EXMAX = extreme maximum temperature; EXMIN = extreme minimum temperature; PCIPSN = precipitation from snow (cm); PCIPTL = total precipitation (cm); DDH = heating degree days; DDG = growing degree days; FFDYS = frost free days; FFS = date of last spring frost; FFA = date of first fall frost.

Table 2. (Continued).

SITE	JUMAX	JAMIN	MNDLY	EXMAX	EXMIN	PCIPSN	PCIPTL	DDH	DDG	FFDYS	FFS	FFA
33	23.6	-22.8	1.7	36.1	-43.8	304.2	705.4	6012.4	1373.6	142.7	135.0	277.7
34	24.0	-23.6	1.3	36.4	-45.4	348.6	709.3	6120.4	1330.1	152.0	130.7	282.7
35	24.0	-24.1	1.1	37.1	-42.8	217.1	725.1	6216.8	1363.4	88.4	157.9	246.2
36	24.1	-22.9	1.7	37.1	-42.5	198.4	707.7	6002.0	1373.5	90.0	157.3	247.4
37	23.8	-25.3	0.4	37.9	-44.3	249.5	736.0	6467.7	1318.6	69.3	162.9	232.2
38	23.8	-25.4	0.3	37.7	-42.9	246.5	737.2	6490.1	1313.5	66.3	164.3	230.7
39	23.8	-25.3	0.4	38.0	-44.6	252.7	736.3	6469.5	1317.1	69.6	162.7	232.3
40	23.6	-25.4	0.4	36.1	-44.3	237.4	784.4	6471.4	1314.8	71.1	167.6	238.7
41	23.9	-25.6	0.1	36.3	-46.0	217.3	761.2	6568.6	1224.2	31.0	185.0	216.0
42	23.8	-25.6	0.2	36.2	-45.5	223.3	769.8	6546.2	1249.4	40.1	181.2	221.3
43	23.8	-24.8	0.9	36.1	-47.6	266.6	755.2	6293.4	1331.6	97.8	157.8	255.6
44	23.4	-23.5	1.2	36.7	-43.4	191.7	704.0	6456.6	1310.0	85.7	160.7	246.2
45	24.1	-24.9	0.7	37.1	-42.7	232.0	735.7	6350.2	1363.1	85.8	158.3	244.2
46	23.7	-25.6	0.3	37.4	-45.4	270.8	739.4	6499.0	1304.6	70.9	162.4	233.3
47	23.6	-26.0	-0.1	36.7	-40.0	255.0	760.3	6617.1	1286.0	68.9	164.3	233.2
48	23.9	-24.4	1.1	36.5	-45.7	292.9	733.2	6191.6	1344.3	114.7	149.1	263.8
49	24.3	-25.0	0.6	37.3	-45.3	222.9	742.0	6399.7	1291.9	51.6	174.2	225.8
50	23.2	-24.8	0.5	37.1	-46.9	229.5	741.8	6404.0	1246.6	43.4	179.2	222.6
51	23.0	-24.4	1.0	36.5	-47.2	297.1	740.6	6236.8	1326.5	114.8	148.9	263.8
52	23.5	-25.6	0.3	35.8	-43.4	238.4	790.7	6486.3	1319.4	74.1	166.7	240.8
53	23.9	-25.0	1.0	36.5	-46.1	258.8	742.5	6225.2	1361.1	94.4	159.6	254.0
54	23.4	-26.6	-0.4	35.9	-37.0	226.7	795.2	6745.2	1262.1	84.3	160.8	245.2
55	23.4	-26.4	-0.3	35.2	-34.9	268.5	804.9	6711.8	1264.8	84.0	161.0	245.0
56	23.4	-26.3	-0.2	35.2	-34.4	266.9	805.7	6674.8	1286.5	89.5	158.5	248.0
57	23.4	-26.2	-0.2	34.8	-33.5	268.5	811.2	6669.4	1280.3	87.4	159.5	247.0
58	23.4	-26.2	-0.2	34.6	-33.1	269.3	813.5	6653.5	1277.0	84.6	160.6	245.2
59	23.4	-26.0	0.0	34.3	-32.3	266.8	817.2	6607.5	1296.7	89.1	159.2	248.2
60	23.5	-26.7	-0.5	36.1	-38.9	265.0	780.4	6756.6	1238.1	71.8	165.6	237.4
61	23.7	-26.3	-0.2	37.4	-42.3	256.0	741.7	6661.4	1274.4	64.5	165.3	229.8
62	23.3	-26.5	-0.1	34.0	-48.0	314.3	770.9	6555.8	1261.8	79.2	163.2	242.5
63	23.1	-26.6	0.0	34.4	-47.8	290.3	749.0	6610.2	1242.8	76.9	164.1	241.1
64	24.0	-24.1	1.1	36.6	-46.5	320.7	727.9	6192.6	1325.3	129.6	161.4	271.0

JUMAX = maximum June temperature; JAMIN = minimum January temperature; MNDLY = mean daily temperature; EXMAX = extreme maximum temperature; EXMIN = extreme minimum temperature; PCIPSN = precipitation from snow (cm); PCIPTL = total precipitation (cm); DDH = heating degree days; DDG = growing degree days; FFDYS = frost free days; FFS = date of last spring frost; FFA = date of first fall frost.

12 needles from each seedling (where possible), 120 needles (where possible) from each site. Although the needles were randomly chosen an effort was made to collect only healthy needles. As the needles were collected, they were bulked by provenance in small plastic bags and stored in a refrigerator at 5° C. Within the next two days the needles of each provenance were carefully separated, moistened with the same amount of distilled water and packaged in plastic bags, 10 needles per bag. Due to the large number of samples, two to three days were required to complete preparation of the needles prior to freezing.

The response criterion used in the present study was the minimum temperature at which approximately 50% of a tested provenance was killed (Weaver and Jackson, 1969; Pomeroy *et al.*, 1970; van den Driessche, 1976; Levitt, 1980). Mazur (1969) called the above temperature "the median lethal temperature" and Warrington and Rook (1980) suggested that despite its several weaknesses, this provides a meaningful way of arranging the response of trees to a range of low temperatures.

In the present study, three test temperatures (Table 3) and a control were used for all freezing tests. Actual temperatures were varied as frost injury patterns became apparent. Three replicates per provenance were used for each of the four temperature treatments. This procedure resulted in 768 bags of 10 needles each (where possible) , 12 bags per provenance.

A 12.0 cubic feet programmable temperature controller freezer from Constant Temperature Control Ltd. (Model AR100) was used for the freezing runs. The freezer was equipped with an interior air circulation fan and with a Honeywell cam temperature programmer. A custom made plastic control cam was attached to the programmer to produce the desired cooling sequence.

In addition to the freezer, a multipoint temperature recorder from SYSCON International INC. (Model 525) was used to monitor experimental conditions. The

Table 3. Temperatures and durations of the freezing trials.

Dates of trials	Temperatures / Durations					
	Treatment I (T1)		Treatment II (T2)		Treatment III (T3)	
09/28/1988 (D1)	-8° C	1 hour	-13° C	1 hour	-18° C	1 hour
09/01/1989 (D2)	-6° C	3 hours	-13° C	1 hour	-18° C	1.5 hours
09/09/1989 (D3)	-9° C	1.5 hours	-13° C	1.5 hours	-19° C	1.5 hours
09/19/1989 (D4)	-9° C	2 hours	-13° C	2 hours	-18° C	2 hours
07/21/1990 (D5)	-2° C	1.5 hours	-3° C	2.5 hours	-6° C	2.5 hours
08/06/1990 (D6)	-1° C	2.15 hours	-3° C	2.5 hours	-6° C	2 hours
09/23/1990 (D7)	-2° C	1.5 hours	-5° C	1.10 hours	-6° C	3 hours

recorder was equipped with an RS-232 printer. Four temperature probes corresponding to four channels in the recorder monitored the air temperature of different locations in the freezer. A printout of all four channels consisting of actual temperature readings for all channels, chart speed, date and time of the readings, was produced.

The four temperature probes were placed in plastic bags with a number of needles and located around the freezer periphery. To help maintain a constant temperature throughout the entire freezer, a portable 7 inch table fan from Holmes Air (Model HAFF-71) was used. Despite its use, slight fluctuations ($\pm 2^{\circ}$ C) in temperature over time and space were still present.

Needles collected in September 1988 were cooled at a rate of no more than 2° C/h to each of three temperatures (-8° C, -13° C and -18° C). The freezer was maintained at each desired temperature for approximately one hour after which the designated samples were removed, and placed back in the refrigerator (5° C) for two to three days. For the first test, sample bags were placed in nine paper bags - one paper bag per replicate, three paper bags per treatment.(i.e., freezing temperature). Controls were left in the refrigerator at 5° C. The bags were randomly placed in the freezer. The results obtained from this first test led to the construction of aluminum racks to improve air circulation in the freezer around the sample bags.

Two aluminum racks were especially designed for these experiments (Appendix XII). The first aluminum rack measured 30" x 16" and 14" high with 40 lines for suspension of the samples. All the samples were randomized and hung from the lines. The reason for this arrangement was to minimize errors from a possible difference in frost damage to needles according to their position in the rack. At each of three successive freezing temperatures a group of samples was removed. To speed removal of the samples, three different coloured clothes pins,

one for each temperature treatment were used for suspending sample bags. The second rack, 30" x 5.2" and 7" high with 13 lines, was used for the controls. Because of limited freezer space the smaller rack was placed on the top of the larger one.

At the start of each freezing trial, all samples were equilibrated at about 5° C for approximately six hours in the freezer. The control group of samples was then removed from the freezer and placed in the refrigerator at 5° C.

In September 1989, because the use of the aluminum racks allowed cool air to circulate freely among samples, the previously used temperatures caused higher degrees of injury. This discrepancy led to a number of preliminary tests at the end of June 1990 using needles originating from local jack pine trees. Although it was known that material collected during summer is less frost hardy than that collected in fall, based on previous experience (range of temperatures used in fall 1988 and fall 1989) it was assumed that temperatures between -1° C and -8° C should give at least one temperature treatment with overall 50% mortality (Table 3).

Viability tests

Freezing injury was evaluated visually. Tissue discoloration (browning) was used as a criterion for rating injury (Rehfeldt, 1980; Rehfeldt, 1985a). For each provenance, the proportion of needles exhibiting injury was recorded at each test temperature for each sampling date.

During the fall of 1988, two weeks after freeze testing, the treated needles were compared to the corresponding controls. Using a methodology adapted from Rehfeldt (1989), injury was assessed on a 5-graded scale from 0.0 - no damage (green needle) to 4.0 - total damage (brown needle) (Table 4). Thus, a value was obtained for each replicate, per treatment, per provenance.

In 1989, treated needles were scored two to three weeks after freeze testing (Table 5). In order to record the different kind of discoloration observed on the

Table 4. Damage classification categories applied on the experiments conducted in 1988.

Value	Description
0	no damage
1	25% of the needle's surface is brown
2	50% of the needle's surface is brown
3	75% of the needle's surface is brown
4	100% of the needle's surface is brown

0 : needle with no damage ; 4 : totally damaged needle

Table 5. Damage classification categories applied on the experiments conducted in 1989.

Value	Description
0.0	no injury
0.1	25% of the needle shows discoloration
0.2	50% of the needle shows discoloration
0.3	75% of the needle shows discoloration
0.4	totally discolored needle
0.5	colours blended together (greyish and brown areas)
0.6	25% of the needle shows severe discoloration
0.7	50% of the needle shows severe discoloration
0.8	75% of the needle shows severe discoloration
0.9	between 75% and 90% of the needle shows severe discoloration
1.0	totally damaged needle

0 : needle with no damage; 1.0 : totally damaged needle

controls the above method of scoring was changed, and the injury was assessed on a 10-graded scale from 0.0 - no damage to 1.0 - total damage. On this scale, grades up to 0.5 apply to the controls and above 0.5 to the freezing trials. At that stage, photographs were taken to illustrate the observed degrees of injury (Figures 2 to 7).

For the last three trials the above procedures were again modified. It was observed that the time needed for full manifestation of the discoloration gradually increased from July to September. Also, prolonged exposure of the needles at room temperature resulted in mold development and needle desiccation which made the scoring ambiguous. The scoring of the needles in July 1990 was done one week after the freeze testing; in August 1990 after twelve days; and in the fall of 1990 after two weeks of storage at room temperature (ca.20° C). To remove bias in scoring freezing damage, the samples were mixed up and scored in a random order. A less detailed, 5 point scoring scale was used for these trials as the finer discriminations seemed too subjective (Table 6).

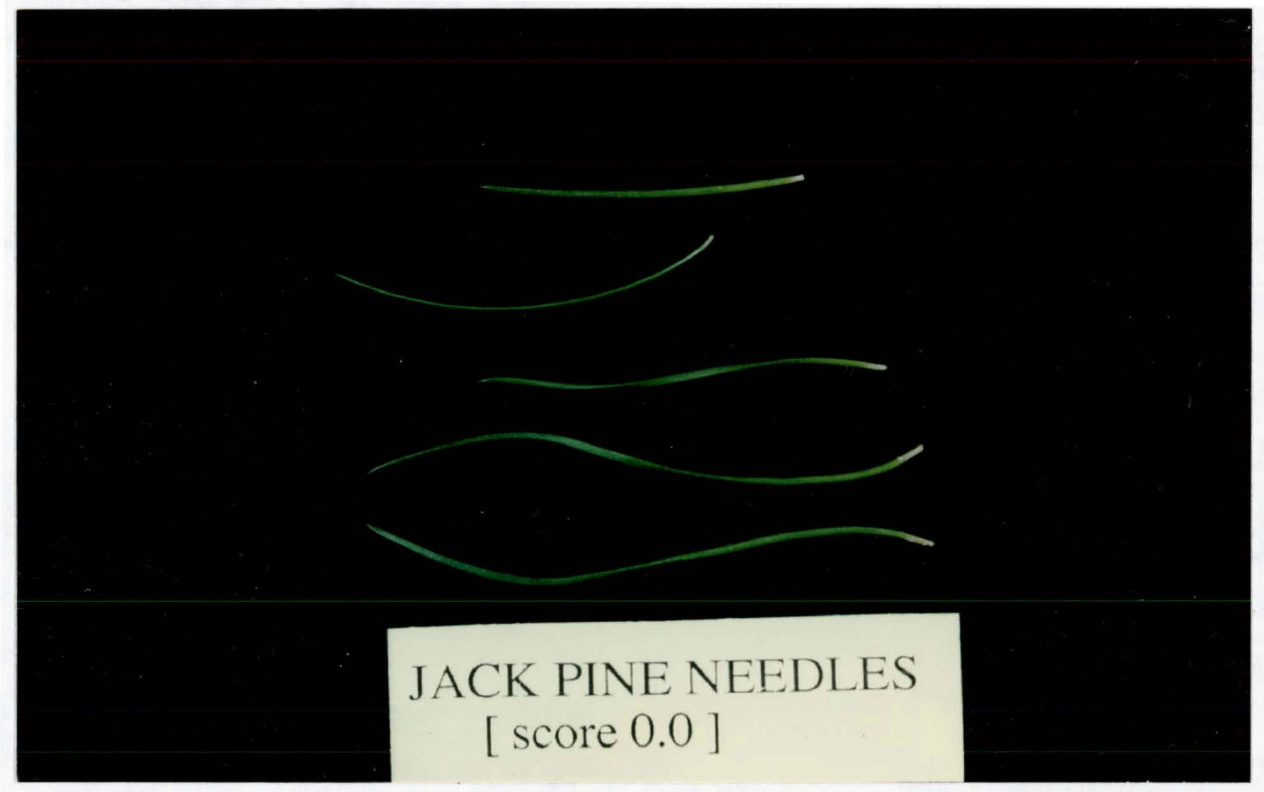


Figure 2 . Jack pine needles exhibiting no discoloration :
score 0 (Table 5)

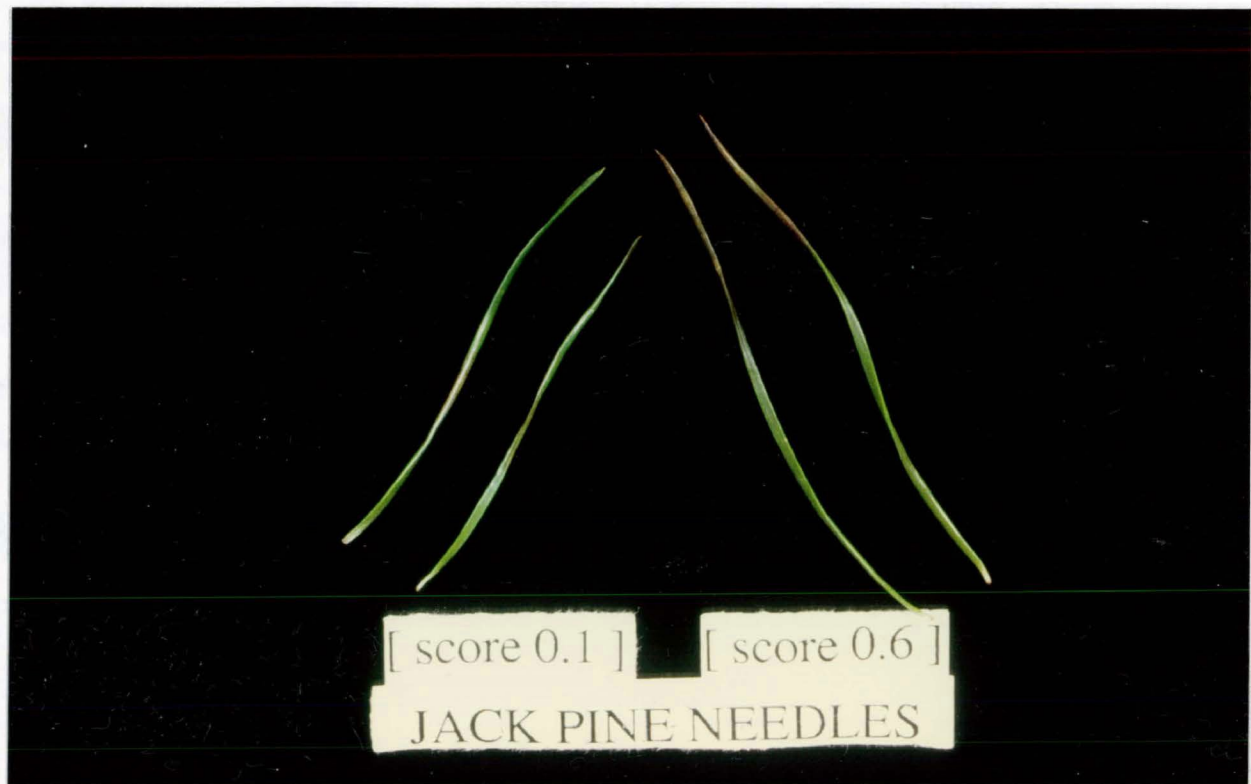


Figure 3. Jack pine needles : comprison between 0.1 (control) and 0.6 (freezing trial) scores of damage (Table5)



Figure 4. Jack pine needles : comparison between 0.2 (control) and 0.7 (freezing trial) scores of damage (Table 5)



Figure 5. Jack pine needles : comparison between 0.3 (control) and 0.8 (freezing trial) scores of damage (Table 5)

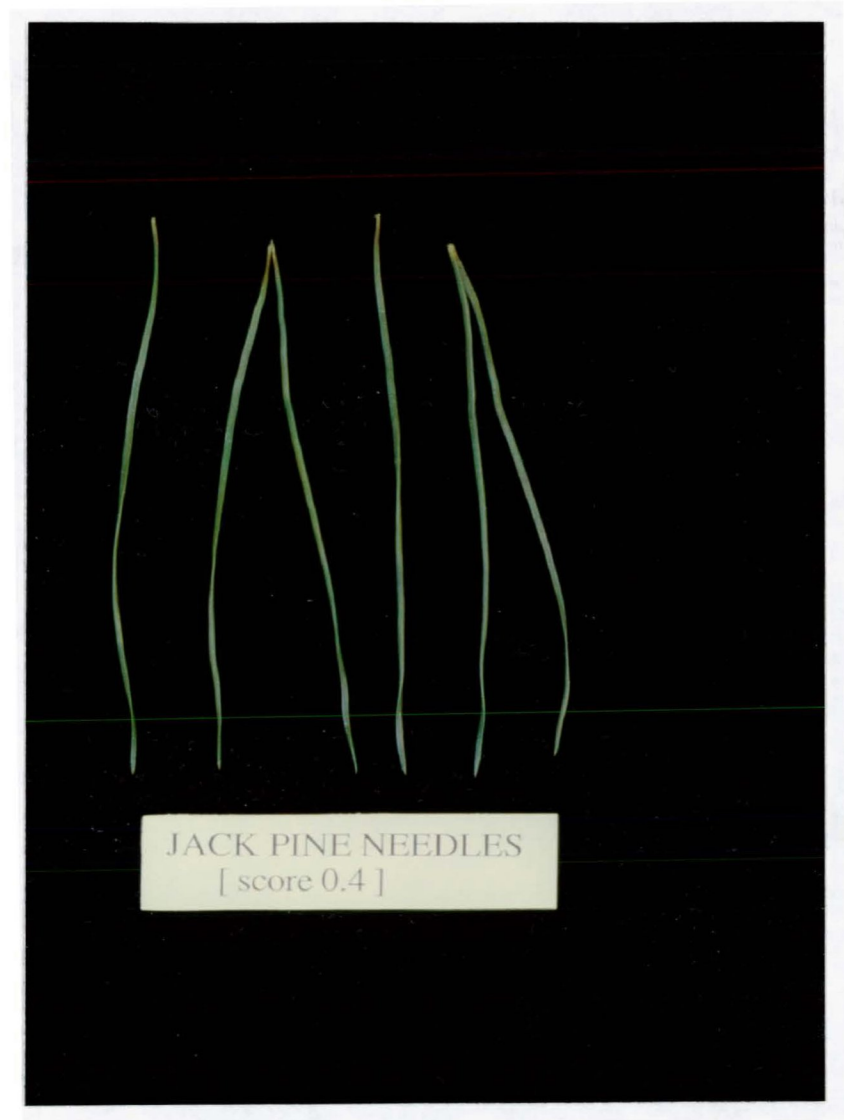


Figure 6. Totally discolored jack pine needles (control) :
score 0.4 (Table 5)



Figure 7 : Totally damaged jack pine needles (freezing trial) : score 1.0 (Table 5)

Table 6 : Damage classification categories applied on
the experiments conducted in 1990.

Value	Description
0.00	no damage
0.25	0% to 25% of the needle's surface is brown
0.50	25% to 50% of the needle's surface is brown
0.75	50% to 75% of the needle's surface is brown
1.00	75% to 100% of the needle's surface is brown

0: needle with no damage; 1.00: totally damaged needle

Statistical analysis

Qualitative data (degree of browning) was transformed to quantitative damage (percentage of injury). Since there was a maximum of ten needles per replicate for each provenance, the maximum score per replicate could be either 40 (Table 4) or 10 (Tables 5 and 6). To facilitate comparisons the data were converted to percentages of maximum damage. The percentage data were arcsin transformed to normalize their distribution. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSSx) on a MICROVAX II computer. The following statistical analyses were made on transformed scores :

- (1) analysis of variance for assessing the magnitude of differences among provenances and the interaction of provenances and freezing temperatures;
- (2) mean comparisons among provenances using the least significant difference at the 0.05 level of probability (LSD0.05);
- (3) correlation analyses relating freezing injury of provenances from one trial to the next;
- (4) simple correlations were used to relate variation among provenances to geographic and climatic criteria of the seed sources. Fifteen independent variables were screened for association with cold hardiness : elevation, latitude, longitude, maximum June temperature, minimum January temperature, mean daily temperature, extreme maximum temperature, extreme minimum temperature, precipitation from snow (cm), total precipitation (cm), heating degree days, growing degree days, frost free days, date of last spring frost and date of the last fall frost (Tables 1 and 2);
- (5) backwards multiple regression, including the above mentioned independent variables, was used to describe the patterns of variation expressed in the data set. Backwards regression analysis is a technique by which frost hardiness values were related to geographic and climatic variables describing the origin of populations

according to a linear backwards regression program for maximizing the goodness of fit, (R square) (Draper and Smith, 1966). Two separate analyses were run. The first concentrated on the relationship between frost hardiness and spatial variables of the place of origin, and the second on climatic variables; and, (6) principal component analysis (PCA) was also used to describe the patterns of variation expressed in the data set. Principal component analysis is a multivariate statistical procedure which reduces many correlated variables to a few meaningful uncorrelated factors (principal components). The goal is to produce a smaller number of these factors which will account for most of the variance in the original set of variables. The variance of each principal component is indicated by its eigenvalue. The principal components are ranked in decreasing order of magnitude of their eigenvalues. An eigenvalue less than 1.00 is interpreted as insignificant (Kaiser, 1960). Eigenvectors or variable loadings listed within each component designate the weight that each variable had in the deterministic equation for a particular component. An arbitrary threshold value of eigenvector > 0.50 was used in the gross interpretation of a single component.

Temperature treatments exhibiting high percentages of samples with either too much (100%) or too little (0%) damage were not included in the principal component analysis as they gave no useful information and introduced noise variables. As a result, the data for 8 out of 21 freezing temperatures met these criteria and were retained for the principal component analysis. Principal component scores for the 64 provenances were calculated for the first three axes (eigenvalues > 1) to serve as new summary variables (Appendix VIII). Further analyses, including simple correlations and backwards multiple regression, were conducted using these three new summary variables to relate variation among provenances to geographic and climatic data of the seed source.

RESULTS

The percentage data for all various sampling dates and temperature treatments are presented in Appendices I to VII. Analysis of variance (Table 7) detected highly significant differentiation among provenances for all sampling dates. Highly significant differences in the interaction of provenances and temperatures were also observed. Temperature treatment differences were also highly significant as expected, but these results are not shown. Lower significant levels were observed for one of the sampling dates (6 August 1990).

Least significant differences (L.S.D.) comparison for each freezing trial/temperature produced inconsistent patterns of provenance differences. An attempt was made to summarize the results of significant differences demonstrated by the L.S.D. procedure for the seven ANOVAs. The detailed L.S.D. matrices are not presented. A summary of significant differences among provenances for the freezing trials is presented in Table 8. A total of thirty two provenances, sixteen with high mean values and sixteen with low, representing relatively extreme high and low degrees of injury were selected for each freezing trial. Mean comparisons using L.S.D. procedure demonstrated generally significant differences between upper and lower groups. It was assumed that provenances which appear three times exhibiting a low degree of damage without showing a high degree of damage for any treatment combination, as well as the ones appearing four times with low damage and a maximum of one time with high were selected as being the most frost hardy. The analogous criteria were applied to the selection of the least hardy provenances (Table 8). According to the above assumption ten provenances were selected as the least hardy and eight as the most hardy (Figure 2). Furthermore, from both Table 8 and Figure 2 the following observations can be made : i) consistency from trial to trial was weak ii) provenances with relatively low

Table 7. Summary of two way Anova's presenting the levels of significance of F-values for the effects of provenances and interaction of provenances with the freezing temperatures

<u>Sampling Date</u>	<u>Significance level</u>	
	<u>Provenance</u>	<u>Interaction</u>
9/28/1988 (D1)	0.000	0.000
9/1/1989 (D2)	0.000	0.009
9/9/1989 (D3)	0.001	0.023
9/19/1989 (D4)	0.000	0.005
7/21/1990 (D5)	0.000	0.000
8/6/1990 (D6)	0.051	0.043
9/23/1990 (D7)	0.000	0.000

Table 8. Summary table showing the sites exhibiting the greatest and least freezing injury for five experimental trials.

SITE NO	TREATMENT 1			TREATMENT 2		TREATMENT 3	
	D1	D2	D4	D1	D5	D1	D7
1				L	H		L
2		L	H	L		L	
3		L			H	H	L
4		L	L		L		
5	L			L	L	L	H
6	L	L		L		L	
7	L		H	L	H	L	
8	L	L	H			L	
9	L		H				
10				H	H	H	H
11			H		L	L	
12	H	L	L	H	H		
13			H	H	L	H	H
14	L	L		L			H
15	H	L		H	H		L
16	L				H	H	
17		H					
18	H			H		H	H
19	H	L				L	H
20	H	H	H		H		
21	L		H	L		H	L
22	H		L		L		H
23	H			H		H	H
24						L	
25	H	H	L		L	L	H
26		L				L	H
27					H	L	
28	H	H	H				
29					H		L
30			H	H	H		
31		H	L			L	H
32				L		H	
33	L	H	H		L		
34		L					
35	L	L	H	L			
36	H		H	L			L
37			L	L		L	L
38	L	H	L		L	H	

D1 = 28 September 1988; D2 = 1 September 1989; D4 = 19 September 1989; D5 = 21 July 1990; D7 = 23 September 1990; L = low degree of injury; H = high degree of injury.

Table 8. (Continued).

SITE NO	TREATMENT 1			TREATMENT 2		TREATMENT 3	
	D1	D2	D4	D1	D5	D1	D7
39						L	
40		H	H	L			L
41	H	H		H			L
42		L			L		L
43	L	L	H	H	L		L
44			H	H	H	H	H
45					L	L	
46	H						
47				L		L	L
48		H	L	H		H	L
49	H	H					
50			L	H	L		
51	H	H	L	H	L		
52	H	H		H			H
53		H			H	H	
54	L	H	L		L	H	
55				H			L
56		H	L	L	H		
57	L		L	L	H		L
58			L		L		L
59	L	L				H	
60				H	L		H
61					H		H
62		L	L				H
63	H		L			H	
64	L					H	

D1 = 28 September 1988; D2 = 1 September 1989; D4 = 19 September 1989; D5 = 21 July 1990; D7 = 23 September 1990; L = low degree of injury; H = high degree of injury.

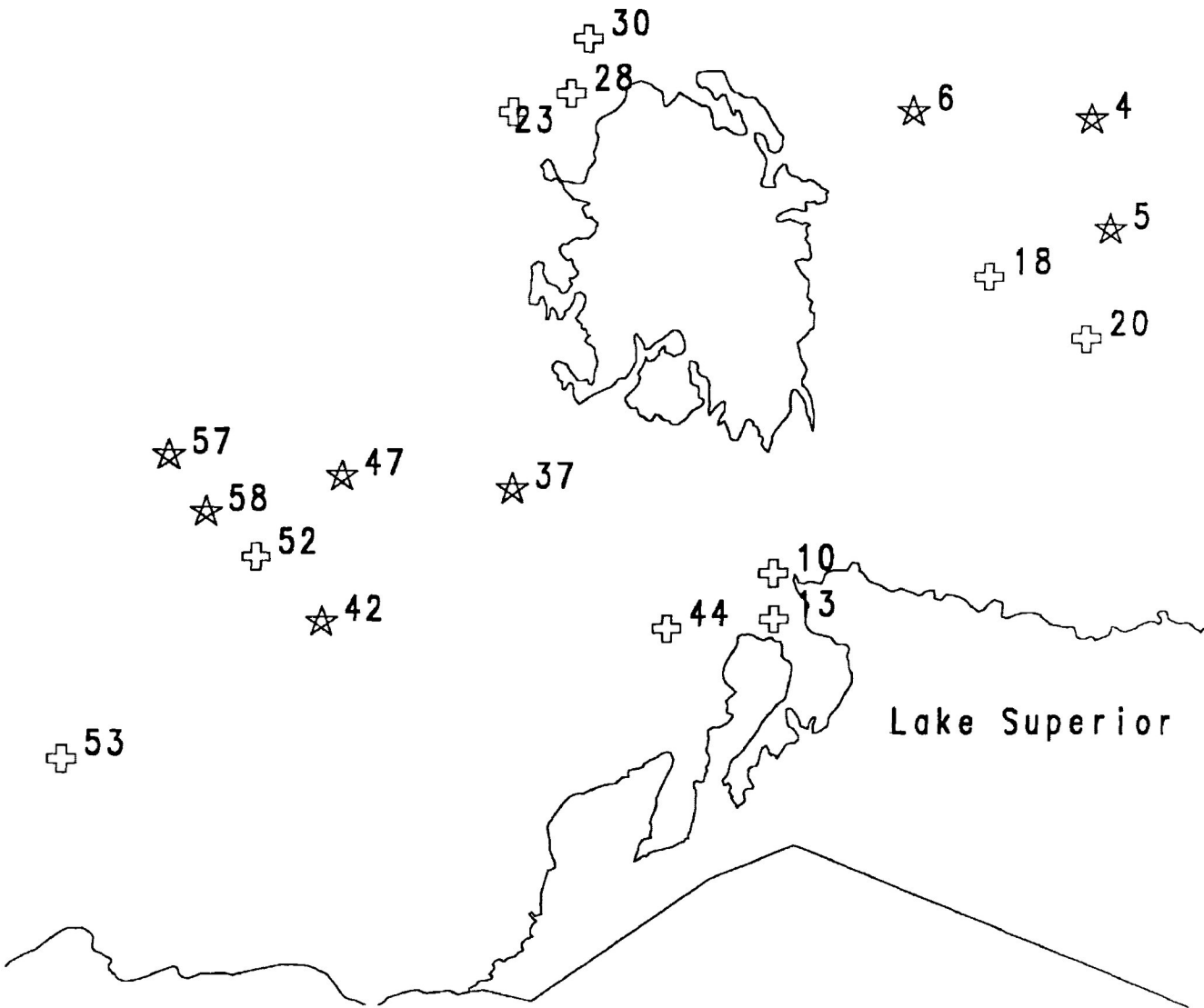


Figure 8. Jack pine provenances exhibiting relatively low (cross) and high (star) degrees of cold hardiness

hardiness, although not randomly distributed, occur in the entire area and iii) provenances with relatively high hardiness are mainly found in northeastern and central western regions of the sampled area of northern Ontario.

The Pearson correlation matrix of freezing injury of populations for all of the various sampling dates and temperatures contains simple correlation coefficients which seem to reflect rather low levels of consistency from one temperature/day combination to another (Table 9). Due to lack of variation there were two cases where coefficients could not be computed i.e. D2T3 and D4T3. These treatments are not presented in Table 9.

Simple correlations of the percent injury with geographic and climatic variables of the seed zone yielded a few statistically significant associations (Table 10). Statistically significant ($\alpha < 0.05$) negative correlation coefficients were found between the elevation of populations and their frost injury rating for D4T1 and D7T3. Even stronger negative correlations ($\alpha < 0.01$) were observed between elevation and D5T2 and elevation and the second principal component. Latitude of origin was the other spatial parameter which explained the variation in the selected critical temperatures. Strong ($\alpha < 0.05$) negative correlation coefficients were observed between latitude and D1T3, D3T1, D4T2 experimental trials. Although, low consistency was found with reference to the majority of climatic variables, strong positive correlations between the number of the growing degree days (DDG) and D3T1 as well as between the number of frost free days (FFDYS) and D7T1, D7T2 were noticed. Both climatic variables approximate growing season length.

From the various combinations of the spatial (independent) variables and dependent variables tested in backwards multiple regression analyses only one, D7T1, turned out to be of importance. Its regression model included two

Table 9. Pearson correlation matrix relating frost injury scores for all seven freezing experiments.

	D1T1	D1T2	D1T3	D2T1	D2T2	D3T1	D3T2	D3T3	D4T1
D1T1									
D1T2	0.493**								
D1T3	-0.003	0.375**							
D2T1	0.061	-0.080	0.116						
D2T2	-0.119	0.128	-0.001	0.093					
D3T1	-0.188	-0.022	0.234	0.149	0.070				
D3T2	-0.342**	-0.116	0.200	0.345**	0.011	0.287*			
D3T3	0.031	0.108	0.294*	0.27*	0.117	0.179	0.207		
D4T1	-0.208	0.040	-0.001	0.000	-0.113	0.125	0.198	-0.073	
D4T2	0.033	0.069	0.086	0.132	-0.007	0.260*	0.149	0.277*	0.341**
D5T1	0.048	0.013	-0.169	0.040	0.009	-0.115	-0.006	0.059	0.128
D5T2	0.062	0.078	-0.009	-0.091	0.037	0.034	-0.086	0.053	0.159
D5T3	0.028	0.059	0.291*	0.001	-0.014	0.001	0.029	0.534**	-0.057
D6T1	0.050	-0.141	-0.241	0.020	-0.217	0.021	-0.083	0.094	-0.226
D6T2	0.034	-0.201	-0.292*	-0.023	-0.061	0.064	-0.023	-0.316*	-0.173
D6T3	-0.045	0.032	-0.056	-0.028	0.226	0.003	0.34	0.076	0.006
D7T1	-0.007	0.110	0.065	-0.016	-0.102	0.150	-0.242	-0.051	0.187
D7T2	0.113	0.277*	0.126	-0.36**	-0.126	-0.052	-0.153	0.019	-0.115
D7T3	0.114	0.161	-0.199	-0.141	0.100	0.033	-0.189	-0.056	0.029

* significant at $\alpha = 0.05$.

** significant at $\alpha = 0.01$.

D1T1 = 09/28/88 -8° C; D1T2 = 09/28/88 -13° C; D1T3 = 09/28/88 -18° C; D2T1 = 09/01/89 -6° C; D2T2 = 09/01/89 -13° C; D3T1 = 09/09/89 -9° C; D3T2 = 09/09/89 -13° C; D3T3 = 09/09/89 -19° C; D4T1 = 09/19/89 -9° C; D4T2 = 09/19/89 -13° C; D5T1 = 07/21/90 -2° C; D5T2 = 07/21/90 -3° C; D5T3 = 07/21/90 -6° C; D6T1 = 08/06/90 -1° C; D6T2 = 08/06/90 -5° C; D6T3 = 08/06/90 -6° C; D7T1 = 09/23/90 -2° C; D7T2 = 09/23/90 -5° C; D7T3 = 09/23/90 -6° C.

Table 9. (Continued).

	D4T2	D5T1	D5T2	D5T3	D6T1	D6T2	D6T3	D7T1	D7T2
D1T1									
D1T2									
D1T3									
D2T1									
D2T2									
D3T1									
D3T2									
D3T3									
D4T1									
D4T2									
D5T1	-0.102								
D5T2	-0.139	0.158							
D5T3	0.086	-0.007	0.050						
D6T1	-0.028	-0.144	-0.141	-0.247*					
D6T2	0.013	-0.137	-0.033	-0.242	0.171				
D6T3	-0.003	0.179	-0.145	-0.022	-0.034	0.114			
D7T1	0.040	-0.073	0.116	0.036	-0.087	-0.112	-0.108		
D7T2	0.029	0.036	0.186	0.143	-0.067	0.127	0.085	0.280*	
D7T3	-0.018	0.016	0.031	-0.060	-0.031	0.091	0.089	0.353**	0.332**

* significant at $\alpha = 0.05$.

** significant at $\alpha = 0.01$.

D1T1 = 09/28/88 -8° C; D1T2 = 09/28/88 -13° C; D1T3 = 09/28/88 -18° C; D2T1 = 09/01/89 -6° C; D2T2 = 09/01/89 -13° C; D3T1 = 09/09/89 -9° C; D3T2 = 09/09/89 -13° C; D3T3 = 09/09/89 -19° C; D4T1 = 09/19/89 -9° C; D4T2 = 09/19/89 -13° C; D5T1 = 07/21/90 -2° C; D5T2 = 07/21/90 -3° C; D5T3 = 07/21/90 -6° C; D6T1 = 08/06/90 -1° C; D6T2 = 08/06/90 -5° C; D6T3 = 08/06/90 -6° C; D7T1 = 09/23/90 -2° C; D7T2 = 09/23/90 -5° C; D7T3 = 09/23/90 -6° C.

Table 10. Simple correlation coefficients relating cold hardiness injury to spatial and climatic variables.

INDEPENDENT VARIABLES (b)	DEPENDENT VARIABLES (a)							
	D1T1	D1T2	D1T3	D2T1	D2T2	D3T1	D3T2	D3T3
LAT	0.059	-0.069	-0.308*	-0.222	0.061	-0.265*	-0.154	-0.222
LONG	-0.030	0.120	0.164	0.262*	0.063	0.185	0.154	0.305*
ELEV	-0.058	-0.091	0.109	0.172	-0.048	0.039	0.029	0.142
JUMAX	0.012	-0.056	-0.022	0.098	-0.112	0.145	0.025	0.122
JAMIN	-0.059	0.073	0.195	0.102	-0.096	0.177	0.184	0.062
MNDLY	-0.062	0.079	0.227	0.159	-0.129	0.249*	0.162	0.170
EXMAX	-0.065	-0.165	-0.265*	-0.181	0.043	-0.238	-0.187	-0.195
EXMIN	0.080	0.177	0.272*	0.194	-0.039	0.247*	0.204	0.216
PCIPSN	-0.055	-0.153	-0.247*	-0.208	-0.012	-0.242	-0.262*	-0.237
PCIPTL	-0.098	-0.179	-0.277*	-0.207	0.059	-0.235	-0.212	-0.226
DDH	0.071	-0.063	-0.231	-0.152	0.136	-0.248*	-0.194	-0.171
DDG	-0.145	-0.032	0.155	0.213	-0.095	0.325**	0.091	0.218
FFDYS	-0.252*	-0.028	0.223	0.039	-0.037	0.146	0.119	0.044
FFS	0.219	0.053	-0.169	-0.061	0.028	-0.131	-0.093	-0.055
FFA	-0.254*	0.005	0.228	0.034	-0.036	0.154	0.126	0.031

* significant at $\alpha = 0.05$.

** significant at $\alpha = 0.01$.

(a) D1T1 = 09/28/88 -8° C; D1T2 = 09/28/88 -13° C; D1T3 = 09/28/88 -18° C; D2T1 = 09/01/89 -6° C; D2T2 = 09/01/89 -13° C; D3T1 = 09/09/89 -9° C; D3T2 = 09/09/89 -13° C; D3T3 = 09/09/89 -19° C; D4T1 = 09/19/89 -9° C; D4T2 = 09/19/89 -13° C; D5T1 = 07/21/90 -2° C; D5T2 = 07/21/90 -3° C; D5T3 = 07/21/90 -6° C; D6T1 = 08/06/90 -1° C; D6T2 = 08/06/90 -3° C; D6T3 = 08/06/90 -6° C; D7T1 = 09/23/90 -2° C; D7T2 = 09/23/90 -5° C; D7T3 = 09/23/90 -6° C; PC1 = first component; PC2 = second component; PC3 = third component.

(b) LAT = latitude; LONG = longitude; ELEV = elevation; JUMAX = maximum June temperature; JAMIN = minimum January temperature; MNDLY = mean daily temperature; EXMAX = extreme maximum temperature; EXMIN = extreme minimum temperature; PCIPSN = precipitation from snow; PCIPTL = total precipitation; DDH = heating degree days; DDG = growing degree days; FFDYS = frost free days; FFS = date of last spring frost; FFA = date of first fall frost.

Table 10. (Continued).

INDEPENDENT VARIABLES (b)	DEPENDENT VARIABLES (a)							
	D4T1	D4T2	D5T1	D5T2	D5T3	D6T1	D6T2	D6T3
LAT	-0.051	-0.286*	0.213	0.245	-0.074	-0.011	0.091	0.182
LONG	-0.203	0.231	-0.268*	-0.347**	0.265*	0.132	-0.003	0.053
ELEV	-0.256*	0.089	-0.096	-0.367**	0.181	0.214	-0.119	-0.055
JUMAX	-0.035	0.053	-0.038	-0.025	0.066	0.120	0.032	-0.125
JAMIN	0.165	0.212	-0.233	-0.019	-0.013	-0.105	-0.028	-0.181
MNDLY	0.203	0.234	-0.202	-0.028	0.000	-0.074	-0.095	-0.236
EXMAX	-0.142	-0.093	0.234	0.050	-0.158	0.127	0.230	0.219
EXMIN	0.116	0.119	-0.267*	-0.067	0.169	-0.118	-0.224	-0.209
PCIPSN	-0.141	-0.170	0.291*	0.048	-0.186	0.106	0.209	0.163
PCIPTL	-0.165	-0.127	0.244	0.057	-0.171	0.115	0.228	0.223
DDH	-0.176	-0.224	0.186	0.047	-0.001	0.070	0.086	0.176
DDG	0.071	0.193	-0.177	-0.136	-0.033	-0.016	-0.078	-0.259*
FFDYS	0.190	0.155	-0.143	-0.095	-0.080	-0.127	-0.030	0.028
FFS	-0.154	-0.152	0.155	0.156	0.08	0.115	0.033	0.018
FFA	0.205	0.15	-0.125	-0.056	-0.091	-0.127	-0.034	0.042

* significant at $\alpha = 0.05$.

** significant at $\alpha = 0.01$.

(a) D1T1 = 09/28/88 -8° C; D1T2 = 09/28/88 -13° C; D1T3 = 09/28/88 -18° C; D2T1 = 09/01/89 -6° C; D2T2 = 09/01/89 -13° C; D3T1 = 09/09/89 -9° C; D3T2 = 09/09/89 -13° C; D3T3 = 09/09/89 -19° C; D4T1 = 09/19/89 -9° C; D4T2 = 09/19/89 -13° C; D5T1 = 07/21/90 -2° C; D5T2 = 07/21/90 -3° C; D5T3 = 07/21/90 -6° C; D6T1 = 08/06/90 -1° C; D6T2 = 08/06/90 -3° C; D6T3 = 08/06/90 -6° C; D7T1 = 09/23/90 -2° C; D7T2 = 09/23/90 -5° C; D7T3 = 09/23/90 -6° C; PC1 = first component; PC2 = second component; PC3 = third component.

(b) LAT = latitude; LONG = longitude; ELEV = elevation; JUMAX = maximum June temperature; JAMIN = minimum January temperature; MNDLY = mean daily temperature; EXMAX = extreme maximum temperature; EXMIN = extreme minimum temperature; PCIPSN = precipitation from snow; PCIPTL = total precipitation; DDH = heating degree days; DDG = growing degree days; FFDYS = frost free days; FFS = date of last spring frost; FFA = date of first fall frost.

Table 10. (Continued).

INDEPENDENT VARIABLES (b)	DEPENDENT VARIABLES (a)					
	D7T1	D7T2	D7T3	PC1	PC2	PC3
LAT	-0.219	-0.070	0.102	-0.046	0.284*	-0.197
LONG	-0.135	-0.093	-0.298*	-0.073	-0.541**	0.042
ELEV	-0.215	-0.222	-0.267*	-0.225	-0.474**	-0.035
JUMAX	-0.097	-0.351*	-0.328**	-0.220	-0.254*	0.044
JAMIN	0.276*	0.310*	0.069	0.175	0.045	0.177
MNDLY	0.320**	0.204	0.046	0.145	-0.003	0.210
EXMAX	-0.211	-0.188	0.001	0.008	-0.228	0.022
EXMIN	0.183	-0.005	-0.047	-0.107	-0.225	-0.004
PCIPSN	-0.182	-0.008	0.013	-0.195	0.098	-0.080
PCIPTL	-0.196	-0.007	0.043	-0.007	0.225	-0.117
DDH	-0.304*	-0.225	-0.048	-0.139	0.012	-0.193
DDG	0.161	-0.024	-0.095	-0.084	-0.166	0.136
FFDYS	0.268*	0.267*	0.075	0.030	0.049	0.220
FFS	-0.267*	-0.276*	-0.097	-0.015	-0.026	-0.142
FFA	0.285*	0.256*	0.069	0.047	0.061	0.243

* significant at $\alpha = 0.05$.

** significant at $\alpha = 0.01$.

(a) D1T1 = 09/28/88 -8° C; D1T2 = 09/28/88 -13° C; D1T3 = 09/28/88 -18° C; D2T1 = 09/01/89 -6° C; D2T2 = 09/01/89 -13° C; D3T1 = 09/09/89 -9° C; D3T2 = 09/09/89 -13° C; D3T3 = 09/09/89 -19° C; D4T1 = 09/19/89 -9° C; D4T2 = 09/19/89 -13° C; D5T1 = 07/21/90 -2° C; D5T2 = 07/21/90 -3° C; D5T3 = 07/21/90 -6° C; D6T1 = 08/06/90 -1° C; D6T2 = 08/06/90 -3° C; D6T3 = 08/06/90 -6° C; D7T1 = 09/23/90 -2° C; D7T2 = 09/23/90 -5° C; D7T3 = 09/23/90 -6° C; PC1 = first component; PC2 = second component; PC3 = third component.

(b) LAT = latitude; LONG = longitude; ELEV = elevation; JUMAX = maximum June temperature; JAMIN = minimum January temperature; MNDLY = mean daily temperature; EXMAX = extreme maximum temperature; EXMIN = extreme minimum temperature; PCIPSN = precipitation from snow; PCIPTL = total precipitation; DDH = heating degree days; DDG = growing degree days; FFDYS = frost free days; FFS = date of last spring frost; FFA = date of first fall frost.

independent variables (latitude and elevation) and produced a coefficient of determination (r^2) of 0.38 ($\alpha < 0.01$).

The multiple regressions of freezing injury against climatic variables resulted in higher correlations than the simple ones (Table 11). Generally, a combination of variables, including environmental gradients in temperature and precipitation, resulted in stronger relationships between dependent and independent variables. The first PC produced a regression model which included the number of growing degree days and the mean daily temperatures with a coefficient of determination (r^2) of 0.13 ($\alpha < 0.05$) (Table 11). The second PC resulted in an equation which included the amount of total precipitation and the extreme minimum temperatures. It produced a coefficient of determination (r^2) of 0.13 ($\alpha < 0.05$). The third PC, with a coefficient of determination (r^2) of 0.10 ($\alpha < 0.05$), was related to the date of the last spring frost and to the number of frost free days. The highest coefficient of determination (r^2) of 0.342 ($\alpha < 0.001$) was observed between D6T3 and the combined effect of the amount of total precipitation, the extreme maximum and mean daily temperatures, the number of heating degree days and the number of frost free days.

Table 12 shows the principal components with their associated eigenvectors or component loadings, eigenvalues and the computed values of each component for each freezing trial. Principal component analysis of the freezing scores generated three significant (eigenvalue greater than 1) principal components that accounted for 22.81 percent, 20.89 percent, and 15.49 percent of the total variation respectively. In eigenvector one, three coefficients had the highest absolute values and were associated with D1T2 (.83), D1T1 (.70), and D7T2 (.57). For eigenvector two, the largest coefficient was negative with an absolute value |.72| and was associated with D2T1. The largest coefficients in eigenvector three, with absolute values |.73| and |.60|, were associated with D1T3 and D4T1 respectively.

Table 11. Summary results of backwards multiple regression of climatic variables and freezing injury.

DEPENDENT VARIABLE (a)	INDEPENDENT VARIABLES (b) IN THE EQUATION	R SQUARE	SIGNIFICANCE OF F-VALUE
D1T1	FFA; PCIPTL	0.111	0.028*
D1T2	FFA; EXMIN; FFDYS	0.124	0.046*
D2T2	JUMAX; PCIPSN; DDG; EXMAX; DDH	0.179	0.040*
D3T2	PCIPSN; EXMAX; DDH; MNDLY	0.175	0.021*
D3T3	JAMIN; MNDLY	0.105	0.034*
D4T1	PCIPTL; EXMIN	0.124	0.017*
D4T2	PCIPSN; EXMAX; MNDLY	0.152	0.019*
D5T1	JUMAX; PCIPSN; JAMIN; DDH	0.190	0.013*

* significant at $\alpha = 0.05$

** significant at $\alpha = 0.01$

(a) D1T1 = 09/29/88 -8°C ; D1T2 = 09/28/88 -13°C ; D2T2 = 09/01/89 -13°C ;
D3T2 = 09/09/89 -13°C ; D3T3 = 09/09/89 -19°C ; D4T1 = 09/19/89 -9°C ;
D4T2 = 09/19/89 -13°C ; D5T1 = 07/21/90 -2°C ; D6T3 = 08/06/90 -6°C ;
D7T1 = 09/23/90 -2°C ; D7T2 = 09/23/90 -5°C ; D7T3 = 09/23/90 -6°C ;

PC1 = first component; PC2 = second component; PC3 = third component.

(b) JUMAX = maximum June temperature; JAMIN = minimum January temperature;
MNDLY = mean daily temperature; EXMAX = extreme maximum temperature;
EXMIN = extreme minimum temperature; PCIPSN = precipitation from snow (cm);
PCIPTL = total precipitation (cm); DDH = heating degree days; DDG = growing
degree days; FFDYS = frost free days; FFS = date of last spring frost;
FFA = date of last fall frost.

Table 11. (Continued).

DEPENDENT VARIABLE (a)	INDEPENDENT VARIABLES (b) IN THE EQUATION	R SQUARE	SIGNIFICANCE OF F-VALUE
D6T3	PCIPSN; EXMAX; DDH MNDLY; FFDYS	0.342	0.000***
D7T1	JAMIN; DDG; MNDLY EXMIN	0.221	0.005*
D7T2	JUMAX; DDH; EXMIN	0.220	0.002**
D7T3	JUMAX; PCIPSN; JAMIN; MNDLY; EXMIN	0.229	0.009**
PC1	DDG; MNDLY	0.127	0.017*
PC2	PCIPTL; EXMIN	0.125	0.018*
PC3	FFS; FFDYS	0.101	0.041*

* significant at $\alpha = 0.05$

** significant at $\alpha = 0.01$

(a) D1T1 = 09/29/88 -8°C ; D1T2 = 09/28/88 -13°C ; D2T2 = 09/01/89 -13°C ;
D3T2 = 09/09/89 -13°C ; D3T3 = 09/09/89 -19°C ; D4T1 = 09/19/89 -9°C ;
D4T2 = 09/19/89 -13°C ; D5T1 = 07/21/90 -2°C ; D6T3 = 08/06/90 -6°C ;
D7T1 = 09/23/90 -2°C ; D7T2 = 09/23/90 -5°C ; D7T3 = 09/23/90 -6°C ;

PC1 = first component; PC2 = second component; PC3 = third component.

(b) JUMAX = maximum June temperature; JAMIN = minimum January temperature;
MNDLY = mean daily temperature; EXMAX = extreme maximum temperature;
EXMIN = extreme minimum temperature; PCIPSN = precipitation from snow (cm);
PCIPTL = total precipitation (cm); DDH = heating degree days; DDG = growing
degree days; FFDYS = frost free days; FFS = date of last spring frost;
FFA = date of last fall frost.

Table 12. Results of principal component analysis for 8 freezing trials of *Pinus banksiana*.

	Components		
	1	2	3
Eigenvalue	1.83	1.67	1.24
% variance	22.81	20.89	15.49
	Component Loadings		
Variabe (a)			
D1T1	0.70	-0.31	-0.30
D1T2	0.83	-0.20	0.20
D1T3	0.30	-0.38	0.73
D2T1	0.03	-0.72	-0.03
D4T1	-0.11	0.46	0.60
D5T2	0.26	0.54	0.19
D7T2	0.57	0.42	0.03
D7T3	0.40	0.45	-0.43

(a) D1T1 = 09/28/88 -8° C; D1T2 = 09/28/88 -13° C; D1T3 = 09/28/88 -18° C; D2T1 = 09/01/89 -6° C; D4T1 = 09/19/89 -9° C; D5T1 = 07/21/90 -2° C; D7T2 = 09/23/90 -5° C; D7T3 = 09/23/90 -6° C.

DISCUSSION

Large amounts of inconsistency were found among the results of the freezing trials. There are potentially many possible factors which might have caused the observed inconsistencies. The adequacy of the visual method of assessment of injury as well as the adequacy of the freezing technique used in this study are two such factors related to experimental technique.

The visual evaluation is a direct and inexpensive method of assessing the degree of frost injury. However, although it is easy to classify the needles as totally damaged or alive, it is challenging and rather subjective to place intermediate degrees of injury in the appropriate classes. The subjectivity of the assessment of the degree of injury can be overcome by using a scale with a small number of classes. Another weakness of this method is the amount of time needed for the development of the symptoms as the conditions of storage may influence the appearance of the needles. Despite the above mentioned weaknesses, several other researchers have found the visual evaluation of frost injury an efficient and reliable method (Rehfeldt, 1980; 1983a; 1986a).

Fluctuations in temperature due to uneven distribution of temperature in the freezer were a source of experimental error. There was a difference of up to $\pm 2^{\circ}$ C among the four corners of the rack. Thus, the random position of the samples in the rack from one trial to another was reflected in "noise" variation which resulted in poor consistency from trial to trial. A smaller number of samples would have facilitated the circulation of air among them and minimized the temperature fluctuations. In addition, four fans within the freezer might have decreased this source of variation.

Another cause for the low levels of consistency among trials might have been the random nature of freezing due to supercooling (Malek, pers. comm. 1992).

Supercooling is defined by Levitt (1980) as the process where the temperature of a plant drops below its freezing point without formation of ice crystals. The supercooling point is the lowest subfreezing temperature before the formation of ice and, contrary to the freezing point which remains constant, it may differ even for a number of tests conducted on the same solution (Levitt, 1980). Burke *et al.* (1976) reported that during controlled freezing, woody plant stems usually supercool to -15° C. Whether or not supercooling should be considered as a component of the freezing resistance mechanism when we expose jack pine needles to temperatures between 0° - 15° C needs further investigation.

Statistically significant provenance and provenance x temperature interactions suggest that jack pine provenances from the study area were genetically differentiated in their response to test temperatures. Thus, different patterns of genetic variation related to the degree of frost hardiness are associated with different test environments. This result implies a certain risk in transferring seed from one environment to another, i.e., lack of adaptation.

The greatest differences among provenances occurred in September and July (Table 7). Variation in frost hardiness during August was minimal. By the 6th of August, appreciable hardening had apparently taken place in the needles of the majority of the provenances tested, and differences among them had disappeared. These seasonal patterns of variation in acclimation of provenances of jack pine are similar to those of provenances of lodgepole pine (*Pinus contorta* var. *latifolia*) from the northern Rocky Mountains reported by Rehfeldt (1980). He reported that the greatest variation among provenances was observed during September and that in the middle of August all provenances were relatively equal in hardiness.

Seasonal variation patterns have been found by several authors. Among them, Glerum (1973), studied the annual trends in frost hardiness for seven conifer species. He observed that jack pine reached the minimum frost hardiness of -3° to -

4° C by June and maintained it until mid-August. The same author in 1985 studied the seasonal frost hardiness trend of white pine (Pinus strobus L.). He found that the minimum frost hardiness of about - 3° C was reached by the end of May and started to increase again in August. Cannell and Sheppard (1982) studied changes in the natural level of frost hardiness of shoots of four provenances of Picea sitchensis.(Bong.) Carr. They reported that during a warm period in August, the shoots were hardy to only -3° C while following cool days in June - July they hardened to about -10° C. They suggested the possibility that shoots might be less hardy following a warm period in August - September than following a cool period in June - July.

Although photoperiod differences due to latitude are considered one of the main factors influencing the initiation of winter hardening in trees (Weiser, 1970) the differences among provenances in frost hardiness were not totally consistent with their current latitudinal distribution. In general, differentiation within the northeastern and central western provenances was more or less arbitrary. The same observation can be made for elevation. This is in contrast with observations made by other authors. In Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco), photoperiod is one of several factors that influence development of cold hardiness (van den Driessche, 1969a). Latitude was the main factor correlated with frost hardiness in Pinus contorta (Jonsson *et al.*, 1980). They found that trees of northern origin or from high elevations developed frost hardiness earlier than those of southern origin and from low elevations. However, Rehfeldt (1982b) reported that the maximum hardiness of buds of 82 provenances of Larix occidentalis Nutt. from the Northern Rocky Mountains was not related to elevation of the seed source. Similar results were found by Rehfeldt (1986a). He investigated the genetic differentiation patterns among 64 provenances of ponderosa pine (Pinus ponderosa) Dougl. ex Laws from central Idaho and reported that although

generally provenance differentiation was closely related to elevation of the seed source, only a weak correlation was found between cold hardiness and elevation. Smithberg and Weiser (1968) pointed out that although latitude is an important factor in phenological events, its influence can either be increased or decreased by local climatic conditions.

The varied significant associations between various degrees of injury and gradients of the climate likely reflect the complexity of the climatic variation and suggest that variation among provenances may be partially attributed to the combined effect of several climatic variables. Data from meteorological stations reinforces the above speculation by indicating strong environmental gradients within the study area (Whitewood and MacIver, 1991). Although the climatological data used in this study originated from a slightly different data base (Maley, 1990), it was derived in the same manner as Whitewood and MacIver (1991). The average annual temperature decreases towards the north with the highest on the northwest shore of Lake Superior. The average annual precipitation increases from the northwest towards the southeast, while the most precipitation occurs on the north-east shore of Lake Superior. The annual total water deficit decreases towards the north with the higher levels of water deficit on the north-west shore of Lake Superior and around the Dorion area. The number of frost-free days increases towards the south with the the largest periods occurring on the north and north-west shore of Lake Superior. The average annual growing degree days increase towards the south whether the highest temperatures occur on the north-west shore of Lake Superior and on the south shore of Lake Nipigon.

Extreme minimum temperatures, precipitation from snow, and mean daily temperature were the climatic gradients which most frequently appeared to be associated with frost hardiness. This is partly in accordance with Alexander *et al.* (1984) who found average annual minimum temperature and annual frost-free

period to be very useful in predicting the degree of cold hardiness for 10 white ash (Fraxinus americana L.) populations from eastern North America. Flint (1972) who studied frost hardiness of twigs of trees of Quercus rubra L. representing 38 different provenances found a strong intercorrelation among climatic gradients. Average annual minimum temperature, extreme minimum temperature, length of frost-free period and biotemperature (definition and calculation of the mean annual biotemperature is given by Flint (1972) were highly correlated with each other and all related to the latitude of the place of origin.

General effects can also be identified by examining the correlations between climatic data and principal component values. The first principal component was closely associated with the number of growing degree days and the mean daily temperatures. The second component was related to the amount of total precipitation and to extreme minimum temperatures, while the third was related to the date of the last spring frost and to the number of frost free days. Since the first three principal component axes account for 59.2 % of the total variation, the correspondence with climatic variables presumably reflects adaptive variation. Trend surface maps based on each principal component axis scores are presented in Appendices IX to XI. The results of the present study suggest that several selective forces appear to be at least partially responsible for differentiation among the tested provenances; however not all findings were in accordance with the above speculation. For example, needles from far northern provenances (provenance no.'s 30, 28 and 23) despite the lower temperatures and very short frost-free period of the place of origin exhibited no higher levels of frost hardiness than those from provenances located at warmer areas with longer frost-free periods.

Three of the provenances in the present study (provenances 44, 13 and 10) which consistently appear with relatively high degrees of damage are located on the north shore of Lake Superior and seem to represent an ecologically specific habitat

type. Given the close proximity of provenance 16, the lack of similarity is surprising and suggests that the following speculations should be considered with scepticism. All above mentioned provenances occur at relatively low elevations (650m -1100m) where the frost-free period varies from 85.7 to 98.9 days (Table 2). Precipitation at these locations averages from 704.0 mm to 768.2 mm (Table 2). Soil moisture stress contributes to the ecological uniqueness of this area (Whitewood and MacIver, 1991). van den Driessche (1969b) found that although moisture stress applied under short days (8 and 12 hours) had no direct effect on cold hardiness in Douglas-fir seedlings, it appeared to decrease the response to photoperiod. Timmis and Tanaka (1976) also found that although mild stress (- 6.5 bars) during long days (16 hours) increased cold hardiness, severe stress (- 10.5 bars) had a reverse effect on frost hardiness. Chen *et al.* (1977) assessed the impact of water stress on the development of frost hardiness in red osier dogwood plants grown under controlled conditions for three weeks. They reported that although water stress for the first week increased the frost hardiness from -3° C (control) to -11° C, further water stress treatment had no significant effect. In the present study, the multiple regressions of freezing injury against climatic variables indicated that precipitation is among the environmental gradients which resulted in the strongest relationships between independent and dependent variables. Although no environmental gradient can be considered in isolation from the other microclimatic variables, it is possible that an adaptation of cold hardiness to drought stress has occurred which is now expressed through the progenies.

According to Carmean (1975), the significance of the microsite influence on the physiological response of the tree should not be overlooked. He pointed out that moisture, nutrients, temperature and humidity are directly or indirectly related to certain soil, topographic and climatic parameters. The above argument emphasizes the need for microclimate studies around the present meteorological stations to

provide more accurate and detailed data and verify the validity of the data used in the present study.

It is possible that the lower elevation, southern location, water body and relatively low moisture supply combine to produce a warmer environment for jack pine on the north shore of Lake Superior. This is in accordance with Joyce (1987) who studied adaptive differentiation in cold hardiness of Larix laricina (DuRoi) K. Koch from 66 populations from northern Ontario. He found that the populations located near Lake Superior were among the ones exhibiting lower levels of frost hardiness.

Maley (1990) assessed phenotypic variation in cone and needle traits of the 10 parent trees sampled for the present study. She found a steep cline around Nipigon area (long. 88° 15'). She concluded that variation among provenances may be due to environmental adaptations as well as to the evolutionary history of the species. The results of this study suggested that there is no similarity between the pattern of variation for morphological characteristics and the pattern of variation for frost hardiness.

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APPENDICES

APPENDIX I**PERCENTAGE DAMAGE OF JACK PINE NEEDLES :
FREEZING TEST NO 1**

DATE : 09/28/1988
TREATMENT 1 : - 8° C
TREATMENT 2 : -13° C
TREATMENT 3 : -18° C

SITE NO	TREATMENT 1			TREATMENT 2			TREATMENT 3		
	1	2	3	1	2	3	1	2	3
1	12.5	10.0	15.0	12.5	7.5	30.0	30.0	77.5	92.5
2	21.9	6.3	9.4	25.0	18.8	6.3	40.6	46.8	46.8
3	10.0	15.0	17.5	60.0	22.5	20.0	55.0	95.0	67.5
4	30.0	10.0	30.0	45.0	25.0	30.0	50.0	65.0	x
5	5.6	11.1	11.1	19.4	8.3	11.1	52.7	33.3	47.2
6	10.7	10.7	10.7	10.7	10.7	7.1	28.0	10.7	35.7
7	11.1	2.8	11.1	22.2	11.1	36.1	44.4	52.7	55.5
8	12.5	12.5	6.3	34.4	25.0	25.0	50.0	68.7	34.3
9	5.0	7.5	12.5	20.0	25.0	32.5	55.0	50.0	75.0
10	11.1	22.2	16.7	50.0	30.6	38.9	55.5	72.2	100.0
11	30.0	15.0	12.5	30.0	42.5	12.5	35.0	45.0	62.5
12	37.5	37.5	40.0	50.0	50.0	50.0	67.5	52.5	65.0
13	22.5	20.0	15.0	62.5	50.0	32.5	72.5	80.0	80.0
14	12.5	9.4	15.6	43.8	12.5	6.3	56.2	68.7	56.2
15	22.5	30.0	30.0	42.5	40.0	42.5	70.0	75.0	65.0
16	10.0	5.0	15.0	32.5	22.5	30.0	65.0	75.0	90.0
17	15.0	12.5	20.0	32.5	22.5	15.0	67.5	70.0	60.0
18	37.5	25.0	25.0	45.0	47.5	60.0	62.5	95.0	80.0
19	37.5	27.5	15.0	30.0	30.0	30.0	35.0	67.5	60.0
20	40.0	30.0	15.0	50.0	15.0	42.5	57.5	67.5	60.0
21	5.6	5.6	0.0	13.9	0.0	19.4	55.5	94.4	75.0
22	38.9	16.7	16.7	36.1	33.3	33.3	75.0	66.6	63.8
23	45.0	30.0	30.0	52.5	37.5	37.5	57.5	80.0	82.5
24	27.5	12.5	20.0	22.5	25.0	40.0	22.5	37.5	20.0
25	35.0	27.5	20.0	30.0	25.0	20.0	17.5	40.0	65.0
26	25.0	25.0	25.0	32.5	32.5	32.5	30.0	30.0	30.0
27	15.0	15.0	35.0	25.0	12.5	35.0	35.0	37.5	75.0
28	25.0	32.5	30.0	30.0	32.5	40.0	72.5	70.0	70.0
29	12.5	22.5	27.5	27.5	27.5	30.0	62.5	82.5	52.5
30	15.0	22.5	12.5	50.0	40.0	45.0	57.5	70.0	75.0
31	16.7	16.7	13.9	36.1	22.2	27.8	22.2	11.1	16.6
32	22.2	11.1	11.1	22.2	22.2	16.7	69.4	91.6	72.2
33	10.0	10.0	12.5	40.0	20.0	12.5	62.5	72.5	57.5
34	25.0	12.5	27.5	32.5	22.5	20.0	70.0	82.5	55.0
35	7.5	15.0	10.0	25.0	22.5	20.0	60.0	55.0	67.5
36	17.5	37.5	25.0	15.0	17.5	25.0	70.0	62.5	72.5
37	25.0	20.0	20.0	15.0	17.5	25.0	30.0	65.0	40.0
38	0.0	20.0	15.0	25.0	20.0	25.0	67.5	77.5	72.5
39	10.0	30.0	15.0	27.5	27.5	15.0	47.5	62.5	57.5
40	25.0	15.0	10.0	25.0	5.0	20.0	62.5	70.0	60.0

SITE NO	TREATMENT 1			TREATMENT 2			TREATMENT 3		
	1	2	3	1	2	3	1	2	3
41	40.0	30.0	40.0	45.0	42.5	35.0	65.0	72.5	45.0
42	20.0	15.0	17.5	45.0	30.0	22.5	55.0	x	82.5
43	5.6	16.7	13.9	38.9	27.8	47.2	55.5	91.6	55.5
44	20.0	25.0	15.0	50.0	42.5	45.0	75.0	70.0	75.0
45	25.0	20.0	20.0	37.5	45.0	30.0	55.0	30.0	70.0
46	45.0	37.5	32.5	30.0	35.0	42.5	47.5	55.0	75.0
47	25.0	5.0	12.5	15.0	17.5	20.0	40.0	62.5	65.0
48	32.5	15.0	17.5	45.0	45.0	40.0	87.5	95.0	100.0
49	45.0	10.0	15.0	25.0	25.0	22.5	65.0	67.5	67.5
50	20.0	27.5	17.5	45.0	40.0	42.5	60.0	75.0	70.0
51	22.2	22.2	25.0	41.7	41.7	30.6	69.4	52.5	75.0
52	27.8	27.8	47.2	44.4	47.2	22.2	33.3	66.6	66.6
53	15.0	17.5	12.5	42.5	15.0	37.5	67.5	65.0	82.5
54	6.3	15.6	9.4	50.0	12.5	43.7	68.7	78.1	75.0
55	10.0	17.5	10.0	57.5	35.0	45.0	65.0	75.0	75.0
56	12.5	17.5	20.0	10.0	27.5	27.5	67.5	67.5	60.0
57	15.0	7.5	0.0	32.5	17.5	10.0	52.5	45.0	70.0
58	20.0	25.0	7.5	25.0	17.5	32.5	67.5	57.5	65.0
59	5.0	0.0	0.0	65.0	20.0	5.0	72.5	75.0	75.0
60	25.0	20.0	25.0	77.5	37.5	25.0	60.0	67.5	67.5
61	25.0	21.9	9.4	37.5	25.0	25.0	75.0	68.7	65.6
62	12.5	15.6	12.5	18.8	34.4	40.6	68.7	59.3	59.3
63	31.3	18.8	31.3	31.3	25.0	18.8	75.0	78.1	75.0
64	12.5	9.4	18.8	31.3	18.8	25.0	81.2	81.2	81.2

APPENDIX II

**PERCENTAGE DAMAGE OF JACK PINE NEEDLES :
FREEZING TEST NO 2**

DATE : 09/01/1989
TREATMENT 1 : - 6° C
TREATMENT 2 : -13° C
TREATMENT 3 : -18° C

SITE NO	TREATMENT 1			TREATMENT 2			TREATMENT 3		
	1	2	3	1	2	3	1	2	3
41	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
42	92.0	16.0	86.0	100.0	100.0	100.0	100.0	100.0	100.0
43	74.0	90.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
44	100.0	100.0	92.0	100.0	100.0	100.0	100.0	100.0	100.0
45	92.0	90.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
46	92.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
47	90.0	100.0	80.0	100.0	100.0	100.0	100.0	x	100.0
48	100.0	97.8	100.0	100.0	100.0	100.0	100.0	100.0	100.0
49	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
50	100.0	98.0	92.0	100.0	100.0	100.0	100.0	100.0	100.0
51	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
52	95.5	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
53	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
54	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
55	96.0	86.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
56	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
57	82.2	82.2	100.0	100.0	100.0	100.0	100.0	100.0	100.0
58	97.8	93.3	100.0	100.0	100.0	100.0	100.0	100.0	100.0
59	94.0	90.0	94.0	100.0	100.0	100.0	100.0	100.0	100.0
60	x	x	x	100.0	100.0	100.0	100.0	100.0	100.0
61	92.0	92.0	100.0	100.0	100.0	100.0	100.0	x	100.0
62	91.1	91.1	80.0	77.8	100.0	100.0	100.0	100.0	100.0
63	88.0	92.0	98.0	100.0	100.0	100.0	100.0	100.0	100.0
64	94.0	96.0	82.0	100.0	92.0	100.0	100.0	100.0	100.0

APPENDIX III**PERCENTAGE DAMAGE OF JACK PINE NEEDLES :
FREEZING TEST NO 3**

DATE : 09/09/1989
TREATMENT 1 : - 9° C
TREATMENT 2 : -13° C
TREATMENT 3 : -19° C

APPENDIX IV

**PERCENDAGE DAMAGE OF JACK PINE NEEDLES :
FREEZING TEST NO 4**

**DATE : 09/19/1989
TREATMENT 1 : - 9°C
TREATMENT 2 : -13°C
TREATMENT 3 : -18° C**

SITE NO	TREATMENT 1			TREATMENT 2			TREATMENT 3		
	1	2	3	1	2	3	1	2	3
1	79.0	96.0	89.0	96.0	100.0	100.0	100.0	100.0	100.0
2	97.5	100.0	100.0	97.5	100.0	91.2	100.0	100.0	100.0
3	98.0	93.0	96.0	99.0	100.0	96.0	100.0	100.0	99.0
4	98.0	85.0	94.0	94.0	98.0	95.0	100.0	100.0	100.0
5	x	98.9	76.7	94.4	100.0	100.0	100.0	100.0	100.0
6	100.0	96.0	93.0	100.0	100.0	100.0	100.0	100.0	100.0
7	100.0	98.0	100.0	100.0	93.0	99.0	100.0	100.0	100.0
8	100.0	100.0	100.0	100.0	100.0	96.0	100.0	100.0	100.0
9	100.0	98.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
10	94.0	100.0	99.0	100.0	100.0	98.0	100.0	100.0	100.0
11	100.0	100.0	95.0	100.0	100.0	100.0	100.0	100.0	100.0
12	96.0	100.0	75.0	92.0	97.0	100.0	100.0	93.0	94.0
13	96.0	100.0	100.0	92.0	100.0	100.0	100.0	100.0	100.0
14	97.0	100.0	98.0	99.0	84.0	100.0	100.0	100.0	100.0
15	100.0	100.0	86.0	98.0	x	100.0	100.0	100.0	100.0
16	88.0	72.0	98.0	100.0	100.0	98.0	100.0	100.0	100.0
17	94.0	100.0	100.0	97.0	100.0	91.0	100.0	100.0	100.0
18	87.0	90.0	96.0	100.0	100.0	98.0	100.0	100.0	100.0
19	97.0	97.0	95.0	100.0	100.0	100.0	100.0	100.0	100.0
20	100.0	100.0	95.0	100.0	100.0	100.0	100.0	100.0	100.0
21	99.0	100.0	100.0	100.0	100.0	99.0	100.0	100.0	100.0
22	79.0	100.0	98.0	100.0	100.0	100.0	100.0	100.0	100.0
23	67.0	100.0	94.0	100.0	100.0	100.0	100.0	100.0	100.0
24	94.0	93.0	100.0	x	100.0	100.0	100.0	100.0	100.0
25	0.0	86.0	97.0	98.0	77.0	100.0	100.0	100.0	100.0
26	100.0	88.0	x	96.0	100.0	100.0	100.0	100.0	100.0
27	100.0	76.7	90.0	100.0	100.0	100.0	100.0	100.0	100.0
28	97.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
29	100.0	100.0	90.0	96.7	100.0	96.7	100.0	100.0	100.0
30	100.0	96.0	100.0	98.0	100.0	91.0	100.0	100.0	100.0
31	100.0	57.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
32	98.0	96.0	97.0	100.0	100.0	99.0	100.0	100.0	100.0
33	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
34	78.0	100.0	100.0	100.0	100.0	98.0	100.0	100.0	100.0
35	100.0	98.9	100.0	100.0	100.0	100.0	100.0	100.0	100.0
36	100.0	99.0	98.0	100.0	100.0	100.0	100.0	100.0	100.0
37	90.0	92.0	98.0	100.0	100.0	99.0	100.0	100.0	100.0
38	88.0	89.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
39	100.0	71.0	80.0	100.0	90.0	79.0	100.0	100.0	100.0
40	100.0	100.0	99.0	100.0	99.0	100.0	100.0	100.0	100.0

SITE NO	TREATMENT 1			TREATMENT 2			TREATMENT 3		
	1	2	3	1	2	3	1	2	3
41	63.0	96.0	89.0	98.0	100.0	91.0	100.0	100.0	100.0
42	87.8	x	93.3	100.0	100.0	100.0	100.0	100.0	100.0
43	100.0	100.0	94.0	100.0	100.0	100.0	100.0	100.0	100.0
44	100.0	100.0	100.0	100.0	96.0	100.0	100.0	100.0	100.0
45	73.0	97.0	98.0	100.0	100.0	100.0	99.0	100.0	100.0
46	95.0	89.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
47	80.0	87.0	98.0	98.0	100.0	92.0	100.0	100.0	100.0
48	92.0	100.0	79.0	100.0	100.0	100.0	100.0	100.0	100.0
49	27.0	100.0	99.0	100.0	100.0	100.0	100.0	100.0	100.0
50	100.0	88.0	93.0	100.0	98.0	100.0	100.0	100.0	100.0
51	96.0	84.0	100.0	99.0	100.0	100.0	100.0	100.0	100.0
52	100.0	78.0	99.0	100.0	98.0	100.0	100.0	100.0	100.0
53	81.1	88.9	97.8	100.0	100.0	100.0	100.0	100.0	100.0
54	76.0	100.0	92.0	98.0	100.0	100.0	100.0	100.0	100.0
55	100.0	100.0	89.0	100.0	100.0	100.0	100.0	100.0	100.0
56	100.0	80.0	95.0	100.0	100.0	100.0	100.0	100.0	100.0
57	98.9	53.3	70.0	95.5	98.7	100.0	100.0	100.0	100.0
58	55.0	21.0	61.0	100.0	83.0	100.0	100.0	100.0	100.0
59	87.0	100.0	95.0	91.0	90.0	100.0	100.0	100.0	100.0
60	100.0	81.0	76.0	100.0	100.0	100.0	100.0	100.0	100.0
61	100.0	83.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
62	90.0	96.0	98.0	93.0	93.0	100.0	100.0	100.0	97.0
63	20.0	83.0	82.0	100.0	98.0	68.0	100.0	100.0	100.0
64	100.0	88.0	100.0	100.0	100.0	99.0	100.0	100.0	100.0

APPENDIX V

**PERCENTAGE DAMAGE OF JACK PINE NEEDLES :
FREEZING TEST NO 5**

DATE : 07/21/1990
TREATMENT 1 : -2° C
TREATMENT 2 : -3° C
TREATMENT 3 : -6° C

SITE NO	TREATMENT 1			TREATMENT 2			TREATMENT 3		
	1	2	3	1	2	3	1	2	3
1	0.0	0.0	0.0	0.0	80.0	20.0	100.0	100.0	100.0
2	0.0	0.0	0.0	0.0	72.5	35.0	100.0	100.0	50.0
3	0.0	0.0	0.0	100.0	100.0	67.5	100.0	100.0	100.0
4	0.0	0.0	0.0	0.0	0.0	0.0	82.5	100.0	100.0
5	0.0	2.5	0.0	0.0	0.0	0.0	50.0	100.0	100.0
6	0.0	15.0	0.0	80.0	50.0	92.5	100.0	100.0	100.0
7	0.0	0.0	0.0	0.0	0.0	75.0	100.0	100.0	100.0
8	0.0	0.0	0.0	60.0	80.0	0.0	100.0	100.0	100.0
9	0.0	0.0	37.5	80.0	0.0	77.5	100.0	100.0	100.0
10	0.0	0.0	0.0	100.0	100.0	45.0	100.0	100.0	100.0
11	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0
12	0.0	55.0	0.0	100.0	50.0	100.0	100.0	100.0	100.0
13	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0
14	0.0	0.0	0.0	0.0	100.0	0.0	100.0	100.0	100.0
15	0.0	0.0	0.0	100.0	100.0	0.0	100.0	70.0	100.0
16	0.0	0.0	0.0	100.0	0.0	100.0	100.0	100.0	100.0
17	97.5	0.0	0.0	0.0	100.0	10.0	100.0	100.0	100.0
18	0.0	0.0	0.0	37.5	0.0	0.0	100.0	100.0	100.0
19	0.0	5.0	0.0	80.0	0.0	100.0	100.0	100.0	100.0
20	0.0	72.5	2.5	2.5	62.5	37.5	80.0	100.0	100.0
21	0.0	2.5	0.0	2.5	65.0	0.0	100.0	100.0	100.0
22	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0
23	0.0	0.0	0.0	52.5	0.0	0.0	100.0	100.0	100.0
24	0.0	0.0	92.5	0.0	2.5	100.0	100.0	100.0	100.0
25	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0
26	0.0	0.0	0.0	0.0	77.5	55.0	100.0	100.0	100.0
27	0.0	0.0	0.0	7.5	0.0	70.0	100.0	100.0	100.0
28	0.0	0.0	2.5	10.0	5.0	0.0	100.0	100.0	100.0
29	0.0	0.0	0.0	0.0	90.0	0.0	100.0	100.0	100.0
30	0.0	0.0	0.0	100.0	40.0	100.0	100.0	100.0	100.0
31	0.0	0.0	0.0	60.0	10.0	0.0	90.0	100.0	100.0
32	0.0	0.0	0.0	0.0	60.0	0.0	100.0	100.0	100.0
33	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0
34	0.0	0.0	0.0	2.5	0.0	0.0	100.0	100.0	100.0
35	0.0	0.0	0.0	0.0	0.0	32.5	100.0	100.0	100.0
36	0.0	0.0	0.0	35.0	0.0	20.0	100.0	100.0	100.0
37	0.0	0.0	0.0	0.0	27.5	0.0	100.0	100.0	100.0
38	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0
39	0.0	0.0	2.5	32.5	0.0	0.0	100.0	100.0	100.0
40	0.0	5.0	0.0	0.0	0.0	100.0	100.0	100.0	100.0

SITE NO	TREATMENT 1			TREATMENT 2			TREATMENT 3		
	1	2	3	1	2	3	1	2	3
41	0.0	0.0	0.0	0.0	70.0	85.0	100.0	100.0	100.0
42	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0
43	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0
44	0.0	0.0	0.0	0.0	92.5	0.0	100.0	100.0	100.0
45	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0
46	0.0	0.0	0.0	0.0	0.0	45.0	100.0	100.0	100.0
47	0.0	0.0	0.0	45.0	0.0	0.0	100.0	100.0	100.0
48	0.0	0.0	0.0	0.0	0.0	10.0	100.0	100.0	100.0
49	0.0	0.0	0.0	45.0	0.0	7.5	100.0	100.0	100.0
50	15.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0
51	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	70.0
52	0.0	0.0	0.0	0.0	65.0	65.0	100.0	100.0	100.0
53	0.0	2.5	0.0	0.0	0.0	70.0	100.0	100.0	100.0
54	0.0	0.0	0.0	0.0	0.0	0.0	100.0	90.0	100.0
55	0.0	0.0	0.0	0.0	0.0	17.5	100.0	100.0	100.0
56	0.0	0.0	0.0	90.0	0.0	0.0	100.0	100.0	100.0
57	0.0	0.0	0.0	0.0	70.0	0.0	100.0	100.0	100.0
58	0.0	0.0	5.0	0.0	0.0	0.0	100.0	100.0	100.0
59	0.0	0.0	0.0	0.0	52.5	0.0	100.0	100.0	100.0
60	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0
61	0.0	0.0	0.0	0.0	0.0	85.0	100.0	100.0	100.0
62	0.0	0.0	80.0	0.0	0.0	0.0	100.0	100.0	100.0
63	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0	100.0
64	0.0	0.0	0.0	x	65.0	17.5	100.0	100.0	100.0

APPENDIX VI

**PERCENTAGE DAMAGE OF JACK PINE NEEDLES :
FREEZING TEST NO 6**

DATE : 08/06/1990
TREATMENT 1 : -1° C
TREATMENT 2 : -3° C
TREATMENT 3 : -6° C

SITE NO	TREATMENT 1			TREATMENT 2			TREATMENT 3		
	1	2	3	1	2	3	1	2	3
1	0.0	0.0	0.0	2.5	0.0	0.0	95.0	97.5	100.0
2	0.0	0.0	0.0	67.5	0.0	0.0	100.0	90.0	82.0
3	0.0	0.0	0.0	0.0	35.0	0.0	100.0	100.0	100.0
4	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	90.0
5	0.0	0.0	10.0	20.0	0.0	0.0	87.5	100.0	100.0
6	0.0	0.0	2.5	0.0	0.0	0.0	100.0	67.5	100.0
7	0.0	0.0	0.0	0.0	0.0	0.0	95.0	100.0	100.0
8	0.0	0.0	0.0	0.0	0.0	0.0	100.0	72.5	100.0
9	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0
10	0.0	0.0	0.0	0.0	0.0	0.0	90.0	97.5	100.0
11	0.0	0.0	0.0	0.0	0.0	0.0	100.0	90.0	100.0
12	0.0	0.0	0.0	0.0	0.0	0.0	40.0	100.0	100.0
13	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0
14	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	90.0
15	0.0	0.0	0.0	0.0	0.0	0.0	97.5	90.0	100.0
16	0.0	0.0	0.0	0.0	0.0	0.0	80.0	80.0	100.0
17	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0
18	0.0	0.0	0.0	0.0	0.0	0.0	97.5	100.0	100.0
19	0.0	0.0	0.0	0.0	0.0	0.0	20.0	90.0	70.0
20	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0
21	0.0	0.0	0.0	0.0	0.0	2.5	100.0	100.0	97.5
22	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0
23	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	97.5
24	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0
25	0.0	2.5	0.0	0.0	20.0	0.0	100.0	100.0	100.0
26	7.5	0.0	2.5	12.5	0.0	0.0	100.0	100.0	80.0
27	2.5	0.0	0.0	0.0	0.0	85.0	100.0	100.0	100.0
28	0.0	0.0	0.0	0.0	2.5	0.0	92.5	100.0	92.5
29	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0
30	2.5	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0
31	0.0	0.0	0.0	0.0	35.0	0.0	100.0	100.0	82.5
32	0.0	2.5	0.0	32.5	0.0	0.0	100.0	70.0	100.0
33	0.0	0.0	5.0	0.0	0.0	0.0	100.0	100.0	100.0
34	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0
35	0.0	2.5	0.0	0.0	0.0	0.0	100.0	100.0	70.0
36	0.0	5.0	0.0	0.0	7.5	0.0	82.5	52.5	97.0
37	2.5	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0
38	0.0	0.0	0.0	0.0	0.0	0.0	85.0	60.0	55.0
39	0.0	0.0	0.0	0.0	0.0	0.0	80.0	100.0	100.0
40	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	80.0

SITE NO	TREATMENT 1			TREATMENT 2			TREATMENT 3		
	1	2	3	1	2	3	1	2	3
41	5.0	0.0	0.0	0.0	0.0	0.0	100.0	70.0	90.0
42	0.0	2.5	0.0	0.0	0.0	0.0	100.0	100.0	97.0
43	0.0	0.0	0.0	0.0	0.0	0.0	100.0	90.0	80.0
44	0.0	0.0	0.0	2.5	0.0	0.0	80.0	100.0	45.0
45	0.0	0.0	0.0	0.0	2.5	0.0	100.0	55.0	100.0
46	0.0	0.0	0.0	0.0	0.0	40.0	100.0	100.0	100.0
47	0.0	0.0	0.0	0.0	0.0	0.0	100.0	60.0	100.0
48	0.0	0.0	0.0	0.0	0.0	0.0	37.5	100.0	100.0
49	0.0	0.0	0.0	7.5	0.0	0.0	100.0	100.0	100.0
50	2.5	0.0	0.0	7.5	0.0	0.0	100.0	100.0	45.0
51	0.0	0.0	5.0	0.0	0.0	7.5	100.0	100.0	100.0
52	0.0	0.0	0.0	2.5	2.5	0.0	100.0	100.0	100.0
53	0.0	0.0	0.0	5.0	0.0	0.0	100.0	100.0	92.0
54	0.0	0.0	2.5	0.0	0.0	0.0	92.5	100.0	100.0
55	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0
56	0.0	0.0	0.0	0.0	0.0	0.0	90.0	100.0	100.0
57	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0
58	2.5	0.0	2.5	0.0	50.0	0.0	100.0	100.0	90.0
59	0.0	0.0	0.0	0.0	0.0	0.0	100.0	92.5	90.0
60	2.5	0.0	0.0	0.0	0.0	2.5	100.0	100.0	100.0
61	0.0	0.0	0.0	7.5	20.0	0.0	100.0	100.0	100.0
62	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0
63	2.5	0.0	0.0	0.0	0.0	0.0	100.0	100.0	40.0
64	0.0	0.0	0.0	15.0	0.0	0.0	100.0	100.0	100.0

APPENDIX VII

**PERCENTAGE DAMAGE OF JACK PINE NEEDLES :
FREEZING TEST NO 7**

DATE : 09/23/1990
TREATMENT 1 : -2° C
TREATMENT 2 : -5° C
TREATMENT 3 : -6° C

SITE NO	TREATMENT 1			TREATMENT 2			TREATMENT 3		
	1	2	3	1	2	3	1	2	3
1	0.0	0.0	0.0	55.0	0.0	2.5	2.5	2.5	2.5
2	0.0	0.0	0.0	50.0	7.5	0.0	55.0	82.5	25.0
3	0.0	0.0	0.0	0.0	100.0	0.0	10.0	2.5	25.0
4	0.0	10.0	0.0	75.0	0.0	0.0	5.0	15.0	20.0
5	0.0	0.0	0.0	2.5	0.0	50.0	60.0	70.0	62.5
6	0.0	0.0	0.0	2.5	25.0	17.5	30.0	65.0	5.0
7	0.0	0.0	0.0	55.0	10.0	10.0	65.0	30.0	65.0
8	0.0	0.0	0.0	5.0	60.0	7.5	30.0	2.5	20.0
9	0.0	0.0	0.0	0.0	42.5	0.0	2.5	0.0	37.5
10	0.0	2.5	15.0	50.0	50.0	57.5	72.5	62.5	52.5
11	10.0	0.0	0.0	50.0	30.0	0.0	25.0	82.5	10.0
12	0.0	0.0	5.0	65.0	65.0	72.5	20.0	27.5	70.0
13	0.0	0.0	27.5	17.5	90.0	60.0	70.0	97.5	0.0
14	15.0	2.5	0.0	20.0	0.0	15.0	35.0	57.5	90.0
15	0.0	0.0	0.0	57.5	0.0	0.0	0.0	10.0	0.0
16	0.0	0.0	10.0	7.5	50.0	90.0	10.0	32.5	55.0
17	0.0	0.0	0.0	0.0	0.0	0.0	7.5	20.0	85.0
18	0.0	0.0	0.0	0.0	20.0	0.0	65.0	57.5	75.0
19	15.0	5.0	0.0	57.5	15.0	0.0	52.5	45.0	95.0
20	0.0	0.0	5.0	45.0	40.0	2.5	30.0	7.5	0.0
21	0.0	0.0	0.0	0.0	5.0	37.5	0.0	0.0	0.0
22	0.0	0.0	0.0	62.5	70.0	50.0	70.0	97.5	67.5
23	0.0	0.0	0.0	0.0	2.5	60.0	0.0	90.0	77.5
24	0.0	0.0	0.0	0.0	17.5	85.0	20.0	0.0	10.0
25	0.0	0.0	0.0	60.0	17.5	45.0	10.0	95.0	90.0
26	0.0	0.0	0.0	0.0	17.5	37.5	5.0	47.5	12.5
27	0.0	0.0	2.5	30.0	25.0	45.0	62.5	25.0	17.5
28	0.0	0.0	0.0	0.0	12.5	0.0	7.5	20.0	0.0
29	0.0	0.0	0.0	0.0	30.0	2.5	10.0	5.0	0.0
30	0.0	0.0	0.0	30.0	57.5	0.0	77.5	60.0	22.5
31	0.0	0.0	7.5	15.0	17.5	0.0	72.5	37.5	80.0
32	0.0	0.0	0.0	60.0	87.5	0.0	20.0	40.0	0.0
33	20.0	0.0	0.0	25.0	12.5	75.0	5.0	45.0	57.5
34	0.0	5.0	0.0	40.0	0.0	0.0	0.0	30.0	15.0
35	0.0	0.0	0.0	27.5	25.0	0.0	40.0	40.0	x
36	0.0	5.0	0.0	32.5	0.0	72.5	0.0	0.0	0.0
37	0.0	0.0	0.0	25.0	5.0	5.0	0.0	12.5	12.5
38	0.0	0.0	0.0	25.0	2.5	0.0	42.5	2.5	2.5
39	0.0	0.0	0.0	5.0	25.0	0.0	25.0	7.5	2.5
40	0.0	0.0	0.0	0.0	0.0	20.0	0.0	0.0	2.5

SITE NO	TREATMENT 1			TREATMENT 2			TREATMENT 3		
	1	2	3	1	2	3	1	2	3
41	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
42	0.0	0.0	0.0	0.0	7.5	0.0	0.0	0.0	5.0
43	0.0	0.0	0.0	32.5	25.0	0.0	10.0	0.0	15.0
44	0.0	0.0	0.0	5.0	20.0	17.5	7.5	20.0	52.5
45	0.0	0.0	0.0	37.5	0.0	50.0	70.0	30.0	62.5
46	0.0	0.0	0.0	72.5	20.0	52.5	25.0	0.0	22.5
47	0.0	0.0	0.0	35.0	0.0	0.0	0.0	0.0	0.0
48	0.0	0.0	0.0	47.5	37.5	17.5	20.0	0.0	0.0
49	0.0	2.5	0.0	0.0	5.0	0.0	2.5	47.5	10.0
50	0.0	0.0	0.0	15.0	15.0	0.0	10.0	25.0	0.0
51	0.0	0.0	0.0	12.5	0.0	0.0	27.5	15.0	12.5
52	0.0	0.0	0.0	0.0	0.0	70.0	32.5	77.5	27.5
53	0.0	10.0	0.0	20.0	0.0	20.0	22.5	22.5	72.5
54	0.0	0.0	0.0	0.0	22.5	12.5	0.0	17.5	15.0
55	0.0	0.0	0.0	0.0	0.0	22.5	2.5	0.0	0.0
56	0.0	0.0	0.0	40.0	0.0	60.0	20.0	27.5	67.5
57	0.0	0.0	0.0	0.0	40.0	30.0	0.0	0.0	0.0
58	0.0	0.0	0.0	40.0	40.0	10.0	10.0	10.0	0.0
59	0.0	0.0	0.0	47.5	5.0	67.5	0.0	25.0	0.0
60	0.0	0.0	0.0	55.0	90.0	72.5	65.0	87.5	5.0
61	0.0	0.0	0.0	10.0	55.0	2.5	70.0	0.0	92.5
62	0.0	0.0	0.0	10.0	35.0	55.0	77.5	40.0	70.0
63	0.0	0.0	0.0	0.0	15.0	x	0.0	45.0	20.0
64	0.0	0.0	0.0	30.0	52.5	0.0	10.0	12.5	40.0

APPENDIX VIII

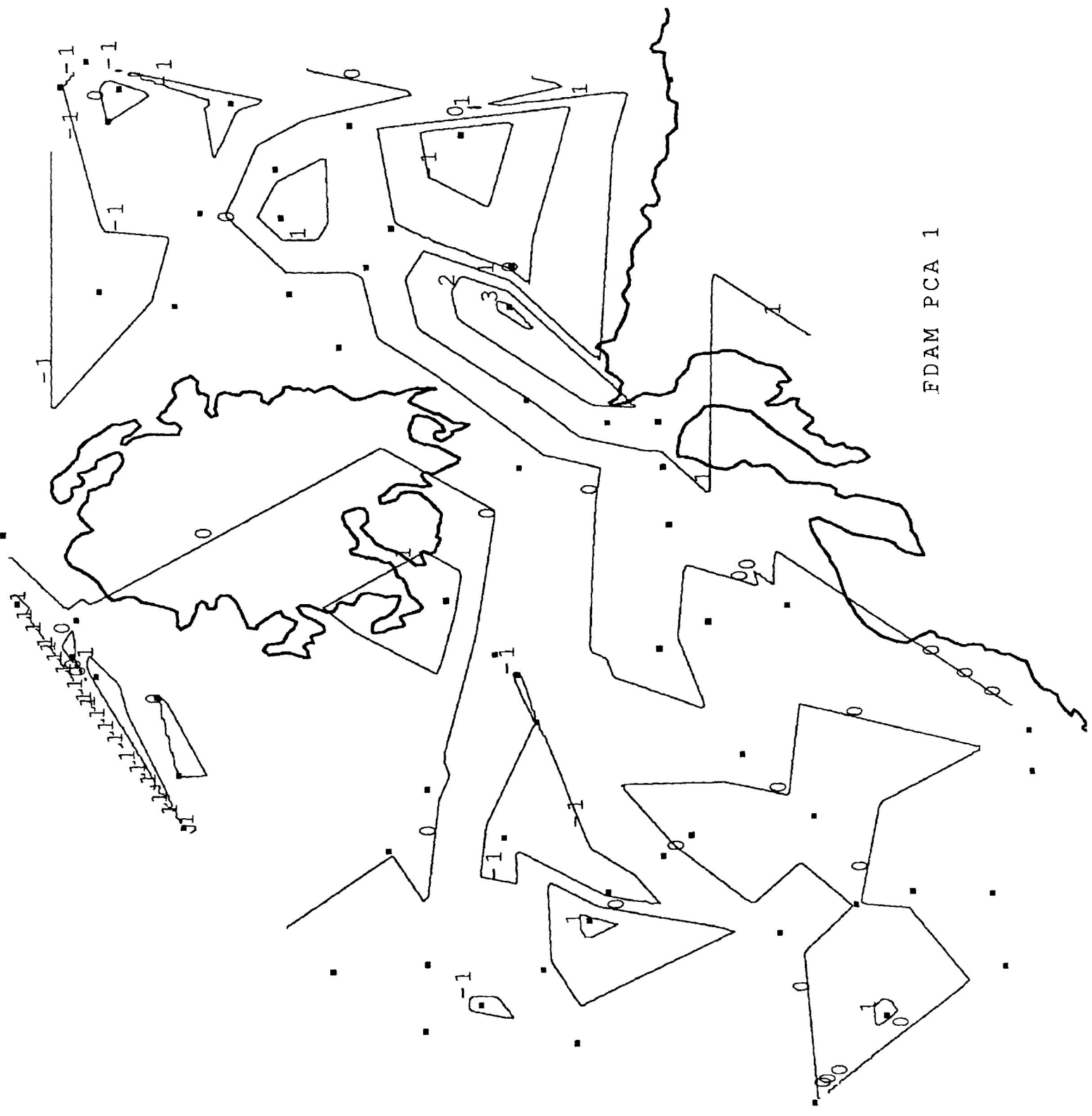
**THREE SUMMARY VARIABLES GENERATED FROM
PRINCIPAL COMPONENT ANALYSIS**

SITE NO	FIRST SUMMARY VARIABLE	SECOND SUMMARY VARIABLE	THIRD SUMMARY VARIABLE
1	-1.098	-0.128	0.219
2	-0.977	2.088	-0.529
3	0.536	1.178	1.467
4	0.020	0.279	-0.268
5	-1.265	0.555	-1.764
6	-1.557	2.168	-1.052
7	-0.761	1.629	-0.116
8	-0.706	1.412	0.830
9	-0.966	0.820	1.004
10	1.764	1.536	0.998
11	-0.264	0.377	-0.563
12	3.283	1.324	-0.169
13	1.806	0.398	0.883
14	-0.677	1.303	-0.151
15	0.924	0.041	0.961
16	0.527	0.867	0.687
17	-0.761	-0.206	0.307
18	1.678	-0.780	-0.063
19	0.773	1.679	-0.724
20	0.660	-0.489	0.393
21	-1.923	0.065	1.652
22	1.691	0.810	-0.866
23	1.673	-0.663	-0.650
24	-0.299	0.721	-0.921
25	1.062	-0.544	-3.024
26	-0.037	0.866	-1.129
27	-0.010	0.370	-1.080
28	0.058	-1.271	0.682
29	-0.431	-0.314	0.584
30	1.275	1.509	0.938
31	-0.510	0.002	-2.658
32	-0.058	0.380	1.013
33	-0.492	-0.018	0.705
34	-0.467	-0.971	0.058
35	-0.930	1.090	0.380
36	-0.199	-0.044	0.679
37	-1.045	-0.557	-0.829
38	-1.016	-1.334	0.398
39	-0.850	-0.686	-0.756
40	-1.301	-0.634	0.871

SITE NO	FIRST SUMMARY VARIABLE	SECOND SUMMARY VARIABLE	THIRD SUMMARY VARIABLE
41	0.839	-1.846	-0.515
42	-0.685	-0.071	0.410
43	-0.353	-0.120	1.014
44	0.650	-0.284	1.499
45	0.600	-0.019	-1.194
46	1.466	-0.503	-0.437
47	-1.380	-0.219	-0.323
48	1.176	-1.810	1.883
49	-0.439	-1.770	-0.884
50	0.227	-1.177	0.407
51	0.016	-1.655	0.013
52	1.274	-0.310	-0.909
53	-0.060	-0.984	0.033
54	-0.481	-1.580	0.611
55	-0.138	-0.879	1.263
56	-0.115	-0.334	-0.266
57	-1.241	-0.050	-0.575
58	-0.287	-1.481	-1.524
59	-0.556	0.212	1.274
60	x	x	x
61	0.271	0.197	-0.075
62	0.139	0.792	-0.566
63	0.124	-1.205	-0.842
64	-0.175	0.273	1.304

APPENDIX IX

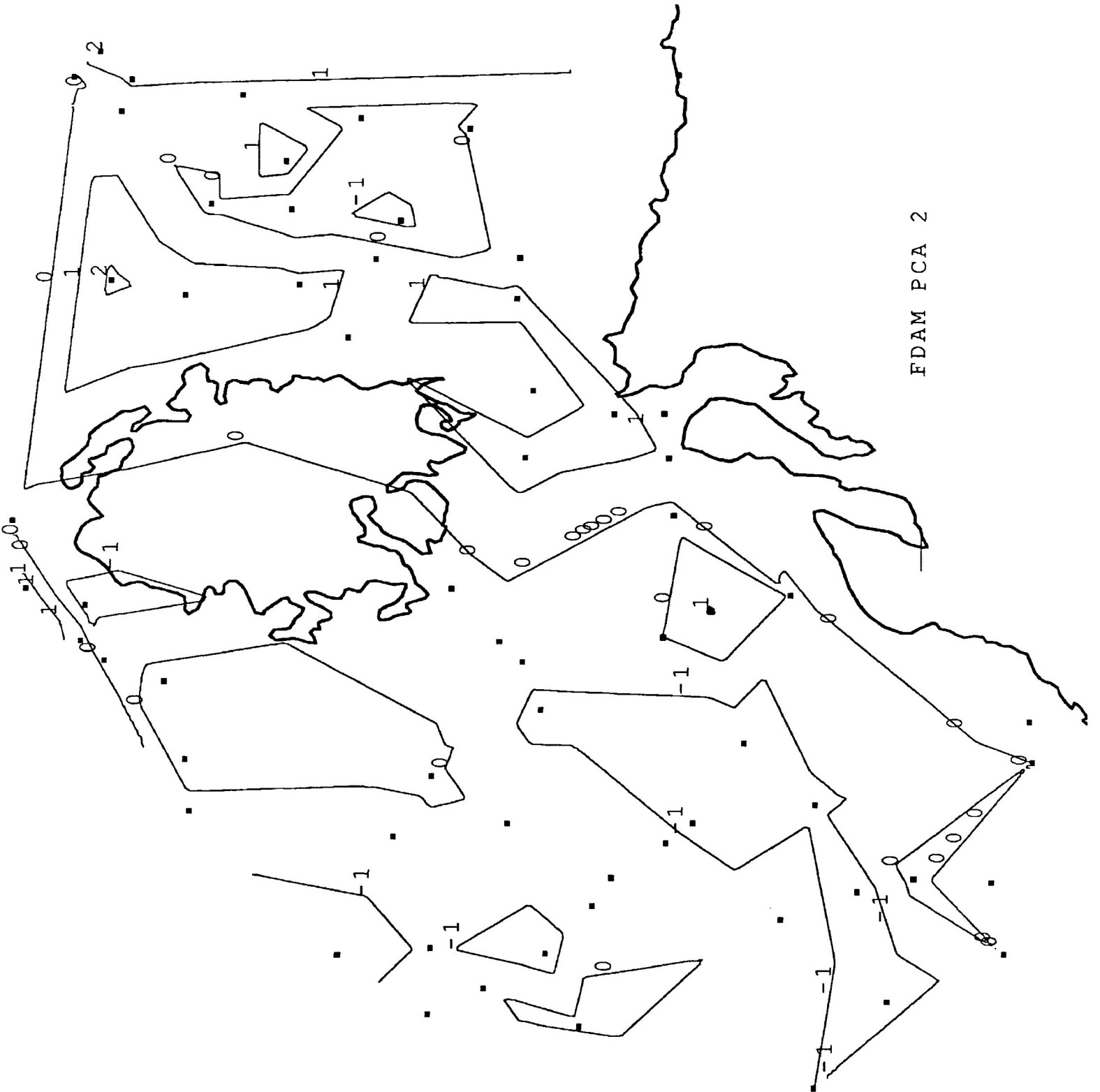
**TREND SURFACE BASED ON THE FIRST PRINCIPAL
COMPONENT AXIS SCORES**



EDAM PCA 1

APPENDIX X

**TREND SURFACE BASED ON THE SECOND PRINCIPAL
COMPONENT AXIS SCORES**



FDAM PCA 2

APPENDIX XI

**TREND SURFACE BASED ON THE THIRD PRINCIPAL
COMPONENT AXIS SCORES**



FDAM PCA 3

APPENDIX XII

ALUMINUM RACKS USED FOR THE SUSPENSION OF THE SAMPLES

