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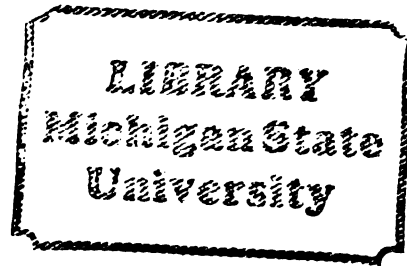
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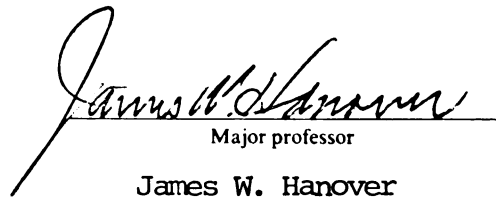
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HONEYLOCUST (Gleditsia triacanthos L.): GENETIC  
VARIATION AND POTENTIAL USE AS AN AGROFORESTRY SPECIES

presented by

Michael Alan Gold

has been accepted towards fulfillment  
of the requirements for

Ph.D. \_\_\_\_\_ degree in FORESTRY \_\_\_\_\_

  
Major professor  
James W. Hanover

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HONEYLOCUST (Gleditsia triacanthos L.): GENETIC VARIATION  
AND POTENTIAL USE AS AN AGROFORESTRY SPECIES

By

Michael Alan Gold

A DISSERTATION

Submitted to  
Michigan State University  
in partial fulfillment of the requirements  
for the degree of

DOCTOR OF PHILOSOPHY

Department of Forestry

1984



## ABSTRACT

### HONEYLOCUST (Gleditsia triacanthos L.): GENETIC VARIATION AND POTENTIAL USE AS AN AGROFORESTRY SPECIES

By

Michael Alan Gold

Honeylocust, Gleditsia triacanthos L., has potential as a multi-purpose tree in agroforestry systems due to a combination of desirable traits including high wood specific gravity, abundant coppicing, a tap-rooted/profusely branched root system, drought tolerance, high carbohydrate pods, and high protein seeds and leaves.

The potential for genetic and/or cultural improvement in the growth and form of honeylocust is unknown. An in-depth study of the geographic and genetic variation in honeylocust was initiated in 1979 with the establishment of a comprehensive rangewide provenance/progeny test at two locations in southern Michigan.

At the end of the second growing season, significant differences among regions and half-sib families-within-regions were found in total height, caliper, thorniness, date of spring flushing, stem dieback, and fall growth cessation. Strong negative correlations were found between latitude of origin and stem dieback. The ranges of

variation in spring flushing, fall growth cessation, leaf retention, and stem dieback appear to follow clinal patterns. Families of northern origin from the Lake States area are the best overall juvenile performers in terms of total height, stem caliper, cold-hardiness and degree of thorniness.

Results of chemical analyses on pod sugars and seed and leaf proteins are reported. Total pod sugar content varied from 13.6 to 30.9 percent. Seed protein content varied from 16.6 to 27.8 percent. Leaf protein content ranged from 13.6 to 28.9 percent. The variation patterns in leaf protein, seed protein, and pod sugars are random with no particular provenance or region being especially high in any given trait. The use of yield components is discussed in relation to breeding strategies for maximizing sugar and protein yields.

Results of two cultural studies on preemergent herbicides and spacings are reported. Ultra short rotation intensive culture systems for growing honeylocust can be successfully accomplished by direct-seeding, followed immediately by application of the preemergent herbicide DCPA (dimethyltetrachloroterephthalate) with no harmful effects on seed germination. Planting direct-seeded honeylocust at three different spacings showed that a spacing of 10 x 15 cm gave the highest biomass yields in the first year after planting.

## ACKNOWLEDGMENTS

I wish to express my sincere appreciation to Dr. James W. Hanover, Chairman, for his insights, support, and assistance which allowed this work to become a reality. I also wish to thank the other members of the Guidance Committee--Drs. R. Bandurski, M. Yokoyama, and J. Hancock--for their assistance during the course of this study.

Special thanks are due to my "little brother"--D. Van Arsdall--who gave me a tremendous amount of help in my field data collection efforts. Thanks are due to all the other members of MICHCOTIP who made this whole experience alot more bearable.

Finally, my heart gives a big and sincere thank-you to my wife, Julie, for her unbelievable patience and encouragement during the long years of this project.

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## INTRODUCTION

The present solutions to many major global problems are destructive, inadequate and shortsighted. We face a future in which new ideas based on the concept of one "global ecosystem" must come to the forefront. There is a need to think toward a long term future of cooperation, permanence, and stability.

Included among the unending list of serious global problems facing the "less developed countries" are food shortages, deforestation, erosion, and a subsequent lack of fuelwood. In the "developed countries" there is a need to diversify the economic base, lessen dependence on fossil fuels and look toward renewable production systems. In all regions there is a need to make fuller, more productive use of marginal and hilly/steep lands.

The solution to these major problems will certainly be a multi-faceted one which will include energy conservation, improved farming practices, and the development of alternative, less energy-intensive technologies. One important facet of the technological solution to these problems may lie in the new field of "agroforestry". This integrated system offers many opportunities and advantages in solving the energy, food, and soil erosion problems. In

agroforestry, forestry is integrated with farming, animal husbandry, and horticulture to achieve both maximum output per hectare of land, and optimum conservation of the land resource on a permanent basis.

Much of the literature discussing the value of agroforestry species and systems consists of speculation drawn from little concrete information. One of the species commonly mentioned as having potential for use as an agroforestry species is honeylocust, Gleditsia triacanthos L. This assertion is based on a very limited data base. In light of this situation, it was obvious that a long-term effort was needed to establish a firm foundation for future research.

Gathering information on the extent of genetic variation in the species is among the first questions which need to be answered . Therefore, a major collection of germplasm was organized to address this question. This now exists as a three year old provenance/progeny test in two locations in southern Michigan.

The main objectives of this study were to begin to discern the extent of genetic variation in honeylocust and demonstrate its potential for use as an agroforestry species. Other objectives included the organization of existing literature on the genetics and agroforestry of honeylocust into a coherent, useful form. And finally, to bring together the literature on agroforestry systems for

the temperate zone, both to further the awareness of ongoing research, as well as describe some potential benefits derived from their use.

## CHAPTER I

### Genetics of Honeylocust

#### INTRODUCTION

Linnaeus erected the genus Gleditsia naming it in honor of Johann Gottlieb Gleditsch (1714-1786) (Sargent 1922). The genus Gleditsia, a member of the Leguminosae family, subfamily Caesalpinioideae, includes about fourteen species and one putative hybrid (Tables 1.1; 1.2). Honeylocust, G. triacanthos L., grows naturally in the eastern half of the United States. It is a minor component in three forest associations: 1) Northern Red Oak - Mockernut Hickory - Sweetgum; 2) Sweetgum - Nuttall Oak - Willow Oak; 3) Sugarberry - American Elm - Green Ash. The first two cover types are edaphic climax associations (Putnam and Bull, 1923). Other common associates of honeylocust are elms, ashes, red maples, blackgum, persimmon, pecan, black walnut, box elder and Kentucky coffee tree. The wood is strong and durable, is used locally for fence posts and railroad ties, and also possesses many desirable qualities such as attractive figure and color, strength, and hardness (Panshin and De Zeeuw, 1970).

Honeylocust was first cultivated by American colonists

Table 1.1 The genus Gleditsia

Family:	Leguminosae	
Subfamily:	Caesalpinioideae	
<u>Latin name</u>	<u>Location</u>	<u>References</u>
<u>G. triacanthos</u> L.	Eastern United States	1
<u>G. aquatica</u> Marsh.	Southern United States	1
<u>G. x texana</u> Sarg.	Mississippi Valley, Texas	1
<u>G. amorphoides</u> (Gris.)Taub.	Southern South America	1
<u>G. caspica</u> Desf.	Caspian Sea, Iran, USSR	1,2
<u>G. assamica</u> Bor.	Northeast India	1
<u>G. japonica</u> Miq.	Japan, Korea, China(PRC)	1,2
<u>G. sinensis</u> Lam.	PRC	1,2,3
<u>G. macracantha</u> Desf.	PRC	1
<u>G. microphylla</u> Gordon	Central, Northern PRC	1
<u>G. delavayi</u> Franch.	South central PRC	1,2
<u>G. australis</u> Hemsl.	Southern PRC	1
<u>G. fera</u> (Lour.)Merr.	Southeastern PRC	1
<u>G. rolfei</u> Vid.	Taiwan, Hainan, Viet Nam, Philippines, Celebes	1
<u>G. melanacantha</u> Tang. & Wang.	PRC	4
<u>Taxonomic references:</u>		

- 1) Gordon, D. 1965. A revision of the genus Gleditsia (Leguminosae). Ph.D. Dissertation. Indiana University, 115p.
- 2) Paclt, J. 1982. On the repeatedly confused nomenclature of Chinese species of Gleditsia (Caesalpinaceae). Taxon 31:551.
- 3) Omei School of Chinese Materia Medica, Szechuan. 1975. What is Gleditsia officinalis Helmsley? Acta. Phytotax. Sinica 13(3):47-50.
- 4) Institute of Botany of the Chinese Academy of Sciences. 1972. Iconographia Cormophytum Sinicorum (2 vols.) Science Press. (Chinese and Latin).

Table 1.2 Interspecific variation in the genus Gleditsia


---

Growth habit	Trees or shrubs ; 3-50 meters in height
Number of recognized species	14
putative hybrids	1
Chromosome number	2N=28
Nodules	absent
Leaf morphology *	pinnate or bipinnate; 1-8 pairs of pinnae; 6-46 leaflets; leaflets extremely variable in all species
Flowers	Small and inconspicuous; polygamo-dioecious; lacking odor; insect pollinated; clustered to unbranched racemes
Thorns	present or absent; simple to multibranched; 1-40cm in length; juvenile trait; arise from supra-axillary or adventitious buds
Geographical distribution	North and South America; temperate and tropical Asia; Malay Archipelago

---

\*

Leaflets are extremely variable between and within all species, often in the same plant. Much of the variation is due to environmental conditions and the differences between juvenile and adult leaves. All attempts to delimit taxa on the basis of leaf characters alone have failed.

more recently has been widely planted as an ornamental replacement for American elm (Harlow and Harrar, 1968). Current interest in honeylocust is in its potential as a multi-purpose agroforestry crop tree for animal and chemical feedstocks. It has become naturalized east of the Appalachian mountains from Georgia to New England in the East, and north to South Dakota in the West (Little, 1953) (Figure 1.1).

Within the natural range of the honeylocust, a large amount of variation exists in both climatic and edaphic conditions. Average annual precipitation varies from 500 mm in South Dakota-Nebraska to 1800 mm in North Carolina. The frost-free period varies from a minimum of 140 days in the northern and northwestern portions of the range, to a maximum of 340 days in the South Central States (USDA, 1941).

Honeylocust is a shade intolerant tree with a strong taproot. It achieves its best growth on fertile, moist, alluvial floodplains and can attain a maximum size of 50 m in height and 1.8 m in diameter, but its normal size range is 18-24 m tall and 0.5-1.0 m in diameter (Harlow and Harrar, 1968). Honeylocust will also grow on soils of limestone origin, is resistant to both drought and salinity (Van Dersal, 1938), coppices vigorously when cut, and is hardy in the Great Plains.

Little is known about the patterns or extent of genetic variation in honeylocust. Similarly, the potentials for genetic and/or cultural improvement in the growth, form and

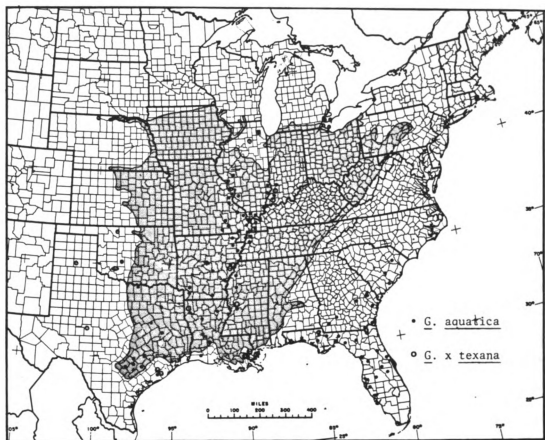


Figure 1.1 The range of honeylocust.



chemistry of honeylocust are unknown.

#### GENERIC RELATIONSHIP

Gleditsia is most closely related to Gymnocladus. Both have polygamous flowers with polysepalous calyces. The tendency toward dioecism is much greater in Gleditsia than in Gymnocladus (Lee, 1976). In both genera a few pinnae of the leaves are often reduced to simple leaflets (Gordon, 1965).

The seeds of Gleditsia and Gymnocladus are smooth, with a hard, impermeable testa and large amounts of endosperm surrounding the embryo. The endosperm is composed of thick walled cells filled with galactomannan gums, which are converted into mucilage upon the addition of water (Sayed and Beal, 1958). The presence of copious endosperm is generally regarded to be a primitive character and furthers the suggestion of a close relationship between the two genera (Lee, 1976).

Both genera are known for their richness in fruit saponins (aglycones), which are triterpenoid in nature. Saponins in the fruit of both genera are commonly used in China as soap substitutes. The presence of structurally similar triterpenoid saponins in the two genera provides supporting chemical evidence of their close affinity, but are of little value in discerning among the species within each genus (Lee, 1976).

Gleditsia and Gymnocladus are known to lack nodule formation in their roots (Allen and Allen, 1936; Grobbelaar, 1964). According to Burkart (1952), this condition is an

indication of the primitive character of both genera, particularly when one considers that both have disjunct geographic distributions and abundant fossil histories.

#### PHYLOGENY

The Caesalpinioideae is predominantly a tropical subfamily of the Leguminosae. Tropical Africa is regarded as the center of diversity for the subfamily, with tropical America regarded as a secondary center of diversity (Lee, 1976).

More than 40 extinct Gleditsia or Gleditsia-like species have been reported in the literature. Fossils of Gleditsia have been located in places outside the present range of the genus (e.g., Europe and Western North America), indicating a much wider distribution of the genus in the past (Robertson and Lee, 1976).

No representative of the genus Gleditsia is recognized with certainty in either the Upper Cretaceous or the Eocene. The genus Gleditsiophyllum from the Upper Cretaceous and the Eocene of North America may be the progenitor of the present day Gleditsia triacanthos (Berry, 1923). Berry (1923) reports on a species resembling Gleditsia found in the Oligocene of Europe, and on the existence of number of undoubted Miocene species. Several of these Miocene species have been described and they include a species (G. columbiana) from the state of Washington in the North-western United States (Prakash and Barghoon, 1961), a

species from Montana (G. montanese) (Prakash and Barghoon, 1962), a species from Japan (Hu and Chang, 1940), and other species from Europe where they range from Greece and Hungary to France (Berry, 1923).

Hu and Chang (1940) recorded Pliocene species in Japan, Europe and Western North America. Berry (1923) recorded a species resembling G. triancanthos, from the early Pleistocene of Kentucky, and an extinct species was found from the interglacial deposits of the Don Valley in Ontario.

The Eastern North American species of Gleditsia are more closely related to species occupying similar temperate areas in Eastern Asia, than to one another (Gordon, 1965). Li (1952) has pointed out that these two areas, temperate Eastern North America and temperate Eastern Asia, are very similar ecologically. Both are quite old geologically and have remained relatively unchanged since the Paleozoic. They do not appear to have been submerged since the end of the Cretaceous.

Li (1952) has interpreted the present isolated and disjunct floras of Eastern Asia and Eastern North America as remnants of a great mesophytic forest that extended over all the Arctic regions in the tertiary. Subsequent geological changes including glaciation have altered and destroyed the floras of many places. The mesophytic forest of the Tertiary in the Northern Hemisphere has survived principally in Eastern Asia and Eastern Northern America. Only scattered relics remain in Southeastern Europe, Western Asia

and Western North America. This interpretation fits in well with the present distribution of Gleditsia, especially if one considers the floristic relationships between the species from these two areas.

#### SEXUAL REPRODUCTION

The flowers of honeylocust are classified as polygamodioecious. Polygamy is defined as the condition of having staminate, carpellate, and hermaphrodite flowers on the same individual (Henderson and Henderson, 1963). In the flowers of Gleditsia, only one type of reproductive organ is usually functional, although a rudimentary or abortive organ of the opposite sex may be present. This phenomenon can be interpreted as incomplete dioecism (Lee, 1976). Although the occurrence of true polygamy is reported in the literature (O'Rourke, 1949; Grisjuk, 1958), individual trees are characterized, in general, as either staminate or pistillate, but perfect flowers are produced on occasion. Therefore, Gleditsia should be considered as a predominantly dioecious genus (Lee, 1976). Pistillate trees rarely produce pollen, but staminate trees frequently produce a few female flowers (Moore, 1948).

When considering the entire range of the species, honey locust flowering phenology differs by as much as six weeks. The average flowering date for honeylocust in the southern limit of the range is May 10; in the north it is June 25 (Lamb, 1915). Flowers appear from the axils of the previous years growth when the leaves are nearly fully elongated

(Sargent, 1922). This lateness to flower helps prevent frost damage to the flowers and subsequent seed crop (Detwiler, 1947). Flowers are nonshowy and pale-yellow to greenish-yellow in color. The staminate inflorescence occurs in short many-flowered pubescent clustered racemes 2" - 2 1/2" in length. The pistillate inflorescence occurs in few-flowered usually solitary racemes 2 1/2" - 3 1/2" in length (Figure 1.2) (Sargent, 1922).

Honeylocust flowers are insect pollinated, although in contrast to flowers of the black locust (Robinia psuedo-acacia L.) with which they are sometimes confused, they are inconspicuous and somewhat less fragrant. Honey bees work freely gathering nectar, but usually not enough to create a surplus honey flow (Pellett, 1947). Pistillate flowers become receptive from the base of the raceme toward the tip. On individual trees, the period of maximum bloom (receptivity) lasts 7-10 days.

Honeylocust tends toward an alternate bearing habit in which good pod/seed crops are produced every second or third year, with different trees producing good crops on alternate years.

The average date of seed ripening varies according to latitude of origin ranging from mid-September to late October (Lamb, 1915). Mature pods begin to drop by mid-September and continue to drop throughout the winter.

In good crop years, yield of sound seed per tree can be quite substantial. A good crop can easily exceed thirty

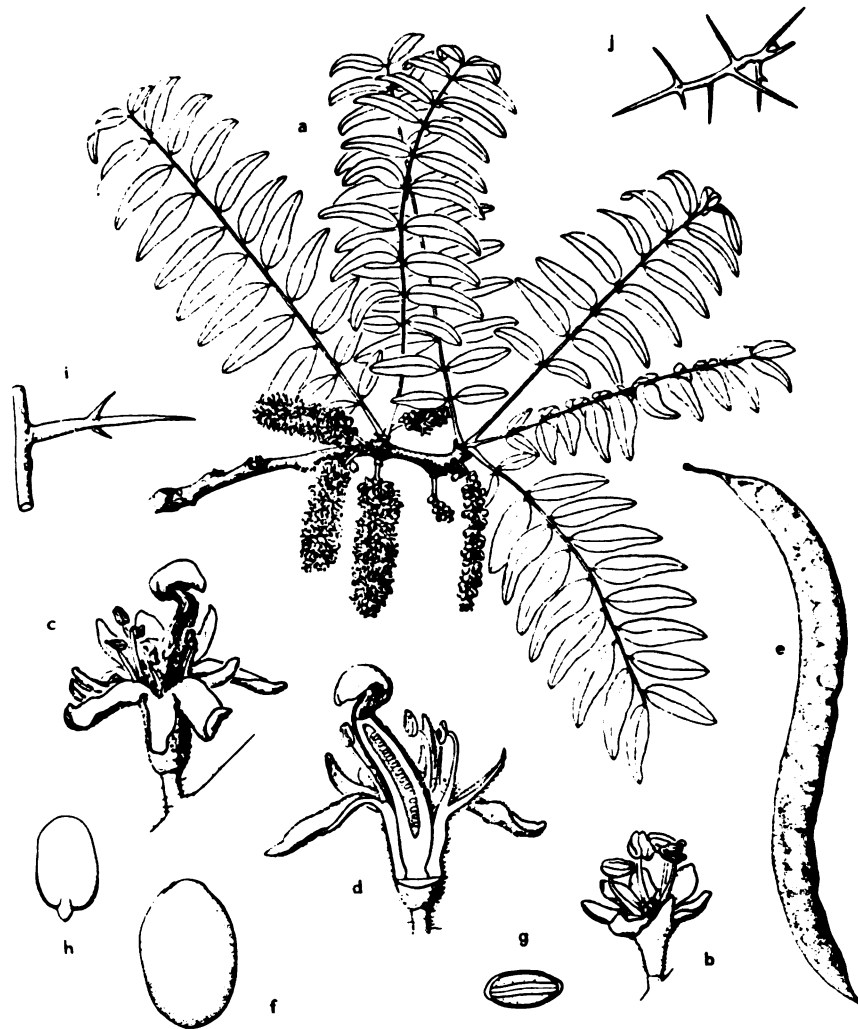


Figure 1.2 *Gleditsia*. a-j, *G. triacanthos*: a, tip of branch with staminate inflorescences--note once pinnately compound leaves on short shoots, X 1/2; b, staminate flower with 4 stamens, X 3; c, carpellate flower--note presence of aborted anthers, X 3; d, same in vertical section--note perigynous insertion of calyx lobes, petals, and stamens X 4; e, mature fruit--northern latitude phenotype, with little pulp, X 1/4; f, seed, X 2; g, soaked seed in cross section, mucilaginous endosperm even-stippled, seed coat and cotyledons unshaded, X 1; h, embryo from soaked seed, X 1; i, supraxillary thorn from branch--note three-pronged thorn, X 1/4; j, adventitious thorn from tree trunk, can be up to 40mm in length, X 1/4.

pounds of cleaned seed per tree (@ 2,800 seeds/lb.). Seed viability is over 90 percent.

Planted from seed, trees begin to bear commercial quantities of seed by age 10 years, with an optimum age of 25 to 75 years (USDA, 1948). Clonally propagated trees from the Tennessee Valley Authority (TVA) tree crops project of the late 1930's- early 1940's such as the Millwood and Calhoun selections, began to bear at age three, bore significant crops by age five, and by age eight, the Millwood clone bore a heavy crop, averaging 180 lbs. dry weight per tree (Moore, 1948). These trees also showed a definite tendency toward alternate bearing, although some pods were produced in the "off" years.

#### Pollination Techniques

Controlled pollination on a small scale has been successfully accomplished by many individuals (Detwiler, 1947; Gordon, 1965; Santamour, 1976). Standard crossing procedures, which include bagging of immature pistillate flowers and introducing pollen, have proven successful. Male flowers close to anthesis are removed from their inflorescences, brought indoors, kept at room temperature until pollen shedding and are then refrigerated at approximately 2<sup>o</sup> C.

Because honeylocust flowers are insect pollinated, requiring a vector to transmit pollen to the female flowers, pistillate flowers chosen for artificial pollination need only be bagged with cheesecloth to prevent natural

pollination before they elongate and become receptive. If staminate flowers on nearby male trees are not yet shedding pollen, the cheesecloth can be temporarily removed and the pollen can be brushed onto a receptive stigma. If pollen shedding is occurring, pollen can be introduced through a syringe. The artificially pollinated flowers are then left bagged in cheesecloth until the staminate flowering has finished, usually within a week to 10 days.

### Hybridization

Evidence of natural interspecific hybridization between Northern American species of Gleditsia, and the fact that the species from North America are more closely related to species occupying similar temperate areas in Eastern Asia than to one another, indicates that interspecific hybridization and crossability are not a barrier to genetic improvement in the honeylocust. Two recognized species of Gleditsia and one natural hybrid are found in the eastern United States. Honeylocust, G. triacanthos, is found throughout most of the eastern United States. Water locust, G. aquatica, is largely ecologically and geographically isolated from honeylocust occurring in swamps, low wet woodlands and the edges of bayous from South Carolina to eastern Texas and northward up the Mississippi and Ohio River valleys to southern Illinois and Indiana (Figure 1.1) (Robertson and Lee, 1976).



Considerable discussion regarding the validity of the hybrid G. x texana is found in Gordon (1965). He concludes that G. x texana is in fact a probable hybrid between the two species. The hybrid is located only in areas where the putative parents occur (Figure 1.1), and is intermediate in nearly every morphological character which separates the parental species. The blooming dates of the parents do occasionally overlap allowing cross pollination to occur.

Support for the putative hybrid comes from Stebbins (1950), who points out that most morphological differences between species of plants depend on multiple factors rather than on single genes. These multiple factors show relatively little dominance and therefore a hybrid can be expected to be intermediate between parents in nearly every character. Further evidence in support of the putative hybrid comes from Santamour (1977), who reports that leaf flavonoids from mature trees and open pollinated seedlings of G. x texana show that G. triacanthos is definitely involved in parentage of the hybrid.

#### Controlled Pollination and Crossability

In 1938 and 1939, the Tennessee Valley Authority (TVA) made controlled pollinations among phenotypically superior, high pod sugar content, honeylocust selections. Attempted crosses were successful and female-male incompatibility was not found (Scanlon 1980). Gordon (1965) artificially crossed G. aquatica x G. triacanthos, and sound seed was produced from the crosses. Interspecific crosses between a

Chinese locust, G. melanacantha, and two ornamental staminate cultivars of G. triacanthos yielded a total of over 100 hybrid pods with an average of 14 sound seeds per pod (Santamour, 1976). Santamour also reported that seed size and seed weight could be significantly influenced by the male parent. Data from Gold and Hanover (1984c) show that 13.5 seeds per pod is an average yield from pods of wild, open pollinated trees, indicating that the controlled pollination caused no reduction in seed set due to compatibility or crossability problems.

#### SEED HANDLING AND NURSERY PRACTICE

##### Collection and Extraction

Mature pods can be collected from the ground soon after they drop, by hitting the branches to jar the pods loose, or by clipping the pods from the branches. Seed extraction, storage, germination and nursery practices are covered in the Woody Plant Seed Manual (USDA, 1948) and the Hardwood Nurseryman's Guide (USDA, 1976). A few additional notes should be added to enhance the ease and success of handling pods and seeds.

After harvest, pods should be stored at or below 0<sup>o</sup> C. This will prevent fermentation of the pods and, if bruchid seed weevils (Amblycerus robiniae) are present in the pods, it will prevent them from continuing to develop, breed, and spread within the pods.

To prepare pods for extraction, place them on trays in a convection/seed drying oven for at least 2 hours at 35<sup>o</sup> C. This will dry out the succulent pulp surrounding the seed chambers which can clog a hammermill. All seed extraction should be done in a well ventilated area because the dust from the ground pods is very irritating to the sinuses.

Dried pods can be put through a hammermill. The resultant pod kibble is placed in a seed tumbler with screens sufficiently large to permit the seed to drop through. Extracted seed can be cleaned in an air blower.

#### Storage and Sowing

Honeylocust seed will remain viable for several years if stored dry at 1-4<sup>o</sup> C. To obtain successful germination of honeylocust seed, they must be scarified and forced to break seed coat dormancy. This can be accomplished with sulfuric acid, hot water, or by mechanical means. To scarify honeylocust seeds with concentrated sulfuric acid, place the seeds into the acid for not less than 60 (maximum 120) minutes. After scarification, rinse the seeds thoroughly. Heit (1967) cites the advantages of sulfuric acid treatment as: 1) Little special or expensive equipment is required; 2) Relatively low cost; 3) It is highly effective; 4) Because there is a great deal of seedlot variation in optimal scarification time, preliminary tests can give the ideal length of acid treatment for each individual seedlot; 5) Following scarification, seeds can be dried, refrigerated, and held for several months without

loss of viability; and 6) Seeds can be sown with mechanical seeders because of their unswollen condition as opposed to seeds treated with hot water.

To determine if seed weevil larvae are present, place the rinsed, acid treated seeds in a refrigerator overnight. Seeds which have minute entry holes due to weevil larvae will imbibe and should be discarded. The remaining should be viable and either dried and stored or sown immediately. Germination of sound seed should be in the range of 75-90 percent. Seeds should be sown  $3/8 - 1/2$ " deep and if properly scarified, complete germination will occur within 21 days of sowing.

#### ASEXUAL REPRODUCTION

In its natural state, honeylocust tends to be an objectionable, thorny nuisance. Interest in honeylocust as a multipurpose pasture/fodder/fuel tree and as an ornamental has led to the development of asexual propagation of thornless genotypes to circumvent the thorn problem. Seed from thornless trees produce 60-80 percent thornless progeny (Chase, 1947; Soutemeyer et al., 1944). In lieu of thorns, thornless trees produce short vestigial shoots which are semi-persistent but not objectionable (Stoutemeyer et al., 1944).

Thorniness exists as a juvenile trait. Trees which will ultimately be very thorny will show this trait in the first season, while trees which will be lightly thorned may

not be detected until the second year. As honeylocust trees increase in age thorn production diminishes and ultimately ceases in the upper crown. Typically, trees 10 years or older show a definite thornless region in the upper and outer areas of shoot growth (Chase, 1947). When thorny trees are used for scionwood, the collection of thornless scionwood from branches which have definitely ceased thorn production will produce thornless trees. As expected, scionwood from thornless trees produce only thornless trees (Stoutemeyer et al., 1944).

#### Budding and Grafting

Many common nursery techniques for budding and grafting are successful in propagation of honeylocust. Due to the geometry of the honeylocust bud, the inverted T-bud technique is used to achieve rapid, simple, vegetative propagation of scionwood. Successful budding can be accomplished with bud from dormant wood collected in the spring or from mature buds in late July or August. Inverted T-budding can result in trees up to 5 feet high in the first year (Stoutemeyer et al., 1944).

Modified cleft grafts as well as whip and tongue bench grafts will yield up to 100 percent take. However, grafted honeylocust tend to sucker profusely, can be time consuming, and are best suited to small scale operations.

Marcavillaca and Garcia (1971) have successfully grafted scionwood from the South American species, G. macracantha Desf., on to honeylocust stock and achieved over

50% take within 45 days of grafting. This interspecific grafting success may prove to be important if varieties of honeylocust are desired which are disease or insect resistant, and adapted to a wide variety of soil types. Also, the development of dwarf varieties would open up new ornamental potentials for the honeylocust (O'Rourke, 1949).

### Rooting of Cuttings

Currently, root cuttings are the best method of propagating honeylocust in large quantities and at reasonable costs. The biggest advantage of root cuttings is that large numbers can be obtained from prunings taken from the roots of young nursery trees. For maximum success, root cuttings over 8 cm in length and in excess of 12 mm in diameter should be used. Cuttings should be taken in early spring and planted directly into the greenhouse or nursery. Root cuttings from mature trees sprout less vigorously and should be avoided when possible (Stoutemeyer et al., 1944).

Marcavillaca and Garcia (1971) reported success in rooting greenwood cuttings taken from a 13-year-old honeylocust tree. Subapical cuttings 30 cm in length, taken 30 cm from the tip of the branch, gave the best results. Dipping the cuttings in 10,000 ppm naphthalene-7-acetic acid (NAA) or 10,000 ppm indole-3-butyric acid (IBA) adsorbed on talc powder as a carrier for the hormone gave the best results, with 77 percent and 87 percent rooting success, respectively. Soaking the cuttings for 24 hours in an

aqueous solution of 50-100 ppm NAA gave 67% rooting success. Concentrations above 100 ppm appear to be phytotoxic.

Stoutemeyer et al (1944) observed that the best material to use for rooting greenwood cuttings originated from stump sprouts or from shoots grown from root cuttings. Hardwood cuttings can also be rooted in the greenhouse but are not useful for large scale production.

To propagate "own-rooted" grafted or budded stock it is best to root greenwood cuttings. When the cuttings have well established root systems, root cuttings can be used to propagate new stock.

### Tissue Culture

With significant advances in clonal micropropagation of woody plants in the past decade, tissue culture techniques hold the greatest promise for commercial scale asexual propagation. Micropropagation is essentially an extension of conventional propagation techniques using aseptic culture. The method of organogenesis consists of the micropropagation of explants from a variety of tissues including leaves, shoots, buds, reproductive structures, etc. (Brown and Somer, 1982).

Past research has shown that specific tissue culture techniques need to be developed for each individual species. Honeylocust has been successfully cloned via organogenesis using stem tissues and regenerative callus culture (Rogozinska, 1968), and more recently regenerated plantlets have been obtained from shoot tips of honeylocust

seedlings (Brown, 1980). This approach may offer the greatest near term potential for commercial production of genetically improved strains of honeylocust.

#### CYTOLOGY AND MUTATION

Honeylocust is a diploid species,  $2N=28$ , and evidence indicates that it is cytologically stable. Chromosome counts have been made on 6 of the 14 known species of Gleditsia and all were undisputed as  $2N=28$  (Gordon 1965). Atchison (1949) states that little variation in chromosome size and morphology can be noted among the species. No polyploidy has been detected within the genus.

Mutation resulting in morphological variants are rare. Counts of half-sib seedling progeny from 400 families of honeylocust indicate that only seven families (1.75 percent of the population) showed any visible mutations. Gold and Hanover (unpublished) found three types of mutations among the seven families. The first type, total albinism in both cotyledons and true leaves, occurred in four families. Three of these four families had approximately four percent albino progeny. One exceptional family had 26 percent albino progeny indicating that a single recessive gene controlled the inheritance of the mutation. A second type of mutation, found in two families, had green cotyledons and albino true leaves. These mutations occurred in eight percent of the progeny. All of the albino-type mutations died within 90 days of germination. A third type of



mutation, showing extremely abnormal growth rate and morphology, survived for two years in the greenhouse. Two families, both originating in Iowa from the same general location, had this type of abnormal progeny. The seedlings had varying intensities of red coloration in the stem and leaves, dark green cotyledons, grew at an extremely slow rate (less than 10 cm. in two years), and had a long, unbranched, nonfibrous root system. In addition, the leaflets failed to open outward but instead folded in upon themselves.

#### GENETICS AND BREEDING

##### Provenance/progeny Testing

Provenance tests are used to evaluate the performance of germplasm from many different locations in a common environment, allowing the inherent genetic differences to be observed and measured. Progeny testing allows for a further degree of refinement, including the understanding of the variation and heritability of any given trait within and between families. The size of a species natural range is a principal factor influencing the amount of geographic variability within a species (Wright, 1976). Honeylocust, with a large natural range and generally continuous distribution, has a great deal of genetic diversity and a continuous pattern of genetic variation (Gold and Hanover, 1984a).

Significant differences among half-sib families exist in stem dieback, spring flushing, fall growth cessation,

thorniness, 2-year stem caliper, and 2-year height growth. The differences were all significant at the 1% level of probability. Additionally, the region x location and family-within-region x location interaction was nonsignificant for all traits indicating consistency of results over locations in southern Michigan.

#### Variation in Date of Spring Flushing

Honeylocust follows commonly cited patterns of variation in spring growth initiation in which families of northern origin (IA, NB, SD, IL, etc.) flush first. Families of more southerly origin were intermediate in their flushing date, and those families which were last to flush were all from southern origins (LA, GA, TX, MS, etc.). The range of variation follows a clinal pattern. According to Mather (1953), clinal variation develops after an initial disruptive selection in a base population migrating from the center of origin of a species. This is followed by stabilizing selection and gene exchange among adjacent populations over the species range (Haldane, 1948; Fisher, 1950). Continental glaciation may have caused a major disruption in the natural selection process in much of the flora of eastern North America, including many species of trees which have clinal patterns of leaf flush.

The means of the earliest and latest flushing families differed by over 11 days, while 21 days separated the earliest and latest flushing individuals. Negative

correlations are found between flushing and latitude of origin ( $r = -0.74$ ). The correlation between flushing and frost free days is positive ( $r = 0.73$ ). These correlations suggest that budbreak is influenced by the temperature distribution patterns at the location of origin. Narrow sense heritability for flushing is  $h^2 = .37$  (Gold and Hanover, 1984a).

#### Variation in Growth Cessation

Photoperiod is thought to be the major factor in the control of growth cessation (Nienstadt, 1974). Families of southern origin which have evolved and adapted to mild climates are the last to stop growth in the fall. Northern sources are capable of responding to decreasing summer day-lengths much earlier than southern sources. Differences in sensitivity to a preset critical daylength, i.e. that day-length which triggers the cessation of growth and onset of dormancy, enables northern sources to go dormant at the proper time when grown in northern locations. For sources of southern origin grown in the north, the critical day-length threshold is not reached until late in the summer. This allows them to grow late into the fall causing frost damage to succulent tissues.

Two year results of Gold and Hanover (1984) show that those families which originate furthest south (below 35°) from the test sites had the lowest percentage of progeny which had ceased growth by mid-October and the greatest subsequent degree of stem dieback. Sources from inter-

mediate latitudes (35<sup>o</sup> -37<sup>o</sup>) had a higher percentage of progeny which had stopped growing and a less severe amount of stem dieback. Sources originating north of 37<sup>o</sup> N. latitude had the fewest number of progeny growing late in the fall (none in most cases) and suffered the least severe degree of stem dieback. Growth cessation is very highly negatively correlated with stem dieback ( $r = -0.88$ ) and frost free days ( $r = -0.78$ ), and is positively correlated with latitude ( $r = 0.79$ ).

#### Variation in Winter Hardiness (Stem Dieback)

Southern Michigan is at the extreme northern limit of the natural range of honeylocust (Figure 1.1). After two seasons in the field, variation in winter hardiness in the form of stem dieback and death from winter injury were strongly evident. Intraspecific differences in winter hardiness of woody plants have been related to climate of geographic origin, latitude of origin, and elevation of origin (Flint, 1974). Differences in cold acclimation of provenances within a species is closely related to phenological differences within the species (Nienstadt, 1974).

With few exceptions, honeylocust families whose source of origin is south of 37<sup>o</sup> N. latitude suffered at least 10 percent stem dieback. Families originating south of 35<sup>o</sup> N. latitude suffered between 30 and 80 percent stem dieback.

Two families of Southern origin, from Georgia and Louisiana, proved to be outliers and did not suffer significant stem dieback. Early empirical evidence points

to other cold tolerant individuals within cold sensitive families. If this holds true, there will be good potential for within family selection for improved cold tolerance in southern sources. Selected individuals would be used for incorporating desired southern traits such as pod size into northern sources. According to Kriebel and Gabriel (1969), one possible explanation for the performance of these families is that relict populations from the Deep South and Mississippi Valley may retain a genetic capacity for winter hardiness normally found solely in trees with northern genotypes.

Honeylocust has a sympodial growth habit and does not set a true terminal bud. Rather, that portion of the stem above the false terminal bud will die back before the onset of growth initiation in the spring. This amounts to 0-40mm natural dieback and is not viewed as the result of a lack of cold hardiness.

Stem dieback is very highly correlated with active shoot growth late into the fall ( $r= 0.89$ ), with frost free days at the point of origin ( $r= 0.77$ ), with cooling degree days ( $r= 0.79$ ) and with date of spring flushing ( $r= 0.88$ ). The high positive correlation between stem dieback and fall growth suggests that photoperiod (in an indirect sense) may play a role in the ability of individual sources to go dormant, to attain a sufficient degree of cold hardiness and to resist subsequent tissue damage.

Stem dieback is negatively correlated with latitude of

origin ( $r = -0.78$ ) and with freeze days ( $r = -0.78$ ). Stem dieback is also negatively correlated with 2-year seedling height ( $r = -0.40$ ) and 2-year seedling caliper ( $r = -0.49$ ).

Narrow sense family heritability in stem dieback based on variance components is  $h^2 = .96$ . This high degree of heritability will be of importance in future breeding programs to incorporate some of the important economic traits inherent in Southern sources into cold hardy, Northern sources.

#### Variation in Thorniness

A common feature of wild, open pollinated honeylocust, is the presence of many sharp, 3-branched thorns occurring singly or in clusters. Thorns are considered to be abortive branches which arise from supra-axillary buds on the branches and from adventitious buds on the trunk (Blaser, 1956). Thorns complete development and lignification in one year and range in size from 2-40cm. Thorniness in honeylocust is thought to have arisen as an evolutionary adaptation to exposure to arid environments. Thorn shoots are thought to curtail transpiration loss (Grisyuk, 1959).

Thorniness is a juvenile trait and the upper branches of thorned trees 10 years and older can be used as thornless scionwood to create "thornless" cultivars. However, the progeny of these grafted "thornless" cultivars will contain thorny seedlings, and this is highly undesirable. Thornless, open pollinated trees of the variety "inermis", produce 60-80 percent thornless progeny. There is a one to

two year lag time between sowing in the nursery and roguing out the thorny seedlings. If only thornless progeny could be assured, seedling production costs would be reduced. The ability to eliminate the thorn trait through selection and breeding will expedite the widespread use of honeylocust. Additionally, the introduction of genetically thornless honeylocust into areas where it is not found locally would totally eliminate the thorn problem.

Addressing this situation, Grisyuk (1959) reports on three years of controlled pollination experiments between thorny and thornless honeylocusts. Crosses were made in all combinations. Results indicate that crossing thornless females with thornless males will produce only thornless progeny (Table 1.3).

Table 1.3 Combined three year results of an experiment to determine the inheritance of the thorn trait in honey locust (Grisyuk, 1959).

<u>Hybrid combination</u>	<u>Number of progeny</u> *		
	<u>Thorny</u>	<u>Thornless</u>	<u>Ratio</u>
T-less female x T-less male	0	303	---
T-less female x Thorny male	25	123	1:5
Thorny female x T-less male	136	147	1:1
Thorny female x thorny male	75	15	5:1

\* All seedlings were examined for thorns for two years.

Testing of  $F_2$  progeny from controlled crosses of the  $F_1$  generation will give conclusive results on a breeder's ability to produce genetically thornless trees which "breed true". This is because the  $F_2$  generation will segregate out all of the genotype combinations. If all  $F_2$  progeny are thornless, a major hurdle will have been crossed in the practical use of the honeylocust. Although data from a rangewide provenance/progeny test show that the degree of thorniness has a moderate negative correlation with latitude ( $r = -0.57$ ) indicating a general decrease in thorniness from south to north, the goal is absolute thornlessness.

#### IMPROVEMENT PROGRAMS

##### Economics

Honeylocust is in an excellent position to benefit from tree improvement efforts. It is rather unique in that its present economic value is derived from its widespread use as an ornamental street tree. An individual ornamental honeylocust cultivar is worth an estimated \$10 per foot for a six foot high sapling, or \$60 per individual (Levenson, 1984). While recognized as possessing many desirable qualities (Panshin and de Zeeuw, 1970), present use and economic value of honeylocust wood is minor, and very local. Further, its potential use as a multi-purpose agroforestry species shows good promise, but it has no economic value whatever at the present time.

Results of an economic analysis of tree improvement research in Michigan indicate that the potential economic



return derived from the development of new ornamental cultivars is much greater than the return gained from the development of genetically improved seed for timber or pulp and paper. This is because the unit value of an ornamental seedling is much greater than the unit value of a tree seedling to be planted for timber or pulp and paper (Levenson, 1984).

A multi-faceted improvement program can work toward many goals simultaneously. If new cultivars are developed, the returns to tree improvement research will provide economic justification on this basis alone, allowing for other tree improvement efforts to move forward. The initial strategy of assembling a rangewide provenance/progeny test, determining basic patterns of genetic variation, heritability, general and specific combining ability, progeny evaluation and selection, will apply to all categories of improvement. Because honeylocust has light, lacy foliage, the potential exists for intercropping of forage or vegetable crops in plantations or seed orchards to enhance the economic viability of the early portion of the testing phase by recovering a significant portion of the initial establishment costs.

To date, honeylocust has not been included in any long term systematic tree improvement research program. Over 70 "chance" selections of honeylocust have been patented as cultivars (Santamour and McArdle, 1983). The original cultivars were selected 50 years ago for high pod sugar

content and for use as stock feed. More recently, selection has been directed toward the development of new ornamental cultivars.

Two current tree improvement research efforts, both established in the field in 1982, include a comprehensive rangewide provenance/progeny test at Michigan State University, and a more limited regional provenance test at the University of Nebraska (Walt Bagley pers. comm., 1981).

Under natural conditions honeylocust rarely grows in well stocked stands, but rather occurs in a scattered distribution pattern. Plantations of honeylocust, even-aged and regularly spaced, offer the best approach to improvement. Honeylocust are insect pollinated and predominantly dioecious. This combination of factors will tend to insure natural outcrossing and prevent excessive inbreeding. While the breeding cycle for honeylocust is not expected to be particularly lengthy compared with many other tree species, an expected 5-10 year generation time demands that initial selection and gain be derived from the vegetative propagation of superior individuals based on the results from the provenance/progeny testing.

Following identification of superior individuals or families in a provenance/progeny test, an improvement program should consist of a controlled breeding program, crossing the most promising individuals. This method, which is preferred to one of allowing open pollination of superior progeny, gives the breeder fullest control of sub-

sequently produced hybrids, and maximizes opportunity for genetic gain. After the first round of controlled pollinations, the first concrete step forward in the overall genetic improvement of honeylocust will be in those traits, such as thornlessness or winter hardiness, which can be selected for at an early age in the nursery. Based on the particular breeding objective and long term goals, both intra and interspecific hybridization may be necessary.

#### Objectives of Honeylocust Improvement Program

Due to the very different end-use goals and objectives for which honeylocust is being considered, a breeding program will be multi-faceted in scope. Major objectives include: 1) Development and selection of new cultivars of ornamental honeylocust; and 2) Development of selections for use in agroforestry systems in temperate and highland tropic areas of the world. A minor objective is the selection of fast growing, straight-stemmed trees for sawlogs and veneer.

#### Breeding for Ornamental Cultivars

A tree improvement program for honeylocust as an ornamental should concentrate on the following traits: 1) Insect and disease resistance, especially resistance to mimosa webworm, an insect which causes severe defoliation in many parts of the range; 2) Faster growth rate; 3) Straight stem form; 4) Thornlessness; 5) Cultivars which are 100% staminate and will not produce pods; 6) Development of additional cultivars with reddish color foliage;

and 7) Development of new dwarf varieties. In all cases attention must be paid to winter hardiness.

It should be noted that many of these traits have been selected for and improved upon in the existing "named" cultivars. These same traits will continue to be important baseline criteria in any ornamental improvement program.

The most serious problem facing honeylocust is the mimosa webworm, Homadaula albizzae Clarke. Since its discovery in the U.S. in 1942, it has become the most serious insect pest of honeylocust. In a study to identify webworm phagostimulants in honeylocust foliage, Peacock (1967) found that the alkaloid triacanthine, isolated in largest concentrations from immature leaves, deters larval feeding. Resistance in G. triacanthos to mimosa webworm has not been reported in the literature.

The screening of native sources for webworm resistance, should be the first step in a search for resistant genotypes. Should resistance be discovered, an identification of the feeding deterrent would be desirable. Another approach suggested by Santamour (1977) is to screen other species within the genus Gleditsia for resistance. If resistance is located, either in G. triacanthos or another species, a program of backcrossing and recurrent parent selection would be needed to incorporate resistant genes into G. triacanthos.

As previously mentioned, initial selection for traits such as thornlessness, reddish foliage color, and winter

hardiness can be accomplished in the nursery as these traits will express themselves within the first two years. Further development of genetically thornless  $F_2$  selections will be possible when the thornless  $F_1$  progeny are sexually mature.

Selection for stem form and growth rate can begin in the nursery, however progeny testing in plantations for longer periods of time will be needed to accurately select for these traits. Narrow sense heritability for growth rate is estimated to be moderately high,  $h^2 = 0.63$ .

Improvement in stem form has already been reported by members of the nursery industry who have released cultivars (i.e. "Green Glory", "Shademaster") which are reported to maintain a central leader and are single stemmed (Pirone, 1978; Santamour and McArdle, 1983). Any improvement in growth rate and stem form would also benefit the use of honeylocust as a timber or veneer species.

As individuals begin to flower, selection for staminate trees will be necessary. In all cases, due to the high commercial potential of honeylocust, outstanding or unusual individuals will be asexually propagated through root cuttings or T-budding for further testing and evaluation.

A long term objective is to make interspecific crosses with exotic species of Gleditsia which are more shrub-like, with the ultimate goal of developing genetically dwarf varieties. Another approach to dwarfism would be through the use of dwarfing rootstocks as is the case in commercial varieties of apple trees.

### Breeding for Agroforestry Systems

While the concept of agroforestry is considered to be a very old idea, the "science" of agroforestry is very new, particularly in the temperate zone, and the development and genetic improvement of multi-purpose species has been rare. Honeylocust has been discussed as an ideal multi-purpose tree since the early days of the century (Smith, 1914). Research conducted between 1934-1947 by the Tennessee Valley Authority and other research stations led to the selection and clonal propagation of "plus trees" with high pod sugar content and sweet taste. As presently envisioned, honeylocust may be of value for the production of an array of chemical and animal feedstocks including ethanol, stock feed and industrial gums (Gold and Hanover, 1984c).

Specific traits which need development and breeding to maximize the value of honeylocust in agroforestry systems include precocious flowering, annual bearing, high pod yields and pod carbohydrates, high seed sets and high levels of seed protein and galactomannan gums, cold hardiness, and resistance to bruchid seed weevils.

If used in the highland tropics of less-developed-countries as sources of fodder, fuelwood and erosion control, additional traits to select and breed for would include high levels of leaf protein, excellent coppice ability, and well developed root systems.

Following the initial establishment of rangewide provenance/progeny testing, the objectives of the two major use categories will be almost 180° opposed to one another. Selection and improvement for a few traits such as insect and disease resistance, cold hardiness and thornlessness will be desired for any use of the honeylocust (other than as living fences). However, based on past experience with nut trees, selection for precocious flowering, annual bearing, high pod yields, etc. are likely to favor trees with broad, branching crowns and poor overall stem form.

The advantage in this two-pronged approach is that the poorest ornamental or timber ideotypes may be superior as agroforestry species. Southern sources of honeylocust are the ones with the largest, heaviest pods, and largest amount of total sugars (Gold and Hanover, 1984c). It may be necessary to hybridize sources of northern and southern origin to capture this type of pod morphology while maintaining winter hardiness. Breeding for resistance to seed weevils will require an approach similar to that proposed for mimosa webworm.

#### CONCLUSIONS AND RECOMMENDATIONS

Controlled breeding in honeylocust is feasible and will be used in improvement programs to upgrade and combine traits from superior selections. The two seed source studies established to date in Michigan and Nebraska are limited by either the range of sources under test and/or by the number of locations in which sources are being

evaluated.

More tests are needed across the range of the species and there is an equally strong need for establishment of comprehensive provenance/progeny tests across the southern range of the species. Within each of these areas outplantings should be made at several locations to more accurately assess the effects of provenance on growth, form, cold tolerance, etc. Concurrently, there is a need for the establishment of a germplasm bank of all of the known species of Gleditsia for future use in interspecific hybridization.



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## CHAPTER II

### Honeylocust half-sib provenance/progeny tests: two-year results

#### ABSTRACT

Honeylocust, Gleditsia triacanthos L., is a minor associate in many natural forest cover types. Due to a combination of desirable traits including high wood specific gravity, abundant coppicing, a tap-rooted/profusely branched root system, drought tolerance, high carbohydrate pods, and high protein seeds and leaves, honeylocust appears to have potential for use as a multi-purpose tree in agroforestry systems.

The potential for genetic and/or cultural improvement in the growth and form of honeylocust is unknown. An in-depth study of the geographic and genetic variation in honeylocust was initiated in 1979 with the establishment of a comprehensive rangewide provenance/progeny test at two locations in southern Michigan.

At the end of the second growing season, significant differences among regions and half-sib families-within-regions were found in total height, caliper, thorniness, date of spring flushing, stem dieback, and fall growth

cessation. Strong negative correlations were found between latitude of origin and stem dieback. The ranges of variation in spring flushing, fall growth cessation, leaf retention, and stem dieback appear to follow clinal patterns. Families of northern origin from the Lake States area are the best overall juvenile performers in terms of total height, stem caliper, cold-hardiness and minimal degree of thorniness.

#### INTRODUCTION

Honeylocust, Gleditsia triacanthos L., is a minor component in many forest associations. The wood is strong and durable and is used locally for fence posts and railroad ties, and also possesses other desirable qualities such as attractive figure and color, strength, and hardness (Panshin and De Zeeuw, 1970). Honeylocust has been widely planted as an ornamental replacement for American elm (Harlow and Harrar, 1968). Its ease of production and culture, fairly rapid growth, and hardiness are among the commendable characters that make it popular for planting in parks, along highways, and in yards and gardens (Li, 1974). Current worldwide interest in honeylocust is based on its potential as a multi-purpose agroforestry crop tree for a variety of animal and chemical feedstocks. It has become naturalized east of the Appalachian mountains from Georgia to New England in the East, and from central Texas north to South Dakota in the West (Little, 1953).

Within the natural range of the honeylocust, a large amount of variation exists in both climatic and edaphic conditions. Average annual precipitation varies from 500 mm in South Dakota-Nebraska to 1800 mm in North Carolina. The frost-free period varies from a minimum of 140 days in the northern and northwestern portions of the range, to a maximum of 340 days in the South Central States (USDA, 1941).

Honeylocust is a shade intolerant tree with a strong taproot. It achieves its best growth on fertile, moist, alluvial floodplains and can attain a maximum size of 50 m in height and 2.5 m in diameter, but its normal size range is 25-32 m tall and 0.8-1.2 m in diameter (Harlow and Harrar, 1968). Honeylocust will also grow on soils of limestone origin, is resistant to both drought and salinity (Van Dersal, 1938), coppices vigorously when cut, and is hardy in the Great Plains where it has been grown successfully in shelterbelts.

Little is known about the patterns or extent of geographic and genetic variation in honeylocust. Similarly, the potential for genetic and/or cultural improvement in the growth and form of honeylocust is unknown. The only provenance test of honeylocust, other than the test being reported on here, is a regional provenance test currently underway at the University of Nebraska (Pers. comm. Walt Bagley, 1981).



An in-depth study of the genetic variation of honeylocust was initiated in 1979. The growth, morphology, ontogeny, phenology, physiology, and chemistry are being studied. This paper will report on the emerging patterns of geographic and genetic variation in honeylocust based on results at the end of the second season in the field.

#### MATERIALS AND METHODS

Honeylocust seed collection requests were sent out in July, 1979. Three groups, including members of the Society of American Foresters Tree Improvement Working Group, State Departments of Natural Resources or their equivalent, and members of the Northern Nutgrowers Association (NNGA), were contacted by mail (Appendix A). Between August 1979 and February 1980, collections were obtained from 467 individual trees in 26 different states covering a majority of the natural and naturalized range of honeylocust. Upon receipt, each collection was assigned an accession number (Appendix B). This number consists of a genus and species code developed by the Michigan State Cooperative Tree Improvement Program (MICHCOTIP) to standardize record keeping for all genera and species used in the breeding program.

All accessed collections were kept refrigerated at 1 - 4 C until extracted and measured. From March to June 1980, seed from all pods were extracted by hand, in order to obtain a maximum number of undamaged seed. During, and subsequent to seed extraction, morphological and chemical measurements were recorded on both pods and seeds. Results

are reported elsewhere (Gold and Hanover, 1984).

Following extraction, seed from individual family seedlots were kept refrigerated until sown. In December 1980, 391 seedlots were sown in a greenhouse at the Michigan State University Tree Research Center. Prior to sowing, all seedlots were scarified in concentrated sulfuric acid to facilitate germination.

Seeds were sown in 30.0 cm x 30.0 cm plastic containers, into which 36 paper plant bands (cells) were inserted and filled with a 1:1:1 soil mixture of peat:perlite:vermiculite. Each cell consisted of a plant band 5.0 x 5.0 x 30.0 cm. A randomized block design with six replications and six seeds per replication was used. The containerized seedlings were grown under 16-hour day lengths with artificial lighting.

Seedlings were removed from the greenhouse in their containers in late July 1981, placed in an outdoor overwintering shelter and hardened off for subsequent spring planting. In mid-April 1982, the half-sib families were outplanted as 1-0 seedlings in a randomized block design at 2 locations in southern Michigan. At each location, three blocks were planted at 7' x 8' spacing, in four-tree linear plots. Location one in East Lansing, Mi., covers 5.1 acres with an average slope of 0-3% and sandy-loam to loam soils (Appendix C). Location two, near Battle Creek, Mi., covers 3.9 acres with slopes from 0-10% and has a sandy-loam soil type (Appendix C).

In the fall of 1982, total height was measured at the end of the first growing season. Data collection in 1983 was limited to the half-sib families which survived in all blocks at both locations. Fifty-seven of these families, representing a cross-section of the entire range, were chosen for data collection and analysis (Table 2.1). Data were collected on the three tallest trees in all plots at both locations. In the spring of 1983, survival, stem dieback, and date of leaf flush were recorded. In the fall of 1983, thorniness, height, caliper, fall growth cessation and leaf retention were recorded.

Dieback was determined by measuring to the highest green portion of the stem. This juncture was clearly delineated after spring flushing commenced. Trees in all 57 families at both locations were observed every three days beginning May 27, 1983 (day one) to determine the date of leaf flush. This was defined as the day when an estimated 50 per cent of the "buds" had expanded to the point at which small leaflets were distinguishable.

In the fall of 1983, the degree of thorniness was scored on a scale from 0, for total absence of thorns, to 4, for very heavy thorniness from base to shoot tips. Growth measurements included total seedling height (cm), and seedling stem caliper (cm) which was measured at 10 cm above the ground. Preliminary observations among half-sib families in the fall of 1982 indicated that growth cessation occurred over a period of more than 60 days. Therefore, in

Table 2.1 Origin data for 57 half-sib families analyzed in 1983, and used in subsequent results and discussion.

Family no.	State	Lat.	Long.	Length of frost free season	Annual ppt.	Heating degree days $\bar{a}$ /coldest month	Mean $T^{\circ}$	Cooling degree days $\bar{b}$ /below 32oF.	No. days
028	GA	33 20	83 24	220	50	2929	43	1722	054
031	GA	33 19	83 41	220	50	2929	43	1722	054
032	GA	33 17	82 58	220	50	2929	43	1722	054
048	GA	33 01	83 33	220	50	2929	43	1722	054
055	GA	33 28	82 31	220	50	2929	43	1722	054
092	KY	38 11	84 53	185	43	4683	34	1268	092
120	OK	35 21	98 39	223	31	3725	37	1949	085
152	TX	30 39	96 24	270	33	1711	50	2908	024
161	PA	40 01	76 19	201	38	5251	30	0948	098
162	PA	40 23	77 54	201	38	5251	30	0948	098
176	MS	33 27	88 50	235	53	2289	47	2321	050
177	MS	33 27	88 50	235	53	2289	47	2321	050
193	SC	34 00	82 14	225	51	3044	45	1573	068
209	SC	34 37	82 50	225	51	3044	45	1573	068
218	SC	34 43	81 14	225	51	3044	45	1573	068
229	SC	34 42	81 37	225	51	3044	45	1573	068
235	SC	33 34	81 44	217	45	2484	46	2087	062
244	KS	37 05	96 31	200	33	5182	29	1361	123
247	KS	37 45	95 10	200	33	5182	29	1361	123
250	KS	39 12	95 33	200	33	5182	29	1361	123
255	LA	32 32	92 39	235	52	1921	46	2538	036
257	LA	32 33	92 56	235	52	1921	46	2538	036
258	LA	32 33	92 56	235	52	1921	46	2538	036
272	IN	40 33	85 40	173	34	6205	25	0748	133
300	VA	38 51	77 19	216	44	3865	38	1353	086
301	IL	42 01	89 21	157	35	6830	21	0714	143
302	IL	42 01	89 21	157	35	6830	21	0714	143
308	IL	41 47	88 00	180	35	6408	22	0893	135
316	NB	41 00	95 52	188	29	6218	22	1148	145
319	NB	41 00	95 52	188	29	6218	22	1148	145

Table 2.1 (cont'd.)

Family no.	State	Lat.	Long.	Length of frost free season	Annual ppt.	Heating degree days a/	Mean T coldest month	Cooling degree days b/	No. days below 32oF.
322	AR	34 41	91 19	244	48	3219	42	2022	081
338	MO	37 56	91 55	190	37	4900	32	1269	107
341	MO	37 56	91 55	190	37	4900	32	1269	107
349	TN	36 12	83 50	200	54	3817	38	1367	083
359	IA	42 02	93 33	150	32	7320	18	0606	149
363	IA	42 02	93 33	150	32	7320	18	0606	149
367	IA	42 02	93 33	150	32	7320	18	0606	149
370	OH	41 27	82 42	145	38	6417	26	0518	136
373	KY	36 50	87 30	195	43	4300	35	1300	092
381	NY	42 23	76 32	154	36	7286	21	0369	148
382	NY	42 23	76 32	154	36	7286	21	0369	148
384	IL	41 25	90 34	180	35	6408	22	0893	135
385	MO	37 42	90 53	190	37	4900	32	1269	107
388	NC	35 45	81 47	230	45	3218	42	1596	071
389	NC	35 45	81 47	230	45	3218	42	1596	071
390	OH	41 22	82 06	153	33	5818	25	0818	127
392	SD	44 23	100 20	146	19	8223	12	0711	173
396	SD	45 31	100 25	132	19	8473	10	0566	180
401	SD	44 34	96 52	151	25	7839	15	0719	169
420	IL	41 38	87 40	191	33	6155	25	0925	120
426	OH	41 17	84 21	160	32	6494	26	0518	136
427	OH	41 30	84 34	160	32	6494	26	0518	136
428	OH	41 17	84 21	160	32	6494	26	0518	136
436	NY	42 35	76 35	154	36	7286	21	0437	136
441	IA	41 44	93 36	162	31	6808	21	0928	136
447	MI	42 45	84 30	154	31	6909	22	0535	151
448	MI	42 45	84 30	154	31	6909	22	0535	151

a/ Sum of negative departures of the average temperature from 65° F.

b/ Sum of positive departures of the average temperature from 65° F.

the fall of 1983 the phenology of growth cessation was studied by recording the number of individual half-sib progeny in each family which were either actively growing or had ceased growth as of October 3, 1983. In effect, a "snapshot" of growth was recorded. Similarly, leaf retention/leaf drop was recorded on October 27, 1983. Individual progeny which still held more than 25 percent of their leaves were scored as retaining, while individuals with less than 25 percent of their leaves were scored as dropped.

For purposes of analysis, the natural range was divided into six regions: Southeast, East-central, Lake States, Northwest, West-central, and Southwest portions of the range (Figure 2.1). A three-level nested ANOVA (Model II) using the combined data set from both plantations was run on 1983 field measurements to test for differences among regions, families-within-regions, and trees-within-families (half-sib progeny) using individual trees as items. For fall growth cessation and thorniness, a two-level nested ANOVA was used using plot means as items (Table 2.2; Table 2.3).

Simple product-moment correlations were calculated between each of fourteen variables, six relating to site of origin, and eight relating to field measurements made in the two plantations (Table 2.4). Family means were used in all correlation analyses. Percent data were transformed using the arcsine square root transformation, and ranked

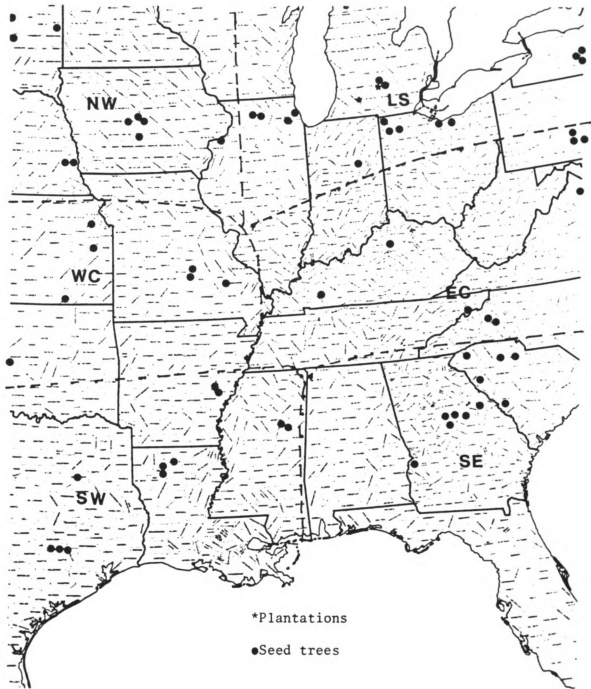


Figure 2.1 Naturalized range of honeylocust and locations of regions, seed trees, and test plantations.

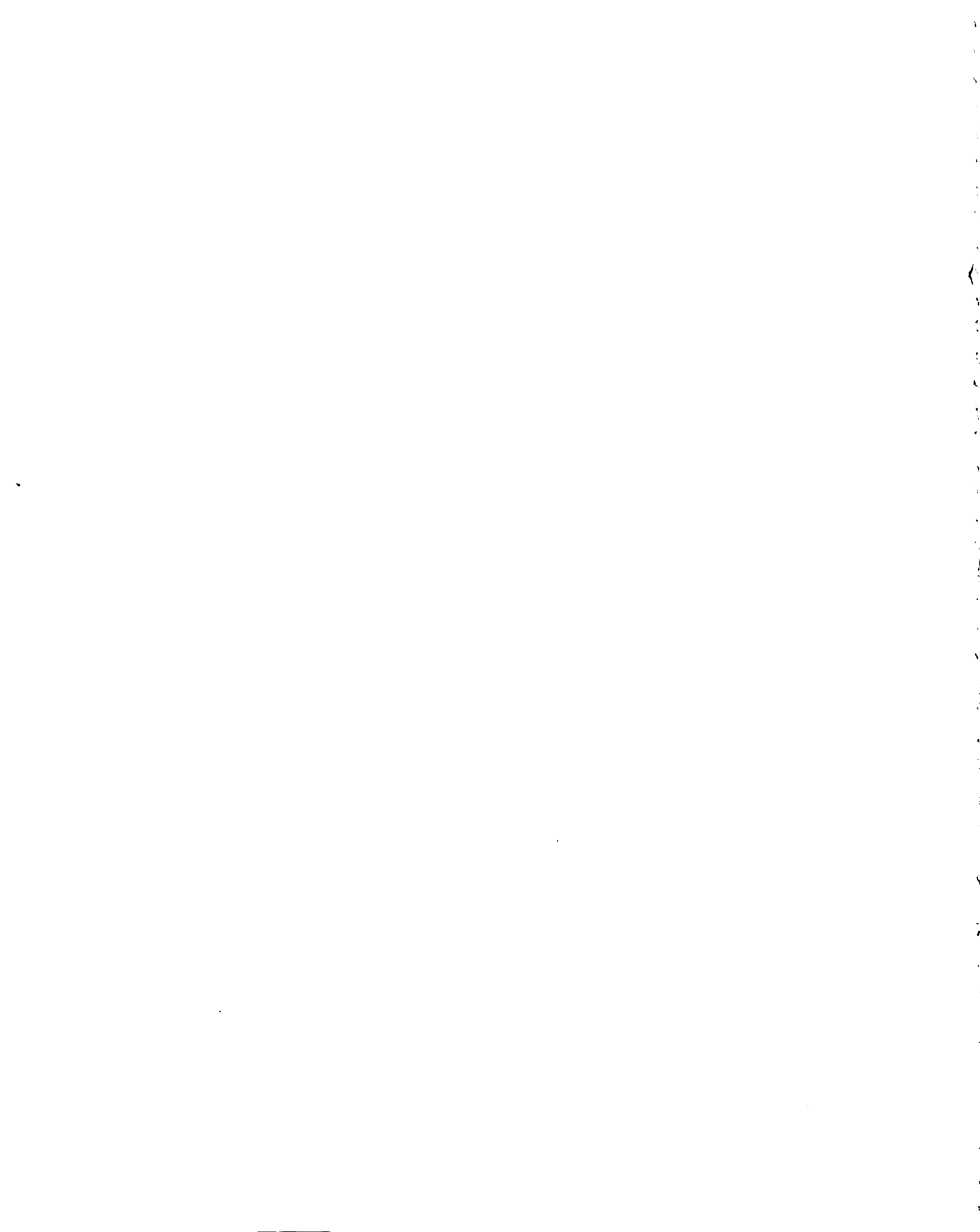




Table 2.2 Expected Mean Squares in 3-level nested ANOVA used to partition out variance components and calculate heritabilities.

<u>Source of variation</u>	<u>Degrees of freedom</u>	<u>Variance components from expected mean squares <sup>1/</sup></u>
Site (S)	S-1	$V_e + NV_b(s)r + NBV_{sr} + NRv_b(s) + NBRV_s$
Block-within-site B(S)	B-1(S)	$V_e + NV_b(s)r + NBV_{sr} + NRv_b(s)$
Region (R)	R-1	$V_e + NV_b(s)r + NBV_{sr} + NBSV_r$
Site x Region	(S-1)(R-1)	$V_e + NV_b(s)r + NBV_{sr}$
B(S) x Region	B-1(S)(R-1)	$V_e + NV_b(s)r$
-----		
Family-within-Region F(R)	F-1(R)	$V_e + NV_b(s)f(r) + NBV_{sf}(r) + NBSV_f(r)$
Site(S) x F(R)	(S-1)F-1(R)	$V_e + NV_b(s)f(r) + NBV_{sf}(r)$
B(S) x F(R)	B-1(S)F-1(R)	$V_e + NV_b(s)f(r)$
Tree(N)-within-plot	N-1(B)(S)(F-1)	$V_e$

<sup>1/</sup> R, F, S, B, N represent the regions, families-within-region, sites, blocks-per-site, and trees-within-plot, respectively.  $V_e$ ,  $V_b(s)f(r)$ ,  $V_{sf}(r)$ ,  $V_f(r)$  are variances due to tree-within-plot, family x block-within-site, family x site, and family respectively.

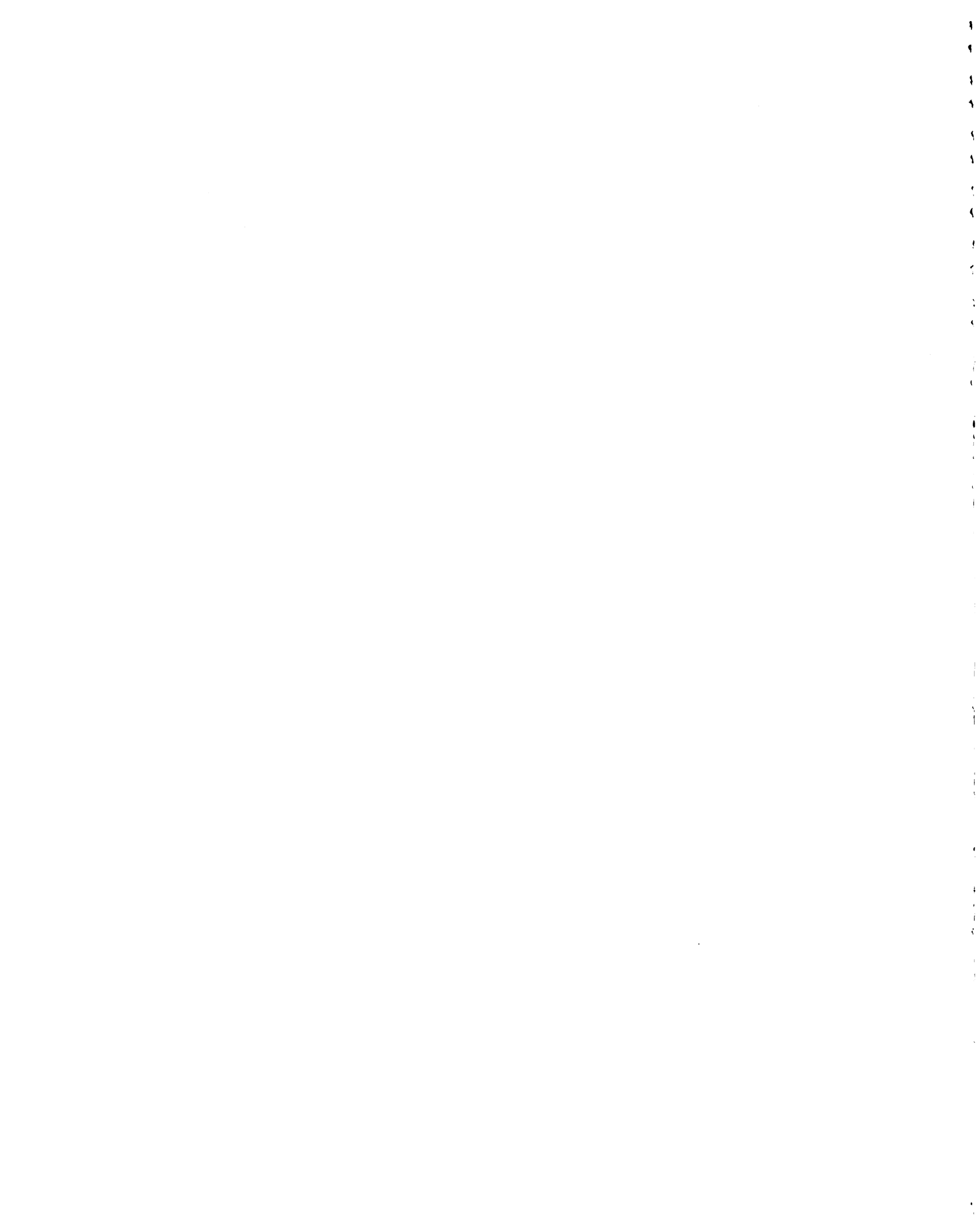


Table 2.3 Three-level nested ANOVA on 57 half-sib families from a rangewide provenance/progeny test at two locations in southern Michigan.

Source of variation	Degrees of freedom	Stem dieback	Leaf flush	Fall growth	2-year caliper	2-year height	Thorniness
Location	1	4690*	1835**	0.19ns	0.52ns	48086**	0.17ns
Rep(location)	4	2581	533	0.12	1.23	14360	0.03
Region	5	52682**	876**	3.25**	1.83**	8948**	2.22**
Loc x Region	5	1357ns	21ns	0.02ns	0.22ns	774ns	0.03ns
Error 1	20	1033	27	0.07	0.15	1294	0.05
-----mean square-----							
Family(region)	51	2267**	56**	0.19**	0.36**	3542**	0.62**
Loc x Fam(region)	51	416ns	29ns	0.03ns	0.20ns	1342ns	0.04ns
Error 2	204	407	30	0.04	0.19	1195	0.05
Sampling	684	286ns	10ns	--	0.06ns	317ns	--

\*

\* Statistically significant at the 5% level.

\*\*

\*\* Statistically significant at the 1% level.

ns

ns Non-significant.

Table 2.4 Simple product-moment correlations on phenological, morphological and growth characteristics of honeylocust. a/

Characteristic	Stem dieback	Spring flush	Thorniness b/	2-year caliper	2-year height	Fall growth	Leaf retention b/
Latitude	-0.78	-0.74	-0.57	---	---	-0.79	-0.78
Longitude	---	---	---	-0.43	-0.42	---	---
Frost-free days <sup>c/</sup>	0.77	0.73	0.49	---	---	0.78	0.75
Cooling-degree days <sup>d/</sup>	0.79	0.74	0.51	-0.41	-0.35	0.77	0.74
Freeze days <sup>e/</sup>	-0.78	-0.75	-0.50	---	---	-0.81	-0.80
Precipitation	0.53	0.48	0.31	---	---	0.55	0.63
Stem dieback	---	0.85	0.51	-0.49	-0.40	0.89	0.83
Spring flush	---	---	0.54	-0.42	-0.44	0.77	0.67
Caliper	---	---	---	---	0.82	0.48	---
Fall growth	---	---	---	---	---	---	0.76

a/ All correlations listed are significant at the 1% level.

b/ Spearman correlation coefficients are presented.

c/ Average length of the frost-free season at point of origin.

d/ Sum of positive departures of the average T<sub>0</sub> from 65° F.

e/ Mean number of days with a minimum T at 32° F or less.

data were subjected to a scalar transformation. (Little and Hills, 1978).

## RESULTS AND DISCUSSION

Patterns of geographic variation can be derived from the results of the ANOVA and correlation analyses. Results of the 3-level nested ANOVA show that trees from different regions differed significantly in all traits analyzed (Table 2.3).

At the end of the second growing season in the field, the mean height and caliper at the East Lansing site were 104.5cm and 1.01cm, respectively. At the Battle Creek site, mean height and caliper were 90.8cm and 0.96cm, respectively. For the 57 families used in the analyses, survival was equal at both locations, averaging 87 percent.

### Variation in Phenological traits

Honeylocust follows the spring growth, fall dormancy patterns common to many other species with large natural ranges (Kriebel, 1957; Sluder, 1960; Bey, 1972). Trees from southern origins, which are adapted to mild climates, are the last to set buds in the fall. Low temperatures are often a major factor in limiting plant distribution because sources native to warm regions cannot often be successfully grown in colder regions. They do not harden off fast enough to survive early cold weather, never develop sufficient hardiness to cold temperatures, lose their hardiness

prematurely and thus are usually damaged or killed by sub-freezing temperatures (Kramer and Kozlowski, 1979).

Studies by Campbell and Sorenson (1973) on phenology and frost damage in Douglas-fir showed that southern sources generally set buds later than northern sources. For provenances that set bud in the same week, southern sources were more frost sensitive than northern sources, with the proportion damaged increased by four percent for each degree of latitude. Each additional week which bud set preceeded frost, the proportion of frost damaged seedlings decreased by approximately 25 per cent.

Southern Michigan is at the northern extreme of the natural range of honeylocust (Figure 2.1), and after two seasons stem dieback and death from winter injury are strongly evident. Stem dieback is very highly correlated with active shoot growth late into the fall ( $r= 0.89$ ), with frost free days at the point of origin ( $r= 0.77$ ), with cooling degree days ( $r= 0.79$ ) and with date of spring flushing ( $r= 0.88$ ). The high positive correlation between stem dieback and fall growth suggests that photoperiod may play a role in the ability of individual sources to enter dormancy (Nienstadt, 1974), to attain a sufficient degree of cold hardiness and to resist subsequent tissue damage (Table 2.4).

Stem dieback is negatively correlated with latitude of origin ( $r= -0.78$ ), with freeze days ( $r= -0.78$ ), and with the mean number of days with a minimum temperature of 32 ° F. or

less ( $r = -0.78$ ). It is also negatively correlated with 2-year seedling height ( $r = -0.40$ ) and 2-year seedling caliper ( $r = -0.49$ ) (Table 2.4).

Based upon the above observations, perhaps the most critical trait to consider in the initial stages of provenance/progeny testing of honeylocust in northern areas, is the degree of winter-hardiness. The presence or absence of winter hardiness is reflected in the varying degrees of severity of stem dieback and mortality. As expected, families originating in northern areas above 40.5° north latitude suffered little or no dieback. The region showing the least overall dieback is the Northwest region, covering the Northern Plains states which have the greatest temperature extremes and harshest climate (Table 2.5).

Table 2.5 Variation among regions in traits analyzed.

Trait	Regions <u>1/</u>					
	SE	EC	LS	NW	WC	SW
Stem dieback (%)	27.9	14.2	4.2	3.0	5.5	51.1
Leaf flush (days)	8.6	7.3	4.4	4.8	4.8	10.0
Fall growth (%) <u>2/</u>	42.8	20.9	2.6	0.0	3.9	60.4
Caliper (cm)	1.09	1.03	1.02	0.98	0.97	0.76
Height (cm)	102.9	103.1	102.2	96.0	93.4	82.8
Thorns (%) <u>3/</u>	83.4	62.5	32.0	44.9	66.7	67.5

1/ Values reported are means for each region. Figure 2.1 indicates the location of each region.

2/ Fall growth (%) represents the number of progeny in any given region which were actively flushing.

3/ Thorns (%) represents the number of progeny in any given region which were thorny.

Families from the Lake States region also proved to be hardy. Additionally, the West-central region encompassing the central Plains States between 35° - 37° north latitudes, was the only region in which families originating south of 37° north latitude proved winter-hardy under Michigan conditions. This is likely due to frequent exposure in this region to severe weather extremes in spite of a long growing season. With the exception of the West-central region, honeylocust families whose source of origin is south of 37° N. latitude suffered at least 10 percent stem dieback. The least winter-hardy region proved to be the Southwest portion of the range. Families from this region suffered an average dieback in excess of 50 percent of total height. In general, families originating south of 35° N. latitude suffered between 30 and 80 percent stem dieback (Table 2.6).

Two families of Southern origin, from Georgia and Louisiana, proved to be outliers and did not suffer significant stem dieback. Field observations point to other cold tolerant individuals within cold sensitive families. If this pattern continues into the future, there will be good potential for within family selection for improved cold tolerance in southern sources. Selected individuals would be used for incorporation of desired southern traits, such as high levels of pod carbohydrates, into northern sources. According to Kriebel and Gabriel (1969), one possible explanation for the performance of these families is that relict populations from the Deep South and Mississippi



Table 2.6 Origin, phenology, and survival data for 57 half-sib families used in analysis and discussion. a/

Family no.	State	Latitude No	Long. No	Frost-free days	b/		Days to flush	c/		Survival %
					Stem dieback	Stem dieback		Fall growth %	Leaf retention %	
359	IA	42 02	93 33	150	2.00	3.75	0.00	20.03	100	
384	IL	41 25	90 34	180	2.25	3.75	0.00	14.07	100	
390	OH	41 22	82 06	153	2.30	5.38	11.72	6.97	100	
319	NB	41 00	95 52	188	2.34	4.64	0.00	10.07	100	
250	KS	39 12	95 33	200	2.39	5.91	0.00	22.40	96	
396	SD	45 31	100 25	132	2.40	4.39	0.00	7.07	96	
401	SD	44 34	96 52	151	2.46	4.63	0.00	7.07	100	
338	MO	37 56	91 55	190	2.52	5.25	0.00	13.97	100	
092	KY	38 11	84 53	185	2.55	3.18	0.00	16.93	92	
301	IL	42 01	89 21	157	2.55	2.23	0.00	19.90	92	
363	IA	42 02	93 33	150	2.56	6.74	0.00	9.93	96	
316	NB	41 00	95 52	188	2.62	7.63	0.00	17.03	100	
381	NY	42 23	76 32	154	2.70	3.87	0.00	7.07	96	
427	OH	41 30	84 34	160	3.05	5.70	6.70	28.57	96	
302	IL	42 01	89 21	157	3.06	4.00	0.00	21.03	96	
426	OH	41 17	84 21	160	3.10	3.74	0.00	33.70	96	
055	GA	33 28	82 31	220	3.30	6.00	0.00	43.03	100	
382	NY	42 23	76 32	154	3.37	3.35	0.00	9.97	96	
367	IA	42 02	93 33	150	3.40	7.00	0.00	0.00	92	
255	LA	32 32	92 39	235	3.58	4.41	0.00	26.23	92	
370	OH	41 27	82 42	145	4.02	4.00	0.00	27.43	96	
428	OH	41 17	84 21	160	4.03	5.96	0.00	8.07	96	
441	IA	41 44	93 36	162	4.06	6.59	0.00	25.63	92	
308	IL	41 47	88 00	180	4.13	3.75	6.53	26.70	100	
447	MI	42 45	84 30	154	4.19	5.57	0.00	24.03	88	
341	MO	37 56	91 55	190	4.56	5.17	6.53	28.57	96	
247	KS	37 45	95 10	200	4.61	4.00	0.00	26.33	100	
436	NY	42 35	76 35	154	4.97	4.60	0.00	11.53	83	
448	MI	42 45	84 30	154	5.17	4.27	0.00	6.97	92	
392	SD	44 23	100 20	146	6.24	5.80	0.00	18.40	83	
272	IN	40 33	85 40	173	6.39	5.75	0.00	7.40	100	

Table 2.6 (cont'd.)

Family no.	State	Latitude No	Long. No	Frost-free days	b/		Fall growth %	c/	Leaf retention %	d/	Survival %
					Stem dieback	Days to flush					
120	OK	35 21	98 39	223	6.94	4.00	7.60	38.97		100	
385	MO	37 42	90 53	190	7.05	4.68	6.53	35.93		92	
420	IL	41 38	87 40	191	8.37	4.50	7.40	38.97		100	
300	VA	38 51	77 19	216	8.53	8.50	6.12	43.03		92	
162	PA	40 23	77 54	201	10.74	8.00	0.00	36.67		88	
244	KS	37 05	96 31	200	10.84	6.48	18.38	35.93		96	
373	KY	36 50	87 30	195	12.13	6.35	6.32	43.03		96	
209	SC	34 37	82 50	225	13.52	9.00	20.22	41.00		100	
193	SC	34 00	82 14	225	14.97	5.63	25.37	33.70		100	
388	NC	35 45	81 47	230	16.76	9.25	41.93	34.63		100	
349	TN	36 12	83 50	200	17.04	9.45	18.42	41.03		92	
229	SC	34 42	81 37	225	19.13	8.11	36.65	36.67		79	
161	PA	40 01	76 19	201	29.07	8.17	44.77	41.03		96	
389	NC	35 45	81 47	230	30.51	8.43	55.83	40.27		88	
235	SC	33 34	81 44	217	31.39	8.50	36.12	41.00		92	
218	SC	34 43	81 14	225	35.63	11.25	54.07	41.00		100	
028	GA	33 20	83 24	220	35.90	8.13	53.27	43.03		100	
031	GA	33 19	83 41	220	38.89	11.00	46.05	43.03		100	
048	GA	33 01	83 33	220	38.91	9.05	64.37	43.03		92	
177	MS	33 27	88 50	235	39.99	10.14	64.07	43.03		88	
322	AR	34 41	91 19	244	46.12	10.88	30.22	40.27		71	
032	GA	33 17	82 58	220	54.61	10.00	64.42	43.03		88	
258	LA	32 33	92 56	235	56.56	9.10	50.78	40.30		83	
176	MS	33 27	88 50	235	61.33	12.70	70.65	43.03		83	
152	TX	30 39	96 24	270	64.32	11.62	75.25	40.27		54	
326	AR	34 41	91 19	244	77.12	7.30	74.88	43.03		42	
257	LA	32 33	92 56	235	81.38	11.50	75.05	43.03		67	

a/ Data presented are overall means from 2-year field data collected in 1983.

b/ Families are ranked on the basis of their percent stem dieback, from lowest to highest.

c/ Fall growth % is equal to the total number of progeny in each half-sib family actively flushing on October 3, 1983.

d/ Leaf retention % is equal to the total number of progeny in each half-sib family which retained 25 or more percent of their leaves on October 27, 1983.

Valley may retain a genetic capacity for winter-hardiness normally found solely in trees with northern genotypes.

Narrow sense family heritability for stem dieback based on variance components is  $h^2 = .96$ . This high degree of heritability will be of important in the future breeding program which will try to incorporate some of the important economic traits inherent in Southern sources into cold hardy, Northern sources.

Similar patterns of regional variation are found when comparing the fall growth cessation patterns with stem dieback patterns (Table 2.6). Very few progeny from families originating in winter-hardy regions were actively growing when scored in October, 1983. In fact, there were no individuals in any family from the Northwest region which were still actively growing by that date. In contrast, 60 percent of the progeny in families from the cold intolerant Southwest portion of the range were actively growing in October, 1983. In general, families originating south of 35° north latitude, with frost-free seasons at their point of origin in excess of 220 days, proved to be the least cold tolerant (Table 2.6).

In the fall, honeylocust drops its leaves over a very short interval. Leaf retention shows a large amount of variation within regions and this may indicate quantitative gene regulation of leaf fall. In general, a definite trend exists toward more rapid leaf fall in families from northern regions. Leaf retention is positively correlated with fall

growth cessation ( $r= 0.76$ ) and stem dieback ( $r= 0.83$ ), and negatively correlated with latitude ( $r= -0.78$ ) and freeze days ( $r= -0.80$ ). Leaf retention was more highly correlated with precipitation at point of origin than any other trait ( $r= 0.63$ ).

An interesting feature of leaf retention is that one of the two southern "outliers", family number 055 from Georgia, which performs like northern families in all other traits, showed a high degree of leaf retention similar to other families from the same latitude. Based on the regional data, it would appear that selection pressure for or against leaf retention is very small, allowing for a great deal of variation within large regional areas.

Honeylocust also follows common patterns of variation in spring growth initiation. Families of northern origin (IA, NB, SD, IL, etc.) flushed first, families of more southerly origin were intermediate in their flushing date, and families which were last to flush were all from southern origins (LA, GA, TX, MS, etc.). The range of variation in spring flushing appears to follow a clinal pattern (Table 2.6).

The earliest and latest flushing families differed by over 11 days, while the earliest and latest individual progeny differed by 21 days. Negative correlations were found between flushing and latitude ( $r= -0.74$ ), and the correlation between flushing and frost free days was positive ( $r= 0.73$ ) (Table 2.4). These correlations suggest

that budbreak is influenced by the temperature distribution patterns at the location of origin. These data agree with the conclusions of Burley (1966a; 1966b) in his study of Sitka spruce. Burley found that when spring flushing is viewed in relation to the nature of the spring temperature distribution at the point of seed origin, a systematic pattern of flushing can be observed among seed sources. Stem dieback is highly correlated with spring flushing ( $r = 0.85$ ) and the relative performance of these two characteristics are highly dependent on latitude and temperature of origin.

#### Regional Variation

The amount of variation accounted for by regional differences varied widely among the traits analyzed (Table 2.7). The variation in phenological traits accounted for by regional differences is larger than the variation accounted for by families-within-regions. This is because the strong effects due to site of origin and genetic background of the various families have already clearly expressed themselves in stem dieback, leaf flushing, and fall growth/dormancy. Effects of origin and genetic background have not become fully evident in growth traits by age two.

Regional variation patterns in height and stem caliper at age two do not follow the distinct patterns shown for phenological traits. Families from the East-central region were tallest and had the largest stem caliper, followed very

Table 2.7 Variation accounted for among regions, families within regions, and trees within families (expressed as percent of total phenotypic variance), and heritability values a/.

Trait	Regions	Families	Trees	Heritability	
				family	single tree
Stem dieback	36.5	16.0	27.1	.96 (.17)	.82 (.04)
Leaf flush	16.9	11.0	25.1	.35 (.15)	.34 (.03)
Fall growth	42.4	25.4	--	--	--
Caliper	7.3	14.8	30.9	.45 (.18)	.32 (.07)
Height	5.0	20.3	24.4	.63 (.19)	.65 (.11)
Thorns	19.5	55.8	--	--	--

a/ Heritability values calculated by method of Wright (1976). Variance components are derived from the expected mean squares in the analysis of variance.

b/ Numbers in parenthesis represent standard error values.

$$\text{Family heritability} = \frac{V_f}{V_e / NBS + V_{fb} / BS + V_{fs} / S + V_f}$$

$$\text{Single tree } h^2 = \frac{V_e + V_{fb} + V_{fs} + V_f}{4(V_f)}$$

N, B, S = The number of trees-within-plot, blocks, and sites, respectively.

$V_e, V_{fb}, V_{fs}, V_f$  = Within-plot variance, error variance, family x site variance, and family variance, respectively.

closely by the Southeast and Lake States regions, respectively (Table 2.5). Considering the data on stem dieback, these early growth patterns are not expected to continue into the future.

Personal field observation reveals that families from the East-central and Southeast regions regrew very vigorously from the point of dieback and therefore were able to equal or exceed the northern families by the end of the second growing season. However, as the trees continue to age, the ability of trees from less cold tolerant regions to "catch up" to northern families each year may diminish over time. Cold-hardy families from the Lake States region and even the slower growing West-central and Northwest regions will add incrementally to their total height each year and eventually surpass families of southern origin, particularly those originating below 37° N. latitude.

At age two, families from the Southwest region were the only ones to exhibit a marked decrease in growth due to lack of cold-hardiness. Many of the progeny in these families died back to the ground level. Negative correlations between stem dieback and height ( $r = -0.40$ ), and dieback and caliper ( $r = -0.49$ ) are already in evidence. These correlations are expected to increase as dieback causes families of southern origin to fall further behind Northern families over time.

A common feature of wild, open pollinated honeylocust, is the presence of many sharp, 3-branched thorns occurring

singly or in clusters. Thorns are considered to be abortive branches which arise from supra-axillary buds on the branches and from adventitious buds on the trunk (Blaser, 1956). Thorns complete development and lignification in one year and become extremely hard, range in size from 2-40 cm, and can be dangerous and difficult to work with (Harlow and Harrar, 1968). Thorniness in honeylocust is thought to have arisen as an evolutionary adaptation to exposure to arid environments. Thorn shoots are thought to curtail transpiration loss (Grisyuk, 1959).

One goal of the honeylocust project is to determine the degree and heritability of the thorn trait, and to develop thornless selections. Thorniness is a juvenile trait and the upper branches of even the thorniest trees 10 years and older can be used as scionwood to create "thornless" cultivars (Chase, 1947). The progeny of these grafted "thornless" cultivars are genetically "thorny" and will contain thorny seedlings, which is highly undesirable. Open pollinated progeny from the thornless "inermis" cultivar produce 60-80 percent thornless progeny. The ability to eliminate the thorn trait through breeding will expedite the widespread use of honeylocust. Additionally, the introduction of genetically thornless honeylocust into areas where it is not found locally would totally eliminate the thorn problem.

Grisyuk (1959) reports on three years of controlled pollination experiments between thorny and thornless



honeylocusts. Crosses were made in all combinations. Results indicate that crossing thornless females with thornless males will produce only thornless progeny. Testing of  $F_2$  progeny from controlled crosses of the  $F_1$  generation should provide the basis for producing genetically thornless trees. If all  $F_2$  progeny are thornless, a major hurdle will have been crossed in the practical use of honeylocust.

Regional differences in thorniness are present, with the Southeast region showing the highest percent of progeny which were thorny, over 80 percent (Table 2.6). A general decrease in number of thorny progeny per region is evident from south to north, with the Lake States region having the least thorny progeny, 32 percent. The negative correlation between latitude and thorniness is moderately high ( $r = -0.57$ ).

#### Family Variation

Family-within-region differences were highly significant for all traits analyzed (Table 2.3). For stem caliper, the family-within-region component accounted for twice as much of the variation as the region component, 14.8 vs. 7.3 percent, respectively (Table 2.7). In terms of total height growth, the family-within-region component accounted for over 20 percent of the variation, four times greater than the region component. The narrow-sense family heritability for height at age two is estimated to be  $h^2 = .63$ , while family heritability for caliper is  $h^2 = .45$ .

Two-year height and caliper were highly correlated ( $r = 0.82$ ). Both characteristics also show a negative correlation with longitude, ( $r = -0.43$ ) for caliper and ( $r = -0.42$ ) for height.

The effect of a relatively high heritability for height growth, coupled with a much larger component of variation among family-within-region than among regions, points to a good potential for capturing genetic gain through selection at the family level. Coefficients of correlation between 1-year and 2-year heights are high ( $r = 0.78$ ), and between 1-year height and 2-year caliper are ( $r = 0.71$ ). Calculated single tree heritabilities are  $h^2 = .65$  for height and  $h^2 = .32$  for caliper.

At the tree-within-family level, the individual half-sib progeny accounted for a large percentage of the variation in both height and caliper, 31.0 and 24.5 percent respectively. If this large amount of tree-within-family variation maintains itself over time, it would permit further genetic gain at the within-family level. The high percentage of variation due to tree-within-family may be due in large part to residual planting effects, initial seedling size differences, age effects, and strong early influence of microsite differences. The magnitude of these differences are expected to decrease as the age of the trees increase. This will reflect a truer picture of the tree-within-family contribution to the overall variation.

The plantation x region interaction, as well as plantation x family-within-region interaction, were nonsignificant for all traits (Table 2.4). This indicates consistency of results over locations in southern Michigan. However, if the rangewide test had been planted at more diverse locations throughout the range, significant location x region and location x family-within-region interactions would likely occur as families would be expected to perform quite differently in more southerly locations.

Significant differences were found between the two plantations for height, days-to-flush, and stem dieback. No significant differences were found between plantations for caliper, fall growth and thorniness (Table 2.3). The mean 2-year height at the East Lansing plantation was 115 percent greater than at the Battle Creek plantation.

Based on 1983 measurements and subsequent data analysis, the most promising families in terms of winter-hardiness, height growth, and caliper growth were compared to the best local family (Table 2.8).

Because the data are based on results at age two in the field, some of the families which suffered significant levels of dieback were able to resprout with enough vigor to show up in the top 5 family rankings. As previously mentioned, it is expected that these less hardy families will fall behind the rest of the more winter-hardy families in overall height, diameter and survival as plantation age

increases. Therefore, families suffering more than 10 percent stem dieback were excluded from the top five ranking. Based on the data, selection on the basis of

Table 2.8 Performance of the 5 best honeylocust half-sib families at age two at two locations in southern Michigan, based on height, caliper, and stem dieback a/.

Family no.	State	Survival %	Height	Caliper
-----	-----	-----	-----	-----
			% of best local source mean	
			<u>b/</u>	
420	IL	100	119 (131)	104 (1.22)
300	VA	92	100 (110)	104 (1.22)
055	GA	100	102 (113)	99 (1.16)
448	MI	92	100 (110)	100 (1.17)
272	IN	100	110 (121)	97 (1.13)
Overall mean values			(098)	(0.99)

a/ Excludes families in which average stem dieback exceeded 10 percent of total seedling height.

b/ Numbers in parenthesis are actual family means. Units shown are in centimeters (cm).

latitude or provenance alone may prove to be an inadequate measure of performance. The data also indicate the presence of variation for hardiness within region.

#### CONCLUSIONS

General recommendations for the selection of superior half-sib families would be to choose families originating north of 40.5° N. latitude, and generally favor the central portion of the range. Due to the great deal of geographic and genetic diversity found in honeylocust, opportunities for genetic improvement are excellent. Empirical observations on stem form, coupled with the measured

variation in thorniness, ultimately will lead to the use of progeny from specific individuals which exhibit an array of positive attributes including high survival, height, diameter, stem form and genetically derived thornlessness.

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## Chapter III

### AGROFORESTRY SYSTEMS FOR THE TEMPERATE ZONE

#### ABSTRACT

The term "agroforestry" refers to land management systems which involve trees, agricultural crops, and domestic animals in any or all combinations. The combinations may be either simultaneous or staggered in both time and space.

The historical development of a permanent agriculture system based on the use of agroforestry in the temperate zone is traced. The reasons for a renewed interest in agroforestry include the end of cheap, subsidized fossil fuels; increased concern about soil erosion and marginal land use; an international awakening as to the dangers of indiscriminant use of pesticides, herbicides and other chemicals; and a need to continuously increase food production to meet growing population demands.

Three agroforestry management systems are reviewed which currently appear feasible for implementation in many of the industrialized countries of the temperate zone. These three systems include: 1) Animal grazing and intercropping under managed coniferous forests or plantations; 2) Multi-cropping of agricultural crops under



intensively managed, high value hardwood plantations; and  
3) Woody/woody intercropping involving nitrogen fixing woody plants.

#### INTRODUCTION

Two of the more pressing problems facing mankind in the 1980's are shortages of food and energy. From a production standpoint, our attempts to feed the world's population have been successful. Modern agriculture now produces more food per acre than at any other period in history. To meet the food shortage challenge, a highly mechanized, fossil fuel dependent, centralized production system has been developed. Farmers rely on fossil fuels for planting their crops, for pesticides, herbicides, harvesting, processing and transporting of our food.

Major problems have arisen in this food production scheme in the past decade. The cost of fossil fuel, the backbone of our modern food production system, has increased from under \$2.00 bbl. to over \$30.00 bbl. Many farmers are finding it difficult to afford the direct and indirect costs of the fossil fuel needed to maintain this large, energy intensive system. Our governmental, industrial, and academic institutions are now looking to the food itself, in the form of ethanol, as an alternate source of energy to replace fossil fuels.

Concurrent with the reality of expensive fossil fuel energy, farmers are becoming totally dependent on the food export trade to sell their crops. The grain embargo of the

U.S.S.R in 1979, after the invasion of Afganistan, led to the development of a massive farm surplus and depressed commodity prices. The multi-billion payment-in-kind (PIK) program of the federal government resulted from a need to decrease farm output and raise crop prices.

A third major problem in the agriculture sector is that of soil erosion. In 1976, the average annual loss of topsoil from agricultural land in the U.S. was approximately 12 tons per acre. The annual fertilizer (N-P-K) losses amount to more than 50 million tons, worth about \$7 billion (Pimentel et al., 1976).

The solution to these major problems will certainly be multi-faceted and will include the development of alternative, less energy-intensive technologies, improved soil conservation practices, and more efficient, diversified farming systems. One important facet of the technological solution to these problems may lie in the field of "agroforestry". Known variously as agri-silviculture, farm forestry, forest farming, tree crops, 3-D forestry, and taungya, this integrated farming system offers many new opportunities and advantages in solving the energy, food, and soil erosion problems.

Agroforestry is an interdisciplinary approach to systems of land use, different from the sum of its two major components, agriculture and forestry. It refers to land management systems involving many interdependent components including trees, agricultural crops, and domestic animals in

any or all combinations. The combinations may be either simultaneous or staggered in both time and space (Lundgren, 1982).

Agroforestry might be considered as the meeting point for a confluence of disciplines, both applied and basic in nature. Within its broadest scope it draws on the accumulated knowledge of many separate disciplines. It draws on forestry, agronomy, animal husbandry and horticulture for its major inputs, with necessary additional inputs coming from soil science, microbiology, ecology, plant breeding, chemistry, economics, sociology, agriculture engineering, and others.

Implicit within the concept of agroforestry systems is the idea of using trees in nonconventional ways. This will demand a rethinking of the design, architecture and role which trees will play. The development of different agroforestry systems will be required for each individual locality based on existing biological, economic and political constraints.

A review of the literature reveals numerous agroforestry systems currently being researched. Some of these systems will entail only slight modifications of current practices, while others will require a more radical change. It is outside the scope of this paper to present a detailed review of agroforestry research in the less developed countries (LDC's), however it should be noted that the bulk of the current research into agroforestry systems is being

conducted in the LDC's (Bene et al., 1977; Huxley, 1983; Anon., 1983; MacDonald, 1981).

While many potential agroforestry systems have been proposed, this review will be limited to three systems which appear close to practical application and implementation. A list of the temperate zone agroforestry systems reviewed in this paper include:

- 1) Systems of animal grazing and intercropping under managed coniferous forests or plantations.
- 2) Multi-cropping of agricultural crops under intensively managed, high value hardwood plantations.
- 3) Systems of woody/woody intercropping, involving nitrogen fixing woody plants.

Another system, using multipurpose trees for energy fuels, chemicals, fiber, animal feed, and soil stabilization, is also considered to have a great deal of potential merit. An in-depth review of this topic is in preparation.

The purpose of this review is to introduce the concept of agroforestry to foresters and hopefully to expand conventional thinking on the uses and potential uses of trees. Some ongoing work in the industrialized countries of the temperate zone will be highlighted.

#### Historical development

The idea of an agriculture based on trees was first outlined in the U.S. by J. Russell Smith, an economic geographer at Columbia University (Smith, 1909; Smith, 1911).

In a lifetime of travel and scientific observation, Smith documented the destructive results of erosion following cultivation of hilly, marginal lands. In his travels to the Mediterranean, Smith observed many examples of a permanent, tree-based agriculture, on steep rocky terrain (Smith, 1950). In the Mediterranean agriculture, chestnuts (Castanea spp.), oaks (Quercus spp.), carob (Ceratonia siliqua), olive (Olea europa), and figs (Ficus spp.) all provided a variety of agricultural and economic products to the people of that region. Smith proposed North American counterparts to each of the crop trees including nut trees (Carya spp., Juglans spp.), oaks (Quercus spp.), persimmons (Diospyros spp.), mulberries (Morus spp.), mesquites (Prosopis spp.) and honeylocust (Gleditsia triacanthos) (Smith, 1914; Smith, 1950).

As early as 1914, Smith was advocating the ideas which were "rediscovered" in the 1970's under the guise of agro-forestry. His ideas included interplanting crop trees with woody legumes coupled with animal grazing to gain maximum benefits from a given site and expand the area of useable lands. He advocated the use of tree crops for human and animal food, economic gain, for improving soil stabilization and increasing soil fertility, and for microclimate amelioration. He encouraged the search for additional candidate cropping trees, and the subsequent breeding of cropping trees to maximize their potential for producing food and wood (Smith, 1914).

Smith was also aware of the resistance of our political and agricultural leaders to a rethinking of our food production system. Therefore, to accomplish this, he proposed the establishment of a privately funded tree crop/hillculture research center for long term, uninterrupted research in to a permanent agriculture based on tree crops (Smith, 1950).

Concurrent with the early writings of Smith, preliminary research was underway on potential tree species suitable for agroforestry. Forbes (1895) and Garcia (1916) documented the feed value of the mesquite (Prosopis juliflora) growing in the arid southwest U.S. Walton (1923) documented the chemistry and feed value of honeylocust and mesquite. Like Smith, these early tree crop researchers felt that the native vegetation merited serious consideration as multi-purpose, well adapted crop trees.

The onset of the Great Depression in the 1930's brought along massive unemployment and the "Dust Bowl" of the Great Plains. This motivated the U.S. government into a temporary rethinking of our agricultural policies. Along with the large scale shelterbelt planting in the Plains States, a series of hillculture/tree crop projects were established in the eastern U.S. The focal point of the research was the Tennessee Valley Authority (TVA).

The TVA began tree crops research in 1934 to provide for reforestation and proper use of marginal lands in the Tennessee Valley. This area of the U.S. was characterized by extensive areas of eroded and poorly utilized land, much

of it steep and unsuited for conventional agricultural use. A system of land management was proposed which would simultaneously protect the soil, prevent soil erosion, and yield a cash crop. The TVA research efforts concentrated on the black walnut (Juglans nigra), but also included Chinese chestnut (Castanea mollissima), filbert (Corylus), hickories, persimmon, and honeylocust (Hershey, 1935; Zarger, 1956). Other projects were located at Virginia Polytechnic Institute, the Alabama Polytechnic Institute (Zarger, 1956), Auburn University (Moore, 1948), and in Ohio (Smith, 1942).

During the 1940's, the tree crops idea surfaced in other areas of the world. Eardley (1945) discussed the suitability of carob, mesquite, and honeylocust as supplementary fodder for livestock in southern Australia. Look (1947) and Jurriaanse (1973) describe many fodder trees useful as stock feed to farmers in South Africa, where large areas were often stricken with drought. In 1947, a publication by the Imperial Agricultural Bureaux detailed the uses and misuses of trees and shrubs as fodder throughout the British Commonwealth. Chemical composition and digestibilities were listed for over 800 species. Additionally, the concept of "protein pastures", in which trees and shrubs are used as fodder, windbreaks, shade trees and soil conditioners, is suggested (Anon., 1947). Schreiner (1959) and Huguet (1979) mention a polyculture system in Italy, "coltura promiscua", in which pollarded poplars are used as vine supports, and also provide fuel and lumber. The

fertile soils support a 3- and occasionally 4-story culture of poplars, grapes, dwarf fruit trees, with an annual crop or forage species at the base.

The early 1950's heralded a post-war economic boom. Cheap fossil-fuels, herbicides, fertilizers, new farm machinery and economic prosperity dominated the thinking of American agriculturalists. One result of this economic climate was that the tree crops projects died a sudden death. According to Zarger (1956), the tree crops projects "had to be abandoned because the cooperating institutions needed the land to meet building program needs." Moore (1948) states, "Hillculture went under in June of 1947, and the Horticulture Department took this work over, and they thought they could not support the honeylocust pasture program in Hillculture, and the plot, of course, was pulled out and planted in peaches." Jurriaanse (pers. comm., 1979) states "The main reason why I dropped the work (fodder tree research) in 1951 was because of lack of support... This was largely due to the fact that there existed a controversy between Agriculture and Forestry as to which department should assume responsibility for this work, neither of them really being interested because no quick results were expected."

The tree crops idea was all but forgotten in the 20-25 year period from the late 1940's-early 1950's until the late 1960's-early 1970's. Four major factors played a role in the renewal of interest in the "tree crops" concept. First,



the environmental and ecological concerns of the late 1960's resulted in the banning of numerous herbicides and pesticides widely used in our agriculture and forestry sectors (Eckholm, 1976). Second, the extremely high productivity achieved by agriculture in the post-war industrialized world was based on the abundant supply and heavy use of subsidized, low-cost energy (Hirst, 1974; Pimentel et al., 1973). The oil embargo of 1973 forced a re-evaluation of input-output costs of our farming systems. The ratio of energy used for each food calorie produced doubled in thirty years (Steinhart and Steinhart, 1974). Third, great concern was again surfacing by the mid-1970's on the effects of continued soil erosion, and the possible dire consequences it held for the U.S. food production capabilities (Carter 1977, Pimental, 1976). Fourth, the awareness of the ever-increasing size of the world's population meant that world food producers would have to continue to increase their output. These events created a search for alternatives to fossil fuels for chemical feedstocks. This precipitated a renewed look at the potential role of trees as one component in the overall solution.

In 1971, a prescient paper was written in which species of the genus Alnus, nonleguminous fixers of atmospheric nitrogen, were recognized as having the potential for use in forest management systems in a similar way to that of legumes in agriculture (Tarrant and Trappe, 1971). The first industrialized country to seriously test the idea of a

combined forestry/agriculture system of management, ie. agroforestry, was New Zealand.

In New Zealand, conflict over land usage between agriculture and forestry, a need to revitalize the rural economy, and the desire to increase timber exports generated an intense interest among researchers to find a way to solve these diverse problems. The idea of "farm forestry" was put forward as a potential solution to many of these problems (Knowles, 1972; Barr, 1973; Olsen, 1974; Farnsworth and Male, 1975). It was determined that widely spaced radiata pine (Pinus radiata), planted at wide initial spacings for maximum growth, could be harvested for sawlogs on 20 year rotations. This led to the further idea of grazing animals in between the trees, fully utilizing the resultant lush understory. Thus, the idea of purposely managing and integrating forestry and agriculture in a two-tier system was re-introduced to western industrialized countries. Up to this time, grazing in forested lands and forest plantations was always considered to be in conflict with or at best an ancillary benefit to the timber crop, but was not a part of a rigorous management systems (Adams, 1975).

A combination of the ideas coming out of New Zealand and the energy/environmental/population/food crises of the mid-1970's led many others to a rediscovery of the ideas of J. Russell Smith. Douglas and Hart (1976) published a book restating and expanding on many of Smith's ideas. Following that, articles advocating the concept of agroforestry in one

form or another were written by Cumberland (1976), Farmer (1976), Hills (1977), MacDaniels and Lieberman (1979), Gordon and Dawson (1979), Felker and Bandurski (1979), Borough (1979), Tustin et al. (1979), Williams (1980), Spurgeon (1980), and others. All of these reviews articulate a need to rethink our agriculture and forestry systems to provide solutions and help alleviate many important local, regional, national, and worldwide problems.

#### Managed conifer sawlog/grazing systems

In New Zealand and Australia forest managers are combining pasture management with open pine plantations. The purpose behind this integration is to diversify from animal husbandry to the more profitable forestry for wood export without losing the advantage of regular income potential provided by animal husbandry. These systems allow high light levels to reach the forest floor, resulting in heavy understory growth which can reduce stand access and increase fire hazard. The concept of deliberately managing this understory for profit by the grazing of animals has been developed into the full integration of agriculture and forestry.

Both animals and pastures may derive benefit from the presence of trees. Animals are able to maintain their body temperature with less energy loss in the modified climate associated with the open tree stands (Farnsworth, 1975). Through their deep root systems and litter fall, trees can tap moisture and cycle nutrients not available to surface-

rooted grasses. Herbaceous plants capable of fixing atmospheric nitrogen can be used to enhance soil fertility and increase the combined productivity of forest and grazing lands. The combination of the nitrogen fixation abilities of pasture legumes with the phosphate releasing powers of tree mycorrhizae may also benefit both trees and pastures (Knowles et al., 1973).

The initial interest in the agroforestry concept came from the New Zealand timber industries who quickly appreciated the advantages of early financial returns from agriculture, easier stand access, reduced fire risk, and simpler (though more closely planned and monitored) stand management. Conventional forestry in New Zealand is known to be a profitable alternative to agriculture but is often unattractive to farmers. Agriculturalists in New Zealand now acknowledge the role of tree crops in diversifying farm production, reducing market and biological risk factors, promoting soil stability, ameliorating microclimate, and making fuller use of farm labor during slack periods, all while maintaining acceptable stock carrying capacity (Tustin, et al., 1979).

A simulation model has been developed, SILMOD, to compare volumes and present net worth (PNW) of radiata pine at final stocking rates of 100, 200, and 400 stems per hectare. While a final stocking rate of 100 stems per hectare gave lower timber yields, the overall profitability was highest at this stocking rate. Results from the model

indicate a high degree of compatibility between agriculture and forestry in New Zealand hill country (Knowles and Percival, 1983).

In Australia, most of the agroforestry research is being conducted in the western part of the country (Borough, 1979). Additional research is ongoing at New South Wales, Victoria, Queensland, and Tasmania. Most of the research has involved plantations of radiata pine underplanted with subterranean clover (Trifolium subterraneum). Several of the trials include species of Eucalyptus (Batini, Anderson and Moore, 1983).

The advantages of agroforestry in Australia are similar to those found in New Zealand. However, in western Australia the greatest potential for agroforestry is viewed as the development of efficient systems for the control of stream salinity in catchment areas, and the control of soil salinity levels on farms (Anonymous, 1978).

In the upland areas of Great Britain there is a need to diversify production in order to improve farm vitality. While agroforestry has not been attempted in the uplands area, the National Farmers' Union of Scotland has recommended the development of a New Zealand type of system. The advantages of agroforestry over conventional forestry are considered to include intermediate returns, shorter rotations, and simpler management, and an end-product (timber) which can be sold when convenient to the farmer (MacBrayne, 1982).

Suggested species for agroforestry in Britain include Scotch pine (Pinus sylvestris), inland (U.S.A.) provenances of lodgepole pine (Pinus contorta) and western larch (Larix occidentalis). Light crowns, adaptation to dry, firm soils, and a deep rooting habit should enable species such as these to withstand stock trampling and resultant root damage. Conservatism is thought to be the biggest obstacle to the inception of agroforestry in Great Britain (MacBrayne, 1982).

In the southern U.S.A. the potential for combined production of timber, livestock, and wildlife is unequalled compared to any other region of equal size in the U.S. The region contains over 80 million hectares of forest land with roughly half considered useable as forest range for livestock (Shiflet, 1980). To date, few examples of successful integrated management exist in the southern U.S., but many situations involving damage from uncontrolled numbers of livestock with little or no management can be found (Pearson, 1983; Adams, 1975).

The key to success in multiple-use management is the maintenance of a careful balance between forage and animals. Grazed firebreaks, nine meters or more wide, are one practical way to integrate forest trees and improved pastures (Halls et al., 1960). Pearson (1982) reported on twenty years of research into cattle grazing, slash pine regeneration and growth, and economics. He found that southern pines appear highly resistant to grazing damage,

that cool season exotic grasses grown under pine stands can provide a source of green forage during the winter when native grasses are dormant, and that pine regeneration can be successful within grazed subclover pastures. The sum of the economic returns from multiple products such as food, fiber, and wildlife are expected to be larger than from a single output. Equally important, the increase in land management flexibility is a key factor in the survival of poor markets for any single output.

Major challenges affecting forest grazing management in the South include the careful planning and development of intensive systems of grazing management compatible with pine regeneration. Other challenges include the design of economical livestock supplemental feeding regimes including the use of improved forages for winter grazing, and the creation of an atmosphere of information exchange to attain social acceptance of multiple-use management (Pearson, 1983).

The objective for integrated land use in the interior regions of the northwest U.S. and southern British Columbia is to increase the sum total of production from all resources on each hectare of land. The Douglas-fir and ponderosa pine zones comprise the main multiple-use areas in this region. Livestock also graze other forest types within the region including grand fir (Abies grandis), western white pine (Pinus monticola), subalpine fir (Abies lasiocarpa), western larch and lodgepole pine. Grazing

management studies support the use of the interior Douglas-fir and Englemann spruce/subalpine fir zones for producing both trees and grass (Mclean, 1983). For integrated management in this region the most critical factors in determining tree-cattle compatibility are careful monitoring of the degree of forage utilization, and the length of time and season in which forage is utilized.

Results of a study using sheep as a silvicultural management tool in the coast range of Oregon, suggest that both brush suppression and acceptable levels of animal production are obtainable. This can be accomplished through the use of a grazing system of light to moderate utilization of clearcuts in the spring, followed by heavier use in areas targeted for brush reduction in the summer and fall. Under this system damage to Douglas-fir is expected to be minimal (Sharrow and Leininger, 1983).

#### Multicropping high value hardwoods with agricultural crops

The deliberate intercropping of agricultural crops with high value tree crops is a practice which can be traced back over 100 years to Burma. In a system which became known as "taungya", agricultural crops such as sweet potatoes, and cotton were interplanted with teak (Tectonia grandis). The function of the agricultural crop was to enable the local population to farm a piece of land and get the benefit of the crop in return for weeding and tending the teak in the critical early years of the rotation (Blanford, 1958).



The advantages of dual cropping include:

1. More intensive use is made of the land. The area between the tree crop, formerly kept free of competition by cultivation or herbicides, is now made use of by an agricultural crop.
2. More acres of high quality land, often closer to processing facilities and markets, can be brought into fiber production.
3. Early returns from the agricultural crop will offset all or part of the establishment costs for the tree crop, greatly improving the return on the investment.
4. The benefits to the agricultural crop derived from tillage, fertilizer and weeding also benefit the tree crop.

The two genera which have received the majority of attention in this type of system are Populus spp., valuable for rapid growth in short rotations and used mainly for pulp, and Juglans nigra, the high value black walnut, grown on long rotations for sawlogs and veneer.

In Italy, a system of tillage and intercropping during the first four years after the establishment of the tree crop, is commonly practiced in conjunction with poplar stands planted at a 6 m x 6 m spacing. Maize is produced the first year, and legumes and grain crops are grown for the next three years. Poplar stands with both tillage and intercropping yield a higher economic return than stands with tillage alone (Sekawin and Prevosto, 1973).

In Australia, a sequential combination of vegetable cropping and grazing is being used in conjunction with widely spaced poplars (6 m x 6 m). In the first two years vine crops such as melon and squash are planted. The vines provide a quick crop and cover the ground to restrict weed

growth. At the end of the second year, permanent pasture is sown beneath the poplars, and cattle are then grazed within the plantation. The prunings from the poplar are used for cattle feed (Anonymous, 1978).

The Crown Zellerbach Corporation is also experimenting with intercropping in their cottonwood (Populus deltoides) plantations on the Mississippi delta. Soybeans and cotton are interplanted between rows of cottonwood and are heavily fertilized. The cottonwood is harvested for pulp on 10 year rotations averaging 23 cm in diameter (dbh) and 25 m in height (Pers. comm. P. Weber, 1983).

In northern Alabama, tree growth in a two-year-old sycamore (Platanus occidentalis) plantation significantly increased during four years in which clover and vetch were grown within the trees (Haines, Haines, and White, 1978).

Dual cropping with Populus is also being practiced in Ontario. Corn (Zea mays) and soybeans (Glycine soja) are being cropped between rows of planted hybrid poplars on Indian reserves (Mergen and Lai, 1982). In eastern Ontario, corn and potatoes (Solanum tuberosum) have been grown successfully between 3 m x 6 m spaced poplar during the first three years of the rotation. Potato yields of 12,000 kg/ha have been obtained in the second year of the rotation. Many other crops have also been successfully grown in the first three years of the project (Raitanen, 1978).

Researchers in the United States Forest Service at Carbondale, Illinois, are currently involved in attempts to

find the proper cover crops to interplant with black walnut in order to enhance the growth of the walnut, utilize the cover crop for hay or fodder, and reduce the use of herbicides and other cultural methods used to control weed competition around the trees. A silvicultural-economic model constructed by Kincaid et al. (1982) indicates that the degree of profitability from an investment in the production of black walnut is directly related to the level of management intensity. The model considered five different management regimes ranging from walnut timber alone, to a multi-crop management system of timber, nuts, soybeans, winter wheat, fescue and grazing. Intercropping with field crops yielded the highest economic returns on the highest quality site (SI-80), while management regimes using forage crops yielded the highest economic returns on sites of intermediate quality (SI-65). Early returns from agricultural production offset the higher initial cost of walnut establishment and yielded a substantial increase in profit. The analysis concluded that multi-crop management offers the greatest returns due to more intensive land use.

Roth and Mitchell (1982) studied the effects of selected cover crops on the growth of black walnut. It was determined that clean cultivated black walnut was significantly larger at age six than walnut growing in any mixed planting system. However, they concluded that the energy necessary to maintain the clean cultivation coupled with the increased potential for soil erosion makes the

clean cultivation system less desirable for long term management.

Underplanting black walnut with leguminous or grass covers can effect positive changes on the phenology of the walnut. Compared with clean cultivation, the maintenance of leguminous or grass covers in walnut plantations may delay bud break 6-12 days, thereby decreasing possible frost damage. Also, underplanting walnut with leguminous winter annuals was found to accelerate the onset of dormancy (Van Sambeek and Rink, 1982).

Van Sambeek and Rietveld (1982) co-established plots of black walnut with leguminous cover crops. Their results show that the seeding of plantations with cool season legumes, both with and without chemical weed control around the walnut seedlings, can accelerate tree establishment and tree growth in intensively managed plantations. This indicates that the planned establishment of leguminous cover crops may be superior to allowing plantations to revegetate naturally.

#### Systems of woody/woody intercropping with N<sub>2</sub> fixing woody plants

Another agroforestry system which has recently been receiving attention consists of intercropping between woody species to maximize overall yields from a given site. In all cases, one of the intercropped species will be a symbiotic nitrogen fixer. With the exception of black locust (Robinia psuedoacacia), tree lupine (Lupinus

arboreus), and a handful of other temperate zone species, most leguminous trees and shrubs are located in the tropics (Allen, Gregory and Allen, 1955). The group of nitrogen fixers considered to have the greatest potential in the temperate zone are non-leguminous actinorhizal woody perennials. Most of these plants occur in temperate regions or in the highland tropics (Dawson, 1983).

Intercropping systems may be either simultaneous or sequential (rotated) cropping systems. The concept of "intercropping vigor", in which dry matter production of mixed cultures exceeds that of pure plantings is now receiving serious attention in the U.S. Good reviews can be found in Tarrant and Trappe (1971), Gordon and Dawson (1979), and Dawson (1983).

The major benefits derived from the use of woody nitrogen fixers include the realization of optimum biomass yields per unit of land area, a reduction or elimination of the need for applied nitrogen fertilizer, improvement in soil fertility and soil physical properties, suppression of soil pathogens, and the improved growth of associated species in mixed cropping systems (Tarrant and Trappe, 1971).

The awareness that intercropping with nitrogen fixing trees will benefit the associated woody crop is not a new one. The beneficial effects of black locust on the growth of associated tree species has been observed on many occasions. Ferguson (1922) and McIntyre and Jeffries

(1932) presented evidence that catalpa (Catalpa speciosa) growing in association with the black locust showed increased diameter and height growth. Chapman (1935) reported that the heights and diameters of certain trees decreased significantly with increased distance from black locust stands. Chapman and Lane (1951) made a study of the growth and survival of hardwood trees growing in association with black locust, shortleaf pine (Pinus echinata) and sassafras (Sassafras albidum). The best survival and growth rates occurred in the association with black locust. Finn (1953) found that yellow poplar (Liriodendron tulipifera), black walnut and black cherry (Prunus serotina) showed a significant increase in both height growth and total foliar nitrogen when interplanted in black locust stands.

Ten year results of a study using European black alder (Alnus glutinosa) as a nurse crop on mine spoils in Kentucky showed that height and diameter growth of hardwoods and pines are accelerated when interplanted at appropriate spacings with European black alder. Nitrogen fixation by European black alder increased foliar nitrogen of the interplanted species (Plass, 1977).

Long rotation mixed cropping systems using nitrogen fixing trees as nurse plants usually require that the nurse crop must be harvested, poisoned, or removed before the final harvest of the timber crop. However, the benefits may outweigh this inconvenience. Funk et al., (1979) found that mixed plantings of the nitrogen fixing tree autumn

olive (Elaeagnus umbellata) stimulated the growth of black walnut. After 10 years, walnut trees grown with autumn olive were 80% taller and 104% larger in diameter than those grown alone. Additionally, the mixed plots were higher in soil nitrogen, lower in soil moisture, and had lower soil and air temperatures.

In a study of a 27 year old Douglas-fir/red alder (Alnus rubra) admixture, Tarrant (1961) found that in addition to increased height growth, the form of the Douglas-fir trees was improved. Total wood volume in the mixed plantation was more than twice that of pure Douglas-fir plantations. Atkinson et al. (1979) examined the feasibility of using red alder as a rotation crop with Douglas-fir. Four sequential cropping systems were analyzed for comparison to a system of continuous cropping of pure Douglas-fir. A net-worth analysis indicated that all the systems are profitable, though systems involving red alder were not as promising as those involving only Douglas-fir. He concluded that expanded markets for red alder, increased efficiency of small tree harvesting, or higher costs of nitrogen fertilizer could tip the balance in favor of alternate cropping systems.

Binkley (1983) studied pure and mixed natural stands of Douglas-fir and Douglas-fir/red alder on sites of high and low fertility. Compared to the pure stand, the presence of red alder on the low fertility site (SI-25 m) increased the average diameter of Douglas-fir. Inclusion of alder biomass

increased the total stand basal area and basal area growth 2.5 fold. Total ecosystem biomass doubled and net primary production tripled when alder biomass was included. In contrast, on the high fertility site (SI-45 m) total ecosystem values were identical between pure and mixed stands, and Douglas-fir biomass and net primary production decreased. He concluded that admixtures of red alder and Douglas-fir have great potential for increasing Douglas-fir growth and ecosystem production on infertile, nitrogen deficient, marginal sites, but have little value on fertile, nitrogen rich sites.

Another intercropping system with great potential is one in which nitrogen fixing shrubs are used as nurse crop plants in the early years of a fiber or timber rotation. Perceived silvicultural advantages to the use of nitrogen fixing shrubs include: 1) Elimination of the problem of nurse crops overtopping the main crop; 2) Elimination of competition for the same area of the canopy (photosynthetic surface); 3) Shrubs never need to be removed, harvested or poisoned; and 4) They may be suitable as fodder for grazing animals.

Harrington and Deal (1982) have recently advocated the use of Sitka alder (Alnus sinuata) as a nitrogen fixing shrub for use on sites of low nitrogen or organic matter content. The early slowdown in height growth, coupled with its low profile, makes the Sitka alder suitable for use with Douglas-fir in mixed stands.



A prime candidate for biological forest fertilization in the southeastern Coastal Plain (USA) is wax myrtle (Myrica cerifera). This naturally occurring shrub is capable of growing well on acid soils in the understory of pine flatwoods. In a study of nitrogen fixation in slash pine plantations, wax myrtle was shown to fix substantial amounts of nitrogen. It is believed that the use of a substantial wax myrtle understory could contribute a significant amount of additional nitrogen to semi-mature slash pine stands (Premar and Fisher, 1983).

Another study compared the growth of pitch (Pinus rigida) and Japanese black (Pinus thunbergii) pines in association with clumps of the nitrogen fixing shrub bayberry (Myrica pennsylvanica). Significantly greater height growth within bayberry patches occurred only in the young pitch pines (Tiffney and Barrera, 1979).

Marrs et al. (1982), studied tree lupine, (Lupinus arboreus), as a nurse crop. Their results indicate that tree lupine could be a very valuable nurse crop for amenity plantings or on marginal lands where the nitrogen status of the soil is low. Advantages of using tree lupine as a nurse crop include rapid establishment and growth, and its natural tendency to die back after 5-7 years, thereby eliminating long term site competition and overtopping problems.

The final intercropping system to be considered consists of short rotation, intensive culture systems for energy, chemical feedstocks, or animal feedstocks, in which

nitrogen fixing trees may be used as an equal component of the final harvest. Mixed plantations of alders and poplars that take advantage of nitrogen-fixing trees are among the most intensively studied silvicultural systems. Both alders and poplars have wood properties which have proven to be acceptable for chip and fiber products (Dawson, 1983).

Hansen and Dawson (1982) demonstrated that the height of 3-year-old hybrid poplar grown in short rotation intensive culture increased significantly with increasing alder (Alnus glutinosa) in the mixtures. Hybrid poplar heights in short rotation intensive culture mixtures containing the highest percentages of alder, were found to be comparable to those obtained from optimal rates of ammonium nitrate fertilization tested on an adjacent plot of pure hybrid poplar.

De Bell and Radwan (1979) found that annual dry matter production in mixed plantings of 2-year-old coppiced black cottonwood (Populus trichocarpa)/red alder was higher than production in pure cultures of cottonwood and alder.

#### CONCLUSIONS

The main benefits which can be derived from the use of agroforestry systems includes: 1) Socio-economic benefits from revitalization of rural areas; 2) Diversification of income sources through risk spreading; 3) Full, productive use of marginal lands; and 4) High quality lands can be brought to their maximum productive capacity.

Agroforestry is a complex applied science requiring knowledge of the environment, agriculture, forestry, horticulture, animal husbandry, and local socio-economic and cultural conditions. Although much is known about the components individually, relatively little is known about the interaction between them. There is a need for basic information on all aspects of agroforestry technologies. This includes a systematic compilation of knowledge on agroforestry systems as well as the development of objective methods to evaluate the systems (Lundgren, 1982).

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## Chapter IV

### Honeylocust (Gleditsia triacanthos L.): Important Chemical Characteristics and Cultural Systems for Use in Agroforestry Systems

#### ABSTRACT

The historical development of honeylocust from an unimportant, minor forest associate to a potentially valuable multi-purpose tree crop for agroforestry systems is reviewed. Various management scenarios for its use are suggested, both for industrialized countries, as well as third world nations. Proposed uses include; 1) As a component in multi-purpose shelterbelt systems; 2) As a perennial crop tree for marginal lands; 3) For use in watershed management systems and for erosion control; 4) In two-tier multi-cropping systems; and 5) In ultra short rotation intensive culture systems.

Results of chemical analyses on pod sugars and seed and leaf proteins are reported. Total pod sugar content varied from 13.6 to 30.9 percent. Seed protein content varied from 16.6 to 27.8 percent. Leaf protein content ranged from 13.6 to 28.9 percent. The variation patterns in leaf protein, seed protein, and pod sugars are random with no particular provenance or region being especially high in any given

trait. The use of yield components is discussed in relation to breeding strategies for maximizing sugar and protein yields.

Two general categories of natural pod phenotypes are described. Pods from northern regions can be characterized as having a papery pericarp fraction, minimal amounts of carbohydrate pulp, and consistently high seed sets. Pods from southern regions have a pulp-filled pericarp fraction, very poor seed sets, with seed chambers often filled with carbohydrate pulp.

Results of two cultural studies on preemergent herbicides and spacings are reported. Ultra short rotation intensive culture systems for growing honeylocust can be successfully accomplished by direct-seeding, followed immediately by application of the preemergent herbicide DCPA (dimethyltetrachloroterephthalate) with no harmful effects on germination of the seeds. Planting direct-seeded honeylocust at three different spacings showed that a spacing of 10 x 15 cm gave the highest biomass yields in the first year after planting.

#### INTRODUCTION

Honeylocust, Gleditsia triacanthos L., is a multi-purpose tree which has potential for use in numerous management scenarios and in many diverse locations throughout the world. A closer look at its potential uses points to the significant role which multi-purpose trees may have as components of agroforestry systems. These uses

include a variety of chemical and animal feedstocks from the pods, high protein food supplements and industrial gums from the seeds, animal fodder/green manure from the leaves, and high caloric value fuelwood. The added values of multi-tiered cropping systems, watershed management and erosion control, and fuller marginal land use must also be considered.

Honeylocust has been advocated as a multiple-purpose crop tree for shelterbelts in the Great Plains (Bagley, 1976), and is suggested for similar use in the province of Heilongjiang in north-eastern China (Pers. comm. Jeff Gritzner, 1983). Because it can provide a source of fodder, protein, energy, and erosion control, honeylocust appears to be the most promising candidate for use as a staple perennial crop tree for marginal land in southern Appalachia (Williams, 1982). For these same reasons and because of its apparent high value leaf fodder, additional interest in testing the honeylocust exists in the Himalayan foothills of India (Pers. comm. P.K. Khosla, 1983) and other areas of the highland tropics (N.A.S., 1983). As a component in a multiple-use integrated farm system it may have value in much of the eastern U.S. (MacDaniels and Lieberman, 1979; Bagley, 1981), New Zealand (Davies and MacFarlane, 1979) and Australia. In Australia honeylocust is being promoted and marketed as a fodder tree for livestock, windbreaks, shade, erosion control and fence posts (Anonymous, 1982).

Other multi-purpose trees which have potential for use in integrated systems include black walnut (Juglans nigra) (Kincaid, et al., 1982), hybrid poplars (Populus spp.) (Lora, and Wayman, 1979; Raitenan, 1978), and a host of other leguminous and non-leguminous nitrogen fixing trees such as the alders (Alnus spp.) (Tarrant and Trappe, 1971; Gordon and Dawson, 1979), black locust (Robinia psuedoacacia) (Keresztesi, 1983), carob (Ceratonia siliqua) (Coit, 1951; Merwin, 1980), and the mesquites (Prosopis spp.) (Parker, 1982). One would be remiss if mention was not made of the genus which has received more attention than all of the others combined, Leucaena. A recent bibliography compiled by the USDA contains over 2,000 citations on Leucaena (Oakes, 1982; Oakes, 1983) covering every imaginable topic from adaptation, to livestock, and utilization. Additionally, the National Academy of Sciences (Anon., 1977) devoted an entire publication to Leucaena, and there are now two journals, Leucaena Research Reports and Leucaena Forum, specifically dedicated to publishing results of Leucaena research.

Two different cultural systems of use are currently envisioned for honeylocust. One system entails the use of a widely spaced, two-tiered orchard with a variety of forage, vegetable or woody crops grown beneath the trees. In addition to the use of the pods for ethanol and stillage for animal feed, and use of the seeds as protein supplements and industrial gums, the return from the annual crops can be

used to increase the overall economic stability and viability of the enterprise. A second system involves growing direct-seeded honeylocust on ultra-short rotations, and at very close spacings, for annual harvest(s) as a chemical or animal feedstock.

#### SILVICULTURE AND GENETICS

Within the natural range of the honeylocust a large amount of variation exists in both climatic and edaphic conditions. The native range extends from central Pennsylvania west to southeastern South Dakota-Northcentral Nebraska, south to central Texas, east along the Gulf to Georgia, and north to Pennsylvania (USDA, 1965). The average annual precipitation within the natural range varies from 500 mm in S. Dakota-Nebraska to over 1800 mm in North Carolina. The frost free period varies from a low of 140 days in the north western extremes to a maximum of over 340 days in southern Louisiana (USDA, 1941). The honeylocust achieves its best growth on fertile, moist, alluvial floodplains, but will also grow on soils of limestone origin and is resistant to both drought and salinity (Howell, 1939).

Results of a study on the genetic variation in growth, phenology and winter-hardiness indicate that a large amount of genetic variation is present among and within regions for all traits analyzed (Gold and Hanover, 1984). In fact, in many of the traits studied, the range of variation is so large that it has proven to be a mixed blessing. Use of

unselected material has given honeylocust an undeservedly bad reputation in some areas (Mostert and Donaldson, 1960), while use of selected sources has led to high expectations in others (Moore, 1948). To maximize the potential benefits of growing honeylocust several factors must be taken into consideration. These include the close matching of ecological requirements to various locations throughout the world, the utilization of the most appropriate ideotypes for each intended use, and careful selection and breeding to develop superior varieties.

#### HISTORICAL BACKGROUND

By early 1900 honeylocust was being touted as a perennial forage tree for the eastern U.S. (Smith, 1914). A detailed chemical analysis of honeylocust pods was first conducted by Walton (1923). The agroforestry potential of honeylocust received broader recognition after Smith (1929), illustrated the potential use of numerous tree crop species in developing a permanent agricultural system for marginal, hilly, and eroded lands. Honeylocust was included in the tree crops/hillculture projects at the Tennessee Valley Authority (TVA), Virginia Polytechnic Institute, and Alabama Polytechnic Institute, which were initiated in 1934 (Hershey, 1936; Zarger, 1956). The honeylocust projects lasted only 14 years, from 1934-1947. Within that brief span of time great strides were made in converting honeylocust from a minor forest component of no commercial



value, into a potentially valuable multi-purpose cropping tree.

A brief review of the TVA'S accomplishments include the location of wild selections of honeylocust with a total pod sugar content exceeding 35 per cent (Detwiler, 1947); development of a technique for propagating thornless trees by the careful selection of scionwood from thorny parent trees (Chase, 1947); vegetative propagation of superior clones followed by the establishment of grafted orchards of these clones (Stoutemeyer et al., 1944; Moore, 1948; Zarger, intercropped pasture tree (Moore, 1948; Zarger 1956); and a determination of the feed value of honeylocust pods through chemical analysis and animal feeding trials (Atkins, 1942; Moore, 1948).

An abrupt change in national priorities following World War II terminated all research efforts on honeylocust except for the maintenance of an archive of superior clones at Norris, Tenn. (Moore, 1948; Zarger, 1956; Scanlon, 1980). A summary of correspondence, general information and specific research results involving honeylocust up to the mid-1940's is available (Detwiler, 1947). An excellent review of all honeylocust research conducted at the TVA can be found in Scanlon (1980). By the late 1940's, interest in honeylocust was also evident as far away as Australia (Eardley, 1945), South Africa (Loock, 1947; Jurriaanse, 1973), and Malawi (Douglas, 1967).

In depth studies on the identification of chemical constituents have provided basic information on the overall chemical composition of honeylocust (Wealth, 1956; Watt and Breyer-Brandwick, 1962). Felker and Bandurski (1976), and Becker (Pers. comm., 1982) analyzed the seed protein and amino acid content. Baertsche (1980) studied the animal feedstock value of intensively cultured seedlings. Walton (1923), National Academy of Sciences (Anon., 1971) and Scanlon (1980) reported on the sugar content of the pods. Each of these studies have focused on a thorough analysis of one or a few individual trees.

Building on these previous studies, and using materials obtained by a rangewide collection of honeylocust germplasm, the approach taken in this study was to identify the range of variation present in useful chemical constituents, namely seed protein content, leaf protein content, and total pod sugar content. Morphological measurements of pods and seeds were determined as a basis for future study of yield components, and for selection and breeding towards development of diverse ideotypes. Finally, results of two cultural studies are presented which will help to lay the groundwork for further research on closely spaced short rotation intensive culture (SRIC) honeylocust for animal and chemical feedstocks.

## MATERIALS AND METHODS

A rangewide collection of honeylocust germplasm was undertaken in the fall of 1979. By February 1980, over 450 accessions had been received, covering a majority of the natural and naturalized range. Collection details and results of a study on the genetic variation in 2-year old honeylocust seedlings are reported elsewhere (Gold and Hanover, 1984). Upon receipt, each collection was stored at 1-4°C. Ten pods from each accession were chosen for further morphological measurements and analysis.

Morphological data

Morphological measurements on the pods included length, width, thickness and weight (oven dry). Pod pubescence was scored on a scale from 0, representing total absence, to 4, representing heavy pubescence. Pubescence was scored to test for possible resistance to Amblycerus robiniae, the bruchid seed weevil, which can severely damage a seed crop.

The total number of seeds per pod was recorded for each source, and these were divided into the number of sound seed and number of insect damaged seed. When possible, seed weight was determined as an average weight of 100 seeds per accession (fresh weight). Seed length, width, and thickness were determined as an average of 10 seeds using a dial guage accurate to 0.0005 millimeter. Seed volume was determined by water displacement.

### Seed protein

Following seed extraction and measurement, seed from 200 sources were analyzed for total protein nitrogen (N) on a whole seed basis. Ten seeds per source were used for N determinations. Prior to analysis, seeds were dried for 48 hours at 50°C. in a convection oven. Each group of 10 seeds was run in two lots of 5 and analyzed in duplicate. Samples were analyzed for total protein N according to the method of Wall and Gehrke (1975). A 40 sample block digester and Technicon Autoanalyzer were used for determination of ammonia nitrogen. Protein was calculated as N x 6.25.

### Pod sugars

Subsequent to completion of morphological measurements on pods and seeds, a subset of 79 sources were frozen at -38°C prior to sugar analyses. Each sample was a composite of 10 pods per source. Pod samples were dried for 24 hours at 50°C in a convection oven and then passed through a Wiley mill with a 20 mesh screen. Reducing and non-reducing sugars were analyzed according to the method of Nelson (1944).

### Leaf proteins

Leaf samples, consisting of recently matured leaves from the upper crown, were collected from the field at the East Lansing test site on August 1<sup>st</sup> and 2<sup>nd</sup> 1982, using the sampling method of Jones, Large, Pflieveder and Klosky (1971). Eighty four sources, representing the entire range

of honeylocust, were chosen for analysis. Two plots per source were sampled and each sample consisted of a bulk of 4 trees within each plot. Immediately following harvest, samples were dried for 24 hours in a convection oven at 65° C and then ground in a Wiley mill through 20 mesh screen. Each sample was analyzed for total protein N employing the same technique used in seed protein analysis (see above). Protein was calculated as N x 6.25.

#### Herbicide study

A weed-free seedbed was prepared at the Michigan State University Tree Research Center, located at E. Lansing, Mi. A 1.5 percent solution of the postemergent herbicide glyphosate (Roundup) was applied to kill existing vegetation. Seven days later the test plot was tilled.

Honeylocust seeds were scarified for 60 minutes in concentrated sulfuric acid ( $H_2SO_4$ ). Scarified seed were planted in a sandy-loam soil at a depth of 1.2 centimeters. Treatments consisted of 25 seeds sown linearly at 20 cm intervals, with three replications of each treatment in a randomized block design. Two preemergent herbicide treatments were tested, DCPA (dimethyltetrachloroterephthalate or Dacthol) at a rate of 9.0 kg active ingredient (ai) per hectare (ha), EPTC (5-ethyldipropylthiocarbamate or Eptam) at a rate of 6.5 kg a.i. per ha., against a control of no herbicide application.

Selection of herbicides and rates of application were chosen based on availability and for comparison with results reported by Warmund, Long, and Geyer (1980), who tested the effects of 10 preemergent herbicides on the germination of honeylocust, black locust, and Kentucky Coffee tree seeds (Gymnocladus dioicus) grown in nursery containers.

Herbicide treatments were applied 24 hours after the seeds were sown. In order to prevent volatilization of EPTC, the plots were irrigated immediately following herbicide application, and at regular intervals for the next 75 days to provide optimal moisture conditions. Germination data were recorded 21 days after herbicide application. Seedling survival and weed control were monitored for 75 days.

#### Biomass yield study

A direct-seed, spacing study was initiated at the M.S.U. Tree Research Center nursery on May 13, 1982. The 18 m x 17 m test site was treated with glyphosate (Roundup), a postemergent herbicide, for removal of existing vegetation. A week later, the test site was tilled. Soil conditions in the nursery at the time of planting were: sandy-loam, pH 6.5, 4% OM, 168 kg available P per ha., and 78 kg available K per ha. Based on results of soil analyses, the test plot was fertilized with one pound of granular 12-12-12, and then raked into the soil. Annual precipitation at the site averages 800mm.

Seed were scarified (as above) on May 20. Following scarification the seeds were soaked in water overnight and the imbibed seeds were sown the following day.

A randomized block design, with 3 blocks and 3 treatments per block, was used to test the effects of different interrow spacings on the biomass yield of direct-seeded honeylocust. Each block was 5.0 m x 5.0 m in size and contained 3 spacing treatments 1 m x 4.5 m in size. Spacing treatment I consisted of 6 rows, spaced at 15 cm intervals, treatment II contained 3 rows spaced 30 cm apart, and treatment III had 2 rows, spaced 45 cm apart. Seeds were spaced at 10 cm intervals within rows in all treatments.

Unfortunately, allowing the seed to imbibe before planting proved disastrous as the seed rotted in the soil and less than 1% germination occurred within the next 21 days. The experiment was repeated beginning on June 16 when the plots were retilled without further herbicide application. Another set of seeds was scarified on June 17, and then sown immediately. Lack of seed caused the elimination of one block on the replanting. Due to the late sowing date, harvest of the seedlings was postponed until the following summer. Hand cultivation was used to control weeds from the time of germination until the plots were harvested.

On August 1, 1983 the 13 month old seedlings were mechanically harvested with a forage harvester. Fresh

weights were recorded for all treatments and 1000 g samples were taken from each treatment for determination of moisture content.

## RESULTS AND DISCUSSION

### Multi-cropping

Total sugar content of the honeylocust pods ranged from 13.6 to 30.9 percent (Table 4.1). Compared with selections located by the TVA, there were no exceptionally high individual tree values for pod sugar content. The sources chosen for total pod sugar analyses represented a cross section of the natural range. Total sugar content is negatively correlated with latitude ( $r = -0.52$ ), the percent pericarp (non-seed) fraction is negatively correlated with latitude ( $r = -0.46$ ), and grams of total sugar per pod (pericarp fraction only) is also negatively correlated with latitude ( $r = -0.41$ ). These correlations indicate that southern sources contain higher levels of total sugar as well as higher percentages of pericarp fraction. Based on these data, regional selection for sources with the highest total sugar content (expressed in grams of sugar per pod) would initially focus on southern sources. Within the southern region, selection would have to be on an individual tree basis due to the very large amount of within-region variation (Table 4.2).



Table 4.1 Variation in morphological and chemical traits of honeylocust parent trees from subsampled rangewide test.

<u>Trait</u>	<u>Mean</u>	<u>Min.</u>	<u>Max.</u>	<u>s.d.</u>
	- - - - Pod - - - -			
<b>Morphological</b>				
Total pod weight (g)	9.3	2.1	22.1	3.5
<sup>1</sup> Weight pericarp fraction only (g)	7.3	3.0	21.4	3.1
<sup>2</sup> Pericarp fraction (%)	73.6	45.4	97.6	11.4
Pod length (cm)	29.0	17.9	41.3	5.0
Pod width (cm)	2.9	1.9	4.7	0.60
Pod thickness (cm)	0.3	0.08	0.65	0.13
<b>Chemical</b>				
Total sugars (%)	22.4	13.6	30.9	3.7
Nonreducing sugars (%)	19.8	12.5	28.2	3.3
Reducing sugars (%)	2.7	0.68	5.0	1.1
<sup>3</sup> Total sugars per pod (g)	1.6	0.41	6.6	0.26
<sup>3</sup> Nonreducing sugars per pod (g)	1.4	0.38	6.0	0.23
<sup>3</sup> Reducing sugars per pod (g)	0.2	0.02	1.1	0.03
	- - - - Seed - - - -			
<b>Morphological</b>				
Total number of seeds per pod	13.5	1.5	30.0	5.4
Weight seed fraction (g)	0.19	0.07	0.30	0.04
Total seed weight per pod (g)	2.5	0.26	5.3	1.1
<sup>4</sup> Seed fraction (%)	26.4	2.4	54.6	11.4
Sound seed per pod	10.3	0.00	25.8	5.0
Damaged seed per pod	3.2	0.00	16.3	3.1
<sup>5</sup> Sound seed (%)	58.2	0.00	100.0	29.7
Seed length (mm)	4.0	3.3	5.1	0.32
Seed width (mm)	2.5	2.0	3.2	0.21
Seed thickness (mm)	1.5	1.1	2.1	0.17
Seed volume (ml)	0.14	0.60	0.20	0.03
Seeds per pound	2,389	6,486	1,513	--
<b>Chemical</b>				
Seed protein (%)	21.6	16.6	27.8	2.0
<sup>6</sup> Seed protein per pod (g)	0.53	0.05	1.05	0.23
1-Pericarp fraction (g) = total pod weight - total seed weight.				
2-Pericarp fraction (%) = (pod weight only/total pod weight) * 100.				
3-Total sugars per pod (g) = (total sugars %/100) * weight pericarp fraction.				
3-The same calculation was used in the determination of grams of reducing and nonreducing sugars.				
4-Seed weight (%) = (total seed weight/total pod weight) * 100.				
5-Sound seed (%) is a measure of damage by seed weevils.				
6-Seed protein per pod = (seed protein %/100) * seed weight.				

Table 4.2 Means and range variation in honeylocust pod sugar content of seed sources among and within regions 1/.

<u>Region 2/</u>		<u>Mean</u>	<u>Minimum</u>	<u>Maximum</u>
SOUTHERN +	SE	23.9	17.7	30.9
	SW	24.2	21.9	27.8
	EC	24.4	17.7	30.6
	<u>EC</u>	<u>24.2</u>		
NORTHERN +	LS	19.1	13.6	24.4
	NW	20.1	16.0	24.9
	WC	19.9	14.5	22.5
	<u>WC</u>	<u>19.7</u>		

1/ Regions are discussed in more detail in Chapter 2 (Gold and Hanover, 1984).

2/ SE,SW,EC,LS,NW,WC represent southeast, southwest, east-central, Lake States, northwest, and west-central regions, respectively.

Differences in sugar content have been documented for trees from the same clone grown in different locations (Scanlon, 1980). Pods from the "Millwood" clone, a selection located by the TVA during its involvement in tree crops research, contained 36.8 percent total sugar when grown in Alabama and 21 percent total sugar when grown in Maryland (Detwiler, 1947). This points to the necessity for testing honeylocust in many diverse locations in order to accurately estimate its performance.

Two general categories of natural pod phenotypes can be described. Pods from northern regions can be characterized as having a papery pericarp fraction, with minimal amounts of carbohydrate pulp, accounting for approximately 50 percent of the total pod weight, and consistently high seed sets. Pods from southern regions have a pulp-filled

pericarp fraction accounting for 75 to 95 percent of total pod weight, and frequently have very poor seed sets. The seed chambers are often filled with carbohydrate pulp.

Crop yields of honeylocust pods, and maximum amounts of extractable carbohydrates on a per tree basis, are two of the important factors to be considered when assessing its cropping potential for chemical and animal feedstocks in two-tier integrated farming systems. The actual pod sugar content (fermentable carbohydrates) per pod is secondary in importance. Although reliable crop data from plantations of honeylocust are scarce, the best available data comes from a cooperative TVA-Auburn University study. A grafted plantation of the superior "Millwood" selection yielded an average of 33 kgs. (dry wt.) per tree between the ages of five and nine. However, due to the biennial bearing habit this average obscures that fact that 9 year old trees produced an average of 82 kgs. (dry wt.) per tree (Moore, 1948). Based on these yield figures, at a 40' x 40' spacing (28 trees to the acre) honeylocust produced over two-and-a-half tons of pods per acre. The average crop of 33 kgs. would yield almost one megagram per acre (dry wt.).

### Seed proteins

Results of analyses for seed protein content among 200 honeylocust sources show a wide range of variation (Table 4.1). Seed protein levels vary from 16.6 to 27.8 percent, with a mean of 21.6. Protein was determined on a

whole seed basis (calculated as  $N \times 6.25$ ). When trying to select for sources which contain maximum overall levels of seed protein, many factors must be considered. Total pod yield per tree, number of seeds per pod, seed weight, and seed size are all potentially important yield components which contribute to maximal seed protein yields per tree.

In order to maximize seed protein production, the most important yield component is the total pod yield per tree. Because most of the collections used in this rangewide study were obtained by mail, an accurate assessment of this component will not be possible until field planted sources begin to flower and fruit. Other yield components were studied which affect total seed protein content on a per pod basis.

Total seed protein yield per pod is determined by the total number of seeds per pod, the total weight of seeds per pod, individual seed size, seed weight and seed protein content. Little relationship was found when correlations between seed weight, seed size, or seed volume were run with seed protein content. Thus, the size, shape, and weight of the seed appears to be totally independent of seed protein content. Also, no relationship was found between protein content per seed, and total seed protein content per pod.

The seed fraction of the pod is negatively correlated with pod weight ( $r = -0.30$ ). Pods from northern latitudes have a higher ratio of seed fraction to pericarp fraction than pods from trees in southern latitudes. In northern

sources, the pericarp consists mainly of a papery exocarp with a minimal amount of carbohydrate filled mesocarp.

In light of these data, variation in total seed protein content per pod appears to be due to the remaining components, i.e. the total number of seed per pod, and individual seed weights. Correlation analyses show that the total number of seeds per pod accounts for 67 percent of the variation in total seed protein content. Individual seed weight accounts for an additional 17 percent of the variation. Based on the results to date, high seed yields are more important to total seed protein yields than individual seed protein content. In the longer term, simultaneous selection for a combination of yield components will be desired to maximize pod yields, seed yields, seed protein content and seed protein quality.

Due to the hard, impermeable seedcoat of the honeylocust seed (Heit, 1942) it has been reported that the unbroken seed would pass directly through the digestive tract of animals such as sheep in which case the protein value of the seeds would be lost (LeRoux, 1959; Mostert and Donaldson, 1960). In a recent study sheep were fed broken, uncrushed pods, with seeds intact (Small, 1983). Results indicated that sheep can digest the whole seed whether consumed alone or in the pods, and that at least 75-90% of the whole seeds were digested. Data from the N.A.S. (Anon., 1971) indicate that 66% of the protein in ground seeds and pods is digestible.

Leaf proteins

Results of crude leaf protein analysis of leaves from 84 different half-sib families, revealed a large amount of variation (Table 4.3). Baertsche (1980) reported a "late harvest" figure of 20.23 percent crude protein for greenhouse grown honeylocust seedlings. Field results of the rangewide analysis of crude protein show that Baertsche's value falls very close to the overall mean for crude leaf protein content, 20.05 percent.

Correlations between leaf protein content and latitude or longitude were nonsignificant. Variation within regions is so large that selection will only be effective at the family and within-family levels (Table 4.3). Based on these preliminary field results, selection for significantly higher levels of leaf protein may be feasible and current data indicate improvements up to 45 percent over the population mean through family selection alone.

Table 4.3 Variation in leaf protein content among geographic regions 1/.

Region	Mean	Minimum	Maximum
SE	20.4	13.6	27.4
EC	20.5	14.0	26.7
LS	20.3	14.7	28.9
NW	20.6	14.1	24.5
WC	18.1	15.0	19.7
SW	20.1	14.8	24.5
Overall mean	20.05	13.6	28.9

1/ Regions are more fully described in Chapter 2 (Gold and Hanover, 1984).

Results of pod sugar and seed/leaf protein analyses indicate that significant improvements in the chemical properties of honeylocust are attainable through selection of appropriate families and/or individuals. Cultural systems will also require further development in order to make SRIC systems economically viable for animal and/or chemical feedstock production.

#### Cultural systems

Results of the ANOVA support the findings of Warmund et al. (1980) that herbicide application one day after planting is a possible alternative to hand-weeding in nurseries. (Table 4.4). Germination of honeylocust seed in DCPA treated plots was not significantly different than the control. Application of EPTC, recommended for use in alfalfa (Medicago sativa) (Tesar, 1980), proved toxic to honeylocust seed germination. After 21 days germination averaged 89.5 percent in the control plots, 77.9 percent in the DCPA treated plots, and EPTC plots showed 100 percent mortality. At the end of 75 days, weed control in both herbicide treatments was 100 percent, while in the control plot it was only 60 percent (Table 4.4).

Table 4.4 Preemergent herbicide effects on germination, survival, and weed control of direct-seeded honeylocust.

Herbicide	Treatment rate	Germination 21 days after treatment	Survival   Weed Control	
			60 days after treatment	
Kg a.i./ha.		%		
DCPA	8.9	77.9a <sup>1/</sup>	77.9a	100a
EPTC	6.5	00.0b	00.0b	100a
Control	---	89.5a	89.5a	60b

<sup>1/</sup> Means for each category followed by the same letter are not significantly different at the 1% level based on Duncan's multiple range test.

A combination of factors may have caused the toxic effects of EPTC. First, immediately following application, the test plots were thoroughly watered to incorporate the EPTC into the soil and prevent its volatilization. As a result, the "effective" application rate may have been too high. Second, the study by Warmund et al. (1980) indicated that EPTC had a detrimental effect on honeylocust seed germination in nursery containers.

Results show that use of the preemergent herbicide DCPA at rates near 10 kg ai/ha will give thorough weed control in the early stages of germination and growth, enabling the seedlings to fully occupy the site. A second study by Warmund et al. (1983) indicates that at least five other preemergent herbicides may also be used for establishment of direct-seeded, field planted SRIC plantations.



### Direct-seed biomass study

Results of a direct-seed, spacing study testing the effects of different spacings on biomass yield of honeylocust, indicate that this technique is worthy of further research. Excellent stand establishment was obtained at all spacings. Significant differences were found between all spacing treatments (Table 4.5). Harvest data collected at the end of one year from seed indicate that the closest spacing - 15 cm x 10 cm - gave the highest yields. While the overall yields were not very high, it should be emphasized that coppice yields are expected to be much higher than growth from seed alone.

Coppice regrowth is an important part of the USRIC concept. The advantages of coppicing include; 1) the avoidance of extensive site preparation and replanting after each harvest; and 2) regrowth from established root systems is often faster than seedling growth. Geyer (1981) reports that coppice yields in 2-year old cottonwood (Populus deltoides) and silver maple (Acer saccharinum) were about 62 percent higher than seedling yields.

Table 4.5 Biomass production in a 1-year old, direct-seeded honeylocust USRIC system 1/.

Interrow <u>2/</u> spacing (cm)	kg/plot <u>3/</u>	% M.C.	Mg Forage yield/ha. (12% M.C.) <u>4/</u>
15	2.5	58.2	1.19a
30	1.6	57.8	0.73b
45	1.1	59.6	0.47c

1/ Treatment means are presented.

2/ Spacing within all rows is 10 cm.

3/ Plot size for all treatments is 15 square meters.

4/ Means followed by a different letter are significantly different at the 1% level using Duncan's test.

When selecting genotypes for use in USRIC systems, stem form becomes irrelevant, while the ability to coppice vigorously takes on an important role. In honeylocust, fast growing sources from southern latitudes of origin will be useful in USRIC systems as they tend to grow vigorously late into the fall (Gold and Hanover, 1984), allowing for fuller use of the growing season and a later fall harvest than would be possible with locally adapted sources. Field observations indicate that sources do not need to be completely winter hardy to be useful in USRIC systems. This is because the stems will be harvested close to ground level on an annual basis. Based on this line of reasoning, the ideotype of a species used in USRIC systems will have a somewhat different set of selection criteria than the ideotype of a species selected for use in a two-tier multi-crop system or other more traditional uses.

Depending on the length of the growing season, one to three harvests per year are anticipated. Planting and

establishment costs should be lower, and the use of forage harvesting equipment is possible. Efficient harvesting and processing should be feasible because of great uniformity in the USRIC material derived from control over the genetic, environmental and cultural systems utilized. In combination with effective use of pre-emergent herbicides, systems of USRIC seem to be a viable alternative for the production of chemical and animal feedstocks.

#### Industrial gums and other specialty chemicals

In order to fully utilize the economic potential of the honeylocust, one must examine all the constituent components of the pods. In addition to sugars and protein, the component which may eventually have the greatest market potential is the galactomannan fraction in the seed endosperm. As mentioned previously, honeylocust pods from sources originating in the northern part of the native range have high seed sets and little carbohydrate pulp in the pericarp fraction. This favors the development of products which are derived from the seed.

Mucilage polysaccharides which swell to a gel in water or gum polysaccharides which dissolve in water, are often associated with legume seed endosperm as a vitreous layer on the inside of the seedcoat. Several, for example those from guar (Cyamopsis tetragonoloba) and carob (Ceratonia siliqua), are of industrial importance. The endosperm of honeylocust seeds is almost pure galactomannan gums. This gum fraction comprises over 30 per cent of the total seed

(Mazzini and Cerezo, 1979). Galactomannan gums are used in the food, bakery, textile, oil drilling, pharmaceutical, cosmetic, and paper industries (Whistler and BeMiller, 1973).

Although no assays for specialty chemicals such as gums were done in the present study, they are a logical next phase in our investigations.

#### CONCLUSIONS AND RECOMMENDATIONS

Initial genetic gains from provenance/progeny testing are expected to markedly improve each trait compared to average, unselected sources. Results of pod sugar and seed/leaf protein analyses indicate that significant improvements in the chemical properties of honeylocust are attainable through selection of appropriate families and/or individuals.

The variation patterns in leaf protein, seed protein, and pod sugars are random with no particular provenance or region being especially high in any given trait. Also, because these traits are yield traits and are composed of many different components, their patterns of inheritance are likely to be complex and as a result, their rate of improvement will be slower.

In the short term, it may be a wise idea to take advantage of natural morphological differences inherent in honeylocust growing in northern vs southern regions of the country. The use of honeylocust in different regions for

different purposes will result in its most efficient use. In the west-central and northern regions, concentrate on seed protein and seed gum production, while in southern regions, take advantage of pod sugar/ethanol/stillage production. In the longer term, simultaneous selection for a combination of yield components will be desired to maximize pod yields, seed yields, seed protein content and seed protein quality.

When developing ideotypes for use in agroforestry systems, selection for maximum pod production will be the key factor, although attention to total sugar content and other important chemical and morphological traits should not be ignored. The ideotype of a honeylocust used in USRIC systems will have a somewhat different set of selection criteria than the ideotype selected for use in two-tier orchard systems. In addition, many cultural techniques are in need of further development in order to make multi-purpose agroforestry systems economically viable as animal/chemical feedstock production systems.

#### The Last Word

When attempting to promote new ways of looking at the potentials of trees, one tends to accentuate the accomplishments and highlight the potential benefits, rather than dwelling on the ever-present and inevitable problems inherent in the development of new systems and technologies. However, many questions need to be answered, and many problems remain to be worked out.

A problem shared in common with all forestry research is that the development of multi-purpose trees and agroforestry systems will require long-term research commitments and project continuity, rare commodities indeed.

Specific problems include the need for testing honeylocust in many diverse locations, determination of optimum spacings, harvesting and processing systems development, intercrop trials, and the development of precise management regimes. Animal damage, insect and disease problems etc., will all have to be overcome.

In contrast with many scientists who work with trees in third world countries, a majority of foresters, agronomists, and others in industrialized countries have been trained to keep their disciplines separate unto themselves and conceive of only a limited role for the use of trees. Multiple-use trees and agroforestry systems such as those described in this paper, are not generally given much thought or credibility. This psychological barrier will have to be removed before these ideas, systems, and methodologies for using trees in new ways are accepted as viable and valid.

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## APPENDICES

**APPENDIX A**

**CORRESPONDENCE AND COLLECTION FORMS**

At Michigan State University we are undertaking a rangewide Honeylocust (Gleditsia triacanthos) seed collection to begin a comprehensive evaluation of the genetic variation which exists within the natural range of the species. This is the first step of a long range project for the genetic improvement of Honeylocust.

Our objectives in this work are to study the potential use of the species for fiber and feedstock production on marginal agricultural lands in the Lake States.

We would like this rangewide study to be a cooperative one consisting of replicated plantations at various locations throughout the range of the species. Would it be possible for you to collect or coordinate a collecting are given on the attached material.

All information obtained from the study will be shared with those who are interested and progress reports will be issued periodically to keep you abreast of the research results.

We will certainly appreciate any assistance you can provide in this effort.

Sincerely,

Michael Gold  
Graduate Student MSU

MG:js

Dear Fellow Nut Growers:

I recently had the pleasure of attending the 70th Annual N.N.G.A. meetings in Wooster, Ohio. Among the various topics discussed was an interesting talk on Agri-silviculture (Forest farming) based on the tree crop idea of Dr. J. Russell Smith. One of the species most often mentioned in the context of tree crops is the Honeylocust.

I am aware of the N.N.G.A. interest in locating the "superior" selections of nut trees in the hopes of finding improved varieties for the northern areas.

I would, therefore, like to ask you to contribute a bit of your time to a similar research project which we are undertaking at Michigan State University. A further explanation of the research is enclosed. Hope to hear from you.

Sincerely,

Michael Gold  
Member N.N.G.A.  
Graduate Student MSU

MG:js

Enc.

Suggestions For Collecting Pods

Pods can be harvested in the fall during the period of natural ripening and will usually yield viable seed. Harvest of the current season's crop is preferred. Ground collections from directly under a tree are acceptable.

From 5 to 25 or more pods may be collected from each of 5 to 10 trees in any location. This is merely a guide: any number of pods, trees, and of course, locations will be useful to us.

Pods from individual trees should be kept separate if possible, then labeled, packaged, and mailed by air or regular mail to the following address:

Prof. J. W. Hanover  
Dept. of Forestry  
Michigan State University  
East Lansing, Michigan 48824

Ship C.O.D. if you wish or indicate shipping charges and we will be happy to reimburse you.

Please complete the enclosed collection information form for each location and mail it with the pods.

Thank you very much for your cooperation!



HONEYLOCUST RANGEWIDE STUDY  
Collection Information

Collector's Name and Address \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Collection Data: Date collected \_\_\_\_ / \_\_\_\_ / \_\_\_\_ County \_\_\_\_\_

Location where collections made (landmarks, roads, etc.) \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Section \_\_\_\_\_ Township \_\_\_\_\_ Range \_\_\_\_\_  
Natural or planted \_\_\_\_\_ Seed crop: Light \_\_\_\_\_ Medium \_\_\_\_\_ Heavy \_\_\_\_\_

Thorns: Present \_\_\_\_\_ Absent \_\_\_\_\_

Site Description: Drainage \_\_\_\_\_ Slope \_\_\_\_\_ Soil \_\_\_\_\_

Tree No. Approx. Height D.B.H. Approx. age  
(feet)

- 1. \_\_\_\_\_
- 2. \_\_\_\_\_
- 3. \_\_\_\_\_
- 4. \_\_\_\_\_
- 5. \_\_\_\_\_
- 6. \_\_\_\_\_
- 7. \_\_\_\_\_
- 8. \_\_\_\_\_
- 9. \_\_\_\_\_
- 10. \_\_\_\_\_

Associated Species \_\_\_\_\_  
\_\_\_\_\_

Additional Information? \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Thanks again for your cooperation in the Honeylocust rangewide collection project. The magnitude of the response is rapidly approaching our goal of 200 sources, broad enough to conduct a thorough evaluation of the genetic variation within Gleditsia triacanthos.

Research results will be issued periodically in the form of Progress Reports and plant materials will be made available as they are developed.

We certainly appreciate your assistance in this effort.

Sincerely,

Michael Gold  
Graduate Student MSU

MG:js

**APPENDIX B**

**ACCESSION RECORD FOR INITIAL HONEYLOCUST RANGEWIDE  
COLLECTION**

Appendix B. Accession record for initial honeylocust  
rangewide collection.

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HONEYLOCUST RANGEWIDE STUDY  
Collection Information

Species: Gleditsia triacanthos

Genus code: 43

Species code: 33

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Accession no.*	State of origin	County of origin	Latitude	Longitude
003	GA	CHATTOOGA	34o.18'N	85o.10'W
004	GA	CHATTOOGA	34o.18'N	85o.10'W
005	GA	CHATTOOGA	34o.18'N	85o.10'W
006	GA	CHATTOOGA	34o.18'N	85o.10'W
007	GA	CHATTOOGA	34o.18'N	85o.10'W
008	GA	MONROE	33o.02'N	83o.58'W
009	GA	MONROE	33o.02'N	83o.58'W
010	GA	MONROE	33o.02'N	83o.58'W
011	GA	MONROE	33o.02'N	83o.58'W
012	GA	MONROE	33o.02'N	83o.58'W
013	GA	MONROE	33o.02'N	83o.58'W
014	GA	OGLETHORPE	33o.31'N	84o.41'W
015	GA	OGLETHORPE	33o.31'N	84o.41'W
016	GA	OGLETHORPE	33o.31'N	84o.41'W
017	GA	CLARKE	33o.57'N	83o.24'W
018	GA	OCONEE	33o.51'N	83o.26'W
019	GA	MORGAN	33o.36'N	83o.38'W
020	GA	MORGAN	33o.36'N	83o.38'W
021	GA	MORGAN	33o.36'N	83o.38'W
022	GA	WALTON	33o.47'N	83o.43'W
023	GA	MARION	32o.18'N	84o.32'W
024	GA	MARION	32o.18'N	84o.32'W
025	GA	TAYLOR	32o.33'N	84o.16'W
026	GA	BALDWIN	33o.04'N	83o.13'W
027	GA	PUTNAM	33o.20'N	83o.24'W
028	GA	PUTNAM	33o.20'N	83o.24'W
029	GA	PUTNAM	33o.20'N	83o.24'W
030	GA	JASPER	33o.19'N	83o.41'W
031	GA	JASPER	33o.19'N	83o.41'W
032	GA	HANCOCK	33o.17'N	82o.58'W
033	GA	HANCOCK	33o.17'N	82o.58'W
034	GA	HANCOCK	33o.17'N	82o.58'W
035	GA	HANCOCK	33o.17'N	82o.58'W
036	GA	STEPHENS	34o.34'N	83o.21'W
037	GA	STEPHENS	34o.34'N	83o.21'W

## Appendix B (Cont'd.)

038	GA	STEPHENS	34 .34 'N	83 .21 'W
039	GA	STEPHENS	34 <sup>o</sup> .34 'N	83 <sup>o</sup> .21 'W
040	GA	HABERSHAM	34 <sup>o</sup> .36 'N	83 <sup>o</sup> .32 'W
041	GA	JASPER	33 <sup>o</sup> .19 'N	83 <sup>o</sup> .41 'W
042	GA	PUTNAM	33 <sup>o</sup> .20 'N	83 <sup>o</sup> .24 'W
043	GA	PUTNAM	33 <sup>o</sup> .20 'N	83 <sup>o</sup> .24 'W
044	GA	JASPER	33 <sup>o</sup> .19 'N	83 <sup>o</sup> .41 'W
045	GA	PICKENS	34 <sup>o</sup> .28 'N	84 <sup>o</sup> .27 'W
046	GA	BARTON	34 <sup>o</sup> .22 'N	84 <sup>o</sup> .42 'W
047	GA	BARTON	34 <sup>o</sup> .22 'N	84 <sup>o</sup> .42 'W
048	GA	JONES	33 <sup>o</sup> .01 'N	83 <sup>o</sup> .33 'W
049	GA	JONES	33 <sup>o</sup> .01 'N	83 <sup>o</sup> .33 'W
050	GA	JONES	33 <sup>o</sup> .01 'N	83 <sup>o</sup> .33 'W
051	GA	JONES	33 <sup>o</sup> .01 'N	83 <sup>o</sup> .33 'W
052	GA	JONES	33 <sup>o</sup> .01 'N	83 <sup>o</sup> .33 'W
053	GA	WARREN	33 <sup>o</sup> .23 'N	82 <sup>o</sup> .40 'W
054	GA	WARREN	33 <sup>o</sup> .23 'N	82 <sup>o</sup> .40 'W
055	GA	MCDUFFIE	33 <sup>o</sup> .28 'N	82 <sup>o</sup> .31 'W
056	GA	MCDUFFIE	33 <sup>o</sup> .28 'N	82 <sup>o</sup> .31 'W
057	GA	MCDUFFIE	33 <sup>o</sup> .28 'N	82 <sup>o</sup> .31 'W
058	GA	BEN HILL	31 <sup>o</sup> .43 'N	83 <sup>o</sup> .16 'W
059	GA	BULLOCH	32 <sup>o</sup> .28 'N	81 <sup>o</sup> .47 'W
060	GA	BULLOCH	32 <sup>o</sup> .28 'N	81 <sup>o</sup> .47 'W
061	GA	BULLOCH	32 <sup>o</sup> .28 'N	81 <sup>o</sup> .47 'W
062	GA	COWETTA	33 <sup>o</sup> .23 'N	84 <sup>o</sup> .48 'W
063	GA	MERIWEATHER	33 <sup>o</sup> .01 'N	84 <sup>o</sup> .50 'W
064	GA	MERIWEATHER	33 <sup>o</sup> .01 'N	84 <sup>o</sup> .50 'W
065	GA	HEARD	33 <sup>o</sup> .13 'N	84 <sup>o</sup> .50 'W
066	GA	NEWTON	33 <sup>o</sup> .35 'N	83 <sup>o</sup> .52 'W
067	GA	NEWTON	33 <sup>o</sup> .35 'N	83 <sup>o</sup> .52 'W
068	GA	NEWTON	33 <sup>o</sup> .35 'N	83 <sup>o</sup> .52 'W
069	GA	NEWTON	33 <sup>o</sup> .35 'N	83 <sup>o</sup> .52 'W
070	GA	NEWTON	33 <sup>o</sup> .35 'N	83 <sup>o</sup> .52 'W
071	GA	NEWTON	33 <sup>o</sup> .35 'N	83 <sup>o</sup> .52 'W
072	GA	NEWTON	33 <sup>o</sup> .35 'N	83 <sup>o</sup> .52 'W
073	GA	NEWTON	33 <sup>o</sup> .35 'N	83 <sup>o</sup> .52 'W
074	GA	NEWTON	33 <sup>o</sup> .35 'N	83 <sup>o</sup> .52 'W
075	GA	HENRY	33 <sup>o</sup> .22 'N	84 <sup>o</sup> .06 'W
076	GA	HENRY	33 <sup>o</sup> .22 'N	84 <sup>o</sup> .06 'W
077	GA	HENRY	33 <sup>o</sup> .22 'N	84 <sup>o</sup> .06 'W
078	GA	HENRY	33 <sup>o</sup> .22 'N	84 <sup>o</sup> .06 'W
079	GA	HENRY	33 <sup>o</sup> .22 'N	84 <sup>o</sup> .06 'W
080	GA	HENRY	33 <sup>o</sup> .22 'N	84 <sup>o</sup> .06 'W
081	GA	HENRY	33 <sup>o</sup> .22 'N	84 <sup>o</sup> .06 'W
082	GA	HENRY	33 <sup>o</sup> .22 'N	84 <sup>o</sup> .06 'W
083	GA	HENRY	33 <sup>o</sup> .22 'N	84 <sup>o</sup> .06 'W
084	GA	HENRY	33 <sup>o</sup> .22 'N	84 <sup>o</sup> .06 'W
085	KY	PENDLETON	38 <sup>o</sup> .48 'N	84 <sup>o</sup> .23 'W
086	KY	SCOTT	38 <sup>o</sup> .20 'N	84 <sup>o</sup> .49 'W
087	KY	OWEN	38 <sup>o</sup> .27 'N	84 <sup>o</sup> .49 'W

## Appendix B (Cont'd.)

088	KY	OWEN	38 .27 'N	84 .49 'W
089	KY	FRANKLIN	38 0.11 'N	84 0.53 'W
090	KY	FRANKLIN	38 0.11 'N	84 0.53 'W
091	KY	FRANKLIN	38 0.11 'N	84 0.53 'W
092	KY	FRANKLIN	38 0.11 'N	84 0.53 'W
093	KY	FRANKLIN	38 0.11 'N	84 0.53 'W
094	KY	GARRARD	37 0.42 'N	84 0.34 'W
095	KY	GARRARD	37 0.42 'N	84 0.34 'W
096	KY	GARRARD	37 0.42 'N	84 0.34 'W
097	KY	GARRARD	37 0.42 'N	84 0.34 'W
098	KY	JESSAMINE	37 0.52 'N	84 0.34 'W
099	KY	JESSAMINE	37 0.52 'N	84 0.34 'W
100	KY	JESSAMINE	37 0.52 'N	84 0.34 'W
101	KY	WARREN	36 0.95 'N	86 0.25 'W
102	KY	HARDIN	37 0.35 'N	85 0.49 'W
103	KY	LYON	36 0.57 'N	87 0.56 'W
104	KY	LYON	36 0.57 'N	87 0.56 'W
105	KY	LYON	36 0.57 'N	87 0.56 'W
106	KY	LYON	36 0.57 'N	87 0.56 'W
107	KY	HICKMAN	36 0.45 'N	89 0.06 'W
108	KY	HICKMAN	36 0.45 'N	89 0.06 'W
109	KY	HICKMAN	36 0.45 'N	89 0.06 'W
110	KY	HICKMAN	36 0.45 'N	89 0.06 'W
111	KY	HICKMAN	36 0.45 'N	89 0.06 'W
112	KY	BATH	38 0.04 'N	83 0.43 'W
113	KY	BATH	38 0.04 'N	83 0.43 'W
114	KY	FLEMING	38 0.20 'N	83 0.39 'W
115	KY	FLEMING	38 0.20 'N	83 0.39 'W
116	KY	FLEMING	38 0.20 'N	83 0.39 'W
117	KY	FLEMING	38 0.20 'N	83 0.39 'W
118	KY	FLEMING	38 0.20 'N	83 0.39 'W
119	KY	GREENUP	38 0.34 'N	82 0.52 'W
120	OK	WASHITA	35 0.21 'N	98 0.39 'W
121	OK	WASHITA	35 0.21 'N	98 0.39 'W
122	OK	GARFIELD	36 0.24 'N	97 0.54 'W
123	OK	GARFIELD	36 0.24 'N	97 0.54 'W
124	OK	GARFIELD	36 0.24 'N	97 0.54 'W
125	OK	GARFIELD	36 0.24 'N	97 0.54 'W
126	OK	MCCURTAIN	33 0.54 'N	94 0.50 'W
127	OK	MCCURTAIN	33 0.54 'N	94 0.50 'W
128	OK	MCCURTAIN	33 0.54 'N	94 0.50 'W
129	OK	MCCURTAIN	33 0.54 'N	94 0.50 'W
130	OK	MCCURTAIN	33 0.54 'N	94 0.50 'W
131	OK	MCCURTAIN	33 0.54 'N	94 0.50 'W
132	OK	MCCURTAIN	33 0.54 'N	94 0.50 'W
133	TX	POLK	30 0.42 'N	94 0.58 'W
134	TX	POLK	30 0.42 'N	94 0.58 'W
135	TX	POLK	30 0.42 'N	94 0.58 'W
136	TX	POLK	30 0.42 'N	94 0.58 'W

## Appendix B (Cont'd.)

137	TX	CHERROKE	31° .59' N	95° .19' W
138	TX	CHERROKE	31° .59' N	95° .19' W
139	TX	ANDERSON	31° .45' N	95° .39' W
140	TX	ANDERSON	31° .45' N	95° .39' W
141	TX	ANDERSON	31° .45' N	95° .39' W
142	TX	ANDERSON	31° .45' N	95° .39' W
143	TX	CHERROKE	32° .59' N	95° .55' W
144	TX	CHERROKE	32° .59' N	95° .55' W
145	TX	CHERROKE	32° .59' N	95° .55' W
146	TX	CHERROKE	32° .59' N	95° .55' W
147	TX	HENDERSON	32° .12' N	95° .51' W
148	TX	WASHINGTON	30° .39' N	96° .24' W
149	TX	WASHINGTON	30° .39' N	96° .24' W
150	TX	WASHINGTON	30° .39' N	96° .24' W
151	TX	WASHINGTON	30° .39' N	96° .24' W
152	TX	WASHINGTON	30° .39' N	96° .24' W
153	TX	WASHINGTON	30° .39' N	96° .24' W
154	TX	WASHINGTON	30° .12' N	96° .37' W
155	TX	WASHINGTON	30° .12' N	96° .37' W
156	TX	WASHINGTON	30° .12' N	96° .37' W
157	DC		38° .55' N	77° .00' W
158	DC		38° .55' N	77° .00' W
159	DC		38° .55' N	77° .00' W
160	DC		38° .55' N	77° .00' W
161	PA	LANCASTER	40° .01' N	76° .19' W
162	PA	HUNTINGTON	40° .23' N	77° .54' W
163	PA	HUNTINGTON	40° .23' N	77° .54' W
164	PA	HUNTINGTON	40° .23' N	77° .54' W
165	PA	HUNTINGTON	40° .23' N	77° .54' W
166	PA	HUNTINGTON	40° .23' N	77° .54' W
167	PA	HUNTINGTON	40° .23' N	77° .54' W
168	PA	HUNTINGTON	40° .23' N	77° .54' W
169	LA	EAST CARROLL	32° .48' N	91° .10' W
170	LA	EAST CARROLL	32° .48' N	91° .10' W
171	LA	EAST CARROLL	32° .48' N	91° .10' W
172	LA	EAST CARROLL	32° .48' N	91° .10' W
173	LA	EAST CARROLL	32° .48' N	91° .10' W
174	LA	EAST CARROLL	32° .48' N	91° .10' W
175	LA	EAST CARROLL	32° .48' N	91° .10' W
176	MS	OKTIBBEHA	33° .27' N	88° .50' W
177	MS	OKTIBBEHA	33° .27' N	88° .50' W
178	MS	OKTIBBEHA	33° .27' N	88° .50' W
179	MS	WINSTON	33° .30' N	88° .50' W
180	MS	WINSTON	33° .30' N	88° .50' W
181	MS	OKTIBBEHA	33° .30' N	88° .50' W
182	MS	OKTIBBEHA	33° .30' N	88° .50' W
183	MS	OKTIBBEHA	33° .30' N	88° .50' W
184	MS	NOXUBEE	33° .30' N	88° .50' W
185	MS	OKTIBBEHA	33° .30' N	88° .50' W

## Appendix B (Cont'd.)

186	MS	OKTIBBEHA	33 .30 'N	88 .50 'W
187	SC	UNION	34o.42 'N	81o.37 'W
188	SC	UNION	34o.42 'N	81o.37 'W
189	SC	YORK	34o.59 'N	81o.14 'W
190	SC	YORK	34o.59 'N	81o.14 'W
191	SC	YORK	34o.59 'N	81o.14 'W
192	SC	ABBEVILLE	34o.00 'N	82o.14 'W
193	SC	ABBEVILLE	34o.00 'N	82o.14 'W
194	SC	ABBEVILLE	34o.00 'N	82o.14 'W
195	SC	ABBEVILLE	34o.00 'N	82o.14 'W
196	SC	ABBEVILLE	34o.00 'N	82o.14 'W
197	SC	ABBEVILLE	34o.00 'N	82o.14 'W
198	SC	PICKENS	34o.37 'N	82o.50 'W
199	SC	PICKENS	34o.37 'N	82o.50 'W
200	SC	OCONEE	34o.53 'N	82o.58 'W
201	SC	OCONEE	34o.53 'N	82o.58 'W
202	SC	OCONEE	34o.53 'N	82o.58 'W
203	SC	OCONEE	34o.53 'N	82o.58 'W
204	SC	ANDERSON	34o.53 'N	82o.58 'W
205	SC	PICKENS	34o.30 'N	82o.39 'W
206	SC	ANDERSON	34o.53 'N	82o.58 'W
207	SC	ANDERSON	34o.53 'N	82o.58 'W
208	SC	PICKENS	34o.37 'N	82o.50 'W
209	SC	PICKENS	34o.37 'N	82o.50 'W
210	SC	FAIRFIELD	34o.00 'N	81o.00 'W
211	SC	FAIRFIELD	34o.00 'N	81o.00 'W
212	SC	FAIRFIELD	34o.00 'N	81o.00 'W
213	SC	FAIRFIELD	34o.00 'N	81o.00 'W
214	SC	FAIRFIELD	34o.00 'N	81o.00 'W
215	SC	CHESTER	34o.43 'N	81o.14 'W
216	SC	CHESTER	34o.43 'N	81o.14 'W
217	SC	CHESTER	34o.43 'N	81o.14 'W
218	SC	CHESTER	34o.43 'N	81o.14 'W
219	NC	GASTON	35o.14 'N	81o.12 'W
220	NC	GASTON	35o.14 'N	81o.12 'W
221	SC	YORK	34o.59 'N	81o.14 'W
222	SC	YORK	34o.59 'N	81o.14 'W
223	SC	YORK	34o.59 'N	81o.14 'W
224	SC	YORK	34o.59 'N	81o.14 'W
225	SC	YORK	34o.59 'N	81o.14 'W
226	SC	YORK	34o.59 'N	81o.14 'W
227	SC	YORK	34o.59 'N	81o.14 'W
228	SC	UNION	34o.42 'N	81o.37 'W
229	SC	UNION	34o.42 'N	81o.37 'W
230	SC	UNION	34o.42 'N	81o.37 'W
231	SC	UNION	34o.42 'N	81o.37 'W
232	SC	UNION	34o.42 'N	81o.37 'W
233	SC	AIKEN	33o.34 'N	81o.44 'W
234	SC	AIKEN	33o.34 'N	81o.44 'W



## Appendix B (Cont'd.)

235	SC	AIKEN	33 .34 'N	81 .44 'W
236	SC	AIKEN	33o.34 'N	81o.44 'W
237	SC	SALUDA	33o.56 'N	81o.33 'W
238	SC	LEXINGTON	33o.56 'N	81o.30 'W
239	SC	CHESTER	34o.43 'N	81o.14 'W
240	SC	CHESTER	34o.43 'N	81o.14 'W
241	SC	YORK	35o.01 'N	81o.18 'W
242	SC	YORK	35o.01 'N	81o.18 'W
243	SC	YORK	35o.01 'N	81o.18 'W
244	KS	CHAUTAUQUA	37o.05 'N	96o.31 'W
245	KS	CHAUTAUQUA	37o.05 'N	96o.31 'W
246	KS	FRANKLIN	37o.45 'N	95o.10 'W
247	KS	FRANKLIN	37o.45 'N	95o.10 'W
248	KS	FRANKLIN	37o.45 'N	95o.10 'W
249	KS	JEFFERSON	39o.12 'N	95o.33 'W
250	KS	JEFFERSON	39o.12 'N	95o.33 'W
251	FL	BAY	30o.10 'N	85o.41 'W
252	LA	BOSSIER	32o.31 'N	93o.44 'W
253	LA	BOSSIER	32o.31 'N	93o.44 'W
254	LA	BOSSIER	32o.31 'N	93o.44 'W
255	LA	LINCOLN	32o.32 'N	92o.39 'W
256	LA	LINCOLN	32o.32 'N	92o.39 'W
257	LA	BIENVILLE	32o.33 'N	92o.56 'W
258	LA	BIENVILLE	32o.33 'N	92o.56 'W
259	LA	BIENVILLE	32o.33 'N	92o.56 'W
260	LA	BIENVILLE	32o.33 'N	92o.56 'W
261	LA	BIENVILLE	32o.33 'N	92o.56 'W
262	LA	NATCHITOCHE	31o.52 'N	93o.12 'W
263	WV	MONONGALIA	39o.38 'N	79o.57 'W
264	IN	PERRY	37o.56 'N	86o.46 'W
265	IN	PERRY	37o.56 'N	86o.46 'W
266	IN	PERRY	37o.56 'N	86o.46 'W
267	IN	OWEN	39o.18 'N	86o.46 'W
268	IN	OWEN	39o.18 'N	86o.46 'W
269	IN	OWEN	39o.18 'N	86o.46 'W
270	IN	LAWRENCE	38o.56 'N	86o.22 'W
271	IN	LAWRENCE	38o.55 'N	86o.37 'W
272	IN	GRANT	40o.33 'N	85o.40 'W
273	AL	LAWRENCE	34o.28 'N	87o.18 'W
274	AL	BIBB	32o.57 'N	87o.11 'W
275	OH	VANWERT	40o.43 'N	84o.06 'W
276	OH	VANWERT	40o.43 'N	84o.06 'W
277	OH	WARREN	39o.26 'N	84o.12 'W
278	OH	WARREN	39o.26 'N	84o.12 'W
279	OH	DELAWARE	40o.23 'N	82o.57 'W
280	OH	DELAWARE	40o.23 'N	82o.57 'W
281	OH	DELAWARE	40o.23 'N	82o.57 'W
282	OH	DELAWARE	40o.23 'N	82o.57 'W
283	OH	DELAWARE	40o.23 'N	82o.57 'W

## Appendix B (Cont'd.)

284	OH	CHAMPAIGN	40° 04' N	83° 34' W
285	WI	DANE	43° 04' N	89° 22' W
286	VA	AUGUSTA	38° 10' N	79° 05' W
287	VA	AUGUSTA	38° 10' N	79° 05' W
288	VA	AUGUSTA	38° 10' N	79° 05' W
289	VA	AUGUSTA	38° 10' N	79° 05' W
290	VA	AUGUSTA	38° 10' N	79° 05' W
291	VA	AUGUSTA	38° 10' N	79° 05' W
292	VA	AUGUSTA	38° 10' N	79° 05' W
293	VA	GILES	37° 19' N	80° 39' W
294	VA	GILES	37° 19' N	80° 39' W
295	VA	GILES	37° 19' N	80° 39' W
296	VA	GILES	37° 19' N	80° 39' W
297	VA	GILES	37° 19' N	80° 39' W
298	VA	GILES	37° 19' N	80° 39' W
299	VA	GILES	37° 19' N	80° 39' W
300	VA	FAIRFAX	38° 51' N	77° 19' W
301	IL	OGLE	42° 01' N	89° 21' W
302	IL	OGLE	42° 01' N	89° 21' W
303	IL	OGLE	42° 01' N	89° 21' W
304	IL	OGLE	42° 01' N	89° 21' W
305	IL	OGLE	42° 01' N	89° 21' W
306	IL	OGLE	42° 01' N	89° 21' W
307	IL	DU PAGE	41° 47' N	88° 00' W
308	IL	DU PAGE	41° 47' N	88° 00' W
309	IL	COOK	41° 48' N	87° 49' W
310	IL	DU PAGE	41° 52' N	88° 00' W
311	IL	LOGAN	40° 10' N	89° 21' W
312	IL	LOGAN	40° 10' N	89° 21' W
313	IL	LOGAN	40° 10' N	89° 21' W
314	IL	LOGAN	40° 10' N	89° 21' W
315	NB	CASS	41° 00' N	95° 52' W
316	NB	CASS	41° 00' N	95° 52' W
317	NB	CASS	41° 00' N	95° 52' W
318	NB	CASS	41° 00' N	95° 52' W
319	NB	CASS	41° 00' N	95° 52' W
320	NB	CASS	41° 00' N	95° 52' W
321	IA	POTTAWATTAMIE	41° 14' N	95° 54' W
322	AR	MONROE	34° 41' N	91° 19' W
323	AR	MONROE	34° 41' N	91° 19' W
324	AR	MONROE	34° 41' N	91° 19' W
325	AR	ARKANSAS	34° 41' N	91° 19' W
326	AR	MONROE	34° 41' N	91° 19' W
327	AR	MONROE	34° 41' N	91° 19' W
328	AR	MONROE	34° 41' N	91° 19' W
329	AR	MONROE	34° 41' N	91° 19' W
330	AR	MONROE	34° 41' N	91° 19' W
331	AR	PRAIRIE	34° 41' N	91° 19' W
332	MO	TEXAS	37° 31' N	91° 51' W

## Appendix B (Cont'd.)

			°	°
333	MO	TEXAS	37 .31'N	91 .51'W
334	MO	TEXAS	37° .31'N	91° .51'W
335	MO	TEXAS	37° .31'N	91° .51'W
336	MO	TEXAS	37° .31'N	91° .51'W
337	MO	TEXAS	37° .31'N	91° .51'W
338	MO	PHELPS	37° .56'N	91° .55'W
339	MO	PHELPS	37° .56'N	91° .55'W
340	MO	PHELPS	37° .56'N	91° .55'W
341	MO	PHELPS	37° .56'N	91° .55'W
342	CO	LARIMER	40° .35'N	105° .05'W
343	TN	UNION	36° .12'N	83° .50'W
344	TN	UNION	36° .12'N	83° .50'W
345	TN	UNION	36° .12'N	83° .50'W
346	TN	UNION	36° .12'N	83° .50'W
347	TN	UNION	36° .12'N	83° .50'W
348	TN	UNION	36° .12'N	83° .50'W
349	TN	UNION	36° .12'N	83° .50'W
350	TN	UNION	36° .12'N	83° .50'W
351	TN	UNION	36° .12'N	83° .50'W
352	TN	UNION	36° .12'N	83° .50'W
353	TN	UNION	36° .12'N	83° .50'W
354	TN	WASHINGTON	36° .12'N	82° .42'W
355	TN	WASHINGTON	36° .12'N	82° .42'W
356	TN	WASHINGTON	36° .12'N	82° .42'W
357	TN	WASHINGTON	36° .12'N	82° .42'W
358	GA	NEWTON	33° .35'N	83° .52'W
359	IA	BOONE	42° .02'N	93° .33'W
360	IA	BOONE	42° .02'N	93° .33'W
Y61	IA	BOONE	42° .02'N	93° .33'W
362	IA	STORY	42° .02'N	93° .33'W
363	IA	STORY	42° .02'N	93° .33'W
364	IA	STORY	42° .02'N	93° .33'W
365	IA	STORY	42° .02'N	93° .33'W
366	IA	STORY	42° .02'N	93° .33'W
367	IA	STORY	42° .02'N	93° .33'W
368	IA	STORY	42° .02'N	93° .33'W
369	IA	STORY	42° .02'N	93° .33'W
370	OH	ERIE	41° .27'N	82° .42'W
371	KY	OHIO	37° .34'N	86° .30'W
372	KY	HOPKINS	37° .16'N	87° .31'W
373	KY	CHRISTIAN	36° .50'N	87° .30'W
374	KY	BUTLER	37° .09'N	86° .54'W
375	WV	MONONGALIA	39° .38'N	79° .57'W
376	NY	SARATOGA	43° .16'N	73° .36'W
377	NY	SARATOGA	43° .16'N	73° .36'W
378	NY	ALBANY	42° .40'N	73° .49'W
379	NY	ALBANY	42° .40'N	73° .49'W
380	NY	ALBANY	42° .40'N	73° .49'W
381	NY	TOMKINS	42° .23'N	76° .32'W

## Appendix B (Cont'd.)

382	NY	TOMKINS	42 .23 'N	76 .32 'W
383	NY	TOMKINS	42° .23 'N	76° .32 'W
384	IL	ROCK ISLAND	41° .25 'N	90° .34 'W
385	MO	IRON	37° .42 'N	90° .53 'W
386	MS	SHARKEY	32° .55 'N	90° .54 'W
387	NC	BURKE	35° .45 'N	81° .47 'W
388	NC	BURKE	35° .45 'N	81° .47 'W
389	NC	BURKE	35° .45 'N	81° .47 'W
390	OH	LORAIN	41° .22 'N	82° .06 'W
391	CT	FAIRFIELD	41° .07 'N	73° .25 'W
392	SD	HUGHES	44° .23 'N	100° .20 'W
393	SD	HUGHES	44° .23 'N	100° .20 'W
394	SD	CORSON	45° .31 'N	100° .25 'W
395	SD	CORSON	45° .31 'N	100° .25 'W
396	SD	CORSON	45° .31 'N	100° .25 'W
397	SD	CORSON	45° .31 'N	100° .25 'W
398	SD	CODINGTON	44° .54 'N	97° .08 'W
399	SD	CODINGTON	44° .54 'N	97° .08 'W
400	SD	CODINGTON	44° .54 'N	97° .08 'W
401	SD	DUEUL	44° .34 'N	96° .52 'W
402	GA	STEWART	32° .03 'N	84° .49 'W
403	GA	STEWART	32° .03 'N	84° .49 'W
404	GA	STEWART	32° .03 'N	84° .49 'W
405	GA	STEWART	32° .03 'N	84° .49 'W
406	GA	STEWART	32° .03 'N	84° .49 'W
407	GA	RANDOLPH	31° .50 'N	84° .52 'W
408	GA	RANDOLPH	31° .50 'N	84° .52 'W
409	GA	HARRIS	32° .44 'N	84° .54 'W
410	GA	HARRIS	32° .44 'N	84° .54 'W
411	GA	HARRIS	32° .44 'N	84° .54 'W
412	GA	HARRIS	32° .44 'N	84° .54 'W
413	GA	HARRIS	32° .44 'N	84° .54 'W
414	GA	HARRIS	32° .44 'N	84° .54 'W
415	GA	HARRIS	32° .44 'N	84° .54 'W
416	GA	HARRIS	32° .44 'N	84° .54 'W
417	GA	HARRIS	32° .44 'N	84° .54 'W
418	GA	HARRIS	32° .44 'N	84° .54 'W
419	MD	PRINCE GEORGES	38° .57 'N	76° .56 'W
420	IL	COOK	41° .38 'N	87° .40 'W
421	IL	COOK	41° .38 'N	87° .40 'W
422	NM	BERNALILLO	35° .05 'N	106° .38 'W
423	NM	BERNALILLO	35° .05 'N	106° .38 'W
424	OH	DEFIANCE	41° .17 'N	84° .21 'W
425	OH	DEFIANCE	41° .17 'N	84° .21 'W
426	OH	DEFIANCE	41° .17 'N	84° .21 'W
427	OH	WILLIAMS	41° .30 'N	84° .34 'W
428	OH	HENRY	41° .17 'N	84° .21 'W
429	OH	DEFIANCE	41° .17 'N	84° .21 'W
431	NY	TOMKINS	42° .26 'N	76° .30 'W

## Appendix B (Cont'd.)

432	NY	SENECA	42 .35 'N	76 .35 'W
433	NY	SENECA	42o.35 'N	76o.35 'W
434	NY	SENECA	42o.35 'N	76o.35 'W
435	NY	SENECA	42o.35 'N	76o.35 'W
436	NY	SENECA	42o.35 'N	76o.35 'W
437	IA	POLK	41o.44 'N	93o.36 'W
438	IA	POLK	41o.44 'N	93o.36 'W
439	IA	POLK	41o.44 'N	93o.36 'W
440	IA	POLK	41o.44 'N	93o.36 'W
441	IA	POLK	41o.44 'N	93o.36 'W
442	IA	POLK	41o.44 'N	93o.36 'W
443	IA	POLK	41o.44 'N	93o.36 'W
444	IA	POLK	41o.44 'N	93o.36 'W
445	IA	POLK	41o.44 'N	93o.36 'W
446	IA	POLK	41o.44 'N	93o.36 'W
447	MI	INGHAM	42o.45 'N	84o.30 'W
448	MI	INGHAM	42o.45 'N	84o.30 'W
449	NB	DOUGLAS	41o.15 'N	96o.00 'W
450	CO	LARIMER	40o.35 'N	105o.05 'W
451	WV	MONONGALIA	39o.38 'N	79o.57 'W
452	WV	MONONGALIA	39o.38 'N	79o.57 'W
453	WV	MONONGALIA	39o.38 'N	79o.57 'W
454	WV	MONONGALIA	39o.38 'N	79o.57 'W
455	WV	MONONGALIA	39o.38 'N	79o.57 'W
456	WV	MONONGALIA	39o.38 'N	79o.57 'W
457	WV	MONONGALIA	39o.38 'N	79o.57 'W
458	WV	MONONGALIA	39o.38 'N	79o.57 'W
460	NY	ST. LAWRENCE	44o.40 'N	75o.01 'W
461	IL	PIATT	39o.48 'N	88o.37 'W
462	IL	PIATT	39o.48 'N	88o.37 'W
463	IL	PIATT	39o.48 'N	88o.37 'W
464	IL	PIATT	39o.48 'N	88o.37 'W
465	IL	PIATT	39o.48 'N	88o.37 'W
466	IL	PIATT	39o.48 'N	88o.37 'W
467	IL	PIATT	39o.48 'N	88o.37 'W
468	IL	PIATT	39o.48 'N	88o.37 'W
469	IL	PIATT	39o.48 'N	88o.37 'W
470	IL	PIATT	39o.48 'N	88o.37 'W

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Add 43,330,000 to all accession numbers

**APPENDIX C**

**PLANTATION MAPS**

MICHOTIP Plantation No.-82.01

*Medicago trisacanthos*

E. Lansing, MI.

Range-wide provenance/half-sib progeny test, Water Quality research area, E. Lansing, MI. 1 year old A-0-0 containerized seedling stock planted bare root by Howe, Miller, Gold, et. al. on 4/12 - 4/15 1952 with new modified multiplierplanter, 7 x 8' spacing. Randomized block design with 3 replications, and 4-tree plots. (FSN, RIA, section 6) Note: add 45,530,000 to all numbers to get complete accession number. Species code=4533.

COLUMNS

1	2-3	6-9	10-13	14-17	18-21	22-25	26-29	30-33	34-37	38-41	42-45	46-49	50-53	54-57	58-61	62-65	66-69	70-73	74-77	78	
x	175	170	123	360	151	370	233	32	398	257	73	439	342	29	388	150	x				
x	140	88	206	298	8	369	268	321	54	194	306	355	297	455	59	106	x				
x	193	157	228	30	168	105	35	589	424	255	469	373	279	161	378	15	x				
x	114	172	295	406	52	365	109	159	221	368	214	229	290	454	354	261	x				
x	163	384	64	78	181	186	354	326	447	333	452	117	348	31	398	222	x				
x	70	467	386	585	218	224	311	424	397	251	274	16	409	48	244	146	x				
x	401	178	276	449	509	377	75	72	451	376	393	307	82	374	466	416	x				
x	66	85	68	118	21	435	159	69	351	141	409	208	319	174	337	49	x				
x	148	280	303	434	65	108	310	304	41	89	187	184	179	237	225	162	x				
x	445	235	414	379	62	462	156	331	93	299	441	362	359	76	17	320	x				
x	396	292	67	66	238	262	135	84	98	104	112	165	74	236	269	248	x				
x	275	394	361	143	259	34	301	167	42	134	296	92	335	291	58	71	x				
x	101	456	28	410	425	195	24	330	431	61	119	423	155	349	463	420	x				
x	322	154	26	382	395	300	325	446	381	180	324	341	421	177	450	120	x				
x	282	213	273	363	234	290	153	364	461	185	316	359	246	278	272	443	x				
x	457	437	283	412	152	436	442	465	302	419	312	260	338	303	429	468	x				
x	55	53	209	392	332	371	345	305	247	171	366	329	258	103	470	56	x				
x	111	182	77	147	27	323	339	427	107	176	23	169	340	122	308	327	x				
x	440	453	100	115	63	90	250	110	448	124	387	353	158	136	245	281	x				
x	235	223	426	404	528	91	35	27	444	184	249	453	422	428	60	403	x				
x	304	78	308	168	301	443	594	255	461	390	380	367	391	160	166	411	x				
x	446	15	279	268	92	463	281	334	369	245	150	328	467	218	169	363	x				
x	428	392	179	64	319	524	65	384	290	251	277	73	278	440	378	388	x				
x	388	374	221	355	337	449	41	448	341	171	300	454	152	153	392	236	x				
x	447	101	269	118	368	397	327	365	187	373	390	24	338	429	160	35	x				
x	377	181	29	62	359	309	236	382	82	122	436	250	31	285	400	366	x				
x	178	21	136	225	93	330	141	147	74	134	108	290	75	112	257	326	x	xxxx			
x	156	114	88	134	117	75	48	58	71	423	424	444	330	401	296	391	136	x			
x	xxxx	222	276	437	393	54	85	139	184	425	54	70	157	342	384	58	470	x			
x	xxxx	166	66	174	123	108	307	69	342	244	69	385	282	364	449	71	224	x			
x	xxxx	xxxx	140	112	86	414	320	25	106	311	383	29	268	310	66	261	60	x			
x	xxxx	xxxx	248	91	401	351	451	98	61	163	393	247	115	376	61	-	80	x			
x	xxxx	xxxx	?	115	55	257	152	386	380	298	174	186	305	156	427	118	209	x			
x	xxxx	xxxx	24	403	416	445	68	406	376	457	184	382	194	304	283	55	249	x			
xxxx	xxxx	xxxx	440	303	160	23	331	89	224	233	234	456	361	229	441	394	359	x			
340	245	133	293	328	456	321	426	278	429	427	258	297	299	76	362	154	100	151	x		
277	431	122	246	209	283	124	249	194	469	379	452	269	389	420	161	23	367	291	x		
373	526	247	367	285	107	391	56	395	251	444	458	272	53	235	273	461	306	170	x		
348	135	148	30	258	458	361	349	55	299	333	110	453	213	280	329	386	180	238	x		
77	70	182	457	282	261	398	8	410	195	275	165	370	48	387	148	172	28	146	x		
422	445	425	63	84	274	352	302	186	90	465	107	508	455	32	147	246	103	465	x		
49	332	17	384	59	291	49	67	250	325	338	312	168	119	443	302	252	450	368	x		
162	214	175	295	464	32	262	311	322	165	345	333	365	439	396	316	463	109	409	x		
419	280	193	76	297	362	441	468	296	310	52	371	193	380	159	176	206	403	177	x		
396	33	226	157	26	453	177	16	381	146	316	34	469	339	63	448	221	92	84	x		
143	203	273	34	260	370	329	292	404	104	462	120	260	214	464	349	173	301	447	x		
411	306	167	387	120	339	208	170	100	159	450	412	185	62	181	322	421	179	303	x		
434	206	259	235	323	234	180	237	421	455	433	33	327	334	64	369	117	292	323	x		
223	244	335	207	366	110	27	300	105	364	423	162	446	295	17	374	253	111	153	x		
28	383	390	154	169	156	60	385	161	185	363	435	77	182	422	352	379	354	49	x		
354	73	229	436	153	213	172	153	111	467	378	377	463	406	30	581	124	52	207	x		
166	109	31	176	171	272	150	470	35	424	371	41	56	67	319	26	414	143	404	x		
xxxx	xxxx	xxxx	xxxx	xxxx	xxxx	233	420	589	454	119	442	305	428	404	140	332	91	248	x		
x	xxxx	xxxx	xxxx	xxxx	xxxx	x	439	410	103	163	218	471	452	25	88	345	451	105	208	x	
x	xxxx	xxxx	xxxx	xxxx	xxxx	x	xxxx	xxxx	xxxx	xxxx	xxxx	xxxx	xxxx	xxxx	xxxx	xxxx	xxxx	xxxx	xxxx	x	
x	309	85	16	595	274	123	312	445	x	x	x	x	x	x	x	x	x	x	x	x	
x	462	437	248	68	27	178	98	225	x	x	x	x	x	x	x	x	x	x	x	x	
x	89	276	416	13	104	167	259	93	x	x	x	x	x	x	x	x	x	x	x	x	
x	325	335	307	426	65	324	395	537	x	x	x	x	x	x	x	x	x	x	x	x	
x	397	419	98	-	80	92	331	371	x	x	x	x	x	x	x	x	x	x	x	x	



MICROTIP Plantation No.-82.06  
 Rangwala provenance/half-albo progeny test, Bergner farm property. This is the second of two plantations in this study, the first being 82.01 at Hator Quality. 1 year old A-O-G-contaminated seedling stock planted here root by Gold, Somerville, England, Alkema, et al. on 4/28-4/29 1982 with the new modified cuttplanter @ 7' x 8' spacing. Randomized block design with 3 replications, and 4-tree plots. (T15, RW, section 9)

Note: add 43,330,000 to all numbers to get complete accession number. Species code = 4333.

+ Blue spruce planting +



ROWS	1	2-5	6-9	10-13	14-17	18-21	22-25	26-29	30-33	34-37	38-41	42-45	46-49	50-53	54-57	58-61	62-65	66-69	70-73	74-77	78-81	82-85	86-89	90	
1	468	436	390	340	370	462	383	168	394	63	166	309	452	176	380	306	428	89	476	34	104	110	110	31	
2	468	26	374	139	119	290	193	428	147	361	437	308	25	451	348	381	179	378	117	335	303	369	295	31	
3	468	175	158	32	298	103	206	426	309	350	392	319	155	49	310	167	447	431	154	143	239	92	329	397	
4	448	389	235	30	170	462	120	462	53	27	168	111	466	364	342	48	447	285	388	166	421	52	304	31	
5	448	472	34	206	384	234	377	446	401	28	109	65	419	67	146	255	55	233	292	455	250	316	304	470	
6	448	60	403	180	251	386	246	208	337	207	56	351	465	178	334	107	91	283	185	331	464	245	100	470	
7	448	186	455	352	305	412	362	320	238	326	322	177	394	33	119	159	281	448	448	32	105	387	175	424	
8	91	171	24	64	457	280	73	414	238	409	331	341	148	345	274	445	184	86	338	140	435	258	339	161	
9	91	452	315	380	110	160	300	423	373	468	444	379	327	367	359	383	214	338	450	435	258	385	339	161	
10	91	441	277	152	209	221	31	249	261	467	456	349	368	153	391	247	440	150	429	151	456	454	470	161	
11	91	396	2	358	163	427	278	390	162	160	433	17	306	420	35	273	169	161	272	272	63	370	218	161	
12	365	184	423	464	367	362	261	429	470	160	420	221	218	454	315	176	365	363	461	287	382	762	220	161	
13	365	436	390	207	90	277	300	445	351	272	148	214	468	368	396	452	456	249	151	171	175	152	163	278	
14	365	124	296	312	109	251	295	432	233	368	376	431	298	363	348	246	153	34	107	259	152	163	278	278	
15	365	443	305	17	32	450	387	119	333	186	55	458	307	374	338	282	111	35	272	389	290	340	245	278	
16	396	339	364	154	370	382	169	166	238	462	409	324	354	373	306	167	64	165	103	388	258	48	49	278	
17	396	179	53	31	262	120	444	170	162	146	453	424	316	328	306	457	447	147	147	159	385	273	159	49	
18	396	208	305	427	337	309	329	247	117	304	209	143	329	244	280	421	437	322	255	404	440	394	369	49	
19	396	359	190	110	60	303	161	349	250	383	310	140	435	244	178	384	345	105	366	446	285	89	23	49	
20	453	365	464	248	342	455	326	361	30	472	168	401	86	180	159	27	35	341	25	283	366	381	324	49	
21	453	301	193	100	466	28	28	281	451	33	419	92	86	67	448	428	431	426	155	155	36	52	395	330	
22	453	379	403	185	235	392	465	327	98	56	257	308	91	352	274	372	386	302	158	423	361	369	455	330	
23	453	364	146	420	247	257	327	292	35	302	162	382	169	176	334	28	53	379	283	316	465	390	357	330	
24	450	454	110	221	445	368	461	392	35	302	162	382	169	170	334	167	274	403	235	366	397	419	395	330	
25	450	250	437	161	341	25	91	392	338	165	421	309	384	426	262	361	285	244	27	100	55	193	436	338	
26	450	17	65	120	179	272	154	388	48	151	359	323	278	451	428	334	41	319	218	373	111	41	466	338	
27	450	171	301	147	447	64	166	65	315	255	273	229	396	305	150	330	49	319	304	370	117	470	462	338	
28	163	349	186	152	152	468	258	261	362	452	155	153	380	457	90	352	329	450	52	378	391	401	456	338	
29	163	335	427	289	183	322	340	404	363	385	92	446	443	56	467	163	89	367	441	298	369	444	98	27	
30	163	440	33	326	239	103	422	168	177	312	412	390	76	119	359	307	281	435	383	277	245	109	103	27	
31	163	177	249	25	64	273	431	304	152	454	160	49	153	48	159	179	158	387	323	246	439	178	458	27	
32																									

ROWS

COLUMNS