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Estimates of Self-pollination in Pecan Orchards in the Southeastern United States

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Abstract. **Self-pollination was estimated in three Georgia pecan *[Carya illinoinensis* (Wangenh.) K. Koch] orchards. Selfing in two large orchards lacking an interplanted complementary pollinizer (one orchard being comprised of 'Curtis' and the other 'Moneymaker') was estimated to be at least 3% and 49%, respectively. A 'Cheyenne' orchard containing 'Stuart' as a complementary pollinizer at 5% density was estimated to have had at least 14% and 42% of ripened nuts derived from selfing in two consecutive years. These estimates suggest self-pollination may reduce yield in pecan orchards in the southeastern United States.**

About two-thirds of the U.S. pecan crop is produced in orchards growing in the southeastern U.S. (Wood et al., 1990). The majority of these orchards lie outside the native range of the species; therefore, little or no pollen is available from wild trees to cross-pollinate cultivated cultivars, and the genetic variation represented by feral trees is relatively low. Pecan is a wind-pollinated heterodichogamous species; however, for practical purposes, compatible trees must have close spacial associations if there is to be cross-pollination (Woodroof and Woodroof, 1927). Clones are therefore typically characterized as either protandrous or protogynous to designate whether pollen shed generally precedes or follows stigma receptivity (Smith and Romberg, 1940; Thompson and Young, 1985). Guidelines for orchard establishment typically suggest interplanting two or more cultivars possessing complementary floral maturation so as to promote cross-pollination (Goff, 1989), because self-pollination diminishes nut weight and volume and can increase fruit abortion (Marquard, 1988; Romberg and Smith, 1946; Sparks and Madden, 1985; Wolstenholme, 1969).

While many pecan clones are cultivated in the southeastern United States, we know of no attempt to quantify the degree of selfing occurring in commercial orchards. Since the type and degree of dichogamy vary with location, year, tree age, and tree vigor of a particular cultivar (Adriance, 1930; Dodge, 1940; Gray, 1973; Smith and Romberg, 1940; Wolstenholme, 1968), opinions vary widely concerning the degree of self-pollination occurring in southeastern orchards. Therefore, one cannot discount selfing as a possible major problem, even within orchards possessing a substantial component of complementary pollinizers.

Mating systems, such as those typical of pecan orchards, can be evaluated using simply inherited biochemical markers. For example, self-pollination has been estimated for 'Western Schley' growing in the arid Southwest using isozyme analysis (Marquard, 1988). Malate dehydrogenase (MDH; EC 1.1.1.37), phosphoglucose isomerase (PGI; EC 5.3.1.9), and phosphoglucomutase (PGM, EC 2.7.5.1) are each known to be controlled by at least one polymorphic gene in pecan (Marquard, 1987, 1991). Loci controlling the respective polymorphisms are designated *Mdh-1, Pgi-2,* and *Pgm-1.* Each locus has at least three alleles, and rare alleles are known (Marquard, 1989, 1991). The objective of this study was to provide, for the first time, a quantitative estimate of selfing in commercial pecan orchards in sectors of the southeastern United States that are outside the natural range of pecan. In this study, we used isozyme analysis to estimate the percentage of self-pollination occurring in three distinctly different orchards in Georgia.

The three study orchards possessed a suitable cultivar mix from which the fertilizing pollen parent could be determined with good reliability from samples of mature nuts. Additionally, all three orchards were under excellent commercial management programs, although nut yields were low in each orchard. The reason for low yields was unknown. 'Moneymaker' and 'Curtis' orchards were selected because both cultivars carry an unusual allele at one isozyme locus. 'Moneymaker' has an *ac* genotype for the *Pgi-2* gene and the frequency of the rare c allele for *Pgi-2* among cultivars is only ≈8% (Marquard, 1987). 'Curtis' has a *bc* genotype for *Pgm-1,* and the rare c allele has a frequency of only ≈1% among surveyed cultivars (Marquard, 1991). A 'Cheyenne' orchard with 'Stuart' as the pollinizer was selected because of size and uniformity of planting.

Nuts were systematically collected at maturity from individual trees in each of the three orchards in 1987 and 1988. Sampling was from individual trees of each row (every second row in the 'Curtis' orchard) on each of four transects (replications) across the vicinity of the minor axis of the orchards (Fig 1). Fresh kernel tissue from individual seeds was assayed to determine the appropriate isozyme genotype and to estimate the selfing

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Table 1. Cultivars and genotypes in study orchards used to estimate self-pollination and expected genotype of the progeny associated with each parental combination.

Cultivar (main crop) and genotype	Potential male parent and genotype	Potential genotype of progeny and expected segregation frequency		Scorable gene
Moneymaker	Schley (*aa*[z])	*aa, ac*	1:1	*Pgi-2*
ac	Stuart (*bb*)	*ab, bc*	1:1	
	Moneymaker (*ac*)	*aa, ac, cc*	1:2:1	
Curtis	Stuart (*aa*)	*ab, ac*	1:1	*Pgm-1*
bc	Desirable (*aa*)	*ab, ac*	1:1	
	Curtis (*bc*)	*bb, bc, cc*	1:2:1	
Cheyenne	Stuart (*ac*)	*ab, bc*	1:1	*Mdh-1*
bb	Cheyenne (*bb*)	*bb*	(100%)	

[z]Genotypes are correctly given for 'Schley' and 'Stuart' and incorrectly reported in an earlier work (Marquard, 1987).

Table 2. Cultivar mixture, enzyme system assayed, number of nuts assayed, and estimate of nuts derived via self-pollination for three study orchards.

Main crop cultivar	Pollinizer cultivar[z]	Year	Enzyme assayed	Trees sampled	Nuts assayed	Estimated self-pollination with SD
Moneymaker (II)	Schley (II)	1987	PGI	27	>2500	≥49.2 ± 18.6
Curtis (II)	Stuart (II) Desirable (I)	1988	PGM	76	>5000	≥3.0 ± 3.4
Cheyenne (I)	Stuart (II)	1987	MDH	35	>2500	≥14.1 ± 7.1
Cheyenne (I)	Stuart (II)	1988	MDH	36	>3200	≥41.9 ± 10.6

[z]The 'Moneymaker' and 'Curtis' orchards had no interplanted pollinizer trees. The listed cultivars are those adjacent to the study orchards and could, theoretically, function to pollenize the target cultivar even though their dichogamies are likely to reduce complimentary flowering overlaps. I = Pollen begins to shed before stigma receptivity; II = stigmas begin to receptive before pollen shed. Since dichogamy is rarely complete, the potential selfing and crossing between cultivars of the same class is substantial.

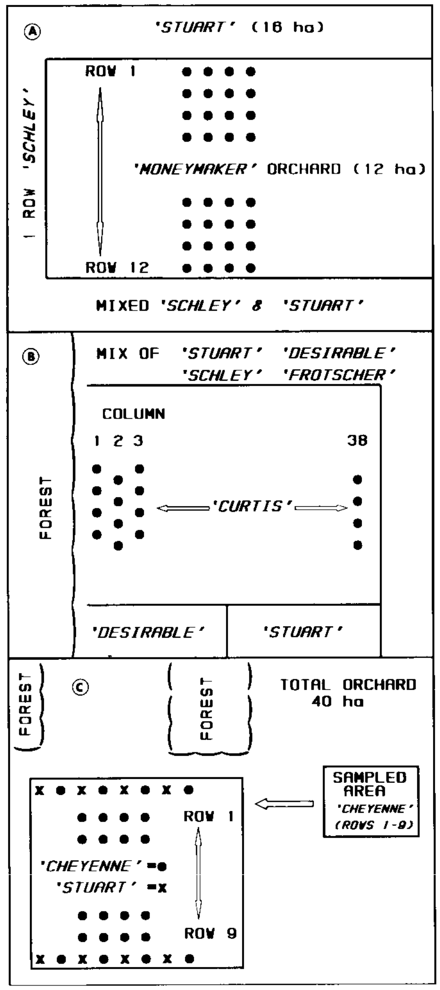

Fig. 1. Diagraminatic representation of the 'Moneymaker' (A), 'Curtis' (B), 'Cheyenne' (C) orchards from which estimates were calculated for self-pollination levels for ripened nuts. Row spacings were 17 × 17 m in the 'Moneymaker', 18 × 18 m in the 'Curtis', and 6 × 12 m in the 'Cheyenne' orchards. Pollinizer trees were immediately adjacent to the trees of the targeted orchard. Winds at these locations are highly variable; however, the prevailing wind is from the southwest (the lower left corner of the above figure).

for each individual tree in each transect. The number of sampled trees and assays performed for each orchard are shown in Table 1. Proteins in cotyledon tissue were extracted, separated by starch gel electrophoresis, and visualized for either MDH, PGI, or PGM as described by Marquard and Skorpenske (1989).

Logic used to calculate selfing can best be illustrated for the 'Cheyenne' orchard (Table 1). For *Mdh-1*, 'Cheyenne' and 'Stuart' have a *bb* and *ac* genotype, respectively (Marquard, 1989). Fruit of 'Cheyenne' that exhibit a *bb* genotype for *Mdh-1* most likely arose from self-pollination. Selfing estimates were determined by dividing the frequency of *bb* types by the total nut number evaluated for each tree. Theoretically, a *bb* genotype could also result from extraneous pollen from a spurious individual in the orchard that carries a *b* allele for *Mdh-1* (although inspection of the orchard revealed no such individuals). Therefore, contamination in this orchard was expected to be very low. Design of the 'Cheyenne' orchard included a repeating pattern with every other tree in every 10th row being a 'Stuart' pollinizer. Fruit with either an *ab* or *bc* genotype likely resulted from pollination by 'Stuart' (a cultivar possessing generally complimentary dichogamy that is sometimes used in combination with 'Cheyenne' to insure cross-pollination). Self-pollination for the orchard was estimated as the average selfing estimated of surveyed trees. Selfing in 'Moneymaker' was determined from the frequency of *cc* genotypes in sampled nuts using *Pgi-2* ('Moneymaker' is *ac* for *Pgi-2)*. The *cc* genotype is expected to occur 25% of the time after self-pollination of 'Moneymaker' in a ratio of 1 *aa* : 2 *ac* : 1 *cc* (Marquard, 1987). Therefore, the selfing estimate was four times the *cc* frequency divided by the total number of evaluated nuts. Selfing in 'Curtis' was estimated from the frequency of *bb, bc,* and *cc* genotypes of *Pgm-1* ('Curtis' is *bc* for *Pgm-1)* divided by the total number of evaluated nuts. The *b* and *c* alleles are uncommon among named cultivars (Marquard, 1991).

'Moneymaker' and 'Curtis' orchards were large, solid blocks of trees surrounded by potential cross-pollinizer trees (Fig. 1). Neighbor trees consisted of cultivars classified in the same dichogamous class as the target cultivar. However, the near absence of complete dichogamy in pecan and phenological variation due to tree vigor, age, and enviroment does not prevent a cultivar from being pollinated by a tree of the same dichogamy class (either protogynous or protandrous). Theoretically, the two targeted cultivars could have been pollinated by other pecan trees in the vicinity. However, none of these cultivars possessed the scorable alleles being evaluated. The 'Moneymaker' and 'Curtis' trees were ≈75 to 80 years old and occupied blocks consisting of ≈12 and 30 ha of orchard, respectively. These two blocks are fairly typical of many old pecan orchards common throughout the southeast with respect to size, spacing, potential pollinizers, and orchard fidelity.

Since few, if any, mature orchards in the southeastern United States are without "off-genotype" trees, there is the possibility that some of the nuts collected from sample trees were pollinated by an off-genotype located within the orchard. The two mature orchards selected for the study had an off-genotype frequency of <2% with no such tree being within two trees of the transects along the minor axis of the orchard. Nuts from either the 'Moneymaker' or 'Curtis' orchards could, theoretically, be contaminated with the *Pgi-2* or *Pgm-1* marker alleles from off-genotype trees. However, the accuracy of our selfing estimate will be inversely proportional to the number of markers used and the frequency of the scorable allele amount off-types. Although the probability would be low [assuming marker gene frequency of (≤0.08) (≤0.02) (≤0.08) (100) = ≤0.16% or ≤1/625 trees)] that such a tree would exist in these particular orchards (316 to 792 trees in these two orchards). Even if such trees were present, pollen produced by off-types would compete with pollen from all other pollen sources, further reducing their chances of fertilizing the flowers of 'Moneymaker' or 'Curtis'. Therefore, potential contamination is likely to be insignificant, and selfing estimates are not inflated by contamination, and the estimated levels of selfing exhibited by the ripe nut are expected to be relatively accurate. Since our method did not account for selfed ovules that aborted or were lost due to biotic or abiotic stress factors (considered random and likely will not increase estimates of selfing) before harvest, the reported estimates are considered conservative.

Estimates of self-pollinated mature fruit found within the three orchards evaluated in this sampling ranged from ≥3% to ≥49% (Table 2). The orchard comprised of 'Cheyenne' and 'Stuart' was estimated to have ≥14% of its mature nuts derived from self-pollination in 1987 and ≥42% in 1988 (Table 2). The yearly variation could be attributed to several factors, including, for example, the differential interaction of the floral phenologies of 'Cheyenne' and 'Stuart' to early spring temperatures. These self-pollination estimates (≥14% and 42%) for this 'Cheyenne' orchard do not fit earlier estimates of selfing derived from the model developed for 'Western-Schley' orchards pollinated by 'Wichita' in the southwestern United States (Marquard, 1988). That model predicts 77% selfing (based on ripened nuts) in an orchard with 5% pollinizers ('Stuart' was present at a 5% density level in the test orchard). This discrepancy may be attributed to the ability of different cultivars to set selfed fruit or the degree of incomplete dichogamy.

The estimates of self-pollination in the 'Moneymaker' and 'Curtis' orchards were ≥49% and ≥3%, respectively (Table 2). This result suggests that the protogynous 'Moneymaker' trees were not adequately cross-pollinated by the surrounding protogynous 'Stuart' and 'Schley' trees but rather were highly self-pollinated. In contrast, trees in the 'Curtis' orchard exhibited ≥3% selfing. This apparently low selfing rate could result from: a) adequate cross-pollination by the adjacent complementary 'Desirable' trees; b) adequate pollination by the adjacent noncomplementary 'Stuart', 'Schley', or 'Frotscher' trees; c) the fact that a high level of selfing did occur but was masked by a high abortion level for selfed fruit; or d) a combination of these factors. The very low orchard yield (also low the previous year) provides circumstantial evidence that abortion may have been high due to selfing. Both 'Moneymaker' and 'Curtis' orchards had light fruit crops during 1987 and 1988. Selfing appeared to be generally consistent across all three orchards and was not substantially different as related to pollinizer proximity.

These estimates of self-pollination of ripened nuts from three distinctly different orchards provide evidence that selfing can be relatively high in some orchards in Georgia and may be a significant problem through the southeastern United States. New orchards should therefore be established with consideration for cross-pollination, and one should not assume that trees will be adequately cross-pollinated by neighboring trees in the vicinity. Additionally, the use of two pollinizing cultivars probably should be seriously considered to increase the likelihood of promoting cross-pollination. Future studies would benefit from the simultaneous use of two or more markers systems, thereby increasing the accuracy of estimates.

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