Reproductive Ecology of Carya (Juglandaceae): Phenology, Pollination, and Breeding System of Two Sympatric Tree Species

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REPRODUCTIVE ECOLOGY OF CARYA (JUGLANDACEAE): PHENOLOGY, POLLINATION, AND BREEDING SYSTEM OF TWO SYMPATRIC TREE SPECIES[1]

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ABSTRACT

Observations and experiments on both shagbark (*Carya ovata*; $2n = 32$) and mockernut (*C. tomentosa*; $2n = 64$) hickories in a New Jersey forest were performed over a 2-year period to determine the mechanisms operating both to maintain reproductive fidelity and to regulate fruit initiation. Phenological observations indicated significant differences between the species for almost all vegetative and reproductive characters examined; however, a large amount of overlap was also observed. Pollination experiments indicated that pollen limitation is not a significant factor in reducing fruit set in these anemophilous species. Pollen-ovule ratios ranged from 1 to 25×10^6. Pollen was 80–90% viable at time of shedding. The large surface area of vegetative structures (e.g., leaves) at the time of pollination may retard the flow of pollen into and out of the canopy and thus increase self-pollination. A full diallel cross, replicated 5 times, was made among 8 trees (4 trees of each species) to characterize combining ability and compatibility relationships. All trees were found to be both geitonogamous and xenogamous to varying degrees. A high frequency of putative interploidal cross-compatibility was also observed. Fruits from these interspecific crosses were found to be germinable. Observations on individual trees suggested that dichogamy may help to reduce selfing and promote outcrossing in this breeding system. While most trees exhibited a temporal separation of genders, the trait was found to be quite plastic (i.e., trees switched between protogyny and protandry between years). Pistillate flowers were receptive for all trees within a species during the same general time period; however, pollen shedding varied considerably among trees and between years. Both species appear to be utilizing a broad array of mechanisms in order to maintain reproductive fidelity and adequate fruit set.

HICKORIES (*Carya* spp., Juglandaceae) are a widespread and important genus in eastern North America (Fowells, 1965). They have been observed to be dominants or codominants in many eastern deciduous forest types (Braun, 1950). Perhaps more importantly, this genus possesses a set of life history characteristics which are shared among many co-occurring North American taxa (e.g., *Carya, Castanea, Fagus, Juglans, Quercus*); all have a similar complex life cycle, pollination syndrome (anemophily), breeding system (monoecy), dispersal syndrome (mammalian predator-dispersers), and regeneration strategy (shade-tolerant, slow growth, etc.). Additionally, many of the species within certain genera (most notably *Quercus*) can and do hybridize (Gleason and Cronquist, 1963), probably due to the nondirectional nature of the pollination process. Perhaps a single characteristic, that of the arboreal habit, has resulted in a dearth of published experimental information on the early reproductive biology (flower to fruit set) of most of these hardwood taxa in natural populations.

We have selected two hickory species (*Carya ovata* and *C. tomentosa*), growing in a natural community, to assess the reproductive characteristics influencing both the transfer of pollen and fertilization. Much significant research has been done with this genus for applied purposes (i.e., forestry and horticulture). The reproductive biology of *Carya illinoensis*, the cultivated pecan, has been well studied (reviewed by Madden and Malstrom, 1975).

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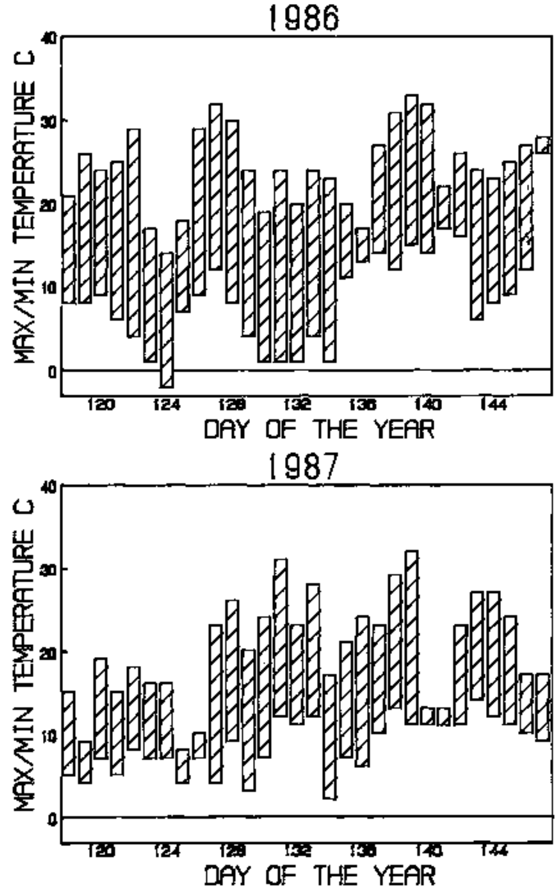


Fig. 1. Daily maximum and minimum temperatures (top and bottom limits of bars) during the peak flowering periods of *Carya* spp. in 1986 and 1987 at Hutcheson Memorial Forest, Somerset Co., NJ.

Studies of *C. illinoensis* cultivars suggest that fruit set may be substantially affected by inviable pollen (Woodroof, 1930; Adriance, 1931); metaxenia (pollen influences), female choice, and heterosis (Romberg and Smith, 1946); and degree of dichogamy (Stuckey, 1916; Smith and Romberg, 1940; Madden and Brown, 1973).

The extent and degree of biotic factors affecting fruit set (i.e., fruit initiation) in natural populations of *Carya*, as well as many other tree species sharing its general life history pattern, are virtually unknown. If we are to understand the demographic dynamics of natural plant populations in an evolutionary context (Harper, 1967), it is imperative that analyses follow the dynamics of individual plants from "zygote to zygote" (Harper, 1977; Solbrig, 1980; Nakamura, 1988). Both genetic and environmental factors are significant sources of mortality affecting the dynamics of populations early in the life cycle of plants (Lee, 1988). Both natural and sexual selection may produce differential mortality patterns favoring the progeny of some members of the population while rejecting others. As pointed out earlier (McCarthy and Quinn, 1989), data on reproductive allocation in long-lived woody species are sorely needed for the development, testing, and generality assessment of many theoretical expectations concerning the reproductive biology of plants (e.g., plasticity of sex expression, factors influencing seed set, mate choice, and mate competition).

We studied the flowering and reproductive biology of single populations of *Carya ovata* and *C. tomentosa* in a New Jersey mixed oak forest to answer the following questions: 1) Are reproductive events temporally partitioned and does phenological separation promote species fidelity? 2) What degree of dichogamy (temporal separation of genders) is expressed within and among individuals of a species? 3) How plastic is this trait (i.e., does dichogamy vary from year to year)? 4) What proportion of the pollen is viable? 5) What is the pollen–ovule ratio? 6) Is pollen limiting fruit set, and what factors might impede successful pollination? 7) Are these two species, differing in ploidy levels, cross-compatible and with what frequency might they cross in nature? 8) Is there any evidence of female choice?

MATERIALS AND METHODS—*Study site*—Field work was conducted at the Hutcheson Memorial Forest (HMF) located in Somerset County, New Jersey (40°30′N, 74°34′W). HMF experiences a subcontinental temperate climate with 112 cm of precipitation annually and a mean annual temperature of 11.7 C (Robichaud and Buell, 1973). Daily high/low temperatures during the peak flowering periods are presented in Fig. 1. Forests of the region are generally considered as mixed oak (Robichaud and Buell, 1973; Forman and Elfstrom, 1975) and were included in the oak-chestnut region of Braun (1950). The forest lies on soils developed from the Triassic red shales of the Piedmont (Wolfe, 1977). These soils may be generally classified as Penn Shaly Silt Loams (Kirkham, 1976). All work was conducted on trees in a wooded hedgerow adjacent to the south end of the forest.

Study species—*Carya ovata* (Mill.) K. Koch (shagbark hickory; $2n = 32$, Stone, 1963) and *C. tomentosa* (Poir.) Nutt. (mockernut hickory; $2n = 64$, Stone, 1963) are common tree species in many eastern North American deciduous forests (Braun, 1950; Fowells, 1965). Both species are sympatric throughout much of their

TABLE 1. *Phenological categories, and associated levels, examined for shagbark and mockernut hickories in 1986 and 1987*

Phenological category	Class levels	Description
Shoot development	No shoot present	Prebud burst, no new shoot growth
	Shoots initiating	Postbud burst, active growth phase
	Shoots elongated	Shoots have reached >90% of primary growth
Leaf development	No leaves present	Prebud burst
	Leaves protruding[a]	Bud burst, leaves appear but blades folded
	Leaves open	Period of leaf blade unfolding and expansion
Catkin development	No catkins present	No visible staminate inflorescences
	Catkins pendant	Postbud burst
	Catkins shedding	Catkins mature, period of pollen release
	Catkin senescence	Postpollen shedding, catkin withers and is shed
Pistillate flower development	No flowers present	No visible pistillate flowers
	Flowers visible	Postshoot initiation, pistillate flowers not developing
	Flowers receptive	Stigmatic surface sticky
	Flower senescence	Either flower is aborted *or* fruit is set (initiated)

range along with several other congeners (McCarthy and Wistendahl, 1988). The southernmost portion of the Hutcheson Forest has *C. ovata* and *C. tomentosa* as the primary cooccurring congeners; *C. ovalis* is present at HMF but with a much lower abundance and is not within 300 m of any study tree. Species identifications were made using Manning (1950) and Gleason and Cronquist (1963). Nomenclature follows Gleason and Cronquist (1963). Voucher specimens have been deposited at the Chrysler Herbarium of Rutgers University.

Four shagbark and four mockernut trees were selected for study. The intensive nature of this study, and the lack of other *Carya* trees in the area with comparable microenvironments and of similar age, size, and reproductive status, precluded larger sample sizes. The eight trees used for this study are the same trees as those described in McCarthy and Quinn (1989; see Table 1). Observations and experiments were conducted from sampling towers erected adjacent to each tree (see McCarthy, 1988).

Carya ovata and *C. tomentosa* are both monoecious and anemophilous. Flowers appear concurrently with the unfolding of leaves (Daniel, Helms, and Baker, 1979); however, pollen may not be shed until leaves are almost fully expanded (personal observation, BCM). The morphology of the staminate inflorescences, pistillate flowers, and fruits has been described in detail by Manning (1940, 1948). The reproductive biology has been summarized in Madden and Malstrom (1975) and McCarthy and Quinn (1989). Briefly, apical buds give rise to a leafy shoot that may differentiate terminal pistillate flowers and 1–12+ proximal (axillary to bud scales) staminate inflorescences (catkins). Thus, these species are herkogamous; the male and female genders are separated by the length of the newly formed shoot. Catkins are initiated in the early summer of the year prior to the year in which they produce pollen (Woodroof and Woodroof, 1927). Pistillate flowers, each bearing a single orthotropic ovule, are borne in a terminal cluster of 1–5 flowers and differentiate in early spring after shoot initiation (Shuhart, 1927).

Phenology observations—In 1986 and 1987, before the buds had swollen in early spring, 10 shoots were randomly selected from that portion of the canopy accessible via the sampling tower of each of the eight study trees. These 80 shoots were then individually evaluated and scored every 24 hr for ca. 50 days. Four phenological categories were recognized: shoot development, leaf development, catkin development, and pistillate flower development (see Table 1). These categories were selected for a determination of both the rate of phenological change and the correlation between stages (e.g., what and how much interference may be contributed by vegetative structures to pollen dissemination) both within and between species. Detailed information on the timing of pollen shedding and stigma receptivity was used to determine the extent and degree of dichogamy.

Pollination observations and experiments—Determining exactly when pollen is shed from the catkins can be difficult. To circumvent this problem we selected 20 shoots from each tree, stratified such that 10 were from the top of the tree and 10 were from the bottom, and bagged only the catkins. We used transparent Slip-Ezy pollination bags (Model 3295, 8 × 5 × 24 cm, available from Corn States, St. Louis, MO).

Carya pollen is bright yellow, and thus we were able to determine exactly when pollen was shed, and to what degree, based on the yellowness of the transparent pollination bags. The pollen from these bags was subsequently used for performing viability tests and experimental pollinations.

At the time of pollen shedding, data were also collected on shoot morphometrics from 10 shoots (5 at each height position). We recorded the length (to nearest 0.1 cm) and width (to nearest 0.1 mm) of the twig, and number of leaves produced. Leaves were then harvested, stored in plastic bags, and brought to the laboratory to determine leaf area (cm^2) using a Li-Cor area meter. All morphometric variables were subsequently used to derive area variables to determine the percentage of nonstigmatic interfering tissues.

At time of pollen shedding, the bagged catkins from 5 shoots were brought to the laboratory for determination of pollen viability. Pollen from each shoot was allocated to two different storage methods: half was stored at room temperature (ca. 20 C), and the other half was stored in a freezer at −10 C. Pollen was analyzed for viability that day, and each day thereafter (for 7 days), at the two pollen storage temperatures. Viability was determined by the fluorochromatic test procedure described by Heslop-Harrison and Heslop-Harrison (1970). Pollen-ovule ratios were determined by collecting matured catkins ca. 1–3 days before anther dehiscence. Individual anthers were excised, squashed on a microscope slide, and stained (using the techniques employed for pollen viability tests) to determine the number of grains per anther. The number of grains per shoot was extrapolated from the number of anthers per catkin and catkins per shoot. Upon collection of the pollen, the number of catkins and pistillate flowers was recorded for each shoot (of the 10 shoots from which morphometric data were collected). Pollen-ovule ratios were determined based on the number of pollen grains and female flowers per flowering shoot.

We performed an experiment in 1987 to determine if pollen was limiting to fruit set by comparing naturally-pollinated flowers to hand-pollinated flowers. Twenty shoots were randomly selected within each canopy; ten were allocated to an experimental pollen-supplementation treatment, while the other ten were allocated to an open-pollination control. Pollinations were performed using pollen collected from the same tree (or nearest conspecific neighbor if dichogamy was close to complete) and applied with a small sable-haired brush. Stigma receptivity is reliably indicated when the stigmatic surfaces become visibly sticky with a mucilaginous substance (Madden and Malstrom, 1975). A pilot project performed the previous year employing two pollen loads, low (<100 grains) and high (saturated with >1,000 grains), did not indicate any difference in fruit set (t-test, $P = 0.50$, $N = 10$). Therefore, we concluded that stigma clogging is probably not a problem and performed the pollinations by saturating the stigma with pollen.

Breeding system experiments—To determine general combining ability (Wright, 1976) and compatibility relationships (within trees, among conspecifics, and between congenerics), we established a full diallel cross among the eight trees (four of each species). Each tree thus served as both a pollen donor (paternal parent) and a pollen recipient (maternal parent) for crosses with itself and every other tree (64 total possible crosses). Forty shoots were randomly selected, emasculated, and bagged on each tree prior to pistillate flower formation. Since the pistillate flowers are subtended by the developing leaves we chose to bag the entire shoot using 40 × 17 × 12 cm Pollen-Tector pollination bags (Model 1250/OT, available from Carpenter Paper, Des Moines, IA). These 40 shoots were subdivided into eight sets of five replicates, one set for each pollen donor tree including itself. Pollen collection and application procedures are the same as described above for the pollination limitation experiment. Here, however, pollinations were always performed before 0900 hr; previous studies have shown that airborne pollen is lowest in the morning (Traub and Romberg, 1933). In this way we are relatively confident that the only pollen arriving on the stigma was that coming from the designated pollen source.

The success of the crosses was determined approximately 60 days later. A cross was considered successful if the flowers set fruit and maturation was initiated (i.e., both ovary wall expansion and kernel filling) for the majority of fruits on that shoot. Unfortunately, these fruits require a maturation period of ca. 150 days and many never reach maturity due to a variety of nongenetic reasons (e.g., shoot resources, environment, predation, etc.). Accordingly, we were unable to collect a sufficient number of matured fruit that year for the further study of offspring quality. We were able to collect small samples of fruits ($N = 10$–15) from self, conspecific, and congeneric crosses (trees pooled) to determine germinability. Seeds

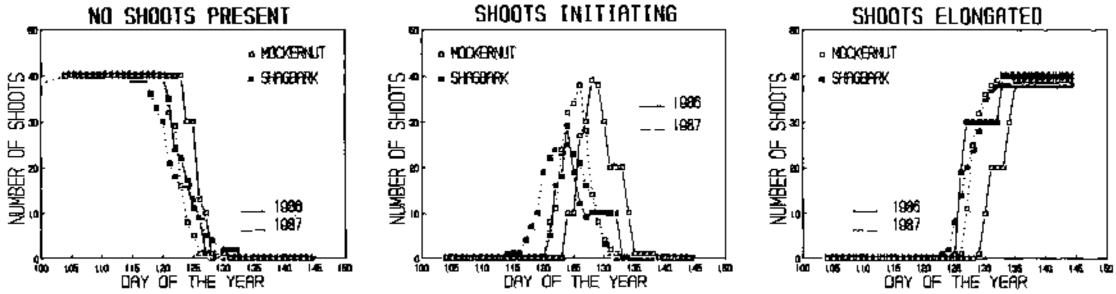


Fig. 2. Shoot phenology patterns for 40 shoots each of *Carya ovata* (shagbark hickory) and *C. tomentosa* (mockernut hickory) in 1986 and 1987. See Table 1 for a description of categories.

were packed in trays of 50:50 vermiculite:sand, moistened, cold-stratified at 5 C for 90 days, and germinated in a greenhouse.

Data analyses and presentation—Phenological data were analyzed using various univariate descriptive and nonparametric test procedures provided by STATGRAPHICS (STSC, 1986). Kolmogorov-Smirnov two-sample tests (Sokal and Rohlf, 1981) were used to analyze differences between species, within years, for each of the categorical levels described in Table 1. Due to the large number of pairwise comparisons we selected an alpha level of $P = 0.01$ in order to reduce the probability of committing Type I errors. Data are presented as distribution functions (by year and species) for each categorical level.

Pollination data were also evaluated using a variety of procedures provided by STATGRAPHICS (STSC, 1986). Differences between species for twig and leaf area were analyzed using *t*-tests after square root transformations. Backtransformed means ±1 SD are presented. Pollen viability and pollen-ovule relationships among trees and between species were compared using the Kruskal-Wallis test. Data from the pollination limitation experiment were analyzed by *t*-test at an alpha of $P = 0.05$.

Due to the small number of matured fruits available, quantitative genetic covariances could not be determined. Instead, data are qualitatively presented in terms of relative crossabilities, based on the number of replicates which successfully initiated fruit (three categories: poor [no fruit set], fair [1 or 2 reps set fruit], good [3+ reps set fruit]).

RESULTS—*Phenology*—Shoots of both species tend to follow a predictable pattern in a phenological sequence. Buds would swell (early April) for ca. 2 weeks prior to bud break. At bud break, the juvenile catkins would emerge first and begin to unfold; the catkins would take 2–5 days to become pendant and an additional 10–20 days to reach maturity (i.e., pollen shed). Generally, any given catkin would release its pollen over a period of 1 day; however, pollen release for the whole tree might last 3–4 days (usually only 1–2 days). Leaves would begin to protrude from the bud 4–8 days after bud break and catkin emergence, and begin to unfold 1–4 days thereafter. As the leaves unfold and the blades expand, shoot growth is initiated, requiring 3–7 days to reach near max-

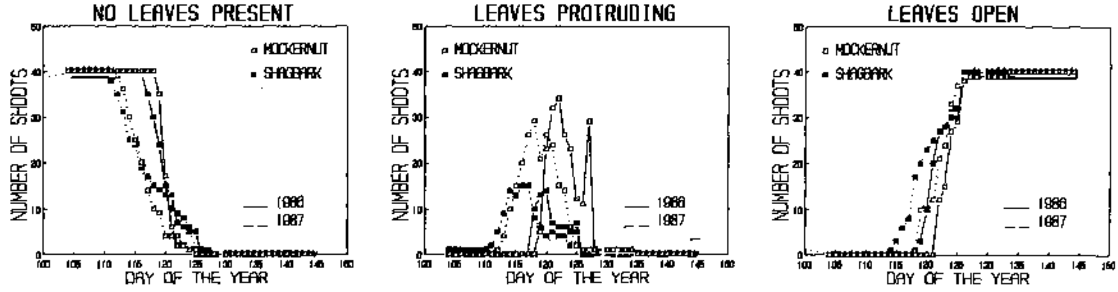


Fig. 3. Leaf phenology patterns for 40 shoots each of *Carya ovata* (shagbark hickory) and *C. tomentosa* (mockernut hickory) in 1986 and 1987. See Table 1 for a description of categories.

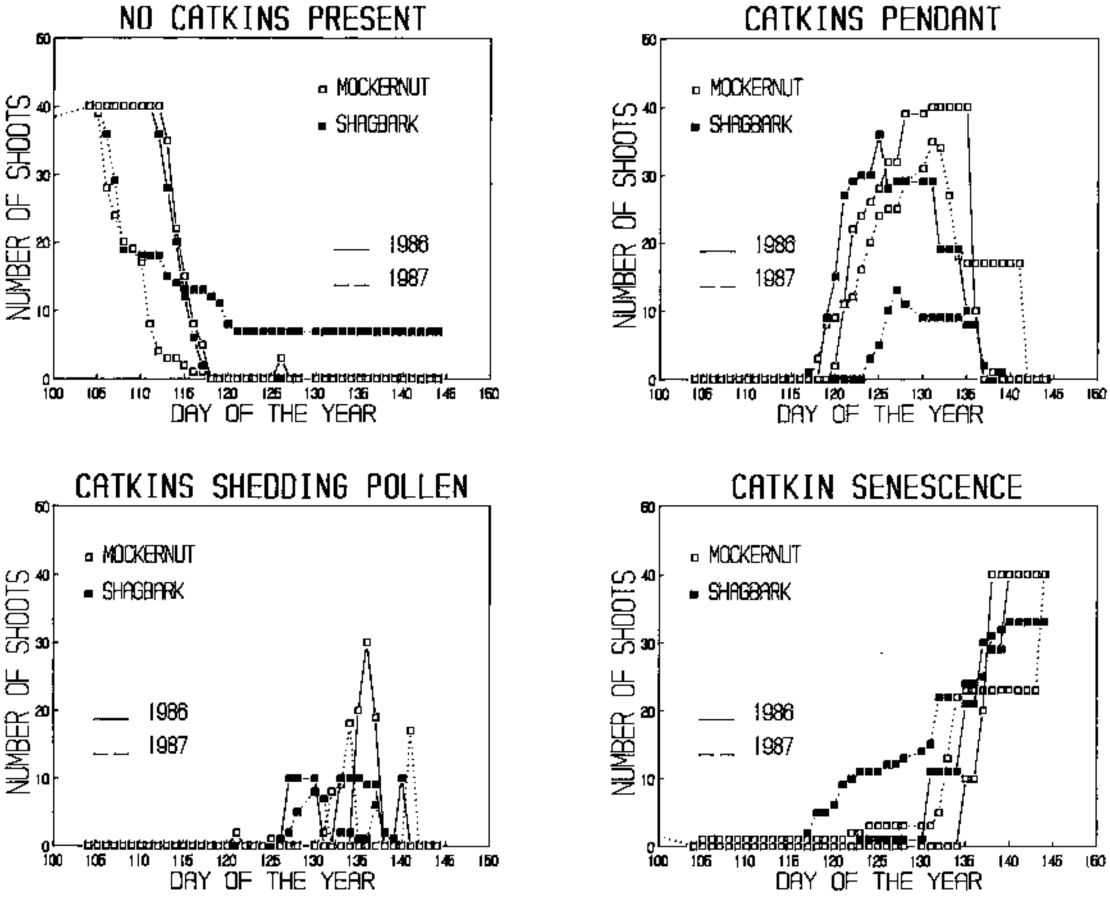


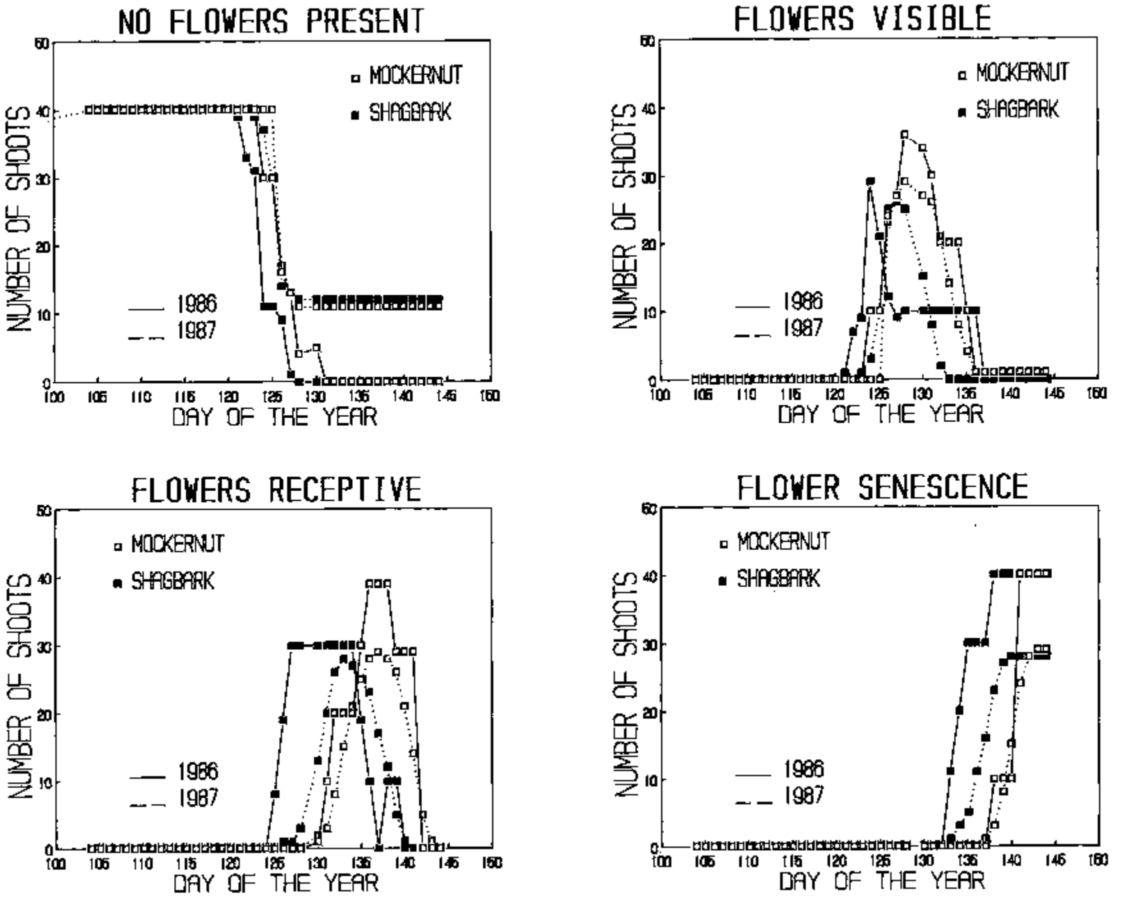
Fig. 4. Catkin (staminate inflorescence) phenology patterns for 40 shoots each of *Carya ovata* (shagbark hickory) and *C. tomentosa* (mockernut hickory) in 1986 and 1987. See Table 1 for a description of categories.

imal elongation. Upon (or slightly before) reaching maximal primary growth of the shoot, the pistillate flowers would appear and require 4–10 days to become receptive. The period of receptivity (determinable by a viscid fluid which covers the stigmatic surface) lasts ca. 1 week.

In each year, both species typically responded in a similar fashion (Fig. 2–5). The phenological sequence of shoot development was at least as similar for different species within the same year as it was for the same species between years. However, in over 90% of the phenological traits examined (Fig. 2–5) there was a highly significant difference ($P < 0.0001$) between species within a year (28 pairwise K-S tests). While there may be a statistical difference in the cumulative distribution frequencies, a considerable amount of overlap (>50%) is also indicated in almost all traits (Fig. 2–5). Airborne pollen and receptive stigmas are present for both species within a similar period.

No striking relationship between pollen shedding and temperature (Fig. 1, 4) is indicated other than the tendency for a tree to shed pollen on a day (or immediately after a day) of relatively cool minimum temperatures. No pollen was observed to be released on rainy or high humidity (>85%) days. The exact time of pollen shedding must be largely induced by the environment because some trees were observed to have anthers with mature pollen many days before actual release.

The response patterns of individual trees (Fig. 6, 7) demonstrate two important points. First, shoot growth and leaf expansion are almost always initiated at or before the time of pollination (except for tree H in 1986). Thus, a considerable amount of leaf and twig area is present at the time of pollination (Table 2). These surfaces represent nontarget (i.e., nonstigmatic) tissues; the total area tends to vary more between years than within a year and is


NO FLOWERS PRESENT

FLOWERS VISIBLE

FLOWERS RECEPTIVE

FLOWER SENESCENCE

Fig. 5. Flower (pistillate) phenology patterns for 40 shoots each of *Carya ovata* (shagbark hickory) and *C. tomentosa* (mockernut hickory) in 1986 and 1987. See Table 1 for a description of categories.

clearly correlated with the other measured phenological traits. There is no clear relationship, however, between the amount of nontarget surface area at the time of pollen shedding and the fruit set for 1986 and 1987 (Table 2).

Second, while shoots of individual trees tend to be synchronous in gender state, the range and extent of dichogamy are variable. Some trees within a species were protandrous, some protogynous; separation of genders within a given species varied both among trees and between years (Fig. 6, 7). The temporal separation of genders was complete (i.e., no overlap) on some trees in some years and incomplete (some overlap) in others. Tree J (shagbark) only produced 9 catkins and 12 pistillate flowers in 1987; there was an insufficient number of catkin-bearing shoots to determine pollen shedding in this year (Fig. 6).

Pollination—Pollen viability determined at the time of shedding in 1987 was found to be quite high (>84%) for all trees of both species (Table 3). Pollen viability decreased ca. 10% ($P = 0.10$) over the 7-day measurement period for both species at both storage temperatures (data not shown).

Pollen-ovule ratios ranged from 670,656 to 21,003,640 and tended to be slightly higher ($Z = 0.883$, $P = 0.377$) for mockernut (Table 4). The difference between species in pollen-ovule ratios is much more pronounced ($Z = 1.746$, $P = 0.081$) if the data for mockernut tree H are deleted; the catkins of this tree consistently exhibited a bizarre development pattern where the distal portion of each catkin was sterile, bearing no anthers or pollen. However, the pollen produced in the functional anthers was viable.

The pollen supplementation experiment indicated that pollen is not a limitation on fruit set for either shagbark or mockernut hickories. While the mean fruit set was slightly higher for pollen-supplemented flowers, there was no sig-

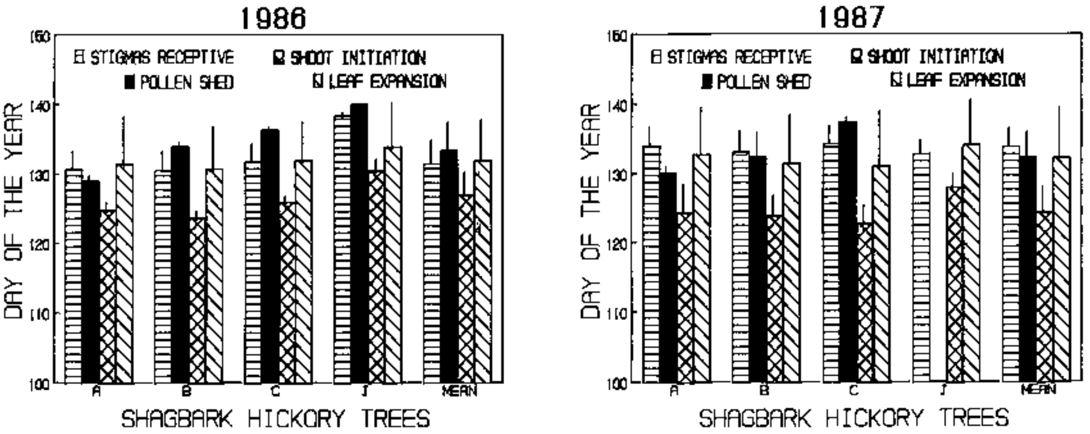


Fig. 6. Mean (±SD) phenological patterns for selected traits among trees of *Carya ovata* in 1986 and 1987.

nificant difference in fruit set (*t*-tests, ns). Mean fruit set was 76% for pollen-supplemented flowers and 70% for flowers receiving a natural pollen load in shagbark; the values were 85 and 70%, respectively, for mockernut.

Breeding system—The diallel cross was not completely balanced for several reasons. The female flowers of tree A (shagbark) were not receptive long enough to make any other crosses (with a great degree of confidence) other than A to itself. As mentioned earlier, tree H did not produce a sufficient amount of pollen, during the peak flowering period, to be used for experimental purposes. Various other crosses were also not possible due to the timing of pollen production and flower receptivity.

The crosses that were made (Table 5) indicate a high degree of compatibility both within and between species. However, one maternal tree in each species (C and G) showed disproportionate incompatibility (i.e., failure to set fruit) regardless of whether the paternal parent was conspecific or heterospecific. No tree performed disproportionately poorly as a paternal parent.

Fifteen nuts, from crosses where the paternal parent was a *Carya ovata,* were collected from various *C. tomentosa* trees. Twelve of these fifteen (80%) seeds germinated and produced a radicle twice the length of the seed. Fourteen nuts, originating from crosses where the paternal parent was a *C. tomentosa,* were collected from various *C. ovata* trees. Eight of these fourteen (57%) seeds germinated and produced a radicle twice the length of the seed. A total of 1,750 nuts, arising from natural pollination, were collected from tree E (i.e., paternity unknown, all at least half-sibs) for other purposes and 1684 germinated (96%) and pro-

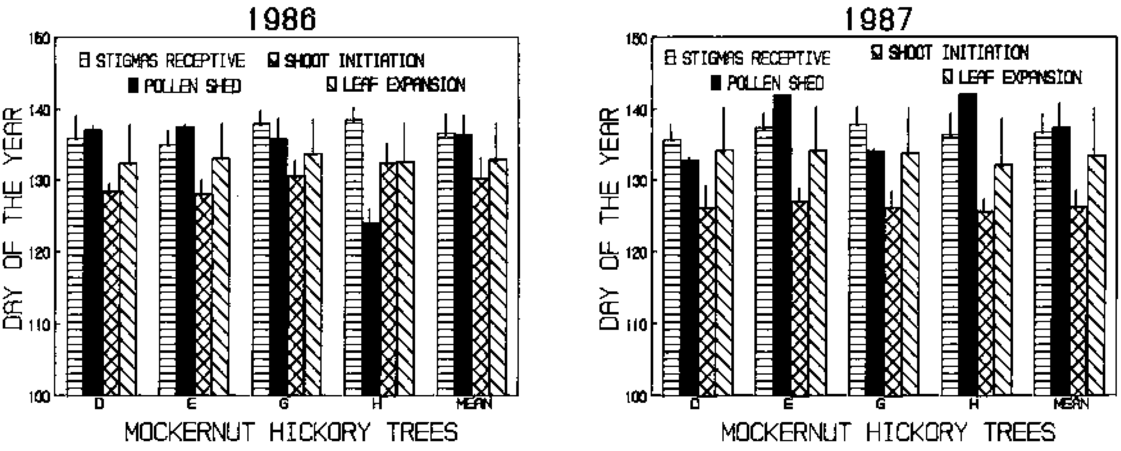


Fig. 7. Mean (±SD) phenological patterns for selected traits among trees of *Carya tomentosa* in 1986 and 1987.

TABLE 2. *Mean (±SD) of vegetative and reproductive characters of shoots (N = 40) of shagbark and mockernut hickories in 1986 and 1987*

Character		1986	1987
No. of catkins[a]	Shagbark	6.0 ± 2.2	4.9 ± 5.6
	Mockernut	6.7 ± 3.5	10.3 ± 8.2
	P	0.2501	0.0004
No. of flowers	Shagbark	2.0 ± 0.8	1.3 ± 1.0
	Mockernut	2.4 ± 1.5	1.9 ± 1.0
	P	0.1401	0.0166
No. of fruits	Shagbark	0.9 ± 0.9	0.9 ± 0.9
	Mockernut	1.4 ± 1.1	1.4 ± 1.1
	P	0.0114	0.0201
No. of leaves	Shagbark	4.2 ± 1.1	4.1 ± 1.1
	Mockernut	6.1 ± 1.8	4.6 ± 0.9
	P	0.0001	0.0477
Leaf area[b] at pollen shedding	Shagbark	227.9 ± 99.5	181.5 ± 78.35
	Mockernut	116.6 ± 45.8	172.3 ± 84.8
	P	0.0884	0.8705
Twig area[c] at pollen shedding	Shagbark	108.2 ± 20.3	60.6 ± 14.8
	Mockernut	241.5 ± 4.31	142.0 ± 70.5
	P	0.0032	0.0374
Total area[d] at pollen shedding	Shagbark	1,094.2 ± 500.6	785.6 ± 375.2
	Mockernut	1,028.9 ± 358.8	949.8 ± 435.4
	P	0.8389	0.5885

[a] Actual number of catkins; divide by 3 to obtain number of peduncles.
[b] Surface area of one leaf in cm^2.
[c] Surface area of twig (cm^2) calculated for an equivalent cylinder of that length and width.
[d] Total surface area per shoot; calculated as TSA = (no. of leaves * leaf area) + twig area.

duced relatively uniform seedlings in the greenhouse, with the exception of one seedling which was an albino.

DISCUSSION—*Flowering phenology*—Both *Carya ovata* and *C. tomentosa* share the same temporal window for reproduction. the ecological and evolutionary consequences of overlapping flowering times have been primarily studied by those interested in zoophilous pollination systems (e.g., see Rathcke, 1983; Real, 1983; Waser, 1983); fewer studies have explored the implications for co-occurring wind-pollinated congeners which may suffer from interspecific pollen transfer (Waser, 1983).

Separation of flowering times is a common isolating mechanism for sympatric anemophilous species. The nondirectional nature of the pollination vector (wind) would logically dictate the expectation that congenerics should be isolated in time; conspecifics should produce pollen and receptive stigmas in a highly synchronous fashion. However, we did not observe nonoverlapping flowering at HMF in either year. Both species share an array of reproductive features deployed during the same general time period resulting in no obvious reproductive isolation. Statistically, between-species comparisons indicated that most of the reproductive events examined were temporally separated; however, the large amount of overlap (usually >50%) among reproductive phases resulted in a complicated reproductive continuum rather than a series of synchronous reproductive episodes. This lack of clear temporal partitioning may result in hybridization. Species identity is surely not being *maintained* totally by temporal differences in flowering, but the degree of temporal differentiation observed clearly *contributes* to species integrity.

Pollination and fertilization—Pollen is produced in enormous quantities by these two species. The production and release of a large number of pollen grains are characteristic of the wind pollination syndrome (Whitehead,

TABLE 3. *Percentage pollen viability at pollen shedding in 1987 for trees of shagbark and mockernut hickory*

Species	Tree	$\bar{x}$ ± SD
Shagbark	A	96.7 ± 1.1
	B	87.0 ± 4.6
	C	93.5 ± 1.2
	I	92.1 ± 1.8
Mockernut	D	83.8 ± 7.4
	E	93.7 ± 4.1
	G	95.0 ± 1.3
	H	89.5 ± 0.9

TABLE 4. *Pollen-ovule ratios for shagbark and mockernut hickories. There was no significant difference between species (Z = 0.883, P = 0.377)*

Species	Tree	P:O for tree	Species mean ± SD
Shagbark			2,211,661 ± 803,118
	A	2,055,061	
	B	1,498,376	
	C	3,081,546	
	J	No data	
Mockernut			9,411,475 ± 8,575,828
	D	9,687,150	
	E	6,284,453	
	G	21,003,640	
	H	670,656	

1969, 1983; Faegri and van der Pijl, 1971; Proctor and Yeo, 1972; Niklas, 1985). Madden, Brison, and McDaniel (1969) determined that the average pecan catkin produces 2.5 million pollen grains. Woodroof (1924) determined the number of pollen grains produced by one tree to be 2.9 × 10^{10}.

Pollen-ovule ratios are a good indicator of the breeding system; wind-pollinated, xenogamous species tend to have high pollen-ovule ratios (10^3–10^4, Cruden, 1977). In this respect, *Carya* is typically representative. Interestingly, our observed P:O ratios ranged from ca. 1–20 × 10^6 and are among the highest cited for any natural or cultivated plant species (Pohl, 1937; Cruden, 1977). To what extent this is characteristic for other upland hardwoods is unclear—there are too few data to make adequate comparisons.

The pollen supplementation experiment indicates that the amount of available pollen imposed no obvious limitations on fruit set during the year we conducted our study. The vast majority of pollen that was produced was quite viable, and this viability was maintained over time (1 week). Presumably, the longer a grain can stay airborne the greater its chances of effecting pollination.

A number of factors lead us to postulate that most hickory trees regularly self-, rather than cross-pollinate, and that gene flow is not particularly extensive within and between these species. First, pollen dispersion has been shown to be strongly leptokurtic for all temperate tree species examined to date (Wright, 1976), including *Carya illinoensis* (Woodroof and Woodroof, 1927). Pollen is rarely disseminated more than 100–150 m from the parent plant, although long-distance dispersal events in *Carya* are not uncommon (Woodroof and Woodroof, 1927). Secondly, our data show hickories to have already leafed out, to various degrees, by the time pollen is shed. This can result in a containment effect because the catkins are borne proximally to the leafy shoots, thus promoting a vertical dribble of pollen within a canopy rather than an angular dispersion of pollen away from the canopy. The overwhelming surface area of nontarget tissues (i.e., leaves and branches) relative to stigmatic surface area (see model by Faegri and van der Pijl, 1971) associated with a tree and its nearest neighbors suggests that, in the absence of strong turbulence, horizontal or vertical pollen movement out of the canopy may be minimal. The presence of so much leafy tissue probably promotes geitonogamy over xenogamy. Thirdly, we note that one of the best nut-producing trees (H; >90% of flowers set fruit) was the most isolated tree (ca. 150–200 m from the nearest conspecific pollen-producer), further confirming our suspicion that fertilization is probably effected foremost through self-pollination. Mature fruits from this tree (H) were almost all germinable and produced vigorous seedlings.

Breeding system—Results from the diallel cross indicate that hickories are highly self-compatible. The morphological considerations discussed above suggest that geitonogamy is favored over xenogamy. Dichogamy, the temporal separation of genders, may however reduce the frequency of self-pollination. Our data indicate that hickory exhibits interfloral, incomplete, synchronous dichogamy (see Lloyd and Webb, 1986). Individual plants may be protandrous in one year, while being protogynous the following year. Dichogamy among cultivars of *Carya illinoensis* has generally been found to be more or less complete (i.e., no overlap of staminate and pistillate flowers) and has posed substantial problems for tree breeders (Smith and Romberg, 1940). Both *C. illinoensis* (Stuckey, 1916) and *Juglans* spp. (Del-

Table 5. *Results of full diallel cross in which every tree served both as a pollen donor and pollen recipient. Each cross was replicated 5 times. Abbreviations: nd = no data (cross was not executable due to complete dichogamy), 0 = all 5 reps failed to set fruit, + = 1–2 reps set fruit, ++ = 3–5 reps set fruit*

Male trees (pollen donors)	Female trees (pollen recipients)							
	Carya ovata				*C. tomentosa*			
	A	B	C	J	D	E	G	H
A	++	++	++	+	0	+	+	+
B	nd	++	++	nd	+	+	0	+
C	nd	+	0	nd	+	0	++	++
J	nd	nd	0	+	++	nd	0	++
D	nd	++	++	+	++	++	+	++
E	nd	+	0	++	+	+	0	++
G	nd	+	0	nd	+	++	++	++
H	nd	nd	nd	nd	nd	nd	nd	nd

pino, 1871, described in Darwin, 1888; Stout, 1928; Gleeson, 1982) have been characterized as having a specialized form of synchronous dichogamy known as heterodichogamy (Lloyd and Webb, 1986), which involves a protandrous-protogynous dimorphism. Average flowering times of the complementary mating types are synchronized to the extent that mating is almost completely disassortative. Our observations on the between-year variation in the timing of gender state would suggest that natural populations of *Carya* are not in fact heterodichogamous but more accurately described as "one-cycle" (Lloyd and Webb, 1986). As Pendleton et al. (1988) point out, the protandrous-protogynous dimorphism in the Juglandaceae has been reported almost exclusively for cultivated varieties rather than from natural populations.

Dichogamy has been considered as a mechanism to promote outcrossing, and in fact we believe this to be the case here. Wyatt (1983) and Lloyd and Webb (1986) have challenged the hypothesis that dichogamy evolved solely as an outcrossing mechanism; they note that there are a large number of self-incompatible species which also exhibit the trait and thus create a paradox. They suggest that dichogamy may have an important role in promoting pollen dispersal and reducing [self] pollen-stigma interference. However, in this case, it is unlikely that dichogamy is needed to reduce pollen-stigma interference because the stigmas were generally saturated with self-pollen when hand-pollinated, and even then they produced excellent fruit set. In addition, neither of these species have optimized morphological characteristics to promote pollen dispersal (e.g., the lack of leaves at pollen shedding). The extent to which herkogamy contributes to outcrossing is unclear, but it may contribute to pollen dispersal and reduce pollen-stigma interference (Webb and Lloyd, 1986).

Previously, we have noted a strong genetic basis to flowering and fruiting in these trees (McCarthy and Quinn, 1989); staminate flowering was found to be more variable than pistillate flowering. Phenological patterns (Fig. 6, 7) indicate that the plasticity of male flowering (particularly time of pollen shedding) determines both the type and degree of dichogamy. The sensitivity of anthers to atmospheric conditions apparently dictates the extent to which a given plant will be protandrous or protogynous. The environmental conditions dictating anther dehiscence seem to be universal (Faegri and van der Pijl, 1971; Whitehead, 1983; Niklas, 1985). Thus, the degree and extent of dichogamy are quite plastic and apparently not under rigid genetic control.

We found some evidence, based on our crossing design, to suggest selective fertilization (Stephenson and Bertin, 1983), i.e., incompatibility of pollen (or pollen tubes) and pistils in self- and cross-pollinations. Some maternal parents would consistently reject crosses with specific paternal parents. The prevalence of maternal choice in natural populations of North American hardwood trees has rarely been examined. Romberg and Smith (1946), studying the effects of cross-, self-, and sib-pollination in cultivars of *C. illinoensis*, found no evidence of pollen-stylar incompatibility or selectivity of pollen tubes in the fertilization of egg cells. There was always good fruit set as long as stigmas were receptive when the pollen was applied. Adriance (1931) also found excellent fruit set among eight cultivars of *C. illinoensis*. However, Romberg and Smith (1946) determined that the quality of nuts was lower for nuts arising from self-pollinations, suggesting that the vigor of seedlings might be reduced due to inbreeding. Due to a lack of matured nuts, our data were not sufficient to determine the effects of inbreeding on offspring quality.

The putative interploidal cross-compatibility of these two species indicates that polyploidy (and the production of sterile hybrids) has not yet led to the evolution of effective prezygotic isolating mechanisms. However, except for the potential production of unreduced gametes by *C. ovata*, progeny of these two species should be triploid (or rarely pentaploid) and sterile. While our interspecific crosses produced filled, germinable nuts, we know nothing about the vigor of seedlings resulting from these crosses; obviously the determination of F_1 or F_2 fertility would require a prohibitive amount of time. We can, however, say that interspecific hybridization in nature is possible.

SIGNIFICANCE FOR FUTURE STUDIES—No single mechanism operates to maintain the reproductive fidelity of these two sympatric species of *Carya*. In the absence of strong temporal separation of flowering or other prezygotic isolating mechanisms, sympatric congeners of anemophilous hardwoods should occasionally produce interspecific hybrids in natural populations. Few studies have empirically addressed the ecological and evolutionary implications associated with nondirectional pollination vectors such as wind—studies of this nature should be encouraged. Our data suggest that a variety of mechanisms may contribute to reproductive fidelity throughout the early stages of zygote production. Further studies of parent-offspring relationships, utilizing complete crossing designs, would be quite profitable in determining what pre- and/or postzygotic mechanisms were operating to regulate offspring quality; our data are equivocal in this respect. Parental regulation of offspring quality might be one of the few ways in which hybridizations among anemophilous congenerics in natural populations might be minimized.

LITERATURE CITED

ADRIANCE, G. W. 1931. Factors influencing fruit setting in the pecan. Bot. Gaz. 91: 144–166.

BRAUN, E. L. 1950. Deciduous forests of eastern North America. Hafner, New York.

CRUDEN, R. W. 1977. Pollen-ovule ratios: a conservative indicator of breeding systems in flowering plants. Evolution 31: 32–46.

DANIEL, T. W., J. A. HELMS, AND F. S. BAKER. 1979. Principles of silviculture. 2d ed. McGraw-Hill, New York.

DARWIN, C. 1888. The different forms of flowers on plants of the same species. John Murray, London.

DELPINO, F. 1871. Ulteriori osservazioni sulla dichogamia, part ii. fasc. ii. Milano, Italy.

FAEGRI, K., AND L. VAN DER PIJL. 1971. The principles of pollination ecology. Pergamon Press, Oxford.

FORMAN, R. T. T., AND B. A. ELFSTROM. 1975. Forest structure comparison of Hutcheson Memorial Forest and eight old woods on the New Jersey Piedmont. William L. Hutcheson Memorial Forest Bull. 3: 44–51.

FOWELLS, H. A. 1965. Silvics of forest trees of the United States. US Dept. Agric. Handb. No. 271, Washington, DC.

GLEASON, H. A., AND A. CRONQUIST. 1963. Manual of the vascular plants of northeastern United States and adjacent Canada. D. Van Nostrand, Princeton, NJ.

GLEESON, S. K. 1982. Heterodichogamy in walnuts: inheritance and stable ratios. Evolution 36: 892–902.

HARPER, J. L. 1967. A Darwinian approach to plant ecology. J. Ecol. 55: 247–270.

———. 1977. Population biology of plants. Academic Press, New York.

HESLOP-HARRISON, J., AND Y. HESLOP-HARRISON. 1970. Evaluation of pollen viability by enzymatically induced fluorescence: intracellular hydrolysis of fluoroscein diacetate. Stain Technol. 45: 115–120.

KIRKHAM, W. C. 1976. Soil survey of Somerset County, New Jersey. US Dept. Agric., Soil Conservation Service.

LEE, T. D. 1988. Patterns of fruit and seed production. *In* J. Lovett Doust and L. Lovett Doust [eds.], Reproductive ecology of plants, 179–202. Oxford University Press, Oxford.

LLOYD, D. G., AND C. J. WEBB. 1986. The avoidance of interference between the presentation of pollen and stigmas in angiosperms. I. Dichogamy. New Zealand J. Bot. 24: 135–162.

MADDEN, G. D., AND E. J. BROWN. 1973. Blossom dates of selected pecans. Pecan Quart. 7: 17–19.

———, AND H. L. MALSTROM. 1975. Pecans and hickories. *In* J. Janik and J. N. Moore [eds.], Advances in fruit breeding, 420–438. Purdue University Press, West Lafayette, IN.

———, F. R. BRISON, AND J. C. MCDANIEL. 1969. Pecans. *In* R. A. Jaynes [ed.], Handbook of North American nut trees, 163–189. Humphrey Press, Geneva, NY.

MANNING, W. E. 1940. The morphology of the flowers of the Juglandaceae. II. The pistillate flowers and fruit. Amer. J. Bot. 27: 839–852.

———. 1948. The morphology of the flowers of the Juglandaceae. III. The staminate flowers. Amer. J. Bot. 35: 606–621.

———. 1950. A key to the hickories north of Virginia with notes on the two pignuts, *Carya glabra* and *C. ovalis*. Rhodora 52: 188–199.

MCCARTHY, B. C. 1988. A method of access into the crowns of subcanopy and canopy trees. Canad. J. Forest Res. 18: 646–649.

———, AND J. A. QUINN. 1989. Within- and among-tree variation in flower and fruit production in two species of *Carya* (Juglandaceae). Amer. J. Bot. 76: 1015–1023.

———, AND W. A. WISTENDAHL. 1988. Hickory (*Carya* spp.) distribution and replacement in a second-growth oak hickory forest of southeastern Ohio. Amer. Midl. Naturalist 119: 156–164.

NAKAMURA, R. R. 1988. Seed abortion and seed size variation within fruits of *Phaseolus vulgaris*: pollen donor and resource limitation effects. Amer. J. Bot. 75: 1003–1010.

NIKLAS, K. J. 1985. The aerodynamics of wind pollination. Bot. Rev. 51: 328–386.

Pendleton, R. L., E. D. McArthur, D. C. Freeman, and A. C. Blauer. 1988. Heterodichogamy in *Grayia brandegei* (Chenopodiaceae): report from a new family. Amer. J. Bot. 75: 267–274.

Pohl, F. 1937. Die Pollenerzengung der windbluter. Beih. Bot. Centralbl. 56: 365–470.

Proctor, M., and P. Yeo. 1972. The pollination of flowers. Taplinger, New York.

Rathcke, B. 1983. Competition and facilitation among plants for pollination. *In* L. Real [ed.], Pollination biology, 305–325. Academic Press, Orlando.

Real, L. 1983. Microbehavior and macrostructure in pollinator-plant interactions. *In* L. Real [ed.], Pollination biology, 287–304. Academic Press, Orlando.

Robichaud, B., and M. F. Buell. 1973. The vegetation of New Jersey. Rutgers University Press, New Brunswick, NJ.

Romberg, L. D., and C. L. Smith. 1946. Effect of cross-pollination, self-pollination and sib-pollination on the dropping, volume and kernel development of pecan nuts and vigour of the seedlings. Proc. Amer. Soc. Hortic. Sci. 47: 130–138.

Shuhart, D. V. 1927. The morphological differentiation of the pistillate flowers of the pecan. J. Agric. Res. 34: 687–696.

Smith, C. L., and L. D. Romberg. 1940. Stigma receptivity and pollen shedding in some pecan varieties. J. Agric. Res. 60: 551–564.

Sokal, R. R., and F. J. Rohlf. 1981. Biometry. 2d ed. W. H. Freeman, San Francisco.

Solbrig, O. T. 1980. Demography and natural selection. *In* O. T. Solbrig [ed.], Demography and evolution in plant populations, 1–20. University of California Press, Berkeley.

Stephenson, A. G., and R. I. Bertin. 1983. Male competition, female choice, and sexual selection in plants. *In* L. Real [ed.], Pollination biology, 109–149. Academic Press, Orlando.

Stone, D. E. 1963. Pollen size in hickories (*Carya*). Brittonia 15: 208–215.

Stout, A. B. 1928. Dichogamy in flowering plants. Bull. Torrey Bot. Club 55: 141–153.

STSC, Inc. 1986. Statgraphics users guide. Rockville, MD.

Stuckey, H. P. 1916. The two groups of varieties of the *Hicoria pecan* and their relation to self-sterility. Georgia Agric. Exp. Stat. Bull. 124.

Traub, H. P., and L. D. Romberg. 1933. Methods of controlling pollination in the pecan. J. Agric. Res. 47: 287–296.

Waser, N. M. 1983. Competition for pollination and floral character differences among sympatric plant species: a review of the evidence. *In* C. E. Jones and R. J. Little [eds.], Handbook of experimental pollination biology, 277–293. Van Nostrand-Reinhold, New York.

Webb, C. J., and D. G. Lloyd. 1986. The avoidance of interference between presentation of pollen and stigmas in angiosperms. II. Herkogamy. New Zealand J. Bot. 24: 163–178.

Whitehead, D. R. 1969. Wind pollination in the angiosperms: evolutionary and environmental considerations. Evolution 23: 28–35.

———. 1983. Wind pollination: some ecological and evolutionary perspectives. *In* L. Real [ed.], Pollination biology, 97–107. Academic Press, Orlando.

Wolfe, P. E. 1977. The geology and landscapes of New Jersey. Crane Russak, New York.

Woodroof, J. G. 1924. The development of pecan buds and the quantitative production of pollen. Georgia Exp. Stat. Bull. No. 144.

———. 1930. Studies of the staminate inflorescence and pollen of *Hicoria pecan*. J. Agric. Res. 40: 1059–1104.

———, and N. C. Woodroof. 1927. The development of the pecan nut (*Hicoria pecan*) from flower to maturity. J. Agric. Res. 30: 1040–1063.

Wright, J. W. 1976. Introduction to forest genetics. Academic Press, New York.

Wyatt, R. 1983. Pollinator-plant interactions and the evolution of breeding systems. *In* L. Real [ed.], Pollination biology, 51–96. Academic Press, Orlando.