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GENETIC AND ENVIRONMENTAL VARIATION IN AN INTENSIVELY CULTURED BLACK LOCUST (*Robinia pseudoacacia* L.) HALF-SIB PROGENY TEST

By

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ABSTRACT

GENETIC AND ENVIRONMENTAL VARIATION IN AN INTENSIVELY CULTURED BLACK LOCUST (*Robinia pseudoacacia* L.) HALF-SIB PROGENY TEST

By

Abibou Gaye

Based on results from a germination test in the laboratory involving 16 half-sib families of black locust, 5 fast-growing and 5 slow-growing families were used in a nursery progeny test intensively cultured to evaluate early genetic differentiation in height, diameter, number of branches, thorn length, insect susceptibility, stem form and photosynthetic efficiency. Also, the relationships between nursery characteristics and 4-year field height were investigated.

The variation patterns for all characteristics indicate large within-family variation compared to family variation; but family heritabilities were much higher than within-family heritabilities suggesting that substantial genetic gains can be made from family and within-family selections. On the other hand, although 4 good families out of 5 figured among the 5 top ranking families in height and diameter in the nursery, the poor correlations observed between nursery traits and 4-year field height did not suggest reliable prediction of future performance.

The effects of seed weight and the implications of the practice of top-pruning black locust seedlings prior to field planting on seedling development were analyzed and discussed.

To my son Papa A.M. Gaye (four years old) who did not understand why I was separated from him for two and one-half years.

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INTRODUCTION

Black locust is one of the most widely planted species around the world (acclimated in most temperate and mediterranean zones of the world) because of its remarkable attributes (Table 1), its ability to utilize a wide range of sites and its multiple uses such as timber, posts, firewood, apiculture, fodder and erosion control.

The species is frequently propagated by seed for economic reasons, but can be easily propagated by root cuttings (Swingle, 1937) and by tissue culture (Keresztesi, 1983; Davis and Keathley, 1987), and thus, its potential for further improvement through cloning and hybridization is great. However, in the U.S.A. where it is native, little attention has been paid to the silviculture and breeding of the species for commercial purposes until recently when fears of petroleum shortage prompted biomass energy research. Thus, comprehensive germplasm collections of half-sib families from the natural and naturalized range in the USA and southeast Canada have been made by Michigan State University (MSU) and the University of Georgia since 1983. Analyses of growth and phenological characteristics from provenance tests in Georgia (Kennedy, 1983) and progeny tests in Michigan (Mebrahtu and Hanover, 1989) have shown no geographic patterns of genetic variation but revealed variation among and especially within families. Therefore, it has been suggested that there is a need for progeny tests to select among and within the populations for genetic improvement of the species

Table 1. Physiological attributes of black locust.

- Rapid growth rate, out-competes weeds
- Indeterminate growth habit
- Nodulated roots, fixes atmospheric N_2
- High density wood
- Good pulping qualities
- Highly resistant to decay fungi
- Tolerates low fertility sites
- Resistant to drought stress
- Resistant to air pollutants
- Resistant to low temperatures
- Resistant to high temperatures
- Very high net photosynthetic rates
- High light saturation
- High leaf area accretion rate
- Long leaf retention time
- Low stomatal diffusive resistance
- High transpiration rate
- Rapid leaf position adjustment to changes in light intensity
- Small leaflets minimize self-shading
- Vigorous sprouting of root cuttings
- Very plastic root system: strong tap and dense fibrous upper roots
- Flowers at early age
- Produces abundant seed crops
- High seed viability and longevity
- Seeds easily cleaned, stored, sown
- Seeds germinate rapidly
- Easily micropropagated
- High leaf protein
- Much genetic variation

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for traits reflecting growth characteristics, stem form and damaging agents.

Since tree breeding, usually achieved through progeny testing, is a long-term process requiring labor, space and money, the basic objective of tree breeders has always been to achieve selection as early as possible, i.e., before the economic maturity of the trait of interest, resulting in shortening the breeding cycle and hence, reduction of direct costs (Magnussen, 1989a; Namkoong et al., 1988). Thus, early selection, particularly in species such as black locust exhibiting substantial growth at early stages of development, will result, if proven efficient, in maximum genetic gain per unit of time (Magnussen, 1989a; Namkoong and Conkle, 1976).

Keeping this general strategy in mind, the following study was designed to investigate the response of black locust open-pollinated families to early selection through laboratory and nursery trials and comparisons with field performance by pursuing these specific objectives:

1. To determine the kind and amount of genetic variation for seed germination, growth, insect damage, stem form and physiological (photosynthesis) characteristics in 10 open-pollinated, progeny tested seed sources of black locust.
2. To examine the relationships between seedling growth characteristics in the nursery and seed weight, seed germination, field performance (first-year, second-year and fourth-year height).
3. To discuss the extent to which nursery bed selection may be reliable with regard to studied traits.

The trait emphasized in the study was height growth because it is a primary component of commercial growth and its pattern changes can be readily analyzed (Namkoong and Conkle, 1976); but since it can be associated with undesirable characteristics (thorniness, damaging agents, poor form), attempts were also made to assess the relationships between growth and other characteristics. Indeed, characteristics can be closely or loosely, positively or negatively interrelated and thus, progress from selection is dependent on the nature and extent of the interrelationships found between characteristics (Matziris and Zobel, 1973).

SECTION I.

FAMILY VARIATION IN SEED CHARACTERISTICS OF BLACK LOCUST (*Robinia pseudoacacia* L.).

ABSTRACT

Eight fast-growing and 8 slow-growing half-sib seedlots of black locust were used in a germination test for the purpose of selecting 10 rapid germinating families (5 good and 5 poor) to be used in a nursery progeny test.

Five days after sowing (following pretreatment of seeds in H_2SO_4 for 50 minutes), the germination percentages per family ranged from 47.70% to 98.17% with 88% versus 83.60% for good and poor families. The ANOVA showed significant differences among families ($P<0.01$) but no statistical differences were found between good and poor families. On the other hand, the correlation results indicate that seed weight did not, apparently, influence seed germination.

INTRODUCTION

For tree breeding to be successful, rapid and uniform germination of seed in the nursery is required; that is, factors influencing germination such as seed dormancy, seed characteristics (size, weight, age) and genetic factors need to be identified. For hard seeds such as those of black locust, it is well known that the primary factor responsible for delayed germination is the dormancy induced by an impervious seed coat rendering seed difficult to germinate under favorable conditions of temperature and moisture unless pretreatments (water, acid, or mechanical) are done to soften the seed coat prior to

sowing. However, aside from the seed coat dormancy, seed characteristics (seed size, seed weight, age) and inherent factors may control the rate of seed germination and thus represent potential factors in delaying or accelerating seed germination.

The following germination test was conducted in the laboratory for the purpose of selecting among 16 seedlots, 10 rapid germinating seed sources to be used for direct seeding in the nursery. We also wanted to determine the relationships between seed germination percentage and seed weight.

MATERIAL AND METHODS

Sixteen half-sib seedlots were used in the germination test and comprised 8 known fast-growing families (good families) and 8 known slow-growing families (poor families), distinguished on the basis of height measurements made in 1989 from a progeny test including 393 families established in East Lansing in 1985. From the height measurements (1989) the top third and the bottom third families were considered, respectively, as good and poor performers in the field. The respective height ranking from the progeny test of the 16 families used in this study is given in Table 2. All seedlots used for the germination test were collected in 1984 except for 2 seedlots which were collected in 1989 (Table 2) but from the same trees in which seed were collected in 1984. Seedlots were stored in paper bags at 1°C.

On May 15, seeds from each of the 16 seedlots were pretreated by immersion in concentrated H_2SO_4 for 50 minutes, then removed and rinsed thoroughly with water, and set to germinate in trays containing moist cotton covered by filter paper. The test was carried out with two

Table 2. Seedlot characteristics and 4-year field height for 16 black locust open-pollinated families.

Seed-lot	Type[1/]	Date of Collec-tion	Weight of 100 seeds (g)	Total No. of Seeds Tested	4-Year Field Mean (cm)	Rank for Height[a]	Rank for Height[b]
282	GF	2-84	2.09	132	543.33	3	25
344	GF	2-84	1.94	124	551.66	2	19
348	GF	2-84	2.88	100	518.75	4	27
380	GF	11-89	2.06	134	602.00	1	14
382	GF	2-84	2.48	134	510.00	7	45
384	GF	2-84	2.24	119	483.86	8	15
385	GF	2-84	2.10	147	518.33	5	46
391	GF	2-84	1.92	146	511.25	6	56
310	PF	2-84	1.75	141	378.83	14	312
347	PF	2-84	2.08	125	396.66	13	230
373	PF	2-84	1.69	115	369.43	16	280
375	PF	2-84	1.80	137	372.50	15	302
383	PF	11-89	1.70	166	417.50	11	270
398	PF	2-84	1.68	169	465.00	9	214
408	PF	2-84	1.68	157	417.47	12	320
411	PF	2-84	1.73	143	425.00	10	252

[1/]GF, PF = good families, poor families, respectively, based upon 4-year height growth in the field.

[a] = ranking for the 16 families.

[b] = ranking for the 16 families relative to the 393 families of the progeny test.

replications at 25°C and continuous light. Germinants were counted during 3 consecutive days beginning 2 days after sowing to furnish the data for the calculation of germination rates. Count of germinants were stopped the 3rd day because germination was nearly completed in most seedlots. In addition, for each seedlot, 100 seeds were weighed to estimate seed weight.

Percent germination data were subjected to an ANOVA using replication means following arcsine transformation of the percentages. Duncan's multiple range test and the family mean heritability were calculated as well as correlations associating rank and means of family seed weight with, respectively, rank and means of family percent germination.

RESULTS AND DISCUSSION

Variation in seed germination

Mean percent family germination is given in Table 4 and illustrated in Figure 1. Five days after sowing, germination percentages per family ranged from 47.70% to 98.17% around a general mean of 85.88%, indicating a high germination rate which is highly desirable for nursery production. Seed from the 8 good families had a higher germination rate than those of the 8 poor families (88.00 vs. 83.60%), but in the 10 families selected for the nursery test (Table 4) the 5 poor families had a slight advantage in germination over the 5 good families (90.50 vs. 88.50%) because 2 rapid germinating good families were not retained for the nursery test due to lack of available seed.

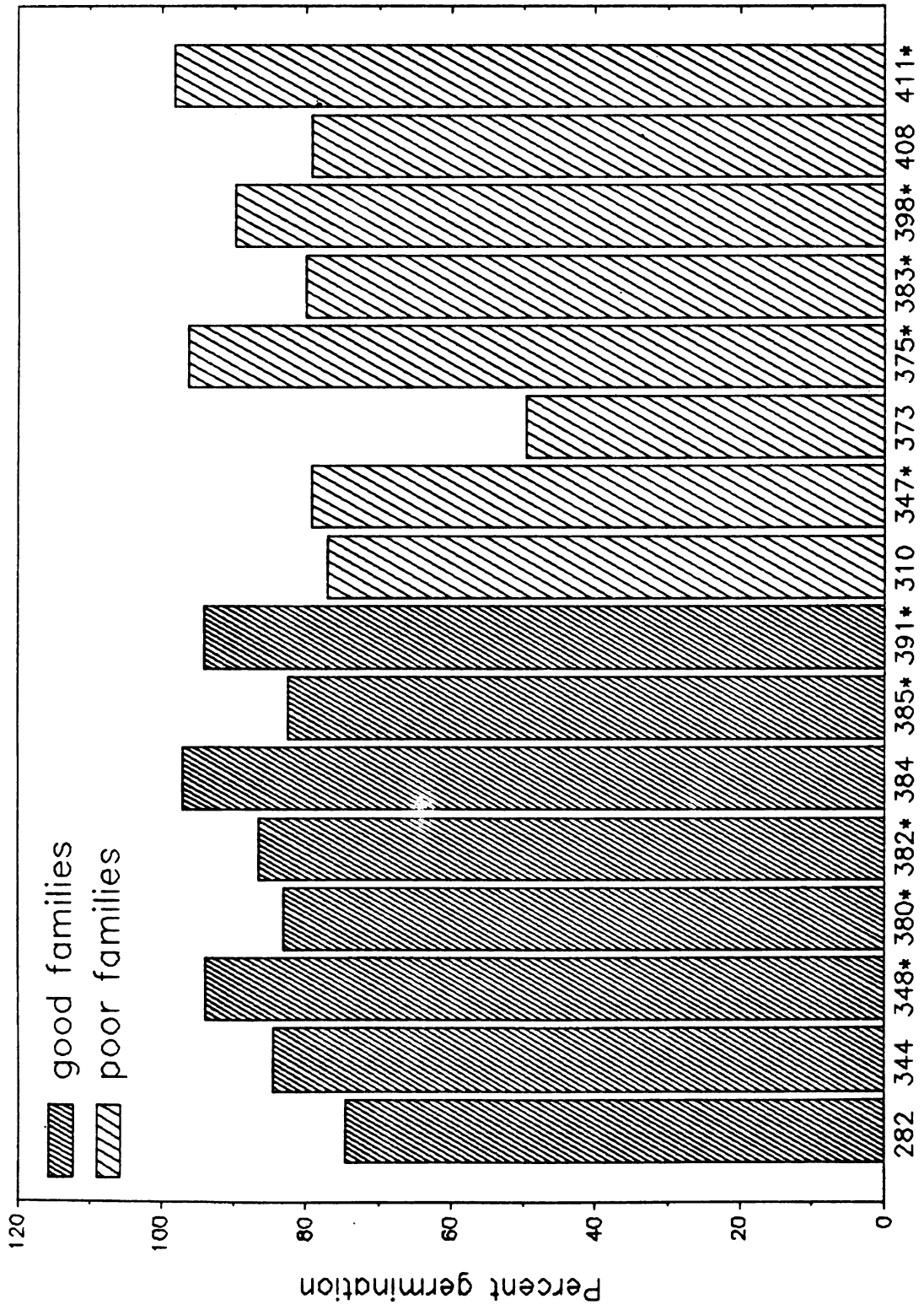


Figure 1. Percent germination for 10 seedlots of black locust, 5 days after sowing in the laboratory. * = families used in the nursery test.

The ANOVA (Table 3) showed significant differences among families ($P<0.01$ for the 16 families and $P<0.05$ for the 10 families) but no differences were found between good and poor families either at the level of the 16 families or at the level of the 10 families. Family differences accounted for about 75% (16 families) or 63% (10 families) of total variation, explaining the high family heritability observed (Table 3). The decrease in family heritability in the 10 families versus the 16 families is due to the decrease of genetic variability in the 10 families as a result of selecting for higher germination rates. The error variance accounted for the remaining variation since variation due to replication was negative and hence, estimated to be zero.

Effect of seed weight

Average seed weight by family varied from 1.68 g/100 seeds (16.8 mg/seed) to 2.88 g/100 seeds (28.80 mg/seed) with a general mean of 1.98 g/100 seeds (19.80 mg/seed). This is somewhat consistent with the results reported by Pathak et al. (1978) who found a variability ranging from 11.02 mg/seed to 26.73 mg/seed in 5 seedlots of black locust. The good families outweighed the poor families in either the 16 families tested or the 10 families selected for the nursery trial (Table 4).

The correlation coefficients calculated between average seed weight or rank seed weight per family and mean percent germination or rank germination per family (Table 4) were low and either positive (16 families) or negative (10 families) suggesting that seed weight did not, apparently, influence seed germination. In contrast seed weight is reported to influence strongly seed germination in sweet gum, white spruce and slash pine (Franklin, et al. 1981), and in bald cypress (Faulkner and Toliver, 1983).

Table 3. Summary of ANOVA for percent germination (in parenthesis, percent of total variation), and correlations among seed germination and seed weight in black locust open-pollinated families, and expected mean squares.

	% germination for all 16 families				% germination for 10 related families			
	df	mean square	F test	variances	df	mean square	F test	variances
Replication	1	22.57	0.84 ns	negative (0)	1	9.632	0.42 ns	negative (0)
Family	15	185.03	6.91**	79.12 (74.71%)	9	101.122	4.44*	39.18 (63.26%)
Good vs. Poor	1	--	ns	--	1	--	ns	--
Error	15	26.78	--	26.78 (25.59%)	9	22.759	--	22.75 (36.74%)
Family heritability (h^2f)	0.855				0.774			
Correlations: mean seed germination vs. seed weight	0.256				0.072			
rank seed germination vs. rank seed weight	0.235				0.238			

ns = not significant * = significant at 0.05 level of probability ** = significant at 0.01 level of probability

Expected mean squares for the ANOVA

Source	EMS
Replication (r)	$\sigma^2_w + f\sigma^2_r$
Family (f)	$\sigma^2_w + r\sigma^2_f$
Error	σ^2_w

Table 4. Mean seed germination, mean seed weight and ranking of black locust open-pollinated families.

	All 16 Families				10 Families Used In Nursery Tests			
Seedlots	**% germination**			**Seed weight(g)**	**% germination**			**Seed weight(g)**
	mean		**rank**	**100 seed**	**rank**		**rank**	**100 seed**	**rank**
411*	98.17	a	1	1.73	12	98.17 a	1	1.73	8
384	97.00	ab	2	2.24	3				
375*	96.33	abc	3	1.80	10	96.33 ab	2	1.80	7
391*	94.00	abcd	4	1.92	9	94.00 abc	3	1.92	6
348*	93.96	abcd	5	2.88	1	93.96 abc	4	2.88	1
398*	89.68	abcde	6	1.68	15	89.68 bcd	5	1.68	10
382*	86.50	bcde	7	2.48	2	86.50 bcd	6	2.48	2
344	84.56	cde	8	1.94	8				
380*	83.12	de	9	2.06	7	83.12 cd	7	2.06	5
385*	82.50	de	10	2.10	4	82.50 cd	8	2.10	3
383*	80.00	de	11	1.70	13	80.00 d	9	1.70	9
347*	79.25	e	12	2.08	5	79.25 d	10	2.08	4
408	79.18	e	13	1.68	15				
310	77.00	e	14	1.75	11				
282	74.62	e	15	2.09	5				
373	49.50	f	16	1.69	14				
All families	85.88			1.98		89.60		2.053	
Good families	88.00			2.21		88.51		2.308	
Poor families	83.60			1.76		90.50		1.798	

*Families used in the nursery test.
Means with the same subscript are not different at the 0.05 level of probability using Duncan's multiple range test.

CONCLUSION

It appears that, when properly pretreated and allowed to germinate under favorable conditions, seed of black locust will germinate well and rapidly but will also exhibit significant differences among genotypes. Nevertheless, the data failed to show significant differences in germination among the known fast-growing and slow-growing families. The results also indicate that percent seed germination is quite independent of seed weight.

Thus, seed weight will influence the early performance of black locust seedling height growth but the effects are random with respect to longer term performance of progenies. Therefore, seed weight cannot be used to adjust for first-year growth rate when comparing genotypes for early performance.

SECTION II.

FAMILY AND WITHIN-FAMILY VARIATION IN EARLY SEEDLING GROWTH OF BLACK LOCUST (*Robinia pseudoacacia* L.)

ABSTRACT

Ten genotypes (5 good and 5 poor) of black locust were progeny tested in the nursery to: (1) investigate early genetic differentiation in height, diameter, number of branches and thorn length; and (2) assess the relationships between nursery growth traits, seed weight and 4-year field height.

Total height in the nursery averaged 99 cm to 128.8 cm per family with 114.3 cm and 110.6 cm, respectively, for good and poor families. However, the ANOVA detected weak family differences ($P<0.1$) and none for good versus poor families. On the other hand, family differences were much stronger in diameter ($P<0.05$) and number of branches ($P<0.01$). The variation patterns for all growth traits were characterized by relatively little family variation and a large amount of within-family variation; but, the family heritabilities were high compared to within-family heritabilities.

Seed weight showed significant relationships with diameter, number of branches and thorn length in the nursery and exerted an important effect on 4-year field height, masking thus, variation in inherent vigor. Therefore, selection should be delayed until after seed effects have ceased.

Results also indicate poor correlations between nursery growth traits of the 10 tested families and 4-year field height of the same 10 families although 4 good families out of 5 were included in the 5 top

ranking families in the nursery. However, these poor correlations are thought to result from the top-pruning of seedlings prior to field planting.

INTRODUCTION

Sluder (1983), Robinson and Van Buijtenen (1979) have reported various studies based on conifers (Zobel et al., 1977; Zaeger 1965; Hunt 1967; Grisby 1975) showing that substantial improvement in growth rate can be made by selecting outstanding nursery seedlings. However, these studies were based on mass selection, i.e., selection of best seedlings regardless of seed sources, which is different from selection among families in progeny tests (Sluder 1983) where genetic effects are to be separated from environmental effects. Thus, as pointed out by Rehfeldt (1983), reliable results in the nursery will require intense cultivation (weeding, spraying, fencing) to control extraneous environmental variation in order to test under conditions in which the phenotype is more a function of the genotype than the environment. But, since genes involved in trait expression in one age or environment may not be the same in another age or environment (Namkoong et al. 1988) assessing the degree of relationships existing between early and later plant characteristics is required to determine whether or not early seedling characteristics can reliably predict future growth.

To my knowledge, genetic studies of black locust based on nursery progeny tests have not been done, and thus, this following study is intended to provide a necessary basis for forecasting relationships between juvenile and mature characteristics.

MATERIAL AND METHODS

Nursery procedures

Seed from the 10 seedlots selected for the nursery test were hand-sown (June 15) after stratification (50 minutes in H_2SO_4) across nursery beds in a single soil series characterized by good internal drainage at the Tree Research Center at Michigan State University. The experimental design was a RCBD with 4 replications of 80-spot rectangular plots (4 rows of 20 sowing spots each) and spacing was 45 x 33 cm within-plot and 110 cm between plots. The plots were fumigated and leveled prior to sowing; the sowing depth was approximately 2 mm and about 2 seeds were used per sowing spot, i.e., 160 seeds per plot or 6400 seeds total.

A germination count conducted 7 days after sowing (June 23) and based on well established seedlings (at least 2 leaves well developed) has shown good results and thus, consistent with the germination test results from the laboratory. A week later seedlings were thinned to one per emplacement and few transplants were made.

Throughout the growing season, the plots were hand-weeded, sprayed with insecticides and watered when necessary, and protected by a fence. However, blocks 3 and 4 were attacked and apparently randomly damaged by rabbits a few days after thinning, prompting the reinforcement of the protective fence around the nursery experiment. Though most of the seedlings maimed by rabbits flushed again, only about 50 seedlings out of 80 per plot (> 2000 total) were selected for observations and measurements by excluding the disadvantaged seedlings (damaged seedlings plus transplants).

Characteristics measured

Height measurements were made to the nearest centimeter at 40 days (July 26), 63 days (August 18), 82 days (September 6), 108 days (October 2) and 137 days (October 31) after sowing with the last measurement occurring after the seedlings had shed their leaves. Also after the growing season, diameter was measured to the nearest millimeter at ground line, the number of branches per seedling counted, and thorn length scored by a visual estimate according to the following procedure: 0 = ≤3 mm; 1 = 3 to 10 mm; 2 = > 10 mm.

Statistical and genetical analyses

All growth traits measured on seedlings were subjected to an ANOVA on an individual seedling basis, assuming the following random linear model:

$y_{ijk} = \mu + R_i + F_j + (RF)_{ij} + E_{ijk}$ where

y_{ijk} = performance of individual k from population j in block i;

μ = overall mean;

R_i = effects of the ith replication (i = 1, 2, ... 4);

F_j = effects of the jth family (j = 1, 2, ... 10);

$(RF)_{ij}$ = effects of the interaction between the ith replication and the jth family;

E_{ijk} = deviation of seedling k from the effect of population j in replication (= within-plot error).

With the assumptions that families are half-sib and that epistasis and dominance are ignored (Namkoong et al. 1988), then family effects are considered to estimate 1/4 of additive genetic variance, and within-

plot error effects 3/4 of additive genetic variance plus environmental variance. The form and expected mean squares of the ANOVA as well as the expected mean cross products of the Analysis of Covariance (ANCOVA) are given in Table 5.

Variance estimates (replications, family, family by replication interaction and within-plot) and narrow sense individual, family and within-family heritabilities were calculated and means were separated by using Duncan's multiple range test whenever the ANOVA appeared significant at 0.05 level of probability. In addition, total (individual), between family, within-family and additive genetic correlations relating growth characteristics were calculated as well as family mean correlations associating growth characteristics with seed germination, seed weight and field height (1-, 2- and 4-year).

Expected genetic gains were not estimated because the heritability values were based on few genotypes growing on a single site rendering their use in predicting genetic gains of limited value (Hicks et al. 1977).

All analyses were made using the Statistical Analysis System (SAS Institute Inc., 1985) and figures were obtained from Plotit (Eisensmith 1985).

RESULTS

I. Height growth

1. Patterns of height growth

Figure 2 illustrates the estimates of daily growth rates as presented in Table 6 and can be summarized as follows: From June 15

Table 5. Analysis of variance (ANOVA) model and expected mean squares (EMS) of ANOVA and analysis of covariance (ANCOVA) based on individual tree data.

ANOVA FORM				EMS	
Source	**df**	**MS**	**F test**	**ANOVA**	**ANCOVA**
Replications (r)	r-1	MSR	MSR/MSFR	$\sigma^2_w + t\sigma^2_{fr} + rf\sigma^2_r$	$\sigma_{w_1w_2} + t\sigma_{fr1fr2} + ft\sigma_{r1r2}$
Family (f)	f-1	MSF	MSF/MSFR	$\sigma^2_w + t\sigma^2_{fr} + rt\sigma^2_f$	$\sigma_{w_1w_2} + t\sigma_{fr1fr2} + rt\sigma_{f1f2}$
Rep x family (fr)	(r-1)(f-1)	MSFR	MSFR/MSW	$\sigma^2_w + t\sigma^2_{fr}$	$\sigma_{w_1w_2} + t\sigma_{fr1fr2}$
Within plot error (t)	rf(t-1)	MSW	--	σ^2_w	$\sigma_{w_1w_2}$
TOTAL	rft-1				

r = number of replications
f = number of families
t = harmonic mean number of seedlings/plot

Adapted from Namkoong et al. (1988).

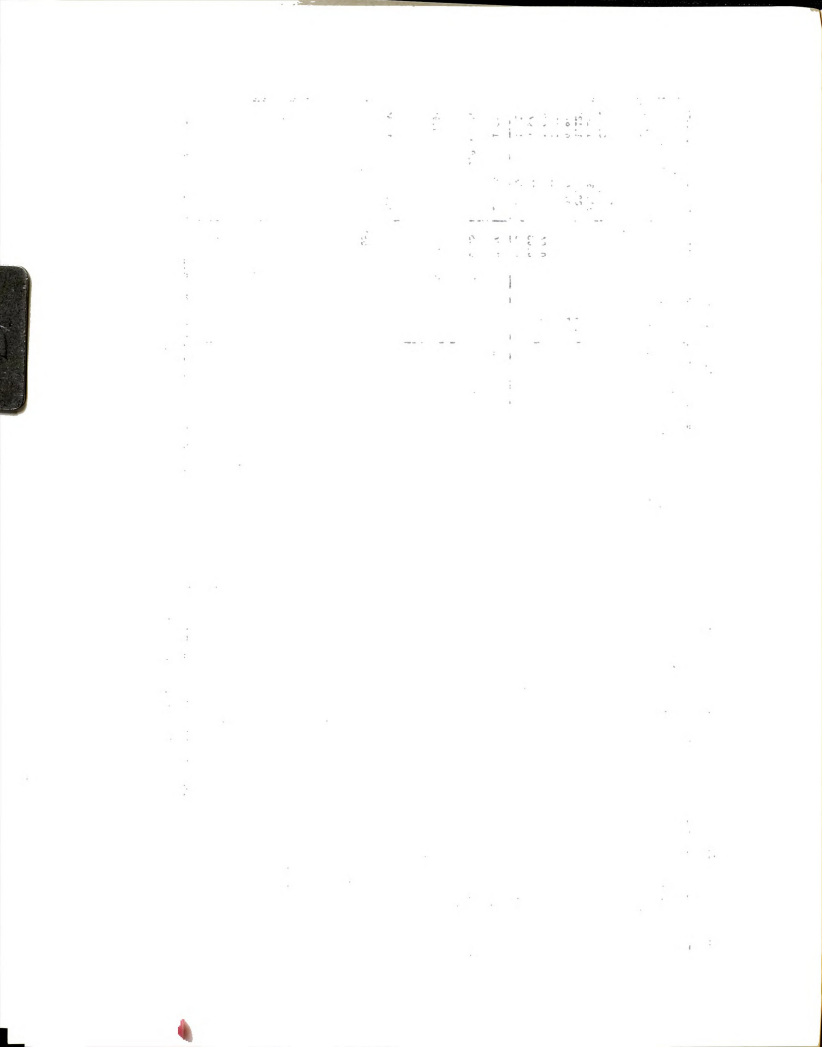
(sowing date) to late July, height growth was relatively slow (2.6 mm to 3.45 mm per day per family); then it increased rapidly during August and peaked at early September (18 mm to 24 mm per day per family) before decreasing sharply and nearly ceasing in late September. The residual growth observed in October averaged less than 1/2 mm per day per family. The slow growth rates observed during July may have been due to adjustment of the genotypes in the environment and/or development of an efficient root system prior to aerial expansion.

Overall, the daily mean growth per family varied from 9.30 to 11.80 mm around a general mean of 10.42 mm. The known fast-growing families (good families) average 10.58 mm per day versus 10.26 mm per day for the known slow-growing families (poor families) while the individual tree values varied from 1.44 mm to 19.5 mm per day. The residual growth accounted for little in total growth since, at the end of September, the proportion of total height growth completed by families ranged from 97.60 to 99.50% with 98.80% versus 98.50%, respectively, for good and poor families. Similar patterns of height growth in natural conditions were reported on black locust by Jester and Kramer (1939) whose results from greenhouse and field experiments showed that long days allow continuous growth while short days result in onset of dormancy in black locust. Wareing (1954 in Kennedy 1983) speculated that the photoperiodic receptor, growth inhibitor substance, is manufactured in mature leaves under short day conditions. However, Waisel and Fahn (1965 in Kennedy 1983) indicated that cambial activity was influenced primarily by T°C. The cambium appeared to be

Table 6. Total and daily height growth rate (cm) in a black locust nursery progeny test.

Family	Ht_1 (41) (June 15-July 26)		Hi_1(23) (July 27-Aug 18)		Hi_2(19) (Aug 19-Sept 6)		Hi_3(26) (Sept 7-Oct 2)		Hi_4(29) (Oct 3-Oct 31)		THi(97) (July 27-Oct 31)	
	Total	rate/day	Total	rate/day	Total	rate/day	Total	rate/day	Total	rate/day	Total	rate/day
347	13.84	0.346	32.02	1.392	46.61	2.453	35.84	1.378	0.614	0.021	115.02	1.185
348	12.39	0.309	30.33	1.318	41.29	2.173	26.93	1.035	1.26	0.043	99.55	1.026
375	12.40	0.310	31.61	1.374	38.50	2.026	24.18	0.930	1.46	0.050	95.89	0.988
380	12.21	0.305	34.56	1.502	43.23	2.275	32.19	1.238	1.25	0.043	111.39	1.148
382	11.75	0.293	27.91	1.213	40.65	2.139	39.05	1.501	1.49	0.051	108.99	1.123
383	7.65	0.191	21.61	0.939	36.07	1.898	31.06	1.194	1.72	0.059	91.41	0.942
385	9.50	0.237	20.30	0.882	36.44	1.917	32.17	1.273	1.26	0.043	90.83	0.936
391	11.91	0.297	27.42	1.192	39.14	2.060	34.42	1.323	1.16	0.040	102.16	1.053
398	10.25	0.256	25.24	1.097	40.48	2.130	27.90	1.073	0.959	0.033	94.80	0.977
411	10.48	0.262	28.11	1.222	41.91	2.205	26.90	1.034	0.904	0.031	98.72	1.017
Means	11.32	0.283	28.06	1.220	40.54	2.133	31.07	1.195	1.199	0.041	101.15	1.042
Range of family means		0.191-0.346		0.882-1.50		1.89-2.45		0.930-1.50		0.021-0.059		0.936-1.18
Range of individual trees		0.025-0.775		0.00-3.26		0.105-4.31		0.00-2.61		0.00-1.41		0.144-1.95
Good families: means	11.57	0.289	28.15	1.22	40.17	2.11	32.98	1.26	1.28	0.044	102.68	1.058
range of family means		0.297-0.309		0.882-1.502		1.91-2.27		1.03-1.32		0.040-0.051		0.936-1.148
range of individual trees		0.025-0.725		0.00-3.26		0.105-4.315		0.00-2.61		0.00-1.41		0.24-1.95
Poor families: means	11.06	0.276	27.96	1.21	40.90	2.15	29.13	1.120	1.10	0.038	99.58	1.026
range of family means		0.262-0.346		0.93-1.39		1.89-2.45		0.93-1.37		0.021-0.059		0.942-1.185
range of individual trees		0.025-0.775		0.00-3.08		0.157-3.789		0.00-2.61		0.00-1.000		0.144-1.917

Ht_1 = initial height Hi( ) = height increment(interval in days) THi = total height increment



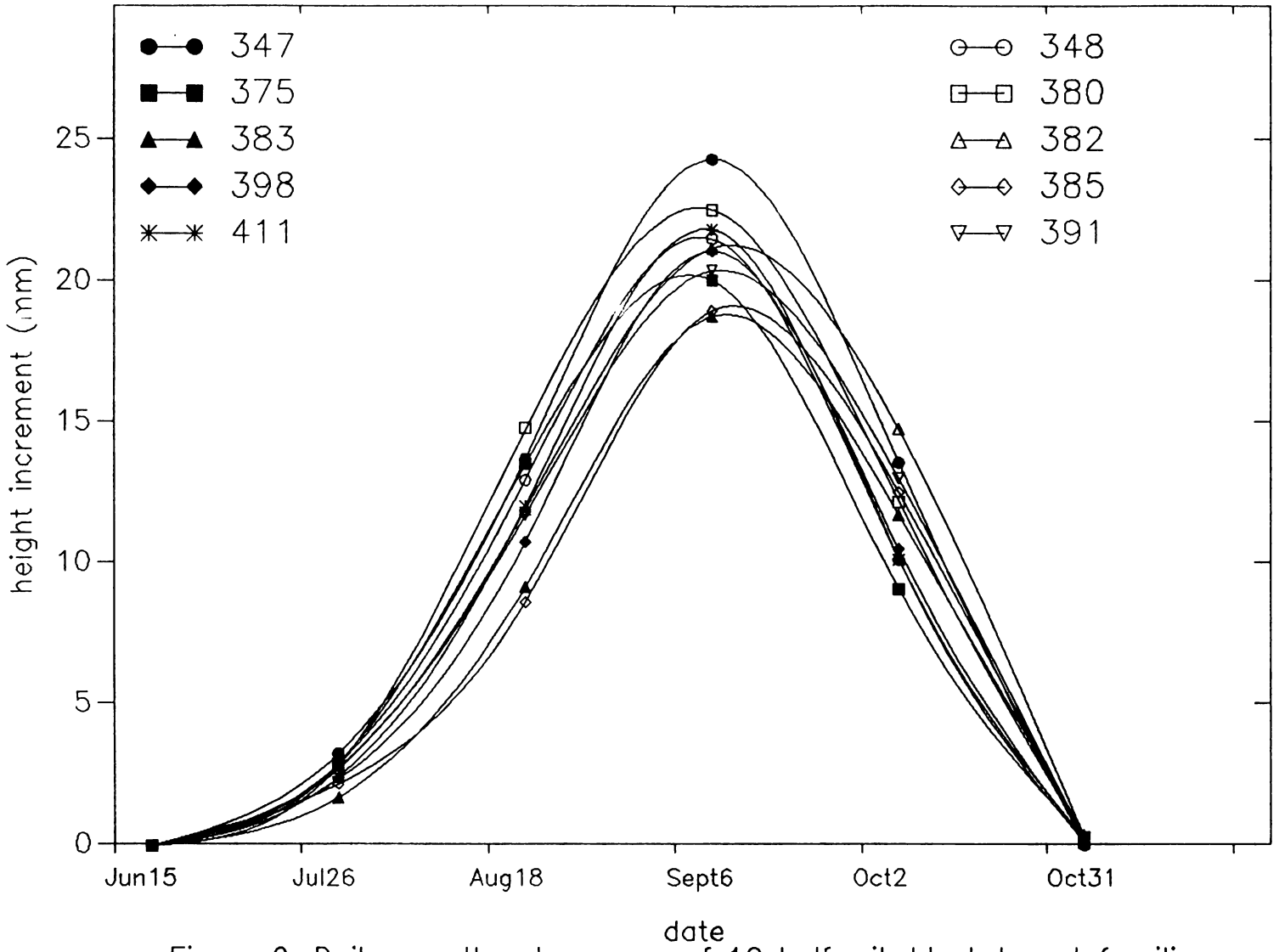


Figure 2. Daily growth rate curves of 10 half-sib black locust families, from sowing to end of growing season, in a nursery progeny test. Good families = 348,380,382,385,391; Poor families = 347,375,383,398,411.

active under short day conditions and 28°C/20°C day/night temperatures, and became dormant under 18°C/12°C day/night temperatures. Therefore, the effect of short day photoperiod in inducing dormancy in black locust may be inhibited by T°C.

On the other hand, Figure 3 illustrates the family growth curves and their rank changes over time. Growth curves are similar in shape (sigmoid) and are characterized throughout the growing season by fluctuations in rankings, indicating that the families did not perform consistently relative to each other over time. However, the rank correlations (Table 8) performed to quantify the degree of association between family mean rankings of height at different measurement ages proved significant in all situations. Further, the correlations increased in significance over time indicating that rankings were more steadily maintained specially after 82 days of age. In fact, as indicated in Table 7, the patterns of rank changes showed a certain stability in family ranking throughout the growing season: 2 families (1 good and 1 poor family) were consistently at the top level whereas 3 families (1 good and 2 poor families) were consistently at the bottom level.

2. Variance trends and heritability estimates

2.a. *Variance trends*. The results of the analysis of variances performed on all height measurement ages are given in Table 9. There were highly significant differences for replications and family by replication interactions at all ages whereas significant but small differences among families ($P<0.1$) were detected only after 82 days of seedling age while the good and the poor families showed no significant

differences at all ages (Figure 4). For the residual growth, only family x replication interaction showed significant ($P<0.05$).

Variance components are given in Table 9 and their trends were plotted over age to examine their pattern changes (Figure 5a).

2.a.1. *Family variation*. The main effect of family increased steadily through time, accounting for 0 to 3.03% of total variation. It reached its maximum absolute value at age 108 days coinciding with the end of the active growing season, and then declined slightly during the residual growth period to account finally for 2.76% of total height variation.

On the other hand, the within-family variation contributed without exception for most of the variation associated with height at all ages. It accounted for 42.6% to 71.35% of the total variation and its trends appeared similar to those of the main effect of family variance. Within-family variation includes both environmental factors (microsites) and additive genetic effects that cannot be separated, but in segregating families a sizeable genetic component can be expected (Ying and Morgenstern, 1979). That is, if family selection is to be made in the nursery stage it should be followed by within-family selection in order to achieve more genetic gain.

These results on family variation are consistent with previous findings on black locust showing relatively little family variation and a large amount of variation within-family (Kennedy 1983; Mebrahtu and Hanover 1989).

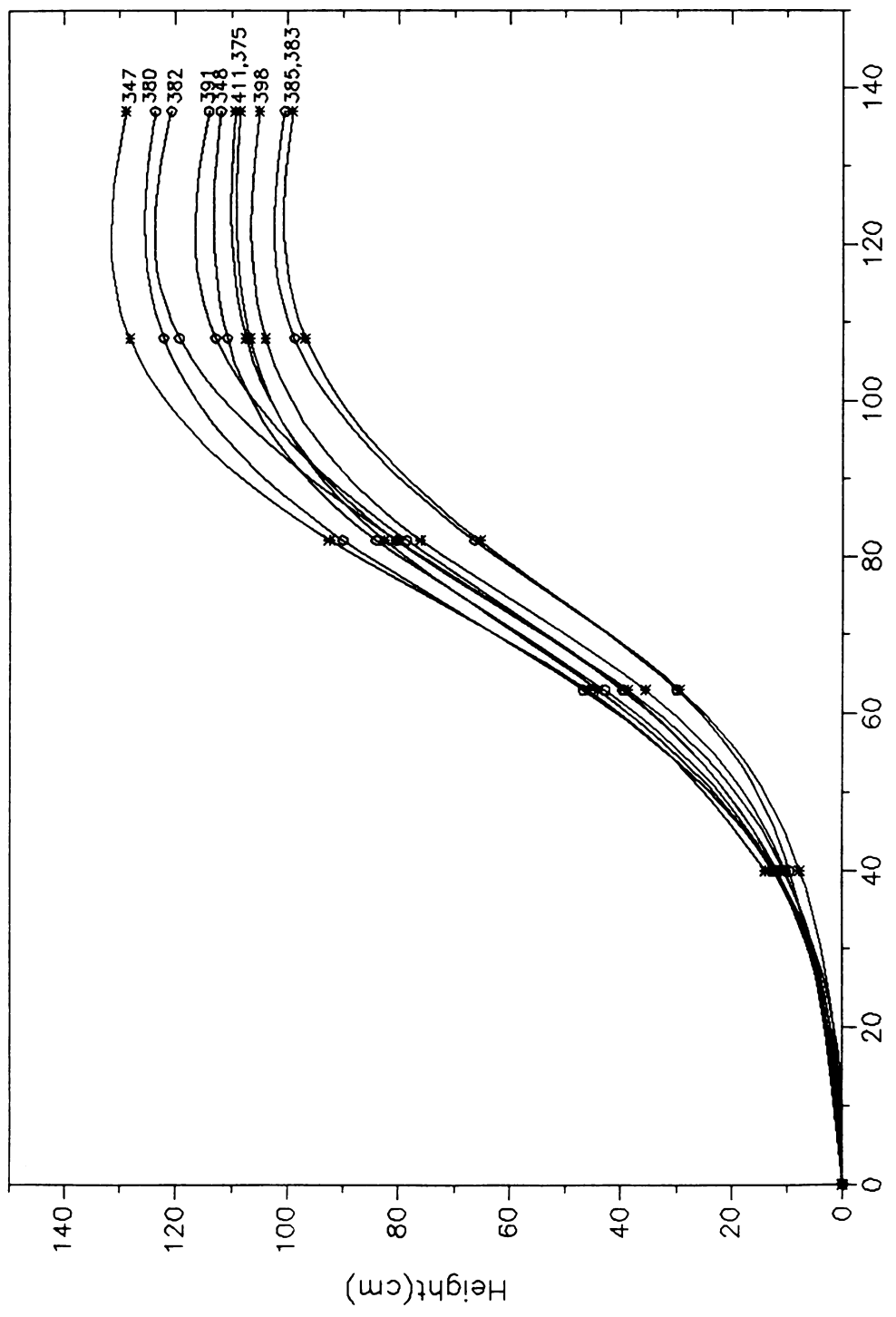


Figure 3. Cumulative height growth curves of 10 half-sib black locust families, from sowing to end of growing season in a black locust nursery progeny test. good families = 348,380,382,385,391; poor families = 347,375,383,398,411

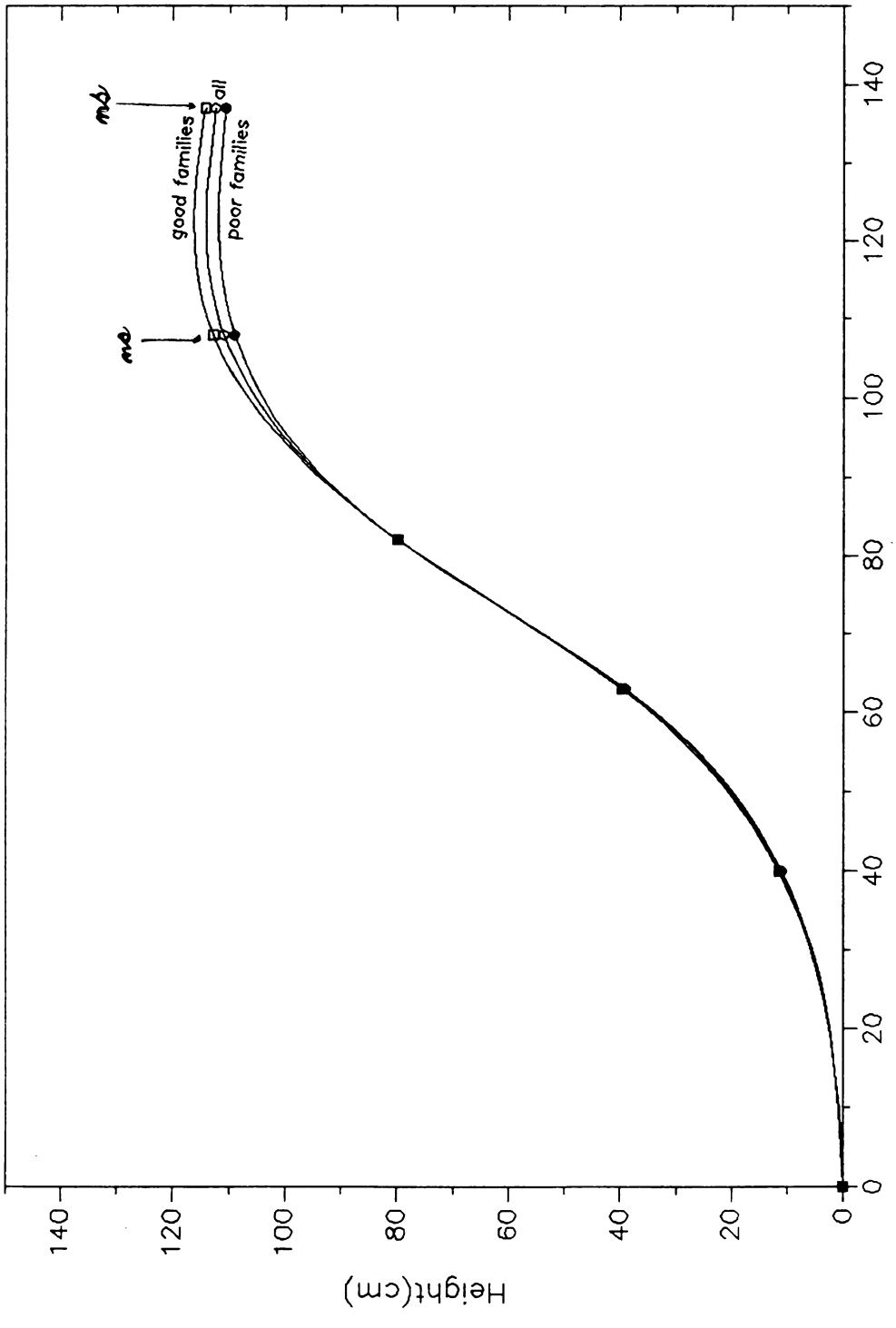


Figure 4. Cumulative height growth curves of 5 good and 5 poor half-sib families in a black locust nursery progeny test.
ns = no significant differences among good and poor families at $p<0.1$.

Table 7. Family means, number of seedlings and individual tree values for nursery growth characteristics in 10 half-sib families of black locust.

RANK	Height day 40 (cm)		Height day 63		Height day 82		Height day 108		Height day 137		Diameter (mm)		Number of branches		Thorn length (score)	
	FAM	Mean	FAM	Mean	FAM	Mean	FAM	Mean	FAM	Mean	FAM	Mean	FAM	Mean	FAM	Mean
1	347	13.84	380	46.77	347	92.47	347	128.85	347	128.87	348	11.84	380	10.26	411	1.578
2	375	12.40	347	45.86	380	90.01	380	122.21	380	123.63	380	11.72	347	9.82	398	1.533
3	348	12.39	375	44.02	348	84.02	382	119.37	382	120.76	382	11.31	391	9.46	382	1.527
4	380	12.21	348	42.73	375	82.52	391	112.84	391	114.04	347	11.28	382	9.03	385	1.510
5	391	11.91	382	39.66	411	80.51	348	110.74	348	111.93	391	11.17	348	8.92	347	1.509
6	382	11.75	391	39.33	382	80.32	411	107.35	411	109.25	375	10.07	383	7.79	383	1.487
7	411	10.48	411	38.59	391	78.48	375	106.74	375	108.38	383	9.96	385	7.64	391	1.448
8	398	10.25	398	35.50	398	75.99	398	103.90	398	105.00	385	9.65	375	6.18	380	1.422
9	385	9.50	385	29.81	385	66.26	385	98.73	385	100.37	411	9.59	398	3.65	375	1.393
10	383	7.65	383	29.26	383	65.34	383	96.74	383	99.09	398	9.22	411	3.28	348	1.268
All fam's:																
Mean		11.32		39.38		79.92		111.02		112.49		10.60		7.61		1.467
Range of fam. means		7.65-13.84		29.26-46.77		65.34-92.47		96.74-128.25		99.09-128.87		9.22-11.84		3.28-10.26		1.268-1.578
Range of ind. trees		1.00-31.00		4.50-96.00		10.00-160.00		16.00-211.00		16.00-211.00		2.55-21.51		6.00-32.00		0.000-3.000
No. trees		1974		1974		1974		1964		1943		1952		1932		1934
Good fam's:																
Mean		11.577		39.73		79.91		112.91		114.27		11.15		9.07		1.434
Range of ind. trees		1.000-29.00		4.50-96.00		10.00-160.00		27.00-211.00		29.00-211.00		2.76-21.51		0.00-32.00		0.000-2.000
No. trees		993		993		993		988		981		989		979		982
Poor fam's:																
Mean		11.063		39.033		79.94		109.10		110.68		10.03		6.12		1.501
Range of ind. trees		1.00-31.00		6.00-92.50		10.00-157.00		16.00-207.00		16.00-207.00		2.55-19.12		0.00-22.00		0.000-3.000
No. trees		981		981		981		976		962		963		953		952

GF = 348, 380, 382, 385, 391 PF = 347, 375, 383, 398, 411

Table 8. Matrix of rank correlations between nursery growth characteristics for 10 half-sib families of black locust.

	RH_{40}	RH_{63}	RH_{82}	RH_{108}	RH_{137}	RDIA	RNBR	RTHL	RPCT	RSWT
Rank height day 40 (RH_{40})	1.000	0.883***	0.866***	0.633*	0.633*	0.649*	0.512	-0.683*	-0.093	
Rank height day 63 (RH_{63})		1.000	0.916***	0.766***	0.766**	0.750*	0.669*	-0.626	-0.217	
Rank height day 82 (RH_{82})			1.000	0.733*	0.733*	0.649*	0.512	-0.484	-0.186	
Rank height day 108 (RH_{108})				1.000	1.000**	0.721*	0.840**	-0.227	-0.373	
Rank height day 137 (RH_{137})					1.000	0.721*	0.840**	-0.227	-0.078	0.490
Rank diameter (RDIA)						1.000	0.797**	-0.694*	-0.163	0.745*
Rank number of branches (RNBR)							1.000	-0.459	-0.503	0.515
Rank thorn length (RTHL)								1.000	-0.030	-0.333
Rank % germination (RPCT)									1.000	-0.200
Rank seed weight (RSWT)										1.000

* = significant at 0.05 level of probability
** = significant at 0.01 level of probability
*** = significant at 0.001 level of probability

Table 9. Results from the analysis of variance for the nursery growth characteristics of 10 half-sib black locust families comprising 5 good families and 5 poor families.

	Height day 40 (cm)		Height day 63		Height day 82		Height day 108		Height day 137	
	df	MS	df	MS	df	MS	df	MS	df	MS
Replication	3	5612.39**	3	33941.08**	3	57640.64**	3	66491.90**	3	66217.14**
Family	9	610.38ns	9	7889.67ns	9	14000.23ns	9	24307.54*	9	23365.45*
GF vs. PF	1	ns	1	ns	1	ns	1	ns	1	ns
Rep x Family	27	562.14***	27	6089.45***	27	12088.23***	27	12660.18***	27	12362.67***
Within-plot	1934	15.83	1934	151.51	1934	421.89	1224	726.62	1903	694.23

	Residual height		Diameter (mm)		Number of branches		Thorn length (score)	
	df	MS	df	MS	df	MS	df	MS
Replication	3	14.090ns	3	139.49ns	3	70.670ns	3	4.085*
Family	9	20.871ns	9	185.307**	9	1215.55***	9	1.609ns
GF vs. PF	1	ns	1	ns	1	**	1	ns
Rep x Family	27	21.514**	27	66.197***	27	130.187***	27	0.930**
Within-plot	1903	12.156	1912	6.814	1892	20.447	1894	0.317

MS = mean squares; GF = good families; PF = poor families
ns = not significant
* = significant at P<0.1 level of probability
** = significant at P<0.05 level of probability
*** = significant at P <0.01 level of probability

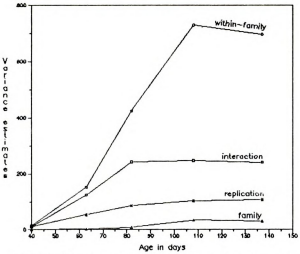


Figure 5 (a). Age trends in variance components of height growth in a black locust nursery progeny test.

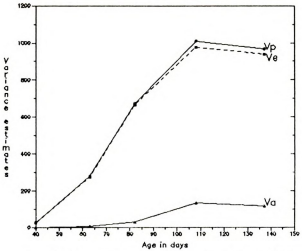


Figure 5 (b). age trends in additive genetic (Va), environmental(Ve),and phenotypic family (Vp) variances of height growth in a black locust nursery progeny test.

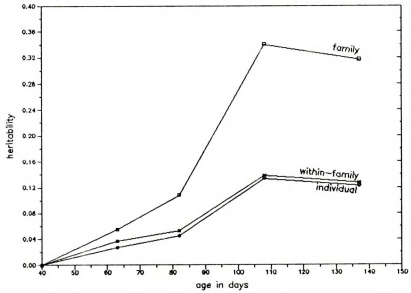


Figure 5 (c). Age trends in individual, family and within-family heritabilities of height growth in a black locust nursery progeny test.

2.a.2. *Family x replication interaction variation*. The family by replication interaction was highly significant for all height measurements including residual growth and accounted for 22.37 to 37.57% of the total variation. From ages 40 days to 108 days old, though the interaction variance increased in absolute terms, its relative magnitude showed a steady decline from 37.57% at 63 days to 22.10% at 108 days (end of active growing season).

The significance of the interaction effects reflects the failure of the genotypes to perform consistently from one replication to another due to random environmental differences among replications (damaging agents, i.e. insects, rabbits; variation in soil moisture and fertilization, etc.). As a result, interaction effects decreased the capability to detect inherent genotype differences. It seems that from age 40 to 82 days (non-significant family differences) and to some extent the following ages (significant family differences at $P<0.1$) the interaction effects were large enough to affect inferences among families (Menzies et al. 1987).

2.a.3. *Replication variation*. The replication variance increased in absolute magnitude throughout the entire growing season but its relative magnitude decreased constantly from age 40 days to age 108 days, and then increased slightly during late season growth. The high significance of the replication effects is an indication that blocking was efficient in increasing the precision of the experiment.

2.b. *Patterns of genetic and environmental variances*. Figure 5b illustrates the patterns of the estimates of additive genetic variance (family variance = 1/4 additive genetic variance), environmental

variance (within-family + interaction variances) and phenotypic family variance (additive + environmental variances).

The additive genetic variance remained at low levels until after 82 days of seedling age when it increased rapidly to reach its maximum at 108 days of age and declined slightly during the late season growth. On the other hand, phenotypic and environmental variances followed a similar pattern, increasing rapidly during the active growth period and decreased a little during the residual growth period. Phenotypic family variance and environmental variance were about the same magnitude until after 82 days of age when they began slowly but steadily to differentiate.

From these results, it could be suggested that the additive genetic effects become well present after 82 days in the nursery experiment to make a clear difference between the phenotypic and environmental variances.

2.c. Heritability estimates. Heritability is defined as the ratio of additive genetic variation (i.e., genetic portion transmitted to the next generation) to phenotypic variation (Gill 1987). But heritability estimates should be used with caution because, first, they apply strictly to the population and test conditions for which they are made (Wright 1962), and second, they are affected by methods of calculation.

Narrow sense heritability estimates were calculated on an individual tree, family and within-family basis (Table 10) and their trends are illustrated in Figure 5c. These heritabilities, deemed appropriate, respectively, for mass selection, family and within-family selection (Squillace and Gansel 1974) followed a similar pattern,

Table 10. Variance components, percent of total variance in parenthesis and heritability estimates for nursery growth characteristics in 10 black locust half-sib families.

	Height day 40 (cm)	Height day 63 (cm)	Height day 82 (cm)	Height day 108 (cm)	Height day 137 (cm)	Diameter (mm)	Number of branches (count)	Thorn length (score)
Variance: Family (σ^2_f)	-- (0.00)	1.87 (0.56)	7.64 (1.000)	33.79 (3.03)	29.69 (2.76)	0.6262 (7.10)	5.7103 (20.15)	0.00356 (1.04)
Replication (σ^2_r)	9.86 (26.33)	53.65 (16.13)	86.46 (11.35)	103.91 (9.32)	107.30 (9.97)	0.1607 (1.82)	-- (0.00)	0.00652 (1.91)
Rep x Family (σ^2_{fr})	11.62 (31.02)	124.97 (37.57)	242.15 (31.79)	246.30 (22.10)	240.67 (22.37)	1.2061 (13.68)	2.2096 (7.80)	0.0127 (3.74)
Within-plot (σ^2_w)	15.97 (42.64)	152.08 (45.72)	425.25 (55.84)	730.24 (65.53)	697.92 (64.88)	6.8179 (77.37)	20.408 (72.04)	0.3175 (93.29)
Heritability: h^2_i	0	0.0269	0.0452	0.133	0.122	0.289	0.806	0.0427
h^2_f	0	0.055	0.108	0.340	0.317	0.650	0.896	0.424
h^2_w	0	0.037	0.053	0.138	0.127	0.275	0.839	0.030

Heritability components formulae from Ernst et al, 1985.

h^2_i = individual tree heritability. $h^2_i = \sigma^2_A / \sigma^2_p = \dfrac{4\sigma^2_f}{\sigma^2_f+\sigma^2_{fr}+\sigma^2_w}$

σ^2_p= phenotypic variance among half-sib families.

h^2_F = family heritability. $h^2_F = \frac{1}{4}\,\sigma^2_A / \sigma^2_p = \dfrac{\sigma^2_f}{\sigma^2_f+\sigma^2_{fr/t}+\sigma^2_{w/rt}}$

σ^2_A= additive genetic variance

h^2_w = within-family heritability. $h^2_w = \frac{3}{4}\,\sigma^2_A / \sigma^2_w = \dfrac{3\sigma^2_f}{\sigma^2_w}$

increasing with age until the end of the active growing season, and then decreasing during the late season growth period.

Family heritability estimates were much larger than the estimates for individual tree and within-family which were about the same magnitude. The maximum values of heritability were obtained at 108 days of seedling age with 0.340, 0.133 and 0.138, respectively, for family, individual and within-family heritabilities. These results suggest that, with selection based on height growth, genetic gain is possible but progress would be more rapid for family selection.

The individual-tree and family heritabilities calculated here for total height (0.122 and 0.317, respectively, in East Lansing) in the nursery were much lower than those reported by Mebrahtu and Hanover (1989) (0.74 and 0.48, respectively, in East Lansing) for first-year field height of black locust genotypes. However, their field heritabilities were, (1) based on plot means and, thus, were inflated by excluding within-family variance from the denominator (see formulae, Table 10), and (2) calculated over 400 families including the 10 families used in the nursery test. Thus, again, heritabilities should be interpreted with caution.

II. Other growth characteristics: diameter, number of branches, thorn length

Measured at the end of the growing season, diameter, number of branches and thorn length averaged per family, respectively, 9.96 to 11.84 mm, 3.28 to 10.26 and 1.26 to 1.57 (score) (Table 7).

Diameter and number of branches exhibited significant differences among families (respectively, $P<0.05$ and $P<0.01$) and for the interaction effects ($P<0.01$) but not for replications while for thorn length, no

significant family differences were detected but the interaction and replication effects were highly significant (Table 9). For diameter, number of branches and thorn length, the variance associated with within-family accounted for most of the total variation with, respectively, 77.40%, 72% and 93.30% of total variation whereas variance components associated with families were rather high for number of branches (20.15%), somewhat important for diameter (over 7%) and small for thorn length (1.04%) (Table 9).

The heritability estimates along with the genetic effects for diameter and number of branches were much higher than those obtained for height, suggesting that selection based on diameter may lead to more genetic gain than selection based on height.

The rank correlations associating total height with diameter and number of branches (Table 8) were high and significant (respectively 0.72 and 0.84), as was also that associating diameter with number of branches (0.79), indicating close family ranks between the three characteristics. In fact, the 5 top families (4 good and 1 poor) for final height were the same for diameter and number of branches. In contrast, the rank correlations relating thorn length to total height, diameter and number of branches were all negative but non-significant except for diameter.

III. Correlations

1. Relationships between nursery growth characteristics

The correlation coefficients between height growth measurements at different ages were high, positive, of similar magnitude at the levels of total, between-family and within-family (Table 11). These

correlations decreased with more distant age pairs and the high within-plot correlations are largely because the variation in size at younger ages are maintained as populations become older (Lambeth et al. 1983). In addition, the genetic correlations, whose estimates are based on genetic effects, were positive, usually greater than the phenotypic correlations and in some cases they exceeded one. Correlations of more than one are explained as due to sampling error and some invalid assumptions upon which their calculations are based (Rink 1984).

On the other hand, the correlations associating total height, diameter and number of branches were also high and positive at the level of total (individual), between-family and within family. The genetic correlations and the total phenotypic correlations were about the same magnitude for height and diameter while the genetic correlations appeared much higher than total phenotypic correlations for total height and number of branches, and for diameter and number of branches. The number of branches per family was more related to diameter than to height.

These results indicate that simultaneous selection for rapid height growth, large diameter and branchier seedlings is attainable, especially at the family level. However, depending on the objective of selection, number of branches may be considered as an undesirable characteristic. In that case, simultaneous selection for height, diameter and less branchiness will be ineffective, but this expectation should not be emphasized until refinement of the method of assessment of branching patterns is carried out because all branches had not the same importance, depending on their size and position on the tree.

Table 11. Phenotypic (between-family, within-family and total), genotypic and replicate correlations among growth characteristics in a black locust nursery progeny test.

Between-Family Correlations (df=9)

	1 Height day 40	2 Height day 63	3 Height day 82	4 Height day 108	5 Height day 137	6 Diameter	7 Number branches	8 Thorn length
1		0.919	0.901	0.832	0.830	0.622	0.428	0.118
2			0.970	0.817	0.818	0.718	0.472	0.183
3				0.882	0.878	0.748	0.453	0.272
4					0.990	0.773	0.472	0.343
5						0.735	0.480	0.345
6							0.862	-0.577
7								-0.428
8								

Within-Family Correlations (df=1921)

	1 Height day 40	2 Height day 63	3 Height day 82	4 Height day 108	5 Height day 137	6 Diameter	7 Number branches	8 Thorn length
1		0.867	0.757	0.665	0.668	0.618	0.435	0.139
2			0.911	0.884	0.827	0.725	0.499	0.214
3				0.947	0.949	0.763	0.483	0.298
4					0.993	0.779	0.468	0.364
5						0.786	0.475	0.367
6							0.649	0.379
7								0.230
8								

Total Correlations (df=1930)

	1 Height day 40	2 Height day 63	3 Height day 82	4 Height day 108	5 Height day 137	6 Diameter	7 Number branches	8 Thorn length
1		0.871	0.769	0.679	0.681	0.622	0.428	0.117
2			0.917	0.823	0.826	0.718	0.472	0.183
3				0.942	0.943	0.748	0.435	0.271
4					0.994	0.774	0.472	0.343
5						0.781	0.480	0.346
6							0.672	0.291
7								0.174
8								

Table 11 (cont'd)

Replicate Effects Correlations (df=3)

1 Height day 40	2 Height day 63	3 Height day 82	4 Height day 108	5 Height day 137	6 Diameter	7 Number branches	8 Thorn length
1	0.943	0.964	0.960	0.961	0.911	0.386	-0.044
	2	0.988	0.996	0.996	0.842	0.121	-0.109
		3	0.997	0.997	0.818	0.134	-0.207
			4	1.000	0.835	0.137	-0.158
				5	0.840	0.144	-0.150
					6	0.618	0.370
						7	0.680
							8

Genetic Correlations

1 Height day 40	2 Height day 63	3 Height day 82	4 Height day 108	5 Height day 137	6 Diameter	7 Number branches	8 Thorn length
1	--	--	--	--	--	--	--
	2	1.301	0.978	0.940	--	--	--
		3	0.851	0.812	--	--	--
			4	1.006	--	--	--
				5	0.770	0.727	-0.546
					6	0.908	-1.033
						7	-0.571
							8

Table 11 (cont.)

In contrast, the correlation coefficients associating thorn length with total height, diameter and number of branches followed different paths depending on the level of correlation. At the family and genetic effect levels, the correlations were negative whereas at total and within-family levels the coefficients between thorn length and height, diameter or number of branches were positive and significant (Table 11). Since thorn length is an undesirable characteristic, it appears that selection of families for tall height and large diameter based on short thorns as criterion is feasible but selection within these families will be ineffective.

In summary, the relationships between growth characteristics as observed in the nursery were strong and positive except for thorn length. In addition to the moderate heritabilities obtained at family level, simultaneous selection at family level for height, diameter and thorn length appear to be reliably feasible. However, whether these favorable relationships will hold over time and in field tests has to be proven before reaching any conclusion.

2. Relationships between nursery growth characteristics, seed weight, percentage seed germination and field height performance (Table 12)

2.a. *Effects of seed weight*. Results from several studies have identified seed weight as a source of variation in seedling size in the nursery and field tests involving several species such as loblolly pine (Sluder, 1979; Bailan et al., 1989), pecan (Adams and Thielges, 1979), Douglas-fir (Silen and Osterhaus, 1979), jack pine (Radscliff, 1981) and red oak (Kriebel, 1967). Since variations in growth rate resulting from

Table 12. Family mean correlations of seedling growth characteristics in the nursery with 1-, 2- and 4-year-old field height, with percent germination and with seed weight of 10 families and in parenthesis of the 8 families of black locust after deletion of 2 unstable families from the 10.

Nursery Measurements	Field Height Performance			Laboratory Determination	
	1-year-old	2-year-old	4-year-old	Percent germination	Seed weight
Height day 40 (cm)	0.227	0.211	0.063	0.188	0.434
Height day 63	0.134	0.110	0.090	0.219	0.319
Height day 82	0.171	0.122	0.075	0.126	0.304
Height day 108	0.357	0.241	0.215	-0.172	0.370
Height day 137	0.346 (0.668**)	0.230 (0.715**)	0.215 (0.781***)	-0.175 (-0.043)	0.365 (0.509)
Total height increment	0.360	0.226	0.240	-0.245	0.336
Diameter (mm)	0.546* (0.693**)	0.454 (0.768***)	0.486 (0.768***)	-0.133 (-0.167)	0.722** (0.799***)
Number of branches (count)	0.498 (0.567*)	0.495 (0.607*)	0.462 (0.669**)	-0.507 (-0.451)	0.548* (0.566*)
Thorn length (score)	-0.211 (-0.239)	-0.289 (-0.434)	-0.238 (-0.249)	-0.238 (-0.124)	-0.599* (-0.639**)
Field height performance:					
1-year-old	1.000			-0.184 (-0.225)	0.563* (0.571*)
2-year-old	0.905*** 0.949***)	1.000		-0.229 (-0.158)	0.545* (0.594*)
4-year-old	0.906*** (0.939***)	0.884*** (0.955***)	1.000	-0.148 (-0.350)	0.464 (0.516)

*significant at 0.1 level
**significant at 0.05 level
***significant at 0.01 level

differences in seed weight appears as environmental effects (Sluder, 1983) and add to inherent variation, its effects will result in decreasing the precision of juvenile selection.

From this study (Table 12), family mean correlations between seed weight and nursery height at different measurement ages did not appear significant and decreased over time (0.434 to 0.365). Regression analyses between seed weight as independent variable and initial height or total height as dependent variables, indicated that 18.85% and 13.35% of the variation, respectively, in initial height and total height were related to the weight of the seed. In contrast, family mean seed weights were positively and significantly correlated with diameter ($P<0.05$) and number of branches ($P<0.1$) whereas the correlation of seed weight and thorn length was negative and significant at 0.1 level of probability.

These results suggest that selection based on seed weight in the nursery will result in larger diameter, branchier, less thorny and to some degree taller families. Results suggest also that, since seed weight functions as environmental effects to mask variation in inherent vigor (Sluder 1983), previous results about heritabilities and correlations were inflated and thus less efficient in predicting later growth.

On the other hand, correlation coefficients between seed weight and field heights were significant for first-year ($P<0.1$), second-year ($P<0.1$), but not significant for 4-year old seedlings ($r=0.464$). Regression analyses between seed weight and field heights showed that 31.80%, 29.79% and 21.57% of total variation, respectively, in 1-year, 2-year and 4-year heights were explained by seed weight. These results

suggest that seed weight still exercised an important effect on seedling size 4 years after field planting and as a result, selection should be delayed until after seed effects have ceased.

2.b. Effects of percent seed germination. Seed germination was not correlated with any growth characteristic in the nursery and seedling size in the field. The only correlation coefficient worth notice was between seed germination and number of branches (-0.507). These low correlations between seed germination and growth characteristics (nursery and field) are indicators that seed germination is independent of growth in black locust.

2.c. Relationships between nursery seedling characteristics and field seedling size of the same families. The family mean correlations found between nursery characteristics and seedling size in the field (Table 12) were not significant and decreased among more distant age pairs. These poor correlations suggest that nursery bed selections could not be considered as reliable prediction methods. Family rank correlations between nursery and field characteristics showed similar trends as family mean correlations, indicating that family rankings were not consistent from the nursery environment to the field environment. Indeed, extreme rank order changes were observed from the nursery to the field and consisted of family 347 (poor family) moving from top level in the nursery environment to about bottom level in the field environment while family 385 (good family) had taken an inverse course. As a result, selection of the 5 top families based on nursery results would have resulted in the two types of error described by Lowe and

Van Buijtenten (1989) as occurring frequently in early selection, namely (1) a family with good field performance could be rejected on the basis of early test data (for example family 385), and (2) a family with good early test performance fails to perform well in later field tests (for example family 347). However, only error 1 appears to be important because genotypes are rejected before subsequent field testing whereas error 2 can be identified in the field and corrected.

When both unstable families (347 and 385) were deleted from the data, the family mean correlations between nursery characteristics (except thorn length) and field seedling size (1-, 2-, 4-year) appeared significant (Figure 6) indicating that reliable selection in the nursery may be expected from those 8 families. But seed weight effects appeared also more pronounced with the 8 families precluding selection based on inherent vigor.

Beyond all these considerations, there is a high level of probability that these observed relationships between nursery test and field performance may not hold since seedling sizes were altered through systematic top-pruning (at about 50 cm above ground) prior to field planting. As a result of the top-pruning, 4 families did not reach, during the entire first year in the field their size obtained from the nursery test: family 375 (-25 cm), 347 (-11.4 cm), 411 (-6.30 cm) and 383 (-0.9 cm) and naturally they become the poor families as revealed by the 4-year height measurement. Negative effects of top-pruning black locust seedlings have been demonstrated in earlier research by Meginnis (1934) who studied different regimes of top-pruning and their effects on seedling development, and concluded that seedlings should not be top-

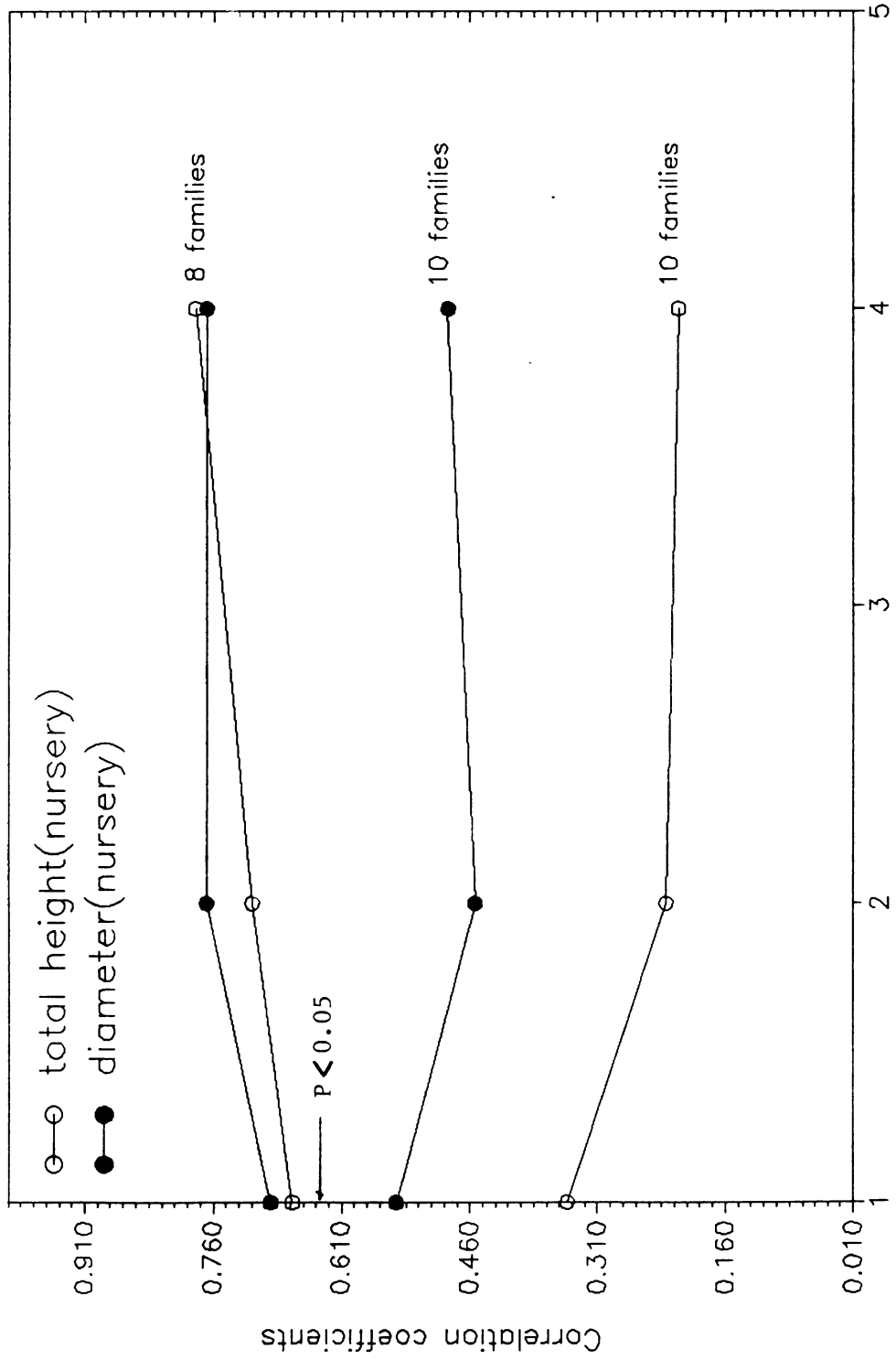


Figure 6. Correlations between nursery height or diameter and field height.
10 families: 347*, 348, 375, 380, 382, 383, 385*, 391, 398, 411.
8 families: * = families removed from the 10.

pruned unless pruning must be done to salvage top-damaged or poorly formed seedlings.

Furthermore, top-breakage of seedlings has been shown to decrease age-age correlations. Lambeth et al. (1983) (loblolly pine) and Magnussen (1989b) (red pine), partioned age-age correlations between size and intrinsic growth rate and found that size was the major contributor component in these correlations. Sluder (1983) reported for slash pine, poor correlations between heights at age 15-year and the other ages as being due to top breakage resulting from an ice storm occurring after the 8-year measurement. Rehfeldt (1983) indicated that, to obtain reliable data for age-age correlations, progeny comparisons or family evaluations, trees with broken tops at any age should be purged from data.

DISCUSSION AND CONCLUSION

The variation patterns in height, diameter, number of branches and thorn length, as observed in the nursery, indicate large within-family variation compared to family variation; but, family heritabilities were much higher than within-family heritabilities, suggesting that substantial genetic gains can be made from both family and within-family selections. Thus, family selection followed by within-family selection is, logically, the first step for improving black locust. Similar variation patterns in growth and phenological characteristics were also demonstrated in black locust field plantings (Kennedy, 1983; Mebrahtu and Hanover, 1989) and are described by Boyle and Yeh (1987) as typical of long-lived trees, wind-pollinated, predominantly outcrossing and widely distributed.

As also indicated by the nursery results, the additive genetic variation and the estimation of heritabilities were much higher for diameter than for height, suggesting that selection based on diameter in the nursery would be more effective than selection based on height. However, the family diameter differences ($P<0.05$) were attributed to seed weight effects since significant relationships were observed between diameter and seed weight ($P<0.05$) and not between height and seed weight. That is, selection based on diameter in nursery would merely be equivalent to selection for seed weight which masks variation in inherent vigor.

On the other hand, all growth characteristics in the nursery showed significant family x block interaction indicating inconsistent response of families among blocks, though attempts were made to effectively control random environmental affects through weeding, insecticide spraying, fertilization and fencing. Apparently, the interaction effects were large enough to affect family inferences in height ($P<0.1$) while they were not in diameter ($P<0.05$) and number of branches ($P<0.01$). These interaction effects have presumably arisen from rabbit damage that occurred in 2 out of 4 blocks about 2 weeks after sowing, and affected in general seedlings that expanded quickly after germination, and hence, offered more nutrients. However, the rabbit attacks were apparently random with regard to families and their incidence in the experiment was minimized by purging the maimed seedlings from the data, even though many of the attacked seedlings recovered quickly and figured among the best performers in the nursery.

Although family means of final height in the nursery ranged from 99 cm to 128.87 cm with 114.3 cm and 110.68 cm, respectively, for good

and poor families, the ANOVA detected weak differences in height ($P<0.1$) and none for good versus poor families.

On the other hand there were poor correlations between nursery growth characteristics of the 10 tested families and 4-year field height of the same 10 families, although the 5 top-ranking families included 4 out of 5 good families. The poor correlations appeared to be caused mainly by extreme rank order changes involving 2 families (1 good and 1 poor family) from the nursery environment to the field environment, since good relationships ($P<0.05$) between nursery growth characteristics and 4-year field height followed when the 2 unstable families were removed from the data (Figure 6). However, nursery bed selection on the basis of the 8 remaining families would result on the rejection of a confirmed good family in the field (family 385 which is the 3rd best family considering the 4-year field height). Furthermore, the good correlations obtained with the 8 families were apparently a reflection of seed weight effects since the relationships between seed weight and 4-year field height appeared also significant ($P<0.05$) and consequently selection based on the 8 families should not be appraised upon removal of the seed weight effect.

The poor correlations between nursery growth characteristics and field height could be attributed to: (1) genetic differences arising from the two different environments (LaFarge 1975); (2) sampling variation since nursery and field measurements were not made on the same progenies (Sluder 1983); and most important (3) the top-pruning of seedlings prior to field planting. Top-pruning of seedlings at about 50 cm above ground is a current practice in black locust to facilitate seedling handling by machine planters, thus reducing planting costs and

also to increase survival. However, there is considerable evidence that top-breakage of seedlings would alter the relationships of nursery growth with later growth ages. Therefore, whole seedlings would be preferred to top-pruned seedlings for establishing plantations. To take advantage of all the nursery growth and still use machine planters, it would make sense to investigate the best date of seed sowing in the nursery that would yield seedlings of about 50-70 cm at the end of the growing season.

SECTION III.

VARIATION IN SEEDLING QUALITY FACTORS AND INSECT DAMAGE IN A HALF-SIB NURSERY TEST OF BLACK LOCUST (*Robinia pseudoacacia* L.)

ABSTRACT

Insect incidence (aphids and twig borers) and seedling form were assessed on seedlings evaluated for growth characteristics in the nursery progeny test. Results showed no statistical differences among families and for good versus poor families, both for insect attacks and stem form. However, significant differences were detected, separately, for crooked stems ($P<0.1$) and for sinuous stems ($P<0.05$), the two components of stem form.

Results also indicate that controlling insect damage in the nursery would indirectly result in significant decrease of crooked stems whereas it would have little influence on stem sinuosity which appears to be under more direct genetical control.

On the other hand, weak correlations were found between nursery growth characteristics or field height and, respectively, insect injuries and stem form.

INTRODUCTION

Insect attacks, pathogenic agents, winter hardiness, drought resistance, tree form, etc., are very important traits to consider in a tree breeding program because they may reduce the capability to detect family differences in growth characteristics (Friedman 1983) and seriously affect wood quality.

ABSTRACT

INTRODUCTION

In this study, insect damage and stem form were assessed on seedlings evaluated for growth characteristics in the nursery test to determine their impact on seedling development.

MATERIAL AND METHODS

Damage by insects as observed in the nursery was mainly due to aphids (various species) and twig borers (*Edytolopha insiticiana*). Aphids were noticed in early stages of seedling development and stayed in the nursery throughout the active growing season, feeding on young shoots and leaves whereas twig borers appeared around mid-August attacking branches and stems. These insects seldom killed the seedlings they attacked but their effects resulted in leader and branch breakages (twig borers) or dieback of shoots and buds (aphids), and hence, stunted and often deformed seedlings. Confronted with an unusual season of aphid proliferations in the nursery insecticide sprays were applied five times during the growing season to avoid heavy damage on seedlings; July 6 (orthene + pentac), July 16 (Maverick), August 15 (Maverick + Triton B_{1956}), August 31 (Diazinon), and September 6 (Diazinon).

Stem form and insect attacks were assessed through scoring procedures. For stem form, 1=straight, 2=sinuous, and 3=crooked; and for insect damages, 1=susceptible, 0=nonsusceptible. Aphid and twig borer effects were not separated since a given seedling may have been susceptible to both insect species.

Data analysis for these two characteristics was done by using two units of ANOVA: (1) ANOVA on an individual-tree basis for scoring data, allowing an estimate of the within-plot variation; and (2) ANOVA on a plot mean basis using percentage data (conversion of scoring data in

percentages) transformed to arcsine square root percent which result in no within-plot estimation. Table 13 presents the expected mean squares for both units of ANOVA (derived from Foster 1986). Variance components and narrow sense heritabilities were calculated as well as correlations with nursery growth characteristics and field height. The means were separated by Duncan's multiple range test if the analysis of variance revealed significant differences (P=0.05) among them. Narrow sense family heritability was calculated only if there were significant differences among families (P<0.1).

RESULTS AND DISCUSSION

Variation in susceptibility to insect attacks and stem form

The ANOVA based on scoring data (Table 14) showed no significant differences among families either for insect attacks or stem form while for both traits family by replication interactions appeared highly significant. The absence of significant family effects in insect damage was probably caused by insecticide sprays which also can explain the non-significance of family variation in stem form since insect injury to the growing points of seedlings often led to deformed seedlings.

From the estimates of variance components, it appeared that the within-family variation accounted for the bulk of total variation, with 92.50% and 95%, respectively, for insect incidence and stem form while family variation accounted for little of total variation in insect incidence (0.79%) and stem form (0.36%).

On the other hand, the ANOVA of percentage data (plot means) allowed a more extensive analysis of stem form which was partioned in crooked stems, sinuous stems and cumulative effects of crooked and

Table 13. Expected mean squares for the ANOVA based on individual-tree and plot mean basis (adapted from Foster, 1986).

Source	Expected Mean Squares		
	df	Individual-tree model (score)	Plot model (percentage)
Replications (r)	r-1	$\sigma_w^2+t\sigma_{RF}^2+tf\sigma_R^2$	$\frac{\sigma_w^2}{t}+\sigma_{RF}^2+f\sigma_R^2$
Families (f)	f-1	$\sigma_w^2+t\sigma_{RF}^2+tr\sigma_F^2$	$\frac{\sigma_w^2}{t}+\sigma_{RF}^2+r\sigma_F^2$
Rep x family	(r-1)(f-1)	$\sigma_w^2+t\sigma_{RF}^2$	$\frac{\sigma_w^2}{t}+\sigma_{RF}^2$
Within-plot	rf(t-1)	σ_w^2	

σ^2_R = variance among replications

σ^2_F = variance among families

σ^2_{RF} = variance for replication x family interaction

σ^2_w = variance of seedlings within-family

t = harmonic number of seedlings per plot

Table 14. Mean squares, variance components, percent total variation in parenthesis and family heritability estimates of insect incidence and stem form in a black locust nursery progeny test.

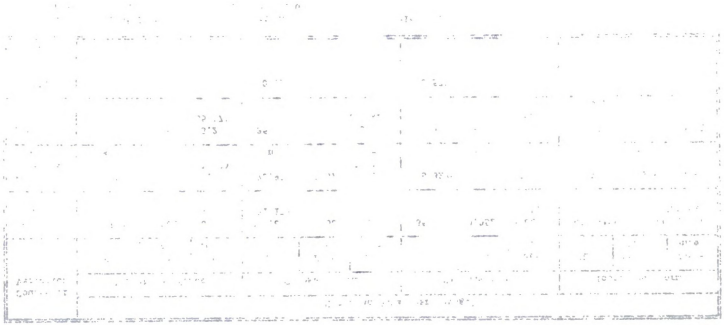
Source of Variation	Individual Tree Data (score)							
	Insect incidence				Stem form			
	df	MS	F-test	Variance	df	MS	F-test	Variance
Replications	3	2.952	7.17**	0.0053(3.47)	3	9.4851	7.2499**	0.0167(2.42)
Families	9	0.658	1.59ns	0.00124(0.79)	9	1.704	1.30ns	0.00248(0.36)
good vs. poor families	1	--	ns	--	1	--	ns	--
Family x replications	27	0.4117	2.28**	0.00554(3.57)	27	1.3083	1.99**	0.0131(1.91)
Within-plot	1925	0.1431	--	0.1429(92.15)	1904			0.6576(95.30)

(This table is continued on next page)

Table 14 (cont'd.)

Source of Variation	Plot Mean Data (percentage)											
	Insect incidence			Crooked stems			Sinuous stems			Total poor form		
	MS	Prob >F	Vari-ance	MS	Prob >F	Vari-ance	MS	Prob >F	Vari-ance	MS	Prob >F	Vari-ance
Replica-tions	312.70***	0.001	26.94 (34.52)	241.89*** (31.17)	0.0016	20.59	85.59**	0.052	5.62 (12.56)	169.59***	0.0039	13.94 (28.61)
Families good vs. poor	74.54 ns	0.132 --	7.81 (10.0) --	73.99* ns	0.0712 --	9.51 (14.40) --	68.32** ns	0.043 --	9.74 (21.77) --	48.77 ns	0.159 --	4.66 (9.56) --
Error	43.294	--	43.29 (55.47)	35.94	--	35.94 (54.42)	29.36	--	29.36 (65.65)	30.128 (61.82)	--	30.12
Family herita-bility (h^2F)		--		0.514			0.570				--	

ns = not significant *significant at 0.1 level **significant at 0.05 level
ms = mean square ***significant at 0.01 level



sinuous form (total poor form). The ANOVA of percent data was consistent with that of scoring data showing no significant differences among genotypes for insect injury and total poor form. However, significant family differences were observed for stem sinuosity ($P<0.05$) and to lesser extent for crookedness of stems ($P<0.1$). Family variation accounted for about 22% and 14%, respectively, for sinuous and crooked stems whereas their respective coefficients of family mean heritability were about the same magnitude (0.570 and 0.514). Family means from the percentage data are presented in Table 15 and illustrated in Figure 7.

The level of susceptibility to insect injury per family ranged from 10.66% to 26.83% around the overall mean of 18.84%. Of the 10 families, 5 performed below the overall mean (3 good families and 2 poor families) and among them 4 families were the best performers in height and diameter in the nursery but the other less susceptible family to insect damage (383) was the worst performer in growth during the entire nursery experiment. That is, the best performers in growth in the nursery were not necessarily the least susceptible to insect damage, as evidenced also by the absence of significant rank correlation between total height and insect incidence.

For stem form, the range of family means was as follows: 10.21 to 31.10%, 7.05 to 20.62% and 25.86 to 43.89%, respectively for crooked stems, sinuous stems and total poor form. As observed for insect injury, there was also no consistency between performance in growth and stem form.

On the other hand, there were no significant differences in either ANOVA unit between the good and poor families.

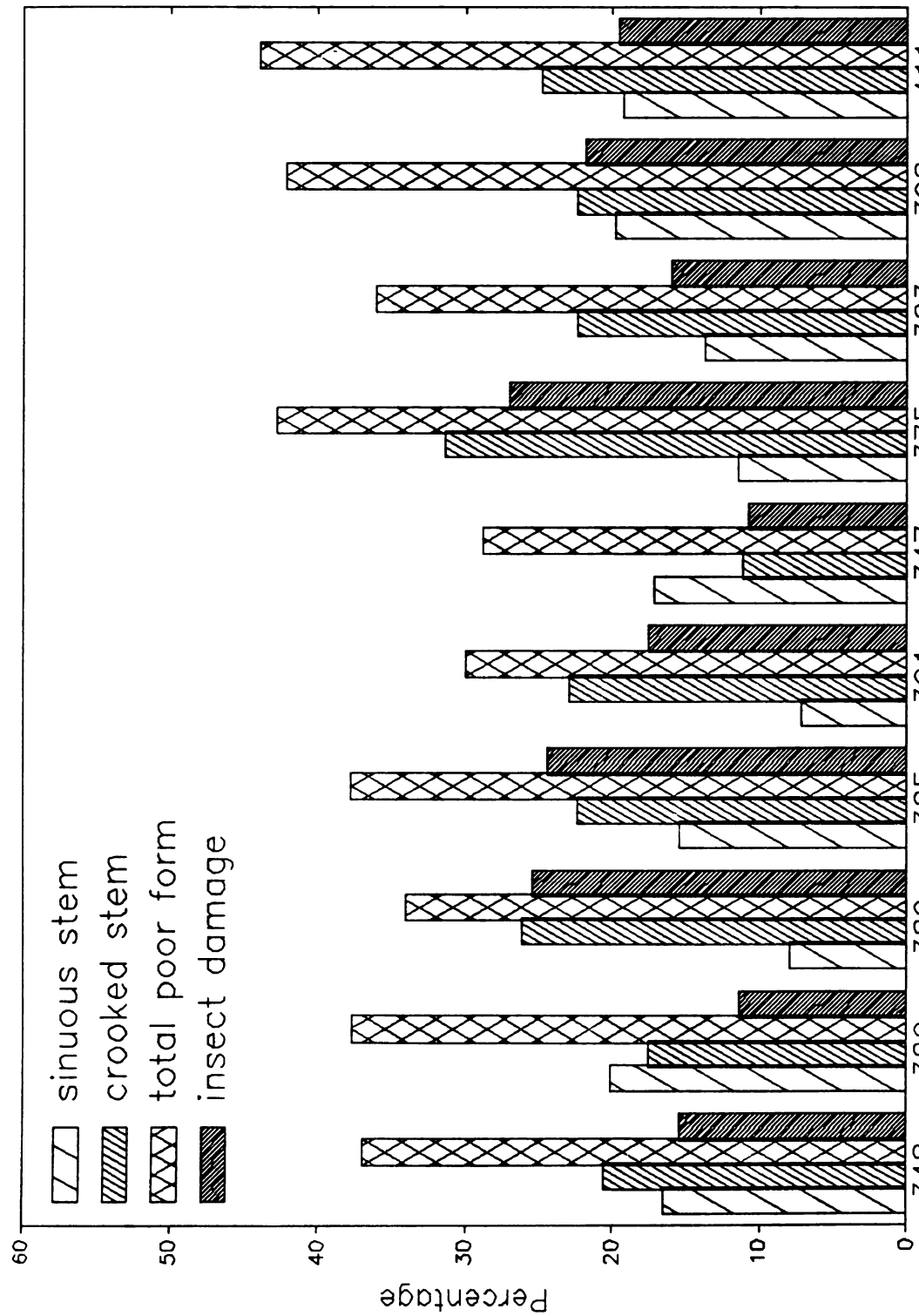


Figure 7. Insect incidence and seedling form in an intensively cultured black locust progeny test.
Good families=348,380,382,385,391; Poor families=347,375,383,398,411.

Table 15. Mean insect incidence and stem form and relative rankings in the nursery for 10 open-pollinated black locust families.

	Scoring Data				Percentage Data							
	Insect Incidence		Stem Form		Insect Incidence		Crookedness		Sinuosity		Total Poor Form	
	Family	Mean	Family	Mean								
1	375	0.269	375	1.74	375	26.83	375	31.10	380	20.62a	411	43.89
2	382	0.253	411	1.68	382	25.25	382	26.08	398	19.82a	375	42.72
3	385	0.243	398	1.64	385	24.63	411	24.56	411	19.33a	398	41.67
4	398	0.217	385	1.61	398	21.86	383	22.66	348	16.51ab	380	37.87
5	411	0.195	382	1.60	411	18.97	385	22.53	347	15.55ab	385	37.30
6	391	0.175	383	1.57	391	17.31	391	22.26	385	14.76abc	348	36.99
7	383	0.159	348	1.57	383	16.70	398	21.84	383	13.46abc	383	36.11
8	348	0.153	380	1.55	348	15.21	348	20.49	375	11.61abc	382	34.51
9	380	0.113	391	1.51	380	10.90	380	17.24	382	8.43bc	391	29.32
10	347	0.16	347	1.40	347	10.66	347	10.21	391	7.05c	347	25.86
Good families		0.187		1.573		18.68		21.72		13.47		35.20
Poor families		0.190		1.611		19.00		22.07		15.95		38.05
All families		0.189		1.592		18.84		21.89		14.71		36.62

Within a column means with the same subscript are not statistically significant at 0.05 (Duncan's multiple range test).

Relationships between insect injury and stem form

The correlation matrix between insect damage and the components of stem form is shown in Table 16.

As anticipated, positive and strong correlations were observed between insect damage and crooked seedlings since the injuries caused by insects to the growing points of seedlings appeared to be a major causative agent of stem crookedness. However, correlations between insect damage and total poor form were positive but not significant at family level while correlations between insect damage and stem sinuosity were negative but not significant (Figure 8). Regression analyses between insect damage (independent variable) and stem form components (dependent variables) showed that 64%, 32% and 18% of total variation, respectively, in crookedness, total poor form and sinuosity of stems were explained by insect injury.

These results suggest that controlling insect damage would indirectly result in a significant decrease of crooked stems whereas it would have little influence on sinuosity of stems. Thus, stem sinuosity appears to be under direct genetical control and is apparently caused by physiological mechanisms related to cambial activity (Franklin and Callaham, 1970).

On the other hand, while crooked and sinuous stems were not correlated at family level, total poor form (crookedness plus sinuosity) revealed significantly related to stem crookedness but not to stem sinuosity. Regression analyses indicated that about 56% of family variation in total poor form was explained by crookedness versus only

Table 16. Correlation matrix between insect damage and stem form components, and correlation coefficients associating insect damage and stem form with nursery growth characteristics in a black locust nursery progeny test.

Percentage Data	Insect Incidence			Crookedness			Sinuosity			Total Poor Form		
	Between family (df=9)	Error (environ -mental) (df=30)	Total (df=39)	Between family (df=9)	Error (environ-mental) (df=30)	Total (df=39)	Between family (df=9)	Error (environ-mental) (df=30)	Total (df=39)	Between family (df=9)	Error (environ mental) (df=30)	Total (df=39)
Insect incidence	1.000	1.000	1.000	0.806**	0.802***	0.802***	-0.330	-0.471**	-0.424**	0.498	0.586***	0.564***
Crookedness				1.000	1.000	1.000	-0.337	-0.425**	-0.395**	0.654*	0.780***	0.746***
Sinuosity							1.000	1.000	1.000	0.460	0.195	0.274
Total poor form										1.000	1.000	1.000
Nursery growth characteristics (plot means):												
Total height	-0.536	0.226	-0.042	-0.562	0.105	-0.070	-0.113	-0.086	-0.093	-0.530	0.023	-0.113
Diameter	-0.579	0.382*	0.066	-0.409	0.344*	0.079	-0.224	-0.089	-0.142	-0.568	0.311	0.019
No.of branches	-0.515	0.466**	-0.011	-0.473	0.349*	-0.065	-0.307	-0.003	-0.162	-0.747*	0.421*	-0.135
Thorn length	0.202	0.245	0.2343	-0.078	0.318	0.208	0.008	0.262	0.180	0.015	0.487**	0.364

*significant at 0.05 level
**significant at 0.01 level
***significant at 0.001 level

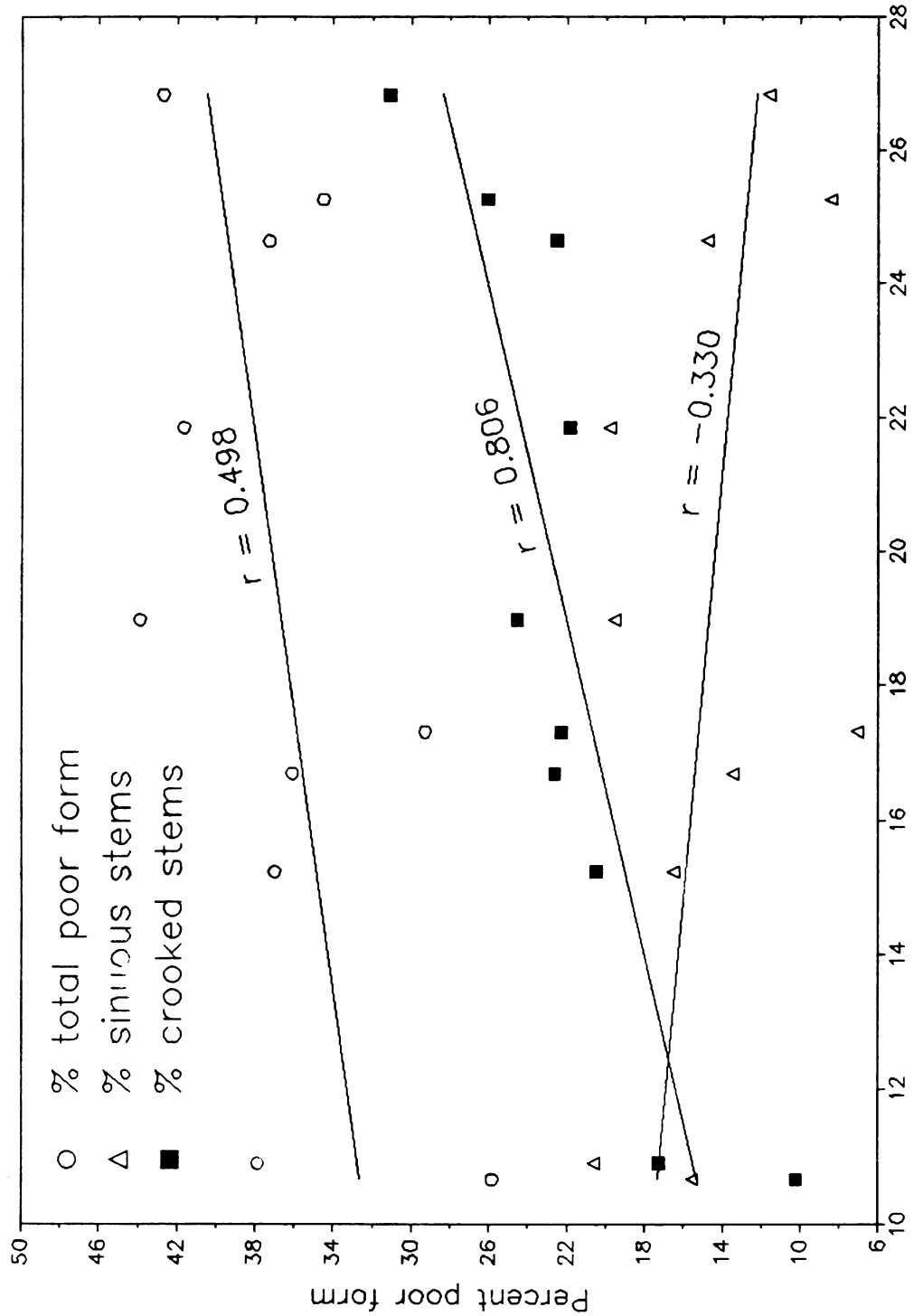


Figure 8. correlation of average % poor stem form of seedlings with average percent seedlings injured by insects in an intensively cultured black locust progeny test.

7.50% explained by sinuosity of stems. Therefore, crookedness could be considered as the dominant factor contributing in total poor form.

Relationships of insect damage and stem form with nursery growth characteristics and field height (Table 16).

Negative and non-significant correlations were found between total height, diameter, number of branches with, respectively, insect injury and stem form components in the nursery test. In contrast, Genys and Harman (1990) reported first-year height and diameter of 25 populations of black locust in the nursery to be positively and significantly correlated to injury by twig borer.

On the other hand, no significant associations were detected for the same families between first-year and fourth-year field height and insect injury (-0.300 and -0.22, respectively), total poor form (-0.339 and -0.083, respectively), crookedness (-0.402 and -0.191, respectively) and sinuosity (0.060 and 0.126, respectively) as evaluated in the nursery. Mebrahtu and Hanover (1989) found from over 400 families of black locust, including the 10 families studied in the nursery, a much lower and positive correlation between first-year field height and injury by twig borer ($r=0.073$).

CONCLUSION

Insect damage and stem form did not appear as a potential tool for nursery selections as evidenced by their non-significant variation among families (probably due to insecticide sprays) and their weak correlations with growth characteristics in the nursery and in the field. However, injury by insects appeared to be a major causative factor of stem crookedness whereas it showed little influence on stem

sinuosity which apparently was caused by lack of apical dominance, and thus, was under more direct genetical control than crookedness. Since the stem borer (*Megacyllene robiniae*), the major threat of black locust field plantings seldom occurs in the nursery, identifying genotypes resistant to insect damage in the nursery does not necessarily mean that these genotypes will be resistant to the stem borer in the field. Therefore, breeding for insect resistance in black locust should be done on field plantings while nursery insects should be strictly controlled to improve the form of seedlings.

SECTION IV.

EFFECTS OF GENOTYPES AND GROWTH POTENTIAL ON GAS EXCHANGE CHARACTERISTICS OF BLACK LOCUST (*Robinia pseudoacacia* L.)

ABSTRACT

In this study, variation in late season photosynthetic efficiency and its relationships with seedling growth characteristics were investigated from the nursery progeny test. Mean net Pn per family ranged from 10.96 μmol $m^{-2}s^{-1}$ to 13.42 μmol $m^{-2}s^{-1}$ but no statistical differences among families or between good versus poor families were found. Family differences accounted for little of total variation (2.88%) while most of the variation was contributed by the within-family variation (66.40%).

On the other hand, poor and inconsistent correlations were found associating late season net Pn with growth characteristics suggesting that late season net Pn is not a potential tool for nursery bed selection.

INTRODUCTION

Physiological and nutritional characteristics (photosynthetic rates, uptake and use of nutrients,) are related to genetic potential of tree growth (Bailan et al., 1989) and thus, theoretically, can be used as a tool to improve progeny testing.

Genetic variability in photosynthesis (Pn) has been demonstrated in several studies involving Scotch pine (Gatherum, 1964), Douglas-fir (Luukanen and Kozlowski, 1972; Campbell and Rediske, 1965), larch species (Ledig and Botkin, 1974), aspen (Gatherum, et al 1967; Foote and

Schaedle, 1978), black walnut (Carpenter, 1972, 1974), sycamore (Ledig and Botkin, 1974), black locust (Mebrahtu, 1989), etc. These studies were either dealing with Pn efficiency (CO_2 uptake per unit leaf surface area per unit time) or Pn capacity of an entire plant (CO_2 absorbed per plant per unit time), both being a determination of net Pn, i.e., gross Pn minus rate of respiration. These studies have shown the weakness of using photosynthetic measurements (either in natural conditions or in controlled environment) as a tool for predicting present and future growth since results were not coherently correlated with growth characteristics and productivity. Indeed, correlations were either weak or strong, positive or negative, inconsistent over environments and time. These incoherent relationships are interpreted as primarily caused by failure to account for seasonal pattern changes of assimilation (Ledig and Perry, 1969; Boltz et al., 1989). Considering this assertion, the objectives of this study were to investigate the potential of Pn efficiency as a tool for nursery bed selections through Pn measurements taken in natural conditions and at different times on the 10 families evaluated for growth characteristics in the nursery. However, this present study is of limited value because, out of three scheduled measurements, only one has been performed (due to technical reasons) and coincided to the late season growth; but it is of interest to investigate the variation in late season net Pn and determine its relationships with seedling growth characteristics.

MATERIAL AND METHODS

To conduct the experiment, 5 healthy seedlings from those measured for growth characteristics in the nursery were randomly selected in each

plot, giving 20 seedlings per family (200 seedlings total). For the selected seedlings, about 7 leaflets from attached leaves of the same degree of development and well exposed to natural light were sampled and their leaf surface area determined by using a LI-3000 area meter (LI-COR, Inc.) prior to Pn measurements. Then net Pn measurements were made during natural light period with a LI-6200 (LI-COR, Inc.) portable porometer comprising a sample chamber.

Due to unfavorable weather conditions (clouds, rain) measurements had been taken during four non-consecutive days (September 20, 24, 25 and 27) with a block (replication) per day, i.e., 50 seedlings per day. Each day of measurement began when direct sun had covered the nursery site and dew had evaporated from the seedlings (9:30 - 10:00 a.m.) and finished when the sun began to leave the site (4:30 - 5:00 p.m.). There were some differences in measurement conditions among blocks (light intensity, ambient CO_2, ambient temperature, relative humidity and rate of air flow), but these conditions were rather uniform within each block.

In addition to the measurement of Pn efficiency, stomatal conductance (Gs) and transpiration rates were obtained from the data. Analysis of variance on the basis of individual-tree data were performed on Pn efficiency, stomatal conductance and transpiration, and their subsequent relationships with nursery growth characteristics were calculated and analyzed.

RESULTS AND DISCUSSION

Variation

There were no significant differences among families or between good versus poor families at 0.05 level of probability for net photosynthesis (net Pn), stomatal conductance (Gs) and transpiration, all measured during late season growth (Table 17). However, family differences were detected at $P<0.1$ for stomatal conductance. As expected, because of the variability in measurement conditions among blocks, significant differences observed for the family by block interaction overshadowed inherent family differences. The significance of the interaction was stronger for Gs and transpiration ($P<0.01$) than for net Pn ($P<0.05$).

Family differences accounted for little of total variation in net Pn (2.88%) and stomatal conductance (6.20%) and none in transpiration rates while most of the variation was contributed by the within-family variation with 66.40%, 72.88% and 66.93% of total variation, respectively, for net Pn, Gs and transpiration (Table 16).

Mean net Pn per family ranged from 10.96 μmol $m^{-2}s^{-1}$ to 13.42 μmol $m^{-2}s^{-1}$ or 89.84% to 109.95% of the study mean with only 5 families performing above the overall mean (Table 18). The 5 best performers averaged about 6% better than the study mean, and among them only one family (382) figured in the 5 top families for final height and diameter in the nursery, indicating a reversal tendency of the order of family ranks between late season Pn and total height and diameter in the nursery. Further, only 3 families out of the 5 best for net Pn and residual growth were common to both and the worst performer in net Pn (family 375) accounted for the 3rd best in the late season growth.

Table 17. Means x summary of ANOVA in 10 open-pollinated families of black locust for late season net photosynthesis, stomatal conductance, transpiration, and late season height in a nursery progeny test.

Characteristics	Mean	Range	GF	PF	Family F-test (% variance)	Interaction F-test (% variance)	Replication F-test (% variance)
Photosynthetic efficiency	12.20±3.03	10.96-13.42	12.1864	12.2259	1.54 (2.88)	1.61* (8.70)	10.93** (21.99)
Stomatal conductance	0.498±0.181	0.3950-0.6505	0.5223	0.4762	1.87* (6.20)	2.02** (14.87)	3.32** (6.03)
Transipiration	0.0041±0.00109	0.00357-0.00465	0.004165	0.004052	1.16 (0.00)	2.55** (18.54)	5.80** (14.51)
Late season Height (cm)	1.1991±3.5106	0.6146-1.7267	1.2869	1.1334	0.970 (0.000)	1.77** (1.34)	0.654 (0.000)

GF= good families PF= poor families
* significant at 0.1 level of probability
** significant at 0.05 level of probability

Table 18. Family mean and percent of overall mean for late season net photosynthesis, stomatal conductance and transpiration in a black locust nursery progeny test.

Rank	Photosynthesis (μ mol m^{-2} s^{-1})			Stomatal Conductance (mol m^{-2} s^{-1})			Transpiration (mol m^{-2} s^{-1})			Late Season Height (cm)	
	FAM	Mean	% overall mean	FAM	Mean	% overall mean	FAM	Mean	% overall mean	FAM	Mean
1	385	13.42	109.95	385	0.65055	130.42	385	0.004655	113.31	383	1.726
2	411	13.37	109.60	383	0.54696	109.65	383	0.004280	104.18	382	1.492
3	383	13.18	108.04	391	0.52026	104.37	375	0.004265	103.81	375	1.462
4	398	12.32	101.01	411	0.49635	99.51	382	0.004265	103.81	385	1.206
5	382	12.28	100.63	382	0.49475	99.19	380	0.004240	103.21	348	1.260
6	348	11.97	98.08	375	0.48453	99.14	398	0.004175	101.62	380	1.253
7	380	11.89	97.43	380	0.47011	94.25	391	0.004010	97.61	391	1.167
8	391	11.31	98.72	348	0.46439	93.10	411	0.003965	96.51	398	0.959
9	347	11.26	92.28	398	0.45812	91.84	348	0.003600	87.63	411	0.904
10	375	10.96	89.84	347	0.39506	79.20	347	0.003575	87.02	347	0.614
Over-all mean		12.2065			0.49875			0.004108			1.199

Therefore, an inconsistent relationship seemed to exist between late season net Pn and growth characteristics as observed in the nursery.

For stomatal conductance, only 3 families performed above the overall mean whereas for transpiration 6 families were found above the study mean.

Correlations

Relationships between physiological characteristics (Table 19).

The correlation coefficients at family level between late season net Pn and stomatal conductance (r=0.623) and/or transpiration (r=0.464) were not significant but moderate while Gs and transpiration appeared strongly related (r=0.813). These results indicate that high stomatal conductance in late season growth would lead to increased rates of transpiration (i.e., loss of water) but not necessarily in high photosynthetic rates.

On the other hand, total (individual-tree) and within-family correlations among the three characteristics were all highly significant ($P<0.01$) suggesting that mass selection (i.e., selection of individuals regardless of families) and within-family selection based on late season Pn efficiency would result in discriminating seedlings with higher Gs and transpiration rates.

Relationships between Pn efficiency and nursery growth characteristics and field height.

Negative family correlations were found associating late season Pn efficiency with initial height, total height, diameter and number of branches (Table 19) while positive and weak correlations were observed between Pn efficiency and late season growth, thorn length, stem form

Table 19. Correlations between physiological characteristics and nursery growth characteristics and field height.

Total between-family and within-family correlations, among physiological characteristics.

	Photosynthesis			Stomatal Conductance			Transpiration		
	Total df=195	Between-family df=9	Within-family df=186	Total df=195	Between-family df=9	Within-family df=186	Total df=195	Between-family df=9	Within-family df=186
Photosynthesis	1.000	1.000	1.000	0.428***	0.623	0.408***	0.470***	0.464	0.470***
Stomatal conductance				1.000	1.000	1.000	0.720***	0.813**	0.714***
Transpiration							1.000	1.000	1.000

(Table 19 continued)

Table 19 (cont'd)

Family mean correlations and rank correlations (in parenthesis) associating physiological characteristics with nursery characteristics and field heights.

Characteristics	Photosynthesis		Stomatal Conductance		Transpiration	
Nursery:						
Initial height	-0.812**		-0.683*		-0.603*	
Total height	-0.592	(-0.600)	-0.692*	(-0.539)	-0.531	(-0.624)
Residual height growth	0.178		0.495		0.560	
Diameter	-0.535	(-0.539)	-0.415	(-0.321)	-0.498	(-0.296)
No. of branches	-0.415	(-0.515)	-0.074	(-0.224)	-0.169	(-0.224)
Thorn length (score)	0.484	(0.624)	0.179	(0.139)	0.355	(0.018)
Insect damage (score)	0.164	(-0.200)	0.462	(-0.381)	0.599	(-0.587)
Stem form (score)	0.263	(-0.284)	0.309	(-0.03)	0.494	(-0.212)
Lab:			-0.106	(0.054)	-0.204	(-0.200)
Percent germination	-0.174	(-0.103)	-0.114	(-0.078)	-0.358	(-0.151)
Seed weight	-0.150	(-0.139)				
Field:			0.0357	(-0.139)	-0.051	(-0.236)
First-year height	-0.0328	(-0.054)	0.226	(0.069)	0.089	(0.023)
Second-year height	0.0222	(0.185)	0.221	(0.103)	0.206	(-0.006)
Fourth-year height	0.102	(0.236)				

*significant at 0.05 level **significant at 0.01 level ***significant at 0.001 level

and insect damage in the nursery. Similar trends of correlations were also observed between nursery growth characteristics with stomatal conductance and transpiration (Table 19). Finally, little association was found between late season Pn efficiency in the nursery and seed weight, percent seed germination, and 1-, 2- and 4-year field height. These poor and inconsistent correlations relating late season Pn efficiency and growth characteristics compare in many respects with other studies on photosynthesis (see Introduction) and are mainly caused by the failure to take into account the seasonal changes in photosynthesis. For example, Logan (1971) found in studying a 7-year old jack pine provenance test (*Pinus banksiana* Lamb.) a favorable relationship between photosynthetic rate and tree height in October and not the other months. Therefore, photosynthetic rates cannot be characterized by measurements at only one period in the growing season (Ledig and Perry, 1969).

CONCLUSION

This study based on late season Pn efficiency has shown: (1) no statistical differences in late season net Pn among genotypes of black locust grown in the nursery; and (2) inconsistent relationships with growth characteristics.

It appeared that photosynthesis is a complex characteristic greatly affected by plant architecture and seasonal changes, and thus cannot be characterized by irregular measurements. However, genetic improvement may result from comprehensive studies of Pn, if in addition other factors such as relation of Pn to respiration and distribution of

photosynthate within trees are taken into consideration (Luukkanen and Kozlowski, 1972).

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