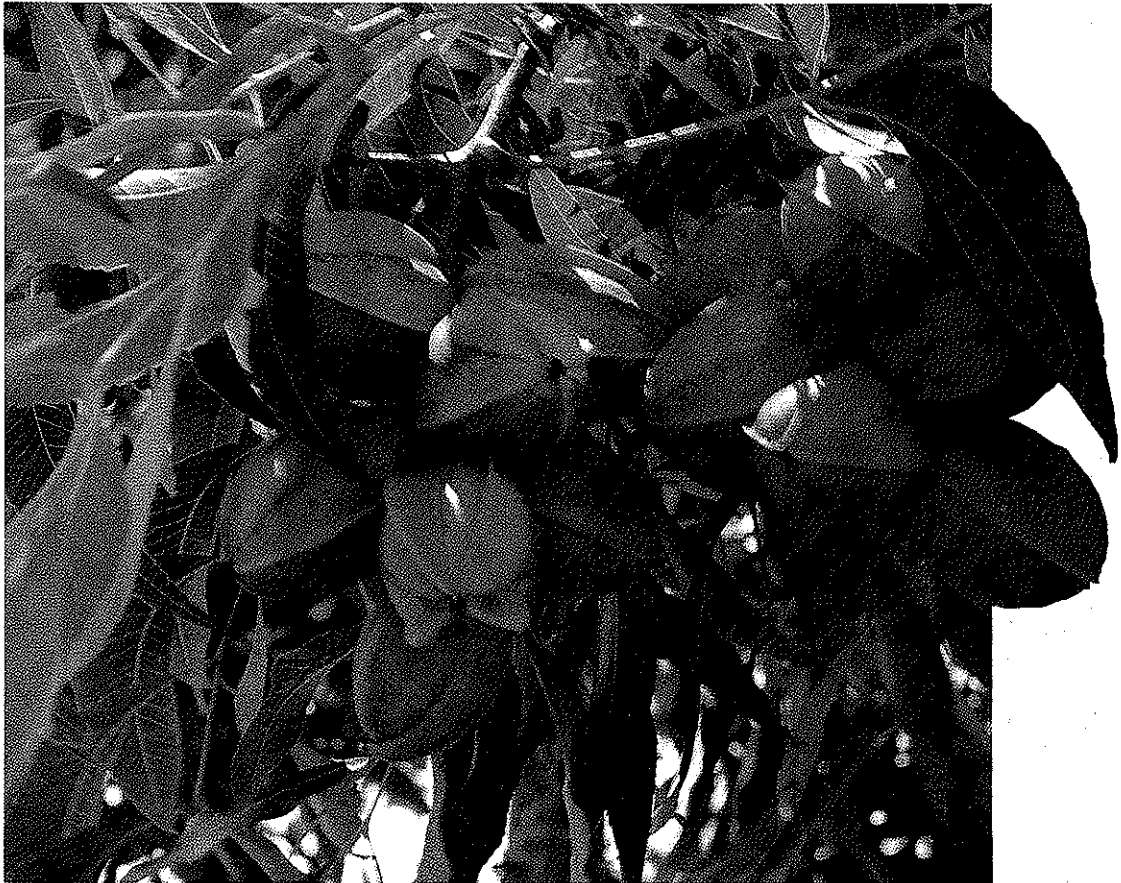

PROGRESS

In Breeding Pecans for Disease Resistance



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Progress in Breeding Pecans for Disease Resistance

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Introduction

Breeding for resistance to disease, with any tree crop, is historically time-consuming and costly. Thus, the initiation of a program with such a goal must be carefully planned, documented with basic understandings, and centered around the latest available plant breeding technologies. In recent years, we have been engaged in a program with the ultimate goal of developing cultivars of pecan [*Carya illinoensis* (Wangenheim) K. Koch.] with lasting resistance to the scab disease caused by *Cladosporium caryigenum* (Ell et Lang) Gottwald, but with several immediate goals. These include (1) definition of factors that may be associated with resistance, (2) identification of quality sources of resistance genes, and (3) development of procedures to expedite incorporation of these resistance genes into horticulturally desirable cultivars.

Consonant with these goals, an effort has been made to create at Mississippi State University a nursery collection of pecan x other hickory species (hicans), walnut x pecan, and pecan x pecan crosses involving only those parental genotypes deemed most promis-

ing for resistance breeding purposes. Present knowledge concerning resistance factors (particularly the phenolics believed important among resistance phenomena), known field resistance for parental choices, and breeding techniques have been utilized in developing this disease resistance breeding nursery. The purpose of this report is to detail progress in nursery development and to catalog the progeny contained in the Mississippi State University pecan disease resistance breeding nursery.

The Search for Resistance

C. caryigenum is known to have a great genetic diversity in nature and an operative mechanism for adaptive genetic reconstitution (1, 33). When one considers the adaptive capability of this fungus, and the range of fungal genotypes present across the pecan belt, the development of quality, lasting resistance in commercial pecan genotypes is a great challenge, most likely requiring the identification and transfer of resistance genes from hickory species other than *C. illinoensis*. We recognize that, in the past, cultivars thought to be resistant to scab later proved to be very



Figure 1. Pecan cluster at left shows symptoms of scab infection caused by *Cladosporium caryigenum* (Ell et Lang) Gottwald. In contrast, the cluster at right shows no signs of scab.

susceptible upon propagation and distribution. Kenknight (21), using scab inoculum from several sources, demonstrated infection on previously "non-scabbing" cultivars in Louisiana.

Street (35), at Mississippi State University, using a technique devised by McNeill (30) amenable to quarantine requirements and employing excised nuts, screened 25 pecan cultivars against 27 isolates of *C. caryigenum*. All cultivars except Baker were susceptible to one or more of the isolates. Minor infection also occurred upon Baker. Thus, once "non-scabbing" trees are vegetatively propagated and dispersed, the odds that the fungal genotypes capable of attacking them will increase prominently are greatly enhanced.

Observations (13) that native pecan populations often exhibit high levels of scab infection, whereas native stands of other hickory species rarely display such infection, suggest that these other species possess resistance factors not prevalent in pecan. Studies relative to resistance factors in pecan and other hickories at Mississippi State University seem to confirm this thesis, though these studies are not complete.

Juglone (5-hydroxy 1,4-naphthoquinone) has been shown to be a chemical host factor associated with resistance of pecan and other members of the Juglandaceae to scab (12, 18, 25). Juglone and hydrojuglone glucoside have also been correlated with resistance in juvenile leaves of black walnut (*Juglans nigra* L.) to anthracnose (*Gnomonia leptostyla* L.) (5).

In a survey of juglone levels in leaves and nuts, walnut trees (*Juglans regia* L. and *J. nigra*) possessed levels consistently higher than the hickories. Certain hickory trees were identified as having levels higher than those in pecans. Differences were noted among pecan cultivars (3). When juglone levels in husk, kernel, and leaflet of black walnut, shagbark hickory (*Carya ovata* (Mill) K. Koch), and four pecan cultivars were compared, the findings were: (husk) walnut > hickory and pecan; (leaflet) walnut > hickory and pecan; and (kernel) walnut > hickory > pecan (4).

Studies of seasonal variations of juglone content in pecan indicate that the level in leaves is higher in June, and decreases during the season. This decrease in leaves is accompanied by increases of juglone in nuts (4, 12, 18, 25). The leaf rachis, twigs, twig bark, trunk, root, root bark, and pollen all had notably lower levels of juglone than found in the leaves and nut tissues (4).

In addition to juglone, many plant phenolic derivatives have been implicated in disease resistance. The flavonoids, which include condensed tannins and isoquercitrin, comprise the largest class of phenolic compounds. Tannins in particular have been demonstrated to exhibit growth retardation of many parasites (7, 11) as well as feeding deterrents to in-

sects and mammals (2, 16, 17, 26, 27, 39). Tannins are widely distributed in woody plants and are usually found in greatest concentration in epidermal tissues.

In 1982, Graves et al. (14) first demonstrated the fungitoxic effect of tannins extracted from pecan to *C. caryigenum* at 4,000 ppm. Subsequently, concentrations of extractible tannins from leaves of the pecan cultivar Van Deman, ranging from 1,700 to 20,000 ppm, have been reported during the course of the growing season (14, 24). Recently, isoquercitrin has been identified in pecan tissue and found to be highly toxic to *C. caryigenum* (19, 22, 23).

Whereas the levels of juglone among pecan cultivars, hickory species, and walnuts have been studied, this has not been done for tannins or isoquercitrin. Cultivars of pecan and/or other hickory species may have differing levels of each phenolic compound. It is also possible that the differential capability of isolates to tolerate phenolic compounds may dictate isolate prevalence on a given host genotype. Further, the respective levels of each of these allelochemicals in combination may determine the quality of resistance.

To attribute disease resistance to chemical factors within host tissues requires more than identification of the factor and establishment of its antimicrobial activity *in vitro*. Considerations of concentration, location, and availability of chemicals in tissues invaded by the pathogen are equally important. Methods for histochemical localization and quantitation of the three principal phenolics in hickories have been developed (8, 9, 10, 15) and research is in progress to fully explore the potential role of phenolics in resistance to *C. caryigenum*.

Considering the time required in disease resistance breeding for a crop such as pecan, it was deemed expedient to begin a collection of pecan/walnut, pecan/hickory, pecan/hican, and pecan/pecan crosses useful in definitive studies, and as parental sources of quality disease resistance for future breeding efforts. It should be noted that progeny of these crosses may, upon evaluation, prove to be horticulturally desirable and useful genotypes for commercial exploitation.

Procedures

A program was begun in 1979 to create a collection of inter- and intraspecific crosses and backcrosses that could be useful for a concerted disease resistance breeding effort. Parental materials employed in the effort were as follows:

Female Parents

Stevens, Stuart, and Odom cultivars growing on the Mississippi State University campus were chosen as

female parents. The logistics of effecting these crosses dictated that female parents must be conveniently located and accessible by a heavy lift truck that would permit making crosses near tops of the trees.

Stevens was chosen because of consistently high levels of juglone in leaves (highest of any pecans assayed), and levels near highest (among pecan cultivars) in fruit (3).

Stuart has scab resistance, good horticultural qualities, and became the most widely planted cultivar in the southeastern United States following its introduction in the 1920s. This cultivar exhibited good scab resistance throughout the southeast until the late 1950s when pockets of scab buildup became noticeable and significant. Scab has since become a problem on Stuart orchards in many areas. Even so, considering the extent of exposure by virtue of the extensive and widespread plantations, the length of time the Stuart cultivar remained scab-free, and the fact that scab is most often less severe where it does occur than on most cultivars, the presence of scab resistance qualities seems evident. It should also be noted that the juglone content in fruit of Stuart was near the highest, though in leaves it was very low (3).

Odom is also an old cultivar that has historically appeared relatively scab-free, although it is not widely planted. It also has exhibited a high level of juglone in both leaves and nuts in early studies (12).

Pollen Sources

From the beginning of the endeavor, an attempt was made to use pollens from as many different hickory and walnut species as possible, and from a representative number of hicans. Pecan cultivars of interest were also included where possible.

Pollen collection was limited by the availability of, and access to, catkin bearing trees, and our ability to collect catkins from trees at critical times. Critical periods for catkin harvest, to achieve successful pollen release, were usually less than 12 hours depending upon temperature, humidity, and wind conditions. Thus, daily inspections were necessary. These were often not possible with our resources, particularly when trees were located long distances from the campus. Pollen sources from which collections were obtained are listed in Table 1.

Breeding efforts were initiated with a pollination technique study in 1980-81 (37, 38). Methods deemed most appropriate as a result of this study were subsequently employed. Female flowers were protected by brown Kraft corn pollination bags (Lawson "Shower-proofd," No. 504). Bags were placed over wire hoops fastened with masking tape to the woody stem behind female flower clusters after catkin removal. The wire hoops were designed to prevent the pollination bags from collapsing on the foliage and flowers. Nonabsorb-

Table 1. Pollen sources from which collections were obtained.

<i>Hickories other than pecan</i>		
Nutmeg	<i>Carya myristicaeformis</i> (Michx.f.) Nutt	
Red	<i>C. ovalis</i> (Wangh.) Sarg.	
Common Shagbark	<i>C. ovata</i> (Mill) K. Koch	
Mockernut	<i>C. tomentosa</i> Nutt	
Pignut	<i>C. glabra</i> (Mill) Sweet	
Sand	<i>C. pallida</i> (Ashe) Engl. & Graebn	
Southern Shagbark	<i>C. carolinae-septentrionalis</i> (Ashe) Engl. & Graebn	
Water	<i>C. aquatica</i> (Michx.f.) Nutt	
Shellbark	<i>C. laciniosa</i> (Michx.f.) Loud.	
Bitternut	<i>C. cordiformis</i> (Wangh) K. Koch	
<i>Walnuts</i>		
Black	<i>Juglans nigra</i> L.	
Persian (English)	<i>J. regia</i> L.	
<i>Hicans</i>		
McCallister	(a natural cross of <i>C. illinoensis</i> and <i>C. laciniosa</i>)	
Hican #1	(uncertain parentage)	
Hican #2	(uncertain parentage)	
<i>Pecan Cultivars</i>		
Cape Fear	Moore	Pabst
Frotscher	Odom	Stevens
Lewis	Owens	Stuart

bent cotton was wrapped around the stems and the bags were secured with masking tape to form a pollen proof seal.

Pollens were collected by spreading mature catkins over aluminum foil in the laboratory. After 24 hours, the pollen was collected and filtered through two layers of cheese cloth to remove large pieces of trash. The pollen was stored in cotton stoppered vials and refrigerated or frozen until needed. Pollens to be used within 14 days of collection were placed in a dessicator and stored at 4°C. Those to be held for longer periods were placed in a dessicator and frozen at -20°C. Pollens released too late for use were held in a dessicator at -20°C and used the following year. Pollination was accomplished with a powder insufflator. A small hole was cut in the bags, the nozzle of the insufflator was inserted, and pollen was expelled in the vicinity of the flowers. Holes in the bags were sealed with masking tape. For controls, the entire procedure was repeated using an empty insufflator.

Bags and wire hoops were removed within 11 to 14 days after pollination. In August, the hoops were reattached to stems and plastic mesh bags were placed over the hoops to catch any nuts that might drop and to protect nuts from pests. Nuts, upon harvest, were carefully labelled, germinated, grown out in

Table 2. Inventory of progeny resulting from intra- and interspecific crosses within the Juglandaceae produced between 1980 and 1987 and currently being grown in a nursery located on the Mississippi State University Plant Science Research Center.

Male parent	Female parent	Total progeny
<i>Pecan x Pecan</i>		
1. Cape Fear	Stuart	1
2. Frotscher	Stuart	2
3. Lewis	Stuart	3
4. Moore	Stuart	2
5. Odom	Stuart	3
6. Owens	Stuart	29
7. Pabst	Stuart	6
8. Stevens	Stuart	9
9. Stuart	Stuart	7
10. Cape Fear	Stevens	1
11. Frotscher	Stevens	26
12. Lewis	Stevens	4
13. Moore	Stevens	2
14. Owens	Stevens	6
15. Stevens	Stevens	5
16. Stuart	Stevens	25
17. Lewis	Odom	1
	TOTAL	132
<i>Other Hickories x Pecan</i>		
1. Nutmeg	Stuart	6
2. Nutmeg	Stevens	3
3. Red Hickory	Stuart	3
4. Red Hickory	Stevens	9
5. Shagbark	Stuart	14
6. Shagbark	Stevens	2
7. Shagbark	Odom	2
8. Southern Shagbark	Stuart	4
9. Sand	Stuart	2
10. Sand	Odom	4
11. Mockernut	Stuart	4
12. Pignut	Stuart	5
	TOTAL	58
<i>Hican x Pecan Backcrosses</i>		
1. McCallister	Stuart	14
2. McCallister	Stevens	2
3. Hican No. 2	Stevens	2
	TOTAL	18
<i>Intergeneric Crosses</i>		
1. Black Walnut	Stuart	5
	TOTAL	5
GRAND TOTAL		213

greenhouse culture and transplanted to a field nursery the following year.

Subsequent to the pollination technique study completed in 1981 (37, 38), approximately 100 terminals were bagged each year through 1987. Successful crosses were achieved each year with the exception of one. Failures were thought to be related to improper timing.

Results and Discussion

A total of 213 successful crosses were achieved during the course of the effort. The progeny are currently growing in a nursery located on the Mississippi State University Plant Science Research Center (Table 2). These include 132 intraspecific crosses, 58 interspecific crosses, 5 intergeneric crosses, and 18 hican x pecan crosses. Of these, 191 are currently large enough to harvest graftwood. The interspecific crosses include those with nutmeg [*Carya myristicaeformis* (Michx.f.) Nutt], red [*C. ovalis* (Wangh.) Sarg.], shagbark [*C. ovata* (Mill) K. Koch], southern shagbark [*C. carolinae-septentrionalis* (Ashe) Engl. & Graebn], sand [*C. pallida* (Ashe) Engl. & Graebn], mockernut [*C. tomentosa* Nutt], and pignut [*C. glabra* (Mill) Sweet]. Failure to achieve successful crosses with water [*C. aquatica* (Michx.f.) Nutt], shellbark [*C. laciniosa* (Michx.f.) Loud.], and bitternut [*C. cordiformis* (Wangh) K. Koch] most likely had nothing to do with compatibility, but rather, difficulties in obtaining viable pollens, and the timing of pollination. A particular disappointment was our failure to produce progeny from the shellbark x pecan cross. We believe this cross has great promise. Unfortunately, harvests from our pollen sources were difficult. We also had difficulty obtaining adequate pollen from our hican sources, because all were located more than 100 miles from campus. We obtained a good harvest from the McCallister hican on one occasion. The McCallister pollen stored well and some successes were achieved the following year using frozen pollen.

Plans are to continue attempts to broaden our collection of interspecific crosses, and to compare these to parental types in our efforts to understand resistance phenomena. These will be compared as to levels of the individual phenolics, and the relationships of these to infection phenomena with *C. caryigenum*. The methods of Diehl et al. (8, 9, 10) and Graves et al. (15); involving histochemical quantitation at the infection site, scanning and transmission electron microscopy, and confirmatory immunofluorescent procedures, will be used for genotypic comparisons. Information from such studies should provide insight in parental choices for resistance breeding purposes, as well as guidance as to inheritance of resistance factors. Isozyme methodologies

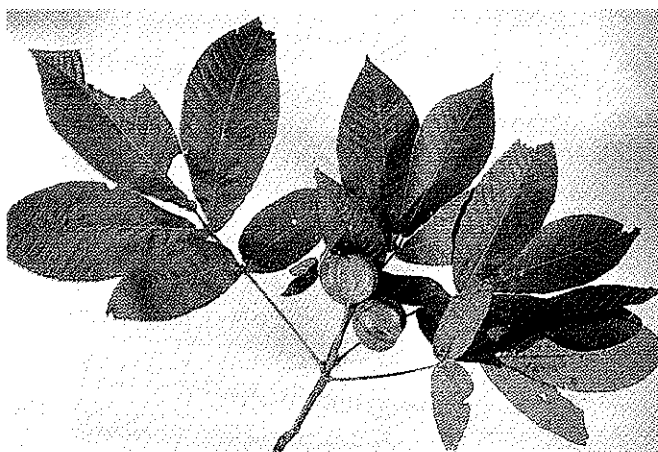
Figure 2. Photographs of some of the major pecan cultivars and hickory and walnut species used in crosses to develop scab resistance in pecan.



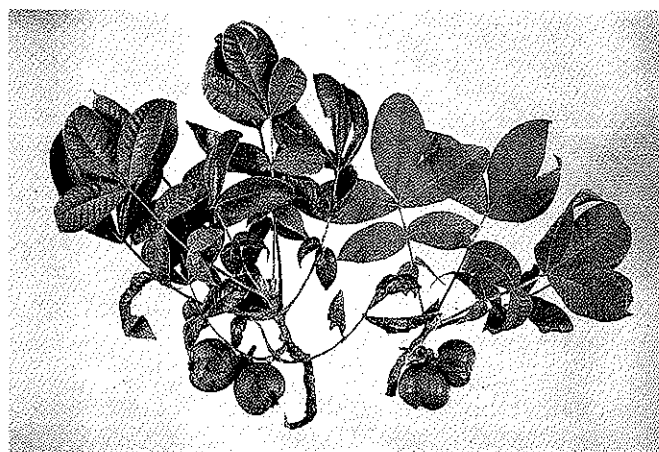
Stuart Pecan



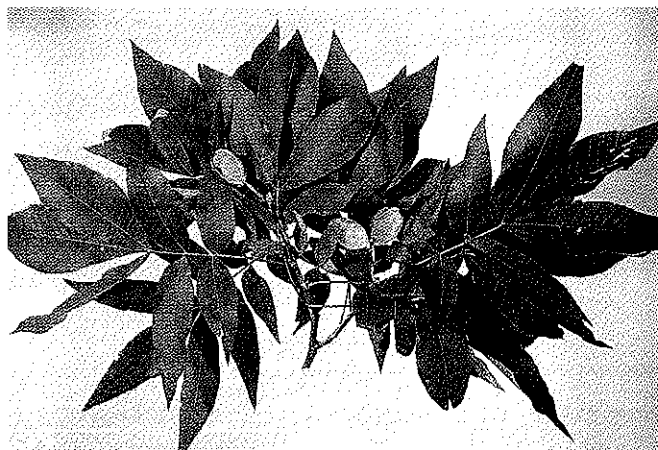
Odom Pecan



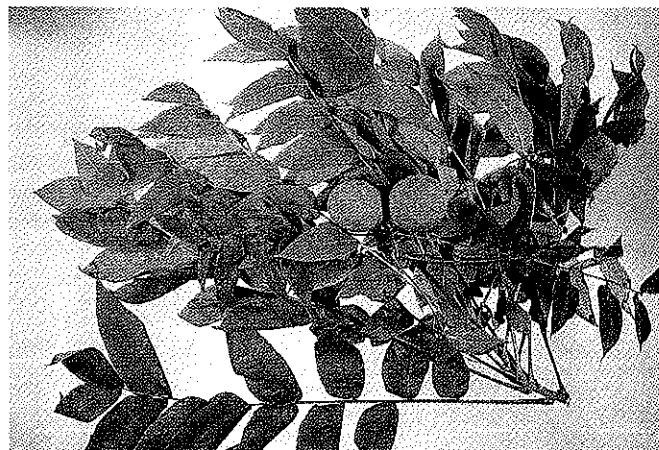
Shagbark hickory



Sand hickory



Nutmeg hickory



Black Walnut



Figure 3. At left is the large McCallister hican harvested from an orchard near Yazoo City, MS. It is more than twice the size of the Stuart pecan on the right.

will be developed to confirm genotypic verity of individual crosses, and as a tool in further breeding work in following inheritance of identifiable resistance factors.

There have been several reports of natural hybrids resulting from crosses between pecan and a number of other hickory species including water (34), shellbark, mockernut, bittersweet (32), and shagbark (6). Additionally, there have been various reports from controlled crosses (20, 28, 29, 36), not to mention verbal reports of hobbyists and growers. This is perhaps the first report of controlled crosses of pecan x nutmeg, red, southern shagbark, sand, and pignut hickories. It is perhaps also the first report of controlled crosses of pecan x black walnut. The hican x pecan backcrosses represent an interesting first step since it is believed that such backcrosses offer the best prospect for introducing improved disease resistance into progeny with acceptable fruit quality.

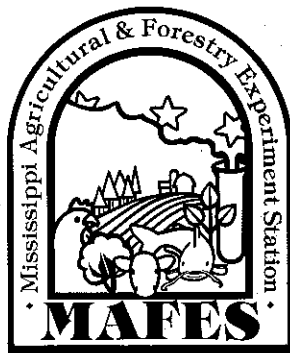
Most hickories (including pecan) have a somatic chromosome number of 32. Some, such as pignut, red, sand, and mockernut, are tetraploids with a chromosome number of 64 (20). Natural crosses between diploids and tetraploids have been reported (31, 32). The resulting triploids are sterile. It is theoretically possible to create fertile hybrids from such crosses by doubling the chromosome number using colchicoid techniques, or perhaps by genetic manipulations that may someday be possible should success be made in achieving somatic embryogenesis through tissue culture methods.

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