GENETIC AFTEREFFECTS OF INCREASED TEMPERATURE IN LARIX

Michael S. Greenwood and Keith W. Hutchison[1]

Abstract: We tested the hypothesis that temperature during gametogenesis and embryogenesis can affect progeny genotype and phenotype. Identical crosses were made among cloned parents of Larix spp. inside and outside a greenhouse, where the temperature inside averaged 4°C above the outside temperature. Significant growth differences as a function of crossing environment were observed. When the crosses were grown in the same environment the phenotypes of crosses made inside tended to resemble more southern ecotypes. In addition, segregation distortion at the chlorophyll- a/b-protein locus as a function of crossing environment was observed. These results support the hypothesis that progeny phenotype and genotype can exhibit aftereffects that are a function of crossing environment.

INTRODUCTION

Modification of progeny performance as a function of parental environment has been reported for both Picea abies (L.) Karst. and Pinus sylvestris L. (Lindgren and Wang 1986, Johnsen 1989a and 1989b, Johnsen and others 1989, and Dormling and Johnsen 1992). For both species, the same crosses were made on cloned parents (of northern origin) in seed orchards about 5-8° latitude apart and the resulting progenies were planted on the same site. Full- and half-sibling progeny produced at the more southern locations consistently grew more than their siblings produced in the north. In addition, progeny produced in the south exhibited less cold hardiness and overall seemed to behave like southern ecotypes. These modifications due to parental environment have been called aftereffects. Similar changes in growth habits of inbred lines of Pisum and Linum were induced by temperature or fertilization regime (Highkin 1958, Durrant 1971), which also appear to be heritable in these two species.

In general, clonal seed orchards located in more southerly regions of a species range flower better, which has resulted in the common practice of locating clonal seed orchards as far south within the range as possible (Schmidtling 1987). In addition, consistently good flowering can be obtained using indoor, potted breeding orchards (e.g. Philipson 1983, Ross 1985, Adams and Greenwood 1992). These practices require further understanding of the effects of breeding environment on progeny performance by forest tree species. In addition, the possibility of rapid global warming raises questions about the ability of long-lived woody perennials to adapt to such changes. Are there short-term, as yet uncharacterized genetic strategies so that better adapted progeny can be selected before seed is even shed? The purpose of this paper is to compare the effect of adjacent indoor (greenhouse) and outdoor environments on height growth and allele frequencies of progeny from identical crosses Larix spp.

MATERIALS AND METHODS

Fourteen parents were selected from a potted, indoor clonal breeding orchard of Larix spp. for creation of identical indoor and outdoor breeding populations. The orchard was established by grafting in 1987. Details of establishment and culture are described by Eysteinsson and Greenwood (1993). The parents, representing three species (five from Larix laricina (DuRoi) K. Koch, five from L. decidua Mill., and four from L.leptolepis Gord.), were each represented by at least four ramets, all of which remained in the greenhouse until mid-November, 1991. At that time two randomly chosen ramets of each parent were moved outside to a pad immediately adjacent to the greenhouse. Crossing began in the spring of 1992 and continued in 1993 (Table 1). The greenhouse remained unheated most of

[1]Ruth Hutchins Professor of Tree Physiology, Department of Forest Ecosystem Science; and Associate Professor of Biochemistry, Department of Biochemistry, Microbiology and Molecular Biology, respectively, University of Maine, Orono, ME 04469.

the winter, so that shoots went through winter dormancy. Temperatures were recorded simultaneously at hourly intervals at both locations using thermocouples (two inside and two out) attached to an ECD model 50 data logger (ECD Inc., Milwaukie, OR 97222-8825).

Twelve pairs of identical indoor-outdoor crosses (nine made in 1992 and three in 1993) yielded enough viable seed for a common garden progeny test. One cross was repeated in 1992 and 1993. Most crosses were intraspecific (four among eastern larch parents, three among European larch, three among Japanese larch) with two interspecific crosses between Japanese and European larch. Pollen always came from the same location as the female parent (see Eysteinsson and Greenwood 1993 for details on pollen collection and pollination). A total of five of the 14 parents were involved in two to three crosses each, but there were no reciprocal crosses between inside and out.

In early January, 1994, seed was germinated in 150x15mm petri dishes on moistened filter paper (Whatman #42) in a growth chamber under a 16h photoperiod (incandescent light only), 28°C day, 20°C night. Germinants (radicle emerged) were transplanted to 65 cm^3 plastic tubes (Ray Leech cells, Stuewe and Sons, Inc. 2290 SE Kiger I. Drive, Corvallis, OR 97333) containing peat moss:builder's sand:vermiculite:perlite (8:4:3:3) with Osmocote 17:6:12 (four mo. timed release) fertilizer at $4g \cdot l^{-1}$ (Grace-Sierra, 1001 Yosemite Dr., Milpitas, CA 95035). Percentage of seed germinating was recorded for each family. Seedlings continued to grow in a heated greenhouse (20-25° day, 15-20° night) under a 16h photoperiod (natural daylight supplemented with high pressure sodium lamps for 16h). Each family was grown as a single block, and trays containing the blocks were shuffled weekly to even out the effects of environmental variation.

In late May 1994, seedlings were transplanted into 11.4 l plastic pots containing peat moss:vermiculite:builder's sand (2:1:1) and Osmocote as described above, and moved into the same fiberglass-covered greenhouse used for the indoor breeding orchard with no supplemental photoperiod. The seedlings were arranged in four blocks containing four replicates, the latter consisting of full-sibling pairs (one sibling from outside, one from inside were placed side by side) of 12 families, for a total of 384 trees. The heights of all the seedlings were measured immediately after repotting. All the seedlings were watered via drip irrigation, assuring that the potting medium was close to field capacity most of the time. The trees were measured again in late September 1994.

In the spring of 1992 and again, in 1993, embryos were carefully excised from seeds gathered in the respective year. DNA was extracted from the seeds as described by Doyle and Doyle (1990). Inheritance of specific alleles at one of the loci encoding a chlorophyll-a/b-binding protein (cab) protein was determined by PCR amplification of the genomic sequence, followed by analysis using single strand conformational polymorphisms (SSCP) (Orita and others 1989).

Statistical analysis was performed using SYSTAT software (Systat, Inc., 1800 Sherman Ave., Evanston, IL 60201-3793).

RESULTS

Temperatures averaged about 4°C warmer inside than out in 1992 but the difference was greatest between January and September (Figure 1). The lack of difference in the fall is probably due to greater cloud cover during the day, which is typical of fall and early winter in the northeastern US. Similar temperature profiles were observed in 1993.

A significant block effect developed during the growing season, probably due to uneven temperature in the greenhouse (Table 1). The effect of cross was highly significant at both measurement times. The effect of crossing environment was not significant at either time, but the cross by location interaction was highly significant at both dates, indicating that the crosses grew differently as a function of crossing environment. In general, crosses made outside among eastern larch grew more, while crosses made inside among the other species tended to grow more (Figure 2). The ANOVA of the height differences between crossing environments reveals that the effect of cross on height difference between crossing environments is highly significant (Table 2). Height difference due to crossing environment was significantly different from zero to five of the 12 crosses at each measurement date ($p < 0.05$).

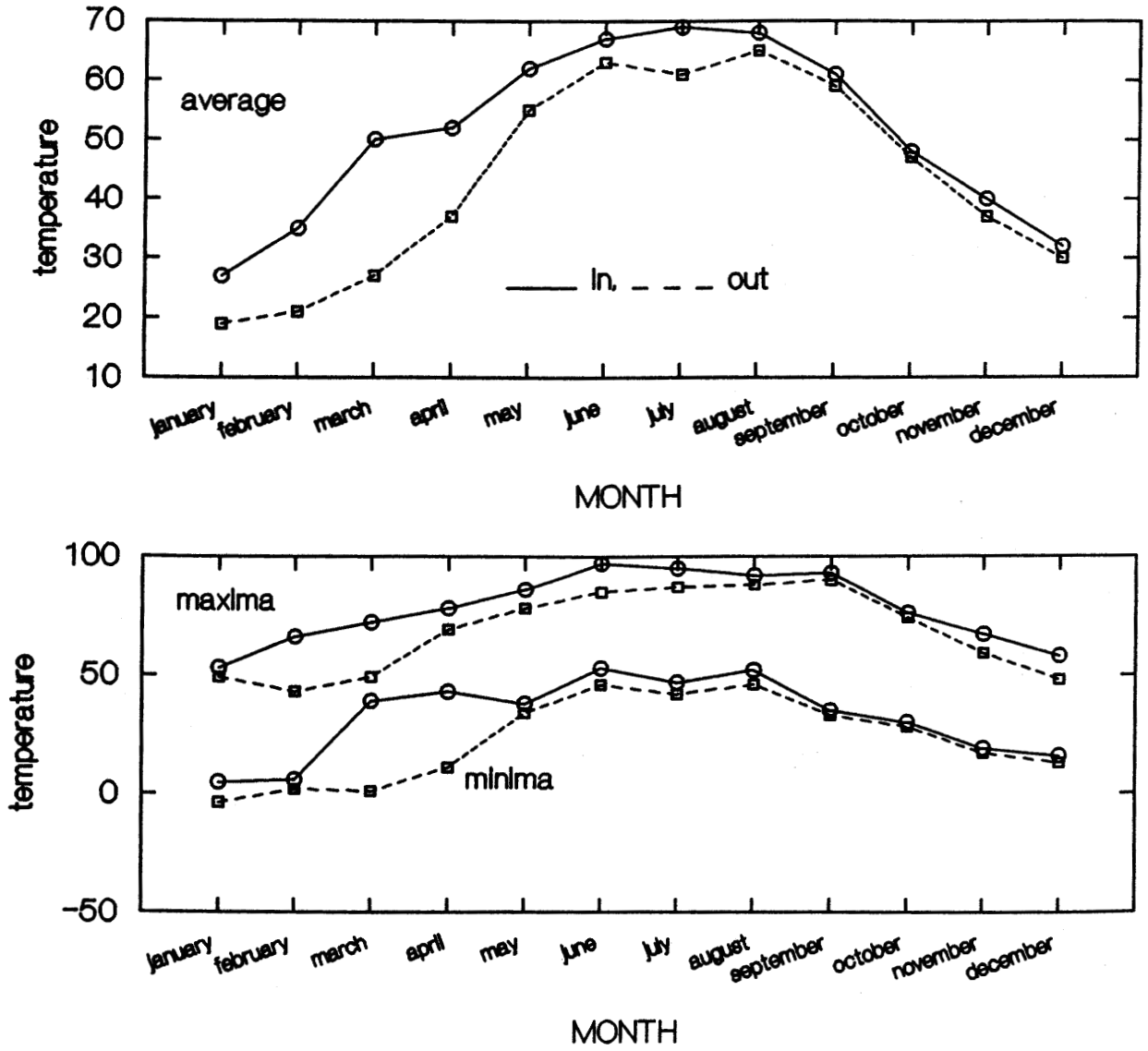

Figure 1. Mean monthly temperatures (1992) at breeding orchards located inside and outside the greenhouse.

Table 1. ANOVAs for heights in May and September.

Source of Variation	DF	May Height		September Height	
		Mean Square	P	Mean Square	P
Block (B)	3	1.91	0.912	2518	0.031
Cross (C)	11	139.43	0.000	23,984	0.000
Location (L)	1	11.80	0.297	644	0.381
BxC	33	11.17	0.422	772	0.591
BxL	3	6.56	0.611	2483	0.032
CxL	11	47.64	0.000	3034	0.000
BxCxL	33	8.36	0.810	888	0.380
Error		10.8		835	

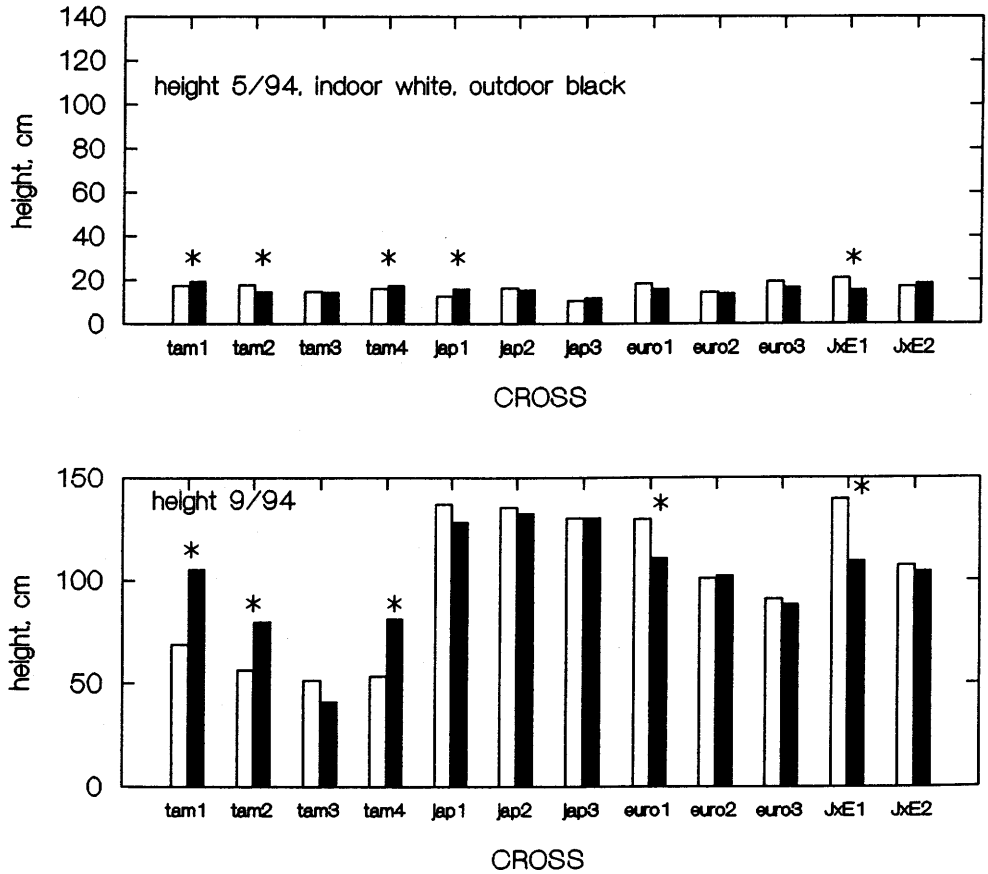

Figure 2. Heights of indoor (white) and outdoor (black) siblings of 12 identical controlled crosses made inside and outside the greenhouse. Abbreviations: tam = Larix laricina, jap = L. leptolepis, euro = L. decidua. Asterisks indicate crosses where indoor-outdoor difference was significantly > zero at $p < 0.05$.

Table 2. ANOVAs for height difference between indoor and outdoor full-sibling heights by cross.

Source of Variation	DF	Mean Square	P	Mean Square	P
Block (B)	3	16.93	0.424	5533	0.011
Cross (C)	11	93.14	0.000	5778	0.000
BxC	33	17.20	0.547	1629	0.305
Error		18.04		1437	

Height differences due to crossing environment caused by differences in seed vigor must be considered because weight and percent germination of seed from crosses made inside tended to be slightly greater. However, there was no significant correlation between percent germination and height at either measurement date (Pearson r=-0.150 in May, r=0.193 in September). Also, height growth rank between crossing environments changed for four of the 12 crosses between the May and September measurements. May and September heights were not significantly correlated (Pearson r= -0.081), providing further evidence that residual effects of seed vigor on seedling height, if they exist at all, are not persistent.

The opposite response to crossing environment by L. laricina crosses may be a function of bud set following repotting. The relatively poor growth of these crosses was due to a tendency to set bud relatively easily after repotting: by midsummer, less than one third of these crosses had been growing continuously (see Table 3). The observation that a slightly higher percentage of the crosses made outside grew continuously explains in part why the crosses made outside grew more. In contrast, most of the trees from the crosses involving the other species grew continuously, with the percentage slightly higher for crosses made inside. The bud set probably occurred because ambient photoperiod at the time of repotting was about 14h, about 2h shorter than the trees had been receiving prior to repotting.

Table 3. The percentage of trees by species on 7-19-94 that grew continuously following repotting the previous May.

	# Trees		Continuously Growing	
Species	In	Out	In	Out
Eastern larch	91	87	27%	32%
Japanese larch	53	53	98%	96%
European larch	70	79	69%	63%
Hybrid (JxE)	42	43	68%	67%

Environmental aftereffects could be the result of either epigenetic or genetic changes. In the latter category, changes could arise either as a result of changes in the mutation rate or in selection for particular alleles or haplotypes within the genome. Selection should be detectable as segregation distortion, or non-Mendelian inheritance. Previous observations while constructing a genetic map for larch suggested that one of the cab loci protein would be a candidate marker for detecting segregation distortion (Hutchison et al. 1995, Unpublished). As shown in Table 4, allele 1 was found at significantly higher frequency in the progeny of an indoor grown L. decidua cross in 1992. Progeny from the outdoor grown clone was not available. This observation was repeated the following year. Though the significance of the segregation distortion for the indoor grown trees was decreased, the trend remained

the same. Of even greater interest is that segregation distortion reverses in the progeny of the clonal replications that were grown outdoors (Table 4).

Table 4. Frequency of occurrence of two alleles at the cab-3 locus in L. decidua grown inside (1992 and 1993) and outside (1993), including χ^2 for deviation from expected 1:1 segregation of the two alleles.

1992-Indoor				1992-Indoor				1993-Outdoor			
Allele 1	Allele 2	χ^2	p	Allele 1	Allele 2	χ^2	p	Allele 1	Allele 2	χ^2	p
37	19	5.79	0.016	34	25	1.37	0.24	18	39	7.74	0.005

DISCUSSION

Significant environmental aftereffects on height growth of five of the 12 full-sib families created inside and outside the greenhouse have been demonstrated, which are not a function of seed vigor. The most likely cause of the aftereffects observed here is temperature, which was consistently higher inside the greenhouse throughout the periods of both gametogenesis and embryogenesis in larch. However, differences in light quality due to the fiberglass covering of the greenhouse were also present. These effects appear to be similar to those demonstrated for Pinus sylvestris and Picea abies, in that crosses made inside the greenhouse behaved like southern ecotypes. The crosses made between L. decidua and L. leptolepis inside tended to grow slightly more, and the majority of the trees among these crosses grew continuously after repotting. In the two crosses where this difference was significant at $p<0.05$, all the trees were growing actively in mid-July. In contrast, the majority of trees among the eastern larch crosses set bud after repotting, and had not resumed growth by mid-July, so the full growth potential of these crosses was not realized. Three of the four larch crosses exhibited significantly more height growth when made outside, and among these crosses, 40 percent of the trees from the outside crosses had been growing actively most of the summer, versus 29 percent for the crosses made inside. The greater frequency of resumption of active growth by the outdoor crosses is consistent with demonstrations that more northerly ecotypes at Picea abies require smaller heat sums before bud burst occurs (e.g. Beuker 1994). Observations on frost hardiness as a function of crossing environment have also been made, but await confirmation by further observations in the spring of 1995.

Environmental aftereffects could potentially be explained by selection for specific alleles or regions of the genome. This selection could take place either during gametogenesis or in the developing embryo. Segregation distortion of the cab locus could be due to linkage to a generalized lethal allele at another locus. Alternatively, it could be due to environmental selection of a specific allele in the elevated temperatures of the greenhouse. Data from inheritance of allele 1 and allele 2 in the indoor vs. outdoor plants provides at least a partial answer to these two possibilities. In the indoor population allele 1 was the 'favored' allele (Table 4). However, the outdoor population of trees showed a strong segregation distortion in the opposite direction, that is, selection for the alternative allele (Table 4). These data strongly suggest that the segregation distortion is not due to a general lethal allele at a locus near the cab locus. If that were the case, the selection should have been for the same allele in both populations. Rather, it appears that environment affect which allele is selected in these two populations.

ACKNOWLEDGEMENTS

Funding for this study provided by Northern Global Change Program (U.S.D.A. Forest Service), and the Co-Operative Forest Research Unit, University of Maine. We thank Christopher Campbell and William Livingston for their helpful comments on the manuscript, and Bill Halteman and William Livingston for their help with the statistical analysis.

LITERATURE CITED

Adams, G.W., and M.S. Greenwood. 1992. Optimization of environmental regimes for flowering in an indoor breeding hall for black spruce, white spruce and jack pine. In anon (ed): Mass production technology for genetically improved fast growing tree species. Proceedings of AFOCEL-IUFRO conference, Bordeaux, France: AFOCEL 77370 Nangis, France, pp. 219-227.

Beuker, E. 1994. Adaptation to climatic changes of the timing of bud burst in populations of Pinus sylvestris L. and Picea abies Karst. Tree Physiology 14: 961-970.

Dormling, I., and O. Johnsen. 1992. Effects of the parental environment on full-sib families of Pinus sylvestris. Can. J. For. Res. 22: 88-100.

Doyle J.J., and J.L. Doyle. 1990. Isolation of plant DNA from fresh tissue. BRL Focus 12: 13-15.

Eysteinsson, T., and M.S. Greenwood. 1993. Management of a prototype indoor orchard for accelerated breeding of larch. Orono, Maine 04469: Misc. report 377, Maine Agricultural Experiment Station, 18pp.

Highkin, H.R. 1958. Temperature-induced variability in peas. Amer. J. Bot. 45: 626-631.

Johnsen, O. 1989a. Phenotypic changes in progenies of northern clones of Picea abies (L.) Karst. grown in a southern seed orchard. II. Seasonal growth rhythm and height in field trials. Scandinavian Journal of Forest Research 4: 331-342.

Johnsen, O. 1989b. Phenotypic changes in progenies of northern clones of Picea abies (L.)Karst. grown in southern seed orchards. I. Frost hardiness in a phytotron experiment. Scandinavian Journal of Forest Research 4: 317-330.

Johnsen, O., J. Dietrichson, and G. Skaret. 1989. Phenotypic changes in progenies of northern clones of Picea abies (L.) Karst. grown in a southern seed orchard. III. Climatic damages and growth in a progeny trial. Scandinavian Journal of Forest Research 4: 343-350.

Lindgren, D., and Q. Wang .1986. Are genetic results influenced by the environment during seed maturation? In (ed): In: Proc. IUFRO conf. on breeding theory, progeny testing and seed orchards, Oct. 13-17, 1986, Williamsburg, VA, USA., pp. 192-199.

Orita M, H. Iwahana, H. Kanazawa, K. Hayashi, and T. Sekiya. 1989. Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. Proc. Natl. Acad. Sci. USA 86: 2766-2770.

Philipson, J.J. 1983. The role of gibberellin A4/7, heat and drought in the induction of flowering in Sitka spruce. J. Exp. Bot. 34: 291-302.

Ross, S.D. 1985. Promotion of flowering in potted Picea engelmanii (Perry) grafts: Effects of heat, drought, gibberellin A4/7, and their timing. Can. J. For. Res. 15: 618-624.

Schmidtling, R.C. 1987. Locating pine seed orchards in warmer climates: benefits and risks. Forest Ecology and Management. 19:273-283.