

GENETIC VARIATION OF BLACK LOCUST SEEDLINGS ON RECLAIMED SURFACE MINE SOILS¹

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Abstract.--Surface mining of coal frequently is done on forestry-based industrial lands in West Virginia. The resulting plant competition following reclamation inhibits the reestablishment of natural hardwood forests. Black locust (Robinia pseudoacacia L.) is a successful, fast growing hardwood when planted on these sites. Studies were established to determine how much genetic variability exists in black locust and which sources are the most desirable for planting on surface mined lands. Three experimental plantings have been established in Greenbrier County, WV, since 1984 from seed collected throughout the range of black locust. The oldest plantation has 30 seedlots from across the species' entire range. Genetic variation has been observed in leaf nutrient and chlorophyll variation. Height variation has also been observed. No geographic patterning has been associated with the variation. Selected seedlots can be identified which are expected to be superior sources.

INTRODUCTION

The state of West Virginia has an abundance of natural resources including forests and minerals. Over 75% of the state is covered with natural hardwood forests (Bones 1978). These forests may be managed for valuable hardwood lumber or converted to pine plantations for fiber production. Coal is another abundant natural resource in the state is frequently surface mined on forestry-based industrial lands.

Surface mines are recontoured soon after the mining operation is completed. The heterogenous artificial soils which cover the site are quickly regenerated with plants to prevent soil erosion. Herbaceous plants which are often used are legumes including vetch, clover, and Lespedeza and

grasses (oats, fescue, and millet). These plants usually colonize and dominate the new sites very quickly, and the resulting plant competition inhibits the reestablishment of natural hardwood forests. Forestry operations on surface mined lands are greatly hampered due to the difficulty of forest regeneration, thus removing large acreages of forestry land from productivity for many years.

Numerous experimental trials of re-planting hardwood and conifer species on reclaimed surfaces have been made. Black locust (Robinia pseudoacacia L.) has been demonstrated to be a successful, fast-growing hardwood when planted on these sites (Brown 1973). One of the most widely planted tree species in the world (Keresztesi 1980), it is a rapidly growing, shade-intolerant species which grows well on a range of soil qualities. The wood is naturally resistant to decay and resists swelling and contracting with moisture changes, and as a legume it improves soil quality by fixing atmospheric nitrogen. Black locust has been used in the forest industry as a source of railroad ties, fenceposts, mine timbers, stakes, boxes, and other items. Fencepost-sized trees can be grown in 15 to 20 years (Kellogg 1933). The wood has a high specific gravity of about 0.73 oven-dry (Panshin and de Zeeuw 1970) and is one of the most efficient wood sources in the paper-pulping process.

¹Paper presented at the 1988 Mine Drainage and Surface Mine Reclamation Conference sponsored by the American Society for Surface Mining and Reclamation and the U.S. Department of the Interior (Bureau of Mines and Office of Surface Mining Reclamation and Enforcement) April 17-22, 1988, Pittsburgh, PA.

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The best growth of black locust has been found in West Virginia (Fowells 1965). Because of its extensive use on strip mined soils, a great opportunity exists to improve the surface mined land quality while concurrently producing wood for wood products by establishing plantings of black locust on these lands.

Most forest tree species exhibit genetic variation in height and volume growth. Considerable gain has been made in recent years by identifying superior sources of many tree species. Kennedy (1983) has reported genetic variation among sources of black locust in seed length, seed length/width ratio, seedling height, rachis length, and timing of spring bud break. Provenance tests of over 100 locust seedlots have been established in West Virginia since 1984. The purposes of these tests are to determine how much genetic variability exists in black locust and which sources are the most desirable for planting on surface mined land. Recommendations from field performance results of these tests will not be available for another 3 to 9 years. Physiological traits which may reflect expected field performance can be measured immediately, however. The basic photosynthetic pigments, chlorophyll A and chlorophyll B, can easily be measured in a laboratory using spectrophotometric techniques (MacKinney 1941). These chemicals are primary components of carbon assimilation, and variation in their concentrations may indicate limits in the amount of possible growth. Concentrations of the basic nutrients N, P, Ca, Mg, and K may also limit seedling development. Studies of these traits were made to provide genetic information in advance of useful growth data. Knowledge of the genetic variation in leaf physiology will lead to a better understanding of tree growth and of ways to improve the species.

PROCEDURES

Plantation Establishment

Seed collections were made throughout the range of black locust during the years 1981 to 1984. Three experimental plantations of seedlings on surface mined lands have been established in Greenbrier County, WV (table 1).

Greenhouse-grown seedlings were produced at Westvaco's West Virginia Research Center in Rupert, WV. The nursery-grown seedlings were produced at the Parsons, West Virginia State Nursery. Each planting is composed of five or six blocks within which all seedlots are represented and randomly distributed. Measurements of height and survival are made annually. Relative heights were expressed as a percentage of the plantation mean.

Plant materials for the physiological studies were obtained from the 1984 Bellburn planting. Table 2 lists geographic

Table 1.--Location, elevation, and establishment data of experimental black locust plantations, Greenbrier County, WV.

| Site | Ele. | Year Estab. | No. of Seedlots | Seedling type |
|-------------|--------|-------------|-----------------|--------------------------|
| Bellburn #1 | 823 m | 1984 | 30 | Greenhouse-grown |
| Clearco | 1070 m | 1985 | 26 | Greenhouse-grown |
| Bellburn #2 | 823 m | 1986 | 117 | Nursery-grown, 1-0 Stock |

origins for each seedlot. Prior to the 1985 growing season, after one year in the field, the seedlings ranged in height from 6 to 120 cm.

Chlorophyll Analysis

Two times during the 1985 growing season, on June 12 and August 23, leaf samples were collected on randomly selected, fully developed leaves to perform chlorophyll concentration analyses. A paper punch was used to make a 0.266 cm² leaf disk of each sampled leaf. Tissues were collected only on the leaflet blade, avoiding the midrib. Leaf disks were immediately stored in a glass vial which was transported to the lab inside an ice chest. All vials were stored in the freezer as soon as they were returned to Westvaco's West Virginia Research Laboratory. Chlorophyll analyses were similar to those described by Arnon (1949). Disks were homogenized in 3 ml of 80% acetone for 10 min. The mixture was transferred to graduated centrifuge tubes, diluted to 10 ml with 80% acetone, and centrifuged for 20 min at high speed. The supernatant was measured at 645 and 633 nm wavelength with a Beckman Spectronic 21 spectrophotometer using an 80% acetone blank (MacKinney 1941). Chlorophyll A (Chl. A) and chlorophyll B (Chl. B) concentrations were determined by the following equations:

$$\text{Chl. A (mg/L)} = 12.7 D_{663} - 2.69 D_{645}$$

$$\text{Chl. B (mg/L)} = 22.9 D_{645} - 4.68 D_{663}$$

$$\text{Total chlorophyll (mg/L)} = 20.2 D_{645} + 8.02 D_{663}$$

where D_{645} and D_{663} are the optical density readings at 645 and 663 nm, respectively. Concentrations in the extract solutions were adjusted for total area of leaf disks used in the extraction. Final leaf concentrations were given as $\mu\text{g/cm}^2$ leaf area.

Table 2.--Geographic origins of seedlots in the 1984 Bellburn #1 black locust seedlot test.

| Seedlot | City | County | State | North Latitude | West Longitude | Elevation, m |
|---------|----------------|------------|-------|-------------------|-------------------|-----------------|
| 683 | Blacksburg | Pulaski | VA | 37° 09' | 80° 33' | 536 |
| 687 | Blacksburg | Montgomery | VA | 37 17 | 80 27 | 689 |
| 688 | Gay | Meriwether | GA | 33 01 | 84 35 | 244 |
| 692 | Gay | Meriwether | GA | 32 58 | 84 41 | 259 |
| 696 | Dadeville | Tallapoosa | AL | 32 50 | 85 48 | 198 |
| 699 | Dadeville | Tallapoosa | AL | 32 50 | 85 48 | 201 |
| 700 | Dadeville | Tallapoosa | AL | 32 50 | 85 48 | 201 |
| 701 | Grant | Marshall | AL | 34 31 | 86 15 | 372 |
| 703 | Gurley | Madison | AL | 34 46 | 86 30 | 238 |
| 707 | Hiawassee | Towns | GA | 34 52 | 83 43 | 622 |
| 712 | Hiawassee | Towns | GA | 34 55 | 83 42 | 597 |
| 715 | Lake City | Campbell | TN | 36 17 | 84 12 | 372 |
| 716 | Caryville | Campbell | TN | 36 18 | 84 13 | 323 |
| 720 | Staunton | Augusta | VA | 38 05 | 79 05 | 469 |
| 722 | Staunton | Augusta | VA | 38 13 | 79 03 | 475 |
| 730 | Umpire | Howard | AR | 34 17 | 94 03 | 268 |
| 732 | Umpire | Howard | AR | 34 15 | 94 04 | 244 |
| 734 | Licking | Texas | MO | 37 34 | 91 53 | 366 |
| 742 | Chestnut Mound | Smith | TN | 36 13 | 85 50 | 213 |
| 743 | Elmwood | Smith | TN | 36 13 | 85 53 | 165 |
| 748 | Robbinsville | Graham | NC | 35 17 | 83 46 | 695 |
| 750 | Bryson City | Swain | NC | 35 25 | 83 28 | 543 |
| 754 | Gneiss | Macon | NC | 35 06 | 83 16 | 884 |
| 756 | Gneiss | Macon | NC | 35 08 | 83 17 | 645 |
| 797 | Univ. Park | Centre | PA | 40 48 | 75 55 | 384 |
| 799 | Univ. Park | Centre | PA | 40 49 | 77 56 | 421 |
| 808 | Frostburg | Allegany | MD | 39 39 | 78 59 | 640 |
| 811 | Frostburg | Allegany | MD | 39 37 | 78 52 | 488 |
| 812 | Elkins | Randolph | WV | 38 54 | 79 42 | 725 |
| 815 | Harman | Randolph | WV | 38 54 | 79 33 | 847 |

Nutrient Analysis

A collection of leaves was made on June 12, 1985, in order to perform a nutrient variation analysis. One fully mature leaf randomly selected from each of the five trees of a seedlot was collected within each block. If leaf samples were too small to make a minimum 1 g oven-dry sample, additional leaves were collected from trees within the block. One hundred and seventy-five samples were collected, dried, and analyzed at the Westvaco Forest Science Laboratory for N, P, Ca, Mg, and K.

Simple correlations of seedlot mean values for nutrient and chlorophyll concentrations and height growth were made to determine possible patterns.

RESULTS

Seedlot Chlorophyll Variation

Chlorophyll A concentrations were consistently higher than chlorophyll B concentrations across all seedlots during both collection times. Chl. A comprised over 60 percent of total chlorophylls, and showed a slight increase in the August collection. Mean seedlot values in the June measurements ranged from 22 to 29 $\mu\text{g}/\text{cm}^2$ for chlorophyll A and from 13 to 19 $\mu\text{g}/\text{cm}^2$ for

chlorophyll B (table 3). The range of August values was 21 to 35 and 13 to 21 $\mu\text{g}/\text{cm}^2$ for chlorophyll A and B, respectively. Significant seedlot differences in Chl. A concentrations existed in both the June and August measurements (table 4). Differences among seedlots for Chl. B were only observed in the August collection. The ratio between Chl. A and Chl. B did not vary between seedlots. This ratio was higher in the August collection, however, due to an increase in Chl. A levels.

For a specific collection period, the values of Chl. A, Chl. B and total chlorophylls were highly correlated. Seedlots number 699 from Alabama and 812 from West Virginia had the highest chlorophyll levels in the June collection. They still had high rankings in the August collection, but seedlot 815 from West Virginia, which had a much lower level in the June collection, had the highest August levels. Seedlot 799 decreased in total chlorophyll from the June to August collections. Analysis of variance indicated no interaction between seedlot and date of collection (table 4). The ratio between Chl. A and Chl. B showed no consistent relationship across seedlots in both collections.

There was no apparent geographic pattern to the chlorophyll measurement variation. The highest June values were in

Table 3.--Concentrations of chlorophylls and the ratio of chlorophyll A to chlorophyll B in the leaves of thirty black locust seedlots.

| Seedlot | Chlorophyll A | | | Chlorophyll B | | | Total Chlorophyll | | | Chl. A/Chl. B Ratio | | |
|----------|---------------|--------|-------|--------------------|--------|-------|-------------------|--------|-------|---------------------|--------|------|
| | June | August | Avg. | June | August | Avg. | June | August | Avg. | June | August | Avg. |
| | | | | µg/cm ² | | | | | | | | |
| 683 | 25a-e* | 24b-e | 25b-h | 15 | 14b | 15cd | 41a-d | 38cde | 40b-h | 1.65 | 1.67 | 1.66 |
| 687 | 22e | 23cde | 22h | 14 | 14b | 14d | 36d | 37cde | 36gh | 1.55 | 1.61 | 1.58 |
| 688 | 23b-e | 21de | 22h | 15 | 13b | 14d | 38bcd | 34de | 36h | 1.55 | 1.59 | 1.57 |
| 692 | 22de | 29a-d | 26b-h | 14 | 18ab | 16bcd | 36cd | 47a-e | 41b-h | 1.59 | 1.68 | 1.63 |
| 696 | 27a-d | 29a-e | 28a-d | 17 | 17ab | 17abc | 44abc | 46a-e | 45a-d | 1.60 | 1.64 | 1.62 |
| 699 | 29a | 33ab | 31a | 19 | 20a | 19a | 48a | 53ab | 51a | 1.59 | 1.61 | 1.60 |
| 700 | 25a-e | 29a-e | 27a-g | 15 | 18ab | 16bcd | 39a-d | 46a-e | 43b-h | 1.67 | 1.64 | 1.65 |
| 701 | 26a-e | 23cde | 25b-h | 16 | 14b | 15bcd | 42a-d | 38cde | 40b-h | 1.66 | 1.64 | 1.65 |
| 703 | 24a-e | 24b-e | 24c-h | 16 | 14b | 15bcd | 40a-d | 39cde | 39b-h | 1.56 | 1.68 | 1.62 |
| 707 | 25a-e | 22cde | 24d-h | 16 | 14b | 15bcd | 41a-d | 36cde | 39c-h | 1.57 | 1.65 | 1.60 |
| 712 | 23b-e | 22cde | 23fgh | 14 | 13b | 14d | 38bcd | 36cde | 37fgh | 1.64 | 1.67 | 1.66 |
| 715 | 24a-e | 26a-e | 25b-h | 15 | 15b | 15bcd | 39a-d | 41b-e | 40b-h | 1.61 | 1.70 | 1.66 |
| 716 | 26a-e | 22cde | 24d-h | 16 | 14b | 15bcd | 42a-d | 36cde | 39b-h | 1.60 | 1.58 | 1.59 |
| 720 | 23b-e | 25b-e | 24d-h | 14 | 15b | 15cd | 36cd | 40b-e | 38d-h | 1.63 | 1.64 | 1.63 |
| 722 | 24a-e | 27a-e | 25b-h | 15 | 16ab | 15bcd | 39bcd | 42a-e | 40b-h | 1.65 | 1.70 | 1.67 |
| 730 | 26a-e | 29a-e | 27a-f | 16 | 17ab | 17bcd | 41a-d | 46a-e | 44a-g | 1.64 | 1.69 | 1.67 |
| 732 | 25a-e | 28a-e | 26b-h | 16 | 18ab | 16bcd | 40a-d | 45a-e | 42b-h | 1.57 | 1.57 | 1.57 |
| 734 | 23b-e | 22cde | 22h | 14 | 13b | 14d | 37bcd | 35cde | 36h | 1.58 | 1.64 | 1.61 |
| 742 | 23b-e | 24b-e | 23d-h | 15 | 13b | 14d | 38bcd | 37cde | 38e-h | 1.57 | 1.75 | 1.66 |
| 743 | 25a-e | 30abc | 27a-g | 16 | 18ab | 17abc | 41a-d | 48abc | 44a-f | 1.54 | 1.65 | 1.59 |
| 748 | 24a-e | 27a-e | 26b-h | 16 | 15ab | 15bcd | 40a-d | 42a-e | 41b-h | 1.57 | 1.71 | 1.65 |
| 750 | 26a-e | 26a-e | 26b-h | 15 | 16ab | 15bcd | 41a-d | 41b-h | 41b-h | 1.67 | 1.67 | 1.67 |
| 754 | 23b-e | 22cde | 23gh | 14 | 13b | 14d | 37bcd | 35cde | 36h | 1.62 | 1.70 | 1.66 |
| 756 | 22de | 25b-e | 23d-h | 14 | 15ab | 15cd | 36cd | 41b-e | 38d-h | 1.55 | 1.63 | 1.58 |
| 797 | 22cde | 29a-d | 26b-h | 13 | 18ab | 16bcd | 35d | 47a-e | 42b-h | 1.68 | 1.63 | 1.65 |
| 799 | 26a-e | 20e | 23e-h | 16 | 13b | 15cd | 42a-d | 33e | 38e-h | 1.62 | 1.56 | 1.59 |
| 808 | 28ab | 27a-e | 27a-f | 16 | 17ab | 17bcd | 44a-d | 44a-e | 44a-g | 1.69 | 1.59 | 1.64 |
| 811 | 28abc | 31abc | 29ab | 16 | 18ab | 17abc | 44a-d | 49abc | 46abc | 1.70 | 1.70 | 1.70 |
| 812 | 28ab | 30a-d | 29abc | 18 | 18ab | 18ab | 46ab | 48a-d | 47ab | 1.59 | 1.67 | 1.63 |
| 815 | 23b-e | 35a | 28a-e | 15 | 21a | 17abc | 38bcd | 55a | 45a-e | 1.57 | 1.70 | 1.62 |
| Over-all | 25 | 26 | 25 | 15 | 16 | 16 | 40 | 42 | 41 | 1.61 | 1.65 | 1.63 |

*Values within a column followed by the same letter are not significantly different at 5% by Duncan's Multiple Range Test.

seedlots from Alabama, West Virginia, and Maryland. The lowest June values were seedlots from Virginia, Georgia, North Carolina, and Pennsylvania. The August values similarly indicated no geographic patterning to the variation.

The lack of a clear-cut geographic pattern to the variation is significant because most tree species do show such variation. It is possible that natural variation has been severely disturbed by artificial regeneration techniques during the past several hundred years.

Leaf Nutrient Variation

Leaf nutrient levels also varied between seedlots. Of the five nutrients routinely analyzed, only calcium showed no seedlot source differences (table 5). Significant block differences for N and Ca levels may have been due to the artificial mixing of soils associated with strip mining.

Correlation analysis of individual samples indicates that nitrogen levels were proportional to phosphorus, calcium, and potassium levels. Phosphorus levels also correlated positively with magnesium and potassium. Although seedlot differences were significant for four nutrients, the ranges of values were not great (table 6). Seedlot 688 from Meriwether County, GA, had the highest values for most nutrients. Seedlot 815 from Randolph County, WV, had the lowest nitrogen levels. As with the chlorophyll variation, no geographic patterns could be determined among the seedlots.

Height Growth Variation

The most recent data are presented in table 7 for 56 seedlots. Statistically distinct differences in growth can be seen in each study. At the Bellburn #1 location, seedlots from Tennessee, Alabama, and West Virginia had the best growth by age 3 yrs. In the Clearco study, lot BN4194,

Table 4.--Analysis of variance of black locust leaf chlorophyll concentrations.

| Source | DF | Chlorophyll A | | Chlorophyll B | | Chl. A/Chl. B | |
|-----------------------|-----|--------------------|---------|--------------------|---------|--------------------|---------|
| | | Type III | F | Type III | F | Type III | F |
| | | Sums of Squares | | Sums of Squares | | Sums of Squares | |
| Spring Collection | | | | | | | |
| Seedlot | 29 | 682.45 | 1.70 * | 232.38 | 1.34 NS | 0.3450 | 0.98 NS |
| Block | 5 | 200.32 | 2.90 * | 84.13 | 2.81 * | 0.1608 | 2.65 * |
| Error | 136 | 1879.02 | - | 814.81 | - | 1.6482 | - |
| Fall Collection | | | | | | | |
| Seedlot | 29 | 2015.60 | 1.85 * | 725.91 | 2.21 ** | 0.3307 | 1.05 NS |
| Block | 5 | 413.69 | 2.20 NS | 80.68 | 1.37 NS | 0.2822 | 5.21 ** |
| Error | 127 | 4782.61 | - | 1498.85 | - | 1.3757 | - |
| Combined Measurements | | | | | | | |
| Seedlot | 29 | 1811.19 | 2.13 ** | 671.50 | 2.32 ** | 0.4135 | 1.16 NS |
| Block | 5 | 138.39 | 1.17 NS | 26.73 | 0.65 NS | 0.1079 | 1.70 NS |
| Error A | 140 | 4109.91 | 1.25 NS | 1398.32 | 1.21 NS | 1.7195 | 0.97 NS |
| Collection | 1 | 115.16 | 4.88 * | 6.76 | 0.82 NS | 0.1132 | 8.94 ** |
| S X C | 29 | 941.14 | 1.38 NS | 333.38 | 1.39 NS | 0.3194 | 0.87 NS |
| Error B | 128 | 3017.97 | - | 1057.76 | - | 1.6212 | - |

*, ** - Differences between means are significant at 5% and 1%, respectively.
NS - No significant differences between means.

Table 5.--Statistical analysis of variance of black locust seedlot leaf nutrient levels.

| Degrees of Freedom | Source | | |
|--------------------|---------------|------------|--------------|
| | Seedlot 29 | Block 5 | Error 140 |
| Nitrogen | | | |
| Type III | | | |
| Sum of Squares | 5.64 | 1.23 | 14.95 |
| F | 1.82* | 2.32* | - |
| Phosphorus | | | |
| Type III | | | |
| Sum of Squares | 0.06 | 0.01 | 0.12 |
| F | 2.23** | 1.29NS | - |
| Calcium | | | |
| Type III | | | |
| Sum of Squares | 0.43 | 0.28 | 1.68 |
| F | 1.24NS | 4.58** | - |
| Magnesium | | | |
| Type III | | | |
| Sum of Squares | 0.13 | 0.01 | 0.26 |
| F | 2.39** | 1.56NS | - |
| Potassium | | | |
| Type III | | | |
| Sum of Squares | 2.15 | 0.06 | 4.11 |
| F | 2.53** | 0.43NS | - |

* - Differences significant at 5%.
** - Differences significant at 1%.
NS - Differences not significant.

which was provided by the Soil Conservation Service, but originated in West Virginia, performed excellently. The Bellburn #2 study showed additional good material from Virginia. Seedlot # 716 from Tennessee performed well in both of the Bellburn plantings. Individual tree selection is necessary for an efficient genetic improvement program.

Although seedlot differences exist, we have not observed any geographic pattern from which we can make collection recommendations. This observation is consistent with those made by other researchers who have concluded that little geographic variation can be associated with genetic variation in black locust (Bongarten 1985; Kennedy 1983). Individual tree selection is necessary for an efficient genetic improvement program.

Relationship Between Traits

Mean seedlot values for each trait measured in this report were compared to determine possible relationships which may exist. Although considerable variation occurred in the data, simple linear regressions did point out some expected and unexpected relationships.

Seedlots with higher levels of nitrogen tended to have higher phosphorus ($r = 0.54$) and potassium levels ($r = 0.37$). Phosphorus levels also paralleled magnesium levels. The results are similar to those observed with individual sample correlations. An unexpected result was the negative relationship between certain nutrients and chlorophyll concentrations and height

Table 6.--Leaf nutrient variation among thirty seedlots of black locust seedlings.

| Seedlot | Nitrogen | Phosphorus | Calcium % Tissue | Magnesium | Potassium |
|---------|----------|------------|---------------------|-----------|-----------|
| 683 | 3.15 a-e | 0.21 a-e | 0.58 | 0.24 cde | 1.62 b-e |
| 687 | 2.80 de | 0.19 cde | 0.51 | 0.22 cde | 1.52 cde |
| 688 | 3.48 a | 0.25 a | 0.62 | 0.30 ab | 1.96 a |
| 692 | 3.04 a-e | 0.19 cde | 0.46 | 0.21 e | 1.80 ab |
| 696 | 3.26 a-d | 0.19 cde | 0.50 | 0.19 e | 1.57 b-e |
| 699 | 3.36 ab | 0.20 cde | 0.53 | 0.20 e | 1.67 bcd |
| 700 | 2.99 b-e | 0.18 de | 0.58 | 0.21 de | 1.59 b-e |
| 701 | 3.07 a-e | 0.18 de | 0.49 | 0.21 e | 1.64 bcd |
| 703 | 3.03 a-e | 0.19 cde | 0.53 | 0.24 a-e | 1.62 b-e |
| 707 | 3.24 a-d | 0.21 a-e | 0.57 | 0.19 e | 1.64 bcd |
| 712 | 3.06 a-e | 0.20 b-e | 0.46 | 0.20 e | 1.57 b-e |
| 715 | 3.15 a-e | 0.19 de | 0.59 | 0.22 cde | 1.76 abc |
| 716 | 3.07 a-e | 0.17 e | 0.51 | 0.20 e | 1.68 bcd |
| 720 | 3.14 a-e | 0.19 de | 0.48 | 0.21 e | 1.67 bcd |
| 722 | 3.36 ab | 0.22 a-d | 0.53 | 0.27 a-d | 1.50 de |
| 730 | 3.01 a-e | 0.21 a-e | 0.51 | 0.20 e | 1.72 bcd |
| 732 | 3.19 a-e | 0.21 a-e | 0.50 | 0.19 e | 1.56 b-e |
| 734 | 3.25 a-d | 0.24 ab | 0.55 | 0.30 a | 1.51 cde |
| 742 | 3.19 a-e | 0.18 de | 0.58 | 0.25 a-e | 1.65 bcd |
| 743 | 3.34 ab | 0.20 cde | 0.59 | 0.22 de | 1.63 bcd |
| 748 | 2.82 cde | 0.20 cde | 0.59 | 0.22 de | 1.43 de |
| 750 | 3.16 a-e | 0.22 a-d | 0.48 | 0.24 a-e | 1.53 cde |
| 754 | 3.12 a-e | 0.23 abc | 0.53 | 0.28 abc | 1.64 bcd |
| 756 | 3.38 ab | 0.23 abc | 0.50 | 0.22 cde | 1.62 bcd |
| 797 | 3.28 abc | 0.21 a-e | 0.57 | 0.24 b-e | 1.69 bcd |
| 799 | 2.99 b-e | 0.19 de | 0.59 | 0.21 de | 1.38 e |
| 808 | 3.20 a-d | 0.21 a-e | 0.63 | 0.23 cde | 1.55 cde |
| 811 | 3.07 a-e | 0.21 a-e | 0.60 | 0.22 de | 1.73 bcd |
| 812 | 2.86 cde | 0.19 cde | 0.56 | 0.22 cde | 1.64 bcd |
| 815 | 2.72 e | 0.18 de | 0.62 | 0.24 a-e | 1.49 de |
| Mean | 3.12 | 0.20 | 0.54 | 0.22 | 1.62 |

* Values within a column followed by a common letter are not significantly different at 5% by Duncan's Multiple Range Test.

growth. Seedlot values for magnesium tended to decrease with increasing chlorophyll levels. Height increments were negatively related to phosphorus ($r = -.60$) and magnesium levels ($r = -.68$).

The various chlorophyll measurements tended to show positive relationships between seedlots, as could be expected. They also exhibited a positive relationship with height growth. The June measurements were more strongly related to overall seasonal growth than August levels. This relationship is supported by the variation in June foliage coloration. Seedlots which had the highest total chlorophyll in June generally had greater growth rates during the season (fig. 1). Some seedlots may produce chlorophyll earlier in the season which may allow some growth advantage. This possible relationship between June chlorophyll levels and overall seasonal growth should be verified as it may be a useful selection tool.

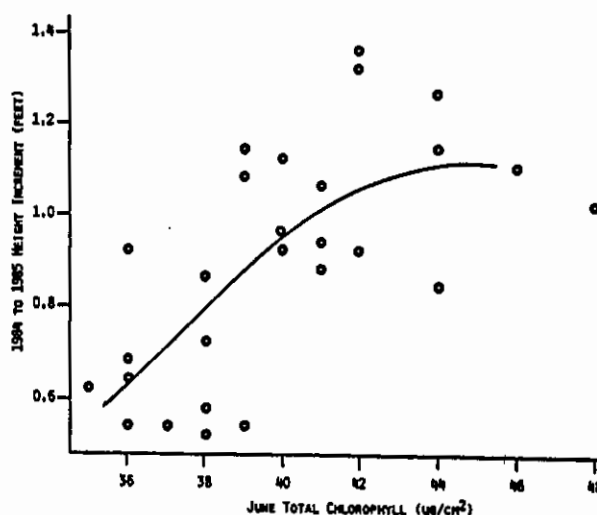


Figure 1.--Distribution of seedlot mean values for total June chlorophyll and height increment during the 1985 Growing season. The curvilinear regression accounts for 50% of the variation.

Table 7.--Relative heights of 56 black locust seedlots planted on 3 sites in West Virginia. Seedlots within a planting site which are followed by a common letter are not significantly different at a 95% probability.

| Seedlot | Bellburn #1 3 yr old | Clearco 2 yr old | Bellburn #2* 1 yr old |
|------------------------------------------------------------------|-------------------------|---------------------|--------------------------|
| -Relative Heights and Differences- Percent of Plantation Mean | | | |
| 683 | 89 A-E | --- | 106 B-Q |
| 687 | 81 CDE | --- | 130 A-D |
| 688 | 92 A-E | --- | 94 E-X |
| 692 | 83 B-E | --- | 109 A-O |
| 696 | 115 A-D | --- | 87 J-X |
| 699 | 106 A-E | --- | --- |
| 700 | 113 A-D | --- | 100 C-S |
| 701 | 121 AB | --- | 71 R-Y |
| 703 | 103 A-E | --- | 124 A-F |
| 707 | 99 A-E | --- | 100 C-S |
| 712 | 103 A-E | --- | 106 B-Q |
| 715 | 105 A-E | --- | 103 B-S |
| 716 | 125 A | --- | 134 AB |
| 720 | 79 DE | --- | 95 E-W |
| 722 | 79 DE | --- | 92 E-X |
| 730 | 105 A-E | --- | 111 A-N |
| 732 | 95 A-E | --- | 84 K-X |
| 734 | 71 E | --- | --- |
| 742 | 109 A-D | --- | --- |
| 743 | 103 A-E | --- | 112 A-N |
| 748 | 105 A-E | --- | 63 T-Y |
| 750 | 85 B-E | --- | 93 E-X |
| 754 | 83 CDE | --- | 90 F-X |
| 756 | 92 A-E | --- | 98 D-S |
| 797 | 100 A-E | --- | 105 B-R |
| 799 | 95 A-E | --- | 125 A-D |
| 808 | 95 A-E | --- | 111 A-N |
| 811 | 113 A-D | --- | 107 B-Q |
| 812 | 117 ABC | --- | 106 B-Q |
| 815 | 100 A-E | --- | 102 B-S |
| 838 | --- | 93 CDE | 60 XY |
| 839 | --- | 112 BCD | 112 A-M |
| 840 | --- | 80 DEF | 85 K-X |
| 841 | --- | 92 C-F | 89 G-X |
| 842 | --- | 90 C-F | 73 Q-Y |
| 845 | --- | 98 CDE | 114 A-L |
| 846 | --- | 98 CDE | 84 K-X |
| 847 | --- | 122 BC | 93 E-X |
| 848 | --- | 79 DEF | 113 A-L |
| 849 | --- | 101 CDE | 95 E-W |
| 852 | --- | 56 F | --- |
| 856 | --- | 71 EF | --- |
| 857 | --- | 85 C-F | --- |
| 858 | --- | 115 BCD | --- |
| 860 | --- | 83 DEF | 120 A-I |
| 861 | --- | 111 BCD | 119 A-I |
| 862 | --- | 72 EF | 96 D-U |
| 863 | --- | 83 DEF | 88 H-X |
| 865 | --- | 93 CDE | 97 D-T |
| 866 | --- | 81 DEF | 62 WXY |
| 867 | --- | 96 CDE | 100 C-S |
| 868 | --- | 92 C-F | 78 N-Y |
| 869 | --- | 85 C-F | 108 A-O |
| 4191 | --- | 138 AB | --- |
| 4192 | --- | 101 CDE | --- |
| 4194 | --- | 157 A | --- |

*Only those seedlots common to the other plantings are listed.

CONCLUSIONS

This research in black locust seedlot variations has led to the following conclusions:

1. The concentrations of chlorophylls A and B in the leaves of black locust seedlings vary seasonally, increasing from low June levels to the highest levels in August.
2. Concentrations of chlorophylls in black locust exhibit considerable seedlot variation. The variation has no geographic patterning associated with it. Chlorophyll A had a consistently higher concentration than chlorophyll B and increased in concentration during the season.
3. There are no significant interactions between seedlot chlorophyll levels and time of collection.
4. The ratio between chlorophyll A and chlorophyll B did not vary between seedlots. It did increase during the growing season as chlorophyll A levels increased and chlorophyll B did not.
5. Leaf nutrient levels of N, P, Mg, and K did vary significantly between seedlots. No geographic patterning was associated with this variation.
6. Simple correlation analysis of the various traits measured indicates a positive relationship between June chlorophyll levels and overall seasonal growth. Seedlot variation in the rates of chlorophyll development may yield a growth advantage and possibly could be used as a selection criterion in black locust improvement.
7. Seedlots do vary significantly in their rate of growth. Several seedlots have been identified which are superior to others when planted on surface mined lands. As the technology to reproduce superior black locust genotypes develops, owners of reclaimed surface mines may be able to select and plant only the best material for their sites.

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