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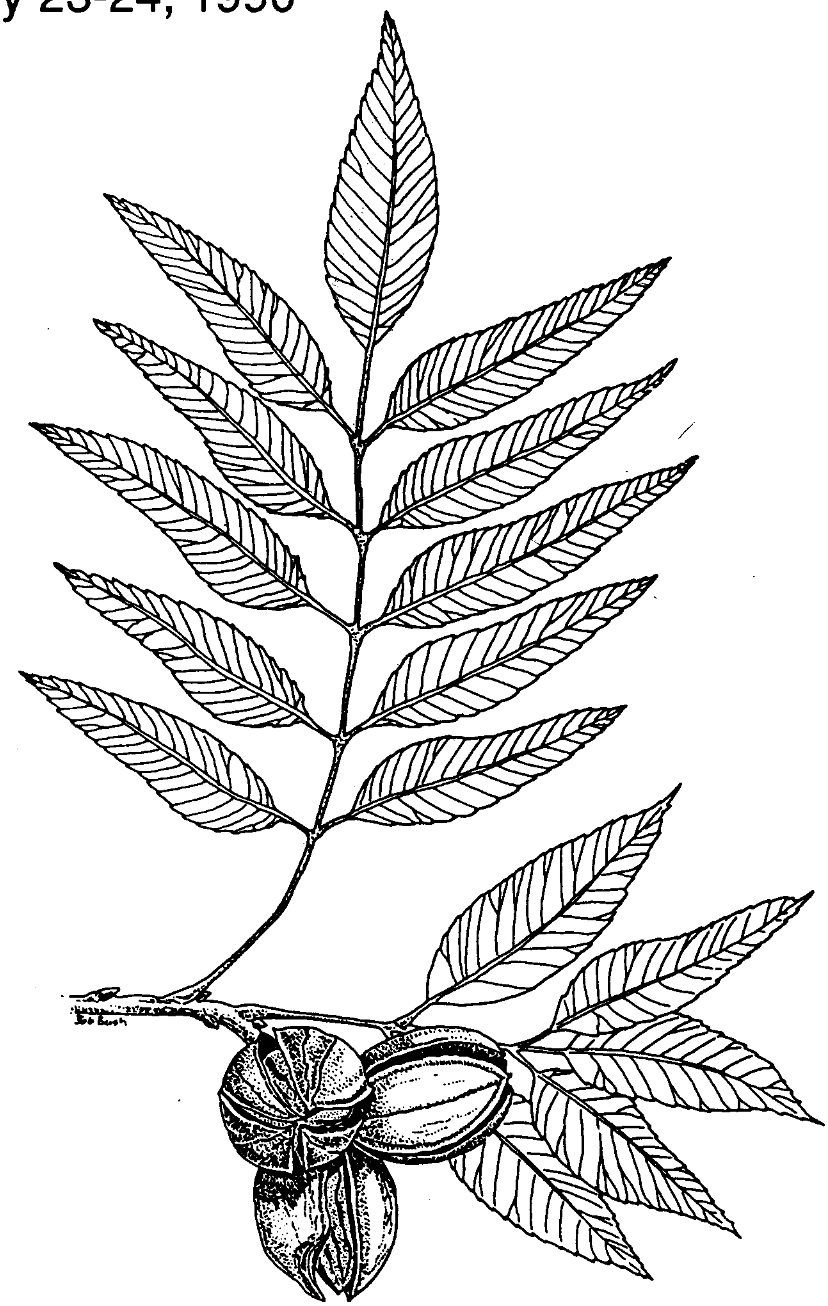

THE CHALLENGES AND PROSPECTS FOR DEVELOPING PECAN CULTIVARS WITH LASTING RESISTANCE TO CROP-LIMITING DISEASES

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ABSTRACT

Disease control comprises a major part of pecan production costs. Resistant cultivars would greatly benefit the industry. Personal observations suggest that quality resistance to many foliar diseases of pecan may be achieved through simple selection processes. Developing resistance to scab, the most serious disease, however, is a more complex problem. Considerable progress has been made in recent years in defining factors associated with scab resistance. Techniques have been devised to facilitate quantitation of these factors. Utilizing these new research findings and techniques, progress is now being achieved toward identification of sources of quality resistance for breeding purposes. Procedures for transfer of quality resistance genes to acceptable pecan types are now available. Development of cultivars with lasting resistance within the near future is now a distinct possibility.

Diseases are limiting factors in pecan (*Carya illinoensis* Koch) culture in the humid southeastern states. Leaf diseases may at times cause complete early defoliation of some cultivars, and often severely limit functional tree leaf area during the growing season. Maintenance of healthy foliage is known to be very important for tree vigor, nut quality, and fruit set on an annual basis. The most significant disease of pecan, however, is scab caused by *Cladosporium caryigenum* (Ell et Lang) Gottwald which most severely affects nut production. Scab may also be prominent on leaves and stems. Under some conditions this disease may cause total crop loss. It exacts a severe penalty every year, if not in crop loss, in the cost of necessary protection measures in most areas of the southeast pecan region.

Control of pecan diseases requires at least seven to eight fungicide applications per season at considerable expense. Concerns over environmental influences of pesticides and constraints caused by increased regulatory activities of the Environmental Protection Agency may severely limit use of fungicides in the future. Further, the failure of commodity prices to keep pace with production costs in recent years has created another serious problem for pecan producers. Disease resistant cultivars would be a decided asset for the pecan industry. We feel that disease resistance is an absolute necessity if pecan production in the humid southeast is to continue to compete. Perhaps most important, following many years of observation and concentrated research into various aspects of scab disease resistance, we are convinced that lasting disease resistant cultivars are attainable, and with concentrated continued research effort, this can be achieved within the next few years. Historically breeding for resistance to disease, with any tree crop, is time-consuming and costly. Thus, initiation of a program with such a goal must be carefully planned, documented with basic research findings, and must employ the latest available plant breeding technologies. We feel that we now have the knowledge, techniques, and plans that will permit significant and immediate progress.

BREEDING FOR SCAB RESISTANCE

Areas of notable research progress in breeding for scab resistance have been centered around several immediate goals, namely: (1) basic understandings of the scab pathogen, variability in nature, adaptive capability, and epidemiology; (2) definition of factors associated with resistance; (3) identification of quality sources of resistance genes; and (4) development of procedures to expedite the incorporation of these resistance genes into horticulturally desirable cultivars.

VARIABILITY AND ADAPTIVE CAPABILITY OF CLADOSPORIUM CARYIGENUM

The scab fungus is known to have great genetic diversity in nature and an operative mechanism for adaptive genetic reconstitution (Alford 1970, Soonthornpoct 1973). There has been a historical pattern of selection and naming of pecan

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cultivars, thought to be resistant to scab, but which, upon mass (commercial) propagation and distribution, have eventually been found to be susceptible to scab. This suggests that there is considerable genetic diversity of the pathogen in nature. KenKnight (KenKnight 1968), using scab inoculum from several sources, demonstrated infection on previously "non-scabbing" cultivars in Louisiana. Street (Street 1972), using a technique devised by McNeill (McNeil and Graves 1970) amenable to quarantine necessities, screened excised nuts of 25 pecan cultivars against 27 isolates of *C. caryigenum*. All cultivars except Baker were significantly infected by one or more of the isolates, and minor infection occurred upon Baker.

Although no true sex stage has been associated with *C. caryigenum*, mechanisms that could account for observed genetic variability and adaptability have been observed. Anastomosing of hyphae, fusion of spores, fusion of newly formed spores with hyphal strands, all of which could play a role in heterokaryosis or parasexual behavior have been observed (Blasingame 1968). Microconidia are produced, in some instances in great profusion, that may function as spermatia in sexual behavior patterns (Blasingame 1968). Somatic hyphae are largely uninucleate, but binucleate hyphae are common. Conidiophores and conidia are multinucleate (Blasingame 1968, Soonthornpoct 1973). Occurrence of genetic recombination can be easily demonstrated in the laboratory employing hyphal-tip or single spore isolates, hyphal-tip or single sporing methods and identifiable cultural markers such as growth rate on standard and minimal media, hyphal and mycelial characteristics, etc. (Alford 1970, Soonthornpoct 1973).

PATHOGENICITY EVALUATIONS

Pathogenicity screening of pecan genotypes for resistance to *C. caryigenum* has posed problems (Graves et al. 1979, McNeill and Graves 1970). First, any screening effort must obviously employ a wide range of isolates representative of the genetic diversity throughout the pecan belt, thus necessitating procedures amenable to quarantine requirements. Secondly, resistance in leaf and nut tissues cannot be correlated, thus screening of seedling foliage, only, is of questionable value. Testing of progeny by plantation throughout the pecan region can be prohibitively expensive, and can at best provide doubtfully adequate exposure in light of obvious genetic diversity and adaptive capability of the pathogen. A thorough understanding of resistance phenomena could provide for the most effective and efficient screening method.

EPIDEMIOLOGY, OCCURRENCE OF SCAB AMONG HICKORIES

Early reports of scab disease have shown that it is not uniformly distributed and does not attack the same pecan cultivars to an equal extent in all locations. The spread of the disease on one cultivar appears to be independent of that on others (personal observation). Buildup of the disease in new plantings has always proceeded slowly, many times requiring years to reach significant levels, and buildup rate has seemingly proceeded independently for each cultivar. The buildup rate in relation to cultivar, however, may differ according to location, suggestive of fungal genotypic variability according to location. There are many native pecans (distinctive genotypes) that do not exhibit scab infection in nature, even though they are located amidst an abundance of "scabbing" trees. Experience suggests that this occurrence is simply related to extent of exposure, i.e., once "non-scabbing" trees are vegetatively propagated, and scattered over the humid Southeastern United States, fungal genotypes capable of parasitizing them will slowly increase. When considering the adaptive capability of the scab fungus, and the range of fungal genotypes apparently present across the pecan belt, the presence of quality, lasting resistance within *C. illinoensis* is subject to question.

Observations (Graves et al. 1982) that native pecan populations exhibit a high level of scab infection, whereas native stands of other hickory species rarely display such infection, suggests that these other species possess resistance factors not prevalent in pecan. It has been our observation that, among native pecan populations of the lower Mississippi Delta region where environmental conditions are conducive to scab development, approximately 60% of the trees will exhibit noticeable scab infection. These observations seem to be borne out by published accounts of KenKnight (1968). In contrast, it was also noted that among native stands of other hickory species in the same region, infection by the scab fungus was rare. This would suggest that these other species possess resistance factors not prevalent in pecan, and that perhaps through interspecific breeding, quality resistance may be linked to desirable horticultural characteristics.

INTERSPECIFIC AND INTRASPECIFIC HYBRIDIZATION

Natural hybrids between hickory species apparently are fairly common. There have been several reports of natural hybrids from crosses between pecan and a number of other hickory species (hicans) including water, *Carya aquatica* (Michx. f.) Nutt. (Stone et al. 1965), shellbark, *C. laciniosa* (Michx.) Loud. (Rehder 1940), shagbark, *C. ovata* (Mill.) K. Koch. (Crane et al. 1937), mockernut, *C. tomentosa* Nutt (Rehder 1940), and bitternut, *C. cordiformis* (Wangh.) K. Koch. (Rehder 1940). There have been published reports of controlled crosses between pecan and other hickory species (Jaynes 1969, McDaniel 1954, McKay 1961, Thielgas et al. 1977, Windham et al. 1981), and likewise there have been verbal reports of such crosses by hobbyists and growers. However, there have been few follow-up reports concerning the results of these crosses and/or the possible use of such hicans in controlled back-crosses with pecan.

Most hickories have a somatic chromosome number of 32, but some, such as pignut, *C. glabra* (Mill.) Sweet, red, *C. ovalis* (Wangh.) Sarg., sand, *C. pallida* (Ashe) Engl. & Graebn, and mockernut are tetraploids with a chromosome number of 64 (Jaynes 1969). Natural crosses between diploids and tetraploids have been reported (Palmer 1937, Rehder 1940). Although the resulting triploids are sterile, it is theoretically possible to create fertile hybrids from such crosses by doubling the chromosome number using colchiploidy techniques, or perhaps by genetic manipulations that may someday be possible should success be achieved in somatic embryogenesis through tissue culture methods.

Current knowledge of resistance has been utilized in parental selections in an ongoing interspecific, intraspecific hybridization program on the Mississippi State University campus. Progeny of these crosses have been established in a nursery to be used for evaluation of resistance phenomena, studies relative to mode of inheritance of resistance factors, and as a possible source of parental materials for a resistance breeding program. An effort has been made to achieve as many crosses between pecan and other hickory and walnut species as possible. To date, a total of 76 interspecific and 5 intergeneric crosses have been accomplished involving 7 *Carya* spp. and 1 *Juglans* spp. In addition, 132 intraspecific crosses involving pecan cultivars of interest have been made (Graves et al. 1989). An effort is currently being made to utilize isozyme methodologies to verify parentage of crosses.

RESISTANCE FACTORS

Resistance to any pathogen is likely to be the result of multiple factors in nature. Physical and chemical deterrents to pathogen spore germination and penetration are components of a plants defense system. Wetzstein and Sparks (Wetzstein and Sparks 1983) correlated pecan leaf resistance to *C. caryigenum* and the presence of fewer glandular trichomes and a greater frequency of collapsed trichomes, in addition to abundant phenolics in the palisade parenchyma and bundle sheath cells. Glandular trichomes were apparent on both the abaxial and adaxial leaf surfaces and occasionally exhibited extruded material. Susceptible cultivars also had a greater diversity in trichome diameter than resistant cultivars. Latham and Rushing (Latham and Rushing 1988) found that among inoculated *C. caryigenum* conidia landing near trichomes, a majority (82.2%) of the conidia upon germination grew to the base of the trichomes where penetration occurred. Influence of trichomes may result in altered patterns of leaf wettability, humidity at the leaf surface, solution retention and conidia penetration (Grauke et al. 1988, Wetzstein and Sparks 1983).

Wood et al. (Wood et al. 1988) noted several phylloplane associated substances that either had an inhibitory, neutral or promotive effect on *C. caryigenum* conidial germination. They hypothesized that pecan susceptibility to scab is partially dependent upon phylloplane composition.

Work having to do with the relationship of plant constituents to scab resistance may be summarized with the following notations:

1. Certain plant constituents common to the Juglandaceae and considered of significance in disease resistance have been identified. Plant phenolic derivatives have been identified as major among these (Hedin et al. 1980, Hedin et al. 1979, Laird et al. 1990, Langhans et al. 1978).

2. Juglone, the condensed tannins, and isoquercitrin have been identified as principal phenolics of pecan and hickory, and they obviously play a role in disease resistance. Juglone, which also occurs in walnuts, has been shown to be a chemical host factor associated with resistance of pecan and other members of the Juglandaceae to scab (Borazjani et al. 1983, Borazjani et al. 1985, Graves et al. 1979, Hedin et al. 1979, Langhans et al. 1978). Juglone and hydrojuglone glucoside have also been

correlated with resistance in juvenile leaves of black walnut to anthracnose by other researchers (Cline and Neely 1984). The condensed tannins and isoquercitrin, extracted from pecan, have been shown to be highly toxic to the scab pathogen, although field correlations have not yet been completed (Laird et al. 1985, Laird et al. 1990).

3. Microspectrophotometric methods for histochemical localization and quantitation of these three principal phenolics in hickories and walnuts have been developed and are being used to ascertain occurrence within tissues and ultimately the full role of phenolics in disease resistance. The respective levels of each of these allelochemicals in combination may determine the quality of resistance (Diehl et al. 1989, Diehl et al. 1990, Graves et al. 1986).

4. Research has indicated a highly significant difference in phenolic levels between infected and noninfected tissues. The host responds to challenge by the pathogen with increased phenolic production. Thus quality resistance cannot be determined solely on the presence of preformed phenolics. Evidence indicates that the total phenolic presence is a better indicator of quality resistance than any of the principal phenolics alone (Diehl et al. 1990).

5. Progress in procedures that will combine the specificity of the antigen-antibody reaction plus a fluorescent probe to localize the phenolics *in situ* has been achieved (Diehl, unpublished). These immunofluorescent procedures together with electron microscopy methods will provide validation of the histochemical procedures. Transmission electron microscopy (TEM) procedures have been developed for localization of phenolics within cell vacuoles (Diehl et al. 1988).

6. Both TEM and scanning electron microscopy (SEM) methods have been developed, and are being utilized to follow fungal infection processes of the scab fungus in pecan which will be related to both preformed phenolic presence and host phenolic responses to the invading pathogen (Diehl et al. 1990, Diehl et al. 1988).

In summary, selection for resistance to many foliar diseases appears to be feasible. However, scab, the most serious disease, poses a more difficult problem. Considerable genotypic diversity of the scab pathogen, *C. caryigenum*, seems obvious in nature. In addition, the fungus exhibits characteristics that would permit necessary genetic recombinations that may account for the obvious adaptive capability present. Historical observations, results of selection attempts, together with the genetic diversity and adaptability of the pathogen suggest that selection of cultivars with lasting resistance to scab within the species *C. illinoensis* is subject to question. By contrast, the low level of scab incidence on other *Carya* spp. and the apparent ease by which hican hybrids can be achieved creates interesting possibilities for transferring quality resistance genes to horticulturally acceptable pecan types.

Factors associated with resistance have been identified. Three principal hickory phenolics identified have been shown to be highly toxic to the scab pathogen, and indications of a strong role in resistance have been detailed. Research has indicated that there is a host response to the pathogen by increased phenolic levels, and that total phenolic levels within challenged tissues greatly influence resistance. Histochemical procedures for quantitation of phenolic response plus enumeration of other resistance factors should permit evaluation of parental sources and subsequent breeding progenies for an intelligent resistance breeding program. The collection of interspecific crosses begun at Mississippi State University may prove a strong resource for such a program.

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