FROST HARDINESS OF BALSAM POPLAR (*POPULUS BALSAMIFERA* L.) DURING THE SPRING DEHARDENING PERIOD

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ABSTRACT

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Keywords: Populus balsamifera, frost hardiness, dehardening, genetic variation, clones.

Changes in the frost hardiness of balsam poplar (*Populus balsamifera* L.) cuttings from four populations along a latitudinal transect from N. Wisconsin to Bearskin L., Ontario, were examined during the spring of 1987. Hardiness levels of dormant stem cuttings from the two extreme populations were examined after various incubation periods, under two different dehardening temperature regimes, with a standard freezing test (freezing temperatures: -3,-11,-19, and -27° C). Northern clones were less susceptible to frost injury than southern clones during the spring dehardening period, and this phenomenon was closely related to the tendency of northern clones to remain dormant longer than southern clones. High within-population variation was also noted in hardiness levels and bud break characteristics. Leaf tissue dehardened more rapidly than stem tissue, and the dehardening process occured more rapidly at the higher incubation temperature.

A second study in which cuttings from the four provenances were subjected to a series of controlled freezing temperatures (-3,-6,-9,-12,-18, and -24° C) at parallel developmental stages revealed that provenance differences in frost injury were essentially a function of differential shoot phenology at the time of freezing. Cuttings were hardy to -18° C when leaf expansion first became visible, and could be subjected to -12° C without injury when the newly expanding shoot became visible, indicating that an attenuated form of hardiness may exist even when the shoots are actively growing.

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INTRODUCTION

This study was initiated to obtain a more comprehensive understanding of the ecological genetics of Populus balsamifera L. This species is presently being investigated at Lakehead University in Thunder Bay, Ontario for potential use in short rotation silvicultural systems . As noted by Rehfeldt (1979), an understanding of the ecological genetics of a species is fundamental to the development of comprehensive silvicultural and tree improvement programs.

The goal of this study was to investigate the susceptibility of balsam poplar to to freezing temperatures which might be encountered during spring growth initiation. Injuries to plants due to low temperature are of great importance where freezing occurs. As a result, the nature of damage caused to plants by freezing has been the topic of a great deal of research. Although the development of cold hardiness (and its' environmental control) has been well studied, less is known about the conditions and rates of dehardening. To date, there have been only limited investigations into the dehardening of boreal hardwood species. The general relationship appears to be as follows (Levitt, 1980):

(1) After physiological dormancy is overcome through the chilling process, plants lose hardiness if exposed to dehardening temperatures.

(2) Wide species variation exists in the nature and rapidity of the dehardening process, and the process appears to proceed more rapidly at higher temperatures.

(3) Genetic variation in frost hardiness has been found to exist within and between natural populations of forest tree species during this period.

In addition to evaluating the dehardening characteristics of balsam poplar, the other main goal of this study was to examine genetic variation in frost hardiness within and between widely separated populations of balsam poplar. In this regard, dehardening was evaluated as a possible adaptive characteristic.

LITERATURE REVIEW

COLD RESISTANCE AND FREEZING INJURY IN PLANTS

Cold injury has been an important factor in the reduction of growth and quality of forest tree species (Plenkema, 1964; Strain, 1966). The nature of injuries caused to plants by freezing has been the subject of a great deal of research . Reviews on the subject have been written by Levitt (1956, 1966, 1980), Olien (1967) and Mazur (1969). According to Levitt (1980), there are two main types of freezing injury: (1) primary direct injury due to intracellular freezing, and (2) secondary freeze-dehydration injury due to extracellular freezing. The former, which is rarely observed in nature (Scarth, 1944), is usually related to the rupturing of cell membranes by ice crystals that form in the protoplasm and distrupt the protoplasm. The latter is most often explained in terms of a freeze-induced water stress resulting from the diffusion of the cell's water to extracellular ice centers.

If a plant is to survive in climates with seasonal freezing temperatures it must minimize damage associated with intracellular and extracellular freezing. In terms of freezing resistance, there are essentially two main adaptive strategies available to the plant - avoidance and tolerance. Levitt (1978) states that the only resistance strategy that must be developed by all vegetative plants, in order to survive the freezing stress of temperate climates, is extracellular freezing tolerance. However, some species combine avoidance strategies (i.e. avoidance of ice formation at freezing temperatures) with the strategy of tolerance of extracellular freezing.

For example, most Eastern deciduous forest species avoid freezing in their xylem ray parenchyma by "deep supercooling" to temperatures as low as -40°c in midwinter (Burke et al., 1977). Supercooling probably occurs because of a lack of nucleating substances in these tissues necessary for ice initiation. In the absence of nucleating centers, pure water can supercool (remain as a liquid) to -38 °C.

FACTORS RELATED TO FREEZING TOLERANCE

The seasonal change in the ability of a tree to resist freezing injury is referred to as the frost-hardiness process. A large number of conflicting observations have been made on the mechanisms controlling the frost-hardiness process (Olien, 1967, Mazur, 1969, and Levitt, 1980). During the late spring and early summer when a plant is actively growing, it has the least resistance to freezing injury (i.e. a non-hardy state). However, in the fall when a plant is in a transitional state to maximum winter hardiness, numerous physiological and biochemical changes occur within the plant. Increased frost hardiness has been associated with general protoplasmic augmentation, including a build-up of substances such as sugars, proteins, lipids, amino acids, and nucleic acids (Weiser, 1970). However, as noted by Glerum (1976), correlations between these substances and frost hardiness levels can rarely be applied simultaneously during hardening, and they are generally considerably poorer during the dehardening period.

Water content is frequently inversely related to hardiness (Levitt, 1956) although some exceptions do exist. The water content of sycamore (Platanus occidentalis L.) twigs fluctuates during the winter in a manner that does not parallel freezing tolerance (Le Saint and Catesson, 1966). Early investigators assumed that the total amount relatively stable "bound water" in close association with biological macromolecules, was an important aspect of the hardiness process. In this regard, bound water plays a decisive role in preserving the structure of membranes and other native macromolecules under freezing temperatures. Subsequent investigations by Heber, (1959), Levitt (1969), and Brown et al. (1970), have cast serious doubts on the importance of bound water to the hardiness process.

In the 1970's, a great deal of research was focused on changes in the cell membrane during cold acclimation. Numerous reports indicate that there is an increase in phospholipids during the hardening process (Siminovitch et al, 1968,

1975; Yoshida, 1969). The build-up of phospholipid reserves may be necessary to replace those degraded during freezing (Yoshida and Sakai, 1974). Low temperature is also known to cause an accumulation of polyunsaturated fatty acids (Gerloff et al., 1966), and unsaturated fatty acids are said to increase the fluidity of cell membranes, presumably making them less susceptible to mechanical damage at lower temperatures (Akamatsu, 1974). Glerum (1976) points out that it is not known to what extent these changes in the quantities of phospholipids and unsaturated fatty acids represent changes in the cellular membrane.

Timmis and Worrall (1974) have provided evidence that the mechanism that controls frost hardiness is localized in nature, occurring in each cell or tissue type. They obtained a 25°C difference in hardiness on different branches of Douglas fir (Pseudotsuga menziesii (Mirb.) Franco var menziesii) located on the same seedling. The localized nature of chilling in inducing hardiness has also been reported by Howell and Weiser (1970). The concept of a translocatable hardiness-promoting factor (i.e. a sugar or growth regulating hormones) was proposed and supported by these investigators.

Levitt (1962) has proposed a theory which suggests a molecular basis for freezing injury and tolerance. According to his sulfhydryl (SH) hypothesis of freezing injury, low temperature causes structural proteins to become reversibly denatured, unmasking reactive SH groups. As freeze- dehydration removes cell water during freezing, these proteins are forced into closer proximity. This compaction causes sulfhydryl groups in adjoining proteins (or in adjoining strands of the same protein) to become linked through the formation of disulfide (SS) bonds. These bonds aggregate the proteins irreversibly, killing the cell upon rehydration during thawing. Levitt suggests that biochemical changes accompanying frost hardening are those which reduce the likelihood of disulfide bond formation (i.e. freezing tolerance involves increases in the resistance toward

the oxidation of SH groups). However, Mazur (1969) points out several difficulties with this hypothesis.

Levitt presents evidence that the number of disulfide bonds increases with freezing injury, but there is no evidence that this is the cause and not the result of freezing injury. Furthermore, the theory has been applied to injury from both intra- and extracellular freezing, despite the fact that it most satisfactorily accounts for injuries observed when higher plants are frozen very slowly and thawed rapidly.

More comprehensive reviews on the factors related to freezing tolerance are given by Mazur (1969) and Levitt (1980).

METHODS OF EVALUATING FROST HARDINESS

Frost hardiness is a general term for the resistance of a plant to freezing injury. It is usually a reflection of freezing tolerance, since freezing tolerance is the major mechanism of frost resistance. Hardiness has been evaluated in terms of the frost killing point, the freezing temperature required to kill 50 percent of the plant (Johansson et al., 1955). Other measures include the "ultimate frost-killing point", resulting in 100 percent killing, or the "incipient frost-killing point" that just begins to cause injury.

Relative differences in the hardiness of trees were originally evaluated in terms of field survival. But this method proved to be slow and inaccurate owing to the many complex relationships involved (Olien, 1967). Artificial freezing tests under controlled conditions are now used to test the hardiness of plants. According to Levitt (1956), the freezing test generally consists of lowering the temperature of the material (i.e. seedlings or tissue samples) at a standard rate, often between 1° - 5°C per hour, to a series of predetermined temperatures.

There are several factors of importance in a freezing test. As demonstrated by Pfeiffer (1933), the rate of cooling may influence the frost killing point of plants. If

cooling occurs too rapidly (5 - 20°C/minute), intracellular ice formation may occur (Levitt, 1980). The length of time for which plant material is maintained at the freezing temperature is also of importance, as pointed out by Day and Peace (1937) and Aronsson and Eliasson (1970). Also, the rate of thawing may influence the development of damage (Iljun, 1934; Levitt, 1966).

Numerous methods for determining the damage caused to the tissues during freezing have been developed. These tests are designed to determine whether: (1) enzyme and metabolic functions have been impaired, or (2) cell membranes have been damaged or destroyed.. A summary of these viability tests was provided by Timmis (1976) and is given in Table 1. Methods for determining whether a cell or tissue is alive or dead have also been covered by Parker (1953).

PLANT DISTRIBUTION AND FROST HARDINESS

The ability of plants to survive subfreezing temperatures is of interest in the study of distribution, succession, and migration of plants, because climate is generally considered the most important environmental factor affecting plant distribution (Alden and Hermann, 1971; Sakai and Weiser, 1973). It has been suggested that seasonal freezing temperature are the single environmental factor that limit the northward migration of various native trees. Studies conducted on willows (Salix spp.) native to warm climates (Sakai, 1970) and loblolly pine (Pinus taeda L.)(Posen, 1967) have shown that some species are capable of developing cold tolerance greater than the minimum temperature of their ecological range.

In spite of evidence indicating that injury from freezing does not limit the range of plants in regions of seasonal subfreezing temperatures, it has been suggested that low temperature is one of the most significant natural environmental factors causing direct plant injury in cold climates (Campana, 1964).

Table 1. Methods for evaluating damage to plant tissues (condensed from Timmis, 1976).

Name of method	Theory for injured tissue	Method of measurement	Reference
(A) METHODS BASED ON IMPAIRMENT OF ENZYME AND METABOLIC FUNCTIONS			
1. Morphological			
Bud tissue browning	Phenol-amine group reactions and subsequent oxidations.	Visual assessment	Alden, 1971
2. Physiological			
Photosynthesis	Cholorplasts break down. Mesophyll diffusion resistance increases.	Infrared gas analysis	Neilson et al., 1972
3. Chemical			
Tri-phenyl tetrazolium chloride	Inactivated dehydrogenases cannot reduce this vacuum infiltrated substance.	Incubation and absorbance of red alcohol extract.	Steponkus and Lanphear, 1967.
(B) METHODS BASED ON DAMAGE TO CELL MEMBRANES			
4. Electrical			
Electrolytic method	Ions leak from cells.	Conductivity of solution.	Dexter et al., 1932, Wilner, 1960
Impedance	Ionic conductance of membrane increases.	Inserted electrodes and impedance bridge circuit.	Greenham and Daday, 1957, van den Driessche,1973

Burke et al. (1976) and George and Burke (1977) have indicated that low temperature extremes affect the range of most Eastern deciduous forest species and fruit tree cultivars. As previously mentioned, these species avoid freezing in some of their tissues by "deep supercooling" to temperatures as low as -40°C in midwinter. As a result, these species are confined to regions where minimum winter temperature does not drop below -40°C.

SEASONAL VARIATION IN FROST HARDINESS

The development of cold hardiness (also known as cold acclimation) has been well studied in woody plants, and most investigators have found that the development of hardiness is a two- or three-stage process (Tumnavov and Krasavtsev, 1959; Weiser, 1970). Weiser (1970) indicates that the first stage of hardening appears to be induced by short days. The second stage is apparently induced by low temperatures (i.e. just below 0°C) and a third stage is induced by low temperatures in the range of -30° to -50°C.

The conditions and rates of dehardening in the spring have not been intensively studied in natural populations of forest trees (see Glerum, 1973). The existing literature (based mainly on horticultural species) suggests that following the fall hardening process, while plants are physiologically dormant, brief exposure to dehardening temperatures (10° - 20°C) will not result in a loss of hardiness (Edgerton, 1954). However, after physiological dormancy is overcome through the chilling process, plants will lose hardiness if exposed to dehardening temperatures (Irving and Lanphear, 1967). After physiological dormancy is overcome, plants may not reharden substantially if reexposed to low temperatures (Hamilton, 1973). However, Howell and Weiser (1970) and Pukacki (1982), have provided evidence that the ability to reharden is not lost with the loss of physiological dormancy.

Under natural conditions in temperate climates, trees tend to lose hardiness over a two-month period in the late winter and early spring. The phenomenon of pre-bud burst shoot dehardening has been well documented. Glerum (1973, 1976) notes that a substantial loss of hardiness in Pinus resinosa Ait, Picea mariana (Mill) B.S.P. and Larix laricina (Du Roi) K. Koch before bud break. Furthermore, he concluded that Larix laricina still maintained considerable hardiness (i.e. between -17° and -11°C) during bud flush. Pinus sylvestris L. has been observed to lose hardiness gradually over a four-week period in the spring when exposed to a constant temperature of 20°C (Aronsson, et al., 1976). Cannell and Sheppard (1982) have reported that Picea sitchensis (Bong.) Carr. begins to deharden in response to warm temperatures several weeks before bud burst.

Minimum frost hardiness generally coincides with rapid cell division and elongation at the time of bud burst, and this is when the shoots are most at risk from frosts. Glerum (1973,1976) has suggested that dehardening may be a two-stage process. Timmis and Worrall (1974) considered the onset of elongation to be a second stage of dehardening, following warm temperature induced dehardening. However, the exact relationship between growth (or conversely dormancy) and frost hardiness is not clearly understood.

The period of minimum frost hardiness occurs at the time of bud burst in Picea glauca (Moench) Voss (Nienstadt and King, 1969), Abies balsamea (L.) Mill. (Lester et al., 1977) and many other conifers. This is not so for Pinus spp. which do not reach minimum hardiness until the needles are rapidly elongating (Glerum, 1973). Similarily, some Larix spp. can tolerate temperatures below -10°C during the early stages of bud burst.

Pelkonen and Glerum (1986) examined clonal variation in the frost hardiness of several poplar species using electrical impedance techniques. With this technique, fatal injuries due to freezing were identified on the basis of low kHz/MHz

impedance ratios (< 2) or a decrease in the KHz impedance. The 1 kHz impedance for all clones was found to increase with hardiness levels towards the end of the fall, reaching an peak on November 23. Throughout the winter, the 1 kHz impedance was found to decrease in frost susceptible clones of P. deltoides X P. euramericana (Dode) Guinier, and remain more or less constant in frost tolerant clones of P. deltoides var. occidentalis (pop. 645) and P. balsamifera. Changes in the electrical impedance trends when cuttings go from a dormant to an actively growing state were also examined in a clone of P. balsamifera and P. deltoides X P. euramericana. A rapid decrease in the 1 kHz impedance was observed in both clones 12 days prior to bud flush, suggesting that both clones begin dehardening several weeks prior to bud burst. However, it should be noted that the impedance values observed in this experiment were not correlated to actual levels of freezing injury.

GENETIC VARIATION IN FROST HARDINESS

Genetic variation in the frost hardiness of North American forest trees has been most frequently examined within the context of provenance investigations. Large provenance differences have been reported in the rate of autumn hardening within Pseudotsuga menziesii (Mirb.) Franco var. menziesii (Scheumann, 1962; Cambell and Jorensen, 1973), Pinus sylvestris L. (Jonsson et al., 1981), Pinus strobus L. (Mergen, 1963), Quercus rubra L. (Flint, 1972), and many other species. In most cases, these large provenance differences can be closely correlated with time of bud set (i.e. due to the adaptive differentiation of the species along an environmental gradient).

However, ecotypic variation in frost hardiness has also been reported. Rehfeldt (1977), has determined that during cold acclimation, progenies of the coastal variety of Douglas fir (Pseudotsuga menziesii (Mirb.) Franco var. menziesii) are of lesser hardiness than those of the Rocky Mountain variety (P. menziesii var. glauca (Beissn.) Franco). Rehfeldt (1979) has also noted high within-population

variance during cold acclimation in P. menziesii var. glauca. Inherent differences in hardiness in the spring can often be explained by differences in the onset of cambial growth and bud burst (Picea glauca, Nienstadt and King, 1969; Abies balsamea, Lester et al., 1977).

THE SILVICS AND ECOLOGICAL GENETICS OF BALSAM POPLAR

Balsam poplar (Populus balsamifera L.) is a deciduous hardwood species, ranging from Newfoundland to the northwestern tip of Alaska. The northern boundary for the species is defined by the tree line, and the southern boundary extends into northern and eastern British Columbia, and east through Alberta, to the southern tip of Lake Michigan and into New York and Maine (Roe, 1958). Most of balsam poplar's range is characterized by a continental climate.

The occurence of balsam poplar is restricted from the very wettest soils, and it rarely grows on dry and exposed sites. The species will grow in pure stands on lowland alluvial and lacustrine deposits associated with river flats, streambanks, sandbars, and the borders of lakes and swamps. Elsewhere, it generally occurs as scattered individuals or in small stands, often in association with aspen.

Shoot growth begins relatively early in the spring. Farmer and Reinholt (1986) used a forcing study to examine the chilling requirements and flushing pattern of balsam poplar along a latitudinal transect from northern Wisconsin to the southwestern shore of Hudson's Bay. It appears that the species requires less chilling to overcome physiological dormancy than most other species examined to date in central North America, and that the chilling requirement for balsam poplar is overcome by early January.

Pelkonen and Glerum (1986) have reported that the time to bud flush after freezing tests was longer and more variable for P. balsamifera clones than for various P. deltoides clones. The work of Farmer and Reinholt (1986) also

suggests that there is a high degree of variability in the flushing pattern of balsam poplar clones. They have observed that 42-48 percent of the total variation in time to bud break can be accounted for by clones within populations. Geographical source accounted for 19 to 12 percent of variance in time to bud break.

Furthermore, time to bud break was observed by Farmer and Reinholt (1986) to decrease from southern to northern material (ie. northern material broke bud earlier than southern material). This geographic trend in days to bud break may have adaptive value for populations growing in areas with shorter growing seasons, or it may be related to the fall dormancy relations of this species. Northern material (Fort Severn, Bearskin Lake) at Thunder Bay set buds in the late summer, several weeks before southern (N. Wisconsin) stock. Therefore, despite the fact that all of the plants received the same amount of chilling before forcing, they may have been in different stages of dormancy induction when the chilling began.

METHODOLOGY

COLLECTIONS

The experimental material used in this study was collected between 1982 and 1983 on a latitudinal transect at Longitude 90°W from northern Wisconsin to Bearskin Lake, Ontario (see Figure 1). Cuttings from approximately 50 balsam poplar ortets were taken from each of the four geographic sources; N. Wisconsin, Thunder Bay, Pickle Lake, and Bearskin Lake. A summary of the spring climatic conditions associated with each of these provenances is given in Table 2.

Table 2. Climatic conditions associated with each provenance (1951-1980) during the spring (Sources: Hare and Thomas; 1979, Chapman and Thomas,1968, Environment Canada, 1982, Vishner,1954).

Source	Mean Annual Temp (°C)	Mean anuual growing degree days above 5.5°C	Mean Daily Temperature (°C)				Mean Annual Frost-Free Days	Mean date of last occurrence of 0°C
			Mar	Apr	May	June		
Bearskin L. 53-54°N	-3.1	700-800	-14.4	-4.4	3.6	11.6	75-85	June 16
Pickle L., 50-51°N	-0.8	900-1000	-10.8	-0.5	6.4	12.9	80-90	June 12
Thunder Bay 48-49°N	2.4	1100-1200	-6.2	2.4	8.3	13.8	95-105	June 6
N. Wisconsin 45-46°N	4.0	1300-1400	-4.0	3.0	11.1	16.8	100-110	May 31

Ortets in each population were located at least 1 km apart to minimize the possibility of selecting ramets from a single naturally occurring clone. Ortets were

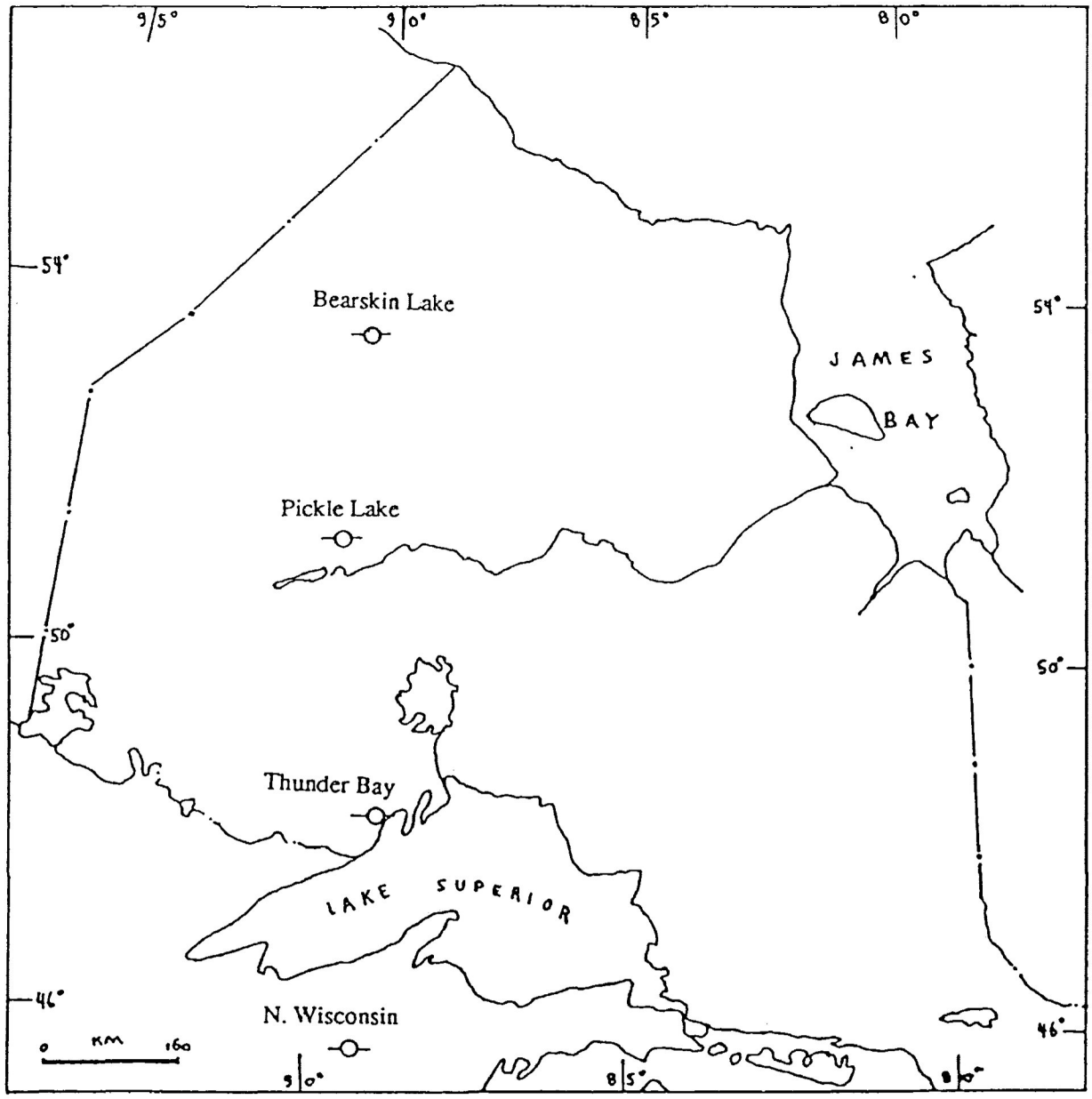


Figure 1. Geographic sources of the balsam poplar clones used in this study.

collected from populations adjacent to roads or rivers. These cuttings were later rooted in containers and transplanted in the Lakehead University nursery (Thunder Bay, Ontario).

Evaluations into the frost hardiness of balsam poplar during the spring dehardening period were conducted in the spring of 1987.

EXPERIMENTAL PROCEDURES

Two experiments were conducted during the course of this investigation. The first experiment (Experiment 1) was a study of the dehardening characteristics of clones of balsam poplar collected from two widely separated populations. It consisted of two trials run in February and March of 1987. Experiment 2 was conducted during April,1987. In this second experiment, an attempt was made to relate the frost hardiness of balsam poplar to shoot morphology during the initial stages of shoot elongation.

EXPERIMENT 1

Experiment 1 was designed to (1) evaluate the nature and rapidity of the dehardening process in balsam poplar, and (2) evaluate genetic variation in frost hardiness within and between two widely separated populations (N. Wisconsin and Bearskin L.) during the spring dehardening period.

This experiment was repeated twice in the spring of 1987. The first trial commenced on February 13, 1987. On this date, a total of 96 10-cm long stem cuttings (one year-old branches, 5-8 mm in diameter, with two buds each) were collected from each of 24 clones established in the nursery (2304 total cuttings).

Twelve of these clones were randomly selected from ortets in the N. Wisconsin (Latitude 46°N, Longitude 90°W) nursery population, and the remaining 12 clones were selected from the Bearskin Lake (Latitude 52-53° N, Longitude

90°W) population. The clones that were selected for each trial of this experiment are listed in Appendix I.

Cuttings from the twelve clones from each source were placed in polyethylene bags containing a small amount of damp peat. The cuttings were then subjected to two different dehardening temperature regimes. Half of the cuttings were placed in model E7 Conviron controlled environment chabmers set at 25°C during the day (14 hours) and 15°C at night, and the remaining half were placed in a chamber with a 15°C day (14 hours)/5°C night temperature regime.

Ten cuttings from each clone were removed from each chamber after 0 (control), 1, 4, 9 and 14 days, and eight of these cuttings were assigned to a series of four freezing temperatures (i.e. two cuttings at each temperature). The freezing temperatures used in this experiment were -3, -11, -19, -27°C. The remaining two cuttings per clone were placed directly in the greenhouse to serve as controls.

Prior to each freezing test, the cuttings were removed from the growth chambers and stored at 5°C for six hours to ensure that the cuttings were at the same temperature at the beginning of each freezing test. The cuttings were removed from the polyethylene bags and placed in wire-mesh baskets according to treatment combination. They were then placed in a chest-type freezer, and cooled at a rate that did not exceed 3°C per hour, until the first specified air temperature (-3°C) was reached. The cuttings were held at this temperature for one hour, after which cuttings assigned to this temperature were removed and thawed for 18 hours at 5°C. While these cuttings were being thawed, the freezing temperature was lowered for the remaining cuttings until the next specified temperature (-11°C) was reached. After an hour at this temperature, the cuttings designated for -11°C were removed and thawed at 5°C (18 hours). This procedure was repeated when the freezer reached the designated -19 and -27°C temperature regimes. A telethermometer with surface probes (attached to the outer bark of the cuttings) and air temperature probes was used to monitor the temperature of the cuttings during

the freezing test. The freezing curve of a randomly selected cutting was recorded using a Houston Instrument Series 4500 microscribe strip chart recorder.

After cuttings from each successive level had thawed, they were planted in Spencer-Lemaire containers [Hillsons], containing a peat:vermiculite (60:40) mixture and randomly placed in a greenhouse under natural lighting conditions. The final freezing test (for the 14-day incubation period) was conducted on February 28, 1987. Once in the greenhouse, date of bud flush was assessed on a daily basis (bud break was said to occur when green leaves were visible through the top of the bud) until the final viability assessment on March 21, 1987. Each cutting was examined and placed into one of the damage categories listed in Table 3.

Table 3. Damage classification categories

Value	Description	Status of Cutting[1]		
		leaf	cambium	rooting
0	no necrosis	0	0	yes
1	necrosis on leaf margin	0	0	yes
2	moderate leaf necrosis	0	0	yes
3	severe leaf necrosis	0	0	yes
4	top bud dead or dormant	0	0	yes
5	all buds dead	1	0	yes
6	leaf and stem tissue alive, no roots	0	0	no
7	buds dead, stem alive, no roots	1	0	no
8	dead	1	1	no

[1] 0 = alive; 1 = dead

Necrosis in stem tissue was assessed using a cut test, in which a small section of the bark was sliced off to reveal the cambium. In dead and damaged cuttings, the cambium was brown. Buds that had not flushed were sliced in half and examined for necrosis. The ability of the cuttings to root from preformed root primordia was also evaluated in the final assessment.

No attempt was made to assess damage to root tissue, since only a small percentage of the cuttings had developed roots at the time of the freezing treatments.

For the ANOVA, the response was based on the percent survival of the eight cuttings per clone subjected to the freezing test. The results from all four freezing temperatures were combined to give a single measure of the hardiness of a particular clone. For example, if live stem tissue was observed on 6 out of the 8 cuttings taken from a clone during the final assessment, then the measure of hardiness for the stem tissue would be 75 percent survival. This response was a somewhat indirect measure of hardiness, since the cuttings were subjected to different freezing temperatures (-3, -11, -19, and -27° C) during the test. However, this parameter was a good indicator of the overall hardiness of a particular clone, since all of the cuttings recieved parallel treatments during the course of the freezing test.

A percentage survival value was computed for both leaf and stem tissue on the basis of the damage categories listed in Table 3. Mortality to leaf tissue was represented by a damage score of 5, 7, or 8; while mortality to stem tissue was indicated by a damage score of 8. In this regard, the hardiness of a particular cutting was based on the point where mortality occured, as opposed to the point where frost injury began to occur. Stem tissues and leaf tissues were considered separately because preliminary observations indicated that stem tissues appear to be more hardy than leaf tissues. Thus, cuttings with only root meristems alive after freezing (ie. damage score = 5) were rarely observed.

The second trial (Experiment 1.2) was initiated with cuttings collected on March 23, 1987. With this trial, an attempt was made to evaluate changes in hardiness just prior to and immediately following bud break, since the first test placed greater emphasis on changes in hardiness preceding bud break. Due to a restriction on the amount of experimental material in the nursery, it was necessary

to randomly re-select (with partial replacement) the 12 clones within each source for the second trial (see Appendix I). The procedures used in the second trial were the same as those used in the first, with the exception of the length of the incubation periods used to promote dehardening. Incubation periods of 0, 2, 5, 8, and 11 days were used in the second trial.

The final freezing test for Experiment 1.2 was conducted on April 3, 1987, and the final viability assessment was made on April 21, 1987 using the same criteria as outlined for the first trial (Table 3).

EXPERIMENT 2

Experiment 2 was designed to (1) relate the frost hardiness of balsam poplar stem cuttings to shoot morphology during the initial stages of shoot elongation, and (2) determine if any provenance differences in frost susceptibility exist amongst cuttings at parallel stages of morphological development (ie. to determine whether or not provenance differences in hardiness are solely a function of provenance differences in the timing of bud-burst and shoot elongation.

All four geographic sources (N.Wisconsin, Thunder Bay, Pickle Lake, Bearskin Lake) were used in the second experiment. Shoot sections (0.5 m in length) with dormant buds were collected from each nursery population, over a two-week period from April 9-23, 1987. They were placed in polyethylene bags containing a small amount of damp peat, and placed in a growth chamber with a 15° C day(14 hours)/ 5° C night temperature regime. These temperatures might typically be encountered during the spring dehardening period at the nursery.

By April 23, 1987, cuttings from each population had progressed into various stages of shoot elongation. Seven morphological stages were arbitrarily identified for this experiment and were assigned values ranging from 1 (immediately prior to bud break) to 7 (new shoot visible; leaves almost perpendicular to the stem axis). A

full description of the developmental stages used in this experiment is given in Table 4. A total of 245 10-cm long stem cuttings (with two buds each) were taken from approximately 20 clones within each population. Clones were not evaluated on an individual basis in this experiment. Thirty-five of these cuttings were associated with each of the seven morphological stages (4 provenances X 7 morphological stages X 35 cuttings = 980 total cuttings).

Cuttings were subjected to six freezing temperatures (-3, -6, -9, -12, -18, and -24° C) during the freezing test, which was conducted on April 23, 1987. Five cuttings (ie. replications) from each of the 28 treatments (4 provenances X 7 morphological stages) were associated with each freezing temperature, including the control. With the exception of the designated freezing temperatures, the procedures used in the freezing test were the same as those outlined in Experiment 1. Frost damage to the cuttings was evaluated on May 13, 1987.

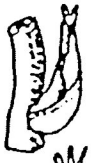
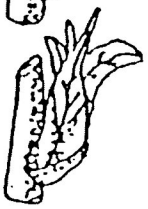
EXPERIMENTAL DESIGN

A combination of parametric (ie. such as standard ANOVA techniques in which in samples have been drawn from normally distributed populations with equal variance) and non-parametric statistics (ie. distribution-free procedures such as the Freidman two-way analysis by ranks) were used in the analysis of the results of the two experiments.

Experiment 1

Both trials of Experiment 1 were set up according to a split-split plot design (see Anderson and McLean,1974) with the two dehardening temperature regimes tested by the whole plot error (∂_i), the five incubation periods and the interaction of dehardening temperature by incubation period tested by the split plot error (w_{ij}), and the remaining effects tested by the split-split plot error (within error, $e_{[ijkl]}$).

Table 4. Codes for the seven morphological stages of shoot elongation used in Experiment 2.

Developmental Stage	Code	Description
		- no external evidence of growth.
	2	- visible swelling of the bud; leaves not yet visible.
	3	- green leaves visible through the top of the bud; extended less than 1 mm; bud scales still intact.
	4	- leaves visible; extended less than 3 mm; no major alteration to the shape of the bud.
	5	- new shoots elongated less than 5 mm; beginning to form a vaselike structure.
	6	- neck of vaselike structure increases in diameter as leaves begin to develop a perpendicular habit.
	7	- new shoot becomes visible; leaves almost perpendicular to the shoot.

The linear model for the experiment is as follows:

$$Y_{ijklm} = \mu + T_i + \delta_{[i]} + P_j + TP_{ij} + \omega_{[ij]} + S_k + TS_{ik} + PS_{jk} + C_{[k]l}$$
$$+ TC_{i[k]l} + PC_{j[k]l} + TPS_{ijk} + TPC_{ij[k]l} + e_{[ijkl]m}$$
$$i=1,2 \;\; j=1,5 \;\; k=1,2 \;\; l=1,12 \;\; m=1$$

where,

Y_{ijklm} = percent survival from the m^{th} experimental unit associated with the i^{th} clone nested within the k^{th} source, the j^{th} incubation period, and the i^{th} dehardening period.

μ = overall mean

T_i = effect of dehardening temperature [fixed]

$\delta_{[i]}$ = first restriction error within the i^{th} dehardening temperature. This term is the result of a restriction on the randomization of the treatments onto the i^{th} dehardening temperature's experimental units (ie. due to the correlation of errors caused by simultaneously running the treatments associated with all five incubation periods in the same growth chamber, under each dehardening temperature). To avoid this error term, each dehardening temperature/ incubation period treatment combination should have been run in a separate growth chamber (see Anderson and McLean, 1974).

P_j = effect of the incubation period [fixed].

TP_{ij} = effect of the temperature/incubation period interaction.

$\omega_{[ij]}$ = second restriction error, zero df. (split plot error).

S_k = effect of the k^{th} source [random].

TS_{ik} = effect of the temperature/source interaction.

PS_j = effect of the incubation period/source interaction.

$C_{[k]l}$ = effect of the l^{th} clone nested within the k^{th} source [random].

$TC_{i[k]l}$ = effect of the clone (nested)/dehardening temperature interaction.

$PC_{j[k]l}$ = effect of the clone (nested)/incubation period interaction.

TPS_{ijk} = effect of the 3-way interaction between dehardening temperature, incubation period, and source.

$TPC_{ij[k]l}$ = effect of the 3-way interaction between dehardening temperature, incubation period, and clone nested within source.

$e_{[ijkl]m}$ = within error, zero df. (split-split plot error).

The expected mean square (EMS) table for this design is found in Table 5. There was only a single response per treatment combination, which resulted in zero degrees of freedom for the error term. Therefore it was necessary to make the assumption that the variance components associated with the three-way interactions are equal to zero (ie. $s_{TPS} = s_{TPC} = 0$). These interactions were used to form the pooled error term shown in Table 5. Valid F-tests cannot be made for factors such as dehardening temperature [T_i], and the two-way interaction [TP_{ij}], since there are zero degrees of freedom associated with the first and second restriction errors (whole plot and split plot errors). In order to make a test onthe Incubation period [P_j], it was necessary to make the assumption that $f_{[TP]} = 0$, which is the usual test for a split plot design. This limitation in the design was deemed acceptable, since detecting source and clone effects and associated temperature interactions were the main objectives of the analysis.

Experiment 2

Experiment 2 was designed to evaluate the hardiness of balsam poplar stem cuttings from four different provenances at seven parallel stages of morphological development. Hardiness was said to be based on the percent survival of the cuttings subjected to the freezing test (6 freezing temperatures X 5 reps = 30 clones for each source/morphological stage combination). The design could be interpreted as a two-way ANOVA with one observation per cell, since all 30 cuttings in the freezing test were used to form a single experimental unit. However, this experiment was evaluated using non-parametric techniques (Table 21, Appendix VI); namely the Friedman two-way analysis by ranks (see Bradley 1968, Lehmann 1975). This technique was used instead of ANOVA techniques due to the complications associated with a single observation per cell (ie. zero degrees of freedom in the error term).

Table 5. Expected Mean Square (EMS) table for Experiment 1.

Source	df	2 F i	5 F J	2 R k	12 R l	EMS
Dehardening Temp.; [T_i]	1	0	5	2	12	$\sigma^2 + 24\,\sigma_\omega^2 + 120\,\sigma_\delta^2 + 120\,\phi[T]$
$\delta_{[i]}$; 1st restriction error	0	1	5	2	12	$\sigma^2 + 24\,\sigma_\omega^2 + 120\,\sigma_\delta^2$
Incubation Period; [P_j]	2	2	0	2	12	$\sigma^2 + 24\,\sigma_\omega^2 + 48\,\phi[P]$
Temp. by period; TP_{ij}	2	0	0	2	12	$\sigma^2 + 24\,\sigma_\omega^2 + 24\,\phi[TP]$
$\omega_{[ij]}$; 2nd restriction error	0	1	1	2	12	$\sigma^2 + 24\,\sigma_\omega^2$
Source; [S_k]	1	2	5	1	12	$\sigma^2 + 10\sigma_c^2 + 120\,\sigma_s^2$
Temp. by Source; $TS_{[ik]}$	1	0	5	1	12	$\sigma^2 + 5\sigma_{tc}^2 + 60\,\sigma_{ts}^2$
Period by Source; $PS_{[jk]}$	2	2	0	1	12	$\sigma^2 + 2\,\sigma_{pc}^2 + 24\,\sigma_{ps}^2$
Clone/Source; $[C_{[k]}]_l$	22	2	5	1	1	$\sigma^2 + 10\,\sigma_c^2$
Temp. by Clone/S; $TC_{i[k]}$	22	0	5	1	1	$\sigma^2 + 5\,\sigma_{tc}^2$
Period by Clone/S; $PC_{j[k]}$	44	2	0	1	1	$\sigma^2 + 2\,\sigma_{pc}^2$
Error and/or TPS, TPC; $e_{[ijkl]m=1}$ (pooled error)	46	1	1	1	1	σ^2
Total	240					

RESULTS

Experiment 1.1

The most outstanding feature of the first trial of Experiment 1 was the large number of cuttings which remained undamaged even after being subjected to the lowest temperature in the freezing test (-27°C), regardless of the dehardening temperature regime (Table 6). A slight increase in the susceptibility of both stem and leaf tissue to frost injury was observed at temperatures below -11°C after nine days incubation. However, only four percent of the cuttings were completely killed by exposure to -27°C after 14 days incubation at 25-15°C. Differences in hardiness levels after each dehardening treatment are shown in Figure 2; which gives the mean hardiness level by source. The hardiness level is defined as the lowest temperature to which cuttings can be subjected without causing 100 percent mortality. Minimal decreases in hardiness levels (in both leaf and stem tissue) were observed during the first trial (Appendix IX, Table 24), and any decreases in frost susceptibility were coincident with bud break. Cuttings which had not visually begun leaf growth could generally be exposed to -27° C without incurring mortality. Percent bud break by clone after 14 days of dehardening is illustrated graphically in Figure 3 (tabular form in Appendix III, Table 15). Bud break was said to occur when green leaves were visible through the top of the bud (Developmental stage 3, Table 4). All of the clones were still dormant after nine days of dehardening, and even after 14 days, 56 percent of the cuttings were still dormant. Cuttings from the Wisconsin source show some variability in percent bud break (ranging from 0 to 75 percent) after 14 days at 25-15°C; however, this variability is found to be lacking in the remaining 14-day treatment combinations.

For the purpose of analysis, percent survival values were calculated for both leaf and stem tissue (Appendix IV, Table 17). Despite the fact that percent survival is a somewhat indirect measure of hardiness, it was considered to be a more sensitive indicator of the susceptibility of the cuttings to frost damage than the hardiness measures (ie. point at which 100 % mortality is observed) used in Figure 2.

Table 6. Percentage of balsam poplar cuttings in each damage category in Experiment 1.1. Cuttings from N. Wisconsin and Bearskin L. were exposed to five dehardening periods, under two dehardening temperature regimes.

Incub. Period	Freezer Temp.	Source	Incub. Temp.	DAMAGE SCORE									% flushed
				0	1	2	3	4	5	6	7	8	
Day 0	5 C	Wisc.	15-5°	100									0
			25-15°	100									0
		Bear.	15-5°	96								4	0
			25-15°	88						8			0
	-3 C	Wisc.	15-5°	96									0
			25-15°	96		4							0
		Bear.	15-5°	63	17	4				13			0
			25-15°	75	13					4	8		0
	-11 C	Wisc.	15-5°	100									0
			25-15°	96		4							0
		Bear.	15-5°	83	13	4							0
			25-15°	91	9					4			0
	-19 C	Wisc.	15-5°	92	8								0
			25-15°	92	8								0
		Bear.	15-5°	83	13	4							0
			25-15°	83	4					9		4	0
	-27 C	Wisc.	15-5°	88	8							4	0
			25-15°	100									0
		Bear.	15-5°	63	13		8			13	4		0
			25-15°	79	8					8	5		0

Table 6. Percentage of balsam poplar cuttings in each damage category in Experiment 1.1. Cuttings from N. Wisconsin and Bearskin L. were exposed to five dehardening periods, under two dehardening temperature regimes.

Incub. Period	Freezer Temp.	Source	Incub. Temp.	DAMAGE SCORE									% flushed
				0	1	2	3	4	5	6	7	8	
Day 1	5 C	Wisc.	15-5°	96						4			0
			25-15°	100									0
		Bear.	15-5°	83	4					8	4		0
			25-15°	79						13	8		0
	-3 C	Wisc.	15-5°	96	4								0
			25-15°	83	4			8		4			0
		Bear.	15-5°	75	8	4				13			0
			25-15°	88						4		4	0
	-11 C	Wisc.	15-5°	92						8			0
			25-15°	83	4		4			8			0
		Bear.	15-5°	70	13	13				4			0
			25-15°	79	4	4				13			0
	-19 C	Wisc.	15-5°	100									0
			25-15°	88	4					8			0
		Bear.	15-5°	79	4	8				4	4		0
			25-15°	63	25	4				8			0
	-27 C	Wisc.	15-5°	83						8	4	4	0
			25-15°	95						5			0
		Bear.	15-5°	71	29								0
			25-15°	63	17	4				17			0

Table 6. Percentage of balsam poplar cuttings in each damage category in Experiment 1.1. Cuttings from N. Wisconsin and Bearskin L. were exposed to five dehardening periods, under two dehardening temperature regimes.

Incub. Period	Freezer Temp.	Source	Incub. Temp.	DAMAGE SCORE									% flushed
				0	1	2	3	4	5	6	7	8	
Day 4	5 C	Wisc.	15-5°	96		4							0
			25-15°	96		4							0
		Bear.	15-5°	71		4	4			13	8		0
			25-15°	92		4				4			0
	-3 C	Wisc.	15-5°	100									0
			25-15°	96									0
		Bear.	15-5°	83	4					13			0
			25-15°	71	4			8		17			0
	-11 C	Wisc.	15-5°	88				4		8			0
			25-15°	100									0
		Bear.	15-5°	70	17					4	9		0
			25-15°	83	4			8		4			0
	-19 C	Wisc.	15-5°	100									0
			25-15°	88	13								0
		Bear.	15-5°	63	29					8			0
			25-15°	67	13	4				13		4	0
	-27 C	Wisc.	15-5°	88	8								0
			25-15°	88	8	4							0
		Bear.	15-5°	67	8	13				8			0
			25-15°	58	17	4	4			17			0

Table 6. Percentage of balsam poplar cuttings in each damage category in Experiment 1.1. Cuttings from N. Wisconsin and Bearskin L. were exposed to five dehardening periods, under two dehardening temperature regimes.

Incub. Period	Freezer Temp.	Source	Incub. Temp.	DAMAGE SCORE									% flushed
				0	1	2	3	4	5	6	7	8	
Day 9	5 C	Wisc.	15-5°	96						4			0
			25-15°	92						8			0
		Bear.	15-5°	67	13	4				17			0
			25-15°	71	8	4				13			0
	-3 C	Wisc.	15-5°	92				4		4			0
			25-15°	92	4					4			0
		Bear.	15-5°	58	25					17			0
			25-15°	79						17			0
	-11 C	Wisc.	15-5°	83	4					13			0
			25-15°	63	13			4		13		8	0
		Bear.	15-5°	50	21	21					8		0
			25-15°	63	8	13				17			0
	-19 C	Wisc.	15-5°	79	13	4					4		0
			25-15°	58	4	4				29	4		0
		Bear.	15-5°	54	25					21			0
			25-15°	42	17	8		4		25	4		0
	-27 C	Wisc.	15-5°	79	8					13			0
			25-15°	21	4	4				38	17	17	0
		Bear.	15-5°	42	21	17				21			0
			25-15°	50	8	4			4	21	8	4	0

Table 6. Percentage of balsam poplar cuttings in each damage category in Experiment 1.1. Cuttings from N. Wisconsin and Bearskin L. were exposed to five dehardening periods, under two dehardening temperature regimes.

Incub. Period	Freezer Temp.	Source	Incub. Temp.	DAMAGE SCORE									% flushed
				0	1	2	3	4	5	6	7	8	
Day 14	5 C	Wisc.	15-5°	79						17	4		4
			25-15°	92						4			25
		Bear.	15-5°	42	4	4				29	8	8	0
			25-15°	75	4	4				13	4		4
	-3 C	Wisc.	15-5°	67	8	4				21			8
			25-15°	79		8				13			8
		Bear.	15-5°	42	4	4				21	21	4	0
			25-15°	50	29	8	8			4			4
	-11 C	Wisc.	15-5°	75	8	4				8			4
			25-15°	67	13		4			17			46
		Bear.	15-5°	52	9	4				26	9		0
			25-15°	42	4	17		12		21	4		4
	-19 C	Wisc.	15-5°	50	13				8	21	8		12
			25-15°	37	4			8	4	17	17	13	46
		Bear.	15-5°	63	21					8	4	4	4
			25-15°	63	8					25	4		8
	-27 C	Wisc.	15-5°	38	13			8		21	21		12
			25-15°	42	21	8				17	4	8	12
		Bear.	15-5°	46	8			4		21	13	8	0
			25-15°	59	4					17	8	4	8

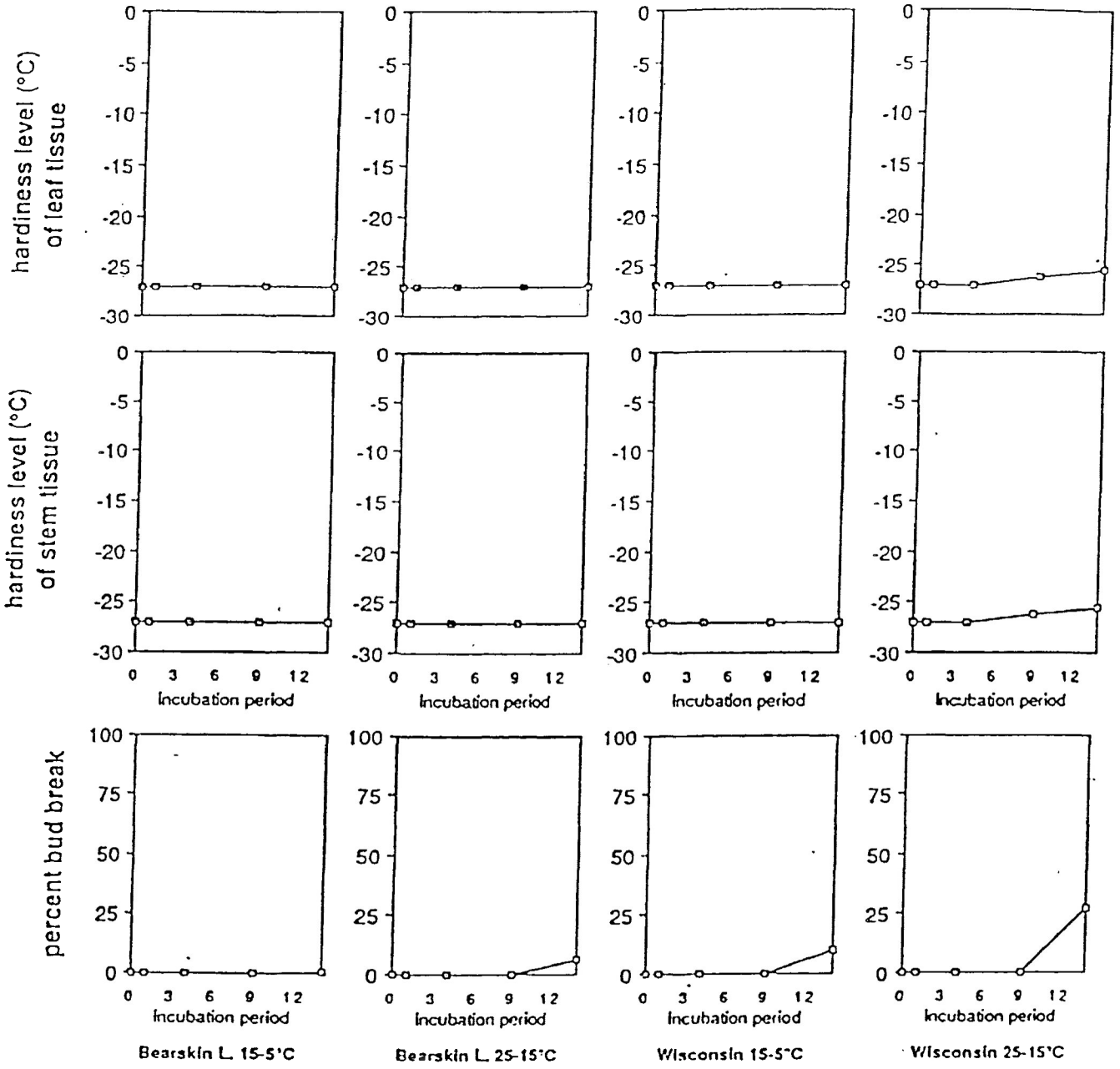


Figure 2. Mean hardiness levels and mean percent bud break (at the time of freezing) of balsam poplar cuttings by source in Experiment 1.1. Cuttings were subjected to five dehardening periods at two dehardening temperatures. The hardiness level was the lowest temperature to which cuttings could be subjected without causing 100 percent mortality to all cuttings in a treatment combination. Mean hardiness levels were calculated for both (a) leaf, and (b) stem tissue, on the basis of the twelve clones within each source.

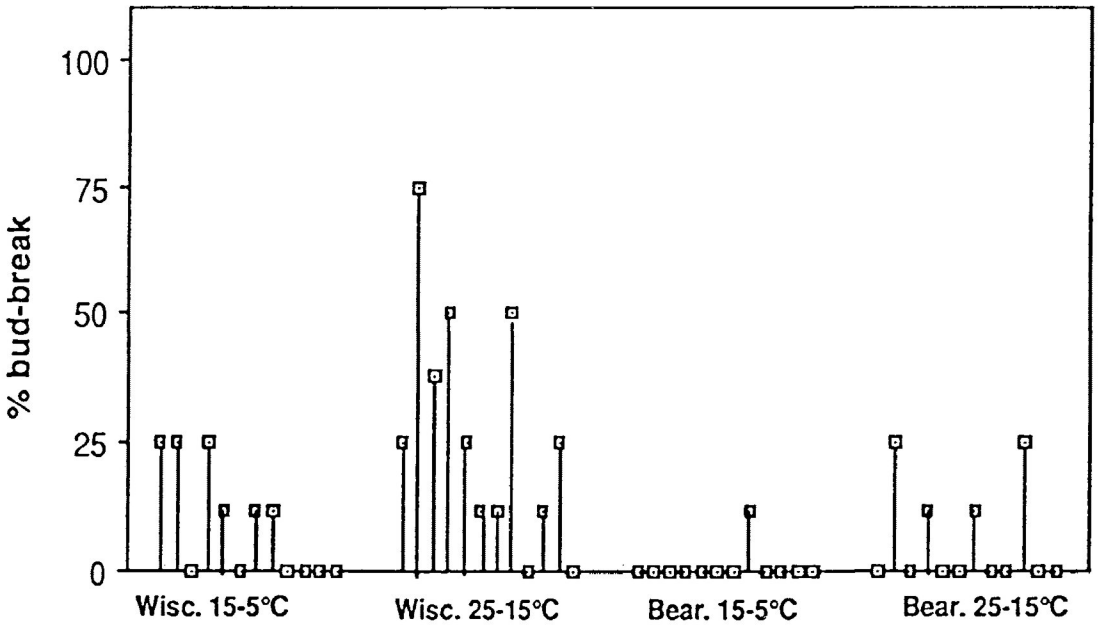


Figure 3. Clonal differences in the percent bud break (at the time of freezing) of balsam poplar cuttings after a dehardening period of 14 days, under two dehardening temperature regimes (15-5°C and 25-15°C). All cuttings were dormant after 0, 1,4, and 9 days of dehardening. Percent bud break is based on the 8 cuttings per clone subjected to the four freezing temperatures used in the freezing test in Experiment 1.1.

Upon examination of the survival trends presented in Figure 2, one might conclude that there is no significant decrease in the hardiness of the cuttings to -27°C, while they are still dormant. However, the data in Table 6 indicates that making this assumption would be an oversimplification of the dehardening process. A large number of cuttings exhibited slight to moderate leaf necrosis (ie. damage scores 1 and 2) throughout Experiment 1.1, suggesting that some freezing injury does occur before bud break, even though it does not result in mortality to the cutting. After each incubation period, the percentage of cuttings in damage categories 1 and 2 increases as the freezing temperature decreases. The data presented in Table 6 also suggests that the rooting characteristics of apparently dormant cuttings are negatively affected by decreasing freezing temperatures. There is a tendency for the number of cuttings in damage category 6 (healthy cuttings with no root development) to increase as the freezing temperature decreases within a given incubation period, suggesting that roots and root primordia are the most susceptible tissue to freezing injury. This trend becomes highly visible after 9 days of incubation. Furthermore, the data suggest that the cuttings are able to maintain considerable hardiness during the initial stages of leaf expansion. After 14 days of incubation, percent bud break had a weak negative correlation with the percent survival of leaf tissue ($r = -0.56$) and the percent survival of stem tissue ($r = -0.45$).

Due to the lack of variability in the hardiness of the cuttings evident in Figures 4 and 5, an ANOVA was not conducted for Experiment 1.1. However, several trends are apparent in the data. Hardiness appears to be lost more rapidly with higher dehardening temperatures (25-15°C as opposed to 15-5°C), although the results of this trial are somewhat inconclusive. Differences in bud break characteristics and percent survival of leaf tissue can be perceived between geographic sources, especially in percent bud break after 14 days incubation (Figure 3). Clonal differences in dormancy release and survival were small, and tended to be more pronounced in the Wisconsin population.

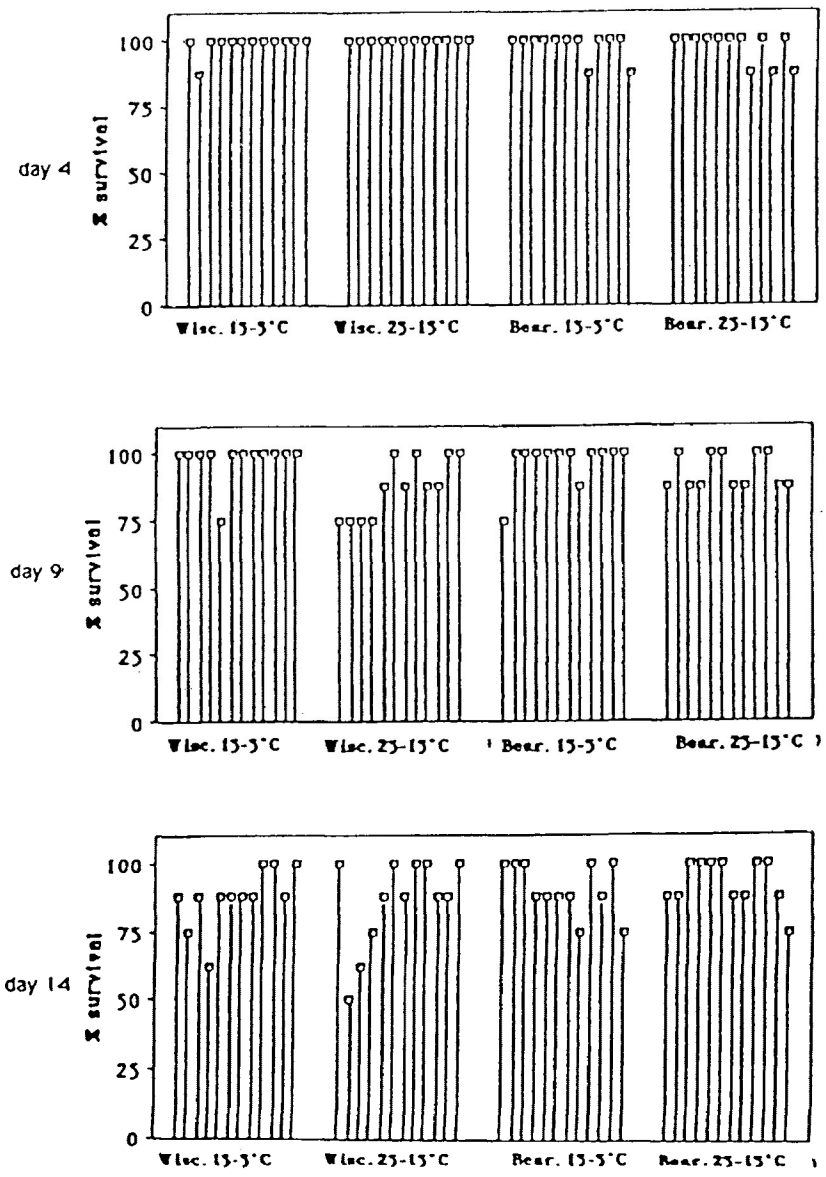


Figure 4. Clonal differences in the percent survival of leaf tissue from balsam poplar cuttings after 4, 9 and 14 days of dehardening in Experiment 1.1. Cuttings from N. Wisconsin and Bearskin L. were exposed to five dehardening periods, under two dehardening temperature regimes. Percent survival is based on 8 cuttings per clone subjected to the four freezing temperatures. Results from the first two dehardening periods were omitted, since no appreciable loss in hardiness (ie. 100% survival to -27°C) was observed prior to 4 days of dehardening.

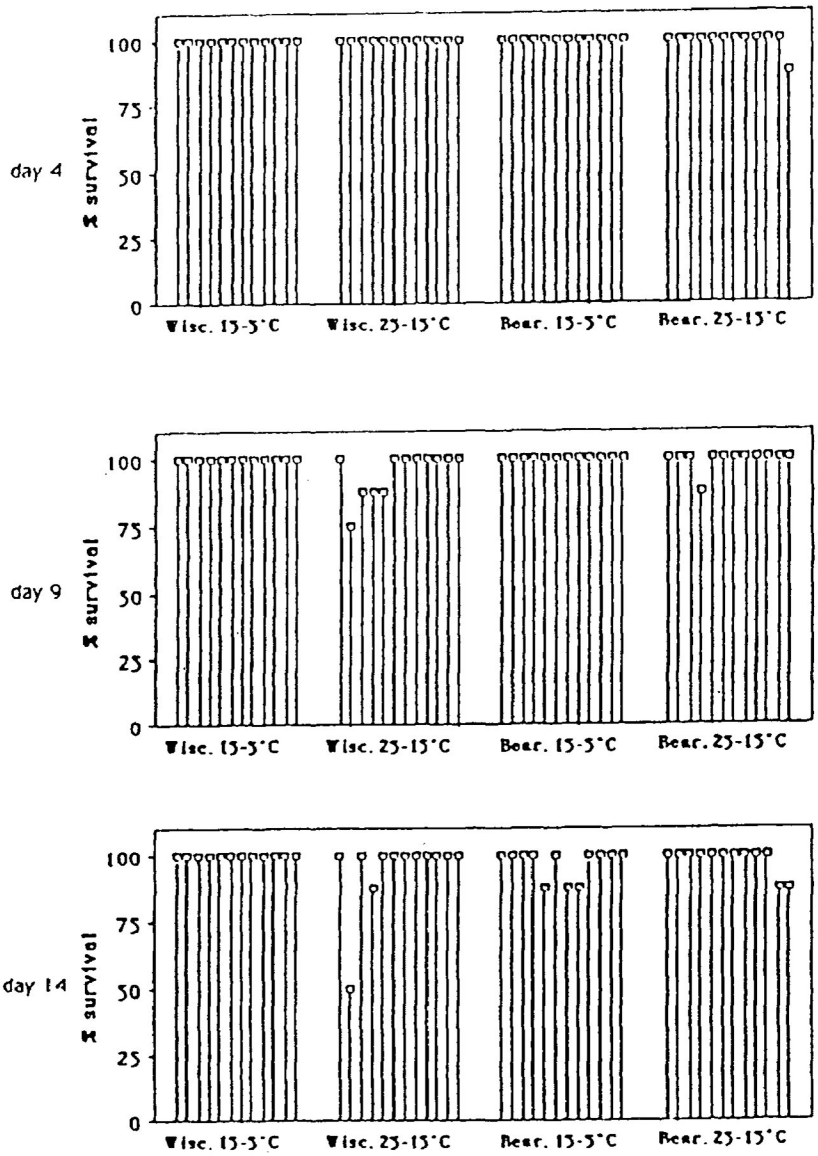


Figure 5. Clonal differences in the percent survival of stem tissue from balsam poplar cuttings after 4, 9 and 14 days of dehardening in Experiment 1.1. Cuttings from N.Wisconsin and Bearskin L. were exposed to five dehardening periods, under two dehardening temperature regimes. Percent survival is based on 8 cuttings per clone subjected to the four freezing temperatures. Results from the first two dehardening periods were omitted, since no appreciable loss in hardiness (ie. 100% survival to -27°C) was observed prior to 4 days of dehardening.

A slight leaf spot outbreak occurred during the greenhouse viability test in both trials of this experiment, but the spread of this fungus was effectively controlled with the application of benomyl (100 p.p.m). Necrosis associated with the fungus was thought to have a small, but insignificant confounding effect with the survival of the cuttings after freezing. Less than 0.6 percent of the controls were completely dead by the end of the viability test.

Experiment 1.2

In the second trial of Experiment 1, leaf tissue became susceptible to frost injury after five days of incubation, while stem tissue did not exhibit an appreciable loss before the eighth day of incubation (Table 7). As was the case in Experiment 1.1, non-lethal freezing damage was observed in dormant stem cuttings. Once again, there was a tendency for the number of cuttings in damage categories 1, 2, and 6 to increase as the freezing temperature decreased within a given incubation period. This tends to suggest that some dehardening is occuring prior to bud break. In Experiment 1.2, this trend was apparent after an incubation period of 2 days.

The data in Table 7 indicates that dehardening proceeded more rapidly at the higher dehardening temperature regime. After exposure to -11°C, 71 percent of the cuttings were found in damage category 8 (all tissue dead) when dehardened for 11 days at 25-15°C; there were no cuttings in category 8 after parallel treatment at 15-5°C. As shown in Figure 6, hardiness also appears to decrease much more rapidly in leaf meristems than it does in the cambium. Leaf meristems were hardy to -27° C while still in a state of dormancy; this condition was exhibited by the cuttings incubated for the shortest incubation periods (0 and 2 days). Hardiness levels decreased with further incubation, and by the 11th day, Wisconsin clones dehardened at 25-15°C had an average hardiness of -5°C (Appendix IX, Table 25). Bearskin Lake clones (which tended to break bud later than the southern clones) were still hardy to -11°C under the same dehardening regime.

Table 7. Percentage of balsam poplar cuttings in each category in Experiment 1.2. Cuttings from N. Wisconsin and Bearskin L. were exposed to five dehardening periods, under two dehardening temperature regimes.

Incub. Period	Freezer Temp.	Source	Incub. Temp.	DAMAGE SCORE									% flushed
				0	1	2	3	4	5	6	7	8	
Day 0	5 C	Wisc.	15-5°	100									0
			25-15°	96						4			0
		Bear.	15-5°	83						13	4		0
			25-15°	83						13	4		0
	-3 C	Wisc.	15-5°	100									0
			25-15°	88				4		4			0
		Bear.	15-5°	83		4				5			0
			25-15°	83		4				4	9		0
	-11 C	Wisc.	15-5°	96									0
			25-15°	96	4								0
		Bear.	15-5°	79	9	4				8			0
			25-15°	71	9	4	8	4		4			0
	-19 C	Wisc.	15-5°	96						4			0
			25-15°	96	4								0
		Bear.	15-5°	58	4			9		21	4	4	0
			25-15°	71	9	4	8	4		4			0
	-27 C	Wisc.	15-5°	92						4		4	0
			25-15°	88			4			4			0
		Bear.	15-5°	54	13	4	4			21		4	0
			25-15°	71	9	8				8	4		0

Table 7. Percentage of balsam poplar cuttings in each category in Experiment 1.2. Cuttings from N. Wisconsin and Bearskin L. were exposed to five dehardening periods, under two dehardening temperature regimes.

Incub. Period	Freezer Temp.	Source	Incub. Temp.	DAMAGE SCORE									% flushed
				0	1	2	3	4	5	6	7	8	
Day 2	5 C	Wisc.	15-5°	88						8		4	0
			25-15°	100									0
		Bear.	15-5°	79						17			0
			25-15°	88						4			0
	-3 C	Wisc.	15-5°	83						17			0
			25-15°	100									0
		Bear.	15-5°	63	9		8	4		4	8		0
			25-15°	83	4		4	4		5			0
	-11 C	Wisc.	15-5°	88						4			0
			25-15°	92						8			0
		Bear.	15-5°	58	8		13	4		13	4		0
			25-15°	88	4					4	4		0
	-19 C	Wisc.	15-5°	71				4		21			0
			25-15°	83						17			0
		Bear.	15-5°	83	4			4		5			0
			25-15°	56	5		4			26	9		0
	-27 C	Wisc.	15-5°	83	9	4				4			4
			25-15°	67	4			17		12			4
		Bear.	15-5°	61		13		4		13	4	5	0
			25-15°	54	17	8	4			17			0

Table 7. Percentage of balsam poplar cuttings in each category in Experiment 1.2. Cuttings from N. Wisconsin and Bearskin L. were exposed to five dehardening periods, under two dehardening temperature regimes.

Incub. Period	Freezer Temp.	Source	Incub. Temp.	DAMAGE SCORE									% flushed
				0	1	2	3	4	5	6	7	8	
Day 5	5 C	Wisc.	15-5°	92						4			17
			25-15°	88						8		4	63
		Bear.	15-5°	79	9		4			8			0
			25-15°	75						25			0
	-3 C	Wisc.	15-5°	88	4					4		4	0
			25-15°	54	4				4	30		4	33
		Bear.	15-5°	71	4	4		4		9	4		0
			25-15°	50		9		4		41			0
	-11 C	Wisc.	15-5°	67	4			4		25			38
			25-15°	30	4	4		4	9	49			50
		Bear.	15-5°	58	4	21	4			13			0
			25-15°	46	9	4	8			25	4		8
	-19 C	Wisc.	15-5°	17			4	13	8	33	29		33
			25-15°	9	9				22	30	30		46
		Bear.	15-5°	25	8	13	8	17		29			13
			25-15°	16	17	4	17			25	21		8
	-27 C	Wisc.	15-5°	33	21	4		4		26	12		21
			25-15°	13	4	4	4		9	8	25	33	54
		Bear.	15-5°	42	8	13	4			29	4		4
			25-15°	21	4				13	21	33	8	21

Table 7. Percentage of balsam poplar cuttings in each category in Experiment 1.2. Cuttings from N. Wisconsin and Bearskin L. were exposed to five dehardening periods, under two dehardening temperature regimes.

Incub. Period	Freezer Temp.	Source	Incub. Temp.	DAMAGE SCORE									% flushed
				0	1	2	3	4	5	6	7	8	
Day 8	5 C	Wisc.	15-5°	92						8			83
			25-15°	83						13			96
		Bear.	15-5°	88	4	4				4			29
			25-15°	75	4	8				13			67
	-3 C	Wisc.	15-5°	83						13			92
			25-15°	27				5		68			96
		Bear.	15-5°	58	9	17		4		8	4		42
			25-15°	38	13	13				28	4		71
	-11 C	Wisc.	15-5°	67	17				8	4			75
			25-15°	4	4			4	9		65	14	96
		Bear.	15-5°	38	17	25		4		8		4	58
			25-15°	8		4		9	21	8	42	4	75
	-19 C	Wisc.	15-5°	9	13	8	4	4	13	8	33		88
			25-15°				4		17		33	46	100
		Bear.	15-5°	25	21	8	4	17		17	4	4	54
			25-15°	9	4		4	4	17	4	50	8	75
	-27 C	Wisc.	15-5°						9	8	38	48	100
			25-15°								4	96	100
		Bear.	15-5°	8	17		17	8	4	12	21	13	58
			25-15°	4		4		4		4	46	38	92

Table 7. Percentage of balsam poplar cuttings in each category in Experiment 1.2. Cuttings from N. Wisconsin and Bearskin L. were exposed to five dehardening periods, under two dehardening temperature regimes.

Incub. Period	Freezer Temp.	Source	Incub. Temp.	DAMAGE SCORE									% flushed
				0	1	2	3	4	5	6	7	8	
Day 11	5 C	Wisc.	15-5°	88						12			100
			25-15°	100									100
		Bear.	15-5°	79	4	4		4		13			63
			25-15°	79	13					8			83
	-3 C	Wisc.	15-5°	71	4	4			4	17			100
			25-15°	29	13	4				21		4	100
		Bear.	15-5°	58	8	13	9	4		8			83
			25-15°	29	25	13		4		29			92
	-11 C	Wisc.	15-5°	29	21	8			8	22	8		92
			25-15°	4						4	21	71	100
		Bear.	15-5°	29	17	21	4	8		21			58
			25-15°	17	4	4	4	4	9	16	33	8	75
	-19 C	Wisc.	15-5°					4	17	8	62	8	100
			25-15°						4		21	75	100
		Bear.	15-5°	13	4		8	4		34	33	3	92
			25-15°	8	4				13		54	21	96
	-27 C	Wisc.	15-5°						13		21	76	100
			25-15°							4	4	92	100
		Bear.	15-5°				4	8	4	4	50	17	92
			25-15°						4		21	75	92

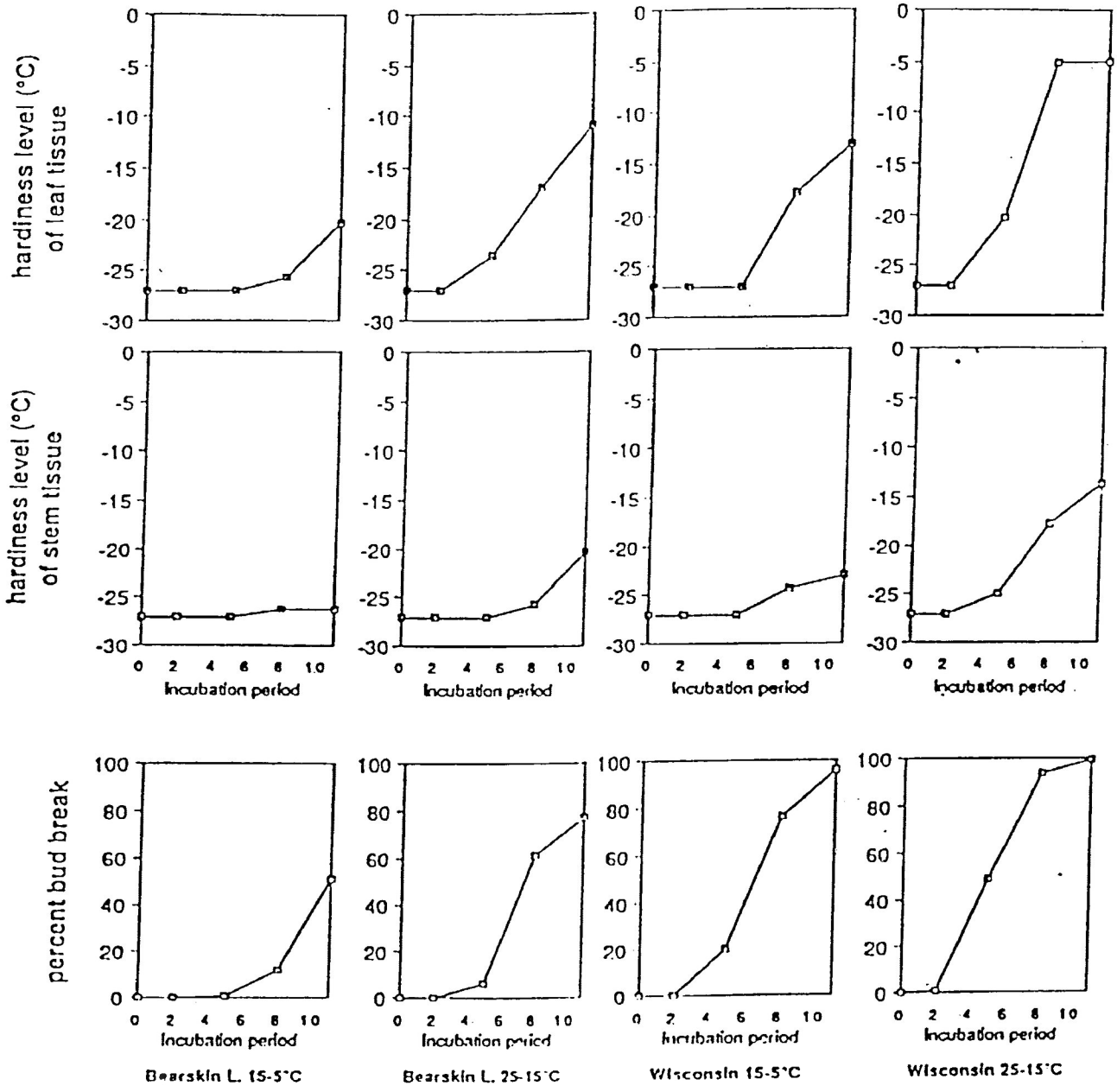


Figure 6. Mean hardiness levels and mean percent bud break (at the time of freezing) of balsam poplar cuttings by source in Experiment 1.2. Cuttings were subjected to five dehardening periods, under two dehardening temperatures. The hardiness level was the lowest temperature to which cuttings could be subjected without causing 100 percent mortality to all cuttings in a treatment combination. Mean hardiness levels were calculated for both (a) leaf, and (b) stem tissue, on the basis of the twelve clones within each source.

Clones from the Wisconsin source invariably showed more susceptibility to frost injury than clones from Bearskin Lake. After 11 days at 25-15°C, the mean survival rate in leaf tissue from southern clones was 27.0 percent, as opposed to 40.6 percent in northern clones (Appendix IV, Table 18). Although percent survival cannot be directly related to an actual level of hardiness (with the exception of 100 percent which corresponds to -27°C), 25 percent survival generally corresponds to a hardiness level of -3°C. Data in Table 7 clearly illustrates that frost injury only begins to occur at temperatures below -3°C, even when the cuttings are actively growing.

Clonal differences in percent bud break at the time of freezing are illustrated in Figure 7. Clones from N. Wisconsin showed a large amount of variation in percent bud break (0 to 100 percent at 25-15°C) after five days of incubation, while Bearskin L. clones were just beginning to break bud (0 to 25 percent bud break) after the same dehardening treatment. Percent bud break gradually increased with further incubation; after 11 days almost all of the Wisconsin clones had flushed, while percent bud break in northern clones was still quite variable (12.5 to 100 percent). Large geographic source differences in the hardiness of leaf and stem tissue are also evident in Figures 8 and 9 after an incubation period of five days. Clonal differences are most evident in the hardiness of leaf tissue after 5 and 8 days of dehardening. In stem tissue, clonal variation is only apparent during the last two dehardening periods (8 and 11 days).

An analysis of variance was conducted for the percent survival after freezing in both leaf and stem tissue, as well as the percent bud break at the time of freezing (Appendix II). Each analysis was based on the response data from the final three dehardening periods (5, 8, and 11 days). The first two dehardening periods were excluded from these analyses, since there was little or no variation associated with these treatments. Tests for the assumptions underlying each ANOVA are found in Appendix VII. On the basis of Cochran's C statistic (Table 22), the assumption of

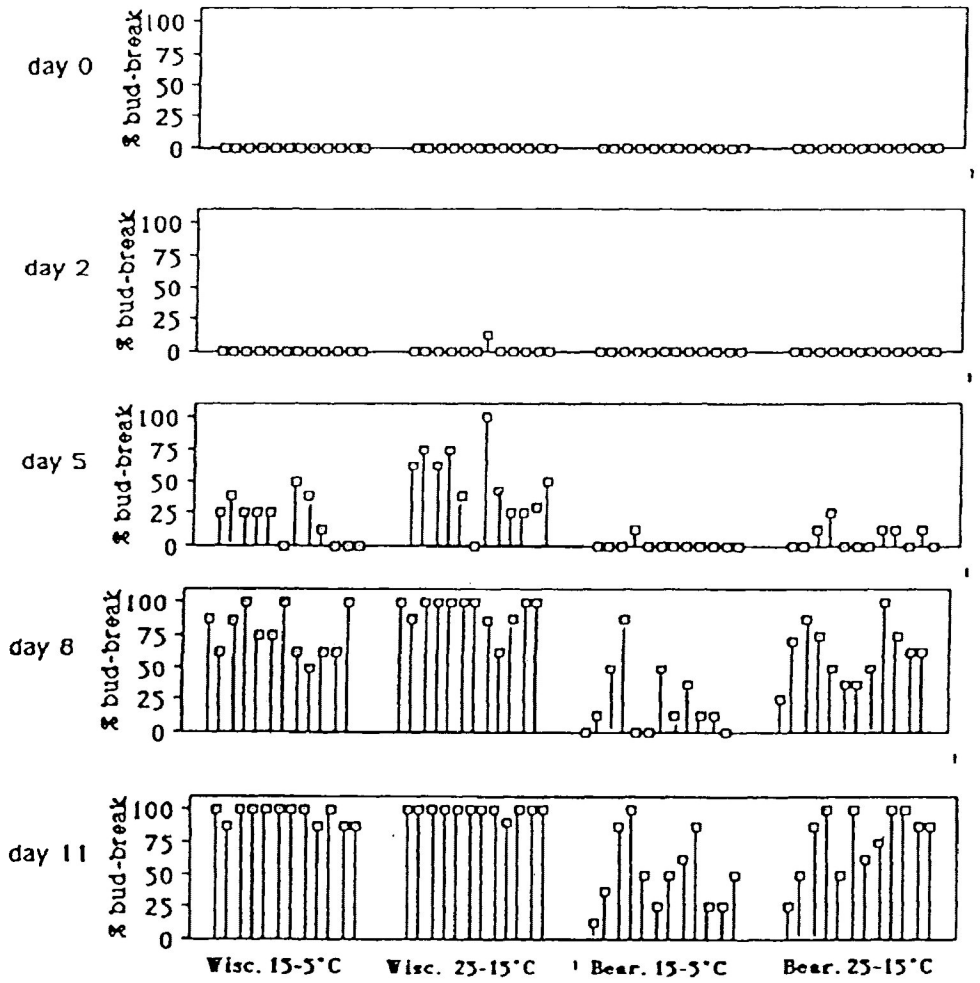


Figure 7. Clonal differences in the percent bud break (at the time of freezing) of balsam poplar cuttings after various dehardening periods (0, 2, 5, 8, and 11 days), under two dehardening temperature regimes (15-5°C and 25-15°C) in Experiment 1.2. Percent bud break is based on the 8 cuttings per clone subjected to the four temperatures (-3, -11,-19, and -27 C) used in the freezing test.

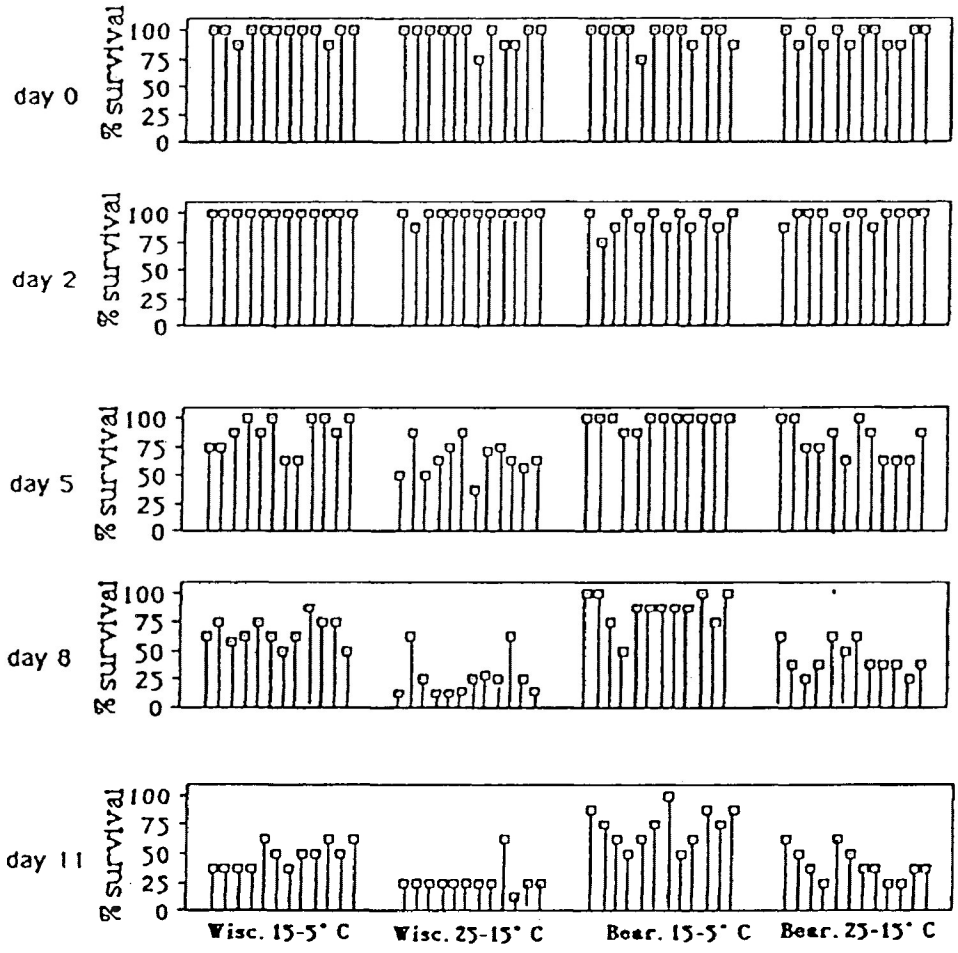


Figure 8. Clonal differences in the percent survival of leaf tissue (at the time of freezing) of balsam poplar cuttings after various dehardening periods (0, 2, 5, 8, and11 days), under two dehardening temperature regimes (15-5°C and 25-15°C) in Experiment 1.2. Percent survival is based on 8 cuttings per clone subjected to the four temperatures (-3, -11,-19, and -27 C) used in the freezing test.

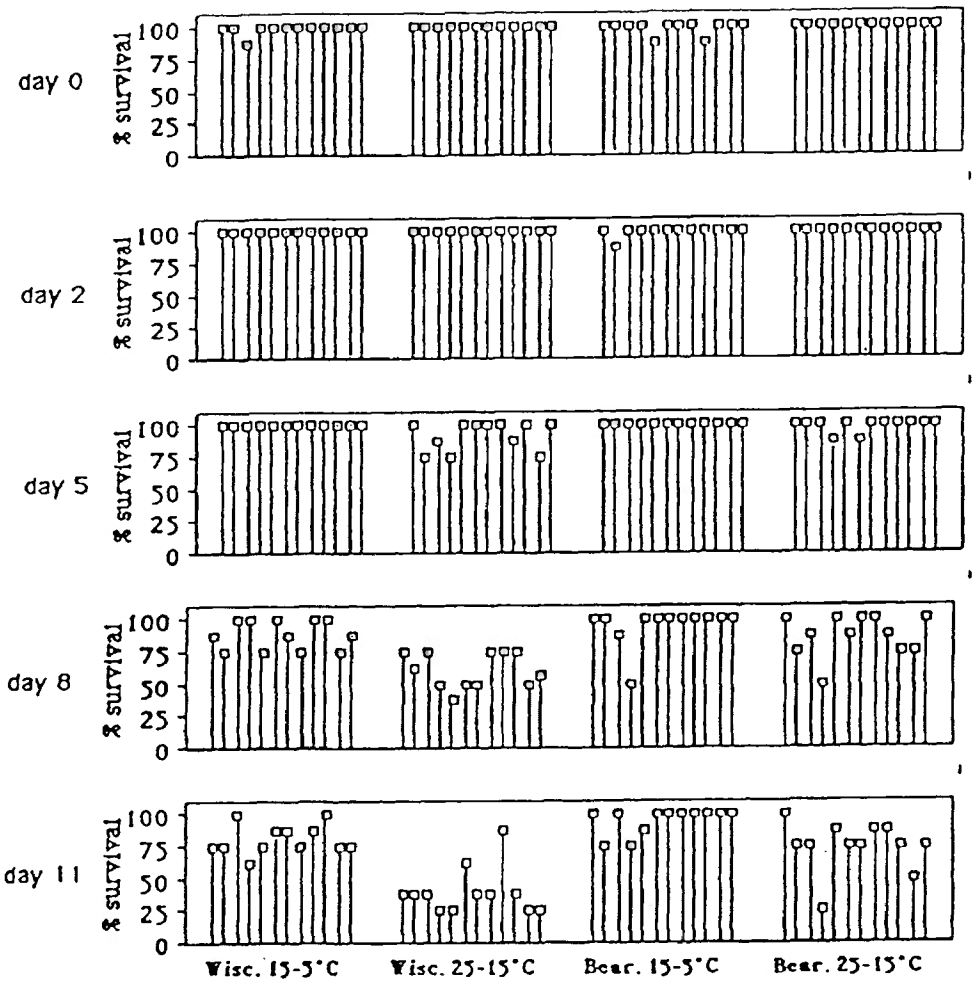


Figure 9. Clonal differences in the percent survival of stem tissue (at the time of freezing) of balsam poplar cuttings after various dehardening periods (0, 2, 5, 8, and11 days), under two dehardening temperature regimes (15-5°C and 25-15°C) in Experiment 1.2. Percent survival is based on 8 cuttings per clone subjected to the four temperatures (-3, -11,-19, and -27 C) used in the freezing test.

homogeneity of variance was not violated in any of the ANOVA's used in this study. The normal probability plots (Figure 11, AppendixVII) indicate that departures from normality do exist in the data set, and that these deviations are mainly due to an excessive number of values far from the mean (ie. there were many cases where either all or none of the cuttings survived). However, F-tests are generally robust to non-normality, and Box and Anderson (1955) have shown that for the values of skewness and kurtosis (Table 22) observed in this experiment, the effects of these departures from normality can be ignored. A summary of F values and associated levels of significance from the three ANOVA's in Experiment 1.2, is found in Table 8.

The ANOVA for the percent survival of leaf tissue (Appendix II, Table 12) indicates a highly significant difference ($P < 0.001$) between geographic sources in this trial. Although the analysis of variance failed to detect any clonal differences in the hardiness of leaf tissue, there were several clones within each population that exhibited superior levels of hardiness (i.e. Wisconsin 235, Bearskin 321). Due to the first restriction error associated with this experimental design, a valid F-test cannot be conducted for the incubation temperature. There is little doubt, however, that the dehardening temperature would be a significant source of variation. Incubation period [P_j] was analyzed according to the conventions of more traditional split-plot designs (i.e. with the incubation temperature/ incubation period interaction [TP_{ij}] as the whole plot error term), and was found to be a significant source of variation.

The analysis of variance for the hardiness of stem tissue (Appendix II, Table 13) yielded slightly different results. Once again geographic sources were highly significant sources of variation ($P < 0.001$); but unlike the previous analysis for the hardiness of leaf tissue, clones within source were also a major source of variation ($P = 0.006$). In further contrast to the ANOVA for leaf hardiness, the dehardening period was not a significant source of variation in the hardiness of stem tissue.

Table 8. Summary of F values and their associated levels of significance for leaf hardiness, stem hardiness, and percent bud break at the time of freezing in Experiment 1.2 (Source: Appendix II).

Source	df	F Value for leaf hardiness	F Value for stem hardiness	F Value for percent bud break
Incubation Temp, $[T_i]$	1	no test	no test	no test
$\delta_{[ij]}$, 1st restriction error	0			
Incubation Period, [Pj]	2	22.0 *	7.4	58.0 *
Temp. X Period, [TPij]	2	no test	no test	no test
$\omega_{[ij]}$, 2nd restriction error	0			
Source $[S_k]$	1	80.9 **	30.8 **	121.4**
Temp. X Source $[TS_{ik}]$	1	2.0	6.5 *	3.6
Period X Source $[PSj_k]$	2	0.1	8.2 **	0.2
Clone/Source $[C_{[k].l]}$	22	0.9	2.4 **	1.7
Temp. X Clone/S $[TC_{i[k]l]}$	22	1.4	1.2	1.0
Period X Clone/S $[PCj_{[k]l]}$	44	0.8	1.2	1.3
Error and/or TPC, TPS	46			
Total	143			

Source interactions with incubation temperature (TS_{ik}) and incubation period (PS_{jk}) were also found to be significant variance components in this analysis.

To a greater extent than in Experiment 1.1, freezing injury was correlated with bud break ($r = -0.82$ for leaf tissue; and $r = -0.71$ for stem tissue). During the first two incubation periods, when the cuttings were still dormant or just beginning leaf expansion, survival rates averaged 97 percent for leaf tissue, and 99.5 percent for stem tissue (Appendix IV, Table 18).

Source differences in bud break characteristics (Appendix II, Table 14) were found to be highly significant. Differences associated with clones within each population were of lesser importance ($P = 0.072$).

The raw data for Experiment 1 can be found in Appendix V.

Experiment 2

During the course of this experiment, a number of the cuttings developed roots from preformed primordia in the stem prior to the freezing test. These root meristems were formed during the initial stages of bud burst and shoot elongation. The presence of roots was noted at the time of the freezing test, along with the developmental stage of the cuttings (see Table 4 for a description of the development stages). A summary of the percent survival of stem tissue with root meristems at various stages of shoot elongation is given by freezing temperature in Table 9. A formal analysis was not conducted on these data for several reasons; missing treatment combinations and unequal sample sizes would have made meaningful conclusions difficult, and there was no way of establishing a cause and effect relationship between the presence of roots and the subsequent survival of stem tissue on the basis of this experiment . In this regard, changes in hardiness might be attributed to other physiological changes concurrent with root initiation. However, the results indicate that even after root elongation has begun, cuttings can be subjected to -19°C without having the ability to develop new rooots impaired even though the existing roots are killed by temperatures below -3°C.

Table 9. Summary of the percent survival of stem tissue with growing roots at the seven developmental stages used in Experiment 2. The number of cuttings (n) on which percent survival values were based is also included in the table.

Freezing temperature		Developmental stage of rooted cuttings[A] 1	2	3	4	5	6	7
+5	% survival:	100	100	100	100	100	100	100
	n =	2	3	1	6	5	14	4
-3	% survival:	100	100	100	50	100	100	100
	n =	1	1	1	2	14	6	8
-11	% survival:	100	100	100	60	30	12	-
	n =	2	1	1	5	10	8	0
-19	% survival:	-	-	0	0	27	0	-
	n =	0	0	1	3	11	2	0
-27	% survival:	0	-	0	0	0	0	0
	n =	1	0	1	2	5	9	4

[a]Description of developmental stages is given in Table 4.

The results of Experiment 2 indicate that an attenuated form of frost hardiness is active long after the cuttings have been released from dormancy. Balsam poplar cuttings are able to maintain considerable levels of hardiness even after bud break and the initial stages of leaf expansion. In Figure 10, the 50% killing point (Tk_{50}) is shown as the indicator of the hardiness of the cuttings. On the basis of the ultimate frost-killing point, each of the four provenances used in this study were able to withstand freezing to -24°C at the second developmental stage (leaves visible, extended less than 2 mm; base concealed by bud scale). Hardiness levels drop rapidly after this point, although considerable levels of hardiness (-9 to -12°C, depending on source) were maintained to the fifth developmental stage (new shoot becomes visible, leaves begin to develop a perpendicular habit). Observations made during this experiment suggest that balsam poplar cuttings can withstand short term exposure to temperatures between -3 and -6° C, during the initial stages of leaf expansion. During the freezing test, exotherms were consistently observed at approximately -4° C, suggesting that cuttings may avoid injury above this temperature by supercooling (ie. they avoid freezing).

Source differences in the percent survival of leaf tissue after freezing (Table 10) were jointly evaluated over all seven developmental stages used in Experiment 2, with a Friedman two-way analysis by ranks (see Appendix VI, Table 21). No significant geographic source differences were noted in the hardiness of leaf tissue ($P = 0.122$). Large geographic source differences were obseved at developmental stage seven (new shoot visible; leaves almost perpendicular to the shoot), at which the Bearskin Lake source exhibited superior hardiness levels over the other geographic sources. This trend was not consistent in all of the developmental stages. Source differences were not statistically evaluated at

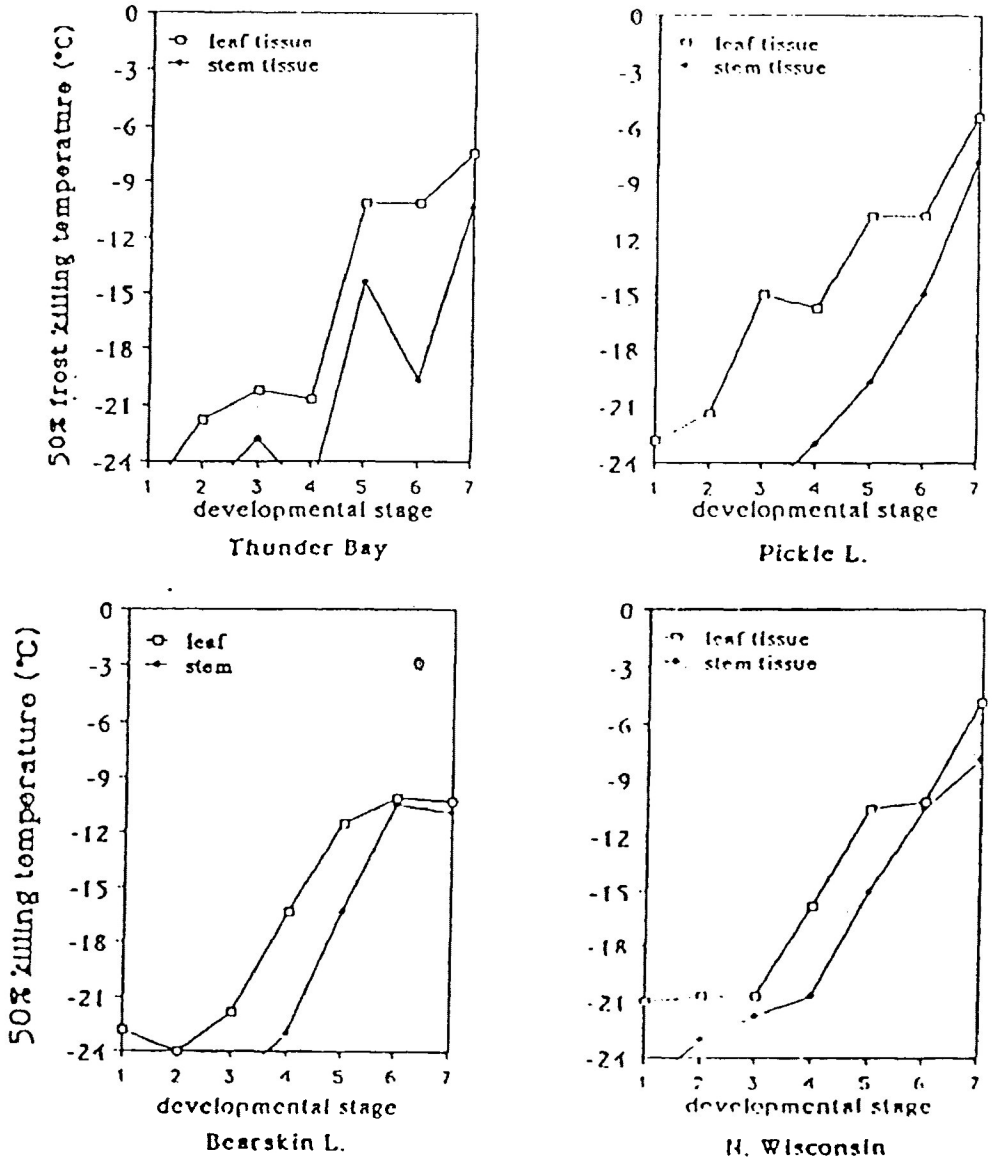


Figure 10. The 50% killing point (Tk_{50}) for the stem and leaf tissue of balsam poplar cuttings from four geographic sources, at various stages of shoot development. The Tk_{50} was the temperature required to kill 50% of the cuttings subjected to the six freezing temperatures (-3,-6,-9,-12,-18, and -24 C) used in the freezing test in Experiment 2.

Table 10. Percent survival of leaf and stem tissue in balsam poplar cuttings in Experiment 2. Survival is given by developmental stage for each of the four geographic sources used in the experiment. Percent survival is based on the 30 cuttings per source subjected to the freezing test.

Source	Frost Hardiness by Developmental Stage[A]						
	1	2	3	4	5	6	7
1. Bearskin L.							
- leaf	83	90	86	77	57	43	46
- stem	96	100	93	93	77	50	50
2. Pickle L.							
- leaf	86	66	66	70	50	50	23
- stem	100	100	100	90	73	66	37
3. Thunder Bay							
- leaf	96	86	77	80	40	43	26
- stem	100	96	86	100	63	73	40
4. N. Wisconsin							
- leaf	83	80	80	70	50	40	20
- stem	93	90	86	80	67	50	37

[A] Developmental stages are listed in Table 4.

individual developmental stages, due to design limitations perceived in Experiment 2. The percent survival values listed in Table 10 could not be analysed using a conventional analysis of variance since there was only a single response for each treatment combination . The nonparametric Sign test was considered for evaluating source comparisons at individual developmental stages, using the damage scores presented in Table 23 (Appendix VII). However, since the power of

this test depends on the number of paired observations in the data set, and the number of paired observations did not remain constant from source comparison to source comparison, the use of the Sign test was rejected. Furthermore, a low number of paired observations (ranging from 7 to 16) were observed in most source comparisons. Steele and Torrie (1980) suggest that the Sign test is most sensitive with 20 or more pairs of observations, and that it is impossible to detect a departure from the null hypothesis (ie. no source differences) with fewer than six pairs of observations.

DISCUSSION

The two experiments conducted in this study will be discussed on an individual basis. The first experiment was broken down into two separate trials (Experiments 1.1 and 1.2).

EXPERIMENT 1.1

Experiment 1.1 was initiated to evaluate potential changes in the cold hardiness of balsam poplar cuttings prior to and immediately following the initiation of leaf expansion. The results of this trial indicated that cuttings were able to maintain hardiness to at least -27°C when in a dormant state. In other words, changes in hardiness levels were always associated with bud break. The vast majority of temperate conifer species examined to date have exhibited rapid dehardening in the spring, and most have shown substantial losses of hardiness prior to bud break. The first trial of this experiment was designed to place the greatest emphasis on hardiness changes prior to bud break, however, since the cuttings were uniformly hardy to -27°C during this period, little variation was observed in hardiness levels. As a result, it was virtually impossible to draw meaningful conclusions on the factors included in the original experimental design (i.e. dehardening temperature, source and clonal difference in hardiness).

EXPERIMENT 1.2

In Experiment 1.2, greater emphasis was placed on the dehardening trends during bud burst and new-shoot elongation. As with the first trial, the cuttings were hardy to -27°C when dormant. After the cuttings began to emerge from dormancy, several trends became apparent. Dehardening proceeded much more rapidly at higher temperatures. The percent survival of cuttings dehardened at 25-15°C was generally 20 to 40 percent lower than that of cuttings dehardened at 15-5°C.

Leaf tissue was more susceptible to frost injury than stem tissue, which suggests that the dehardening process is initiated in leaf tissue in advance of stem tissue. Timmis and Worrall (1974), have suggested that translocatable factors from the expanding shoot are involved in the stimulation of cambial division and the loss of short-day induced hardiness in the previous year's foliage of Douglas-fir seedlings. Wareing (1951) has indicated that the cambia of diffuse porous trees may require the presence of buds for renewed growth. Although there is no evidence in this experiment of a translocatable dehardening factor at work in balsam poplar during the spring, such a hypothesis might be useful in explaining the differential hardiness observed between leaf and stem tissue. On the other hand, the possibility that the dehardening process is independently regulated in both stem and leaf tissue has to be considered. In Experiment 1.2, significant clonal variation was noted in the hardiness of stem tissue, but not in the hardiness of leaf tissue. This phenomenon was thought to be related to the nature and rapidity of the dehardening process in each of these tissues. Not only is hardiness lost earlier in leaf tissue, it also appears to be lost more rapidly (see Figure 6). As a result, variable injury among clones to leaf tissue was only observed for a fairly short time period. For example, the greatest variability in the percent survival of leaf tissue was observed in the Wisconsin population after the eight-day dehardening treatment; by the eleventh day clones dehardened at 25-15°C uniformly exhibited 25 percent survival (clone 235 was the exception). In this regard, the low level of clonal variation in leaf hardiness (Table 8) reflects both the rapid dehardening rate observed in leaf tissue, and the small number of hardiness evaluations during the period of highest clonal variation in leaf hardiness within each population. This design limitation was unavoidable since there was a restriction in the amount of clonal material available in the nursery.

An interaction between dehardening temperature and geographic source (TS_{ij}) was noted in the analysis of variance for stem hardiness. This interaction was related to the ability of clones from the Bearskin source to maintain considerable hardiness levels throughout the experiment, at both dehardening temperature regimes. Clones from Wisconsin exhibited a considerable loss of hardiness after 11 days at 25-15°C. This interaction is merely a reflection of differential timing in the loss of hardiness between the two sources.

The pattern of genetic variation in cold hardiness corresponds closely to that of the climate of northern Ontario in the spring (see Table 2). Northern clones were less susceptible to frost injury than southern clones throughout the spring dehardening period, and this phenomenon was closely related to the tendency of northern clones to remain in a state of imposed dormancy longer than their southern counterparts, ie., the two populations appeared to respond differently to degree days during the dehardening period. Selection against early flushing genotypes seems apparent in the Bearskin Lake source, and this may be related to a longer period of environmental uncertainty during the spring. The assessment of population differentiation in this study was based on two adaptive traits; frost hardiness and bud break characteristics. Since loss of hardiness was coincident with bud flush, the two traits tended to be correlated. Rehfeldt (1984) points out that when population differentiation in conifers has been detected for a single adaptive trait, correlated patterns have been observed for other functionally related or linked traits.

Considering the unpredictable nature of the weather during the spring dehardening period, one might expect the clones within each population to exhibit fairly uneven dehardening characteristics. This trend was observed in this experiment; high within-population variance in hardiness level of stem tissue was observed in the two sources. Early flushing (Wisconsin 239, Bearskin 302) and late

flushing genotypes (Wisconsin 220, Bearskin 321) were clearly found in both populations. Natural selection appears to be operating on both distant and local populations of balsam poplar. Although there is no direct evidence from this study, the clonal variation within local populations has been explained in terms of microsite heterogeneity that permits co-existence of clones through diversifying selection (Ellstrand and Roose, 1987). For example, trees may be more susceptible to frost damage in low-lying areas (i.e. frost pockets) than on upland sites. Local adaptive variation due to topography and air currents has been demonstrated in Sitka spruce (Picea sitchensis (Bong.) Carr.) by Burley (1966). However, before selection can be implicated on a local level with balsam poplar, the exact nature of any local adaptions remain to be established.

Farmer and Reinholt (1986), who examined dormancy relations in balsam poplar cuttings from the same provenances used in this study, observed a tendency for northern clones to break bud earlier than southern clones, although differences in timing were not statistically significant. The tendency for northern clones to break bud earlier than southern clones was more pronounced in Experiment 1.2. Source differences in bud break characteristics were thought to be solely related to differences in the response to spring temperature in this study, since the chilling requirement for the cuttings was assumed to have been met by early January. However, in their forcing study, Farmer and Reinholt (1986) suggested that variation in bud break probably reflected genetic differences in both the degree to which the chilling requirement had been met, and the response to the forcing conditions. The authors hypothesized that the clinal geographic trend observed in their study might have been an artifact of difference in the time of growth cessation due to differential photoperiodic response. Therefore, while all plants were exposed to the same chilling period, they may have been in different stages of dormancy induction when the chilling began. The fact that cuttings collected in late winter (which became dormant and obtained their chilling requirement under natural conditions) exhibited

a different pattern of variation than earlier collections, tends to support this hypothesis. It would appear that the relationship between dormancy induction and spring dehardening patterns is a topic that merits further investigation.

In both trials of Experiment 1, cuttings were presumed to be in a state of imposed dormancy when collected. Farmer and Reinholt (1986) have reported that balsam poplar exhibits unconditional autumn dormancy which is overcome by a relatively short chilling period. Usually this chilling requirement is overcome by January. However, bud break occurred more rapidly in cuttings collected in March, than those collected in February. This suggests that the buds may have been active in the period from February to March. Perry (1971) cites numerous examples of species in which metabolic activity occurs while the plants are supposedly dormant. It was not possible to discern any changes in hardiness levels between the two collection dates (February 13 and March 23) in Experiment 1, since cuttings collected on both dates were hardy to a least -27°C.

An interesting trend was noted in rooting characteristics of balsam poplar cuttings during the initial stages of new shoot expansion. As the freezing temperature to which the cuttings were exposed decreased, so did the rooting ability of cuttings which otherwise showed no visibile sign of damage (i.e. the number of cuttings in damage category 6 increased as the freezing temperature decreased). This trend may indicate that the preformed root primordia in the stem are more sensitive to frost injury than other tissues in the stem (i.e. cambium) during this period. However, other possible explanations exist. In a study with several Populus clones, Bloomberg (1963) determined that a cutting's moisture content was positively correlated with it's rooting ability. The critical nature of cutting moisture content to rooting ability and subsequent survival has also been demonstrated in poplar hardwood cuttings by Phipps et al. (1983). Considering that freeze-induced dehydration has long been known to increase with decreasing temperature (see

Levitt, 1980), and that moisture content has been strongly associated with rooting ability, the above trend may be explained in these terms. Decreased rooting ability from water stress might also have resulted from the environment of the growth chamber during the incubation treatments. A high percentage of cuttings in the control group (not subjected to freezing) fall into damage category 6 after 14 days of incubation (Table 6, page 32). For any propagation program, a damage score of 6 (ie. no roots) means that the plant will not survive even though leaves and stem still have live tissue.

Cuttings in this experiment were essentially hardy to -27°C when dormant. Therefore, one might expect near-perfect correlation between survival after freezing and percent bud break at the time of freezing. The fact that there was a weak correlation between these two variables in Experiment 1.1, and only a moderately high correlation in Experiment 1.2, is useful in emphasizing that balsam poplar cuttings were able to maintain considerable hardiness levels during bud flush and the initial stages of new-shoot expansion. These observations and other observations in the existing literature with boreal conifers (Glerum, 1976; Cannell and Sheppard, 1982) suggest that although the loss of dormancy and hardiness are initiated at the same time, the frost hardiness mechanism remains active well after dormancy release.

EXPERIMENT 2

Experiment 2 was designed to evaluate changes in the cold hardiness of balsam poplar cuttings during and immediately following bud flush and to relate levels of hardiness to the developmental stage. Furthermore, an attempt was made to evaluate provenance differences in the hardiness of cuttings at parallel developmental stages. The results of this experiment indicate that considerable

hardiness was maintained at the point of bud break; on the basis of the 50% killing point, cuttings from each of the four provenances were able to withstand freezing to -18°C without damage to the foliage, and -24°C without damage to the stem tissue (Figure 10, page 56). Once again, stem tissue appeared to deharden after leaf tissue, and a substantial loss of hardiness was not observed in stem tissue until the new shoot had extended 5 mm , and the bud began to form a vaselike structure (see Table 4). At this point, the foliage was still hardy to -9°C, and the stem tissue was hardy to approximately -15°C. When the newly expanding stem became visible, and the leaves were almost perpendicular to the shoot, the difference in hardiness between leaf tissue and stem tissue had been considerably reduced (-6°C for leaf tissue and -9°C for stem tissue). These experiment results indicate that frost injury rarely occurs at temperatures above -3°C. It should be noted that cuttings were held at the designated freezing temperatures for a one hour period. Greater damage might have resulted if the cuttings were held at each temperature for an extended period. Cuttings may have avoided injury above this temperature by deep supercooling, and this hypothesis is consistent with the fact that during the freezing test, exotherms (caused by the heat of fusion) were consistently observed at approximately -4°C.

Under natural conditions, the developmental stages used in Experiment 2 generally covers the period from May 2 to June 12 (depending on geographic source). Roe (1958) reports that in northern Michigan, the average date for flowering to begin is May 2 with full bloom reached on May 9; the average date for swelling of leaf buds is May 2, beginning leaf formation May 13, and full leaf June 10. The same general trend was observed in the clonal nursery population the year of the study. However, the spring of 1987 was extremely mild, and some of the clones from Wisconsin flushed during the last week of April. It appears that balsam poplar has a fairly high general tolerance of freezing temperatures throughout this period.

The results of the Friedman two-way analysis by ranks indicates that differences in frost susceptibility amongst provenances were not significant when buds of similar developmental stage were compared. A similar test by Lester et al. (1977) with Abies balsamea (L.) Mill also failed to detect provenance differences in frost susceptibility when developmental stage was taken into consideration. There was undoubtedly a fair amount of "experimental noise" associated with the design used in Experiment 2. The main premise behind the experimental design was that cuttings from different sources were evaluated at parallel discrete developmental stages; however, shoot development actually proceeds along a continuum. Although it is extremely unlikely (since significant source differences have been previously unreported in the literature when buds of similar developmental stage were compared), it is possible that the non-parametric test used in this experiment was not powerful enough to detect source differences in hardiness. The analysis was calculated to be 79.6 percent as efficient as a conventional parametric F-test (Bradley, 1968). A total of five cuttings (ie. replications) from each treatment combination were subjected to the six freezing temperatures (-3, -6, -9, -12, -18, and -24°C) used in the freezing test. The design would have been much stronger, and might have allowed for reliable geographic source comparisons at each developmental stage, if the number of replications at each freezing temperature was greatly increased. More replications would have been used, had they been available from the nursery population.

The Friedman two-way analysis was not conducted with data on stem tissue damage since the developmental stages used in this study were based solely on the newly expanding shoots; therefore, the assumption that cuttings from each source were tested at parallel developmental stages could only be applied to leaf tissue. Nonetheless, the results of Experiment 2 suggest that frost injury to the buds and shoots of balsam poplar cuttings was essentially a function of the stage of shoot growth at the time of freezing.

FURTHER COMMENTS

There was a limited amount of clonal material available for this study, which imposed some limitations on this study. One of the main weaknesses was that only two cuttings per clone were used at each of the four temperatures in the freezing test. This limitation resulted in the use of a somewhat indirect measure of hardiness. The percent survival of the eight cuttings per clone (two cuttings per clone at each of the four freezing) used in the freezing test was still thought to be a good indicator of the overall hardiness of a particular clone. Clonal differences might have been easier to elucidate if a wider range of temperatures had been used in the freezing test.

Highly significant differences in hardiness levels and bud break characteristics were noted between the two populations studied in Experiment 1. Bearskin Lake clones were less susceptible to frost injury than N. Wisconsin clones through the dehardening period, and the differential hardiness was closely related to the tendency of northern clones to remain dormant longer than their southern counterparts. Selection against early flushing genotypes is possible in the Bearskin Lake source, and this appears to be related to a longer period of environmental uncertainty in the spring. The differential timing of developmental events between these two populations suggests adaptive differentiation associated with latitude. However, it is difficult to suggest an adaptive cline on the basis of only two populations. More populations would have been evaluated in Experiment 1, if the clonal material had been available.

The results of Experiment 2 also indicate that frost injury to the buds and shoots of balsam poplar cuttings was a function of the stage of shoot growth (ie. phenological stage) at the time of freezing. Provenance differences in hardiness levels at parallel developmental stages seem unlikely, but some evidence of superior hardiness levels was observed in the Bearskin Lake source.

During the freezing test, the temperature in the chest-type freezer was decreased using the manual control on the freezer. Although the freezing curves obtained through this laborious procedure were quite similar, the lack of an automatic control for decreasing temperature was likely a source of experimental error. There is a significant difference (up to 5 °C) between the top and bottom of the freezer. Cuttings were place on the same level on the bottom of the freezer, where the temperature remained relatively stable, even when the lid of the freezer was opened to remove cuttings.

CONCLUSIONS

This study was initiated to examine the susceptibility of balsam poplar cuttings to freezing temperatures which might be encountered during the spring dehardening period. In addition, genetic variation in cold hardiness was examined within and between four widely separated populations of the species, in order to evaluate dehardening as a possible adaptive characteristic. The following conclusions were made:

1. Generally, balsam poplar stem cuttings were subjected to -27° C without mortality when dormant. Some localized non-lethal freezing injury was observed in cuttings subjected to freezing prior to bud break, suggesting that some dehardening occurs immediately prior to bud flush. In Experiment 1.2, a good correlation was found between freezing injury and percent bud break ($r = -0.82$ for leaf tissue; and $r = -0.71$ for stem tissue).

2. During bud flush and the initial stages of new-shoot expansion, cuttings were able to maintain substantial hardiness. This attenuated form of hardiness may be synonomous with a second stage of dehardening (with the first stage being the loss of deep mid-winter hardiness). At the point of bud break, cuttings from all four provenances were able to withstand freezing to -18° C without damage to the foliage, and -24° C without damage to the stem tissue. Even in more advanced stages of new-shoot development, cuttings survived freezing to -6° C without injury.

3. Dehardening occurred much more rapidly under the 25-15° C temperature regime than under the 15-5° C temperature regime, because developmental processes related to shoot phenology proceededmore rapidly under the higher temperature regime.

4. The dehardening process appears to be initiated in the meristematic regions of leaf tissue in advance of the cambium of stem tissue. In Experiment 1.2, leaf tissue became susceptible to frost injury after five days of incubation, while stem tissue did not exhibit an appreciable loss of hardiness before the eigth day of incubation.

5. Highly significant differences in hardiness levels and bud break characteristics were noted between the two populations studied in Experiment 1. Bearskin Lake clones were less susceptible to frost injury than N. Wisconsin clones through the dehardening period, and the differential hardiness was closely related to the tendency of northern clones to remain dormant longer than their southern counterparts. Selection against early flushing genotypes is possible in the Bearskin Lake source, and this could be related to a longer period of environmental uncertainty in the spring. The differential timing of developmental events between these two populations suggests adaptive differentiation associated with latitude. However, it is difficult to define an adaptive cline on the basis of only two populations.

6. The results of Experiment 2 indicate that frost injury to the buds and shoots of balsam poplar cuttings was a function of the stage of shoot growth at the time of freezing. Provenance differences in hardiness levels at parallel developmental stages seem unlikely, although some evidence of the possibility exists.

7. Relatively high within-population variance was also observed in hardiness levels and bud break characteristics. A number of early flushing (Wisc. 239, Bear. 302) and late flushing (Wisc. 220, Bear. 321) genotypes were observed in each population.

LITERATURE CITED

Akamatsu, Y. 1974. Osmotic stsbilization of unsaturated fatty acid auxotrophs of E. coli. J. Biochem. 76: 553-561.

Alden, J. 1971. Freezing resistence of tissues in the twig of Douglas fir. Ph.D. Thesis, Oregon State Univ., Corvallis, Oregon.

Alden, J. and R.K. Hermann. 1971. Aspects of the cold hardiness mechanism in plants. Bot. Rev. 37: 37-142.

Anderson, V.A., and R.A. McLean. 1974. Design of experiments: a realistic approach. Marcel Dekker, Inc. New York. 418 pp.

Aronsson, A., and J. Eliasson. 1970. Frost hardiness in Scots pine. I. Conditions for test on hardy plant tissues. Studia Forestalia Suecica 77:1-25.

Aronson, A., Ingsted, T. and L. Loof. 1976. Carbohydrate metabolism and frost hardiness in spruce and pine seedlings grown at different photoperiods and thermoperiods. Physiol. Plant. 36:127-132.

Bloomberg, W.J. 1963. The significance of adventitious roots in poplar cuttings and the effects of certain factors on their development. For. Chron. 39(3); 279-289.

Box, G.E.P., and S.L. Anderson. 1955. Permutation theory in the deviation of robust criteria and the study of departures from assumptions. J. of Royal Stat. Soc., Series B, 17:1-34.

Bradley, J.V. 1968. Distribution free statistical tests. Prentice-Hall, Inc. Englewood Cliffs, N.J. 388 pp.

Brown, J.H., Bula, R.J., and F.P. Low. 1970. Physical properties of cytoplasmic protein-water extracts from roots of hardy and nonhardy Medigo sativa ecotypes. Cryobiology 6: 309-314.

Burke, M.J., Gusta, L.V., Quamme, H.A., Weiser, C.J. and P.H. Li. 1976. Freezing and injury in plants. Ann. Rev. Plant Physiol. 27: 507-528.

Burley, J. 1966. Provenance variation in the growth of seddling apices of Sitka spruce. For. Sci. 12:170-175.

Campana, R. 1964. Noninfectious tree diseases. I. Effect of cold injury and freezing. Weed and Turf 3(8): 10-11, 22-23.

Campbell, R. K., and F. C. Sorensen. 1973. Cold acclimation in seedling Douglas fir related to phenology and provenance. Ecology 54:1148-1151.

Cannell, M.G.R. and L.J. Sheppard. 1982. Seasonal changes in the frost hardiness of provenances of Picea Sitchensis in Scotland. Forestry 55(2): 137-153.

Chapman, L.J. and M.K. Thomas. 1968. The climate of northern Ontario. Dept. of Transport, Meterological Branch. Climatological Studies No. 6. 58 pp.

Clausen, J. and W.M. Hiesey. 1960. The balance between coherence and variation in evolution. Proc. Natl. Acad. Sci. U.S.A. 46: 494-506.

Day, W. R. and T. R. Peace. 1937. The influence of certain accessory factors on frost injury to forest trees. Forestry 11:13-29.

Dexter, S.T., Tottingham, W.E. and L.F. Graber. 1932. Investigations on the hardiness of plants by measuring electrical conductivity. Plant Physiol. 7: 63-78.

Edgerton, L. G. 1954. Fluctuation in the cold hardiness of peach flower buds during rest period and dormancy. Proc. Amer. Soc. Hort. Sci. 64:178-180.

Ellstrand, N.C. and M.L. Roose. 1987. Patterns of genotypic diversity in clonal species. Amer. J. Bot. 74(1): 123-131.

Environment Canada. 1982. Canada climate normals 1951-1980. Temperature and precipitation. Can. Climate Prog., Atmospheric Environment Serv. 254 pp.

Farmer, R. E. Jr. and R.W. Reinholt. 1986. Genetic variation in dormancy relations of balsam poplar along a latitudinal transect in Northwestern Ontario. Silvae Genetica 35(1): 38-42.

Flint, H. L. 1972. Cold hardiness of twigs of Quercus rubra L. as a function of geographic origin. Ecology 53: 1163-1170.

George, M.F. and M.J. Burke. 1977. Cold hardiness and deep supercooling in the xylem of shagbark hickory. Plant Physiol. 59: 319-325.

Glerum, C. 1973. Annual trends in frost hardiness and electrical impedance for seven coniferous species. Can. J. Plant Sci. 53: 881-889.

Glerum, C. 1976. Frost hardiness of forest trees. In Tree physiology and yield improvement. MGR Cannell and F. T. Last, eds. Academic Press, New York, pp 403-419.

Greenham, C.G. and H. Daday. 1957. Electrical determination of cold hardiness in T. repens and M. sativa. Nature, Lond. 180: 541-543.

Gerloff, E.D., Richardson, T., and M.A. Stahmann. 1966. Changes in fatty acids of alphalfa roots during cold hardening. Plant Physiol. 41: 1280-1284.

Hare, F.K. and M.K. Thomas. 1979. Climate Canada. John Wiley and Sons, Toronto. 230 pp.

Hamilton, D.F. 1973. Factors influencing dehardening and rehardening of Forsythia x intermedia stems. J. Amer. Soc. Hort. Sci. 98(2): 221-223.

Heber, U. 1959. Beiziehungen Zwischen der Grosse von Chloroplasten und ihrem Gehalt an loslichen Eiweissen und Zuckem im Zusammenhang mit dem Frostresistenz Problem. Protoplasma 51: 284-298.

Howell, G., and C.J. Weiser. 1970. The environmental control of cold acclimationin apple. Plant Physiol. 45: 390-394.

Iljun, W. S. 1934. The point of death of plants at low temperatures. Bull. Ass. Russe. Rech. Sci. Prague Sect. Sci. Nat. Math. 1(6):135-160.

Irving, R. M. and F. O. Lanphear. 1967. Dehardening and the dormant condition in Acer and Viburnam. Amer. Soc. Hort. Sci. 6:81-99.

Johansson, W. O., Albertson, C. E. and T. Mansson. 1955. Field trials with a portable refrigerator. Acta Agr. Scand. 6:81-99.

Lehmann, E.L. 1975. Nonparametrics: statistical methods based on ranks. McGraw-Hill Book Co., New York. 457 pp.

Le Saint, A.M., and A.M. Catesson. 1966. Variations simultaness des teneurs en eau, en sucre solubles, en acid amines et de la pression osmotique dans la phloeme et la cambium de sycamore pendant les periodes de repos et de reprise de la croissance. C. R. Acad. Sci. 269:1463-1466.

Lester, D. T., Lindow, S. E. and C. D. Upper. 1977. Freezing injury and shoot elongation in balsam fir. Can. J. For. Res. 7:584-588.

Levitt, J. 1956. The hardiness of plants. Academic Press, New York. 278 pp.

Levitt, J. 1962. A sulfhydrl-disulfide hypothesis of frost injury and resistance in plants. J. Theoret. Biol. 3: 355-391.

Levitt, J. 1966. Winter hardiness in plants. In Cryobiology, H. T. Merryman ed. Academic Press, New York. pp 495-563.

Levitt, J. 1969. The effect of sulfhydrl agents on freezing tolerance of hardened and unhardened cabbage cells. Cryobiology 5: 278-280.

Levitt, J. 1978. An overview of freezing injury and survival, and its interrelationship to other stresses. In Plant Cold Hardiness and Freezing Stress, P. H. Li and A. Sakai, eds. Academic Press, New York. pp 3-15.

Levitt, J. 1980. Responses of plants to environmental stress. I. Chilling, freezing and high temperature stresses. Academic Press, New York. 497 pp.

Mazur, P. 1969. Freezing injury in plants. Ann. Rev. Plant Physiol. 20:419-448.

Mergen, F. 1963. Ecotypic variation in Pinus strobus L. Ecology 44: 716-727.

Neilson, R.E., Ludlow, M.M., and P.G. Jarvis. 1972. Photosynthesis in Sitka spruce. II. Response to temperature. J. Appl. Ecol. 9: 721-745.

Nienstaedt, H. and J. P. King. 1969. Breeding for delayed bud break in Picea glauca (Moench) Voss. Proc. Sec. World Consult. For Tree Breeding. FAO-IUFRO, Washington, D.C. pp 61-80.

Olien, C.R. 1967. Freezing stress and survival. Ann. Rev. Plant Physiol. 18:387-408.

Parker, J. 1953. Criteria for life: Some methods for measuring viability. Amer. Sci. 41: 614-618.

Pelkonen, P. and C. Glerum. 1986. Poplar clonal variation in frost hardiness and electrical impedance during the fall. Ontario Tree Improvement and For. Biomass Inst., Min. Nat. Res., For Res. Report No. 112. 12 pp.

Perry, T. O. 1971. Dormancy of trees in winter. Sci. 171:29-36.

Pfeiffer, M. 1933. Frostuntersuchugen an Fichtentrienben. Tharandter Forstl. Jahrb. 84: 664-695.

Phipps, H.M., Hansen, E.A., and A.S. Fege. 1983. Pre-plant soaking of dormant *Populus* hardwood cuttings. U.S.D.A. For. Serv. Res. Paper NC-241. 8 pp.

Plenkema, D.L. 1964. Some effects of freeze injury on the development of Douglas fir. Northwest Sci. 38: 14-17.

Posen, C.E. 1967. Natural regeneration of loblolly pine 230 miles north of it's natural range. J. For. 65: 732.

Pukacki, P. 1982. Freezing tolerance of Polish Norway spruce. Arboretum Kornickie 26: 149-161.

Rehfeldt, G. E. 1979. Variation in the cold hardiness among populations of Pseudotsuga meziesii var glauca. USDA For. Serv. Res. Paper INT-233. 11 pp.

Rehfeldt, G. E. 1977. Growth and hardiness of intervarietal hybrids of Douglas fir. Theor. Appl. Genetics 50:3-15.

Rehfeldt, G.E. 1984. Seed transfer zones in the northern Rockies. In: Progeny testing. Proc. Servicewide genetics workshop, Dec. 5-9, 1983. Charleston, S.C., U.S.D.A. For. Serv.

Roe, E.I. 1958. Silvical characteristics of balsam poplar. U.S. For. Serv., Lakes States For Expt. Sta., Sta. Paper 65. 17 pp.

Sakai, A. 1970. Freezing resistances in willows from different climates. Ecology 51: 485-491

Sakai, A., and C.J. Weiser. 1973. Freezing resistance of trees in North America with reference to tree regions. Ecology 54: 118-126.

Scarth, G.W. 1944. Dehydration injury and resistance. Plant Physiol. 16: 171-179.

Scheumann, W. 1962. Moglichkeiten und Ergebnisse der Frostresistenz-prufung in der Douglasien und Larchenzuchtung. Tag. Ber. Dt. Akad. Landwirtsch-Wiss. 69:189-199.

Siminovitch, D., Rheaume, B., Pomeroy, K. and M. Lepage. 1968. Phospholipids, protein and nucleic acid increases in the protoplasm and membrane structures associated with the development of extreme freezing resistance in black locust tree cells. Cryobiology 5: 202-225.

Siminovitch, D., Singh, J. and I.A. de la Roche. 1975. Studies on membrane in plant cells resistant to extreme freezing. I. Augmentation of phospholipidsand membrane substance without changes in unsaturation of fatty acids during hardening by black locust bark Cryobiology 12: 144-153.

Steele, R.G.D. and J.H. Torrie. 1980. Principles and procedures of statistics: a biometrical approach. McGraw-Hill Book Co., New York. 633 pp.

Steponkus, P.L. and F.O. Lanphear. 1967. Refinement of the triphenyl tetrazolium chloride method of determining cold injury. Plant Physiol. 42:1423-1427.

Strain, B.R. 1966. The effect of late spring frost on the radial growth of quaking aspen. For. Sci. 12: 334-337.

Timmis, R. 1976. Methods of screening tree seedlings for frost hardiness. In:Tree physiology and yield improvement. Cannell, M.G.R. and F. T. Last, eds. Academic Press, New York. pp 421-435.

Timmis, R. and J. Worrall. 1974. Translocation of dehardening and bud-break promoters in climatically split Douglas-fir. Can. J. For. Res. 5:464-477.

Tumanov, I.I., and O.A. Krasavtsev. 1959. Hardening of northern woody plants in temperatures below zero. Sov. Plant Physiol. 6: 654-667.

van den Driessche, R. 1973. Predictions of frost hardiness in Douglas fir seedlings by measuring electrical impedance in stems at different frequencies. Can. J. For. Res. 3: 256-264.

Visner, S.S. 1954. Climatic atlas of the Unites States. Harvard Univ. Press, Cambridge. 403 pp.

Wareing, P.F. 1951. Growth studies in woody species. IV. The initiation of cambial growth in ring porous species. Physiol. Plant. 4(3): 546-562.

Weiser, C. J. 1970. Cold resistance and injury in woody plants. Science 169:1269-1268.

Wilner, J. 1960. Relative and absolute electrolytic conductance tests for frost hardiness of apple varieties. Can. J. Plant Sci. 40: 630-637.

Winer, B.J. 1971. Statistical principles in experimental design. McGraw-Hill. New York. 907 pp.

Yoshida, S. 1969. Studies on the freezing resistance in plants. II. Seasonal changes in phospholipids in the bark of black locust trees. Low Temp. Sci. Series B27: 119-124.

Yoshida, S. and A. Sakai. 1974. The frost hardiness process of woody plants. XII. Relation between frost resistance and various substances in the stem of the bark of black locust. Low Temp. Sci. Ser. Biol. Sci. B25: 29-44.

APPENDIX I

TABLE 11. SUMMARY OF CLONES USED IN EXPERIMENT 1

Table 11. Clones used in Experiment 1.1 and 1.2.

	Experiment 1.1		Experiment 1.2	
	N. Wisconsin	Bearskin Lake	N. Wisconsin	Bearskin Lake
1.	246	359	246	321
2.	245	326	247	345
3.	230	345	229	320
4.	253	342	238	302
5.	242	325	227	305
6.	240	356	220	308
7.	247	337	239	322
8.	235	320	204	312
9.	229	330	235	313
10.	241	355	282	317
11.	233	334	253	316
12.	239	333	228	342

APPENDIX II

Table 12. ANOVA table of percent survival in balsam poplar leaf tissue after exposure to the freezing test in Experiment 1.2. (Analysis is restricted to the final three dehardening treatments: 5, 8, and days).

Source	df	SS	MS	F	Sig. of F
Incubation Temp, $[T_i]$	1	16610.9	16610.9	no test	
$\delta_{[ij]}$, 1st restriction error	0				
Incubation Period, $[P_j]$	2	18820.4	9410.2	22.0	0.047
Temp. X Period, $[TP_{ij}]$	2	855.7	427.8	no test	
$w_{[ij]}$, 2nd restriction error	0				
Source $[S_k]$	1	6314.9	6314.9	80.9	0.000
Temp. X Source $[TS_{ik}]$	1	239.2	239.2	2.0	0.157
Period X Source $[PS_{jk}]$	2	17.3	8.6	0.1	0.916
Clone/Source $[C_{[k].l}]$	22	1716.8	78.0	0.9	0.566
Temp. X Clone/S $[TC_{i[k]l}]$	22	2639.0	120.0	1.42	0.155
Period X Clone/S $[PC_{j[k]l}]$	44	3155.0	71.7	0.8	0.705
Error and/or TPC, TPS	46	3880.4	84.4		
Total	143				

Table 13. ANOVA table of percent survival in balsam poplar stem tissue after exposure to the freezing test in Experiment 1.2. (Analysis is restricted to the final three dehardening treatments: 5, 8, and 11 days.)

Source	df	SS	MS	F	Sig. of F
Incubation Temp, $[T_i]$	1	9707.2	9707.2	no test	
$\delta_{[i]}$, 1st restriction error	0				
Incubation Period, $[P_j]$	2	14055.5	7027.8	7.42	0.120
Temp. X Period, $[TP_{ij}]$	2	1895.1	947.5	no test	
$w_{[ij]}$, 2nd restriction error	0				
Source $[S_k]$	1	6072.3	6072.3	30.83	0.000
Temp. X Source $[TS_{ik}]$	1	641.4	641.4	6.54	0.020
Period X Source $[PS_{jk}]$	2	1607.2	803.6	8.17	0.007
Clone/Source $[C_{[k].l}]$	22	4332.9	196.9	2.41	0.006
Temp. X Clone/S $[TC_{i[k]l}]$	22	2157.7	98.1	1.20	0.294
Period X Clone/S $[PC_{j[k]l}]$	44	4329.5	98.4	1.20	0.267
Error and/or TPC, TPS	46	3758.8	81.7		
Total	143				

Table 14. ANOVA table of percent bud break (at the time of freezing) in balsam poplar cuttings in Experiment 1.2.

Source	df	SS	MS	F	Sig. of F
Incubation Temp, $[T_i]$	1	9908.5	9908.5	no test	
$d_{[ij]}$, 1st restriction error	0				
Incubation Period, $[P_j]$	2	61555.8	30827.9	58.0	0.020
Temp. X Period, $[TP_{ij}]$	2	1063.7	531.8	no test	
$w_{[ij]}$, 2nd restriction error	0				
Source $[S_k]$	1	36995.3	36995.3	121.37	0.000
Temp. X Source $[TS_{ik}]$	1	673.0	673.0	3.57	0.077
Period X Source $[PS_{jk}]$	2	81.3	40.7	0.17	0.831
Clone/Source $[C_{[k].l}]$	22	6705.6	304.8	1.67	0.072
Temp. X Clone/S $[TC_{i[k]l}]$	22	4144.4	188.4	1.03	0.450
Period X Clone/S $[PC_{j[k]l}]$	44	10213.7	232.1	1.27	0.212
Error and/or TPC, TPS	46	8404.9	182.7		
Total	143				

APPENDIX III

Table 15 . Percent bud break at the time of freezing by clone in Experiment 1.1. Percent bud break is based on the 8 cuttings per clone subjected to the freezing test.

Incub. Period	Incub. Temp.	PERCENT BUD BREAK: WISCONSIN												mean	Sd
		246	245	230	253	242	240	247	235	229	241	233	239		
0	15-5°C	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0.00
	25-15°C	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0.00
1	15-5°C	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0.00
	25-15°C	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0.00
4	15-5°C	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0.00
	25-15°C	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0.00
9	15-5°C	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0.00
	25-15°C	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0.00
14	15-5°C	25	25	0	25	13	0	13	13	0	0	0	0	9.40	10.80
	25-15°C	25	75	38	50	25	13	13	50	0	13	25	0	27.00	22.50
		PERCENT BUD BREAK: BEARSKIN LAKE													
		359	326	345	342	325	356	337	320	330	355	334	353		
0	15-5°C	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0.00
	25-15°C	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0.00
1	15-5°C	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0.00
	25-15°C	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0.00
4	15-5°C	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0.00
	25-15°C	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0.00
9	15-5°C	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0.00
	25-15°C	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0.00
14	15-5°C	0	0	0	0	0	0	0	13	0	0	0	0	1.00	3.60
	25-15°C	0	25	0	13	0	0	13	0	0	25	0	0	6.30	10.00

Table 16 . Percent bud break at the time of freezing by clone in Experiment 1.2. Percent bud break is based on the 8 cuttings per clone subjected to the freezing test.

Incub. Period	Incub. Temp.	PERCENT BUD BREAK: WISCONSIN 246	247	229	238	227	220	239	204	235	282	253	228	mean	Sd
0	15-5°C	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0.00
	25-15°C	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0.00
	15-5°C	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0.00
	25-15°C	0	0	0	0	0	0	13	0	0	0	0	0	1.00	0.10
4	15-5°C	25	38	25	25	25	0	50	38	13	0	0	0	11.80	17.20
	25-15°C	63	75	63	75	38	0	100	43	25	25	29	50	48.80	27.70
9	15-5°C	88	63	86	100	75	75	100	63	50	63	63	100	76.80	17.50
	25-15°C	100	88	100	100	100	100	100	86	63	88	100	100	93.70	11.40
14	15-5°C	100	88	100	100	100	100	100	100	88	100	88	88	95.80	6.15
	25-15°C	100	100	100	100	100	100	100	100	90	100	100	100	99.20	2.90
		PERCENT BUD BREAK: BEARSKIN LAKE 321	345	320	302	305	308	322	312	313	317	316	342		
0	15-5°C	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0.00
	25-15°C	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0.00
1	15-5°C	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0.00
	25-15°C	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0.00
4	15-5°C	0	0	0	13	0	0	0	0	0	0	0	0	1.00	0.10
	25-15°C	0	0	13	25	0	0	0	13	13	0	13	0	6.20	8.40
9	15-5°C	0	13	50	88	0	0	50	13	38	13	13	0	11.50	16.40
	25-15°C	25	71	88	75	50	38	38	50	100	75	63	63	61.10	22.10
14	15-5°C	13	38	88	100	50	25	50	68	88	25	25	50	51.00	28.40
	25-15°C	25	50	88	100	50	100	63	75	100	100	88	88	77.10	24.90

APPENDIX IV

Table 17. Percent survival by clone for each source in Experiment 1.1. Percent survival is based on the 8 cuttings per clone subjected to the freezing test.

Incub. Period	Incub. Temp.	PERCENT SURVIVAL : WISCONSIN 246	245	230	253	242	240	247	235	229	241	233	239	mean	Sd
Leaf tissue:															
day 0	15-5°C	100	100	100	100	100	100	100	88	100	100	100	100	98.90	3.60
	25-15°C	100	100	100	100	100	100	100	100	100	100	100	100	100	0.00
day 1	15-5°C	100	100	100	100	100	100	100	100	100	71	100	100	97.60	8.40
	25-15°C	100	100	100	88	100	100	100	88	100	100	100	100	97.90	4.90
day 4	15-5°C	100	88	100	100	100	100	100	100	100	100	100	100	98.90	3.60
	25-15°C	100	100	100	100	100	100	100	100	100	100	100	100	100	0.00
day 9	15-5°C	100	100	100	100	75	100	100	100	100	100	100	100	97.90	7.20
	25-15°C	75	75	75	75	88	100	88	100	88	88	100	100	98.50	11.30
day 14	15-5°C	88	75	88	63	88	88	88	88	100	100	88	100	87.50	10.70
	25-15°C	100	50	63	75	88	100	88	100	100	88	88	100	86.50	16.40
Stem tissue:															
day 0	15-5°C	100	100	100	100	100	100	100	88	100	100	100	100	98.90	3.60
	25-15°C	100	100	100	100	100	100	100	100	100	100	100	100	100	0.00
day 1	15-5°C	100	100	100	100	100	100	100	100	100	88	100	100	98.90	3.60
	25-15°C	100	100	100	100	100	100	100	100	100	100	100	100	100	0.00
day 4	15-5°C	100	100	100	100	100	100	100	100	100	100	100	100	100	0.00
	25-15°C	100	100	100	100	100	100	100	100	100	100	100	100	100	0.00
day 9	15-5°C	100	100	100	100	100	100	100	100	100	100	100	100	100	0.00
	25-15°C	100	75	88	88	88	100	100	100	100	100	100	100	94.80	8.40
day 14	15-5°C	100	100	100	100	100	100	100	100	100	100	100	100	100	0.00
	25-15°C	100	50	100	88	100	100	100	100	100	100	100	100	94.80	14.60

Table 17. Percent survival by clone for each source in Experiment 1.1. Percent survival is based on the 8 cuttings per clone subjected to the freezing test.

Incub. Period	Incub. Temp.	PERCENT SURVIVAL: BEARSKIN L. CLONES 359	326	345	342	325	356	337	320	330	355	334	333	mean	Sd
Leaf tissue:															
day 0	15-5°C	100	88	88	100	100	100	100	100	100	100	100	100	97.90	4.90
	25-15°C	100	100	100	100	100	100	100	88	88	88	100	88	95.80	6.20
day 1	15-5°C	100	100	100	100	100	100	100	100	88	100	100	100	98.90	3.60
	25-15°C	100	100	100	100	100	100	100	100	100	100	100	88	98.90	3.60
day 4	15-5°C	100	100	100	100	100	100	100	100	100	100	100	88	98.90	3.60
	25-15°C	100	100	100	100	100	100	100	100	100	88	100	88	97.90	4.90
day 9	15-5°C	75	100	100	100	100	100	100	100	100	100	100	100	97.40	7.20
	25-15°C	88	100	88	88	100	100	88	100	100	100	88	88	93.80	6.50
day 14	15-5°C	100	100	100	88	88	88	88	75	100	88	100	75	90.60	9.40
	25-15°C	88	88	100	100	100	100	88	88	100	100	88	75	92.70	8.40
Stem tissue:															
day 0	15-5°C	100	100	100	100	100	100	100	100	100	100	100	100	100	0.00
	25-15°C	100	100	100	100	100	100	100	100	100	100	100	100	100	0.00
day 1	15-5°C	100	100	100	100	100	100	100	100	100	100	100	100	100	0.00
	25-15°C	100	100	100	100	100	100	100	100	100	100	100	88	98.90	3.60
day 4	15-5°C	100	100	100	100	100	100	100	100	100	100	100	100	100	0.00
	25-15°C	100	100	100	100	100	100	100	100	100	100	100	88	98.90	3.60
day 9	15-5°C	100	100	100	100	100	100	100	100	100	100	100	100	100	0.00
	25-15°C	100	100	100	88	100	100	100	100	100	100	100	100	98.90	3.60
day 14	15-5°C	100	100	100	100	88	100	88	88	100	100	100	100	96.90	5.60
	25-15°C	100	100	100	100	100	100	100	100	100	100	88	88	97.90	4.90

Table 18. Percent survival by clone for each source in Experiment 1.2. Percent survival is based on the 8 cuttings per clone subjected to the freezing test.

Incub. Period	Incub. Temp.	PERCENT SURVIVAL: WISCONSIN CLONES 246	247	229	238	227	220	239	204	235	282	253	228	mean	Sd
Leaf tissue:															
day 0	15-5°C	100	100	88	100	100	100	100	100	100	88	100	100	97.90	4.90
	25-15°C	100	100	100	100	100	100	75	100	88	88	100	100	95.80	8.10
day 2	15-5°C	100	100	100	100	100	100	100	100	100	100	100	100	100	0.00
	25-15°C	100	88	100	100	100	100	100	100	100	100	100	100	98.90	3.60
day 5	15-5°C	75	75	88	100	88	100	63	63	100	100	88	100	86.40	14.60
	25-15°C	50	88	50	63	75	88	38	71	75	63	57	63	64.80	15.20
day 8	15-5°C	63	75	57	63	75	63	50	63	88	75	75	50	66.20	11.39
	25-15°C	13	63	25	13	13	14	25	29	25	63	25	14	26.60	17.80
day 11	15-5°C	38	38	38	38	63	50	38	50	50	63	50	63	47.90	10.40
	25-15°C	25	25	25	25	25	25	25	25	63	13	25	25	27.00	11.70
Stem tissue:															
day 0	15-5°C	100	100	88	100	100	100	100	100	100	100	100	100	98.90	3.60
	25-15°C	100	100	100	100	100	100	100	100	100	100	100	100	100	0.00
day 2	15-5°C	100	100	100	100	100	100	100	100	100	100	100	100	100	0.00
	25-15°C	100	100	100	100	100	100	100	100	100	100	100	100	100	0.00
day 5	15-5°C	100	100	100	100	100	100	100	100	100	100	100	100	100	0.00
	25-15°C	100	75	88	75	100	100	100	100	88	100	75	100	91.70	11.10
day 8	15-5°C	88	75	100	100	75	100	88	75	100	100	75	88	88.50	11.30
	25-15°C	75	63	75	50	38	50	50	75	75	75	50	57	61.00	13.60
day 11	15-5°C	75	75	100	63	75	88	88	75	88	100	75	75	81.20	11.30
	25-15°C	38	38	38	25	25	63	38	38	88	38	25	25	39.60	18.30

Table 18. Percent survival by clone for each source in Experiment 1.2. Percent survival is based on the 8 cuttings per clone subjected to the freezing test.

Incub. Period	Incub. Temp.	PERCENT SURVIVAL: BEARSKIN L. 321	345	320	302	305	308	322	312	313	317	316	342	mean	Sd
Leaf tissue:															
day 0	15-5°C	100	100	100	100	75	100	100	100	88	100	100	88	95.80	8.10
	25-15°C	100	88	100	88	100	88	100	100	88	88	100	100	94.80	6.40
day 2	15-5°C	100	75	88	100	88	100	88	100	88	100	88	100	92.70	8.40
	25-15°C	88	100	100	100	88	100	100	88	100	100	100	100	96.90	5.70
day 5	15-5°C	100	100	100	88	88	100	100	100	100	100	100	100	97.90	4.90
	25-15°C	100	100	75	75	88	63	100	88	63	63	63	88	80.20	15.50
day 8	15-5°C	100	100	75	50	88	88	88	88	88	100	75	100	86.50	14.50
	25-15°C	63	38	25	38	63	50	63	38	38	38	25	38	42.70	13.50
day 11	15-5°C	88	75	63	50	63	75	100	50	63	88	75	88	72.90	15.80
	25-15°C	63	50	38	25	63	50	38	38	25	25	38	38	40.60	13.20
Stem tissue:															
day 0	15-5°C	100	100	100	100	88	100	100	100	88	100	100	100	97.90	4.90
	25-15°C	100	100	100	100	100	100	100	100	100	100	100	100	100	0.00
day 2	15-5°C	100	88	100	100	100	100	100	100	100	100	100	100	98.90	3.60
	25-15°C	100	100	100	100	100	100	100	100	100	100	100	100	100	0.00
day 5	15-5°C	100	100	100	100	100	100	100	100	100	100	100	100	100	0.00
	25-15°C	100	100	100	88	100	88	100	100	100	100	100	100	97.90	4.90
day 8	15-5°C	100	100	88	50	100	100	100	100	100	100	100	100	94.80	14.60
		100	75	88	50	100	88	100	100	88	75	75	100	86.50	15.50
day 11	15-5°C	100	75	100	75	88	100	100	100	100	100	100	100	94.80	9.90
	25-15°C	100	75	75	25	88	75	75	88	88	75	50	75	74.00	19.60

APPENDIX V

Key for Appendix V: Tables 19 and 20:

Flush codes: - flush code I was used to indicate the flushing status of cutting 1, flush code II represent cutting 2.

- a number indicates that the cutting had flushed prior to freezing, and a letter/number combination was used to indicate the month and day that the cutting flushed after the freezing test (F= February, M= March, A= April).

- the number used to indicate that the cutting had flushed prior to freezing, represented the developmental stage of the cutting at the time of freezing. The developmental codes used in these tables are similar to those found in Table 4, page 21, with the following exceptions:

Code in Table 4	Code in Appendix V
2	0
3	1
4	2
5	3
6	4
7	5
-	6
-	7

- in Appendix V, a flush code of 7 was used to indicate that the newly expanding leaves were perpendicular to the stem; flush code 6 represented the developmental stage in which the leaves were not quite perpendicular to the stem (ie. approximately 80° to the stem).

- when an 'R' proceded the flush code, the cutting had roots at the time of freezing

- Rep I and Rep II do not represent two different replications; they represent cuttings I and II, respectively.

Table 19. Raw data for experiment 1.1

Source/Clone: BEANSKIN 334

Incub. time	Incub. temp.	Freez. time	Flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	Flush code II
day 0	15.5°	5	F28	0	0	F23
		.3	F28	0	8	M20
		.11	F27	0	0	F28
		.19	F28	0	0	F24
		.27	M3	0	8	M21
	25.15°	5	F28	0	0	F28
		.3	F28	0	0	F28
		.11	F27	0	0	M4
		.19	F27	0	0	M4
		.27	M2	0	0	F26
day 1	15.5°	5	M15	6	6/4	
		.3	F26	0	6	F26
		.11	F28	0	0	F26
		.19	M1	0	0	F28
		.27	M5	0	0	F28
	25.15°	5	F28	0	7	
		.3	F24	0	0	F28
		.11	F26	0	0	F28
		.19	M8	0	0	F27
		.27	F28	0	8	M4
day 4	15.5°	5	F28	0	0	F28
		.3	F28	1	0	M3
		.11	M4	0	0	M4
		.19	M5	0	0	M4
		.27	M4	6	0	M5
day 4	25.15°	5	F28	0	0	F28
		.3	M20	4	6	
		.11	M1	0	0	
		.19	F28	1	0	
		.27	F27	0	0	
day 9	15.5°	5	M8	0	0	
		.3	M7	1	1	
		.11	M6	0	0	
		.19	M4	0	0	
		.27	M4	0	0	
	25.15°	5	M5	0	6	
		.3	M1	0	0	
		.11	M4	0	0	
		.19	M12	0	6	
		.27	M1	7	0	
day 14	15.5°	5		7	7	
		.3	M15	6	4	
		.11	M11	0	0	
		.19	M11	1	0	
		.27	M8	6	4	
	25.15°	5	M11	0	0	
		.3	M11	0	0	
		.11	M13	1	6	
		.19	M13	6	0	
		.27	M12	1	6	

Table 19. Raw data for experiment 1.1

Source/Clone: BEANSKIN 355

Incub. time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	Flush code II
day 0	15.5°	5	F24	0	0	F24
		.3	F26	0	6	F28
		.11	F24	0	0	F28
		.19	F25	0	0	F28
		.27	F28	0	3	M4
	25.15°	5	F28	0	0	F25
		.3	F26	0	0	F28
		.11	F28	0	0	F24
		.19	F26	0	0	F26
		.27		7	0	F28
day 1	15.5°	5	F25	0	0	F25
		.3	F25	0	0	F28
		.11	F25	0	0	F24
		.19	F28	0	0	F27
		.27	M1	0	0	F27
	25.15°	5	F26	0	0	F26
		.3	F26	0	0	F26
		.11	F28	0	6	F26
		.19	F28	1	0	F28
		.27	M2	0	6	M4
day 4	15.5°	5	M2	8	7	
		.3	F25	0	0	F26
		.11		7	0	M2
		.19	M3	0	0	M3
		.27	F28	0	0	M5
day 4	25.15°	5	F28	0	0	
		.3	F28	0	0	
		.11	M2	0	4	
		.19	M4	0	0	
		.27	F28	0	0	
day 9	15.5°	5	M13	0	0	
		.3	M1	1	0	
		.11	M7	0	0	
		.19	M6	1	0	
		.27	M4	2	0	
	25.15°	5	F28	0	0	
		.3	F28	0	6	
		.11	F27	0	0	
		.19	M2	6	0	
		.27	M3	0	0	
day 14	15.5°	5	M8	6	6	
		.3	M10	0	1	
		.11	M11	0	0	
		.19	M10	1	0	
		.27		7	1	
	25.15°	5	M6	0	0	
		.3	M8	0	0	
		.11	M8	0	6	
		.19	M11	0	6	
		.27	M11	0	0	

Table 19. Raw data for experiment 1.1

Source/Clone: BEARSKIN 320

Incub. time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	Flush code II	Incub time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II
day 0	15.5°	S	F24	0	0	M8	day 4	25.15°	S	F28	0	0
		-3	F28	2	0	M11			-3	F28	0	0
		-11	M4	0	0	F28			-11	F27	0	0
		-19	M18	0	0	F26			-19	F26	0	0
		-27	F20	0	0	F20			-27	M2	6	1
	25.15°	S	M4	8	0	F24						
		-3	F28	0	7		day 9	15.5°	S	M1	6	1
		-11	F28	0	0	M6			-3	M3	6	0
		-19	F26	0	0	F27			-11	M10	0	1
		-27	M3	0	8	F20			-19	M4	1	8
									-27	M3	0	0
day 1	15.5°	S	F28	0	0	F25		25.15°	S	F28	0	1
		-3	F26	0	0	F26			-3	M3	0	0
		-11	F28	0	1	F20			-11	M1	0	0
		-19	F28	0	0	M11			-19	M3	0	8
		-27	M16	1	0	M4			-27	M4	8	8
	25.15°	S	F28	0	0	F28						
		-3	F28	0	0	F27	day 14	15.5°	S	M11	1	0
		-11	F28	0	0	F27			-3	M9	0	0
		-19	F28	1	0	F26			-11	M10	0	8
		-27	F27	0	0	F26			-19		7	8
									-27	M10	6	0
day 4	15.5°	S	F24	0	0	F24		25.15°	S	M8	0	0
		-3	M3	0	0	F28			-3	M4	0	0
		-11	M2	0	0	F26			-11	M7	6	2
		-19	M4	0	0	F28			-19	M8	6	0
		-27		4	0	F28			-27		7	8

Table 19. Raw data for experiment 1.1

Source/Clone: BEARSKIN 320

Incub. time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	Flush code II	Incub time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II
day 0	15.5°	S	F24	0	0	M8	day 4	25.15°	S	F28	0	0
		-3	F28	2	0	M11			-3	F28	0	0
		-11	M4	0	0	F28			-11	F27	0	0
		-19	M18	0	0	F26			-19	F26	0	0
		-27	F20	0	0	F20			-27	M2	6	1
	25.15°	S	M4	8	0	F24						
		-3	F28	0	7		day 9	15.5°	S	M1	6	1
		-11	F28	0	0	M6			-3	M3	6	0
		-19	F26	0	0	F27			-11	M10	0	1
		-27	M3	0	8	F20			-19	M4	1	8
									-27	M3	0	0
day 1	15.5°	S	F28	0	0	F25		25.15°	S	F28	0	1
		-3	F26	0	0	F26			-3	M3	0	0
		-11	F28	0	1	F20			-11	M1	0	0
		-19	F28	0	0	M11			-19	M3	0	8
		-27	M16	1	0	M4			-27	M4	8	8
	25.15°	S	F28	0	0	F28						
		-3	F28	0	0	F27	day 14	15.5°	S	M11	1	0
		-11	F28	0	0	F27			-3	M9	0	0
		-19	F28	1	0	F26			-11	M10	0	8
		-27	F27	0	0	F26			-19		7	8
									-27	M10	6	0
day 4	15.5°	S	F24	0	0	F24		25.15°	S	M8	0	0
		-3	M3	0	0	F28			-3	M4	0	0
		-11	M2	0	0	F26			-11	M7	6	2
		-19	M4	0	0	F28			-19	M8	6	0
		-27		4	0	F28			-27		7	8

Table 19. Raw data for experiment 1.1

Source/Clone: BEARSKIN 337

Incub. time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	Flush code II	Incub. time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II
day 0	15.5°	5	F27	0	0	F28	day 4	25.15°	5	M3	0	0
		-3	M1	0	1	F27			-3	M5	1	0
		-11	F28	0	0	M2			-11	M4	0	0
		-19	M4	0	0	M2			-19	M4	1	6
		-27	M2	0	1	M4			-27	M6	0	6
	25.15°	5	F27	0	0	F28						
		-3	M1	0	0	M3	day 9	15.5°	5	M7	8	8
		-11	M2	0	M				-3	M5	0	0
		-19		M	0	M4			-11	M11	0	0
		-27	M4	0	0	M5			-19	M11	0	6
									-27	M10	1	2
day 1	15.5°	5	F26	0	0	F28		25.15°	5	M5	0	0
		-3	M4	0	6	M5			-3	M4	0	6
		-11	M1	0	8	M2			-11	M5	0	6
		-19	M11	0	0	M3			-19	M8	0	4
		-27	M20	0	0	M8			-27	M1	1	0
	25.15°	5	F27	8	0	F27						
		-3	F28	0	0	M2	day 14	15.5°	5	M20	0	0
		-11	M3	0	0	M10			-3		7	0
		-19	M5	0	2	M3			-11	M14	8	0
		-27	M5	8	1	M4			-19	M14	0	8
									-27		8	8
day 4	15.5°	5	F28	8	8	F28		25.15°	5	M9	0	0
		-3	M1	0	0	M3			-3	M8	1	3
		-11	M8	0	0	M8			-11		7	0
		-19	M18	8	0	M5			-19	M12	0	0
		-27	M7	0	1	M6			-27	M14	0	0

Table 19. Raw data for experiment 1.1

Source/Clone: BEARSKIN 358

Incub. time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	Flush code II	Incub. time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II
day 0	15.5°	5	F24	0	0	F24	day 4	25.15°	5	F22	0	0
		-3	F25	0	0	F28			-3	F23	0	0
		-11	F24	0	0	F25			-11	F28	0	0
		-19	F24	1	0	F22			-19	F26	0	0
		-27	F27	0	8	F26			-27	F27	0	8
	25.15°	5	F24	0	0	F22						
		-3	F26	1	0	F28	day 9	15.5°	5	F28	0	0
		-11	F28	0	0	F25			-3	M1	0	0
		-19	F25	0	0	F24			-11	M3	1	1
		-27	F28	0	1	F28			-19	M7	0	1
									-27	F28	1	0
day 1	15.5°	5	M2	0	0	M9		25.15°	5	F28	8	0
		-3	F28	0	0	F26			-3	F28	0	0
		-11	F27	0	0	F26			-11	F27	0	0
		-19	F27	0	0	F26			-19	F28	0	0
		-27	F26	0	0	F28			-27	3	0	6
	25.15°	5	F22	0	0	F25						
		-3	F23	6	0	F22	day 14	15.5°	5		8	0
		-11	F27	0	8	F28			-3		7	0
		-19	F25	0	0	F26			-11		7	M
		-27	F24	0	0	F28			-19	M20	0	0
									-27	M10	0	0
day 4	15.5°	5	F28	0	0	F24		25.15°	5	M6	2	0
		-3	F23	8	0	M3			-3	M8	0	1
		-11	F28	0	0	F26			-11	M12	0	6
		-19	M17	8	0	M4			-19	M12	0	0
		-27	F28	0	0	F28			-27	M8	2	0

Table 19. Raw data for experiment 1.1

Source/Clone: BEARSKIN 325

Incub. time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	Flush code II	Incub. time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II
day 0	15-5°	5	F23	0	0	F22	day 4	25-15°	5	F28	0	0
		-3	F22	1	0	F24			-3	F27	0	0
		-11	F23	0	1	F23			-11	F28	0	1
		-19	F28	0	0	F28			-19	F25	8	0
		-27	F26	0	1	F28			-27	F26	0	3
	25-15°	5	F22	0	0	F22						
		-3	F28	0	0	F24	day 9	15-5°	5	M5	8	2
		-11	F24	8	0	F24			-3	F28	0	8
		-19	F26	0	0	F28			-11	M1	0	0
		-27	F28	0	1	F28			-19	M2	1	1
									-27	M10	0	1
day 1	15-5°	5	F24	0	0	F22		25-15°	5	F28	1	0
		-3	F24	0	1	F24			-3	F20	0	0
		-11	F24	0	0	F24			-11	F28	1	0
		-19	F23	1	0	F28			-19	F27	1	8/4
		-27	F28	0	1	F27			-27	M4	0	0
	25-15°	5	F23	0	0	F28						
		-3	F24	0	0	F24	day 14	15-5°	5	M19	8	8
		-11	F28	0	1	F23			-3		8	0
		-19	F28	1	0	F28			-11	M7	8	6
		-27	F25	0	6	M11			-19	M7	0	0
									-27	M11	0	7
day 4	15-5°	5	F24	0	0	F24		25-15°	5	M7	8	1
		-3	F28	0	0	F28			-3	M8	1	1
		-11	F28	1	1	F28			-11	M8	0	4
		-19	F28	1	0	F28			-19	M8	0	0
		-27	F28	0	2	F28			-27	M12	0	8

Table 19. Raw data for experiment 1.1

Source/Clone: BEARSKIN 342

Incub. time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	Flush code II	Incub. time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II
day 0	15-5°	5	F25	0	0	F24	day 4	25-15°	5	F24	0	0
		-3	F26	0	0	F26			-3	F28	0	0
		-11	F28	0	0	F26			-11	F26	0	0
		-19	F26	2	0	F25			-19	F28	0	0
		-27	F27	0	0	F26			-27	F27	0	0
	25-15°	5	F26	0	0	F25						
		-3	F20	0	8	F26	day 9	15-5°	5	F28	0	0
		-11	F25	0	0	F28			-3	M2	0	0
		-19	F28	0	0	F26			-11	M5	0	0
		-27	F26	0	0	F27			-19	M4	0	0
									-27	M5	0	1
day 1	15-5°	5	F26	0	0	F26		25-15°	5	F28	0	0
		-3	F26	0	0	F27			-3	M2	0	0
		-11	F28	0	0	F25			-11	M3	0	0
		-19	F28	0	0	F28			-19	M2	0	1
		-27	F28	0	0	F26			-27		8	0
	25-15°	5	F24	0	0	F25						
		-3	F26	0	0	F23	day 14	15-5°	5	M12	0	0
		-11	F20	0	0	F25			-3	M10	0	0
		-19	F28	0	6	M2			-11	M10	0	0
		-27	M1	0	0	F27			-19	M8	0	1
									-27	M12	8	7
day 4	15-5°	5	F25	0	3	F26		25-15°	5	M6	0	0
		-3	F25	0	0	F26			-3	M7	0	0
		-11	F28	0	0	F27			-11	M9	0	8
		-19	F26	1	0	M1			-19	M6	0	1
		-27	F28	0	0	F28			-27	M8	0	8

Table 19. Raw data for experiment 1.1

Source/Clone: BEARSKIN 345

Incub. time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	Flush code II	Incub. time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II
day 0	15.5°	5	M8	0	0	F27	day 4	25.15°	5	F28	0	0
		-3	F27	0	4				-3	F27	6	0
		-11	M5	0	0	F28			-11		4	0
		-19	F27	0	0	F28			-19	M5	0	1
		-27	F27	0	0	F27			-27	M8	0	0
	25.15°	5	F26	2	0	F27						
		-3	F28	1	0	F28	day 9	15.5°	5	M8	0	1
		-11	F27	0	0	F27			-3	M12	0	0
		-19	F28	0	0	F28			-11	M4	2	2
		-27	M1	0	0	M4			-19	M10	6	1
									-27	M5	6	6
day 1	15.5°	5	F27	0	0	M1		25.15°	5	F27	0	0
		-3	F28	1	0	F28			-3	M8	2	0
		-11	F28	0	2	M2			-11	M3	2	2
		-19	M2	0	2	M6			-19	M6	2	7
		-27	M5	0	1	M4			-27	M8	0	8
	25.15°	5	F28	0	0	F26						
		-3	M5	0	0	M1	day 14	15.5°	5	M8	0	0
		-11	M4	0	0	M3			-3		7	7
		-19	M3	1	0	M3			-11	M20	8	0
		-27	M3	2	0	M5			-19	M12	0	1
									-27	M3	0	0
day 4	15.5°	5	F28	0	2	F25		25.15°	5	M12	0	0
		-3	M1	0	0	M9			-3	M8	2	1
		-11	M1	1	0	M5			-11	M9	2	0
		-19	M5	1	0	M5			-19	M14	6	0
		-27	M4	0	2	M8			-27	M12	0	0

Table 19. Raw data for experiment 1.1

Source/Clone: BEARSKIN 326

Incub. time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	Flush code II	Incub. time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II
day 0	15.5°	5	F26	0	0	F27	day 4	25.15°	5	F28	0	2
		-3	F28	0	1	M4			-3	F24	0	0
		-11	F28	1	2	M3			-11	F28	0	0
		-19	F25	1	0	F24			-19	F28	0	2
		-27		7	0	F27			-27	F28	1	6
	25.15°	5	F22	0	0	F28						
		-3	F28	1	0	F28	day 9	15.5°	5	M4	0	0
		-11	F25	0	0	F24			-3	M6	0	0
		-19	F28	6	0	F25			-11	M6	2	0
		-27	M10	0	0	F27			-19	M10	0	6
									-27	M6	6	2
day 1	15.5°	5	M1	0	1	F24		25.15°	5	M1	0	2
		-3	F24	6	2	F25			-3	M8	0	6
		-11	F28	1	2	F27			-11	M2	6	0
		-19	F28	2	0	F26			-19	M5	0	2
		-27	F26	0	0	F28			-27	M5	2	1
	25.15°	5	F28	0	6	F24						
		-3	F25	0	0	F24	day 14	15.5°	5	M14	6	6
		-11	F28	6	0	M2			-3	M13	6	0
		-19	M1	0	0	M2			-11	M13	6	0
		-27	M1	1	0	F28			-19	M20	0	0
									-27		0	0
day 4	15.5°	5	F27	0	0	F28		25.15°	5	M9	0	0
		-3	M3	0	0	F26			-3	M10	0	2
		-11	F28	0	1	F27			-11	M12	2	0
		-19	F25	1	0	F28			-19	M13	0	6
		-27	F27	0	2	M1			-27	F24	0	7

Table 19. New data for experiment 1.1

Source/Clone: BEANSKIN 359

Incub. time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	Flush code II	Incub. time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II
day 0	15.5°	5	M13	0	0	F24	day 4	25.15°	5	F28	6	0
		-3	F27	6	0	F28			-3	F26	0	0
		-11	F28	0	0	F28			-11	F25	0	0
		-19	F28	0	0	F26			-19	F20	0	0
		-27	F26	1	3	F28			-27	F27	2	0
	25.15°	5	F23	0	0	F24						
		-3	F28	0	0	F24	day 9	15.5°	5	M3	6	0
		-11	F24	0	0	F27			-3	M1	6	6
		-19	F25	0	1	F23			-11		7	7
		-27	F24	0	0	F26			-19	M1	0	0
									-27	M8	6	6
day 1	15.5°	5	F25	0	0	F25		25.15°	5	M4	0	0
		-3	F24	0	0	F24			-3	F20	0	0
		-11	F25	0	0	F28			-11	M1	0	1
		-19	F25	0	0	F26			-19	M3	1	6
		-27	F26	1	1	F28			-27		7	0
	25.15°	5	F28	7	0	F23						
		-3	F25	0	4		day 14	15.5°	5	M11	0	6
		-11	F24	0	2	F25			-3	M10	6	6
		-19	M14	1	6	F28			-11	M12	0	1
		-27	F28	1	0	F28			-19	M10	0	1
									-27	M8	0	1
day 4	15.5°	5	F28	7	0	F28		25.15°	5	M7	7	6
		-3	F28	6	0	F25			-3	M9	3	6
		-11	F26	6	0	F28			-11		4	0
		-19	M2	0	1	M5			-19		7	0
		-27	M2	0	0	F27			-27	M8	0	0

Table 19. New data for experiment 1.1

Source/Clone: WISCONSIN 229

Incub. time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	Flush code II	Incub. time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II
day 0	15.5°	5	F23	0	0	F22	day 4	25.15°	5	F22	0	0
		-3	F22	0	0	F23			-3	F23	1	0
		-11	F22	0	0	F22			-11	F26	0	0
		-19	F22	0	0	F23			-19	F24	1	1
		-27	F23	0	0	F24			-27	F26	0	0
	25.15°	5	F23	0	0	F22						
		-3	F22	0	0	F23	day 9	15.5°	5	F27	0	0
		-11	F22	0	0	F23			-3	F26	0	0
		-19	F22	1	0	F24			-11	F26	0	0
		-27	F23	0	0	F22			-19	F27	1	0
									-27	M1	0	0
day 1	15.5°	5	F22	0	0	F22		25.15°	5	F26	0	0
		-3	M	0	0	F22			-3	F20	0	0
		-11	F23	0	0	F24			-11	F24	1	0
		-19	F24	0	0	M1			-19	F27	6	0
		-27		8	7				-27		7	0
	25.15°	5	F23	0	0	F22						
		-3	F22	0	0	F22	day 14	15.5°	5	M8	0	6
		-11	M11	0	0	F25			-3	M5	0	0
		-19	F26	0	0	F24			-11	M6	1	1
		-27	F24	0	0	F26			-19	M6	0	0
									-27	M7	0	0
day 4	15.5°	5	F23	0	0	F23		25.15°	5	M5	0	0
		-3	F23	0	0	F24			-3	F28	0	0
		-11	F25	0	0	F24			-11	M5	0	0
		-19	F28	0	0	F24			-19	M18	6	7
		-27	F26	1	0	F26			-27	M6	2	1

Table 19. Raw data for experiment 1.1

Source/Clone: WISCONSIN 235

Incub. time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	Flush code II	Incub. time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II
day 0	15-5°	S	F26	0	0	F28	day 4	25-15°	S	F24	0	0
		-3	F26	0	0	F26			-3	F26	0	0
		-11	F26	0	0	F28			-11	M4	0	0
		-19	F27	0	0	F25			-19	F28	0	0
		-27	F26	0	0	F27			-27	M1	0	0
	25-15°	S	F26	0	0	F28						
		-3	F26	0	0	M2	day 9	15-5°	S	M4	0	0
		-11	F26	0	0	F25			-3	M4	0	0
		-19	F27	0	0	F27			-11	M3	0	6
		-27	F28	0	0	F27			-19	F28	0	0
									-27	M5	0	0
day 1	15-5°	S	F26	0	6	F24		25-15°	S	F28	0	0
		-3	F26	0	0	F26			-3	F28	0	6
		-11	F28	6	6	F28			-11	M3	0	6
		-19	F27	0	0	F27			-19	M4	6	0
		-27	F26	6	0	M1			-27	M10	0	7
	25-15°	S	F27	0	0	F26						
		-3	F27	0	0	F25	day 14	15-5°	S	M3	0	6
		-11	F24	6	0	F28			-3	M3	0	0
		-19	M2	0	6	F28			-11	M5	6	0
		-27	M1	6	0	M1			-19	M8	6	0
									-27	M12	6	1
day 4	15-5°	S	F26	0	0	F26		25-15°	S	M6	0	0
		-3	F27	0	0	M1			-3	M7	0	0
		-11	M3	0	0	F28			-11	M8	6	6
		-19	F27	0	0	M1			-19	M10	0	0
		-27	M4	0	6	M3			-27	M11	0	6

Table 19. Raw data for experiment 1.1

Source/Clone: WISCONSIN 239

Incub. time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	Flush code II	Incub. time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II
day 0	15-5°	S	F25	0	0	F24	day 4	25-15°	S	F26	0	0
		-3	F26	1	0	F28			-3	F26	0	0
		-11	F28	0	0	F28			-11	F26	0	0
		-19	F26	1	0	F26			-19	F26	0	0
		-27	F28	1	8				-27	M4	2	1
	25-15°	S	F26	0	0	F26						
		-3	F20	0	2		day 9	15-5°	S	F27	0	0
		-11	F26	0	2	F26			-3	F26	0	0
		-19	F28	0	1	F23			-11	F27	0	0
		-27	F27	0	0	F28			-19	M4	2	1
									-27	M4	1	1
day 1	15-5°	S	F26	0	0	F26		25-15°	S	M4	0	0
		-3	F26	0	0	F26			-3	F28	0	0
		-11	F26	0	0	F26			-11	F27	1	0
		-19	F26	0	0	M3			-19	F27	2	1
		-27	F20	0	0	F28			-27	M4	8	2
	25-15°	S	F24	0	0	F26						
		-3	F28	0	4		day 14	15-5°	S	M7	0	0
		-11	F28	1	0	M7			-3	M3	2	1
		-19	M1	0	1	F27			-11	M4	0	2
		-27	F28	0	0	F27			-19	F28	7	0
									-27	M8	1	6
day 4	15-5°	S	F26	0	0	F24		25-15°	S	M3	0	0
		-3	F26	0	0	F28			-3	F28	0	1
		-11	M2	8	6	M2			-11	F28	0	0
		-19	F26	0	0	F28			-19	M8	8	8
		-27	M3	0	0	F28			-27	M7	1	0

Table 19. Raw data for experiment 1.1

Source/Clone: WISCONSIN 247

Incub. time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	Flush code II	Incub. time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II
day 0	15-5°	5	F27	0	0	F20	day 4	25-15°	5	F22	0	0
		-3	F24	0	0	F24			-3	F24	0	0
		-11	F23	0	0	F24			-11	F24	0	0
		-19	F28	0	0	F25			-19	M1	0	0
		-27	F24	0	0	F24			-27	F28	0	0
	25-15°	5	F22	0	0	F22						
		-3	F23	0	0	F22	day 9	15-5°	5	F20	8	0
		-11	F22	0	0	F23			-3	F20	0	0
		-19	F24	0	0	F23			-11	M1	8	0
		-27	F23	0	0	F22			-19	M3	0	0
									-27	M1	0	0
day 1	15-5°	5	F24	0	0	F28		25-15°	5	F20	0	0
		-3	F24	0	0	F22			-3	F26	0	0
		-11	F23	0	0	F26			-11	F20	0	0
		-19	F25	0	0	F26			-19	M3	0	6
		-27	F28	0	0	F26			-27		8	1
	25-15°	5	F20	0	0	F26						
		-3	F28	0	0	F24	day 14	15-5°	5	M9	0	8
		-11	F24	0	0	F24			-3	F28	8	0
		-19	F26	0	0	F22			-11	M4	0	0
		-27	F25	0	0	F26			-19	M8	8	0
									-27		7	0
day 4	15-5°	5	F23	0	2	F23		25-15°	5	M11	0	0
		-3	F25	0	0	F25			-3	F28	8	0
		-11	F25	0	0	F26			-11	M5	8	8
		-19	F24	0	0	F25			-19	M8	4	1
		-27	F28	0	0	F26			-27	M5	0	2

Table 19. Raw data for experiment 1.1

Source/Clone: WISCONSIN 242

Incub. time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	Flush code II	Incub. time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II
day 0	15-5°	5	M1	0	0	F28	day 4	25-15°	5	F25	0	0
		-3	M4	0	0	F28			-3	M8	0	0
		-11	M1	0	0	M3			-11	M4	0	0
		-19	M4	0	0	M2			-19	M5	0	0
		-27	F28	0	0	M4			-27	M4	0	0
	25-15°	5	M2	0	0	F28						
		-3	M2	0	0	F20	day 9	15-5°	5	M2	0	0
		-11	M2	0	0	M2			-3		4	0
		-19	M4	0	0	M4			-11	M4	0	0
		-27	M5	0	0	M8			-19		7	0
									-27	M10	8	8
day 1	15-5°	5	F20	0	0	F28		25-15°	5	M2	0	0
		-3	F28	0	0	F27			-3	M2	0	0
		-11	M4	0	0	M3			-11		8	0
		-19	M5	0	0	M2			-19	M8	0	0
		-27	M8	0	0	M4			-27	M9	0	8
	25-15°	5	M3	0	0	F28						
		-3	F27	0	0	M4	day 14	15-5°	5	M10	0	0
		-11	M4	0	0	M3			-3	M8	0	0
		-19	M8	8	0	M2			-11	M12	1	0
		-27	M5	0	0	M5			-19	M6	8	0
									-27		5	0
day 4	15-5°	5	F27	0	0	F20		25-15°	5	F29	0	0
		-3	F27	0	0	M2			-3	M6	0	0
		-11	M2	0	0	M4			-11	F28	0	0
		-19	M4	0	0	M5			-19	M13	0	7
		-27	M8	0	0	M6			-27	M10	0	8

Table 19. Raw data for experiment 1.1

Source/Clone: WISCONSIN 253

Incub. time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	Flush code II	Incub. time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II
day 0	15.5°	5	F23	0	0	F23	day 4	25.15°	5	F24	0	0
		-3	F24	0	0	F26			-3	F25	0	0
		-11	F25	0	0	F26			-11	F26	0	0
		-19	F25	0	0	F26			-19	F28	0	0
		-27	F25	0	0	F25			-27	F27	0	0
	25.15°	5	F24	0	0	F23						
		-3	F24	0	0	F24	day 9	15.5°	5	F26	0	0
		-11	F26	0	0	F25			-3	F28	0	0
		-19	F25	0	0	F24			-11	M1	0	6
		-27	F27	0	0	F26			-19	F28	0	0
									-27	M3	0	0
day 1	15.5°	5	F23	0	0	F27		25.15°	5	F26	0	0
		-3	F26	0	0	F25			-3	F28	0	0
		-11	F26	0	0	F25			-11	M1	0	6
		-19	F26	0	0	F26			-19	M2	0	0
		-27	F27	0	0	F26			-27		8	7
	25.15°	5	F22	0	0	F26						
		-3		4	0	F23	day 14	15.5°	5	M2	0	0
		-11	F26	0	0	F28			-3	M3	0	0
		-19	F28	0	0	F26			-11	F28	7	0
		-27	F26	0	0	F26			-19	F28	1	7
									-27		7	0
day 4	15.5°	5	F25	0	0	F24		25.15°	5	M5	0	0
		-3	F24	0	0	F26			-3	M8	1	0
		-11	F26	0	0	F26			-11	M3	0	0
		-19	F28	0	0	F28			-19		7	8
		-27	F26	0	0	F27			-27	M11	8	1

Table 19. Raw data for experiment 1.1

Source/Clone: WISCONSIN 220

Incub. time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	Flush code II	Incub. time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II
day 0	15.5°	5	F26	0	0	F28	day 4	25.15°	5	F25	0	0
		-3	F28	0	0	F27			-3	F28	0	0
		-11	F28	0	0	F28			-11	M1	0	0
		-19	F27	0	0	M5			-19	M3	0	0
		-27	M2	0	1	M2			-27	M3	0	0
	25.15°	5	F26	0	0	F27						
		-3	F26	0	0	M2	day 9	15.5°	5	F28	0	0
		-11	M4	0	0	F28			-3	M4	0	0
		-19	M1	0	0	F26			-11	M6	0	0
		-27	M1	0	0	F27			-19	M2	0	0
									-27	M5	6	0
day 1	15.5°	5	F26	0	0	F26		25.15°	5	M4	0	0
		-3	F26	0	1	M14			-3	F26	0	0
		-11	M2	0	0	F26			-11		8	4
		-19	M4	0	0	F28			-19	M3	0	0
		-27	M1	0	0	M1			-27	M10	6	6
	25.15°	5	F27	0	0	M1						
		-3	F28	6	0	F26	day 14	15.5°	5	M4	0	0
		-11	F27	3	0	M1			-3	M5	0	6
		-19	F28	0	0	M2			-11	M4	1	0
		-27	M4	0	0	M4			-19	M7	0	0
									-27	M10	6	4
day 4	15.5°	5	F27	0	0	F26		25.15°	5	M2	0	0
		-3	F28	0	0	F26			-3	M4	0	0
		-11	F27	0	0	M2			-11	M7	0	3
		-19	M3	0	0	M4			-19		5	7
		-27	M3	1	0	M3			-27	M14	0	7

Table 19. Raw data for experiment 1.1

Source/Clone: WISCONSIN 246

Incub. time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	Flush code II	Incub. time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II
day 0	15.5°	5	F19	0	0	F20	day 4	25-15°	5	F22	0	0
		-3	F22	0	0	F23			-3	F24	0	0
		-11	F22	0	0	F22			-11	F24	0	0
		-19	F20	0	0	F22			-19	F23	0	0
		-27	M4	0	0	F22			-27	F20	0	0
	25-15°	5	F22	0	0	F20						
		-3	F25	0	0	F22	day 9	15.5°	5	F27	0	0
		-11	F22	0	0	F23			-3	M2	0	0
		-19	F23	0	0	F22			-11	F28	0	0
		-27	F24	0	0	F24			-19	F28	0	0
									-27	F20	0	0
day 1	15.5°	5	F22	0	0	F19		25-15°	5	F26	0	0
		-3	F20	0	0	F20			-3	F25	0	0
		-11	M1	0	0	F24			-11	F28	0	0
		-19	F25	0	0	M9			-19	F28	7	0
		-27	F28	0	0	F25			-27	M4	6	7
	25-15°	5	F22	0	0	F22						
		-3	F20	0	0	F20	day 14	15.5°	5	M4	0	0
		-11	F22	0	0	F23			-3	F28	0	6
		-19	F24	0	0	F23			-11	M8	8	0
		-27		M	0	F24			-19	M4	1	0
									-27		7	0
day 4	15.5°	5	F22	0	0	F22		25-15°	5	F28	0	0
		-3	F23	0	0	F22			-3	M5	0	8
		-11	F25	0	0	F24			-11	M4	0	1
		-19	F27	0	0	F28			-19	M7	0	0
		-27	F24	0	0	F25			-27	M6	1	8

Table 19. Raw data for experiment 1.1

Source/Clone: WISCONSIN 245

Incub. time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	Flush code II	Incub. time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II
day 0	15.5°	5	F24	0	0	F28	day 4	25-15°	5	F28	0	0
		-3	F25	0	0	F23			-3	F23	0	0
		-11	F24	0	0	F23			-11	F25	0	0
		-19	F26	0	0	F20			-19	F25	0	1
		-27	M1	0	0	F20			-27	F28	0	0
	25-15°	5	F22	0	0	F24						
		-3	M1	0	0	F28	day 9	15.5°	5	M1	0	0
		-11	F28	0	0	F27			-3	M3	0	0
		-19	F24	0	0	F24			-11	M3	1	1
		-27	F26	0	0	F28			-19	M2	0	0
									-27	F28	0	0
day 1	15.5°	5	F24	0	0	F24		25-15°	5	F28	0	0
		-3	F24	0	0	F22			-3	F28	0	0
		-11	F24	0	0	F25			-11	F26	0	0
		-19	F23	0	0	F20			-19	F27	0	0
		-27	F25	0	0	F28			-27		8	8
	25-15°	5	F24	0	0	F25						
		-3	F25	0	0	F22	day 14	15.5°	5	M4	0	0
		-11	F24	0	0	F28			-3	M8	1	0
		-19	F26	0	0	F26			-11	M2	0	0
		-27	F26	0	0	F28			-19	F28	5	1
									-27	F28	7	1
day 4	15.5°	5	F24	0	0	F24		25-15°	5	M3	0	0
		-3	F28	0	0	F28			-3	M3	0	0
		-11	F28	0	4				-11	M5	1	0
		-19	F28	0	0	F28			-19	F28	8	8
		-27	F28	0	0	F26			-27		8	8

Table 19. Raw data for experiment 1.1

Source/Clone: WISCONSIN 241

Incub time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	Rep. II	Flush code II	Incub. time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	Rep. II
day 0	15.5°	5	F25	0	0	F23	day 4	25.15°	5	F25	0	0
		-3	F26	0	0	F26			-3	M2	0	0
		-11	F26	0	0	F27			-11	F26	0	0
		-19	F26	0	0	F27			-19	F20	0	0
		-27	F27	0	0	F25			-27	F28	0	0
	25.15°	5	F25	0	0	F28						
		-3	F26	0	0	F26	day 9	15.5°	5	M4	0	0
		-11	F25	0	0	F24			-3	M4	0	0
		-19	F20	0	0	F28			-11	M2	0	0
		-27	F26	0	0	F26			-19	M3	0	0
									-27	M3	0	0
day 1	15.5°	5	F26	0	0	F25		25.15°	5	F26	0	0
		-3	F26	0	0	F25			-3	M3	0	0
		-11	M1	0	0	F26			-11	M4	6	0
		-19	F26	0	0	F27			-19	M2	6	0
		-27	F26	0	0	F28			-27	M6	0	6
	25.15°	5	F26	0	0	F23						
		-3	F26	0	0	F26	day 14	15.5°	5	M9	0	0
		-11	F26	0	0	F26			-3	M1	0	0
		-19	F26	0	0	F25			-11	M10	0	0
		-27	F26	0	0	F27			-19	M9	6	5
									-27	M11	0	0
day 4	15.5°	5	F25	0	0	F24		25.15°	5	M3	0	6
		-3	F24	0	0	F26			-3	M2	6	0
		-11	F26	0	0	F25			-11	M8	0	0
		-19	M1	0	0	F26			-19	M4	4	0
		-27	F26	0	0	M2			-27	M11	0	1

Table 19. Raw data for experiment 1.1

Source/Clone: WISCONSIN 233

Incub. time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	Rep. II	Flush code II	Incub. time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	Rep. II
day 0	15.5°	5	F26	0	0	F23	day 4	25.15°	5	F26	0	0
		-3	F26	0	0	F26			-3	F26	0	0
		-11	F24	0	0	F26			-11	F26	0	0
		-19	F20	0	1	M4			-19	F26	0	0
		-27	F27	0	0	F28			-27	M3	0	0
	25.15°	5	F20	0	0	F24						
		-3	F24	0	0	F26	day 9	15.5°	5	F28	0	0
		-11	F26	0	0	F26			-3	F27	0	0
		-19	F26	0	0	F26			-11	M4	0	0
		-27	F26	0	0	F28			-19	M3	0	1
									-27	M4	0	0
day 1	15.5°	5	F28	0	0	F26		25.15°	5	F28	6	6
		-3	F28	0	0	F23			-3	F26	0	0
		-11	F24	0	0	F27			-11	F26	1	0
		-19	F28	0	0	F26			-19	F28	0	0
		-27	F27	0	6/4				-27	M3	0	6
	25.15°	5	F28	0	0	F26						
		-3	F26	0	0	F26	day 14	15.5°	5		7	0
		-11	F27	0	6	F26			-3	M4	0	6
		-19	F24	0	0	F26			-11	M6	0	0
		-27	F27	0	0	F26			-19	M7	0	0
									-27	M12	6	0
day 4	15.5°	5	F28	0	0	F28		25.15°	5	M6	0	2
		-3	F27	0	0	F26			-3	M6	0	0
		-11	F27	0	0	F28			-11	M6	0	0
		-19	F28	0	0	F27			-19	M10	0	0
		-27	F28	0	0	M1			-27	M12	0	0

Table 20. Raw data for experiment 1.2

Source/Clone: YISCONSIN 228

Incub. time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	Flush code II	Incub. time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	Flush code II
day 0	15-5°	5	M31	0	0	M29	day 5	25-15°	5	A2	0	0	1
		-3	A1	0	0	M30			-3	1	0	0	A3
		-11	A2	0	0	A2			-11		5	0	2
		-19	A3	0	0	M31			-19	0	5	7	1
		-27	A1	0	0	M29			-27	A11	2	0	A5
	25-15°	5	M31	0	0	M29							
		-3	M31	0	0	A2	day 8	15-5°	5	0	0	0	2
		-11	M29	0	0	M31			-3	1	0	0	1
		-19	A4	0	0	M30			-11	0	0	0	1
		-27	A6	0	0	M29			-19	1	5	5	0
									-27	1	7	0	1
day 2	15-5°	5	M31	0	0	A3		25-15°	5	3	0	0	A3
		-3	A2	0	0	A2			-3	0	0	0	2
		-11	A1	0	0	A1			-11	3	7	5	3
		-19	A3	0	4	A4			-19	1	8	5	0
		-27	A5	0	0	A7			-27	3	0	8	2
	25-15°	5	A2	0	0	M31							
		-3	A4	0	0	A1	day 11	15-5°	5	3	0	0	4
		-11	N/A	0	0	A3			-3	3	0	0.	4
		-19	A6	0	0	A4			-11		4	0	A6
		-27	A6	0	0	A5			-19	0	4	7	1
									-27	0	8	8	1
day 5	15-5°	5	A1	0	0	M31		25-15°	5	5R	0	0	4R
		-3	A3	0	1	A8			-3	5R	0	6/2	4R
		-11	A5	0	0	1			-11	3R	8	8	3R
		-19	A4	0	4	2			-19	2R	0	8	3R
		-27	A6	0	1	A7			-27	5R	8	8	4R

Table 20. Raw data for experiment 1.2

Source/Clone: YISCONSIN 239

Incub. time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	Flush code II	Incub. time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	Flush code II
day 0	15-5°	5	M29	0	0	M30	day 5	25-15°	5	3	0	0	3
		-3	M29	0	0	M30			-3	2	6/0	0	3
		-11	M28	0	0	M29			-11	3	0	5	3
		-19	M30	0	0	M29			-19	2	5	5	2
		-27	M29	0	0	A2			-27	4	7	7	2
	25-15°	5	M28	0	0	M28							
		-3	A3	0	6/0	M30	day 8	15-5°	5	4	0	0	4
		-11		5	0	M31			-3	3	0	0	3
		-19	M28	0	0	M29			-11	2	4	0	2
		-27	M29	0	7				-19	1	7	5	1
									-27	5	8	7	4
day 2	15-5°	5	M31	6/0	0	M30		25-15°	5	5	0	0	5
		-3	A2	0	0	A2			-3	4	0	0	0
		-11	M30	0	0	M31			-11	5	7	8	5
		-19	M28	0	0	M31			-19	3	8	5	4
		-27	A2	0	0	A2			-27	4	8	8	4
	25-15°	5	M29	0	0	M30							
		-3	M29	0	0	M30	day 11	15-5°	5	5	0	0	5
		-11	M30	0	0	M30			-3	4R	0	0	5R
		-19	A4	0	0	A2			-11	4	7	1	3
		-27	A3	4	4	0			-19	3	7	5	3
									-27	3	8	5	3
day 5	15-5°	5	M31	0	0	M31		25-15°	5	6R	0	0	6R
		-3	A4	0	0	A1			-3	7R	0	1	6R
		-11	A13	0	4	1			-11	5R	7	8	7R
		-19	0	7	7	1			-19	5R	8	8	4R
		-27	1	6/4/00	7	1			-27	6R	8	8	6R

Table 20. Raw data for experiment 1.2

Source/Clone: YISCOFISN 253

heat. time	heat. temp.	freez. temp	flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	flush code II	heat. time	heat. temp.	freez. temp	flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	flush code II
day 0	15-5°	5	M30	0	0	M29	day 5	25-15°	5	1	0	0	2
		-3	M31	0	0	M30			-3	A2	0	0	A2
		-11	A1	0	0	M30			-11	N/A	N/A	6\1	A3
		-19	A4	0	0	A2			-19	A7	1	3	
		-27	A1	0	0	M31			-27	1	8	8	0
	25-15°	5	M31	0	0	M31							
		-3	M31	0	0	M30	day 8	15-5°	5	1	0	0	2
		-11	N/A	6/0	0	A4			-3	1	0	6\4	1
		-19	M30	0	0	A4			-11	0	1	1	0
		-27	A4	0	0	A2			-19	0	4	6\4	0
									-27	1	8	0	1
day 2	15-5°	5	A2	0	0	A2		25-15°	5	A3	0	0	2
		-3	A3	0	0	A3			-3	4	0	0	3
		-11	A5	0	0	A5			-11	4	7	0	4
		-19	A6	1	6/0	A10			-19	0	7	3	0
		-27	A4	0	0	A4			-27	3	8	8	4
	25-15°	5	A1	0	0	A2							
		-3	A2	0	0	A2	day 11	15-5°	5	4	0	0	3
		-11	A3	0	0	A4			-3	A9	0	6/0	3
		-19	A7	0	0	A3			-11	1	0	8	0
		-27	A4	0	0	A3			-19	0	7	8	0
									-27	0	8	8	0
day 5	15-5°	5	A3	0	0	M31		25-15°	5	SR	0	0	SR
		-3	A5	0	0	A2			-3	5	6/0	6/0	SR
		-11	A5	1	0	A4			-11	SR	8	8	SR
		-19		7	6\4	0			-19	4R	8	8	4R
		-27	A9	1	2	A8			-27	SR	8	8	SR

Table 20. Raw data for experiment 1.2

Source/Clone: YISCOFISN 204

heat. time	heat. temp.	freez. temp	flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	flush code II	heat. time	heat. temp.	freez. temp	flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	flush code II
day 0	15-5°	5	M31	0	0	M31	day 5	25-15°	5	0	0	0	M30
		-3	M31	0	0	A1			-3	A2	0	0	A3
		-11	M30	0	0	M31			-11	A14	6/0	6/0	1
		-19	A1	0	0	A1			-19	N/A	N/A	7	0
		-27	A3	0	0	M31			-27	0	7	3	A13
	25-15°	5	A1	0	0	M28							
		-3	M31	0	0	M31	day 8	15-5°	5	0	0	0	0
		-11	M31	0	0	M30			-3	0	0	2	0
		-19	A6	0	0	M30			-11	A6	0	3	A10
		-27	A2	0	3	M31			-19	0	7	6\4	0
									-27	0	8	8	1
day 2	15-5°	5	A5	0	0	A2		25-15°	5	4	0	0	4
		-3	A5	0	0	A2			-3	4	6/0	6/0	4
		-11	A4	0	0	A5			-11	4	5	N/A	N/A
		-19	A7	0	6/0	A4			-19	1	7	7	
		-27	A7	1	0	A6			-27	4	8	8	3
	25-15°	5	A3	0	0	A2							
		-3	A4	0	0	A3	day 11	15-5°	5	2	0	0	3
		-11	A2	0	0	A5			-3	1	6/0	0	0
		-19	N/A	6/0	6/0	A13			-11	1	6\4	6\4	0
		-27	A4	0	0	A6			-19	0	7	7	1
									-27	0	8	8	0
day 5	15-5°	5	A4	0	0	A3		25-15°	5	SR	0	0	SR
		-3	A4	0	0	A2			-3	1	6/0	1	SR
		-11	A2	6	0	A5			-11	4R	8	8	4R
		-19	1	5	7	0			-19	4R	8	7	1
		-27	A7	0	7	0			-27	SR	8	8	SR

Table 20. Raw data for experiment 1.2

Source/Clone: YISCONSIN 202

heat. time	heat. temp.	freez. temp	flush code I	DAMAGE SCORE Rep. I	Rep. II	flush code II	heat. time	heat. temp.	freez. temp	flush code I	DAMAGE SCORE Rep. I	Rep. II	flush code II
day 0	15-5°	5	M30	0	0	A1	day 5	25-15°	5	1	0	0	1
		-3	M31	0	0	M30			-3	A1	8	5	M31
		-11	M31	0	7				-11	A2	0	6/0	1
		-19	M30	0	0	A3			-19	A6	6\4	6\2	A5
		-27	A2	0	0	A2			-27	A5	0	7	1
	25-15°	5	M31	0	0	A4							
		-3	M31	3	0	A10	day 8	15-5°	5	A2	0	0	0
		-11	M29	7	0	A2			-3	1	0	0	0
		-19	M31	0	0	M31			-11	A5	0	0	0
		-27	A2	0	0	A1			-19	A6	0	1	0
									-27	1	7	7	0
day 2	15-5°	5	A2	0	6/0	A4		25-15°	5	1	0	0	A3
		-3	A4	0	0	A3			-3	5	6/0	6/0	1
		-11	A5	0	4	A4			-11	4	4	1	1
		-19	A3	0	0	A2			-19	3	7	1	A12
		-27	A6	0	2	A6			-27	8	8	8	3
	25-15°	5	A2	0	0	A2							
		-3	A3	0	0	A4	day 11	15-5°	5	3	0	0	4
		-11	A4	0	0	A2			-3	3	0	0	4
		-19	A4	0	0	A5			-11	0	0	1	0
		-27	A3	0	0	A3			-19	0	6\4	7	0
									-27	0	7	5	0
day 5	15-5°	5	M30	0	1	A7		25-15°	5	5R	0	0	5R
		-3	A3	0	0	A2			-5	3R	8	0	4R
		-11	A3	0	0	A3			-11	3R	7	8	4R
		-19	A4	0	0	A4			-19	4R	7	8	4R
		-27	A6	0	1	A5			-27	0	8	8	1

Table 20. Raw data for experiment 1.2

Source/Clone: YISCONSIN 233

heat. time	heat. temp.	freez. temp	flush code I	DAMAGE SCORE Rep. I	Rep. II	flush code II	heat. time	heat. temp.	freez. temp	flush code I	DAMAGE SCORE Rep. I	Rep. II	flush code II
day 0	15-5°	5	A1	0	0	A1	day 5	25-15°	5	A4	0	0	M31
		-3	M31	0	0	A1			-3	A4	0	6/0	A7
		-11	M31	0	0	A2			-11	A2	0	0	1
		-19	A1	0	0	M31			-19	A3	6/0	1	A4
		-27	A1	0	0	A2			-27		5	8	1
	25-15°	5	M30	6/0	6/0	M31							
		-3		0	0	M31	day 8	15-5°	5	A3	0	6/0	1
		-11	A3	0	0	A1			-3	3	6/0	0	A4
		-19	M30	0	0	A2			-11	0	6/0	1	0
		-27	M31	6/0	6/0	M31			-19	A6	6\1	7	0
									-27	1	6/0	6/4	1
day 2	15-5°	5	M31	0	0	A2		25-15°	5	A4	0	0	3
		-3	A4	6/0	0	A5			-3	A4	6/0	6/0	3
		-11	A2	0	0	A3			-11	3	7	5	3
		-19	A2	0	0	A3			-19	2	5	7	4
		-27	A4	0	0	A5			-27	0	8	8	3
	25-15°	5	A2	0	0	M31							
		-3	A1	0	0	A2	day 11	15-5°	5	6	0	6/0	2
		-11	A4	0	0	A2			-3	4	6/0	6\1	4
		-19	A4	0	0	A5			-11	1R	0	1	A4
		-27	A4	0	0	A6			-19	0	5	7	0
									-27	0	8	7	1
day 3	15-5°	5	A2	0	0	A4		25-15°	5	5R	0	0	5R
		-3	A3	0	0	A4			-5	4R	0	0	6R
		-11	A2	0	6\2	1			-11	1	6	0	0
		-19	A7	6/0	6/0	A6			-19	0	5	7	0
		-27	A5	1	4	0			-27	5R	8	6/0	0R

Table 20. Raw data for experiment 1.2

Source/Clone: WISCONSIN 246

Incub. time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	Flush code II	Incub. time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	Flush code II
day 0	15-5°	5	M30	0	0	M31	day5	25-15°	5	2	0.	6/0	1
		-3	M28	0	0	M20			-3	M31	0	6/0	0
		-11	M27	0	0	M27			-11	1	6/0	7	0
		-19	M28	0	0	A1			-19	0	7	7	0
		-27	M30	0	0	A2			-27	A4	1	7	2
	25-15°	5	M27	0	0	M29							
		-3	M28	0	0	A1	day 8	15-5°	5	2	0	0	4
		-11	M29	0	0	M31			-3	3	0	0	3
		-19	M29	0	0	M28			-11	2	5	0	0
		-27	M30	0	0	A6			-19	0	2	2	1
									-27	0	7	0	4
day 2	15-5°	5	M30	0	0	M31		25-15°	5	5	0	0	4
		-3	M31	6/0	0	A5			-3	3	4	7	2Ω
		-11	A2	0	0	A5			-11	3	7	7	4
		-19	A5	0	6/0	A1			-19	4	8	7	2
		-27	A3	0	0	A5			-27	4	7	8	5
	25-15°	5	M31	0	0	M31							
		-3	A1	0	0	A1	day 11	15-5°	5	3	0	0	A4
		-11	A1	0	0	M31			-3	4	0	6/0	3
		-19	A4	0	0	A6			-11	1	6	5	2R
		-27	A2	1	0	A4			-19	0	7	0	0
									-27	0	8	7	0
day 5	15-5°	5	1	0	0	1		25-15°	5	5R	0	0	6R
		-3	A2	0	8	M31			-3	5R	3	1	4
		-11	1	0	0	1			-11	4	0	7	4R
		-19	A2	6/0	6/0	A7			-19	4	0	8	3R
		-27	A6	0	7	N/A			-27	5R	0	8	1

Table 20. Raw data for experiment 1.2

Source/Clone: WISCONSIN 247

Incub. time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	Flush code II	Incub. time	Incub. temp.	Freez. temp	Flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	Flush code II
day 0	15-5°	5	M28	0	0	M27	day5	25-15°	5	A5	0	8	1
		-3	M20	0	0	M31			-3	1	6/0	0	A3
		-11	M28	0	0	M27			-11	1	6/0	6/0	2
		-19	M31	0	0	M31			-19	0	6/0	0	A3
		-27	M29	0	0	M29			-27	1	8	8	3
	25-15°	5	M31	0	0	M27							
		-3	M29	0	0	M29	day 8	15-5°	5	2	0	0	2
		-11	M28	0	0	M29			-3	1	0	0	2
		-19	M27	0	1	0			-11	A2	0	0	A4
		-27	M31	0	0	M31			-19	A4	6/0	7	0
									-27	1	8	8	1
day 2	15-5°	5	M31	0	0	M31		25-15°	5	3	6/0	0	3
		-3	M31	0	0	M31			-3	4	0	6/0	3
		-11	A6	0	0	A2			-11	A1	0	7	3
		-19	A3	0	0	A3			-19	3	7	8	3
		-27	A1	0	0	A2			-27	4	8	8	3
	25-15°	5	M31	0	0	M31							
		-3	M31	0	0	M31	day 11	15-5°	5	0	0	0	4
		-11	A2	0	0	A3			-3	4	0	0	4
		-19	A4	0	0	A3			-11	3	5	1	0
		-27	0	7	6/0	M31			-19	2	7	7	1
									-27	2	8	8	2
day 5	15-5°	5	A2	0	0	A11		25-15°	5	5R	6/0	0	5R
		-3	A3	0	0	A3			-3	4R	0	6/0	4
		-11	2	0	0	A3			-11	4R	8	8	4R
		-19	A3	6\3	7	1			-19	3R	8	7	4R
		-27	0	6\4	6/0	A2			-27	4R	8	8	4

Table 20. Raw data for experiment 1.2

Source/Clone: YISCONSIN 229

heat. time	heat. temp.	freez. temp	flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	flush code II	heat. time	heat. temp.	freez. temp	flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	flush code II
day 0	15-5°	5	M29	0	0	M30	day 5	25-15°	5	2	0	0	2
		-3	M28	0	0	M28			-3	A2	1	0	1
		-11	M29	0	0	A1			-11	2	4	7	3
		-19	M29	0	0	M31			-19	0	7	5	0
		-27	M29	0	6/0	A2			-27	2	0	6/0	A2
	25-15°	5	M28	0	0	M31							
		-3	M28	0	0	M29	day 8	15-5°	5	5	0	0	3
		-11	M29	0	6/0	M28			-3	1	0	0	3
		-19	M29	0	0	M30			-11	0	0	0	0
		-27	M29	0	0	M29			-19	0	7	7	0
									-27	N/A	N/A	5	3
day 2	15-5°	5	M31	0	0	N/A		25-15°	5	3	0	0	5
		-3	M31	0	0	M31			-8	2	6/0	6	5
		-11	A4	0	0	A1			-11	4	7	7	4
		-19	A4	0	0	A2			-19	3	7	7	4
		-27	A2	0	0	A3			-27	4	0	0	5
	25-15°	5	M30	0	0	M30							
		-8	M31	0	0	M31	day 11	15-5°	5	4R	0	0	4R
		-11	M31	0	0	A5			-3	4R	0	2	4R
		-19	A7	0	0	A4			-11	3R	7	6\4	4R
		-27	N/A	4	6/0	A1			-19	1	5	7	0
									-27	1	7	7	1
day 5	15-5°	5	M31	0	0	M31		25-15°	5	5R	0	0	6
		-3	M31	0	0	A1			-3	6R	0	0	7R
		-11	0	6\4	6\4	0			-11	5R	8	8	5R
		-19	0	7	6\4	1			-19	4R	8	7	4R
		-27	A1	0	0	A2			-27	6R	8	8	6R

Table 20. Raw data for experiment 1.2

Source/Clone: YISCONSIN 238

heat. time	heat. temp.	freez. temp	flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	flush code II	heat. time	heat. temp.	freez. temp	flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	flush code II
day 0	15-5°	5	M31	0	0	M29	day 5	25-15°	5	2	0	0	2
		-3	M31	0	0	M29			-8	A2	0	6/0	0
		-11	M29	0	0	M30			-11	A4	6/0	2	2
		-19	M30	0	0	M28			-19	1	6\4	7	1
		-27	M29	0	0	M28			-27	1	8	8	2
	25-15°	5	M29	0	0	M30							
		-3	M30	0	0	M29	day 8	15-5°	5	2	6/0	0	3
		-11	M28	0	0	M30			-3	2	0	0	3
		-19	A14	0	0	M29			-11	1	0	0	1
		-27	A4	0	0	M29			-19	0	7	3	0
									-27	3	7	7	2
day 2	15-5°	5	M30	0	0	M31		25-15°	5	5	0	0	5
		-3	A4	6/0	0	A1			-8	4	6/0	7	4
		-11	A2	1	0	A3			-11	4	7	7	4
		-19	A2	0	6/0	A2			-19	3	8	8	3
		-27	A3	0	6/0	A2			-27	4	8	8	3
	25-15°	5	M31	0	0	M31							
		-3	M30	0	0	M31	day 11	15-5°	5	3	0	6/0	5
		-11	A2	0	6/0	A1			-3	4	5	0	3
		-19	A5	6/0	0	A2			-11	4	1	0	3
		-27	M31	6/0	0	A2			-19	1	7	8	1
									-27	2	8	8	2
day 5	15-5°	5	1	0	0	M31		25-15°	5	5R	0	0	5R
		-3	A1	0	0	A1			-3	6R	0	0	5
		-11	1	6/0	6/0	1			-11	4R	8	8	5R
		-19	A10	3	4	A2			-19	5R	8	8	5
		-27	A13	6\4	0	A5			-27	3	8	8	5

Table 20. Raw data for experiment 1.2

Source/Clone: WISCONSIN 227

heat. time	heat. temp.	freez. temp	flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	flush code II	heat. time	heat. temp.	freez. temp	flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	flush code II
day 0	15-5°	5	M30	0	0	A1	day 5	25-15°	5	1	0	6/0	A3
		-3	M30	0	0	M31			-8	1	4	6/0	M31
		-11	M29	0	0	M31			-11	A6	0	0	A3
		-19	M30	0	6/0	M29			-19	A3	0	7	0
		-27	A2	0	0	A3			-27	A10	0	7	0
	25-15°	5	M29	0	0	A4							
		-3	M31	0	0	M30	day 8	15-5°	5	A2	0	0	2
		-11	M29	0	0	M30			-5	1	0	6/0	0
		-19	M31	0	0	M29			-11	0	0	0	0
		-27	M31	0	0	A2			-19	0	1	1	0
									-27	0	8.	8	1
day 2	15-5°	5	A2	0	0	A2		25-15°	5	4	6/0	6/0	3
		-3	A2	0	6/0	A2			-3	4	6/0	7	3
		-11	A3	0	0	A4			-11	4	7	8	4
		-19	A4	0	6/0	N/A			-19	1	8	8	1
		-27	A6	1	0	A4			-27	2	8	8	4
	25-15°	5	A2	0	0	A2							
		-3	0	0	0	A2	day 11	15-5°	5	3	0	0	3
		-11	A4	0	0	A4			-3	3	0	0	3
		-19	A4	0	6/0	A5			-11	0	6/0	2	0
		-27	A3	0	4	0			-19	0	7	6\4	0
									-27	1	8	8	0
day 5	15-5°	5	M31	0	0	A3		25-15°	5	3	0	0	SR
		-3	A1	6/0	0	A3			-5	4	0	0	SR
		-11	A2	0	0	A2			-11	SR	8	8	SR
		-19	1	7	4	0			-19	1	8	8	2
		-27	A7	1	0	A3			-27	0	8	8	0

Table 20. Raw data for experiment 1.2

Source/Clone: WISCONSIN 220

heat. time	heat. temp.	freez. temp	flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	flush code II	heat. time	heat. temp.	freez. temp	flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	flush code II
day 0	15-5°	5	A2	0	0	A2	day 5	25-15°	5	A2	0	0	A2
		-3	A1	0	0	M31			-8	A6	6/0	0	A3
		-11	A2	0	0	M31			-11	A4	0	0	A5
		-19	A5	0	0	A1			-19	A6	6/0	6\1	A7
		-27	A2	0	0	A2			-27	A7	6\3	5	0
	25-15°	5	M30	0	0	M31							
		-3	A2	0	0	A1	day 8	15-5°	5	A4	0	0	A3
		-11	M31	0	0	M31			-3	0	0	0	0
		-19	A9	0	0	A2			-11	A3	0	1	0
		-27	A2	0	0	A3			-19	0	7	0	A6
									-27	0	7	5	0
day 2	15-5°	5	A2	0	0	A6		25-15°	5	3	0	0	2
		-3	A4	0	0	A1			-3	3	6/0	N/A	N/A
		-11	A2	6/0	0	A3			-11	3	7	7	0
		-19	A5	0	0	A5			-19	0	8	8	0
		-27	A3	0	0	A5			-27	4	8	8	0
	25-15°	5	A2	0	0	A2							
		-8	A4	0	0	A2	day 11	15-5°	5	4	6/0	0	2
		-11	A4	6/0	0	A4			-3	0	1	0	0
		-19	A7	0	0	A6			-11	0	2	0	0
		-27	A4	0	0	A4			-19	0	7	7	0
									-27	0	5	8	0
day 3	15-5°	5	A4	6/0	0	A4		25-15°	5	4R	0	0	4R
		-3	A4	0	0	A4			-3	3	0	0	4
		-11	A5	0	0	A3			-11	4	7	7	4R
		-19	0	4	0	A7			-19	0	8	8	1
		-27	A3	6/0	6/0	A3			-27	0	7	8	4R

Table 20. Raw data for experiment 1.2

Source/Clone: BEARSKIN L. 343

heat. time	heat. temp.	freez. temp	flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	flush code II	heat. time	heat. temp.	freez. temp	flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	flush code II
day 0	15-5°	5	A5	0	0	A4	day 5	25-15°	5	A4	0	0	A7
		-3	A3	0	0	A5			-3	A5	6\2	0	A7
		-11	A2	0	1	A4			-11	A10	6/0	0	A7
		-19	A3	0	4				-19	A10	1	6/0	A9
		-27	A13	0	1	A5			-27	A8	0	0	A6
	25-15°	5	A2	0	0	A4							
		-3		7	0	A2	day 8	15-5°	5	0	1	0	A5
		-11	A3	0	4				-3	A6	0	0	0
		-19	A6	1	0	A5			-11	0	2	1	0
		-27	A7	0	0	A4			-19	A8	6/0	2	A6
									-27	0	6\3	6\3	0
day 2	15-5°	5	A10	0	0	A5		25-15°	5	0	0	2	0
		-3	A9	0	3	A13			-3	A6	0	0	A4
		-11	A7	0	7	A6			-11	0	7	5	0
		-19	A8	0	0	A6			-19	0	8	7	0
		-27	A9	6/0	8				-27	0	8	2	0
	25-15°	5	A9	0	4	A3							
		-3	A9	0	0	A4	day 11	15-5°	5	0	0	0	A7
		-11	A6	0	0	A4			-3	0	0	0	A3
		-19	A14	0	6/0	A8			-11		0	2	0
		-27	A10	0	1	A9			-19	0	6\3	1	0
									-27	0	8	8	0
day 5	15-5°	5	A5	0	0	A7		25-15°	5	1	6/0	0	0
		-3	A3	0	6/0	A4			-3	0	6/0	4	0
		-11	A6	2	6/0	A6			-11		0	2	0
		-19	A8	6\3	0	A5			-19	0	7	8	0
		-27	A6	2	6/0	A13			-27	1	7	8	0

Table 20. Raw data for experiment 1.2

Source/Clone: BEARSKIN L. 320

heat. time	heat. temp.	freez. temp	flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	flush code II	heat. time	heat. temp.	freez. temp	flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	flush code II
day 0	15-5°	5	A2	2	0	A4	day 5	25-15°	5	A8	0	0	A7
		-3	M31	0	0	A1			-3	A1	6/0	6/0	A6
		-11	M31	0	0	A1			-11	A2	3	1	A5
		-19	A2	6/0	6/0	M31			-19	0	4	4	A6
		-27	A2	0	0	A5			-27		7	5	0
	25-15°	5	A1	0	0	A1							
		-3	A9	0	0	M31	day 8	15-5°	5	M29	0	2	0
		-11	A2	0	0	M31			-3	0	0	7	0
		-19	A6	1	1	A1			-11	0	2	2	0
		-27	A5	2	2	A4			-19	0	6\1	6/0	0
									-27	0	8	3	A2
day 2	15-5°	5	A6	0	6/0	A5		25-15°	5	1	2	1	0
		-3	A7	1	4				-3	2	6/0	0	0
		-11	A4	1	6/0	A4			-11	3	7	5	3
		-19	A8	0	1	A8			-19	0	5	5	0
		-27		7	2	A5			-27	1	8	7	0
	25-15°	5	A4	3	0	A2							
		-3	A3	0	0	A2	day 11	15-5°	5	0	0	0	ER
		-11	A3	0	0	A4			-3	0	1	2	1
		-19	A8	8	0	A9			-11	0	4	1	0
		-27	A4	6/0	1	A9			-19	0	7	6\4	0
									-27	0	7	7	0
day 5	15-5°	5	A5	0	0	A6		25-15°	5	ER	0	1	2
		-3	A3	0	0	A5			-3	ER	0	6\1	4
		-11	A4	2	0	A4			-11	1	6\4	7	3
		-19	A5	0	2	A5			-19	5	7	7	0
		-27	A1	3	1	A4			-27	2R	8	8	4R

Table 20. Raw data for experiment 1.2

Source/Clone: BEARSKIN L. 342

hrdn. time	hrdn. temp.	freez. temp	flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	flush code II	hrdn. time	hrdn. temp.	freez. temp	flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	flush code II
day 0	15-5°	5	A1	0	0	A2	day 5	25-15°	5	A1	0	0	A3
		-3	M31	0	5				-3	A8	2	0	A2
		-11	M31	0	0	A2			-11	A4	6\2	0	A6
		-19	M31	6/0	6/0	A6			-19	A6	1	0	A14
		-27	A2	0	1	A2			-27		7	1	A8
	25-15°	5	M31	0	0	A4							
		-3	A7	6/0	0	A12	day 8	15-5°	5	A5	0	0	3
		-11	M31	0	2	A2			-3	0	2	0	0
		-19	M31	0	0	A2			-11	A3	6/0	0	A9
		-27	A2	0	0	A6			-19	0	0	2	0
									-27	0	1	1	A9
day 2	15-5°	5	A3	0	0	A2		25-15°	5	2	0	0	M31
		-3	A4	0	0	A3			-3	2	1	2	2
		-11	A9	0	0	A3			-11	2	5	5	2
		-19	A2	0	0	A3			-19	0	5	7	0
		-27	A4	0	0	A3			-27	0	7	0	A8
	25-15°	5	A2	0	0	A12							
		-3	A3	0	0	A3	day 11	15-5°	5	0	6/0	0	A6
		-11	A5	0	0	A4			-3	0	0	0	A7
		-19	A6	1	0	A4			-11	A6	0	0	A6
		-27	A6	1	0	A3			-19	0	0	0	0
									-27	A11	0	7	0
day 5	15-5°	5	A4	0	0	A3		25-15°	5	3R	0	0	3R
		-3	A4	0	0	A3			-3	0	0	6/0	1
		-11	A8	0	0	A4			-11	4	5	4	4
		-19	A3	2	2	A4			-19	0	7	7	0
		-27	A6	0	0	A6			-27	2	8	8	0

Table 20. Raw data for experiment 1.2

Source/Clone: BEARSKIN L. 302

hrdn. time	hrdn. temp.	freez. temp	flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	flush code II	hrdn. time	hrdn. temp.	freez. temp	flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	flush code II
day 0	15-5°	5	A2	0	0	A8	day 5	25-15°	5	M31	0	0	M31
		-3	A12	0	0	M30			-3	A2	6/0	0	A3
		-11	M28	0	0	M27			-11	0	0	0	A3
		-19	M30	0	0	A8			-19	A2	0	4	A3
		-27		4	6\4				-27	1	8	5	1
	25-15°	5		7	0	M28							
		-3	M31	0	0	M31	day 8	15-5°	5	A1	0	0	2
		-11	M30	0	0	M29			-3	1	1	2	1
		-19	M28	0	0	M31			-11	0	0	8	0
		-27		7	0	N/A			-19	1	4	8	0
									-27	2	8	8	2
day 2	15-5°	5	A1	0	0	A2		25-15°	5	1	0	0	1
		-3	A1	0	1	M31			-3	3	6/0	0	0
		-11	A2	1	0	A2			-11	4	8	5	4
		-19	M31	0	0	M29			-19	2	7	8	4
		-27	A2	0	2	A2			-27	3	8	8	3
	25-15°	5	A4	0	0	M30							
		-3	A1	0	0	M31	day 11	15-5°	5	0	0	0	3
		-11	A1	0	0	A1			-3	3	0	0	4R
		-19	A2	0	0	A5			-11	1	1	6\1	1
		-27	A1	0	2	A1			-19	0	8	7	1
									-27	0	7	8	1
day 5	15-5°	5	A2	0	0	A6		25-15°	5	2R	0	0	4R
		-3		7	0	A4			-3	4R	1	1	4R
		-11	A7	0	0	A7			-11	4R	8	8	4
		-19	0	4	4				-19	4R	8	8	4R
		-27	A2	2	0	A5			-27	5R	8	8	3

Table 20. Raw data for experiment 1.2

Source/Clone: BEARSKIN L. 316

heat. time	heat. temp.	freez. temp	flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	flush code II	heat. time	heat. temp.	freez. temp	flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	flush code II
day 0	15-5°	5	A2	0	7		day 5	25-15°	5	A2	6/0	0	A4
		-3	A4	0	0	M30			-3	A4	0	0	A4
		-11	M31	0	0	A5			-11	A7	0	0	A6
		-19	M29	6/0	0	A8			-19	0	7	1	A7
		-27	A2	6/0	6/0	A1			-27		7	3	
	25-15°	5	N/A	0	6/0	M31							
		-3	A1	0	0	M29	day 8	15-5°	5	A3	0	0	0
		-11	A14	0	0	A4			-3	0	0	1	0
		-19	A2	0	0	A2			-11	0	1	0	0
		-27	A2	0	0	A4			-19	A7	1	1	0
									-27	0	7	7	0
day 2	15-5°	5	A4	0	0	N/A		25-15°	5	2	0	6/0	3
		-3	A3	0	0	A2			-3	1	1	3	0
		-11	A4	0	6/0	A4			-11	1	7	7	0
		-19	A5	0	0	A8			-19	0	7	7	0
		-27	A6	0	0	A4			-27	1	8	8	0
	25-15°	5	A1	0	0	A3							
		-3	A3	0	0	A4	day 11	15-5°	5	0	0	0	0
		-11	A5	0	0	A4			-3	0	1	0	0
		-19	A4	0	0	A5			-11	0	1	6\1	0
		-27	A3	6/0	0	A4			-19	0	6/0	6\3	0
									-27	0	7	7	0
day 5	15-5°	5	A2	0	0	A6		25-15°	5	2	0	0	4
		-3	A4	0	0	A4			-3	3	0	1	4R
		-11	A2	6/0	2	A3			-11	4	2	1	A9
		-19	A3	1	0	A3			-19	8	8	8	1
		-27	M31	0	0	A4			-27	4R	8	0	4

Table 20. Raw data for experiment 1.2

Source/Clone: BEARSKIN L. 317

heat. time	heat. temp.	freez. temp	flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	flush code II	heat. time	heat. temp.	freez. temp	flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	flush code II
day 0	15-5°	5	A2	0	0	A2	day 5	25-15°	5	A5	0	0	A5
		-3	A5	0	8	A5			-3	A6	2	0	A3
		-11	M30	0	6/0	A7			-11	A5	1	6\3	A6
		-19		4	1	M31			-19	A7	6/0	7	A10
		-27	A6	0	6/0	A6			-27		7	7	0
	25-15°	5	A2	0	0	A1							
		-3	A2	0	0	A3	day 8	15-5°	5	1	0	0	0
		-11	M31	0	0	M31			-3	A7	2	0	A7
		-19	A2	0	4	A6			-11	0	2	4	0
		-27	A4	1	0	A10			-19	0	1	4	0
									-27	0	3	4	
day 2	15-5°	5	A3	0	6/0	A2		25-15°	5	5	0	0	1
		-3	A6	2	3	A4			-3	2	6\3	0	2
		-11	A3	0	2	A7			-11	A7	7	7	0
		-19	A7	0	4	A8			-19	0	3	7	0
		-27		4	0	A5			-27	0	7	8	0
	25-15°	5	A6	0	0	A5							
		-3	A3	0	1	A2	day 11	15-5°	5	0	2	0	0
		-11	A4	6/0	0	A8			-3	0	4	0	0
		-19	N/A	N/A	6/0	A6			-11	0	3	2	0
		-27	A8	8	6/0	A9			-19	0	6\1	7	0
									-27	0	8	4	0
day 5	15-5°	5	A8	3	0	A7		25-15°	5	0	0	0	1
		-3	A6	1	0	A8			-3	4R	0	2	2R
		-11	A11	0	2	A8			-11	1	7	7	8
		-19	A5	8	0	A12			-19	0	7	5	1
		-27	A10	6/2/80	0	N/A			-27	3	8	8	8

Table 20. Raw data for experiment 1.2

Source/Clone: BEARSKIN L. 312

Incub. time	Incub. temp.	Freez. temp	Rush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	Rush code II	Incub. time	Incub. temp.	Freez. temp	Rush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	Rush code II
day 0	15-5°	5	A3	0	0	A1	day 5	25-15°	5	A3	6/0	6\1	A6
		-3	M28	0	0	A2			-3	A3	6/0	0	A4
		-11	A1	1	0	A1			-11	A4	6/0	6\0	A16
		-19	A2	0	0	A14			-19	A8	0	6/0	A6
		-27	A2	0	1	A3			-27	0	6\4	7	
	25-15°	5	A1	6/0	0	A2							
		-3	A2	2	0	M31	day 8	15-5°	5	0	0	0	A9
		-11	A1	2	0	A4			-3	0	2	0	A3
		-19	A2	0	0	A3			-11	A4	1	6/0	A5
		-27	A2	6/0	0	A2			-19	0	1	4	0
									-27	0	6/0	7	0
day 2	15-5°	5	A7	0	6/0	A5		25-15°	5	1	0	6/0	1
		-3	A5	0	6/0	A5			-3	0	6/0	7	0
		-11	A5	0	0	A4			-11	0	7	0	A8
		-19	A4	0	6/0	A4			-19	0	7	7	0
		-27	N/A	N/A	0	A6			-27	A12	6/0	7	0
	25-15°	5	A4	0	0	A5							
		-3	A2	0	0	A4	day 11	15-5°	5	A5	0	6/0	0
		-11	A4	0	1	A10			-3	0	0	6/0	0
		-19	A8	0	7				-11	0	6/0	0	A6
		-27	A7	0	0	A6			-19	0	7	7	0
									-27	0	7	7	0
day 5	15-5°	5	A4	0	1	A4		25-15°	5	1R	0	6/0	1R
		-3	A4	6/0	0	A4			-3	A5	6/0	6/0	1
		-11	A4	0	0	A5			-11	OR	7	7	4
		-19	A5	6/0	6/0	A5			-19	0	7	1	0
		-27	A5	2	1	A6			-27	2	8	7	1

Table 20. Raw data for experiment 1.2

Source/Clone: BEARSKIN L. 313

Incub. time	Incub. temp.	Freez. temp	Rush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	Rush code II	Incub. time	Incub. temp.	Freez. temp	Rush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	Rush code II
day 0	15-5°	5	M29	0	0	M31	day 5	25-15°	5	A1	0	6/0	A2
		-3	A1	0	0	A2			-3	A4	6/0	6/0	A6
		-11	M31	0	0	M30			-11	1	7	6/0	A4
		-19	M31	0	0	A1			-19	0	6/0	7	0
		-27	M31	0	8				-27	A9	6\3	7	0
	25-15°	5	M30	0	0	A3							
		-3	M30	0	0	M29	day 8	15-5°	5	A3	0	0	4
		-11		7	0	M30			-3	3	0	0	A7
		-19	M30	6\1	0	M31			-11	0	0	0	0
		-27	A2	0	2	A2			-19	0	6\3	1	0
									-27	0	5	4	0
day 2	15-5°	5	M31	0	1	A4		25-15°	5	4	0	0	A5
		-3	A3	0	7				-3	2	1	6/0	3
		-11	A3	6/0	0	A5			-11	1	6/0	7	1
		-19	A2	0	0	A2			-19	0	7	5	0
		-27	A13	0	6/0	A4			-27	1	7	8	1
	25-15°	5	A4	0	0	A2							
		-3	A8	0	0	A2	day 11	15-5°	5	2	0	0	4
		-11	A2	0	0	A3			-3	3	6/0	0	4
		-19	A10	6/0	0	A7			-11	1	1	6\1	0
		-27	A7	0	0	A7			-19	0	7	6\3	0
									-27	0	5	7	0
day 5	15-5°	5	A2	0	0	A3		25-15°	5	1R	0	0	0
		-3	A6	0	0	A3			-3	4R	1	6\2	4R
		-11	A4	6/0	0	A3			-11	2R	7	5	3
		-19	A7	6/0	4	0			-19	0	7	7	1
		-27	A4	6/0	6/0	A6			-27	4	8	7	5R

Table 20. Raw data for experiment 1.2

Source/Clone BEARSKIN L. 308

hrdn. time	hrdn. temp.	freez. temp	flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	flush code II	hrdn. time	hrdn. temp.	freez. temp	flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	flush code II
day 0	15-5°	5	A2	0	6\4	M27	day 5	25-15°	5	A6	6/0	6/0	A6
		-3	A1	0	6/0	A2			-3	A5	6/0	0	A6
		-11	A2	0	6/0	A3			-11	A14	3	4	0
		-19	N/A	0	0	A2			-19	A11	6\3	7	
		-27	A3	0	2	A7			-27		7	8	
	25-15°	5	A5	0	0	A2							
		-3		7	0	A2	day 8	15-5°	5	A14	7	6/0	A7
		-11	A4	0	0	A4			-3	A5	0	6/0	A5
		-19	A10	3	2	A3			-11	A6	2	0	A3
		-27	A2	0	6/0	A2			-19		7	0	A6
									-27	A8	1	0	M31
day 2	15-5°	5	A6	0	0	A4		25-15°	5	1	0	0	0
		-3	A4	0	0	A3			-3	A14	2	6/0	0
		-11	A8	3	0	A8			-11	A6	6/0	6\4	1
		-19	A1	0	0	A5			-19	0	7	7	
		-27	A8	0	6/0	A7			-27	0	8	7	0
	25-15°	5	A2	6/0	0	A4							
		-3	A4	0	6/0	A4	day 11	15-5°	5	0	6/0	1	0
		-11	A10	0	0	A6			-3	0	3	0	0
		-19	A13	6/0	6/0	A7			-11	A10	2	6/0	A7
		-27	A6	6/0	1	A10			-19	0	3	3	0
									-27	0	7	7	0
day 5	15-5°	5	A9	6/0	6/0	A10		25-15°	5	3	1	1	5
		-3	A6	2	4				-3	2	1	1	1R
		-11	A4	0	2	A6			-11	1	6\1	6/0	0
		-19	A1	3	1	A8			-19	0	7	7	0
		-27	A8	6/0	6\2	A7			-27	4	0	8	2R

Table 20. Raw data for experiment 1.2

Source/Clone: BEARSKIN L. 322

hrdn. time	hrdn. temp.	freez. temp	flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	flush code II	hrdn. time	hrdn. temp.	freez. temp	flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	flush code II
day 0	15-5°	5	A4	0	2	A4	day 5	25-15°	5	M30	0	0	A6
		-3	A4	0	2	A12			-3	A7	4	0	A2
		-11	A13	0	2	A4			-11	A9	2	0	A10
		-19	A5	0	0	A4			-19	A14	6/0	4	A13
		-27	A7	0	6/0	A7			-27	A8	6/0	0	A5
	25-15°	5	A5	0	0	A7							
		-3	A8	0	0	A2	day 8	15-5°	5	A13	0	0	A6
		-11	A4	0	0	A2			-3	0	6\4	0	A6
		-19	A3	0	3	A5			-11	0	2	3	0
		-27	A2	0	0	A5			-19	0	0	4	
									-27	0	3	7	0
day 2	15-5°	5	A4	6/0	0	N/A		25-15°	5	0	0	6/0	A4
		-3	A10	0	0	A6			-3	1	2	0	0
		-11	A6	0	0	A6			-11	A13	2	7	
		-19	A10	0	7				-19	0	7	0	A14
		-27	A6	0	0	A9			-27	0	4	7	0
	25-15°	5	A5	0	0	A7							
		-3		4	3	A5	day 11	15-5°	5	A7	0	0	0
		-11	A6	0	0	A6			-3	0	2	3	0
		-19	A12	6/0	0	A13			-11	0	2	4	0
		-27	A10	0	0	A9			-19	0	6/0	6\1	0
									-27	0	2	4	0
day 5	15-5°	5	A5	0	1	A6		25-15°	5	OR	0	0	OR
		-3	A5	0	0	A5			-3	1	2	6/0	0
		-11	A9	1	3	A11			-11	1	7	3	A14
		-19	A14	6\4	0	A14			-19	0	3	7	0
		-27	A13	0	0	A8			-27		8	8	0

Table 20. Raw data for experiment 12

Source/Clone: BEARSKIN L. 321

hard. time	hard. temp.	freez. temp	flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	flush code II	hard. time	hard. temp.	freez. temp	flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	flush code II
day 0	15-5°	5	A2	0	0	A2	day 5	25-15°	5	A3	0	0	A6
		-3	A2	0	0	A6			-3	A8	0	0	A9
		-11	A2	0	0	A3			-11	A8	0	0	A7
		-19	A3	0	0	A4			-19	A8	1	0	A7
		-27	A4	0	0	A2			-27	A7	0	6\3	A18
	25-15°	5	A4	0	6/0	A4							
		-3	A4	0	0	A3	day 8	15-5°	5	A6	0	0	A3
		-11	A4	0	0	A2			-3	A7	0	0	A4
		-19	A2	0	0	A3			-11	A6	0	0	A6
		-27	A6	0	0	A6			-19	A10	0	0	A8
									-27	A8	1	0	A6
day 2	15-5°	5	A2	0	0	A3		25-15°	5	A4	0	0	A4
		-3	A4	0	0	A4			-3	A6	0	0	A3
		-11	A4	0	0	A4			-11	A6	7	0	A6
		-19	A12	0	0	A3			-19	A7	0	6/0	A10
		-27	A3	0	0	A9			-27	0	7	7	0
	25-15°	5	A2	0	0	A4							
		-3	A3	0	0	A4	day 11	15-5°	5	A3	0	0	A8
		-11	A3	0	0	A7			-3	A8	0	0	0
		-19		7	0	A7			-11	A7	0	0	A7
		-27	A4	0	0	A8			-19	A13	0	7	0
									-27	1	6\4	1	A10
day 5	15-5°	5	A4	0	0	A3		25-15°	5	A6	0	0	A4
		-3	A6	0	0	A6			-3	A6	0	0	A7
		-11	A3	0	0	A6			-11	A6	0	0	A10
		-19	A6	6/0	6/0	A6			-19	A10	0	5	0
		-27	A6	0	0	A10			-27	-	5	7	1

Table 20. Raw data for experiment 12

Source/Clone: BEARSKIN L. 300

hard. time	hard. temp.	freez. temp	flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	flush code II	hard. time	hard. temp.	freez. temp	flush code I	DAMAGE SCORE Rep. I	DAMAGE SCORE Rep. II	flush code II
day 0	15-5°	5	M30	0	0	M30	day 5	25-15°	5	A4	0	0	A3
		-3	M31	0	0	A4			-3	0	6\4	0	A13
		-11	A2	0	0	M31			-11	A5	0	0	A6
		-19		8	7				-19		7	2	A6
		-27	A3	0	0	M31			-27	A6	0	6/0	A8
	25-15°	5	A5	0	0	N/A							
		-3	A2	0	0	M31	day 8	15-5°	5	A4	0	0	A3
		-11	M31	0	0	M31			-3	0	0	0	A3
		-19	M31	0	0	A10			-11	0	0	1	0
		-27	A13	1	0	A2			-19	0	0	3	0
									-27	0	3	7	0
day 2	15-5°	5	A2	0	0	A3		25-15°	5	0	0	0	A4
		-3	A2	0	7				-3	0	0	6/0	A2
		-11	A3	2	2	A3			-11	A8	4	4	0
		-19	A4	0	0	A4			-19	0	1	4	0
		-27	A3	2	0	A3			-27	0	7	7	0
	25-15°	5	A3	0	0	A4							
		-3	A9	0	0	A3	day 11	15-5°	5	A4	0	0	A4
		-11	A4	0	7				-3	0	0	2	1
		-19	A6	0	0	A9			-11	A8	2	0	A6
		-27	A8	0	2	A7			-19	0	7	4	0
									-27	0	8	7	0
day 5	15-5°	5	A5	0	0	A6		25-15°	5	0	0	0	DR
		-3	A4	0	3	A3			-3	DR	0	2	0
		-11	A5	0	0	A4			-11	A6	0	6/0	A6
		-19		4	2	A4			-19	0	0	7	0
		-27		7	0	A6			-27	1	7	8	DR

APPENDIX VI

TABLE 21. SUMMARY OF FREIDMAN ANALYSIS FOR SOURCE DIFFERENCES IN LEAF HARDINESS AT THE VARIOUS DEVELOPMENTAL STAGES USED IN EXPERIMENT 2.

Table 21. Summary of Freidman two-way analysis by rands for source differences in the hardiness of leaf tissue at various developmental stages.

source	sum of ranks (R_i^*)
Bearskin L.	23.0
Pickle L.	15.0
Thunder Bay	19.5
N. Wisconsin	12.5

$$\Sigma R_i^{*2} = 1290.5$$

$$\Sigma\Sigma d_{ij}^3 - d_{ij} = 18.0$$

$$Q^* = 5.86$$

Prob.= 0.122

The ststistic Q^* is defined by (Lehmann,1975):

$$Q^* = \frac{[12/Ns(s+1)]\Sigma R_i^{*2} - 3N(s+1)}{1 - \Sigma\Sigma (d_{ij}3 - d_{ij})/Ns(s^2-1)}$$

where Q^* = Freidman's Q statistic (with the correction for ties)
N = numberof blocks (morphological stages)
s = treatments (geographic sources)
dij= the number of observations tied for a given block

The hypothesis of no differences among sources is rejected if:

$$Q^* > c$$

and the critical value c, is determined by the c^2-distribution with s-1 degrees of freedom.

APPENDIX VII

TABLE 22. TESTS FOR HOMOGENEITY OF VARIANCE, SKEWNESS, KURTOSIS

FIGURE 11. NORMAL PROBABILITY PLOTS FOR EACH ANOVA IN EXPERIMENT 1.2

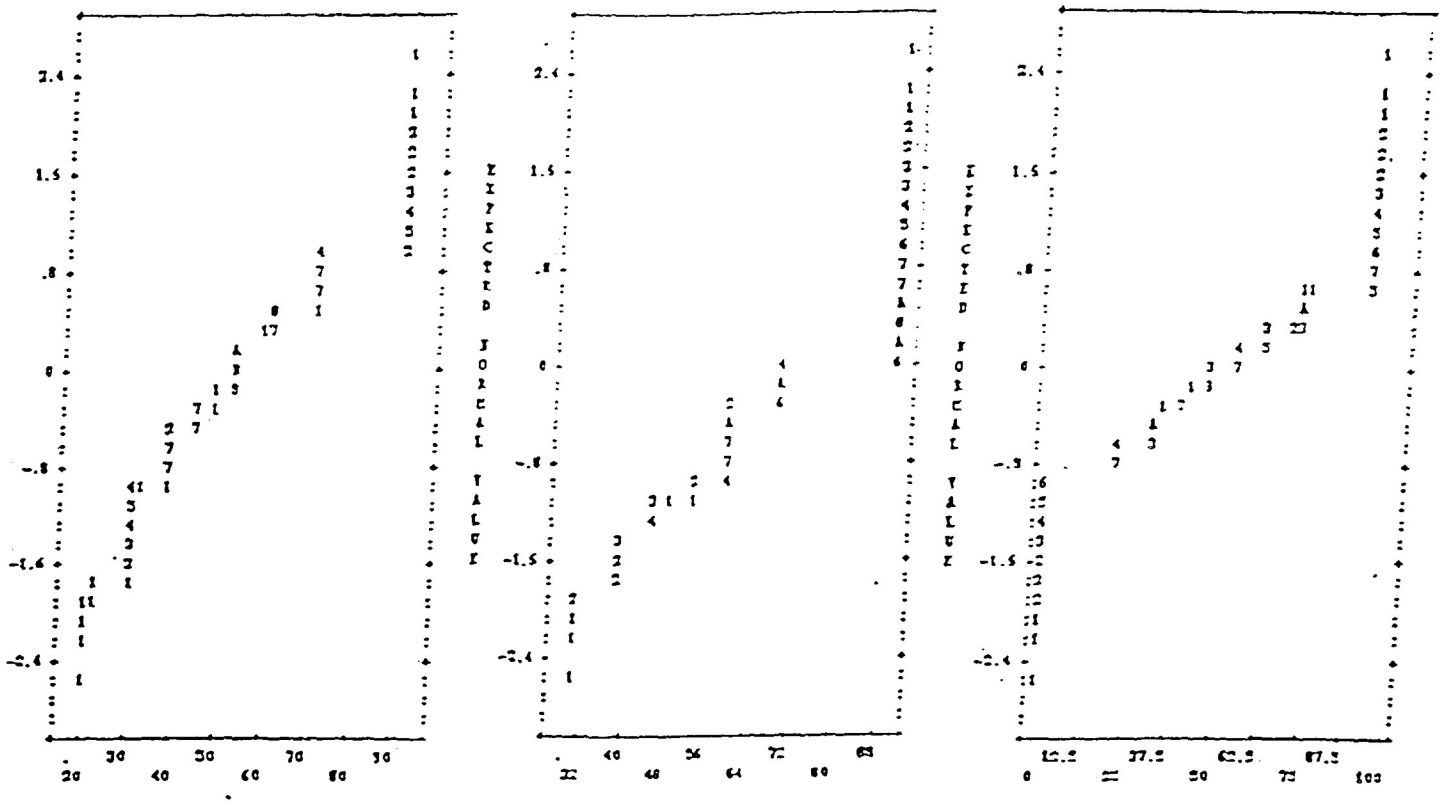
Table 22 Tests for homogeneity of variance, skewness, kurtosis

ANOVA	Homogeneity of Variance Cochrans' C (11,12)[a]	Skewness	Kurtosis
% survival leaf tissue	0.174; P=0.152	-0.415	-0.730
% survival stem tissue	0.158; P=0.331	-0.681	-0.771
% bud break	0.164; P=0.254	-0.165	-1.281

[a] Cochran's C is based on the following algorithm (Winer,1971):

$$C = \frac{s^2_{largest}}{\Sigma s_j^2}$$

The parameters of the sampling distribution of this statistic are k, the number of treatments, and n-1, the degrees of freedom for each of the variances. Tables for the C statistic are given by Winer (1971).



(a) % survival: leaf tissue (b) % survival: stem tissue (c) percent bud break

Figure 11. Normal probability plots for each ANOVA in Experiment 1.2.

APPENDIX VIII

TABLE 23. DAMAGE SCORES BY DEVELOPMENTAL STAGE FOR EACH PROVENACE IN EXPERIMENT 2.

Table 23 Damage scores by developmental stage for each provenance in Experiment 2. A full description of the damage categories is given in Table 4, page 22.

Source	Develop. Stage	Freezer Temp.	DAMAGE SCORE BY REPLICATION I	II	III	IV	V
Wisconsin	1	5	0	0	0	0	0
		-3	0	0	0	0	0
		-6	0	0	0	0	0
		-9	0	1	1	0	0
		-12	0	0	0	1	4
		-18	4	3	2	4	3
		-24	8	7	8	7	7
Wisconsin		5	0	0	0	0	0
		-3	0	0	0	0	0
		-6	1	1	0	0	0
		-9	0	0	0	0	0
		-12	0	0	1	0	0
		-18	6/0	4	0	5	2
		-24	8	8	7	8	5
Wisconsin		5	0	0	0	0	1
		-3	0	0	0	0	0
		-6	0	1	0	0	0
		-9	0	0	0	0	0
		-12	0	0	0	0	0
		-18	0	1	4	7	1
		-24	8	7	8	8	8
Wisconsin		5	0	0	0	0	0
		-3	0	0	0	0	0
		-6	6/0	6/0	0	0	0
		-9	0	0	0	0	1
		-12	0	0	0	6/0	6/0
		-18	5	5	4	7	8
		-24	8	8	8	8	8
Wisconsin		5	0	0	0	0	0
		-3	0	6/0	6/0	0	0
		-6	0	0	1	6/1	2
		-9	0	6/0	0	4	3
		-12	5	5	7	7	7
		-18	8	8	8	8	8
		-24	8	8	8	8	8
Wisconsin		5	6/0	6/0	6/0	6/0	6/0
		-3	0	6/0	0	6/0	0
		-6	0	0	1	6/1	7
		-9	7	7	1	6/2	6/2
		-12	8	8	8	8	8
		-18	8	8	8	8	8
		-24	8	8	8	8	8
Wisconsin		5	6/0	6/0	6/0	6/0	6/0
		-3	6/0	6/0	6/0	6/1	6/2
		-6	7	7	7	7	6/3
		-9	8	8	7	8	8
		-12	8	8	8	8	8
		-18	8	8	8	8	8
		-24	8	8	8	8	8

Table 23 Damage scores by developmental stage for each provenance in Experiment 2. A full description of the damage categories is given in Table 4, page 22.

Source	Develop. Stage	Freezer Temp.	DAMAGE SCORE BY REPLICATION I	II	III	IV	V
Thunder Bay	1	5	0	0	1	0	0
		- 3	0	0	0	0	0
		- 6	0	0	0	0	3
		- 9	0	0	1	0	0
		-12	0	0	0	0	0
		-18	0	0	0	0	2
		-24	6/3	3	3	7	6/3
Thunder Bay		5	0	0	0	0	0
		- 3	0	0	0	0	0
		- 6	0	0	4	0	2
		- 9	0	0	0	0	2
		-12	0	3	1	3	1
		-18	0	0	3	2	2
		-24	8	7	7	4	7
Thunder Bay		5	0	0	0	0	0
		- 3	0	0	0	0	0
		- 6	0	0	0	0	0
		- 9	6/0	0	0	0	0
		-12	0	0	6/0	0	0
		-18	8	5	1	6/2	6/2
		-24	8	7	7	8	8
Thunder Bay		5	0	0	0	0	0
		- 3	0	0	0	0	0
		- 6	0	0	0	0	0
		- 9	0	0	0	0	0
		-12	0	0	0	0	0
		-18	2	2	7	6/2	6/2
		-24	7	7	7	7	7
Thunder Bay		5	0	0	0	0	0
		- 3	0	0	0	1	0
		- 6	0	0	1	2	0
		- 9	5	6/3	4	7	7
		-12	5	7	8	7	5
		-18	8	8	8	8	8
		-24	8	8	8	8	8
Thunder Bay		5	6/0	6/0	6/0	6/0	0
		- 3	0	6/0	6/1	0	6/0
		- 6	0	6/0	6/0	6/1	6/1
		- 9	5	7	6/1	6/1	6/1
		-12	8	5	7	7	7
		-18	7	7	8	7	8
		-24	8	8	8	8	8
Thunder Bay		5	6/0	6/1	6/0	6/0	6/1
		- 3	0	8	6/0	6/0	6/0
		- 6	7	6/1	7	6/1	6/1
		- 9	8	7	7	8	6/3
		-12	8	8	8	8	8
		-18	8	8	8	8	8
		-24	8	8	8	8	8

Table 23 Damage scores by developmental stage for each provenance in Experiment 2. A full description of the damage categories is given in Table 4, page 22.

Source	Develop. Stage	Freezer Temp.	DAMAGE SCORE BY REPLICATION I	II	III	IV	V
Pickle Lake	1	5	0	0	0	0	0
		-3	0	0	0	0	0
		-6	0	0	1	0	0
		-9	0	0	0	4	3
		-12	0	0	0	0	0
		-18	4	0	0	1	4
		-24	5	3	7	7	7
Pickle Lake		5	0	0	0	0	0
		-3	0	0	4	2	0
		-6	0	1	1	0	0
		-9	0	3	2	0	0
		-12	0	5	4	1	2
		-18	1	5	5	5	5
		-24	5	5	7	5	7
Pickle Lake		5	0	0	0	0	0
		-3	0	0	0	0	0
		-6	1	0	0	0	0
		-9	0	0	0	6/2	6/0
		-12	0	0	1	1	1
		-18	5	7	7	7	7
		-24	7	7	7	7	7
Pickle Lake		5	0	0	0	0	0
		-3	0	0	0	3	0
		-6	0	0	0	0	0
		-9	4	0	0	1	2
		-12	0	0	1	0	0
		-18	7	7	3	5	5
		-24	8	8	8	7	7
Pickle Lake		5	0	0	0	6/0	0
		-3	0	0	0	6/0	6/0
		-6	0	6/0	0	0	0
		-9	5	6/1	6/1	6/1	6/1
		-12	8	8	5	5	4
		-18	7	8	7	7	7
		-24	8	8	8	8	8
Pickle Lake		5	0	0	6/0	6/0	0
		-3	0	6/0	4	0	0
		-6	6/0	8	6/3	0	1
		-9	3	3	6/2	7	6/1
		-12	7	3	7	7	7
		-18	8	8	8	8	8
		-24	8	8	8	8	8
Pickle Lake		5	6/0	6/0	6/0	6/0	6/0
		-3	6/0	6/0	6/1	6/0	6/0
		-6	6/3	6/3	7	7	7
		-9	3/8	7	3/8	8	8
		-12	8	8	8	8	8
		-18	8	8	8	8	8
		-24	8	8	8	8	8

Table 23 Damage scores by developmental stage for each provenance in Experiment 2. A full description of the damage categories is given in Table 4, page 22.

Source	Develop. Stage	Freezer Temp.	DAMAGE SCORE BY REPLICATION I	II	III	IV	V
Bearskin Lake	1	5	0	0	0	0	0
		-3	0	0	0	2	0
		-6	0	0	0	1	4
		-9	1	0	0	0	0
		-12	0	0	0	1	1
		-18	0	1	1	2	7
		-24	6/2	5	8	7	7
Bearskin Lake		5	1	0	0	0	6/0
		-3	0	0	0	0	1
		-6	0	2	1	6/0	6/2
		-9	0	0	0	2	0
		-12	0	2	0	0	1
		-18	3	6/3	1	4	7
		-24	5	4	6/2	5	4
Bearskin Lake		5	0	0	0	0	0
		-3	0	0	0	0	0
		-6	0	0	0	0	0
		-9	0	0	1	0	0
		-12	0	0	0	1	4
		-18	4	3	3	1	1
		-24	6/3	5	7	8	8
Bearskin Lake		5	0	0	0	0	0
		-3	0	0	6/1	0	6/0
		-6	0	0	0	0	1
		-9	0	0	6/0	6/0	0
		-12	0	0	0	1	1
		-18	4	6/0	6/1	5	5
		-24	1/8	5	7	8	8
Bearskin Lake		5	0	0	6/0	6/0	0
		-3	0	0	0	6/0	6/0
		-6	0	0	0	1	6/0
		-9	0	6/0	6/1	6/2	6/2
		-12	5	7	7	6/3	6/3
		-18	7	7	8	8	7
		-24	8	8	8	8	8
Bearskin Lake		5	6/0	6/0	6/0	6/0	6/0
		-3	6/0	0	6/0	6/0	6/0
		-6	6/0	6/1	6/1	6/1	6/0
		-9	6/1	6/1	6/3	7	7
		-12	8	8	8	8	8
		-18	8	8	8	8	8
		-24	8	8	8	8	8
Bearskin Lake		5	6/0	6/0	6/0	6/3	6/0
		-3	6/0	6/1	6/0	6/0	6/1
		-6	6/2	6/3	6/3	6/2	6/3
		-9	6/3	6/3	6/3	6/3	7
		-12	3/8	8	8	8	8
		-18	8	8	8	8	8
		-24	8	8	8	8	8

APPENDIX IX

Table 24. Summary of leaf hardiness, stem hardiness, and percent bud break in Experiment 1.1.

Source	Incub. Period	Incub. Temp.	leaf hardiness	stem hardiness	% bud break
N. Wisconsin	0	15-5°C	-27.00	-27.00	0.00
		25-15°C	-27.00	-27.00	0.00
	1	15-5°C	-27.00	-27.00	0.00
		25-15°C	-27.00	-27.00	0.00
	4	15-5°C	-27.00	-27.00	0.00
		25-15°C	-27.00	-27.00	0.00
	9	15-5°C	-27.00	-27.00	0.00
		25-15°C	-26.30	-26.30	0.00
	14	15-5°C	-27.00	-27.00	9.40
		25-15°C	-25.60	-25.60	27.00
Bearskin L.	0	15-5°C	-27.00	-27.00	0.00
		25-15°C	-27.00	-27.00	0.00
	1	15-5°C	-27.00	-27.00	0.00
		25-15°C	-27.00	-27.00	0.00
	4	15-5°C	-27.00	-27.00	0.00
		25-15°C	-27.00	-27.00	0.00
	9	15-5°C	-27.00	-27.00	0.00
		25-15°C	-27.00	-27.00	0.00
	14	15-5°C	-27.00	-27.00	1.00
		25-15°C	-27.00	-27.00	6.30

Table 25 Summary of leaf hardiness, stem hardiness, and percent bud break in Experiment 1.2.

Source	Incub. Period	Incub. Temp.	leaf hardiness	stem hardiness	% bud break
N. Wisconsin	0	15-5°C	-27.00	-27.00	0.00
		25-15°C	-27.00	-27.00	0.00
	2	15-5°C	-27.00	-27.00	0.00
		25-15°C	-27.00	-27.00	0.00
	5	15-5°C	-27.00	-27.00	0.00
		25-15°C	-20.30	-25.00	0.00
	8	15-5°C	-17.70	-24.30	0.00
		25-15°C	-5.00	-17.70	0.00
	11	15-5°C	-13.00	-23.00	9.40
		25-15°C	-5.00	-13.70	27.00
Bearskin L.	0	15-5°C	-27.00	-27.00	0.00
		25-15°C	-27.00	-27.00	0.00
	2	15-5°C	-27.00	-27.00	0.00
		25-15°C	-27.00	-27.00	0.00
	5	15-5°C	-27.00	-27.00	0.00
		25-15°C	-23.70	-27.00	0.00
	8	15-5°C	-25.70	-26.30	0.00
		25-15°C	-17.00	-25.70	0.00
	11	15-5°C	-20.30	-26.30	1.00
		25-15°C	-11.00	-20.30	6.30