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A COMPARISON OF LEAF TERPENES IN PERSEA SUBGENUS PERSEA

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ABSTRACT

Leaf essential-oil components of 10 avocado cultivars and two closely related species were analyzed by gas chromatography. Three-dimensional computer analyses gave highly reproducible results which, however, provided dubious additional elucidation of taxonomic relationships. But the 13 preponderant terpenes together suggested relationships that were internally consistent, that agree well with known morphological differences, and that point to a clarification of *Persea* subgenus *Persea* taxonomy. Cultivars of the so-called West Indian and Mexican horticultural races had a similar distribution pattern of major terpenes. The Guatemalan horticultural race had a different distribution pattern, much like that of both *P. nubigena* and *P. schiedeana*. On the basis of these and other observations, it is proposed that the West Indian, Mexican, and Guatemalan horticultural races be classified as botanical varieties, with the designations *P. americana* var. *americana, P. americana* var. *drymifolia,* and *P. americana* var. *guatemalensis* (var. nov.), respectively. *Persea nubigena* should probably be relegated to varietal status: *P. americana* var. *nubigena*. This seems reasonable also for *P. floccosa,* which would then be *P. americana* var. *floccosa*. *Persea schiedeana* is of uncertain status. Possible phylogenetic relationships are outlined.

Introduction

Kopp's (1966) monograph of *Persea* divided the genus into the subgenera *Eriodaphne* and *Persea* (formerly *Gnesiopersea*). She stated: "There is a very sharp demarcation between the two subgenera." This agrees with results of our attempts to graft and hybridize various of the species; subgeneric intracompatibility has been complete and intercompatibility nil. The subgenus *Persea* contains the commercial avocado, *P. americana* Mill., which is the basis of a developing pomological industry in tropical and subtropical regions around the world. Only one other *Persea* species, *P. schiedeana* C. G. Nees, is cultivated, and only on a small scale, in Mexico. *Persea nubigena* L. O. Williams and *P. steyermarkii* C. K. Allen both have a thin layer of pulp that is edible but not very palatable. These four taxa are all in the subgenus *Persea*. None of the *Eriodaphne* species is palatable.

Persea americana has long been divided into three botanically distinguishable groups designated as horticultural races, namely Mexican, Guatemalan, and West Indian (from what was earlier thought to be their respective centers of origin). According to Bergh (1969), they are typified as follows:

> *Mexican race.* Leaves anise-scented; their under-surfaces more glaucous than those of other races. Flowers generally more pubescent; bloom earliest in the season (fall to spring in California). Fruits small. Fruit skin thin to membraneous (rarely over 1/32 inch). Seed relatively large to very large, and often loose. Fruit pulp commonly rich to strong in flavor, sometimes with anise aroma; often fibrous. About six months from flowering to fruit maturity. The most cold hardy of the avocado races; also more resistant to heat and low humidity. The least tolerant of soil salinity. Rarely does well in a coastal environment.
>
> *West-Indian race.* No anise leaf scent. Fruits small to large. Fruit skin leathery, seldom over 1/16 inch. Seed relatively large; [usually with thicker seed coats]; sometimes loose in its cavity. Pulp mild to watery [and somewhat sweeter] in flavor; lower oil content than the other two races. About six months from flowering to fruit maturity. [The foliage is often a paler green.] The least hardy of the three races to cold and to low humidity, not adapted anywhere in California. The most tolerant of soil salinity, as either rootstock or top. . .
>
> *Guatemalan race.* No anise leaf scent. Young foliage more commonly reddish. [Blooms latest: spring to start of summer in California.] Fruits small to large. In adaptation and tolerance to soil and climate, intermediate between the above two races. But fruit skin usually thick leathery to woody [often brittle and/or pebbled], sometimes over 1/4 inch. Also, while all gradations of seed proportion are found, this race has more genes for small relative seed size, and the seed is almost never loose. Also, the fruit may require a year to achieve maturity (to 18 months in the less tropical climate of California).

An interesting analysis of some 67 characters involving differences among these three races has been made by Rhodes et al. (1971).

Material and methods

In *Persea americana,* five cultivars of the Guatemalan race (Hass, Nabal, Routh, Ryan, and Thille) were studied, as were three Mexican race cultivars (Duke, Mexicola, and Topa Topa), a hybrid of these two races (Bacon), and the only locally available cultivar of the West Indian race (Waldin). Two other species in subgenus *Persea* were analyzed also: *P. nubigena* (considered by Kopp [1966] to be a botanical variety of *P. americana*), and *P. schiedeana*. Most of these were growing at the University of California, Riverside, with the remainder at the nearby University South Coast Field Station. Mature normal leaves were harvested from each entity, macerated, steam distilled, and then analyzed by gas chromatography using the procedures described earlier by Scora, Duesch, and England (1969). Component retention time varies with the individual molecular structure, as well as with the operating parameters.

Results

The resulting temperature-programmed gas chromatographs contained 36 visible peaks, each of which comprised at least 0.01% of the total essential oils of each of the 12 *Persea* entities. Of these peaks, 16 were identified by infrared, ultraviolet, and nuclear magnetic resonance. Table 1 simplifies the data for ease of comparison by deleting all except the 13 major oil components (those averaging at least 1% over all 12 cultivars or species), by deleting all individual values less than an arbitrary 1.5%, and by rounding the remaining values to the nearest integer. These 13 terpenes together comprise from 83% to 98% of the total essential leaf oil; for only two of the 12 taxa do they amount to less than 90% of the leaf total.

Some of the oil constituents proved very difficult to identify. Eight major identified constituents are listed with the respective retention times in table 1. Also identified were the following minor constituents, with average relative retention time: α-pinene (590); sabinene (1,060); β-myrcene (1,350); allo-ocimene (1,800); β ocimene (1,970); 1,8 cineole (2,100); farnesol (4,180); and geranyl acetate (6,140).

Table 1 indicates that the taxa divide into two distinct groups on the basis of leaf terpenes. One group consists of the West Indian and the three Mexican cultivars; these have oil that is predominantly estragole (in agreement with the results of Lozano, Oltra, and Torres [1969]), plus a little caryophyllene and still less ε-cadinene. The second group comprises the Guatemalan cultivars plus *P. nubigena* and *P. schiedeana;* these have considerable caryophyllene, with the remainder of their essential leaf oil much more widely distributed than was true of the first group (table 1).

Intermediate between these two groups was the terpene distribution of the Bacon cultivar, which our progeny testing (unpublished data) has shown to be a hybrid of the Mexican and Guatemalan races. It has several times as much estragole as

TABLE 1

Percentage composition of the 13 major leaf terpenes in Persea americana cultivars and related "species"

	Relative retention times												
Cultivar (or species)	800–1,106 (β-pinene)	2,216–2,509 (ρ-cymene)	3,887–4,086	4,465–4,600	5,412–5,593 (caryophyllene)	5,806–5,822 (farnesene)	5,912–5,991 (humulene)	6,005–6,050 (estragole)	6,053–6,080 (ϵ-cadinene)	6,432–6,541 (anethol)	6,575–6,737	6,732–6,876	7,111–7,383
Guatemalan race:													
Hass	2	10	4	4	21	3	16	4	4	3	4	3	7
Nabal	...	...	2	5	46	2	6	7	3	3	...	14	5
Routh	...	...	2	3	44	7	9	3	7	3	3	9	7
Ryan	...	...	...	4	23	3	4	3	...	3	41	11	3
Thille	...	19	2	7	26	5	5	4	5	3	3	4	7
Guatemalan × Mexican hybrid:													
Bacon	18	2	...	...	6	...	...	40	5	...	20	...	...
Mexican race:													
Duke	...	...	...	...	9	...	...	85	4	...	...	...	...
Mexicola	...	...	...	...	6	...	...	81	5	...	...	...	...
Topa Topa	...	...	...	...	8	...	...	85	5	...	...	...	...
West Indian race:													
Waldin	...	...	...	...	10	...	...	79	5	...	...	...	...
P. nubigena	2	2	5	...	49	...	6	19	6	...	4	4	2
P. schiedeana	16	3	...	...	24	6	3	7	7	3	4	6	4

Note.—Blanks indicate trace amounts—less than 1.5%.

any Guatemalan cultivar (table 1), but only half as much as the Mexicans. Its remaining oil is more widely distributed than was true of the Mexicans, but less so than any of the Guatemalans.

A three-factor computer analysis of all essential leaf oil constituents had earlier proved very effective in differentiating a much broader group of *Persea* and related taxa (SCORA et al. 1970); therefore, a similar analysis was applied to the present raw data involving all 36 peaks (fig. 1). The three dimensions are left, right, and vertical, with knobs used to clarify vertical sign and length. Taxa 4–9 are a rather uniform group, and, in fact, they refer to *P. schiedeana, P. nubigena,* and four of the five Guatemalan cultivars. But Nabal (11) is somewhat separated from that group. More seriously, Mexicola (1) and Topa (3) are markedly separated from the third Mexican cultivar, Duke (12), which is quite similar to the West Indian (Waldin, 10). And the Guatemalan-Mexican hybrid, Bacon (2), shows no Guatemalan affinities whatsoever. A second and markedly different data feed-in manipulation completely changed the numerical values of the three dimensions and taxon location in the computerized pictorialization. But the very same relative groupings remained. Such consistency speaks well for the soundness of the computerization method of pictorializing data, but emphasizes that the method apparently did not provide superior taxonomic clarification in the present instance. However, in spatially separating Duke from the other two Mexican race cultivars, the computerization may have detected minor component differences not shown in table 1; Duke is a somewhat anomalous cultivar in resistance to *Phytophthora* and in other respects.

Discussion

This sort of chromatographic analysis provides an additional, chemical, dimension, which can be useful in helping to determine taxonomic relationships. One must always bear in mind that this is merely a new trait, to be considered with other diagnostic characters. Also, conclusions must be drawn and generalizations made with great caution because of the genetic individuality (RASMUSSEN 1969) and environmental influence (THOMPSON et al. 1971) factors. We are planning to analyze these taxa under other edaphic and climatic conditions and to examine additional cultivars of the West Indian race especially. However, the clear-cut separation of the taxa into two definite groups, with a race hybrid intermediate, permits some reasonable deductions.

The so-called Guatemalan and West Indian horticultural races have always been placed in the same taxon and contrasted with the Mexican horticultural race which is separated into a distinct botanical variety (KOPP 1966) or even into a valid species, *Persea drymifolia* Schlecht. & Cham. (EYNARD 1970). But as we (BERGH and STOREY 1964) noted earlier, "The genetic behavior [suggests] that the three [horticultural races] do belong to the same species, and that the Mexican

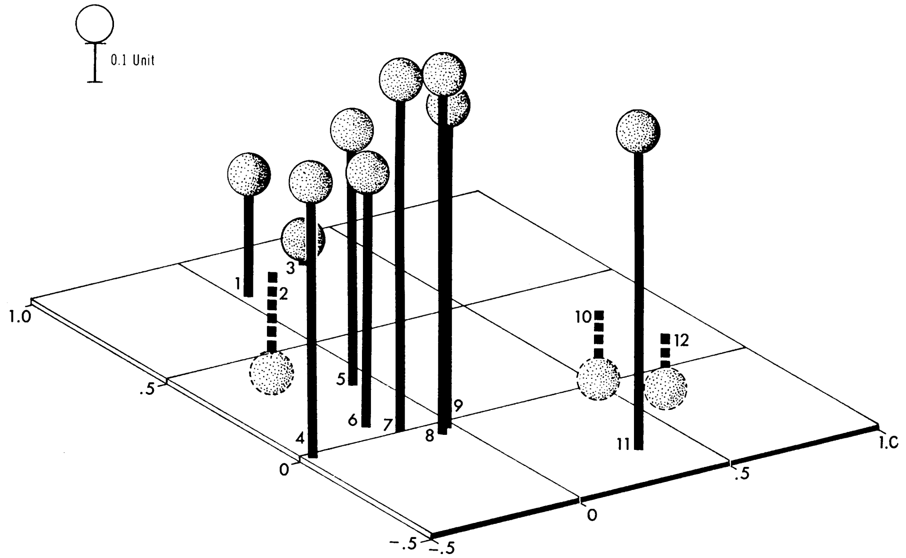

FIG. 1.—Pictorialized 3-D computer analysis of all leaf terpenes in *Persea americana* cultivars and related "species." Guatemalan: Hass, 8; Nabal, 11; Routh, 7; Ryan, 9; Thille, 6; Guatemalan × Mexican hybrid: Bacon, 2; Mexican: Duke, 12; Mexicola, 1; Topa Topa, 3; West Indian: Waldin, 10; and *P. nubigena*, 4; and *P. schiedeana*, 5.

race is no more distinct from the Guatemalan and West Indian races than they are from each other." This was based on segregation for various morphological characters, plus the absence of sterility barriers between the Mexican and the other two races. In fact, the only such barrier known in *P. americana* is time of bloom, a character in which trees of the West Indian race average about the same as Mexican race trees—and earlier than Guatemalan types. Our present results add a new character, leaf oil, and the only West Indian cultivar tested for this was much more like Mexican cultivars than it was like the Guatemalan cultivars with which it would be placed on the basis of established taxonomic systems. These two points of similarity strengthen such Mexican-West Indian fruit likenesses as early maturity, large seed, and thin skin.

But this does not imply that it is rather the Guatemalan race which should be separated taxonomically from the other two. In the important matter of climatic adaptation (or ecological niche), it is intermediate. Instead, all of the morphological, behavioral, biochemical, adaptational, and pre-Columbian distributional traits now known, together indicate that each of the three races is about equally distinct from the other two. Evidently they are approximately equidistant adaptive peaks based on gene constellations selected by nature, by man, or by both, out of a continuously variable genetic background. Early botanists apparently encountered more of the lower-elevation forms intermediate between Guatemalan and West Indian prototypes and so came erroneously to regard this heterogeneous group as distinct from the Mexican type, whose variability was less observed. But forms intermediate between typical Mexicans and typical Guatemalans are now known to be fairly numerous in parts of Mexico (in fact, the Fuerte avocado, long the world's leading cultivar, originated as a selection from such a group growing at Atlixco).

The question arises as to how the taxonomy of the three more or less equivalent horticultural races can best be delineated. As noted by HARLAN, DE WET, and RICHARDSON (1969), "The magnitude of a barrier required to establish species limits is, perhaps, more a matter of subjective opinion than a scientific conclusion." Or, as ORNDUFF (1969) put it, ". . . alternative taxonomies are feasible for various groups of plants. . . . " One possibility is to treat the horticultural races simply as one polytypic species, *P. americana,* or as a variety of that species in contrast to other possible varieties (see below). At the opposite pole from such lumping would be a splitting into three separate species. We have concluded that neither extreme is justified by the available data and that the taxonomic situation is best reflected by a recognition of three botanical varieties. Thereby, the Mexican race remains *P. americana* var. *drymifolia.* The Guatemalan and West Indian races are then separated. The West Indian race (actually now known to be indigenous to central and northern South America, and to have been introduced into the West Indies only in post-Columbian times [BERGH 1969]) probably has priority on the designation *"americana,"* since it is the form prevalent along the seacoast and so was the first form described botanically; it retains the designation *P. americana* var. *americana.* The Guatemalan race therefore requires a new varietal name. Considering its common racial name, which accurately reflects its center of indigenousness, it logically becomes *P. americana* var. *guatemalensis.*

Persea nubigena was reduced by KOPP (1966) to varietal status: *P. americana* var. *nubigena.* Our leaf-oil results support this classification and suggest an affinity to *P. americana* var. *guatemalensis.* In fact, morphologically, its thick fruit skin especially is like the Guatemalan race to a degree unique among all of the 80 or so known *Persea* species, except for *P. schiedeana* and, to a lesser extent, *P. floccosa. Persea gigantea* (WILLIAMS 1953) may be a distinct botanical variety, but KOPP's (1966) relegation of it to a mere variant form of *P. americana* var. *nubigena* seems reasonable.

KOPP (1966) retained *P. floccosa* as a valid species, but she acknowledged that *P. americana* var. *nubigena* "links *P. americana* var. *americana* with *P. floccosa.*" She noted further that "*P. floccosa* exhibits the typical floral and inflorescence structure of *P. americana.* . . . " Our unpublished results repeatedly indicated that there are no sterility barriers between *P. floccosa* and *P. americana* varieties: F_2 and back-cross segregations have been typical of quantitative inheritance, involving complete fertility. The morphological differences are somewhat greater than those between most *P. americana* botanical varieties, but hardly significantly so. Varietal status, *P. americana* var. *floccosa,* would seem to reflect best the biological realities.

Persea schiedeana is, as KOPP (1966) noted, "the most easily distinguished species in the *Persea americana* group." As indicated above, the most reasonable approach now seems to be to regard the other members of the group as varieties of a single species. It may well be that to treat *P.*

schiedeana as a second species overstates its distinctiveness. Kopp (1966) suggests affinity with *P. americana* var. *nubigena*. Our leaf-oil results support such an affinity and, at the same time, would link both entities with *P. americana* var. *guatemalensis*. One character is, of course, quite inadequate for establishing certainty of relationships. The morphological differences are marked. Until further characters are analyzed, and genetic tests made, we prefer to leave the status of *P. schiedeana* open.

No certain conclusions as to phylogenetic relationships within the subgenus are as yet possible. The simplest hypothesis is that the common ancestor was a small-fruited form of the "wild" avocado discovered in Honduras and Costa Rica by Popenoe (Bergh and Storey 1964). Relatively minor modifications, including loss of anise scent would produce the *nubigena* form in one direction; further selection, primarily for larger fruit and smaller seed proportion plus delayed maturity, would readily give rise to the *guatemalensis* form which must concurrently have been selected for adaptation to a less tropical climate. By presumably human selection in a different direction and to a greater degree, the *nubigena* type (or a yet more primitive ancestral form) could likewise give rise to the *schiedeana* form. The *floccosa* form may have been naturally selected from the *nubigena* type, or it could be the precursor of *nubigena,* or (more likely) both could share a not-very-distant common ancestor.

The *americana* and *drymifolia* forms could also be obtained from the "wild" type discovered by Popenoe through extensive pre-Columbian human selection for larger fruit size (especially in the *americana* form), plus human or natural selection for a thinner fruit skin. Only the *drymifolia* form retained the anise biochemistry, and it also became gradually adapted to the least tropical climate of any known *Persea*. The only remaining taxon in *Persea* subg. *Persea,* the *steyermarkii* form, could conceivably represent an early step in the evolution of the *americana* variety; more likely it is a slight divergence from a common ancestor of the two forms.

Associated with these assumed selective forces have been various concomitant morphological and biochemical changes, due to simultaneous selective pressures, to genetic linkage, or to the chance fixation of "neutral" characters. All of these suggested relationships must be regarded as still tentative.

Acknowledgment

Trees of the taxa designated *Persea nubigena* and *Persea schiedeana* were collected by Dr. George A. Zentmyer, University of California at Riverside, and sampled from his collection at the South Coast Field Station.

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