Environmental Preconditioning and Variance in Early Growth of Balsam Poplar

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Summary

To estimate effects of environmental preconditioning in balsam poplar, primary ramets of 16 clones were subjected to moisture stress and control treatments for one growing season. Secondary ramets were taken from these primary ramets, rooted and grown for 80 days. The analysis of variance was used to evaluate variation in growth due to treatment, clones, and primary ramets within treatments. Environmental preconditioning by moisture stress had a minor influence on growth of secondary ramets. However there was major variance associated with primary ramets within preconditioning treatments.

Key words: "C" effects, clones, genetic variance.

Zusammenfassung

Um den Einfluß von Umweltbedingungen auf Balsampappeln zu schätzen, wurden von 16 Klonen primäre Stecklinge geschnitten und diese für eine Vegetationsperiode unter kontrollierten Bedingungen einem Feuchtigkeitsstress ausgesetzt. Von diesen primären Stecklingen wurden wiederum sekundäre Stecklinge bewurzelt und 80 Tage lang angezogen. Daraufhin wurde mit Hilfe einer Varianzanalyse die Variation im Wachstum der Klone während der Behandlung untersucht. Die Umweltbeeinflussung durch Feuchtigkeitsstress hatte auf das Wachstum der sekundär bewurzelten Stecklinge weniger Einfluß. Bei den primären Stecklingen stellte sich jedoch die Feuchtigkeitsstressbehandlung als Hauptursache für die Variation heraus.

Introduction

Environmental preconditioning (Rowe 1964), "C" effects (Lerner 1958), or "dauermodification" (Jollos 1934,1935) all refer to variance among progeny induced by the environment under which maternal parents (or ortets in the case of clones) develop and produce progeny. In sexual progeny, "C" effects may be confounded with maternal influences unrelated to environment. For example seed size, which may be a strongly inherited maternal characteristic, is known to contribute to variance in seedling performance. In contrast to maternal effects, "dauermodification" may be expressed in several generations of progeny (e.g. Hoffman 1927, Hill 1967). It has been suggested that organelle mutation and/or organelle selection under stress may be the basis of the effect (Grun 1976). In contrast to estimating "C" effects in sexual progeny, analysis of vegetatively propagated populations is relatively straightforward, and some estimates have been reported (e.g. Went 1959, Libby and Jund 1962, Wilcox and Farmer 1968, Foster *et al.* 1984, 1985). However, though the potential importance of environmental preconditioning has been noted (Rowe 1964, Baskin and Baskin 1973), and genetic tests with forest trees are usually designed to reduce its influence, there are few published estimates of "C" effects for trees. In this study with a vegetatively propagated population of balsam poplar (*Populus balsamifera* L.) we have used moisture stress to induce "C" effects and a design that allows evaluation of variance associated with clones, with stress treatment and with differences among primary ramets in a population of secondary ramets.

Methods

In late April 1983, dormant shoots were collected from sixteen balsam poplar ortets located near Thunder Bay, Ontario (Lat. 48°35'N, Long. 88°30'W). These plants were located at least 1 km apart to reduce the probability of obtaining two plants from the same natural clone. In most cases the plants were seedlings and all were less than 4 m in height. On April 25, four plants (primary ramets) were propagated by cuttings from each of these ortets. The cuttings for these primary ramets were taken from one or two lateral branches on ortets. They were grown in 8-liter plastic pots on two adjacent benches in the center of a greenhouse where environmental variation was minimal. The ramets of each clone were randomly located on the benches. The medium was peat-vermiculite (3:1) supplemented weekly with a soluble fertilizer (20:20:20, 100 ppp) including micronutrients. Natural photoperiods were used, and the greenhouse temperature typically ranged from 25° C in the day to 18° C at night.

On June 20, 1983, two of the four ramets in each clone were randomly selected to receive a moisture stress treatment. This treatment consisted of withholding water from plants until they were wilted, then watering to saturation. Since destructive sampling was undesirable, internal water potential was not measured, but at wilting petioles were vertical and leaves had clearly lost turgidity. Watering was done on an individual plant basis, and records indicated that stressed plants were rewatered at 5- to-10 day intervals depending on plant size and clone. Control plants were watered daily. Treatment was discontinued on August 20, at which time all plants were moved out-of-doors for hardening in preparation for fall temperatures. At this time stressed plants averaged about 60 cm in height and control plants 80 cm; this difference was statistically significant at the .05 level of probability.. All plants were stored over winter in a lath house.

On March 28, 1984, eight 10-cm-long cuttings (secondary ramets) were taken from each of the four primary ramets in each clone. These cuttings were from both the main shoot and lateral branches. Four cuttings from each primary ramet were planted in each of two 8-liter pots filled with a peat-vermiculite medium supplemented with the soluble fertilizer noted above. The resulting 128 pots of secondary ramets were randomly located on a single greenhouse bench under the same environment noted above. Date of bud break was recorded for each cutting. On April 27, pots were thinned to the single rooted cutting nearest pot center after recording the number of rooted cuttings in each pot. The arc sine transformation of rooting percent for each pot was used in the analysis of variance in rooting percent. The height of the single cutting in each pot was recorded weekly until June 18, 1984, when all plants were harvested

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Table 1. — Outline of experimental design.

Source of Variation	Degrees of Freedom	Expected Mean Square
Clones	15	$\nabla_e^2 + 2\nabla_p^2 + 8\nabla_\delta^2 + 8\nabla_c^2$
Restriction Error	0	$\nabla_e^2 + 2\nabla_p^2 + 8\nabla_\delta^2$
Conditioning Treatment	1	$\nabla_e^2 + 2\nabla_p^2 + 4\nabla_{cs}^2 + 64\phi(s)$
Clone x Treatment	15	$\nabla_e^2 + 2\nabla_p^2 + 4\nabla_{cs}^2$
Primary Ramets within Treatments	32	$\nabla_e^2 + 2\nabla_p^2$
Secondary Ramets	64	∇_e^2
Total	127	

and oven dry weight of root, shoot and leaves determined. By this time roots on the larger plants had totally occupied the pots and continuation of the test would have resulted in complications due to root restriction.

An analysis of variance of the form outlined in *Table 1* was used to determine the amount of variation associated with each of the experimental factors. It is recognized that a "restriction error" in the sense of ANDERSON and MCLEAN (1974) occurs due to the fact that moisture stress treatment is randomized within clones rather than across all primary ramets. Estimates of clonal variance include its effects, if any.

Results

Bud break occurred 5 to 13 days after planting. Mean date of bud break was the same for both control and moisture stress treatment *(Table 2)*. While there was significant clonal variation in bud break date, primary ramets within clones accounted for one-half of the variance in this character. There was no relationship between the mean date of bud break of clones and their growth performance.

Rooting percent *(Table 2)* was reduced slightly by donor-plant moisture stress, but was generally high; there were no missing entries in the experimental design due to rooting failure. Primary ramets accounted for the largest amount of variance in rooting percent.

Length of the dominant new shoot on cuttings increased from an average of 3 cm on April 27, to around 60 cm at harvest on June 18, at which time clone means ranged from 37 to 79 cm *(Table 2)*. Moisture stress applied to primary ramets significantly (.05 level) influenced shoot length only in early May when it accounted for 9 percent of total variance in this character. Clonal variance in shoot length increased to 38 percent of total variance by June 18. On April 27, primary ramets did not contribute to variance in height, but this source of variation increased to 22 percent of variance by May 4, then gradually decreased to harvest date.

At harvest, clonal effects accounted for 32 to 38 percent of variance in dry weight, and moisture stress effect was nonsignifikant except for root weight. Root weight variation departed from that of top weights and total weight in that effects of primary ramets were not significant at the .05 level of probability. However, variation in the number of roots on cuttings was strongly influenced by primary ramets, which accounted for 68 percent of the variance in this character.

Discussion

As expected, we observed wide clonal variation in most of the characteristics evaluated. Moreover there was increasing dominance of clonal variance in height as the test plants developed. However, subjection of parent plants to cycles of moisture stress during development of cutting material had only a relatively small effect upon performance of vegetative progeny, though some residual treatment effects in rooting percent and early growth were observed. The main finding was that primary ramets had a substantial effect, which was apparently related to some influence not associated with moisture stress. This effect was particularly noteworthy for number of roots, percent rooting, and bud break date. There is some indication that its influence on growth was declining with time relative to clone effects. Primary ramet effects could be related either to position and/or physiological condition of cutting material on the ortet, as demonstrated by FOSTER *et al.* (1984), or to conditioning during growth of the primary ramets. Since physiologically similar cuttings from relatively juvenile ortets were used in our study, we believe that most of the primary ramet variance was due to conditioning during their development.

These results represent the first reported effort in a vegetatively propagated tree species to separate effects of an induction treatment from primary ramet effects of other origin. In the studies of WILCOX and FARMER (1968) and FOSTER *et al.* (1984) with *Populus deltoides* BARTR. and *Tsuga heterophylla* (RAF.) SARG. respectively, no environmental preconditioning treatment was imposed on primary ramets. In LIBBY and JUND's (1962) work with *Mimulus guttatus* FISCH. only single primary ramets were subjected to each of the several conditioning environments. In our study the stress treatment represented a harsh condition which would not normally be encountered in production of cuttings. Thus it was expected that such a treatment would cause greater variance relative to primary ramets within treatments. While a long-term test of primary ramet effects will be required to fully evaluate this influence of cloning, these results suggest that simply obtaining cuttings from several ramets in a relatively uniform environment may introduce considerable within-clone variance in balsam poplar. As noted by WILCOX and FARMER (1968) and FOSTER *et al.* (1984) estimates of heritability may be influenced either by failure to use primary ramets, in which case conditioning is confounded with clone, or by failure to account for primary ramets once they are used. In the case of final weight in our test, broad sense heritability based on clonal and environmental variances alone was .51, close to that (.47) reported by WILCOX and FARMER (1968) for shoot weight. However, if primary ramet variance were included in the environmental term (due to failure to account for ramets) then $h^2 = .40$. If these effects were confounded with clone (i.e. included in clonal variance) by failure to use primary

Table 2. — Summary of results.

Character	Test Mean		Range of clone means	Variance[1] due to				
	Control	Stress		Treat.	Clone	Clone x treat.	Primary Ramets	Secondary Ramets
Date of bud break, days from planting	9	9	5-13	± 0	21*	11	50*	18
Rooting percent	99	91	81-100	12*	11	8	42*	27
Shoot length, cm								
April 27	3	3	2-4	4	6*	7	± 0	83
May 4	6	5	3-8	9*	13*	1	22*	55
May 11	11	9	6-13	9*	15*	< 1	21*	54
May 25	25	23	16-32	4	27*	< 1	20*	49
June 8	46	42	28-57	4	34*	2	15*	46
June 18	61	56	37-79	4	38*	± 0	15*	43
At harvest:								
No. primary roots	11	12	7-20	± 0	14	2	68*	15
Leaf weight, g	5.47	5.06	1.64- 8.37	< 1	32*	± 0	26*	41
Stem weight, g	3.00	2.56	.88- 5.14	3	38*	± 0	25*	34
Root weight, g	.91	.77	.29- 1.73	4*	38*	± 0	8	50
Total weight, g	9.54	8.21	2.86-15.78	3	38*	± 0	22*	36

[1]) Variance components expressed as percent of total variance.
* Source of variance significant at .05 level of probability.

ramets, heritability might have been on the order of .62.

In this test, clonal differences were a major source of variance, and the treatment × clone interaction was never significant. Therefore it is unlikely that a major error in selection would occur due to neglect of primary ramet effects. Moreover, if the purpose of a clonal test is to compare genetic variances of populations (e.g. as in a provenance test), mixing of material from primary ramets should not biase estimates as long as all clones are similarly produced. However, if the purpose of investigation is to estimate genetic variance and predict genetic gain, then the effect of cloning must be accounted for in experimental design.

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Allozyme Variation Among North Carolina Populations of Liriodendron tulipifera L.[1])

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Abstract

Genetic variation among three mature-tree and six seedling populations of yellow-poplar *(Liriodendron tulipifera* L.) was investigated by analyzing six polymorphic allozyme loci. The populations analyzed were from six separate locations in North Carolina. They were found to be highly differentiated with respect to allele frequencies in both seedling and mature-tree populations. Mature-tree and seedling populations from the same location were found to have different genotypic distributions although they did not differ in allele frequencies. Frequency of homozygotes in the seedling populations was found to be greater than expected under the assumption of Hardy-Weinberg distribution of genotypes. In the mature-tree populations, however, frequency of homozygotes did not differ from Hardy-Weinberg expectations. Outcrossing rates (t) were estimated for one mountain population and two coastal plain populations and these values verify that yellow-poplar has a mixed mating system. The coastal plain populations had substantially lower values than the mountain population (t = 0.55 vs. t = 0.86) indicating that mating behavior varies among populations.

Key words: Population differentiation, outcrossing rate, yellow-poplar, isozyme, genotypic distribution, allele frequency.

Zusammenfassung

Es wurde die genetische Variation zwischen 3 Altbaum- und 6 Sämlingspopulationen von *Liriodendron tulipifera* L. untersucht, indem 6 polymorphe Allozym-Loci analysiert wurden. Die untersuchten Populationen stammten von 6 separaten Standorten in Nord-Carolina. Es wurde herausgefunden, daß sie hinsichtlich der Allozymfrequenzen sowohl bei den Sämlings- als auch bei den Altbaumpopulationen stark unterschiedlich sind. Altbaum- und Sämlingspopulationen vom gleichen Standort hatten unterschiedliche genotypische Verteilungen, obwohl sie sich in den Allozymfrequenzen nicht unterschieden. Die Häufigkeit der Homozygoten in den Sämlingspopulationen war größer, als unter der Voraussetzung der Hardy-Weinberg-Verteilung für Genotypen erwartet wurde. Für eine Gebirgspopulation und zwei Populationen in der Küstenebene wurde die Fremdungsrate (t) geschätzt, wobei diese Werte bestätigen, daß *Liriodendron tulipifera* ein gemischtes Kreuzungsschema hat. Die Küstenpopulationen hatten erheblich geringere Werte als die Gebirgspopulation (t = 0,55 gegenüber t = 0,86), was anzeigt, daß das Kreuzungsverhältnis stark variiert.

Introduction

Forest tree species are commonly reported to be polymorphic with multiple alleles at many electrophoretically variable enzyme loci (Tigerstedt, 1973; Lundkvist and Rudin, 1977; Yang *et al.*, 1977; Hamrick *et al.*, 1979; Lundkvist, 1979). Information about the genetic structure of tree populations is therefore available and can be used to infer the evolutionary history of trees. The patterns of allele frequency variations among and within populations can indicate if stands are genetically divergent or essentially uniform, and whether the variation patterns are consistent in different areas or in succeeding generations.

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