species (Linhart *et al.*, 1981; Szmidt and Yazdani, 1984; Pitel *et al.*, 1987).

GDH showed very little variation. This is in accordance with all other conifer species studied up to now. One diallelic GDH locus is also described for *Abies balsamea* (Neale and Adams, 1981), *Picea spec.* and *Pinus spec.* (Lundkvist, 1979; Moran *et al.*, 1980; Yeh and El-Kassaby,, 1980; Woods *et al.*, 1983; Cheliak and Pitel, 1984; Cheliak, 1985; Pitel *et al.*, 1987).

The enzyme systems IDH as well as 6-PGD were found to be under the genetic control of two loci with two and three alleles each. Moller (1986) also found two IDH loci and 5 alleles in *A. alba.* There seems to be more variation in IDH than found in numerous other conifer species, e.g. *Picea, Pinus, Larix, Pseudotsuga.* In general there is reported only one locus with one to three alleles (Guries and Ledig, 1978; O'Malley *et al.*, 1979; neale and Adams, 1981; Cheliak and Pitel, 1984; and others). The same authors also describe a dimeric IDH quaternary structure like it is supposed in this investigation for *Abies alba.* The variation of 6-PGD in *A. alba* is comparable to data reported for other conifer species: in Picea as well as in Pinus several authors have found two loci and 4 to 6 alleles usually.

No linkage was found between four loci of the enzyme systems IDH and 6-PGD. This is in agreement with studies on other conifer species, e.g. by O'Malley *et al.* (1979; ponderosa pine) and El-Kassaby *et al.* (1982; Douglas-fir).

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Genetic Variances and Covariances in Freezing Tolerance of Lodgepole Pine During Early Winter Acclimation

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Summary

Nine-year-old *Pinus contorta* trees representing nine families from each of four seed sources were used to assess genetic variances in cold hardiness during mid-winter and to evaluate the covariance between cold hardiness and tree height. Leaves from about 20 trees within each family were collected in late autumn when acclimation was well advanced and were frozen to five temperatures in a laboratory freezing chamber. Differences in freezing tolerance of families were statistically significant, but additive genetic variances and heritabilities were low. Consequently, genetic gains from tree improvement would accumulate slowly. Although a relatively high genetic correlation (0.74) linked freezing injury and height at age 7, the coefficient of genetic prediction was extremely low, 0.04. These statistics suggest that strong selection for rapid growth would result in a correlated decrease in mid-winter freezing tolerance, but the size of the decrease would be negligible.

Key words: Tree breeding, quantitative genetics, cold acclimation.

Zusammenfassung

Neunjährige *Pinus contorta* Bäume, die neun Familien von jeder der vier Samenquellen repräsentieren, wurden benutzt, um die genetischen Veränderungen von Kaltwiderstandsfähigkeit mitten im Winter einzuschätzen. Sie wurden auch benutzt, um die Koveränderung zwischen Kaltwiderstandsfähigkeit und Höhe auszuwerten. Blätter von ungefähr 20 Bäumen jeder Familie wurden im Spätherbst gesammelt, als die Akklimation wohl fortgeschritten war. Sie waren im Laboratorium in fünf verschiedenen Temperaturen eingefroren worden. Unterschiede in der Frostresistenz von Familien waren statistisch bedeutsam, aber genetischen Veränderungszusätze und Vererbbarkeiten waren gering. Folglich werden sich die genetischen Gewinne der Verbesserung der Bäume langsam ansammeln. Auch zeigten die Analysen eine relativ hohe genetische Wechselbeziehung von 0,74 zwischen Frostverletzung und Höhe beim 7jährigen Baum, aber sie zeigten auch einen außergewöhnlich niedrigen Koeffizient bei der genetischen Vorhersage, 0,04. Diese Statistiken deuten an, daß eine starke Auswahl für “schnelles Wachstum” resultieren würde in einer wechselnden Verminderung mitten im Winter in Frosttoleranz, aber die Menge der Verminderung würde geringfügig sein.

Introduction

For lodgepole pine (*Pinus contorta* var. *latifolia*), a temperate zone species with an economic distribution in montane, subalpine and boreal environments (Critchfield, 1980), cold hardiness is a subject pertinent to tree improvement. Cold hardiness must either be maintained or increased if the gains in productivity that seem readily attainable (Rehfeldt, 1985b; Ying *et al.*, 1985; Fries and Lindgren, 1986) are to be realized. In fact, if the negative correlations between growth potential[1]) and freezing tolerance that have been repeatedly demonstrated between populations (Rehfeldt, 1980, 1983, 1985a; Jonsson *et al.*, 1981) also exist within populations, cold hardiness will constrain tree improvement. Nevertheless, the quantitative genetics of cold hardiness and the genetic relationships between cold hardiness and growth potential are poorly understood.

Frost hardiness during dormancy can be considered in three separate stages, all of which may be of concern to tree breeding. Two of the stages, phases one and two of Weiser (1970), involve (1) hardiness in early autumn after growth cessation but before cold acclimation has been induced by frosts and short photoperiods, and (2) hardiness during acclimation when tissues are becoming capable of withstanding extreme cold. A third stage involves hardiness during deacclimation when plants are becoming physiologically prepared for spring growth.

Of the studies that have addressed genetic variances in freezing tolerance of lodgepole pine, most have been concerned with the first stage, during which additive genetic variation seems pronounced. Thus, Jonsson *et al.* (1986) detected differences among families within seed sources and reported repeatabilities as high as 0.50. Genetic correlations involving growth potential and cold hardiness during the first stage have not been addressed directly, but cold hardiness at this time is closely related to the cessation of development (Glerum , 1976; Weiser, 1970). Consequently, a strongly positive genetic correlation between 7-year height and the cessation of shoot elongation implies that growth potential and freezing tolerance during the first stage are also strongly and negatively correlated (Rehfeldt, 1985b).

Genetic variation during stages other than the first have received little attention. Whereas Jonsson *et al.* (1986) could not detect variation during deacclimation, genetic variation in freezing tolerance during the second stage has not been assessed. No studies have dealt with the relationship between growth potential and cold hardiness either during the acclimative stage or during deacclimation.

This purpose of this paper is to (1) elucidate the quantitative genetics of freezing tolerance during the second or acclimative stage and (2) assess the genetic relationship between growth potential and freezing tolerance during the same stage.

Methods

Detached leaves from 692 trees that represented nine wind-pollinated families from each of four seed sources were subjected to laboratory freezing. Each family was represented by about twenty 9-year-old trees growing in an experimental planting. Although the design of the planting was described previously (Rehfeldt, 1985b), thinning at age 7 removed 75% of the trees and increased the distance between rows to 2 m, while about 1 m separated trees within rows. After thinning, families were represented by two to four seedlings in five to seven row plots randomly distributed throughout a rectangular planting. Because the seed sources differ greatly in growth and adaptive traits, they are considered to represent different breeding populations.

In mid-October, approximately 3 weeks after the first frost, a lateral branch from the terminal whorl of the current year's growth of each tree was cut, moistened, packaged in plastic, and transported within coolers to the laboratory at Moscow, Idaho. Here the branches were placed in storage at 2° C. Within 10 days, five sets of 10 leaves were removed from each shoot, moistened, and packaged in plastic bags. One set from each tree was then frozen at the rate of 5° C/h to one of five test temperatures. A preliminary freezing test was used to select temperatures that would injure approximately 50% of the leaves from each seed source. Because of differences in hardiness, each seed source required a different range of temperatures to induce 50% injury (*Table 1*).

After reaching the desired temperature, leaves were removed from the freezer, thawed at 2° C for 24 hours, and stored at 5° C for as long as 2 weeks before being scored visually for injury. An aggregate score between 0 and 100 was given to the 10 leaves in each bag. The score was intended to reflect both the proportion of leaf area injured and the degree of structural injury (judged by bending the leaves):

Score	*Color*	*Structure*
< 5	bright green	rigid
25	dark green	rigid
50	green-brown	pliant
75	brown-green	pliant
> 95	light brown	flaccid

These procedures produced scores of freezing injury to five test temperatures for each of the 692 plantation trees. Analyses of variance (SAS, 1982) were performed according to a model in which test temperatures were assumed to be fixed variates, but all other variates were assumed to

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[1]) Growth potential: An environment-specific expression of the innate capability to produce carbohydrate and to assimilate wood in the absence of biotic and abiotic effects (insects, diseases, frost, rodents, etc.) that mask the genotype. Note that this definition assumes adaptedness.

Table 1. — Range in freezing temperatures and range in family injury scores according to seed source.

Seed source	Range in temperatures (°C)		Mean score	Range in family mean scores		Standard deviation
	High	Low		Low	High	
201	-29.0	-33.0	49	40	55	20
202	-33.0	-36.0	58	45	65	23
218	-31.5	-36.3	41	28	49	27
220	-37.1	-41.6	48	42	52	21

Table 2. — Results of the analysis of variance of freezing injury scores.

Source of variance		Expected components	Degrees of freedom	Mean square	Component
Seed sources	(S)		3		
Temperatures in sources	(T,S)	$\sigma^2_W + 3.3\ \sigma^2_{TxP,F,S} + 19.5\ \sigma^2_{TxF,S} + 173\ \sigma^2_{T,S}$	16	7427.05**	40.71
Families in sources	(F,S)	$\sigma^2_W + 16.4\ \sigma^2_{P,F,S} + 95.9\ \sigma^2_{F,S}$	32	3096.13**	14.94
Plots in families	(P,F,S)	$\sigma^2_W + 15.2\ \sigma^2_{P,F,S}$	189	1561.89**	77.77
Temperatures x families	(TxF,S)	$\sigma^2_W + 3.3\ \sigma^2_{TxP,F,S} + 19.2\ \sigma^2_{TxF,S}$	128	383.73	.27
Temperatures x plots	(TxP,F,S)	$\sigma^2_W + 3.0\ \sigma^2_{TxP,F,S}$	756	381.71	-12.78
Residual	(W)	σ^2_W	2335	420.52	420.52

** Statistical significance at the 1% level of probability.

be random (*Table 2*). An analysis was made on both original and transformed scores. Transforming scores to standard deviates within seed sources eliminated the confounding introduced by exposing each seed source to a different range of temperatures and thereby allowed the effects of seed sources to be estimated without bias. Analyses of original scores, however, were most readily interpreted. These two analyses produced essentially the same results and led to the same conclusions. Only the results of analyses made on original scores are presented because the effects of seed sources were superfluous to the primary objectives, and therefore transformations unnecessarily complicated interpretation of quantitative statistics. The effects of seed sources are ignored hereafter.

The genetic correlation between the 7-year height and the mean score of freezing injury for each tree was calculated according to FALCONER (1960); the coefficient of genetic prediction was calculated according to BARADAT (1976). For both of these statistics, scores of each tree were averaged for the five temperatures.

Results and Discussion

Even though leaves were collected as little as 3 weeks after the first frost, temperatures below −30° C were required to provide a mean injury score of 50. While these results might imply rapid acclimation in an extremely short time, one should recognize that for the mean injury score to reach 50, minor injury would occur to some leaves at much warmer temperatures. In addition, tolerance assessed after a brief exposure to freezing temperatures in a laboratory chamber is relative to but nevertheless overestimates tolerance to natural freezing which occurs under much different conditions (LEVITT, 1980).

Although families differed significantly in injury from freezing, the analysis of variance was dominated by the residual variance and by variance among plots within families (*Table 2*). Because of the size of these two error variances, individual and family heritabilities were relatively low (*Table 3*). Nevertheless, these results illustrate genetic variability in freezing tolerance during the acclimative stage and therefore suggest that freezing tolerance could be improved by means of direct selection.

If improving cold hardiness is the primary objective of tree improvement, progress from direct selection is readily calculated (NAMKOONG, 1979) from the formula for genetic gain, $\triangle_G = i\ \sigma_{TF}\ h^2_F$, where i is the selection intensity and other symbols are defined in *Table 3*. For each unit of i, gains from family selection would amount to 2.3 score units. Likewise, selection within selected families at $i = 1$ would provide an additional gain of (0.75) $\sigma_T\ h^2_I = 1.7$ score units. This means that a comprehensive program of combined selection operating at $i = 1$ for family selection and at $i = 2$ for selection of individuals within selected families would produce genetic gains of only 5.7 score units per generation.

Although it was impossible to determine the amount of loss in growth that would occur per unit increase in score, a score of 25 was associated with pronounced color changes that undoubtedly reflected either destruction or impairment of the photosynthetic apparatus. For a comprehensive breeding program to decrease score by only 5.7 units per generation, a value only one-fifth of the distance

Table 3. — Quantitative genetic statistics generated from the analysis of variance (*Table 2*).

Statistic	Symbol	Value
Additive genetic variance†	σ^2_A	$3.6\ \sigma^2_{F,S} = 53.78$
Total phenotype variance	σ^2_T	$\sigma^2_{F,S} + \sigma^2_{P,F,S} + \sigma^2_W = 513.23$
Phenotypic variance of family means	σ^2_{TF}	$\sigma^2_W/95.9 + \sigma^2_{P,F,S}/5.85 + \sigma^2_{F,S} = 32.62$
Individual heritability	h^2_I	$\sigma^2_A/\sigma^2_T = 0.10$
Family heritability	h^2_F	$0.25\ \sigma^2_A/\sigma^2_{TF} = 0.41$

† Adjusted for inbreeding assumed at F = 0.10.

between zero injury and pronounced chlorophyll destruction, suggests that genetic gains of practical value would accumulate slowly. Even if programs emphasized juvenile selection and rapid turnover of generations, at least four generations seem necessary for improvement to be substantial.

It seems, therefore, that the most rapid gains in cold hardiness will accrue from selection of provenances, between which genetic variation is ample (REHFELDT, 1980, 1983; JONSSON *et al.*, 1981), and for which hardiness during the first and second stages is highly correlated (REHFELDT, 1980). But, because of a negative relationship between growth potential and cold hardiness of populations (REHFELDT and WYKOFF, 1981; REHFELDT 1983, 1985a), selecting hardy provenances generally means accepting lesser productivity.

The genetic correlation between the score of freezing injury and height at age 7 was high, $r_A = 0.74$. Although this means that strong selection for height alone would inadvertently alter freezing tolerance during the acclimative stage, BARADAT's (1976) coefficient of genetic prediction for these two traits equaled 0.04. A high genetic correlation coupled with a low coefficient of genetic prediction suggests that the level of coinheritance is high, but, as evidenced by the low heritabilities of *Table 3*, the level of genetic variation is low.

The coefficient of genetic prediction implies that for each standard deviation that height is improved, a correlated increase of 0.04 standard deviations occurs in the breeding value of freezing tolerance. Because the pooled standard deviation within sources was 22.7 units (*Table 1*), the correlated response corresponds to a change in score of $(0.04) \cdot (22.7) = 9.1$ units, which, as reasoned previously, is a small decrease. This small decrease, moreover, would be associated with a gain in height (21%), which is unrealistic in a single generation of selection (REHFELDT, 1985b). Together, these statistics suggest that six to seven generations of intensive selection for height would be necessary to reduce freezing tolerance by 25 score units, the amount associated with pronounced chlorophyll destruction. It appears, therefore, that correlated responses in freezing tolerance during the acclimative stage would be negligible for the first few generations of intensive selection for increased growth.

Conclusions

The results show that additive genetic variance for freezing tolerance during the acclimative stage of cold hardiness is detectable but weak. Progress from direct selection for increased freezing tolerance therefore would be slow. Despite a relatively high and negative genetic correlation between freezing injury and height at age 7, the amount of correlated response to selection for height seems negligible.

The results also contribute to an understanding of genetic variation in cold hardiness. This understanding is complicated by the fact that dormant trees are susceptable to freezing injury during one of three stages. Although few in number, experimental results are beginning to clarify the manner by which variation in freezing tolerance influences tree breeding programs. During the first stage, tolerance depends on when growth ceased (GLERUM, 1976; WEISER, 1970); and genetic variation is pronounced with a strongly negative relation with growth potential (REHFELDT, 1985b). As shown herein, additive genetic variation during acclimation seems to be of little practical importance. And finally, genetic variation in hardiness during deacclimation has not been detected (JONSSON *et al.*, 1986) perhaps because deacclimation occurs extremely rapidly (GLERUM, 1976). Consequently, frost tolerance during early autumn seems to be the stage in which direct and indirect response to selection are pronounced and thus may be of greatest consequence to tree improvement.

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Are Pollination Bags Needed for Controlled Pollination Programs with Yellow-poplar ?[1])

By D. B. Houston and K. A. Joehlin[2])

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Summary

Differences among trees in seed production ranged from 7% to 75% germinable seed after controlled pollination, with mean control pollinated seed set exceeding that of open pollinated seed set by 600%. Pollination bags did not have a consistent effect on filled seed percentages, or on the number of two- embryo seeds. Male parent did not have a significant effect on filled seed percentages. Removal of petals and stamens (*i.e.*, emasculation) reduced the observed number of seeds produced by insect pollination to less than 1%. Contamination of seed lots produced by controlled pollination of emasculated, non-bagged flowers will be negligible.

Key words: Liriodendron tulipifera, controlled pollination, pollination bags, emasculatin.

Introduction

Yellow-poplar is an insect-pollinated species with a large, perfect flower. The gynoecium, consisting of 60 to 100 pistils, is located in the center, surrounded by a ring of 20 to 40 stamens. The corolla is comprised of six petals, with an orange band at their base in which the nectaries are located (Wilcox and Taft, 1969). The fruit is an elongated cone composed of 60 to 100 overlapping carpels (samaras). Individual samaras have the potential for producing two seeds, but in most cases one of the embryos aborts. Natural seed sets average only about 10 percent (Bonner and Russell, 1974).

In controlled breeding studies with yellow-poplar, precautions are usually taken to prevent unwanted pollination. Flowers which are unopened, but reproductively mature, are emasculated by hand by removing the sepals, petals and stames. The desired pollen, collected by forcing flowers in the laboratory, is then applied to the gynoecium with a small paint brush, and a pollination bag is placed over the flower to prevent subsequent visits by unwanted pollinators. Even though the average period of receptivity of individual flowers is only 12 to 24 hours (Kaeiser and Boyce, 1962), pollination bags are usually removed several days later.

Bagging is an expensive and time-consuming process, especially if an extensive breeding or seed production effort is contemplated. However, if petals and nectaries are removed during the controlled pollination process, insects theoretically should have little reason to visit the emasculated flower, thus drastically reducing the probability of unwanted pollination, the necessity of bagging, and the time required to complete a given set of crosses.

The objectives of this study were to (1) determine the effect of the pollination bag on seed set following controlled pollination, and (2) estimate how much pollination would occur if emasculated flowers were not protected by pollination bags.

Materials and Methods

Branches bearing unopened flower buds were cut from selected trees (*Table 1*) and transported to the laboratory in an ice chest. Pollen was collected by forcing the flowers in water culture. When the anthers dehisced (generally within 24 hrs), pollen was extracted, dried at room temperature (24° C) for 30 min, and stored in vials at 4° C until needed for pollination. Pollen lots were kept separate by male parent.

Pollinations were accomplished in early June. Flower buds were selected for use if they were unopened, and a space could be detected between the petals and the gynoe-

Table 1. — Codes and location of the yellow-poplar trees used in the pollination experiment.

Tree No.	Location
1, 2, 3, 4	Ohio Agricultural Research and Development Center, Wooster,OH
5, 6	Millersburg, Ohio
7, 8	Holden Arboretum, Mentor, Ohio

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