IDENTIFICATION AND PERFORMANCE OF JUGLANS HYBRIDS

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This is to certify that the

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Jonathan W. Wright
Jonathan W. Wright
Major professor

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ABSTRACT

IDENTIFICATION AND PERFORMANCE OF *JUGLANS* HYBRIDS

By

David Truman Funk

For many years, the potential timber value of hybrid walnuts has been acknowledged, but they are hard to produce artificially, rarely encountered in nature, and difficult to identify with certainty. The objectives of this study are to relate morphological and chemical characteristics of suspected hybrid walnut seedlings and trees to corresponding characteristics of presumed parent species, and to determine whether characteristics measured in the hybrids are intermediate, exceed parental values, or perhaps represent unique hybrid traits or combinations of traits.

Twenty-three traits of leaf and branchlet morphology were analyzed in an attempt to determine those that are most suitable for characterizing *Juglans* species and distinguishing hybrids. For several traits in *J. nigra*, an analysis of variance indicated a larger component for leaves within trees than for trees within families or among families. It appears that it will be necessary to analyze perhaps 3 to 6 leaves per tree in order to obtain reliable mean values for foliar characteristics of individual black walnut trees.

The following traits of leaf morphology were selected for distinguishing *Juglans nigra*, *J. regia*, and putative hybrids between the two species: leaflets per leaf, marginal serrations per centimeter,

position of the longest leaflet pair on the leaf, leaflet length:width index, and leaf pubescence. A weighted hybrid index was calculated that separates Persian from black walnut trees and can be used satisfactorily to classify most putative hybrids. Variation of hybrid index values within seedling families of putative hybrid walnut parents is greater than in pure-species families, an additional confirmation of parental hybridity.

Other traits such as leaflet length and lopsidedness, rachis pubescence and branchlet pith color were used to differentiate *J. sieboldiana*, *J. cinerea*, and their hybrids.

To supplement the morphological analysis, paper chromatography was used to analyze *Juglans* foliage extracts for presence of polyphenols. Among the several chromatograms prepared, a total of 79 different spots was distinguished. Some spots tended to be more prevalent in one taxon than in the others, but the distinction was not usually absolute. In order to quantify the differences, 'diagnostic values' were developed for *Juglans nigra*, *J. regia*, and their hybrid. The diagnostic values were computed from a table of decimal fractions indicating the relative frequency of occurrence of the compounds in all the chromatograms for each species or the hybrid. The biochemical diagnostic values were well correlated with morphological hybrid index values; the more expensive chromatographic techniques will probably be used only when necessary to resolve doubtful classification.

Measurements of young progeny-test plantations in Michigan and Illinois suggest that there may be an opportunity to make simultaneous genetic selection for rapid height growth and improved form in hybrid walnuts. The increased variability in these hybrid populations is desirable since it is assumed to indicate increased genetic diversity which in turn allows greater opportunities for selection and tree improvement. If recombinations yielding improvement in both growth and form prove to be common among hybrid walnut progeny, an expanded program of hybrid breeding will be easily justified.

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HYBRID WALNUTS AND HYBRID ANALYSIS

Over the past century, at least 13 combinations of hybrid walnuts involving 8 *Juglans* species have been described.[1] Many of these hybrids are noted for outstanding growth rate, pest resistance, or other desirable traits (Table 1).

Despite the continuing interest in interspecies walnut hybrids, they are usually difficult to produce artificially (McKay, 1957; Shchepot'ev, 1960); the 'Paradox' hybrid (*J. hindsii* x *regia*) introduced by Burbank (Howard, 1945) is perhaps the only walnut hybrid in commercial production. This hybrid is usually produced by collecting seed from the Hinds walnut, *J. hindsii*, growing near a Persian walnut (*J. regia*) pollen source; it is also possible to propagate 'Paradox' hybrid clones by trench layering and rooting of cuttings (Lynn and Hartmann, 1957).

Hybrid walnuts are also difficult to identify in the field (Manning, 1960; Gervais, 1963). Experienced nurserymen sometimes recognize walnut hybrids in seedbeds of otherwise pure species, but

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1/ Tree taxonomy throughout the text follows Little (1953) for native species, and Rehder (1940) for exotics; names of species' authors are omitted.

Table 1.--Hybrid walnuts mentioned in literature

Parental Species	Remarks	Reference
J. cinerea x *J. nigra*	Reported several times, but questionable	Gervais, 1963; Shchepot'ev, 1951
J. cinerea x *J. mandshurica*	28 percent hybrid fruit	Yablokov, 1936
J. cinerea x *J. regia*		
J. cinerea x *J. sieboldiana*		
J. hindsii x *J. nigra*	'Royal' hybrid produced by Burbank	Howard, 1945
	Bears large viable seed by age 5	Whitson, John, and Williams, 1915
J. hindsii x *J. regia*	'Paradox' hybrid produced by Burbank	Howard, 1945
	Used as Persian walnut rootstock	Serr and Forde, 1951
	Resistant to root lesion nematode injury	Serr and Day, 1949
J. mandshurica x *J. regia*	Vigorous, early fruiting, winter hardy	Ozol, 1962
J. mandshurica x *J. sieboldiana*	Growth rate faster than seedlings of either parent species	Yablokov, 1936
J. mandshurica x *J. nigra*	46 percent crossability	Shchepot'ev, 1960
J. neotropica x *J. regia*		Manning, 1960
J. nigra x *J. regia*	Many varieties based on nut shape	Carriere, 1860 in Reed, 1937; Rehder, 1940
	Difficult cross to make	McKay, 1957
	Resistant to spring frost damage	McKay, 1966
	More winter hardy than *J. regia*	Shchepot'ev, 1960
	Vigorous, *Phytophthora* resistant	Schad and Grente, 1955
J. regia x *J. sieboldiana*	Vigorous growth exceeds both parent species	Ozol, 1962
J. regia x Peruvian walnut	Resistant to *Pseudomonas juglandis*	Devoto, 1939

the hybrids are infrequent and J. regia x nigra and J. cinerea x sieboldiana are probably the only ones encountered with any regularity.

Thus we have a situation in which the potential value of hybrid walnuts is acknowledged, but they are hard to produce artificially, rarely encountered in nature, and difficult to identify with certainty.

Some of the problems of identification begin with Juglans species which are themselves variable and sometimes poorly defined. For instance, J. major, the Arizona walnut, has at various times been treated as a race of J. microcarpa, little walnut, a distinct variety of little walnut, or a separate species (Sudworth, 1934). Similarly, J. hindsii has been considered to be included within J. californica, the California walnut (Manning, 1957; Sudworth, 1967), a variety of the California walnut (Jepson, 1908), or a separate species (Sargent, 1965; and many other authors). Anyone attempting to delimit walnut species by reference to herbarium collections must recognize that any specimen might well have been determined according to species definitions that are no longer accepted.

Only two interspecific hybrids involving J. nigra have been authenticated, those with J. regia and J. hindsii. The hybrid with J. regia was first described by Carriere in 1863 (Reed, 1937). Several forms of this hybrid have been named, usually based on nut shape (Rehder, 1940). Black x Persian walnut hybrids are not at all common in nature, however, partly because *J. regia* usually flowers about 2 weeks earlier than *J. nigra*, and also because of apparent incompatibility. For instance, McKay (1957) was able to produce only 12 seeds from 5,000 controlled pollinations using Persian walnut pollen on black walnut pistillate flowers. Some *J. regia* x *nigra* hybrids, such as the vigorous 17-year-old trees described by Wellington (1931), flower profusely but never bear many seed.

A possible natural intersectional hybrid between *J. nigra* and *J. cinerea*, butternut, has been reported from southern Quebec (Gervais, 1963), but since the fruit dimensions were well within the range of black walnut seed size and no other morphological information was given, it seems prudent to be skeptical about this tree's hybridity.

Soviet tree breeders have been active in walnut hybridization for more than 50 years. They have recorded some spectacular successes that deserve attention and attempts at verification by western breeders. Among these are reports of 46 percent crossability between black walnut and Manchurian walnut, *J. mandshurica*, and 16 percent crossability between black walnut and mockernut hickory, *Carya tomentosa*, (Shchepot'ev, 1960). Shchepot'ev (1951) also reported a hybrid between black walnut and butternut and suggested that the cross was more easily made if vigorous young black walnut trees were used as the female parents. Additional information on black walnut hybridization and genetics has been summarized in a previous paper (Funk, 1970).

Taxonomic analysis of suspected plant hybrids must begin with a clear definition of possible parent species, including not only the species' taxonomic holotypes, etc., but also representatives of the normal range of variation. Systematic hybrid analysis of this type received its first real impetus from field studies by Edgar Anderson, who developed the well-known hybrid index. As originally outlined by Anderson (1936) and Riley (1938), morphological characters were selected which appeared reliable in distinguishing two parent species. All these characters were defined in a simple, often dichotomous, way (glabrous vs. pubescent, 2-3 nodes vs. 6-8 nodes, etc.). Conditions characteristic of one parent species were assigned "0" values while typical individuals of the other species were usually scored "2"; values for several traits were summed to compute the total index. Hybrids were expected to have intermediate index values, possessing 0 value for some characters, 2 for others, and 1 for many.

Anderson's methods proved effective for analysis of many hybrid populations, especially when combined with graphical representations of morphological character combinations which he suggested "as a device for helping the eye to aid the mind." (Anderson, 1957). Nevertheless, he acknowledged that while "the pictorialized scatter-diagram methods... yield quicker and more reliable results than any pure biometrical method which has yet been devised, they should be considered as temporary expedients." (Anderson, 1954).

The 'new systematics' that has developed in recent years is usually based on consideration of biochemical and anatomical as well as morphological characters and often includes complicated statistical analyses of metric data. Some numerical taxonomists have insisted that a minimum of 50 to 100 characters must be measured in order to assure a reasonably accurate differentiation of taxa. Nevertheless, there are instances in which inclusion of irrelevant characters in a hybrid index simply increases the variation in an otherwise clear set of data. In such situations, it seems appropriate to omit some characters. Taxonomists often have found it necessary to assign weights to the several characters that may be included in an index, and Hatheway (1962) proposed the following logical criterion: "The contribution of a character to an index should be in proportion to its usefulness in demonstrating a known or suspected relationship."

Several types of multivariate analyses have been used in statistical taxonomic studies, including canonical analysis of correlated traits, principal component analysis of uncorrelated variables, and discriminant analysis. As pointed out by Mergen and Furnival (1960), "If one knows the degree of genetic control of a single character, and if it differs sufficiently between the parental species, one character is sufficient to distinguish the hybrid. However, if little information is available on the variation pattern and its degree of genetic control, ...it is advisable to use a combination of several diagnostic characteristics." Discriminant analysis has been used several times to distinguish both natural and artificial hybrid trees from their parent species. The analysis indicates the combinations of characters that best discriminate among the groups of progeny being considered. The principal drawback to all such multivariate analyses is that they virtually necessitate use of computers.

Another aspect of hybrid analysis treats the progeny of parent trees that are themselves suspected of being hybrids. One possible objective is to verify or reject the putative hybridity of the parent through analysis of the progeny; another objective is to define the progeny as being F_1-selfed, F_2, or backcross hybrids. Ledig, et al. (1969) used discriminant analysis to meet both objectives in a study of hybrid oaks in North Carolina, and Clifford (1954) has shown that hybridity in *Eucalyptus* can be confirmed by analyzing progeny. Seedlings from 'intermediate' trees in suspected hybrid swarms of *Eucalyptus goniocalyx* and *E. elaeophora* exhibited markedly greater variance for some morphological traits than progeny of parents 'typical' for the two species. Presumably the greater variation among the hybrid progeny was due to presence of F_2 and backcross seedlings (Clifford, 1954).

OBJECTIVES

The objectives of this study are to relate morphologic and chemical characteristics of suspected hybrid walnut seedlings and trees to corresponding characteristics of presumed parent species, and to determine whether characteristics measured in the hybrids are intermediate, exceed parental values, or perhaps represent unique hybrid traits or combinations of traits. Information obtained in this study will be pertinent to walnut tree improvement programs that depend on hybridization to create additional genetic variation.

CHAPTER II

MORPHOLOGICAL ANALYSES OF WALNUT SEEDLINGS

In order to provide a baseline for study of variation within and between *Juglans* species, a number of morphological characteristics were tabulated (Appendix Table A-1) using taxonomic reports (Hegi, 1957; McMinn and Maino, 1951; Manning, 1952, 1957, and 1960; Ohwi, 1965; Rehder, 1940; Sargent, 1965; Sudworth, 1934), and my own measurements. The information summarized in this table has been useful in selecting traits for further study, especially among those species considered as possible parents of putative hybrid walnuts. Obviously, such a table can only indicate the patterns of variation within the several walnut species. General taxonomic works cannot devote much space to discussion of extreme intraspecific variation or to suspected-but-unconfirmed hybrids. Referring to specimens from a number of herbaria may provide adequate geographic coverage, but it does not ensure that specimens were collected and prepared in a uniform manner.

Any definitive study of hybrid plants must eventually depend on producing a quantity of control-pollinated progeny that can be compared with their own parents, and through statistical analysis, compared with like populations of non-hybrid seedlings and putative hybrids of uncertain origin. In the case of tree species, several problems make the task difficult.

The initial problem is the delay in reaching reproductive maturity. With any plants, it is convenient to assemble breeding populations in one or a few nearby areas; it is especially desirable for tree breeding since the large size of trees necessitates the use of equipment that is cumbersome to move far. But establishment of breeding arboreta typically begins with collecting seed, preferably representing a reasonable fraction of the geographic range of the species; seedlings are grown in the nursery and then transplanted to the arboretum, eventually to flower and bear fruit. In the case of black walnut, there are occasionally a few flowers and nuts on 5- or 6-year-old trees, but seed production is not dependable, even on very good sites, until the trees are at least 10 years old from seed. The Forest Service has a few small plantations approaching this age, and soon I hope to be able to begin systematic intraspecific crossing in *J. nigra*.

As for other species, since 1968 I have received 107 *J. regia* and 20 *J. sieboldiana* seedlots, mostly from indigenous or naturalized stands; 43 and 7 seedlots, respectively, germinated well enough to establish at least small outplantings. Within another 2 or 3 years these trees should have recovered from transplant shock and grown large enough to be used for analyses of leaf morphology that would be comparable with those based on mature trees. Study of flower and fruit characteristics will have to wait for a few more years. The Forest Service also has a small field plantation of J. major, representing several Arizona locations, but efforts to establish an assortment of J. microcarpa and J. cinerea genotypes in the field have been frustrated. From 6 J. microcarpa collections, no seedlings survived the winter. In an attempt to augment our 7 J. cinerea seedlings grown from commercial seed of unknown origin, I obtained several good collections from Iowa and West Virginia; squirrels destroyed every nut in the nursery. Identification of putative J. microcarpa x nigra and J. sieboldiana x cinerea hybrids will continue to be uncertain until more material can be accumulated.

METHODS

Twenty-three traits of leaf and branchlet morphology were analyzed in an attempt to determine those that are most suitable for characterizing Juglans species and distinguishing hybrids. Trees in six plantations in Michigan and Illinois were used for analysis. All trees had been outplanted for at least one season prior to collecting samples, and large leaves from exposed portions of the crown and vigorous, fully mature branchlets were selected for analysis. Herbarium specimens were pressed and dried and data were tallied as follows:

Leaf length - cm.

Number of leaflets

Longest leaflet:

Position - of pair containing longest leaflet, counting from tip

Petiolule length - mm.

Blade length - mm.

Blade width - mm.

greatest width

at 1/3 blade length from tip

at 2/3 blade length from tip

Serrations/cm. - beginning at the broadest part of the longest leaflet

Curvature - greatest deviation from straight line between tip and base of blade - in mm.

Base shape - average for several leaflets, adjacent to longest leaflet

1 = cordate

3 = truncate

4 = obtuse

5 = right angle

6 = tapering (less than 90° angle)

Lopsidedness - unequal extension of base of leaflet blade along petiolule - mm.

Pubescence - upper leaf surface) 0 = none
)
- lower leaf surface) 2 = along mid veins only

- rachis 4 = heavier in vein axils

6 = pubescent

8 - tomentose

Rachis redness (mature, fresh leaves) - 0 = none

1 = faint

3 = reddish pink

5 = maroon

Foliage color (mature, fresh leaves) - 1 = pale green

3 = "average"

5 = dark green

Branchlet color (Fully mature, current year's shoot growth)

constituent colors:	Green	Gray	Brown
none	0	0	0
light	1	1	1
medium	2	2	2
dark	3	3	3

Branchlet scurf - 0 = none

5 = heavy

Pith color (1- to 1 1/2-year-old wood) - 0 = very light tan

5 = dark brown

Trichomes at upper edge of previous

year's leaf scar - 0 = absent

5 = heavy

Data were recorded from more than 500 trees, although not all traits were determined for each tree. Parent trees and clones are described in Appendix Table A-1a.

BLACK WALNUT MORPHOLOGY

Because provenance test plantations and breeding arboreta had already been established for J. nigra, considerably more data were available for this species than for other walnuts (Appendix Table A-2). Note that these data were collected with the objective of determining mean values and the variability of several morphological traits within the species as a whole. Therefore, some plant material was collected from unreplicated arboretum plantings, and even when taken from adequately designed test plantations, specimens were often subjectively selected as being representative, rather than being chosen at random. Thus, the data are not suitable for rigorous statistical analysis, such as regression on various climatic and geographic variables that characterize the seed source. Indeed, it should not be inferred that the tabulated values accurately represent the areas from which the seed originated, since in some instances only a single specimen was scored. Therefore, seed source locations are listed only to show that sample trees came from throughout the species' natural range, and thus to provide assurance that the overall average values given are probably close to the true mean for black walnut.

In order to determine the sampling intensity that would be necessary to make adequate estimates of morphological characteristics in subspecific J. nigra units (families, clones, etc.), 6 half-sib families were analyzed. Parent trees are located in Iowa, Illinois, Indiana, and Ohio; 2 leaves per tree and 3 seedlings per family were analyzed for the following traits: position of longest leaflet, leaflet index, serration, leaflet curvature, base shape, and pubescence. Analysis of variance followed this format:

Source of variation	d.f.	Expected m.s.
Families	5	$\sigma_L^2 + 2\sigma_T^2 + 3\sigma_F^2$
Trees in families	12	$\sigma_L^2 + 2\sigma_T^2$
Leaves in trees	18	σ_L^2

Components of variance are shown in Table 2.

The relative contributions of families, individual trees, and leaves within trees varied considerably, depending on the trait evaluated. The variation related to seed source/parent tree and to seedlings within families was expected, but it appears that it will be necessary to analyze perhaps 3 to 6 leaves per tree in order to obtain reliable mean values for foliar characteristics of individual black walnut trees.

Table 2.--Components of variance for selected traits

in *Juglans nigra* leaves

Trait	Relative variation due to: Families	Trees in families	Leaves in trees
Longest leaflet	0.001	0	0.004
Leaflet index	0	0	0.08
Serration	0.33	0.10	1.88
Leaflet curvature	0.07	0.37	3.19
Base shape	0	0.75	0.36
Pubescence	1.01	1.07	2.19

MORPHOLOGY OF PERSIAN WALNUT AND PERSIAN X BLACK WALNUT HYBRIDS

A few *J. regia* seedlots representing parents or stands with good timber form or supposed hardiness to climatic damage produced seedlings large enough to be used in morphological analyses. Additionally, some grafted Persian walnut varieties were planted simply because of their reported early or late flowering habit. Data for these seedling families, stand collections or ramets are given in Table A-3.

Morphological data similar to those for *J. regia* and *J. nigra* are given in Table A-4 for putative *J. regia* x *nigra* hybrids. As might be expected, variation was greater in the hybrids than in either parent species in nearly all traits for which it was calculated.

COMPARING SPECIES AND HYBRID POPULATIONS

Without actually making a rigid discriminant analysis, I have tried to achieve the same results by examination of the statistics in Tables A-2, A-3, and A-4. The objectives were to select those traits in the two species and their hybrid that differ widely in their mean values, but at the same time do not vary much within taxa (Kendall and Stuart, 1966). Based on these requirements, characters such as leaflet length and base shape were not useful because means for both parent species and the hybrid were too close to be distinctive. The index of leaflet curvature values were also similar and furthermore, variability was rather high, with coefficients of variation ranging from 38 to 54 percent. Other traits scored, such as leaf length, leaflet lopsidedness, rachis redness, foliage and branchlet color, branchlet scurf, pith color, and leaf scar trichomes were not subjected to any sort of formal statistical analysis because of obvious excessive variability within the species (such as leaf length and coloration), virtual absence from all samples (scar trichomes) or insufficient data (pith color).

Initially, I thought that branchlet color might be a useful diagnostic characteristic since Persian walnut seedlings seemed to have a notably greenish tinge while black walnuts tended to be more brown, but seasonal changes hampered effective scoring. I assumed that branchlet color changes would be completed during the autumn, at least by the time of leaf fall. But when scoring a _J. nigra_ plantation, I tallied 2 blocks in mid-November and 4 blocks in early December. Surprisingly, analysis of variance showed the effect of 'blocks' to be significant for the green component of branchlet color. The trees scored in November were 54 percent more green than those rated in December; there was no seasonal change in the brown or gray components. As recently pointed out by Perry (1971), hardwood seedlings contain sufficient chlorophyll in their bark and buds to carry on some photosynthesis throughout the 'dormant' season. Apparently if differences in branchlet color are to be used in _Juglans_ taxonomic analysis, any populations to be compared will have to be scored at the same time and place.

Calculating a Hybrid Index

The following five traits of leaf morphology were selected as likely to be useful in distinguishing *Juglans regia* and *J. nigra* seedlings and putative hybrids between these two species:

leaflets per leaf

marginal serrations per centimeter

position of the longest leaflet pair on the leaf

leaflet length:width index

pubescence, combining both leaf surfaces plus the rachis

For each of these traits, *J. nigra* seedlings tended to have higher scores, with *J. regia* low and hybrids intermediate. They were thus directly and easily usable in calculating a hybrid index.

Many modifications have been suggested to improve and refine Anderson's (1936) original concept of the hybrid index which he acknowledged to be crude (Anderson, 1949). Essentially, the proposed changes involve using metric data or scales expanded beyond the few values used by Anderson, incorporating more than a few traits in the index, and weighting the index, either to 'equalize' the contributions of the several components, or to adjust their weight in proportion to their assumed relative usefulness. I have tried to make all three kinds of improvements in deriving an index useful for differentiating Persian x black walnut hybrids. As previously described, I used measurement or count values, or at least a 5-point scale to score each trait, and selected 5 traits to make up the index. Rather than select some arbitrary basis, it seemed logical to weight each trait according to its relative constancy within the parent species; that is, inversely to its relative variability. Several statistics describing sample variation might well have been used, such as the standard deviation, variance, and standard error of the mean. I chose standard error of the mean for weighting since it tends to minimize differences in sample size and should be equally reliable whether used in indexes based on family or clonal means or in those representing individual trees. Weighting values were derived as shown in Table 3, followed by the computation of a hybrid index for the overall average of *J. regia* and *J. nigra* as examples.

Weighted hybrid indexes were calculated for *J. regia* half-sib seedling families and grafted ramets, *J. nigra* seedling families, and an assortment of suspected *J. regia* x *nigra* hybrids. Individual seedling and ramet index values are shown in Figure 1, and family mean indexes as well as those for individual trees are given in Table A-5. The index does a good job of separating Persian from black walnuts and the distribution for both species appears to be reasonably normal.

Separation of Hybrids from Parent Species

The putative hybrids show considerable overlap of both of the presumed parent species. With only a couple of exceptions, those designated as F_1 ramets were provided by Dr. John W. McKay from the Agricultural Research Service walnut collection at Beltsville, Maryland; four of them were produced by control-pollination, and I am inclined to take his word

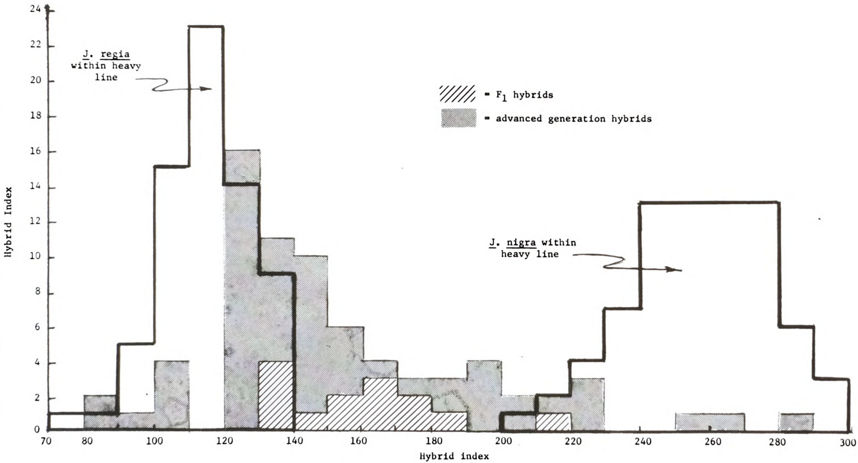


Figure 1.--Weighted hybrid index distribution for seedlings and ramets of _Juglans regia_, _J. nigra_, and putative _J. regia_ x _nigra_ hybrids.

Table 3.--Statistics used in computation of hybrid index for *Juglans regia* and *J. nigra*

	Leaflets per leaf		Serrations per cm		Longest leaflet position		Leaflet index		Pubescence	
	regia	nigra	regia	nigra	regia	nigra	regia	nigra	regia	nigra
$\bar{x}$	8.72	18.36	0.77	4.88	.135	.218	2.13	2.69	0.49	10.29
$S_{\bar{x}}$	0.14	0.51	0.14	0.17	.008	.009	.032	.031	0.04	0.30
Avg. $S_{\bar{x}}$	0.33		0.16		0.009		0.032		0.17	

Hybrid index value for average *J. regia* = 8.72/0.33 + ...0.49/0.17

= 26.42 + ...2.88

= 115.67

Hybrid index value for average *J. nigra* = 18.36/0.33 + ...10.29/0.17

= 55.64 + ...60.43

= 254.95

for the authenticity of the remainder. But Dr. McKay expressed doubts about the parentage of two of the hybrids, including our number NC-5959, the Fort Hunter walnut. This tree has a hybrid index of 215, greater than that for three of the black walnut trees sampled, and I suggest that it may be a backcross to black walnut. The other hybrid that McKay questioned is NC-5954, the Hillgate walnut; an index of 159 puts it squarely in the middle of the other hybrids.

The four F_1 hybrids with index values that overlap the range of Persian walnut (index between 130 and 140) include one of McKay's pedigreed J. regia x nigra crosses (NC-5924), so we cannot confidently state that a walnut with a hybrid index in the 130's is "not an F_1 hybrid."

The 60 trees designated as advanced generation hybrids are progeny of open-pollinated F_1's. Such trees are usually referred to as F_2's, but the designation is not necessarily correct since the parent trees may have been self-pollinated, backcross-pollinated by a tree of one of the parent species, or conceivably outcrossed to a third Juglans species. The three hybrids with the highest indexes (250+) all came from Beltsville where there was great opportunity for the hybrid parent trees to backcross with black walnuts. Four of the six hybrids with an index of less than 110 came from a hybrid tree owned by a nut grower in Indiana. It is easy to imagine that this tree backcrossed to his Persian walnuts, perhaps to a single cultivar that shed pollen at the appropriate time.

It is tempting to suggest that the large group of advanced generation hybrids with indexes between 120 and 150 may be comprised of F_2's.

This suggestion is purely speculative but seems not illogical, especially considering the arrangement of the hybrid walnut plantations at Beltsville in which grafted ramets of many F_1's were planted in adjacent rows and could easily intercross. At any rate, analysis of the progeny tended to confirm the hybridity of the parents (presumed to be F_1's), as shown, for instance, by greater variation within hybrid half-sib families than is found in the intraspecific half-sib families (Table 4). This simple table conforms to the pattern of increased phenotypic variance that would be expected if the hybrid families represent segregation among an increased number of genotypes (Clifford, 1954). For each size of family, the mean range between lowest and highest hybrid index is greater for seedlings of hybrid parentage than for seedlings of either single species. Another indication of parental hybridity is poor germination; seedlots from 25 *J. regia* x *nigra* parent trees averaged only 32 percent in three nursery tests. This low germination is not surprising, considering the reported infertility of Persian x black walnut hybrids (McKay and McKay, 1941); it is well below average germination values reported for black walnut, which range from 60 to 75 percent (Newbold, 1967; U.S.D.A., 1948).

Table 4.--Range of hybrid index within half-sib walnut families; table values show the difference between high and low hybrid index in each family

Taxon of parent tree	Number of seedlings per family: 2	3	4	5	6	8
Juglans regia	28	29	26	26	42	
	13	25	19	32	25	
	4	31			52	
		11				
		9				
Mean range	15	21	23	29	40	
J. nigra	12	28	22		54	
	26		40		50	
	25		58		46	
	1		13		33	
	1				39	
	12				26	
	11					
	7					
	1					
	3					
Mean range	10	28	33		41	
J. regia x *nigra*	39		56	56	59	44
	35			20		
				98		
Mean range	37		56	58	59	44

BUTTERNUT AND JAPANESE WALNUT MORPHOLOGY

As previously mentioned, in the Forest Service research plantations in southern Illinois there are only a few small trees of *Juglans cinerea*, butternut, and *J. sieboldiana*, Japanese walnut. There are also a couple of grafted heartnut (*J. sieboldiana cordiformis*) varieties, and I collected foliage from several trees in Michigan in an attempt to characterize the two species. Morphological data are summarized in Appendix Table A-6.

Even though the data were limited, butternut and Japanese walnut could be distinguished by several morphological features, but not the same ones used to differentiate Persian and black walnuts. Such traits as number of leaflets, position of the longest leaflet, serrations per cm, and leaflet index were quite similar for *J. cinerea* and *J. sieboldiana*; others, such as leaf length and leaflet curvature appeared to differ between the two species, but these data varied too widely to be reliable. I selected leaflet length (column 7 in Table A-6) and relative lopsidedness (14), rachis pubescence (15c), and pith color (16) as being most useful for distinguishing butternut and Japanese walnut and calculated a weighted hybrid index using the statistics in Table 5. Hybrid index values for the average of the two species are also shown. Most traits had lower values for Japanese walnut than for butternut, but since pith color scores were higher in Japanese walnut, they were subtracted when the indexes were calculated.

Table 5.--Statistics used in computation of hybrid index for *Juglans sieboldiana* and *J. cinerea*

	Leaflet length		Lopsidedness		Rachis pubescence		Pith color	
	sieb.	cin.	sieb.	cin.	sieb.	cin.	sieb.	cin.
$\bar{x}$	145.6	101.3	.020	.008	6.6	3.5	0.85	2.9
$S_{\bar{x}}$	9.7	4.0	.003	.001	0.80	0.23	0.13	0.82
Avg. $S_{\bar{x}}$	6.9		.002		0.52		0.48	

Hybrid index value for average *J. sieboldiana* $= 145.6/6.9 + .020/.002 + 6.6/.52 - 0.85/0.48$

$= 21.1 + 10.0 + 12.69 - 1.77$

$= 42.02$

Hybrid index value for average *J. cinerea* $= 101.3/6.9 + .008/.002 + 3.5/0.52 - 2.9/0.48$

$= 14.68 + 4.0 + 6.7 - 6.04$

$= 19.37$

BUTTERNUT X JAPANESE WALNUT HYBRIDS

Foliage was collected from several putative F_1 hybrids as well as from their open-pollinated (F_2?) seedlings, and from advanced-generation (F_3?) progeny of the seedlings. According to the owner of the F_1 parent trees NC-5827 through NC-5831, these trees are themselves half-sibs; interpollination among them must result in some inbreeding. Data representing the traits selected for inclusion in the weighted hybrid index are given in Appendix Table A-7 along with h.i. values for each collection.

In the five lines for which I have data from both parent trees and progeny, h.i. was reduced in each successive generation, but as shown in Figure 2, the index values for each generation group overlapped considerably. Furthermore, the index values for parents and progeny are not correlated ($r = -0.17$). My small sample of 34 seedlings may include more than the expected proportion of extreme segregants, and of course, hybrid index as such is not a heritable characteristic, but since the index is based on real morphological features, it seemed reasonable to expect a stronger parent-progeny correlation than was shown.

One possibility is that a tree with a low h.i., such as NC-5830, might have served as pollen parent for many of the seedlings. I visited the trees during flowering season and estimated the probable pollinator for each of the trees; my conclusions are shown in Table 6. Plainly, I picked the 'wrong tree' as the probable pollinator to verify my hypothesis. Indeed, the estimated and actual h.i. values for the progeny had a

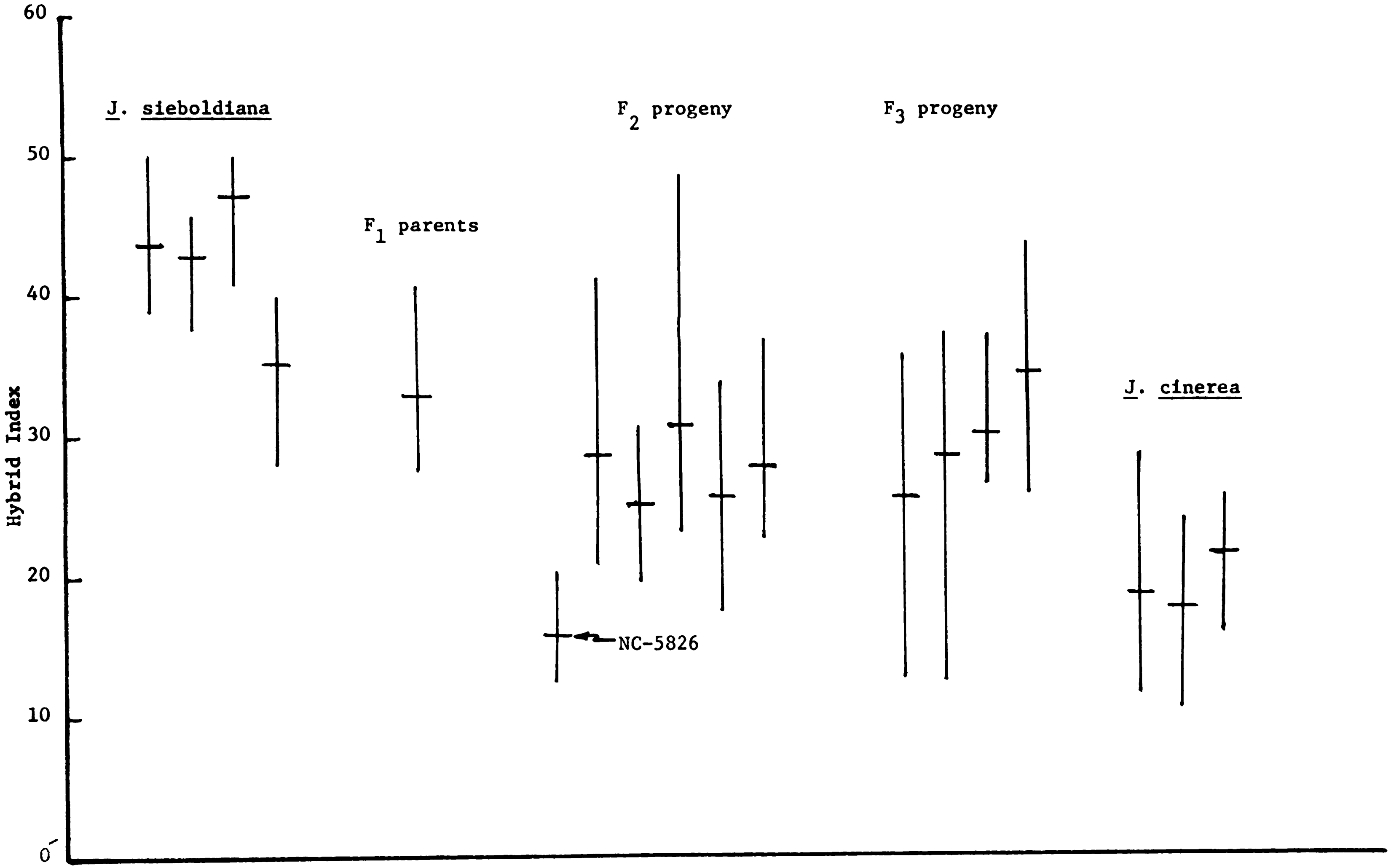


Figure 2.--Range and mean of hybrid index values for *Juglans sieboldiana*, *J. cinerea*, and three generations of putative *J. sieboldiana* x *cinerea* hybrids.

Table 6.--Hybrid index comparisons for hybrid walnut seedling families and assumed parental combinations

Female parent		Assumed male parent		Progeny H.I.	
NC No.	H.I.	NC No.	H.I.	Estimated from parents	Actual
5827	35.2	5828	40.5	37.8	28.6
5828	40.5	5828	40.5	40.5	25.0
5829	31.9	5827 and 5828	37.8	34.8	30.6
5830	27.4	5827 and 5828	37.8	32.6	25.4
5831	29.0	5828	40.5	34.7	27.6

correlation coefficient of -0.21, worse than before.

I believe that the five hybrid trees in Michigan were not only intercrossing and self-pollinating but also to some extent backcrossing to local butternuts. Since the average index of the presumed F_1 hybrids was 32.8, while their open-pollinated progeny averaged 27.5, and as previously calculated, the h.i. for average butternut was 19.4, a simple equation can be solved to estimate the proportion of butternut pollen introduced into the 'F_2' generation.

Let x = the proportion of butternut pollen

Then $19.4x + 32.8\ (1-x) = 27.5$

and $x = .395$

Assuming that intercrossing among the five parent hybrids 'averaged out', I conclude that almost 40 percent of the seedlings are butternut-backcross progeny.

Finally, the low h.i. values for all five trees in family NC-5826 (Figure 2) which averaged only 15.7, lead me to believe that the parent tree was not a hybrid at all, put a pure butternut.

BLACK WALNUT HYBRIDS WITH OTHER SPECIES

The walnut hybrids described so far may be considered as intrasectional hybrids, with *Juglans regia* and *J. nigra* being in Dode's (1906, 1909) section Rhysocaryon, and *J. cinerea* and *J. sieboldiana* in section Cardiocaryon. Dode did in fact place *J. cinerea* in a separate section, Trachyocaryon, but he is a noted taxonomic 'splitter', and Manning (1957) "does not consider [Trachyocaryon] distinct from Dode's section Cardiocaryon of Asia, an opinion shared by Nagel, 1914...."

Black Walnut x Butternut Hybrids

A natural intersectional cross between *J. nigra* and *J. cinerea* is certainly possible since the two species have overlapping ranges through a large area extending from Minnesota and Missouri to New York and North Carolina. But as discussed by Wright (1962), "as a general rule species which occupy the same sites in the same region do not cross with each other. Otherwise, how would the species distinction have been maintained?" This rule seems to hold for black walnut and butternut; even though the two species are morphologically and ecologically similar, there are no published accounts of naturally occurring hybrid swarms, and as previously mentioned, only a few references to artificially produced hybrids.

Considering this situation, I was skeptical about the actual hybridity of two lots of seed from putative *J. nigra* x *cinerea* hybrids in Cass County, Michigan. I searched for the parent trees in 1970 and

found that NC-5801 (= MSFG 848) had apparently been cut. At the location given for NC-5802, I could find no trees that I would consider to be other than ordinary black walnuts, but could not be certain that I had really located tree NC-5802. I imagine that the parent trees were suspected as hybrids primarily because they produced rather elongate nuts.

As can be seen in Tables A-2 and A-6 and in most dendrological keys, leaf characters of black walnut and butternut do not differ sufficiently to distinguish the two species. Instead, they are usually differentiated on the basis of flower morphology, fruit and nut texture, mature bark pattern, pith color, and presence of the 'moustache' of trichomes above the previous year's leaf scar. In our young plantations, only the latter two branchlet characters could be used in taxonomic analysis; they are the same two used in Harlow's (1948) Twig Key. In Table 7, average values for these two characters are compared for black walnut, butternut, and the NC-5801 and NC-5802 seedlings.

For both pith color and the presence of leaf scar trichomes, the NC-5801 seedlings scored even lower than the average values for black walnut. I suggest that the parent tree was pure _J. nigra_, but certainly an interesting seed tree as discussed under progeny test results in Chapter IV. NC-5802 can still be classed as a putative hybrid. Although pith color was as dark as or darker than that of the butternut collections I sampled, the average value for leaf scar trichomes was higher than any recorded for black walnut, but lower than any of the butternuts. This seedling family deserves further study, but one intermediate

Table 7.--Branchlet characters for *Juglans nigra*, *J. cinerea*, and progeny of two putative hybrid parent trees

Taxon	Sample size	Pith color $\bar{x}$	Pith color $S_{\bar{x}}$ 1/	Leaf scar trichomes $\bar{x}$	Leaf scar trichomes $S_{\bar{x}}$ 1/
J. nigra	overall average	1.45	0.27	0.04	0.01
J. cinerea	"	2.93	0.82	3.50	0.49
NC-5801	31	0.95	0.11	0	--
NC-5802	10	3.65	0.18	1.25	0.13

1/ Standard error of seedlot mean.

characteristic is hardly sufficient to make a case, and positive classification must be deferred until the trees have flowered and fruited.

Black Walnut x Heartnut Hybrids

Our plantations include seedlings from two trees designated as *J. nigra* x *sieboldiana* *cordiformis*. The parent tree for seedlot NC-5845 was apparently a grafted ramet and the nuts were labelled as having been collected from the hybrid variety 'Leslie Burt'. Only two seedlings survived from seedlot NC-5848, collected from 'a second-generation hybrid'. Selected data from Tables A-2, A-6, and the two hybrid seedlots are assembled in Table 8. The tendency for the progeny to resemble black walnut in some traits, Japanese walnut in others, and to be intermediate in about one-fourth of the statistics, provides good verification for the hybridity of both parents. As shown in Chapter IV, NC-5845 seedlings were also characterized by the combination of good form and above average growth rate in Illinois test plantations.

Table 8.--A comparison of morphological characters for *Juglans nigra*, *J. sieboldiana*, and progeny of two hybrid trees

Taxon	*J. nigra*		*J. siebold.*		NC-5845		NC-5848
Sample size	overall mean		overall mean		5		2
Statistic	$\bar{x}$	$S_{\bar{x}}$ 1/	$\bar{x}$	$S_{\bar{x}}$ 1/	$\bar{x}$	$S_{\bar{x}}$ 1/	range
Leaflets (no.)	18.4	0.5	14.7	1.0	17.8	0.5	9- 15
Leaflet length (mm)	118	3.6	146	9.7	113	7.8	88-110
Serrations per cm	4.9	0.2	3.5	0.3	3.6	0.7	2-2.5
Base shape	3.7	0.1	2.8	0.3	2.4	0.4	2-3.5
Pith color	1.5	0.1	0.9	0.1	2.1	0.6	1-3.5
Leaf scar trichomes	0.04	0.01	3.0	0.6	0.6	0.4	0- 2
Rachis pubescence	2.8	0.2	6.6	0.8	5.2	0.9	0- 4

1/ Standard error of seedlot mean.

CHAPTER III

CHROMATOGRAPHIC ANALYSES OF WALNUT FOLIAGE EXTRACTS

Paper chromatography has proved to be a useful adjunct to morphological analysis in several taxonomic studies (Carter and Brehm, 1969; Dugle, 1966; Hunter, 1967; McHale and Alston, 1964), and in some studies of plant hybridization, chemical analysis has successfully been used alone (Alston and Hempel, 1964; Alston and Simmons, 1962; Hanover and Wilkinson, 1970; Williams, 1955). In some cases, species-specific compounds representing both parental species were found to accumulate in the hybrids (Alston, *et al.*, 1962), while with other species, unique compounds appeared in the hybrid that were not present in either parent (Alston, *et al.*, 1965).

Paper chromatography was used to analyze *Juglans* foliage extracts for presence of polyphenols following the methods of Hanover and Hoff (1966). More than 90 chromatograms were developed, using both fresh and dry leaves as the starting material, preparing extracts of several different volumes and concentrations, and using several combinations of spot position and solvent flow direction on the chromatography paper. In preliminary analyses, fresh and dried leaves both generated satisfactory extracts, using 20 gm and 10 gm samples, respectively. Diluting the concentrated butyl alcohol fraction to only 1 ml sometimes yielded

an extract so viscous that when it was applied to the paper the spot failed to dry. Using more alcohol to dilute such heavy extracts to 1 1/2 to 2 ml produced solutions and spots that dried promptly. Even so, several chromatograms were excessively streaked. A series of tests using aliquots of between 20 and 50 µl of the concentrated extract, indicated that 20 or 25 µl was sufficient to produce a chromatogram that exposed a large number of compounds and one in which streaking was not severe enough to prevent distinguishing most spots. In the course of these repeated trials, the concentrated extracts of the butyl alcohol fraction yielded good chromatograms even after cold storage of the extract for 8 months or more.

Finally, I compared the quality of papers produced by spotting in all 8 possible positions (each of 4 corners, front and back), and also by running the first-direction solvent across the short dimension of the paper and the second solvent the long way rather than the conventional long-way-first procedure. Spotting the extract on the upper side of the paper in the upper-right-hand corner (Figure 3) produced the best chromatogram.

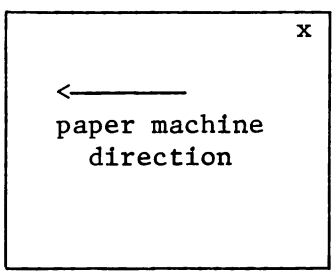


Figure 3.--Applying concentrated foliage extract at "x" produced the best chromatograms.

Running the first-direction solvent across the short dimension of the paper produced no better chromatograms, but different compounds were occasionally exposed. Among other idiosyncracies of the technique, I finally had to accept the fact that papers placed in the front rack of the cabinet would not make good chromatograms; putting a blank paper in the front did seem to improve the quality of the four papers behind it.

CHROMATOGRAPHIC TECHNIQUE

The following technique produced the best spot separation and definition.

1. Collect 20 gm fresh leaves or 10 gm air-dry leaves from herbarium specimens. Remove rachis and coarse portions of petiolules before weighing.
2. Extract for 2 minutes in 100 ml boiling water acidified to 0.05 percent HCl.
3. Macerate in semi-micro blender for 1 minute at high speed.
4. Suction-filter through Büchner funnel containing Whatman No. 4 filter paper plus 1/4-inch pad made of filter paper macerated in water.
5. Return macerated leaves, pad, and filter paper to beaker, add 100 ml boiling water and boil 2 minutes more.
6. Macerate as in 3.
7. Refilter as in 4.
8. Wash macerated leaves and filter paper with 50 ml boiling water and filter to dryness. Discard solid residue.
9. Allow filtrate to cool in separatory funnel. Gently wash this water extract 5 times with 50 ml ethyl ether to remove waxes and fats. Discard ether extract.
10. Wash remaining water extract 5 times with 50 ml normal butyl alcohol. Remove emulsion by decanting and by centrifuging at 4000 r.p.m.

11. Reduce butanol fraction in rotary film evaporator at 50-55° C. When virtually dry, take up in 1 ml n butyl alcohol.

12. Spot Whatman 3MM chromatography paper in the upper right hand corner as it comes from the box with 25 μl of concentrated extract and place in cabinet for descending chromatography.

13. Enter first-direction solvent, made in proportions of 4 butyl alcohol: 1 glacial acetic acid: 5 water. Solvent must be mixed in advance and allowed to stand in separatory funnel all day; the water/acetic acid (lower) layer is then discarded and a few drops of butyl alcohol added to minimize dissolution. Develop first-direction down the long dimension of the paper in the same direction as is shown on the box as "machine direction". A typical run requires 15 to 19 hours to descend to bottom of paper.

14. Remove papers, dry them in fume hood and return them to cabinet.

15. Enter second-direction solvent--10 sodium formate: 200 water: 1 formic acid. Typical second-direction run requires 4 to 6 hours.

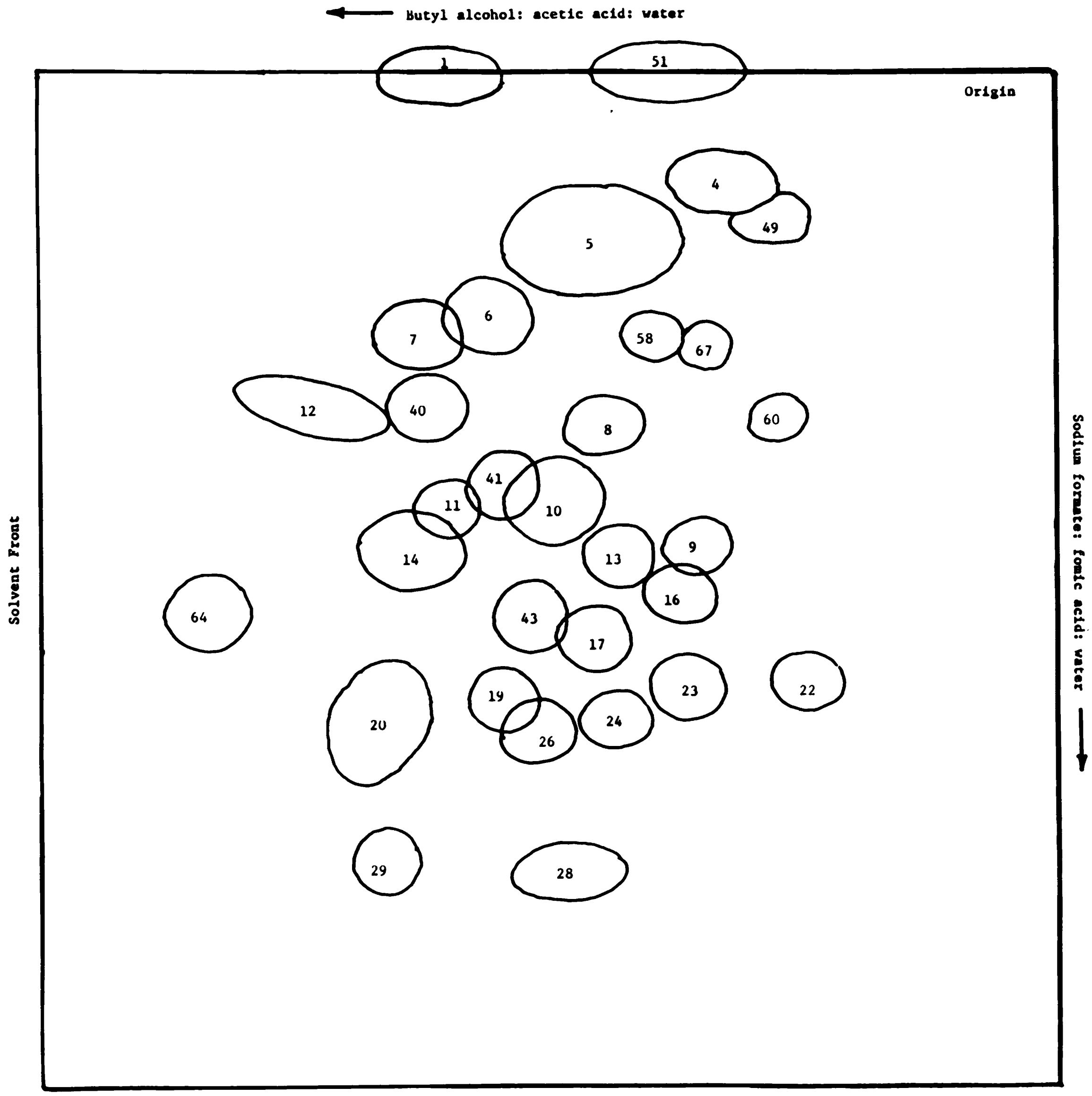
16. Remove papers and dry as before. Outline spots and label as to color shade under visible and long-wave ultraviolet light.

17. Expose papers to ammonia fumes for one hour. Outline spots and label as before under both visible and ultraviolet light.
18. Spray papers with freshly prepared diazotized sulfanilic reagent; outline and label spots.
19. Spray promptly with 2N sodium hydroxide; outline and label spots.

PAPER CHROMATOGRAPHY AS AN AID IN IDENTIFYING PERSIAN X BLACK WALNUT HYBRIDS

Paper chromatograms prepared from 32 foliage samples proved to be usable in the attempt to distinguish *Juglans regia*, *J. nigra*, and putative hybrids between the two species. In the several chromatograms, a total of 79 different spots was distinguished. The average position of these spots on the paper chromatograms is shown in Figures 4, 5, and 6, and their characteristic color under visible and ultraviolet light, following exposure to ammonia fumes and after two reagent sprays is given in Table A-8. I made no attempt to identify the compounds that were represented by co-chromatography with known pure chemicals, elution of the spots followed by spectral analysis, or any other method. However, I do speculate that spot 10 which was present on every paper may be hydrojuglone glucoside or a related juglone derivative.[2/]

2/ Hess, Charles E. The vegetative propagation of black walnut (*Juglans nigra* L.). A report of research conducted under Cooperative Agreement Supplement No. 16 to the Master Memorandum of Understanding of June 1, 1953, between the Forest Service and the Purdue Agricultural Experiment Station, Contract No. A9fs-12234, 11 pp.



Solvent Front

Figure 4.—Composite chromatogram of butyl alcohol soluble compounds in *Juglans regia* foliage.

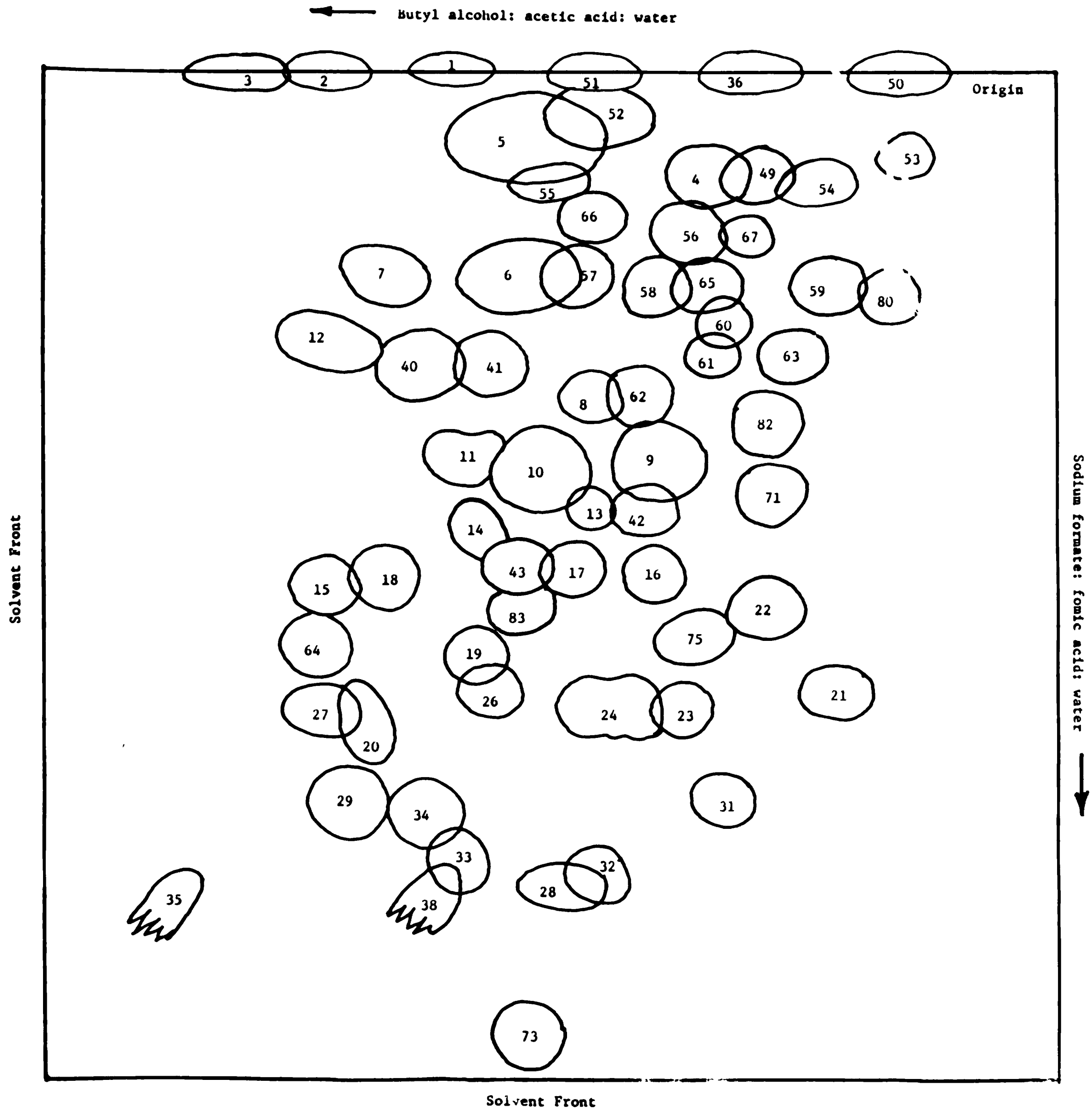


Figure 5.--Composite chromatogram of butyl alcohol **soluble compounds in** _Juglans nigra_ **foliage.**

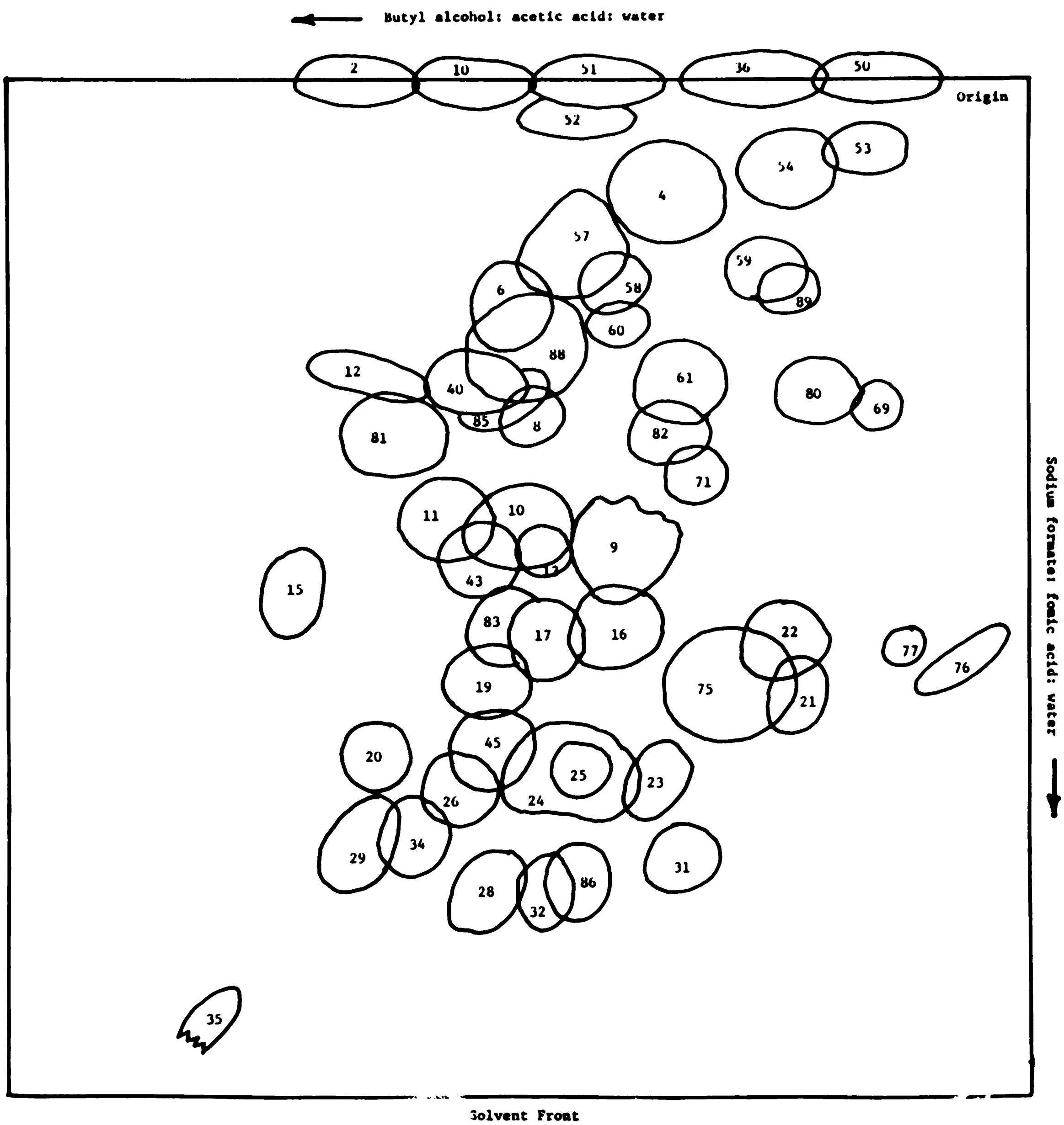


Figure 6.--Composite chromatogram of butyl alcohol soluble compounds in *Juglans regia* x *nigra* foliage.

Paired Affinity Index

I calculated paired affinity (PA) indexes for several pairs of chromatograms (Table 9). The PA index was designed "to compare pairs of species in terms of chromatographic affinity (or presumptive biochemical affinity)" (Ellison, et al., 1962), and I assumed that it might be suitable for individual chromatograms. The index was calculated as follows:

$$PA = \frac{\text{spots common to A \& B}}{\text{total spots in A + B}} \times 100$$

There seemed to be an upper limit to PA values of about .65 to .70 using my technique, with interspecific comparisons expectably lower, in the .35 area. For instance, two chromatograms spotted with the same extract must 'contain' the same compounds; but the analytical technique was not adequate to expose all of them. Such discrepancies may be at least partially explained by differences in environment within the developing cabinet and by the fact that some spots were simply overlooked when the chromatogram was being scanned. The relatively low correspondence between papers run with two samples of the same extract suggested that a composite value might be appropriate. Thus, when chromatograms 70-1, 70-9, and 70-10 were 'pooled' (they were all derived from the same tree), they had a total of 62 spots. Similarly, 70-17 and 70-17-S together had 33 spots, and 70-18 and 70-18-S had 36. When PA indexes were calculated for these pooled chromatograms, the values tended to be somewhat higher as shown in Table 10. Pooling apparently allowed a closer approach to exposing all the polyphenols present in an extract, and should minimize 'differences' between two chromatograms that are due

Table 9.--Paired affinity index for several *Juglans* chromatograms

Chromatograms compared	Spots: Common	Spots: Total	PA	Remarks
70-1 : 70-9	37	59	.62	Dry leaves vs. fresh leaves,
70-1 : 70-10	39	60	.65	same population
70-9 : 70-10	35	54	.65	Two papers from one extract
70-17 : 70-17-S	23	33	.70	Changed paper orientation
70-18 : 70-18-S	24	36	.67	Changed paper orientation
70-10 : 70-15	26	50	.52	Comparisons among 5 *Juglans nigra*
70-10 : 70-16	27	48	.58	collections (10 pairs of chro-
70-10 : 70-17	26	49	.53	matograms)
70-10 : 70-18	22	50	.44	
70-15 : 70-16	23	39	.59	
70-15 : 70-17	25	37	.68	
70-15 : 70-18	22	37	.59	
70-16 : 70-17	25	36	.69	
70-16 : 70-18	17	41	.41	
70-17 : 70-18	17	40	.43	
7b : 8b	23	46	.50	Two *J. regia* samples
7b : 70-10	24	56	.43	*J. regia* : *J. nigra* comparisons
7b : 70-17	16	49	.33	(4 pairs of chromatograms)
8b : 70-10	21	57	.37	
8b : 70-17	16	47	.34	

Table 10.--Comparison of paired affinity index with and without pooling of similar chromatograms

Pooled chromatograms compared	Spots: Common	Spots: Total	Pooled PA	Regular PA	Single chromatograms compared
70-1, -9, -10 : 70-17, -17-S	33	63	.52	.53	70-10 : 70-17
70-1, -9, -10 : 70-18, -18-S	36	62	.58	.44	70-10 : 70-18
70-17, -17-S : 70-18, -18-S	27	42	.64	.43	70-17 : 70-18

to weak or overlooked spots rather than truly distinctive compounds. Whenever possible, two chromatograms should be prepared from each extract and a composite table of R_F values prepared. Even by following this precaution, I doubt that the PA index can be used satisfactorily to assess the hybridity of individual suspect trees; the technique seems more appropriate for classification of populations such as seedling families or hybrid swarms.

Diagnostic Values

Some spots, such as 1 and 10, were found on all papers for both Persian walnut, black walnut, and their hybrid, and therefore could not be used to distinguish the taxa for this study. Others, such as 50 and 54, were found in all black walnut chromatograms and those for most of the hybrids, but never in the Persian walnut papers, and might be classified as good indicators of 'non-*regia*' trees. Other spots tended to be more prevalent in one taxon than in the others, but the distinction was not usually absolute.

In order to quantify these differences, I developed 'diagnostic values' based on the simple presence or absence of the 79 spots on each chromatogram. The values were computed from a table of decimal fractions indicating the relative frequency of occurrence of the compounds in all the chromatograms for each species or the hybrid (Table A-9). These decimal fractions, designated 's.f.' for spot frequency, were used to calculate spot frequency differences (s.f.d.) for each spot as being more characteristic of *J. nigra* (s.f.d. relatively high), *J. regia* (s.f.d. relatively low), or _J. regia_ x _nigra_ hybrids (intermediate). To obtain the appropriate s.f.d. values when comparing two taxa, the calculations were made by subtracting spot frequencies for hybrids from those for _J. nigra_; similarly, _J. regia_ spot frequencies were subtracted from hybrid frequencies and _J. regia_ fractions were subtracted from _J. nigra_. Thus, to calculate the s.f.d. values given for spot 2 in Table A-10, spot frequencies were taken from Table A-9 as shown below:

nigra - _regia_: .53 - .22 = .31

nigra - hybrid: .53 - .38 = .15

hybrid - _regia_: .38 - .22 = .16

Spot frequency differences for each spot that was present were summed to arrive at the diagnostic value (d.v.) for one chromatogram. Thus, the diagnostic value indicating that chromatogram 70-1 represents a black rather than a Persian walnut was calculated as follows:

$$\text{d.v.}_{70-1} = \text{s.f.d.}_{1} + \text{s.f.d.}_{3} + \text{s.f.d.}_{4} \ldots + \text{s.f.d.}_{83}$$
$$11.6 = 0 + .25 - .33 \ldots + .47$$

Note that s.f.d.'s for spots 2, 12, 14, etc., are not included in the calculation since as shown in Table A-9, these spots were not identified on paper 70-1.

Diagnostic values for 32 chromatograms are given in Table 11 along with hybrid index values in each instance that hybrid index was calculated for the same material. Spot frequency differences were initially calculated on the basis of relative frequency of the compounds in chromatograms representing black and Persian walnuts. Diagnostic values computed from these spot frequency differences separated the two species

Table 11.--Diagnostic values for *Juglans nigra*, *J. regia*, and *J. regia* x *nigra* based on paper chromatograms of foliage extracts, and hybrid index values based on morphological data.

(Hybrid index values from Table A-5 are given for comparison)

Chromatogram number	Accession number	Diagnostic values: *n.* vs. *r.*	Diagnostic values: *n.* vs. *h.*	Diagnostic values: *h.* vs. *r.*	Hybrid index
J. nigra					
70- 1	6156	11.6	9.1		
- 9	6156	12.1	8.4		
-10	6156	11.1	7.5		
-15	6137	9.9	7.4		
-16	6160	8.4	5.4		268
-17	6163	9.3	4.5		254
-18	6175	9.0	4.2		
-19	6166-4	9.0	6.6		257
-20a	6163-6	9.0	7.4		253
-20b	6163-6	10.3	7.8		253
-22	6159-4	7.6	6.9		258
-23	6163-9	13.3	9.3		245
-24	6138-5	11.7	8.4		264
-25	6160-10	9.1	5.7		280
-26	6138-7	9.7	9.3		261
J. regia					
7b		-1.6		-5.0	
8b		-0.9		-5.9	
71- 1	5892	-1.2		-3.8	124
- 2	5899	-0.9		-3.5	119
- 3	5902	-3.4		-3.9	119
- 5	5907	-4.9		-5.6	126
- 6	5908	-5.8		-5.8	111
- 7	5909	-4.7		-6.1	113
- 8	6196	-3.8		-6.2	
J. regia x *nigra*					
71- 9	5881	8.4	-4.8	13.2	187
-10	5927	9.0	-4.5	13.4	165
-11	5954	6.1	-5.2	11.8	159
-12	5959	7.9	-4.6	11.7	215
-13	5846	0.8	-5.6	6.4	128
-14	5880	1.3	-3.6	4.9	159
-15	5919	3.1	-5.2	6.2	
-16	5928	3.9	-5.6	8.9	159

quite well, with *J. nigra* diagnostic values ranging from 7.6 to 13.3, and values for *J. regia* between -0.9 and -5.8. When the *nigra* vs. *regia* spot frequency differences were applied to hybrid walnut chromatograms, they tended to have nicely intermediate diagnostic values, but the range from 0.8 to 9.0 included three values overlapping the range for black walnut; one of them was the questionable Fort Hunter hybrid, NC-5959. The diagnostic value of 7.9 calculated for this clone adds support to the prospect that this hybrid is a backcross to black walnut. The Hillgate hybrid (NC-5954) was once again satisfactorily separated from either putative parent species with a diagnostic value of 6.1.

Since the diagnostic values calculated from *nigra* vs. *regia* spot frequency differences did not completely separate the hybrids from the two species, I also calculated diagnostic values based on *nigra* vs. hybrid and *regia* vs. hybrid differences. These species vs. hybrid values fell into two quite distinctive groups (Table 11).

As might be expected, the diagnostic values based on paper chromatograms were correlated with the hybrid index values based on leaf morphology. Correlation coefficients are given in Table 12 and show that hybrid index was highly correlated with overall diagnostic values, no matter which pair of taxa was used to compute spot frequency differences. When the hybrid index:diagnostic value correlation was made for the hybrids only, the diagnostic values based on *nigra* vs. *regia* spot frequency differences were best correlated with hybrid index, even though these values were the ones that showed some hybrid clones overlapping

Table 12.--Correlation between hybrid index and diagnostic values for *Juglans nigra*, *J. regia*, and *J. regia* x *nigra* hybrids

Taxa compared to compute spot frequency differences	Correlation coefficients based on:	
	All samples	Hybrids only
J. nigra vs. *J. regia*	.88	.71
J. nigra vs. hybrids	.91	.35
J. regia vs. hybrids	.85	.57

black walnut values. Clearly, it will be necessary to sample more trees, especially pedigreed F_1's and backcrosses, before either scoring system can be used with complete confidence. Nevertheless, the generally high correlations between the two systems should enable us to rely for the most part on the morphological hybrid index; the more expensive chromatographic techniques and calculations of diagnostic values will probably be used only when necessary to resolve doubtful classification.

CHAPTER IV

PLANTATION PERFORMANCE

Seedlings from 17 of the putative hybrid walnuts have been outplanted in small replicated progeny tests in Barry County, Michigan and Alexander County, Illinois. Twelve seedlots are common to both plantations, but each lacks standard or check seedlings, such as good black walnut of local origin, against which to measure possible superiority of the hybrid trees.

Height data from measurements taken in January, 1971, in Michigan and July, 1971, in Illinois are presented in Table 13. The nominal butternut x Japanese walnut F_3 hybrids (NC-5835 and NC-5836) were among the taller progenies in both states, while Persian x black walnut hybrids were relatively short. Spearman's rank correlation test (Snedecor, 1957) for height of the 12 seedlots common to both locations was highly significant with $r_s = 0.83$.

In an attempt to assess tree form, the trees in Michigan were measured for leader deflection from vertical (indicating crook plus lean); in Illinois we tallied the number of main stems, defined as being at least 85 percent as tall as, and 2/3 the diameter of the leader. According to analysis of variance, differences due to seedlot were statistically significant in the Illinois plantation, but after the Michigan data were adjusted to show leader deflection as a percent of

Table 13.--Relative height and tree form traits (family mean as a percentage of plantation mean) of seedlings from open-pollinated putative hybrid walnut trees

NC-number	Presumed *Juglans* taxon of open-pollinated mother tree	Plantation location: Michigan[1/] Height	Michigan[1/] Form	Illinois[1/] Height	Illinois[1/] Form
5801	*nigra* x *cinerea*[2/]			116	75
5822	*regia* x *nigra*	63	81	67	72
5826	*sieboldiana* x *cinerea*[3/]	100	86	95	95
5827	*sieboldiana* x *cinerea*	126	110	100	90
5828	*sieboldiana* x *cinerea*	93	114	96	127
5829	*sieboldiana* x *cinerea*	100	117	94	119
5830	*sieboldiana* x *cinerea*			91	149
5831	*sieboldiana* x *cinerea*	97	101	112	108
5832	*sieboldiana*	141	104		
5833	*regia* x *nigra*			94	79
5834	*cinerea*	130	85	114	116
5835	*sieboldiana* x *cinerea*	110	98	136	117
5836	*sieboldiana* x *cinerea*	132	98	121	93
5842	*regia* x *nigra*	85	97		
5843	*regia* x *nigra*	65	98	67	76
5845	*nigra* x *sieboldiana*	104	106	120	78
5846	*regia* x *nigra*	68	90	70	106
Mean height, cm.		111		220	
Mean leader deflection (cm. per cm. height)			0.125		
Mean number of 'main stems'					2.32

1/ Trees in Michigan plantations were 5 growing seasons old from seed when measured; trees in Illinois were 6 years old.

2/ Probably *J. nigra*, see page 32.

3/ Probably *J. cinerea*, see page 30.

total height, there was no appreciable difference among seedlots (Table 13). Ranking among the families for the two form characters was not closely correlated ($r_s = 0.33$).

Seedlot NC-5822, comprised of seedlings from three Persian x black walnut trees in Pennsylvania, had the least leader deflection in Michigan and fewest main stems in Illinois. But since these seedlings were also the shortest in both plantations, they don't seem to offer much promise as timber trees. The correlation between height and form was quite weak, with r = +.19 in Illinois and +.27 in Michigan, so there should be a respectable opportunity to select and breed for improvement in both traits simultaneously. For instance, NC-5836, a butternut x Japanese walnut F_3(?) family, grew notably taller than average in both plantations with somewhat better form (less than average leader deflection and fewer than average main stems). Other families also performed well, including NC-5801 and NC-5845 in Illinois. As previously mentioned, the parent tree for NC-5801 is apparently a black walnut rather than a hybrid; progeny of this tree also grew well in earlier tests in Michigan.

CHAPTER V

CONCLUSIONS AND SUGGESTIONS FOR FURTHER RESEARCH

The research reported in this paper has involved a certain amount of 'debunking', since some of the hybrid walnut trees and nuts pointed out or sent to me have been found to be pure *Juglans* species. My results tend to verify the generally accepted conclusion that naturally occurring walnut hybrids are quite rare. Continued skepticism regarding putative hybrids appears to be justified, especially when hybridity is postulated on the basis of nut form that is unusual or perhaps intermediate between presumed parent species. Fruit shape varies greatly within *Juglans* species and many taxonomic forms have been described and named on the basis of little more than distinctive shell thickness or ridging (Rehder, 1940). Other less variable traits of vegetative morphology need to be incorporated into any satisfactory system of hybrid analysis.

On the other hand, many of the advanced generation seedlings that I studied still cannot be identified with certainty. As they mature, flower and fruit characteristics can be added to hybrid indexes and more precise determinations should be possible. Since I had to rely on vegetative traits to characterize the immature trees of the presumed hybrids, it was necessary to use the same traits when constructing hybrid indexes based on pure species. But with the exception of the twig key (Harlow, 1948) used to distinguish butternut from black walnut, none of the references that I have seen (Funk, 1966) attempts to differentiate Juglans species without reference to flowers and fruit. Future efforts to identify putative hybrids should include the use of multiple samples of leaves within trees and trees within families since hybrid seedlots have been found to be quite variable in both morphological and biochemical analyses.

The increased variability of hybrid populations is basically desirable since it can reasonably be assumed to result from increased genetic diversity. And increased diversity is the logical objective of hybridization programs. The examples of hybrid progeny with greater than average height growth and better than average form are encouraging since rapid growth and poor form have been shown to be strongly correlated in Juglans nigra progeny tests (Bey, et al., In press). If recombinations which yield simultaneous improvement in both traits prove to be common among hybrid walnut progeny, an expanded program of hybrid breeding will be easily justified.

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VITA

David Truman Funk

Candidate for the Degree of
Doctor of Philosophy

Final Examination: October 14, 1971

Guidance Committee: Drs. M. Wayne Adams, August DeHertogh, James W. Hanover, Stanley K. Ries, and Jonathan W. Wright, Chairman.

Thesis: Identification and Performance of *Juglans* Hybrids

David T. Funk was born in Greensburg, Indiana on October 17, 1929, and spent his youth in small southern Indiana towns where his father was a school teacher and administrator. He was educated in the public schools, graduating from the high school at Osgood, Indiana. Funk received B.S. and M.S. degrees in Forestry from Purdue University in 1951 and 1956. Mr. Funk married the former Nancy Lou Ross in 1956; they have a daughter, Sara Jane, age 8.

Funk has worked continuously for the U.S. Forest Service for the past 20 years except for active duty in the U.S. Navy from 1951-1955 and time spent at graduate school. He has been assigned to silviculture and tree improvement research projects in Ohio, Indiana, and Illinois and has authored more than 25 publications on reforestation, propagation, and genetics. He presently serves as Sponsoring Scientist for two Public Law 480 tree breeding research projects in Yugoslavia.

Funk is listed in the 1969 edition of "American Men of Science." He is a member of the American Association for the Advancement of

Science, the American Society of Plant Taxonomists, the International Plant Propagators' Society, the Society of American Foresters, Sigma Xi, and Xi Sigma Pi. He is presently chairman of the Society of American Foresters national tree improvement committee, the Central States Tree Improvement Conference, and the tree improvement committee of the Walnut Council.

APPENDIX

Table A-1.--Morphological characteristics of all *Juglans* species

Juglans species	Flowers: Catkin length (cm)	Flowers: No. pistillate flowers	Fruit: Glabrous (0) vs. pubescent (3)	Fruit: Fruits/cluster	Fruit: Width (cm)	Fruit: Length (cm)	Nut: Surface (smooth = 0; deep, sharp ridges = 5)	Nut: Width (cm)
nigra	5-12	2- 5	1	1- 3	3.5-8.	4.5-6.	5	2.8-5.
microcarpa	7-11	1- 3	1		1.2-2.2		2	1. -1.9
m. var. *Stewartii*					1.8-2.5			
major	7-17	1- 4	2		1.9-3.5			1.8-3.
m. var. *glabrata*		1- 3	1		3.6	4.	3	3. -4.0
hindsii	8-10	1- 3	2		3. -5.		1	
californica	5- 7	1- 3	2		1. -3.2	3.3-3.6	2	
hirsuta	6-10	1- 3	3		3. -4.7		4-5	2.5-4
pyriformis	18-22				4.3-4.7	4.5-5.	3-4	3.5-4.5
mollis	8-16	1- 3	1		3 -5	3.5-6	2	2.5-4.5
olanchana	15-30	2- 5	0		4.3-5.5	4.7-5.8		3.4-4.5
o. var. *Standleyi*						3.±	2	2.4-3.5
steyermarkii		3	3		4	4.5	5	
neotropica	10-25	5-10	1		3.5-6.	3.5-6.	3	3. -4.5
venezuelensis	5-10	6- 7	3				4	2.3
boliviana	10-16	3- 6					3-4	3.5-4.5
soratensis	15	5			4.5	5	5	3.1-3.4
jamaicensis	10-13	5- 8	1		2.6-4.	3.5	3	1.9-3.1
australis	6-15	3- 6	1-2		2.5-3.5	3 -3.3	1	1.7-3.0
regia	5-15	1- 5	0	1- 3	4. -5.	-4.4	0	2.3-3.9
cathayensis	20-30		3	6-10		3 -4.5	5	
mandshurica	10	5-10				4.5-5.5	5	
stenocarpa							5	
sieboldiana	10-30	10-20	3	5-12		2.5-3.5	5	
s. var. *cordiformis*				2- 6	3.5-4.5	4.5-5.0	0	3.
cinerea	5- 8	5- 8	3	2- 5		4 -6.5	5	

Table A-1 (Cont'd.)

Juglans species	Leaves: Glabrous (0) vs. pubescent (3)	Leaflet number: Minimum	Leaflet number: Maximum	Leaf length (cm)	Leaflet: Length (cm)	Leaflet: Width (cm)	Base shape (key, p.11)	Branchlets: Trichomes at upper edge of leaf scars	Branchlet color (gray, red, brown, yellow)	Pith color (tan to dark brown)	Mature tree: Height (meters)	Bark color (gray, brown, striped)
nigra	3	13	23	30-70	6-17	3 -6.2	3-4	0	g-B	Tan	50	B
microcarpa	1	13	31	10-23	4- 8	1. -1.5	4	0	r-Y	Lt.B	10	
m. var. Stewartii		17	32			1.1-1.9						
major	2-3	9	19	30-36	3- 9	1.7-3.4	4-5	0	r-B	Tan	20	gB
m. var. glabrata	0	9	17	30-45	8-15	2.3-5.5	5	0				
hindsii	1	15	23		6-10			0			15-25	
californica	0	11	19	30	4- 7	1.5-2.2	2	0	B	Lt.B	16	
hirsuta	3	14	23		15	2.5-6	3-4			Lt.B	large	
pyriformis	1	18	31	40-60	10-15	3 -4.5			B	Lt.B	tall	
mollis	2	8	16		6-15	3 -5	4		dk.B	Lt.B	med.	
olanchana	1	17	23	45-65	14-17	5 -6	5	0		Tan	55	B
o. var. Standleyi	3		19		12	4 -4.5	4-5	0				
steyermarkii	3	16	18	60	14-17	5 -8	4				17	G
neotropica	3	12	19	34-60	10-16	4 -8	2-3			Lt.B	30	B,G
venezuelensis	2	16	20	43	9-11	3.5-4	5				low	
boliviana	1	14	18		13-17	5 -6.5	6	0		Lt.B	35	dk.B
soratensis	1	15	17	42-44	8-11	3.5-5.5	3			Lt.B	9-10	lt.G
jamaicensis	1	12	22	30-55	7-11	2.5-4.5	2-3			Lt.B	45	
australis	1	14	20	30-64	8-16	3 -5	3	0	r-B	Lt.B	7-18	
regia	0	5	13	20-45	8-17	4.0-7.3	3-5	0	b-G	Tan	30	G
cathayensis	3	9	17	40-90	8-22	5. -	1-2	+			12-25	G
mandshurica	3	9	17	30-40+	7-18	4. -	2-3	+		Brn.	20	
stenocarpa	1	9	17					+				
sieboldiana	2	9	21	55-60	8-18	3 -7	3-4	+		Tan	20	
s. var. cordiformis	2	9	17	35-45	11-15	4 -6	2-3	+				
cinerea	3	11	19		6-12	3 -5	3-5	+		dk.B	30	S

Table A-1a.--*Juglans* seed trees and clones used as sources of experimental walnut seedlings and grafted trees

Number	Plant material and origin
846	*J. cinerea* herbarium specimens from plantation MSFGP 4-62, Fred Russ Forest, Decatur, Mich.
1101	*J. nigra* seed, Stone Co., Ark.
1102	*J. nigra* seed, Scott Co., Ark.
1601	*J. nigra* seed, Union Co., Ill.
1605	*J. nigra* seed, Will Co., Ill.
1708	*J. nigra* seed, Perry Co., Ind.
1709	*J. nigra* seed, Greene Co., Ind.
1803	*J. nigra* seed, Delaware Co., Ia.
1805	*J. nigra* seed, Polk Co., Ia.
2004	*J. nigra* seed, Laurel Co., Ky.
2005	*J. nigra* seed, Madison Co., Ky.
2601	*J. nigra* seed, Scott-Smith Co., Miss.
2602	*J. nigra* seed, Chickasaw Co., Miss.
2708	*J. nigra* seed, Bates Co., Mo.
3101	*J. nigra* seed, Caldwell Co., N. C.
3102	*J. nigra* seed, Graham Co., N. C.
3105	*J. nigra* seed, Buncombe Co., N. C.
3803	*J. nigra* seed, Union Co., Tenn.
3806	*J. nigra* seed, Hardin Co.. Tenn.
3901	*J. nigra* seed, Freestone Co., Tex.
4101	*J. nigra* seed, Rockbridge-Amhurst Co., Va.
5801	*J. nigra* (?) seed, Cass Co., Mich. (=MSFG 848)
5802	*J. nigra* x *cinerea* o.p. seed, Cass Co., Mich.
5810	*J. regia* seed, Hardy Russian strain P.I. 264373
5811	*J. regia* seed, Hardy Russian strain P.I. 264376
5816	*J. regia* seed from 6 sib trees originating in Stryj, Galicia
5822	*J. regia* x *nigra* o.p. seed from 3 trees near Lancaster, Pa.
5826	*J. cinerea* (?) seed, Berrien Co., Mich. (=MSFG 841)
5827	*J. sieboldiana* x *cinerea* o.p. seed, Berrien Co., Mich. (=MSFG 862)
5828	*J. sieboldiana* x *cinerea* o.p. seed, Berrien Co., Mich. (=MSFG 863)

Table A-13 (Cont'd.)

Number :	Plant material and origin
5829	*J. sieboldiana* x *cinerea* o.p. seed, Berrien Co., Mich. (=MSFG 864)
5830	*J. sieboldiana* x *cinerea* o.p. seed, Berrien Co., Mich. (=MSFG 865)
5831	*J. sieboldiana* x *cinerea* o.p. seed, Berrien Co., Mich. (=MSFG 866)
5832	*J. sieboldiana* seed, Berrien Co., Mich. (=MSFG 867)
5833	*J. regia* 'Colby' x *nigra* o.p. seed of hybrid walnut cv. 'Illinois xx'. Parent probably pollinated by *J. regia*.
5834	*J. cinerea* seed from pooled progeny of MSFG 842, Kalamazoo Co., Mich.
5835	*J. sieboldiana cordiformis* o.p. seed from pooled progeny of MSFG 852, Kalamazoo Co., Mich.
5836	*J. sieboldiana cordiformis* o.p. seed from pooled progeny of MSFG 858, Kalamazoo Co., Mich.
5837	*J. sieboldiana cordiformis* o.p. seed from tree C-40 of seedlot MSFG 858, Kalamazoo Co., Mich.
5839	*J. regia* x *nigra* o.p. seed, Berrien Co., Mich.
5842	*J. regia* 'Hansen' x *nigra* o.p. seed, Marion, Ind.
5843	*J. regia* 'Pomeroy' x *nigra* o.p. seed, Marion, Ind.
5844	*J. regia* (Carpathian) x *nigra* o.p. seed, Marion, Ind.
5845	*J. nigra* x *sieboldiana* cordiformis hybrid cv. 'Leslie Burt' o.p. seed
5846	*J. regia* x *nigra* o.p. seed, Genoa, Ohio
5848	*J. nigra* x *sieboldiana* cordiformis o.p. seed from a second-generation hybrid, Genoa, Ohio
5850	*J. regia* 'Alpine' o.p. seed
5851	*J. regia* 'Crath 5' o.p. seed
5852	*J. regia* 'Jacobs' o.p. seed
5853	*J. regia* 'McKinster' o.p. seed

Table A-1a (Cont'd.)

Number	Plant material and origin
5854	J. regia o.p. seed from a 'Crath 5' seedling
5858	J. cinerea commercial seed of unknown origin
5859	J. sieboldiana commercial seed of unknown origin
5862	J. nigra x regia grafting scions, Linton, Ind.
5880	J. nigra x regia o.p. seed, Coloma, Mich.
5881	J. nigra x regia o.p. seed, Coloma, Mich.
5892	J. regia seed, Shogram, West Pakistan, 34°30' N, alt. 8500 feet
5899	J. regia seed, Lahore, West Pakistan, 34°35' N, alt. 6000 feet
5900	J. sieboldiana seed, Tokyo, Japan
5902	J. regia seed, Sarlat-Dordogne, France
5903	J. regia seed, Brive(Corrèze), France
5904	J. regia seed, Vézère Valley, France
5905	J. regia seed, Terrasson (Dordogne), France
5906	J. regia seed, Saint-Rabiner (Dordogne), France
5907	J. regia seed, Ayen (Corrèze), France
5908	J. regia seed, Terrasson (Dordogne), France
5909	J. regia seed, Payzac (Dordogne), France
5919	J. regia x nigra hybrid cv. '3rd Avenue' grafting scions
5920	J. regia x nigra hybrid cv. 'Galley' grafting scions
5921	J. regia x nigra hybrid cv. 'Stegall' grafting scions
5922	J. regia x nigra hybrid cv. 'Haig' grafting scions
5924	J. regia x nigra grafting scions (=Beltsville 41-24-8)
5925	J. regia x nigra grafting scions (=Beltsville 41-24-9)
5926	J. regia x nigra grafting scions (=Beltsville 41-24-12)
5927	J. regia x nigra grafting scions (=Beltsville 38-96-3)

Table A-1a (Cont'd.)

Number	Plant material and origin
5928	*J. regia* x *nigra* (o.p.) grafting scions (=Beltsville A2)
5932	*J. regia* x *nigra* (o.p.) grafting scions (=Beltsville 49-03-11)
5933	*J. regia* x *nigra* (o.p.) grafting scions (=Beltsville 49-04-3)
5934	*J. regia* x *nigra* (o.p.) grafting scions (=Beltsville 52-06-1)
5935	*J. regia* x *nigra* (o.p.) grafting scions (=Beltsville 49-10-5)
5936	*J. regia* x *nigra* (o.p.) grafting scions (=Beltsville 49-10-11)
5937	*J. regia* x *nigra* (o.p.) grafting scions (=Beltsville 50-11-5)
5938	*J. regia* x *nigra* (o.p.) grafting scions (=Beltsville 50-12-5)
5939	*J. regia* x *nigra* (o.p.) grafting scions (=Beltsville 51-01-4)
5940	*J. regia* x *nigra* (o.p.) grafting scions (=Beltsville 51-02-3)
5941	*J. regia* x *nigra* (o.p.) grafting scions (=Beltsville 51-02-4)
5942	*J. regia* x *nigra* (o.p.) grafting scions (=Beltsville 52-06-4)
5943	*J. regia* x *nigra* (o.p.) grafting scions (=Beltsville 52-10-7)
5944	*J. regia* x *nigra* (o.p.) grafting scions (=Beltsville 55-81-5)
5945	*J. regia* x *nigra* hybrid cv. 'Coye' o.p. grafted nursery stock
5948	*J. regia* x *nigra* hybrid cv. 'Norris' o.p. grafted nursery stock
5949	(*J. regia* x *nigra* F_2) x 'Norris" grafted nursery stock
5951	*J. regia* x *nigra* hybrid cv. 'LaPlata' o.p. grafted nursery stock (=Beltsville 42-14P-4)
5954	*J. regia* x *nigra* (?) hybrid cv. 'Hillgate' grafting scions
5956	*J. regia* x *nigra* hybrid cv. 'O'Connor' o.p. grafting scions (=Beltsville 49-05-5)

Table A-1a (Cont'd.)

Number	Plant material and origin
5957	_J. regia_ x _nigra_ hybrid cv. 'O'Connor' o.p. grafting scions (=Beltsville 49-05-12)
5959	_J. regia_ x _nigra_ (?) hybrid cv. 'Fort Hunter' grafting scions
6133	_J. nigra_ seed, Sewanee, Tenn.
6135	_J. nigra_ seed, Connersville, Ind.
6137	_J. nigra_ seed, Abbington, Ind.
6138	_J. nigra_ seed, Amana, Ia.
6143	_J. nigra_ seed, Bedford, Ind.
6144	_J. nigra_ seed, Ewing, Ind.
6145	_J. nigra_ seed, Jasper, Ark.
6146	_J. nigra_ seed, Franklin, Ind.
6148	_J. nigra_ seed, Covington, Ind.
6149	_J. nigra_ seed, New Carlisle, Ind.
6150	_J. nigra_ seed, Decatur, Mich.
6154	_J. nigra_ seed, Orange Co., Ind.
6155	_J. nigra_ seed, Crawford Co., Ind.
6156	_J. nigra_ seed, Mendota, Ill.
6157	_J. nigra_ seed, LeRoy, Ill.
6159	_J. nigra_ seed, Montpelier, Ohio
6160	_J. nigra_ seed, Crawfordsville, Ind.
6163	_J. nigra_ seed, Linden, Ind.
6166	_J. nigra_ seed, Elizabeth, Ind.
6170	_J. nigra_ seed, Wooster, Ohio
6171	_J. nigra_ seed, Troy, Ohio
6173	_J. nigra_ seed, Columbus, Ohio
6174	_J. nigra_ seed, Delphos, Ohio
6175	_J. nigra_ seed, Fairfield Co., Ohio
6179	_J. nigra_ seed, Paris, Ky.
6182	_J. nigra_ seed, Saline Co., Mo.
6183	_J. nigra_ seed, Palmyra, Mo.
6190	_J. regia_ cv. 'Illinois 3' grafted nursery stock
6191	_J. regia_ cv. 'Lake' grafted nursery stock
6192	_J. regia_ cv. 'Helme 2' grafted nursery stock
6193	_J. regia_ cv. 'Caesar' grafted nursery stock
6194	_J. regia_ cv. 'Colby' grafted nursery stock
6195	_J. regia_ cv. 'Hansen' grafted nursery stock

Table A-1a (Cont'd.)

Number	Plant material and origin
6196	J. regia seedlings, unknown origin
6197	= 6190
6198	J. sieboldiana cordiformis cv. 'Rhodes' grafted nursery stock
6199	J. sieboldiana cordiformis cv. 'Wright' grafted nursery stock
6200	J. regia x nigra o.p. seed
6201	J. nigra seed, Otoe Co., Nebr.
6202	J. nigra seed, Fairfield Co., Ohio
6208	J. regia x nigra hybrid cv. 'Lorenz' scionwood
6210	J. regia seed, Barkob Research Range, India. Altitude 7000-8000 feet
6471	J. cinerea herbarium specimens, Kellogg Forest plantations, Augusta, Mich.

Table A-2.--*Juglans nigra* morphological data

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
			Leaflet						
					Blade				
Seed source No.	Location	Sample size 1/	No. leaflets per leaf	Position	Length (mm)	Width (mm)	Serrations (per cm)	Curvature (mm)	(5)/(4)
1601	Ill.	4,6	18.0	2.8	136.8	50.8	4.5	5.3	0.16
1605	Ill.	2,6	16.5	4.0	102.5	39	5.5	3.5	.24
1709	Ind.	2,6	17.0	3.0	142.5	54.5	3.5	3.5	.18
1805	Ia.	2,6	15.0	2.0	100.5	39.5	5.0	6.0	.13
2004	Ky.	2,6	15.0	2.0	135.5	53.5	5.5	8.5	.13
2005	Ky.	2,6	17.5	5.5	135.0	54.0	5.0	6.0	.32
2602	Miss.	4,6	18.5	3.5	121.5	48.0	5.3	3.8	.19
2708	Mo.	2,6	15.5	3.5	112.5	43.0	5.	5.	.23
3101	N.C.	2,6	16.0	3.5	117.5	40.5	4.5	3.5	.22
3102	N.C.	2,6	17.0	3.0	160.0	56.5	4.	5.	.18
3105	N.C.	3,6	17.7	3.7	115.	43.0	6.	5.0	.21
3803	Tenn.	3,6	16.0	3.3	121.3	51.	4.3	3.	.21
3901	Tex.	3,6	20.3	5.0	113.3	41.3	4.3	6.3	.25
1101	Ark.	1,6	23.0	6.0	117.	47.	7.	4.	.26
1803	Ia.	2,6	20.5	4.5	106.	38.	4.5	5.	.22
2601	Miss.	1,6	23.0	5.0	105.	39.	5.	0	.22
3806	Tenn.	1,6	23.0	4.0	152.	58.	5.	5.	.17
4101	Va.	2,6	22.0	6.0	124.	46.5	5.	9.	.27
6138	Ia.	6,10	18.2	5.3	104.2	35.3	4.3	5.7	.25
6156	Ill.	0,9							
6157	Ill.	6,5	18.5	4.5	112.5	38.3	3.7	4.7	.24
6159	Ohio	6,8	19.3	4.3	91.5	33.2	6.5	3.3	.22
6160	Ind.	6,9	19.3	4.0	104.3	37.2	4.5	3.3	.21
6163	Ind.	6,9	17.8	4.3	104.7	36.5	4.8	4.7	.24
6166	Ind.	6,5	16.0	4.7	103.3	37.3	4.3	3.2	.29
$\bar{x}$ 2/			18.36	4.1	118.3	44.2	4.88	4.7	.218
s^2			6.27		302.3		.67		.002
$s_{\bar{x}}$ 3/			.51		3.55		.17		.009
CV			.13		.15		.17		.21

1/ First value gives N for columns 4-14; second value for columns 15-19.

2/ Unweighted mean for columns 1-16; weighted for columns 17-19.

3/ Standard error of seedlot means.

Table A-2 (Cont'd.)

(1) Seed source No.	(2) Seed source Location	(11) Leaflet index (6)/(7)	(12) (9)/(6)	(13) Base shape	(14) Pubescence--sum of upper and lower leaf surfaces and rachis	(15) Rachis redness	(16) Foliage color	(17) Scurf	(18) Pith color	(19) Leaf scar trichomes
1601	Ill.	2.69	0.039	3.3	9.8			1.67	1.33	0
1605	Ill.	2.63	.034	4.0	9.0			2.00	.90	0
1709	Ind.	2.61	.025	4.0	8.5			1.92	1.83	0
1805	Ia.	2.54	.060	4.5	9.0			2.08	1.50	0
2004	Ky.	2.53	.063	3.0	8.5			1.83	2.30	0
2005	Ky.	2.50	.044	3.0	9.0			2.17	1.70	0
2602	Miss.	2.53	.031	3.8	9.3			2.08	1.83	0
2708	Mo.	2.62	.044	4.5	11.0			2.08	0.62	0.08
3101	N.C.	2.90	.029	4.	8.5			1.92	1.42	0
3102	N.C.	2.83	.031	4.	11.5			1.58	1.90	0
3105	N.C.	2.67	.043	3.0	10.3			1.92	1.80	0
3803	Tenn.	2.38	.025	3.3	10.3			2.00	1.40	0
3901	Tex.	2.74	.056	2.3	14.0			2.33	1.33	.17
1101	Ark.	2.49	.034	3.	9.			2.08	1.17	0
1803	Ia.	2.79	.047	3.5	11.			1.92	1.70	.08
2601	Miss.	2.69	0	4.	13.			1.75	2.08	0
3806	Tenn.	2.62	.033	5.	11.			1.83	1.58	.08
4101	Va.	2.67	.072	4.	10.5			1.92	2.17	0
1102	Ark.							2.33	1.42	0
1708	Ind.							2.00	2.00	0
6133	Tenn.					1.22	3.00	1.83	1.14	.33
6135	Ind.					2.14	2.43	1.40	0.92	0
6138	Ia.	2.98	.055	3.8	10.0	1.20	3.05	1.60	1.43	0
6156	Ill.					1.22	2.87	1.83	1.33	.11
6157	Ill.	2.94	.042	4.0	10.5	1.60	2.80	1.90		0
6159	Ohio	2.76	.036	4.2	9.8	1.17	2.83	1.50	1.50	0
6160	Ind.	2.80	.032	3.2	12.0	1.7	2.5	1.4	.70	.05
6163	Ind.	2.87	.045	4.5	9.2	1.4	2.8	1.4	.75	.17
6166	Ind.	2.77	.031	3.2	12.2	1.60	2.90	1.90		0
6176	Va.					.70	2.60	1.80	.25	.05

Table A-2 (Cont'd.)

(1) Seed source No.	(2) Location	(11) Leaflet index (6)/(7)	(12) (9)/(6)	(13) Base shape	(14) Pubescence--sum of upper and lower leaf surfaces and rachis	(15) Rachis redness	(16) Foliage color	(17) Scurf	(18) Pith color	(19) Leaf scar trichomes
MSFG										
178	Mich.?							2.14	1.14	.05
6173	Ohio					.5	2.5	1.0	1.0	0.5
6179	Ky.					3.0	3.0	1.8		0
6143	Ind.					3.0	3.5	1.8	2.0	0
6202	Ohio					2.5	2.5	2.5	1.	0
6145	Ark.					4.	2.5	1.		0
6144	Ind.					2.5	2.5	2.0	1.5	0
6170	Ohio					2.0	2.8	2.0	1.	0
6146	Ind.					1.5	2.5	1.	1.0	0
6148	Ind.					0.5	2.5	1.8	2.	0
6174	Ohio					3.0	2.5	1.8	0.8	0
6154	Ind.					1.5	2.8	1.5	3.	0.3
6183	Mo.					3.0	3.0	2.	2.	0
6155	Ind.					3.0	2.8	1.3	1.5	0
6201	Neb.					2.0	2.5	2.0	1.3	0
6149	Ind.					3.5	3.5	1.5	1.3	0
6171	Ohio					3.0	3.0	1.3	1.3	0
6150	Mich.					2.0	2.5	1.8	2.0	0
6182	Mo.					2.	2.5	2.	-	0
$\bar{x}$ 2/		2.69	0.040	3.71	10.29	1.96	2.76	1.80	1.45	0.040
S^2		.024	.0002	.404	2.16			0.11	.272	.010
$S_{\bar{x}}$ 3/		.031	.003	.130	.300			0.047	.079	.014
CV		.06	.38	.17	.14			.18	.36	2.45

Table A-3.--*Juglans regia* morphological data

(1) No.	(2) Seed source: Location or cultivar	(3) Sample size	(4) Leaflets/ leaf	(5) Leaflet: Position	(6) Leaflet: Length	(7) Leaflet: Width	(8) Leaflet: No. serrations	(9) Leaflet: Curvature	(10) (5)/(4)	(11) (6)/(7)	(12) (9)/(6)	(13) Base shape	(14) Pubescence	(15) Pith color	(16) Leaf scar trichomes
5810	Soviet Union	3	7.4	0.3	121.3	54.0	1.0	3.0	.041	2.25	.025	4.3	0.2		
5811	Soviet Union	1	9.	1.	129.	57.	2.	16.	.111	2.26	.124	4.0	0.5		
5816	Galicia	6	8.3	0.5	95.8	46.7	0.9	2.8	.060	2.05	.029	1.8	0.3		
5850	'Alpine' open pollinated	1	9.	1.	130.	54.	0	7.	.111	2.41	.053	4.0	0.5		
5851	'Crath 5' o.p.	1	9.	1.	122.	62.	0	8.	.111	1.97	.065	3.0	0.5		
5852	'Jacobs' o.p.	6	8.8	1.5	128.5	50.8	0.7	4.3	.170	2.53	.033	3.4	0.3		
5853	'McKinster' o.p.	3	9.0	1.3	116.7	57.7	1.0	2.7	.144	2.02	.023	3.0	0.5		
5854	'Crath' seedling	2	9.5	1.5	114.5	63.5	1.3	3.0	.158	1.80	.026	2.5	0.4		
5892	W. Pakistan	5	7.8	1.6	122.8	58.2	1.9	2.2	.205	2.11	.018	3.8	0.4		
5899	W. Pakistan	4	9.0	1.3	129.5	59.5	1.0	5.5	.144	2.18	.042	3.8	0.3		
5902	France	3	9.0	1.3	100.3	45.3	0.7	2.0	.144	2.21	.020	2.7	0.3		
5903	France	6	9.0	1.0	107.8	54.2	0.3	4.5	.111	1.99	.042	3.3	1.0		
5904	France	3	9.0	1.0	110.0	52.3	1.3	5.0	.111	2.11	.045	3.3	0.5		
5905	France	3	9.0	1.3	125.7	61.0	1.7	6.3	.144	2.06	.050	3.7	0.7		
5906	France	5	9.0	1.2	119.0	60.0	1.4	6.0	.133	1.98	.050	3.6	0.4		
5907	France	2	9.0	1.5	94.0	45.5	2.3	7.0	.188	2.07	.074	4.0	0.3		
5908	France	2	9.0	1.0	109.0	54.5	0	8.5	.111	2.00	.078	3.0	0.5		
5909	France	4	8.5	1.0	104.0	52.5	1.4	4.5	.118	1.98	.043	3.0	0.5		
6196	Commercial	14	9.4	1.4	110.2	53.6	0.5	3.7	.149	2.06	.034	3.7	0.9	1.3	0
6210	India	1	9.	1.	133.	58.	1.5	2.	.111	2.29	.015	3.0	0		

Table A-3 (Cont'd.)

(1) No.	(2) Seed source: Location or cultivar	(3) Sample size1/	(4) Leaflets/leaf	(5) Leaflet: Position	(6) Leaflet: Length	(7) Leaflet: Width	(8) Leaflet: No. serrations	(9) Leaflet: Curvature	(10) (5)/(4)	(11) (6)/(7)	(12) (9)/(6)	(13) Base shape	(14) Pubescence	(15) Pith color	(16) Leaf scar trichomes
6189	'School'	1,3	9.0	1.	130.	69.	0	13.	.111	1.88	.100	3.0	0.5	1.0	0
6190,97	'Ill. 3'	3,7	7.7	1.0	118.7	50.7	0	5.7	.130	2.34	.048	2.3	0.7	1.0	0
6191	'Lake'	2,3	9.0	2.0	107.0	49.0	0	8.0	.222	2.18	.075	4.0	0.5	0.8	0
6192	'Helme 2'	4,5	9.5	1.8	106.7	46.0	0	6.5	.189	2.32	.061	4.0	0.5	0.9	0
6193	'Caesar'	2,3	9.0	1.0	92.5	41.5	0	1.5	.111	2.23	.016	3.8	0.5	1.3	0
6194	'Colby'	2,3	6.0	1.0	119.5	59.5	0	6.0	.167	2.01	.050	4.0	0.5	0.8	0
6195	'Hansen'	4,5	8.5	1.3	115.0	53.0	0	6.3	.153	2.17	.055	3.8	0.8	0.6	0
$\bar{x}$			8.72		115.2		.77		.135	2.13	.048	3.4	0.49	0.96	0
S^2			.55		138.4		.55		.002	.03	.001	.38	.04	.06	
$S_{\bar{x}}$ 2/			.14		2.3		.14		.008	.03	.005	.12	.04	.09	
CV			.08		.10		.96		.29	.08	.54	.18	.43	.25	

1/ Second value gives N for columns 15 and 16 when different from columns 4-14.

2/ Standard error of seedlot means.

Table A-4.--*Juglans regia* x *nigra* morphological data

(1) No.	(2) Sample size	(3) Leaflets/ leaf	(4) Position	(5) Leaflet Length	(6) Leaflet Width	(7) Leaflet No. serrations	(8) Leaflet Curvature	(9) (4)/(3)	(10) (5)/(6)	(11) (8)/(5)	(12) Base shape	(13) Pubescence
5822	8	9.9	1.0	118.4	49.8	2.1	6.5	.101	2.42	.055	4.3	0.4
5824	2	13.0	1.0	108.0	40.0	3.5	2.0	.077	2.70	.018	2.5	1.0
5839	1	7.	1.	85.	36.	2.	4.	.142	2.36	.047	4.	0
5842	5	9.8	1.0	101.6	47.4	2.5	3.2	.102	2.15	.031	3.3	0.1
5843	6	9.7	1.0	107.8	53.2	1.3	3.3	.103	2.02	.031	2.8	0.1
5844	3	12.7	2.7	113.0	46.7	4.5	5.7	.212	2.42	.050	3.5	5.5
5846	5	10.6	1.4	116.0	49.2	1.2	9.2	.132	2.36	.079	3.9	0.4
5862	1	13.	2.	90.	41.	3.	4.	.154	2.19	.044	3.	0.5
5880	5	11.8	2.0	125.2	45.2	3.3	3.6	.169	2.77	.029	2.6	1.8
5881	4	11.5	2.3	96.0	42.3	3.4	4.0	.200	2.27	.042	3.5	6.4
5920	2	12.0	2.0	103.5	39.5	3.0	3.0	.167	2.62	.029	4.0	2.8
5921	2	12.5	2.0	123.5	45.0	2.0	11.5	.160	2.75	.093	3.5	0.5
5922	2	11.0	2.0	110.5	51.5	2.5	6.5	.182	2.15	.059	3.5	0.5
5924	3	11.7	2.3	112.0	56.0	1.7	6.7	.196	2.00	.060	3.5	0.5
5925	1	13.	2.	138.	49.	2.5	5.	.154	2.82	.036	3.5	2.0
5926	1	17.	5.	139.	53.	2.	7.	.294	2.62	.050	4.	0.5
5927	7	11.1	2.6	108.3	48.4	2.9	6.3	.234	2.24	.058	3.4	2.9
5928	3	12.3	2.3	153.3	68.0	3.0	5.3	.187	2.25	.035	4.5	2.0
5932	2	13.0	2.5	120.0	49.0	3.5	12.0	.192	2.45	.100	3.0	0
5933	4	9.8	2.0	96.8	44.0	1.0	5.5	.204	2.20	.057	3.4	0.1
5934	3	10.0	2.0	128.3	62.0	0.7	6.0	.200	2.07	.047	3.0	5.0
5935	5	11.0	2.0	98.8	37.8	4.0	7.6	.182	2.62	.077	3.4	5.8
5936	1	7.	0	109.	48.	2.	14.	.000	2.27	.128	4.5	0.5
5937	2	14.0	3.5	119.5	47.5	3.0	14.0	.250	2.52	.117	4.3	0.8
5938	2	10.0	2.5	107.0	41.0	5.8	10.0	.250	2.61	.093	4.5	7.8
5939	3	14.7	3.7	87.3	38.7	5.3	3.7	.252	2.26	.042	3.3	8.5
5940	3	17.7	5.7	86.0	33.7	5.7	4.0	.322	2.55	.046	3.7	7.7
5941	2	14.5	3.0	117.5	59.0	4.0	1.5	.207	1.99	.013	3.3	8.0
5942	5	20.0	6.0	97.8	31.8	4.5	4.2	.300	3.08	.043	4.1	7.1
5943	3	19.0	5.7	97.6	33.0	5.7	7.7	.300	2.96	.079	1.0	11.8
5944	1	15.	3.	113.	46.	2.	5.	.200	2.47	.044	3.5	6.5
5945	1	11.	1.	116.	58.	1.	4.	.090	2.00	.034	1.0	6.0
5948	2	10.0	1.0	106.5	51.0	2.0	3.5	.100	2.09	.033	2.5	1.0
5949	1	19.	3.	119.	44.	4.	4.	.158	2.70	.034	3.0	3.5
5951	2	11.0	2.0	83.0	35.0	4.5	4.5	.182	2.37	.054	3.5	1.0

Table A-4 (Cont'd.)

(1) No.	(2) Sample size	(3) Leaflets/ leaf	(4) Position	(5) Leaflet Length	(6) Leaflet Width	(7) Leaflet No. serrations	(8) Leaflet Curvature	(9) (4)/(3)	(10) (5)/(6)	(11) (8)/(5)	(12) Base shape	(13) Pubescence
5954	3	12.3	2.3	116.0	48.7	3.2	2.0	.187	2.38	.017	4.7	1.2
5956	1	13.	2.	97.	43.	2.	4.	.154	2.26	.041	1.5	0.5
5957	1	9.	1.	92.	34.	0	3.	.111	2.70	.033	2.5	0
5959	4	14.0	3.5	115.8	42.0	3.3	4.8	.250	2.75	.041	4.3	6.5
6200	1	15.	3.	102.	43.	3.	5.	.200	2.37	.049	2.5	0.5
6208	1	9.	1.	95.	43.	1.	2.	.111	2.21	.048	3.0	1.0
$\bar{x}$		12.40		109.0		2.87		.180	2.41	.052	3.34	2.90
S^2		9.12		233.6		2.01		.005	.08	.0007	.74	10.02
$S_{\bar{x}}$ 1/		.47		2.4		.22		.011	.04	.004	.14	.49
CV		.24		.14		.49		.37	.11	.50	.26	1.09

1/ Standard error of seedlot mean.

Table A-5.--Weighted hybrid index (h.i.) values for seedling families of *Juglans regia*, *J. nigra*, and *J. regia* x *nigra* hybrids

Juglans regia seedling families			*Juglans regia* seedling families		
Accession number	h.i.	Family mean h.i.	Accession number	h.i.	Family mean h.i.
5810	123		5902	110	
	97			139	
	94	105		108	119
5811	126	126	5903	119	
5816	95			82	
	119			108	
	78			109	
	120			134	
	111			102	109
	92	103	5904	114	
5850	118	118		113	
5851	104	104		124	117
5852	110		5905	128	
	122			119	
	110			120	122
	107		5906	114	
	132			124	
	127	118		107	
5853	134			92	
	118			113	110
	109	120	5907	119	
5854	130			132	126
	102	116	5908	109	
5892	121			113	111
	134		5909	108	
	138			105	
	113			113	
	112	124		124	113
5899	116		6210	121	121
	107				
	119				
	133	119			

Table A-5 (cont'd.)

Juglans nigra seedling families			*Juglans nigra* seedling families		
Accession number	h.i.	Family mean h.i.	Accession number	h.i.	Family mean h.i.
1101	273	273	4101	275	
1601	231			272	274
	232		6138	237	
	252			291	
	253	242		262	
1605	253			260	
	241	247		269	
1709	212			281	267
	238	225	6157	246	
1803	254			249	
	279	267		252	
1805	230			294	
	229	230		244	
2004	223			274	260
	224	224	6159	277	
2005	257			266	
	245	251		242	
2601	286	286		274	
2602	230			262	
	225			288	268
	253		6160	264	
	265	243		269	
2708	243			266	
	256	250		248	
3101	245			281	
	238	242		279	268
3102	253		6163	241	
	252	253		248	
3105	245			243	
	273			262	
	258	259		252	
3803	208			280	254
	214		6166	259	
	266	229		271	
3806	266	266		250	
3901	278			263	
	287			276	
	291	285		270	265

Table A-5 (cont'd.)

Hybrid seedling families			Hybrid seedling families		
Accession number	h.i.	Family mean h.i.	Accession number	h.i.	Family mean h.i.
5822	127		5844	168	
	130			133	141
	105		5846	120	
	123			126	
	141			124	
	149			130	
	144			140	128
	129	131	5880	122	
5824	180			128	
	141	161		127	
5839	123	123		194	
5842	126			225	159
	132		5881	183	
	87			156	
	143			212	
	131	124		198	187
5843	108				
	83				
	122				
	102				
	92				
	142	108			

Table A-6.--Morphological data and weighted hybrid index for

Juglans sieboldiana and *J. cinerea*

(1) Seed source: No.	(2) Seed source: Location or cultivar	(3) Sample size 1/	(4) Leaf length (cm)	(5) No. leaflets	(6) Longest leaflet: Position	(7) Longest leaflet: Length (mm)	(8) Longest leaflet: Width (mm)	(9) Longest leaflet: Serrations per cm	(10) Longest leaflet: Curvature (mm)	(11) Longest leaflet: Base shape	(12) Longest leaflet: Lopsidedness
	J. sieboldiana										
5832	Michigan	6,1		15.7	3.5	146.2	55.7	3.1	3.5	2.8	2.8
5859	Commercial	9,9	57.2	18.0	4.0	154.1	58.2	4.6	6.2	3.9	3.9
5900	Tokyo	3,20		13.0	2.3	173.0	63.3	3.0	3.3	2.7	
6198	'Rhodes'	6,16	40.2	12.7	2.8	141.3	57.5	3.3	7.0	2.1	3.5
6199	'Wright'	3,3	38.7	14.0	3.0	113.3	47.3	3.3	4.0	2.7	1.3
$\bar{x}$			45.4	14.7	3.1	145.6	56.4	3.5	4.8	2.8	2.9
S^2			105.6	4.8	.43	471.3	33.8	.42	2.8	.43	1.3
$S_{\bar{x}}$ 2/			5.9	1.0	.29	9.7	2.6	.29	.75	.29	.57
CV			.23	.15	.21	.15	.10	.19	.35	.23	.40
	J. cinerea										
MSFG 846	Michigan	19,16	38.8	15.6	3.2	104.8	37.9	4.1	2.9	3.6	0.8
5858	Commercial	7,7	30.6	16.1	4.1	93.3	44.3	3.9	2.3	2.3	0.9
6471	Michigan	11,2	39.9	16.0	3.7	105.7	37.0	4.2	1.8	4.5	0.6
$\bar{x}$			36.4	15.9	3.7	101.3	39.7	4.1	2.3	3.5	.77
S^2			25.8	.07	.20	47.8	15.8	.02	.30	1.2	.02
$S_{\bar{x}}$ 2/			2.9	.15	.26	4.0	2.3	.09	.32	.64	.09
CV			.14	.02	.12	.07	.10	.04	.24	.32	.20

1/ First value gives N for columns 4-15c, second value for columns 16-17.

2/ Standard error of seedlot means.

Table A-6 (Cont'd.)

(1) No.	(2) Seed source: Location or cultivar	(13) (10)/(7)	(14) (12)/(7)	(15a) Pubescence: Leaf: Upper	(15b) Pubescence: Leaf: Lower	(15c) Pubescence: Rachis	(16) Pith color	(17) Leaf scar trichomes	(18) Hybrid index
	J. sieboldiana								
5832	Michigan	.024	.019	2.3	8.0	7.8	1.	5.	43.6
5859	Commercial	.040	.025	0.9	6.4	5.4	1.1	2.7	42.9
5900	Tokyo	.019		3.5	5.7	4.0		1.4	
6198	'Rhodes'	.050	.025	2.5	7.7	8.0	0.5	3.0	47.3
6199	'Wright'	.029	.011	2.0	8.0	7.7	0.8	2.8	35.1
$\bar{x}$		.032	.020	2.2	7.2	6.6	0.85	3.0	
s^2		.0002	.00004	.88	1.1	3.2	.07	1.7	
$s_{\bar{x}}$ 2/		.006	.003	.42	.47	.80	.13	.58	
CV		.39	.33	.42	.15	.27	.31	.43	
	J. cinerea								
MSFG 846	Michigan	.028	.008	2.3	5.9	3.7	3.7	3.6	18.6
5858	Commercial	.025	.010	2.4	3.7	3.7	3.8	2.6	17.7
6471	Michigan	.017	.006	2.4	5.5	3.0	1.3	4.3	21.4
$\bar{x}$		.023	.008	2.4	5.0	3.5	2.9	3.5	
s^2		.00003	.000004	.003	1.4	.16	2.0	.73	
$s_{\bar{x}}$ 2/		.003	.001	.03	0.7	.23	.82	.49	
CV		.24	.25	.02	.23	.12	.48	.24	

Table A-7.--*Juglans sieboldiana* x *cinerea* morphological data for traits selected for inclusion in hybrid index

Accession number	Sample size	Leaflet length	Relative lopsidedness	Rachis pubescence	Pith color	H.I.
NC 5826						
F_2(?) seedlings	5	88.4	.007	4.0	4.0	15.7
NC 5827 = MSFG 862						
F_1 parent	3	133.7	.013	7.0	2.	35.2
F_2(?) seedlings	10	96.0	.019	4.8	1.9	28.6
NC 5828 = MSFG 863						
F_1 parent	4	124.5	.031	5.8	2.	40.5
F_2(?) seedlings	7	112.0	.023	1.7	2.8	25.0
NC 5829 = MSFG 864						
F_1 parent	4	120.3	.007	6.8	1.	31.9
F_2(?) seedlings	6	98.0	.027	3.7	1.5	30.6
F_3(?) seedlings	5	91.6	.026	3.2	3.5	25.3
NC 5830 = MSFG 865						
F_1 parent	4	102.8	.010	5.5	1.5	27.4
F_2(?) seedlings	4	112.0	.017	2.5	1.9	25.4
NC 5831 = MSFG 866						
F_1 parent	4	130.8	.006	5.3	1.5	29.0
F_2(?) seedlings	7	112.9	.016	3.6	1.9	27.6
NC 5835						
F_3(?) seedlings	8	108.5	.018	4.0	1.9	28.4
NC 5836						
F_3(?) seedlings	6	106.2	.019	5.0	2.5	30.0
NC 5837						
F_3(?) seedlings	9	103.3	.029	4.5	2.0	34.4

Table A-8.--Position and description of spots on paper chromatograms of leaf extracts of Juglans regia, J. nigra, and putative J. regia x nigra hybrids[1/]

Spot no.	J. regia		J. nigra		J. regia x nigra		Color[3/] UV (or visible)	
	R_F-1[2/]	R_F-2	R_F-1	R_F-2	R_F-1	R_F-2	Initial	NH_3
1	.61	.00	.60	.00	.54	.00	vis. yel	
2			.73	.00	.66	.00	gold	vis. yel.
3			.81	.00			dk. purp-brn	vis. gra-brn
4	.33	.11	.35	.10	.36	.11	orange to dk. brown	vis. orange
5	.46	.16	.52	.07			grown	
6	.57	.24	.53	.20	.50	.23	purp-brn	vis. tan
7	.64	.26	.67	.20			lt. blu-gray	
8	.45	.35	.46	.32	.49	.33	peach	
9	.36	.47	.39	.39	.41	.46	brt. grn	
10	.50	.42	.51	.40	.50	.44	lavender	vis. lav-gra
11	.61	.43	.59	.38	.57	.43	blu-gra	
12	.74	.33	.72	.27	.65	.29		
13	.44	.48	.46	.43	.48	.46	pink	
14	.64	.47	.57	.45				vis. tan
15			.72	.51	.72	.50	blue	
16	.38	.52	.40	.50	.41	.54	grn-gra	
17	.46	.56	.48	.49	.48	.55	vis. gra-grn	
18			.66	.50			gray	
19	.55	.62	.57	.58	.53	.59		
20	.68	.64	.68	.64	.64	.67	blu-wh	
21			.22	.61	.23	.61	blue	
22	.25	.60	.29	.53	.25	.56		
23	.37	.61	.37	.63	.36	.69	faint blue	gray
24	.44	.64	.44	.63	.45	.68	gray	

1/ Occasional infrequent spots were not tabulated if R_F values were too variable to be confident of average.

2/ Relative migration in relation to solvent front--R_F-1 for first direction, R_F-2 for second direction.

3/ See text, pages 40-41 for description of development procedure.

Table A-8 (Cont'd.)

Spot no.	Color 3/ Visible (or UV) 4/ Diaz. sulf.	NaOH	Remarks
1			Runs together with 2, 3, 36, 50, and 51 along upper margin.
2	gray-tan		
3	brown		
4			
5			Large spot; overlaps 52 and 55 on *J. nigra* papers.
6		yel-grn (UV)	Blurs into 57 on *J. nigra* papers.
7			Sometimes abuts 6 or 40.
8			
9			Sometimes abuts 13, 16, 17 or 82; often a large spot with irregular margin.
10	plum	purple	Hydrojuglone glucoside? Overlaps 43; sometimes abuts 11, 13, 41.
11			Sometimes abuts 10, 14, 40 or 81; irregular upper margin.
12	peach	lavender	Oblong spot.
13			Small spot.
14	yellow		Overlaps 11 on *J. regia* papers; blurs into 43 on *J. nigra* papers.
15			
16			Overlaps 9 on *J. regia* papers.
17			
18			
19	pink		Overlaps 83 on *J. nigra* papers.
20		v. lt. yel.	Abuts 29, blurs into 64 on *J. nigra* papers.
21	yellow	tan	
22	pink	lavender	
23	yellow		
24			Occasionally with irregular lower margin; sometimes abuts 17, 45.

4/ Visible under ultraviolet light only after reagent spray.

Table A-8 (Cont'd.)

Spot no.	J. regia R_F-1[2]	J. regia R_F-2	J. nigra R_F-1	J. nigra R_F-2	J. regia x nigra R_F-1	J. regia x nigra R_F-2	Color[3] UV (or visible) Initial	Color[3] UV (or visible) NH_3
25					.44	.68		gray
26	.52	.65	.56	.61	.56	.70	gray or blue	
27			.72	.63			blue	ultra.
28	.49	.79	.48	.80	.53	.80	rose	
29	.67	.78	.70	.72	.65	.75	blue	gray
30	.81	.69						
31			.33	.72	.34	.77	blue	gray
32			.46	.79	.47	.80	ultra.	
33			.59	.78			orange	lt. pink
34			.62	.73	.61	.75	blue	
35			.82	.87	.80	.92	faint	
36			.31	.00	.27	.00	tan	
37					.65	.11	light	
38			.61	.81			lt. tan	
40	.63	.33	.63	.28	.54	.30	tan	gray
41	.55	.41	.56	.28	.49	.23	grn-gra	vis. yellow
42			.41	.43			peach	
43	.52	.54	.53	.49	.53	.46	purple	vis. tan
45			.50	.61	.53	.66	gray	blue-gray
48	.11	.09	.07	.10			lavender	
49	.29	.14	.30	.10			peach	
50			.16	.00	.15	.00	brt. blue	
51	.38	.00	.46	.00	.42	.00	gold, vis. yel.	vis. green
52			.46	.05	.45	.04		gray
53			.15	.08	.16	.07	blu-gra	
54			.24	.11	.24	.09	vis. v. lt. yel., brt. blu-green	

Table A-8 (Cont'd.)

Spot no.	Color[3/] Visible (or UV)[4/] Diaz. sulf.	NaOH	Remarks
25		lt. yel.	
26	yellow	tan	
27			
28			
29	yellow	tan	Abuts 20 on _J. nigra_ papers.
30		pink	Fades away.
31			
32			Overlaps 86 on hybrid papers.
33			
34			
35	yellow	tan	Blurs into solvent front.
36		gray-tan	
37			
38		tan	Trails into solvent front.
40	yellow		Sometimes abuts 11 on _J. regia_ papers.
41			
42			
43			
45			
48			
49			
50		gray	
51		tan	Overruns 52.
52	plum	lavender	Oblong spot; overlaps 5; overrun by 51.
53			
54			

Table A-8 (Cont'd.)

Spot no.	*J. regia* R_F-1[2]	*J. regia* R_F-2	*J. nigra* R_F-1	*J. nigra* R_F-2	*J. regia x nigra* R_F-1	*J. regia x nigra* R_F-2	Color[3] UV (or visible) Initial	Color[3] UV (or visible) NH_3
55			.50	.11				
56			.37	.16				
57			.48	.20	.47	.17	brown	
58	.40	.26	.40	.21	.41	.20	ultra.	
59			.23	.21	.26	.19	gray	
60	.28	.34	.33	.25	.40	.24	pink to cream	
61			.34	.28	.35	.30		
62			.42	.32			gray	
63			.26	.28	.29	.27	purple	
64	.79	.53	.73	.57			blue	blu-gray
65			.35	.21			blue	
66			.46	.14				faint
67	.35	.27	.31	.16	.34	.13	pink	
68			.68	.42			blue	
69					.15	.32	light	
71			.28	.42	.33	.39	lav.-gra.	
73			.52	.95			light	
75			.36	.56	.30	.59	gray	
76					.07	.57	dark	
77					.12	.56	dark	
80			.17	.22	.21	.31	ivory	
81					.63	.35	blue-gray	
82			.29	.35	.36	.35	vis. yel.	
83			.53	.53	.52	.54	grn.-gray	
85					.52	.31	gray	
86					.45	.79		
87					.46	.67	gray	
88					.50	.27	tan vis. yel.	
89					.24	.20	dark	

Table A-8 (Cont'd.)

Spot no.	Color[3/] Visible (or UV)[4/] Diaz. sulf.	NaOH	Remarks
55	pink	plum	Small oblong spot; overlaps 5.
56		lt. tan	
57		orange	Often overlaps 6.
58			
59	lav.-gra.		Irregular lower margin.
60	yellow		Sometimes abuts 61 in _J. nigra_ papers.
61	tan		Sometimes abuts 60 in _J. nigra_ papers.
62			
63			
64			
65			
66			
67			Small spot.
68			
69			
71			
73			
75			Abuts 22, 23, 24 on hybrid papers.
76			Oblong spot.
77			Small spot.
80			
81			Abuts 11.
82	gray		Sometimes abuts 9 on hybrid papers.
83			Overlaps 19 on _J. nigra_ papers.
85			
86	yellow		Overlaps 32.
87		yellow	Yellow fades away.
88		tan	
89			

Table A-9.--Relative frequency of occurrence of 79 spots on paper chromatograms prepared from leaf extracts of *Juglans regia*, *J. nigra*, and *J. regia* x *nigra* hybrids

Chromatogram number

J. regia

Spot no.	7b	8b	71-1	71-2	71-3	71-5	71-6	71-7	71-8	Sum	Spot freq.
1	1[1/]	1	1	1	1	1	1	1	1	9	1.00
2	1	1	0	0	0	0	0	0	0	2	.22
3	1	1	0	0	0	0	0	0	0	2	.22
4	1	1	1	1	1	1	1	1	1	9	1.00
5	1	1	1	1	1	1	1	1	1	9	1.00
6	1	1	0	0	0	0	0	1	1	4	.44
7	1	1	1	1	1	1	1	1	1	9	1.00
8	1	1	1	1	0	0	0	0	1	5	.56
9	1	0	1	1	1	1	1	1	1	8	.89
10	1	1	1	1	1	1	1	1	1	9	1.00
11	1	1	1	1	1	1	1	1	1	9	1.00
12	1	1	1	1	1	1	1	1	1	9	1.00
13	1	1	1	1	1	1	1	1	1	9	1.00
14	1	1	0	0	0	1	1	1	1	6	.67
15	1	1	0	0	0	0	0	0	0	2	.22
16	1	1	1	1	0	1	1	1	1	8	.89
17	1	1	1	1	0	1	1	1	1	8	.89
18	1	0	0	0	0	0	0	0	0	1	.11
19	1	0	1	1	1	1	0	1	1	7	.78
20	1	1	1	1	1	1	1	1	1	9	1.00

J. nigra

Spot no.	70-1	70-9	70-10	70-15	70-16	70-17	70-18	70-19	70-20a	70-20b	70-22	70-23	70-24	70-25	70-26	Sum	Spot freq.
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	15	1.00
2	0	1	1	1	0	0	1	0	0	0	1	1	1	0	1	8	.53
3	1	1	1	0	0	0	0	0	1	0	1	1	1	0	0	7	.47
4	1	1	1	0	1	1	0	1	1	1	0	0	1	1	0	10	.67
5	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	14	.93
6	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	14	.93
7	1	1	1	0	0	0	0	1	0	0	1	0	0	0	1	6	.40
8	1	1	0	1	1	1	0	1	1	1	1	1	1	1	1	13	.87
9	1	1	1	1	1	1	0	1	0	0	1	1	1	0	1	11	.73
10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	15	1.00
11	1	1	1	1	1	1	1	0	0	0	1	1	1	1	1	12	.80
12	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	2	.13
13	1	0	1	1	1	0	1	0	1	0	1	0	1	1	1	10	.67
14	0	0	0	0	1	1	0	0	1	1	1	0	0	0	1	6	.40
15	1	1	1	0	0	0	0	0	0	0	1	0	0	0	1	5	.33
16	1	1	1	0	1	1	0	0	1	0	0	0	1	1	1	9	.60
17	1	1	1	1	1	1	0	0	0	0	0	0	0	1	0	7	.47
18	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	4	.27
19	1	1	1	0	0	0	0	1	1	1	1	1	1	1	1	11	.73
20	1	1	1	1	0	0	0	1	0	1	1	1	1	1	1	11	.73

J. regia x *nigra*

Spot no.	71-9	71-10	71-11	71-12	71-13	71-14	71-15	71-16	Sum	Spot freq.
1	1	1	1	1	1	1	1	1	8	1.00
2	0	1	1	0	0	1	0	0	3	.38
3	0	0	0	0	0	0	0	0	0	0
4	1	1	1	1	1	1	1	1	8	1.00
5	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	1	1	0	0	2	.25
7	0	0	0	0	0	1	0	0	1	.12
8	1	1	1	1	0	0	0	0	4	.50
9	1	1	1	1	1	1	1	1	8	1.00
10	1	1	1	1	1	1	1	1	8	1.00
11	1	1	1	1	1	1	1	1	8	1.00
12	0	0	1	1	1	1	1	0	5	.62
13	0	0	1	1	0	0	0	0	2	.25
14	0	0	0	0	0	0	0	0	0	0
15	1	0	0	0	0	0	0	0	1	.12
16	1	0	1	0	1	0	1	0	4	.50
17	1	1	1	1	1	1	1	1	8	1.00
18	0	0	0	0	0	0	0	0	0	0
19	0	1	0	0	1	1	0	0	3	.38
20	1	1	1	1	1	0	0	0	5	.62

Table A-9 (cont'd.)

Chromatogram number

J. regia

Spot no.	7b	8b	71-1	71-2	71-3	71-5	71-6	71-7	71-8	Sum	Spot freq.
21	1	0	0	0	0	0	0	0	0	1	.11
22	1	0	0	1	0	0	0	0	0	2	.22
23	1	1	0	0	0	1	0	0	0	3	.33
24	1	1	1	0	1	1	1	1	1	8	.89
25	0	0	0	0	0	0	0	0	0	0	0
26	1	1	0	1	0	1	1	1	1	7	.78
27	1	0	0	0	0	0	0	0	0	1	.11
28	1	0	0	0	1	1	1	1	1	6	.67
29	1	1	0	0	0	1	0	0	1	4	.44
30	1	1	0	0	0	0	0	0	0	2	.22
31	1	0	0	0	0	0	0	0	0	1	.11
32	1	1	0	0	0	0	0	0	0	2	.22
33	1	0	0	0	0	0	0	0	0	1	.11
34	1	1	0	0	0	0	0	0	0	2	.22
35	1	1	0	0	0	0	0	0	0	2	.22
36	0	1	0	0	0	0	0	0	0	1	.11
37	1	1	0	0	0	0	0	0	0	2	.22
38	0	1	0	0	0	0	0	0	0	1	.11
40	0	1	1	0	0	0	1	1	1	5	.56
41	0	1	0	0	0	0	0	0	1	2	.22

J. nigra

Spot no.	70-1	70-9	70-10	70-15	70-16	70-17	70-18	70-19	70-20a	70-20b	70-22	70-23	70-24	70-25	70-26	Sum	Spot freq.
21	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	14	.93
22	1	1	1	0	1	0	1	1	1	1	0	1	1	1	0	11	.73
23	1	0	1	0	1	1	1	1	1	0	0	0	1	1	0	9	.60
24	1	1	0	1	1	1	1	1	0	1	0	1	1	1	0	11	.73
25	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	.07
26	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2	.13
27	1	0	0	1	1	0	0	0	0	0	0	1	0	0	1	5	.33
28	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	.07
29	1	1	1	0	0	0	0	0	0	0	1	0	0	1	0	5	.33
30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31	0	1	0	0	0	0	0	1	0	0	0	1	1	1	1	6	.40
32	1	0	0	1	0	1	1	0	0	0	1	1	1	0	1	8	.53
33	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2	.13
34	1	1	1	1	1	1	0	0	0	0	0	0	0	0	1	7	.47
35	1	1	1	1	0	0	1	0	0	0	1	0	0	0	0	6	.40
36	1	1	1	1	1	1	1	0	0	0	1	1	1	0	1	11	.73
37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
38	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	2	.13
40	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	3	.20
41	1	1	1	0	0	0	0	1	0	1	1	1	0	0	1	8	.53

J. regia x *nigra*

Spot no.	71-9	71-10	71-11	71-12	71-13	71-14	71-15	71-16	Sum	Spot freq.
21	1	1	1	1	0	1	1	1	7	.88
22	0	1	0	0	0	1	0	0	2	.25
23	0	0	1	1	1	1	1	0	5	.62
24	0	1	0	0	0	1	1	1	4	.50
25	0	0	0	1	0	0	1	0	2	.25
26	0	0	0	0	0	0	0	1	1	.12
27	0	0	0	0	0	0	0	0	0	0
28	1	1	1	1	1	1	1	0	7	.88
29	1	1	1	1	0	0	0	0	4	.50
30	0	0	0	0	0	0	0	0	0	0
31	1	0	0	0	0	0	0	0	1	.12
32	1	1	0	1	0	0	0	1	4	.50
33	0	0	0	0	0	0	0	0	0	0
34	1	0	0	1	0	0	0	0	2	.25
35	1	0	1	0	0	0	0	0	2	.25
36	0	0	1	0	0	0	1	0	2	.25
37	0	0	1	0	0	0	0	0	1	.12
38	0	0	0	0	0	0	0	0	0	0
40	0	1	1	0	0	0	0	1	3	.38
41	0	0	0	0	0	0	0	0	0	0.

Tabl● A-9 (cont'd.)

Spot no.	J. regia											J. nigra																	J. regia x nigra									
	7b	8b	71-1	71-2	71-3	71-5	71-6	71-7	71-8	Sum	Spot freq.	70-1	70-9	70-10	70-15	70-16	70-17	70-18	70-19	70-20a	70-20b	70-22	70-23	70-24	70-25	70-26	Sum	Spot freq.	71-9	71-10	71-11	71-12	71-13	71-14	71-15	71-16	Sum	Spot freq.
42	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	3	.20	0	0	0	0	0	0	0	0	0	0
43	0	1	1	0	0	0	0	0	1	3	.33	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	15	1.00	1	1	1	1	0	0	0	0	4	.50
45	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	1	0	0	0	0	0	1	0	1	0	7	.47	1	1	1	0	1	0	1	0	5	.62
48	0	0	0	1	0	0	0	0	0	1	.11	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	.07	0	0	0	0	0	0	0	0	0	0
49	0	0	0	0	0	1	1	0	0	2	.22	0	0	0	1	0	1	0	0	0	0	0	1	1	0	0	4	.27	0	0	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	15	1.00	1	1	1	1	0	1	1	1	7	.88
51	0	0	1	1	0	0	0	0	0	2	.22	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	14	.93	1	1	1	1	1	1	1	1	8	1.00
52	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	14	.93	1	1	1	1	0	0	0	1	5	.62
53	0	0	1	1	0	0	0	0	0	2	.22	1	1	1	1	1	1	1	0	1	1	0	1	1	1	1	13	.87	1	1	0	1	0	0	0	0	3	.38
54	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	15	1.00	1	1	1	1	0	0	1	1	6	.75
55	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	1	1	1	1	1	1	1	0	10	.67	0	0	0	0	0	0	0	0	0	0
56	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	3	.20	0	0	0	0	0	0	0	0	0	0
57	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	1	0	0	0	0	0	0	0	0	0	5	.33	1	1	1	1	1	1	1	1	8	1.00
58	0	0	1	1	0	1	0	1	0	4	.44	1	1	1	1	1	1	0	0	1	1	1	1	1	0	1	12	.80	1	1	0	1	0	0	0	0	3	.38
59	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	0	1	1	0	1	1	1	1	13	.87	1	1	1	1	0	0	0	0	4	.50
60	0	0	1	1	0	1	0	1	0	4	.44	1	1	1	1	0	0	1	1	1	1	1	0	1	1	1	12	.80	0	1	1	1	1	0	0	0	4	.50
61	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	1	1	0	1	0	6	.40	0	1	1	0	0	0	0	0	2	.25
62	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	2	.13	0	0	0	0	0	0	0	0	0	0
63	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	1	1	0	0	1	0	1	1	1	0	10	.67	1	0	0	0	0	0	0	0	1	.12
64	0	1	0	0	0	0	0	0	1	2	.22	1	1	1	1	0	0	1	0	0	0	1	0	0	0	1	7	.47	0	0	0	0	0	0	0	0	0	0

Table A-9 (cont'd.)

	Chromatogram number																																					
	J. regia											J. nigra																	J. regia x nigra									
Spot no.	7b	8b	71-1	71-2	71-3	71-5	71-6	71-7	71-8	Sum	Spot freq.	70-1	70-9	70-10	70-15	70-16	70-17	70-18	70-19	70-20a	70-20b	70-22	70-23	70-24	70-25	70-26	Sum	Spot freq.	71-9	71-10	71-11	71-12	71-13	71-14	71-15	71-16	Sum	Spot freq.
65	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	1	1	1	6	.40	0	0	0	0	0	0	0	0	0	0
66	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	.13	0	0	0	0	0	0	0	0	0	0
67	0	0	1	0	0	1	1	1	0	4	.44	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	3	.20	1	0	0	0	0	0	0	0	1	.12
68	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	.07	0	0	0	0	0	0	0	0	0	0
69	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	1	0	1	6	.75
71	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	1	0	1	0	1	1	0	1	8	.53	1	1	0	1	0	0	1	1	5	.62
73	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2	.13	0	0	0	0	0	0	0	0	0	0
75	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	3	.20	1	1	0	1	1	1	1	1	7	.88
76	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	4	.50
77	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	3	.38
80	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0	0	0	0	0	1	0	0	4	.27	1	1	1	1	1	1	0	1	7	.88
81	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	.07	1	1	1	0	0	0	0	0	3	.38
82	0	0	0	1	0	0	0	0	0	1	.11	0	1	1	1	0	0	1	1	1	1	0	0	1	1	0	9	.60	0	1	1	1	0	1	0	0	4	.50
83	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	1	0	1	1	7	.47	0	0	1	0	0	0	0	0	1	.12
85	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	1	4	.50
86	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	.07	1	1	1	1	1	0	1	1	7	.88
87	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1	1	0	0	5	.62
88	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	1	1	7	.88
89	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	2	.25

1/ 1 indicates that spot was present on listed chromatograms; 0 indicates spot was absent.

Table A-10.--Spot frequency difference (s.f.d.) values for 79 spots found on paper chromatograms prepared from leaf extracts of Juglans nigra, J. regia, and J. regia x nigra hybrids

Spot no.	Spot frequency difference: nigra-regia	nigra-hybrid	hybrid-regia	Spot no.	Spot frequency difference: nigra-regia	nigra-hybrid	hybrid-regia
1	0	0	0	42	.20	.20	0
2	.31	.15	.16	43	.67	.50	.17
3	.25	.47	-.22	45	.47	-.15	.62
4	-.33	-.33	0	48	-.04	.07	-.11
5	-.07	.93	-1.00	49	.05	.27	-.22
6	.49	.68	-.19	50	1.00	.12	.88
7	-.60	.28	-.88	51	.71	-.07	.78
8	.31	.37	-.06	52	.93	.31	.62
9	-.16	-.27	.11	53	.65	.49	.16
10	0	0	0	54	1.00	.25	.75
11	-.20	-.20	0	55	.67	.67	0
12	-.87	-.49	-.38	56	.20	.20	0
13	-.27	.48	-.75	57	.33	-.67	1.00
14	-.27	.40	-.67	58	.36	.42	-.06
15	.11	.21	-.10	59	.87	.37	.50
16	-.29	.10	-.39	60	.36	.30	.06
17	-.42	-.53	.11	61	.40	.15	.25
18	.16	.27	-.11	62	.13	.13	0
19	-.05	.35	-.40	63	.67	.55	.12
20	-.27	.11	-.38	64	.25	.47	-.22
21	.82	.05	.77	65	.40	.40	0
22	.51	.48	.03	66	.13	.13	0
23	.27	-.02	.29	67	-.24	.08	-.32
24	-.16	.23	-.39	68	.07	.07	0
25	.07	-.18	.25	69	0	-.75	.75
26	-.65	.01	-.66	71	.53	-.09	.62
27	.22	.33	-.11	73	.13	.13	0
28	-.60	-.81	.21	75	.20	-.68	.88
29	-.11	-.17	.06	76	0	-.50	.50
30	-.22	0	-.22	77	0	-.38	.38
31	.29	.28	.01	80	.27	-.61	.88
32	.31	.03	.28	81	.07	-.31	.38
33	.02	.13	-.11	82	.49	.10	.39
34	.25	.22	.03	83	.47	.35	.12
35	.18	.15	.03	85	0	-.50	.50
36	.62	.48	.14	86	.07	-.81	.88
37	-.22	-.12	-.10	87	0	-.62	.62
38	.02	.13	-.11	88	0	-.88	.88
40	-.36	-.18	-.18	89	0	-.25	.25
41	.31	.53	-.22				