Phytochemistry, 1970, Vol. 9, pp. 2502 to 2507. Pergamon Press. Printed in England.

THREE FACTOR ANALYSIS OF ESSENTIAL LEAF OILS IN SELECTED *PERSEA* SPECIES

R. W. Scora, B. O. Bergh, W. B. Storey and J. Kumamoto

Departments of Horticultural Science and Vegetable Crops, University of California, Riverside, U.S.A.

(*Received* 9 *December* 1969, *in revised form* 16 *February* 1970)

Abstract—A three-dimensional computer correlation of the components of the leaf oils of *Persea* established a clear association between the profiles of distribution of these components with taxonomic relationships based on morphology and on graft-and-hybridization compatibilities.

INTRODUCTION

Persea is one of some 50 genera in the aromatic Lauraceae family. Nearly all of the 1000 or more laurel species are tropical. A few are subtropical, however, and a very few are of mild temperate adaptation—such as *Sassafras* and *Lindera* in the Eastern United States, *Umbellularia* in California, and the classic laurel (*Laurus*) from the Mediterranean region. The only genera appreciably cultivated are the more tropical *Persea* and *Cinnamomum*. *Persea borbonia* and *P. indica* are grown as ornamentals; the fruit of *P. schiedeana* is consumed in parts of Central America; and *P. americana*, the avocado, is an important fruit crop in tropical and subtropical regions around the world. Lauraceae genera most closely related to *Persea* include *Beilschmiedia*, *Nectandra*, *Ocotea* and *Phoebe*.

Very little is known concerning phylogenetic relationships in the family as a whole and some aspects of *Persea* taxonomy are still unclear and controversial. The only modern monograph on the genus is by Kopp[1] but this is limited to species indigenous to the Western Hemisphere and based largely on herbarium specimens.

In order to obtain an additional dimension of taxonomic characters for the elucidation of phylogenetic relationships, gas chromatography was used to separate as many essential oil components as possible. Gottlieb *et al.*[2] had found (–)- and (+)-camphor (in a ratio of 3:1) and methyleugenol in the related *Ocotea pretiosa*, and studied the effect of geographical and climatic range upon these oils. Fujita established a classification and phylogeny of the genus *Cinnamomum* on the basis of essential oil constituents,[3] and supported the theory of Continental Drift viewed from the chemical constituents of *Ocotea*.[4] Mollan[5] studied the essential oil components of Sassafras wood, bark and leaves and established the presence of linalool. Gibelli and Gigante[6] studied the leaves of "*Persea drymis*" in clinical and pharmacological test procedures.

---

[1] L. E. Kopp, *Mem. N.Y. Botan. Gard.* **14**, 1 (1966).
[2] O. R. Gottlieb, M. Fineberg and M. T. Magalhaes, *Perfumery Essent. Oil Record* **53**, 219 (1962).
[3] Y. Fujita, *Bot. Mag. Tokyo* **80**, 261 (1967).
[4] Y. Fujita, *J. Japan. Bot.* **42**, 278 (1967).
[5] T. R. Mollan, *Perfumery Essent. Oil Record* **52**, 284 (1961).
[6] C. Gibelli and D. Gigante, *Minerva Med., Torino* **34**, 295 (1943).

In this investigation the following *Persea* species were studied: ***P. americana*** var. ***nubigena, P. borbonia, P. caerulea, P. donnell-smithii, P. indica, P. lingue, P. schiedeana*** and ***P. skutchii***. In addition ***P. pachypoda***, which Kopp regarded as possibly better classified in the genus ***Phoebe***, and one species of ***Beilschmiedia, B. miersii***, were analyzed. Except for *P. indica*, which is indigenous to the Canary and neighboring islands, all of these are New World species.

RESULTS

The gas chromatographic separations of the steam-distilled leaf oils showed about thirty individual components. The ten most prominent terpenes are: (1) sabinene, (2) β-myrcene, (3) β-ocimene, (4) octanal, (5) farnesol, (6) caryophyllene, (7) α-humulene, (8) a sesquiterpene, $C_{15}H_{24}$, (9) ϵ-cadinene, and (10) an isomer of myristicin. The bar histograms using these numbers are shown in Fig. 1.

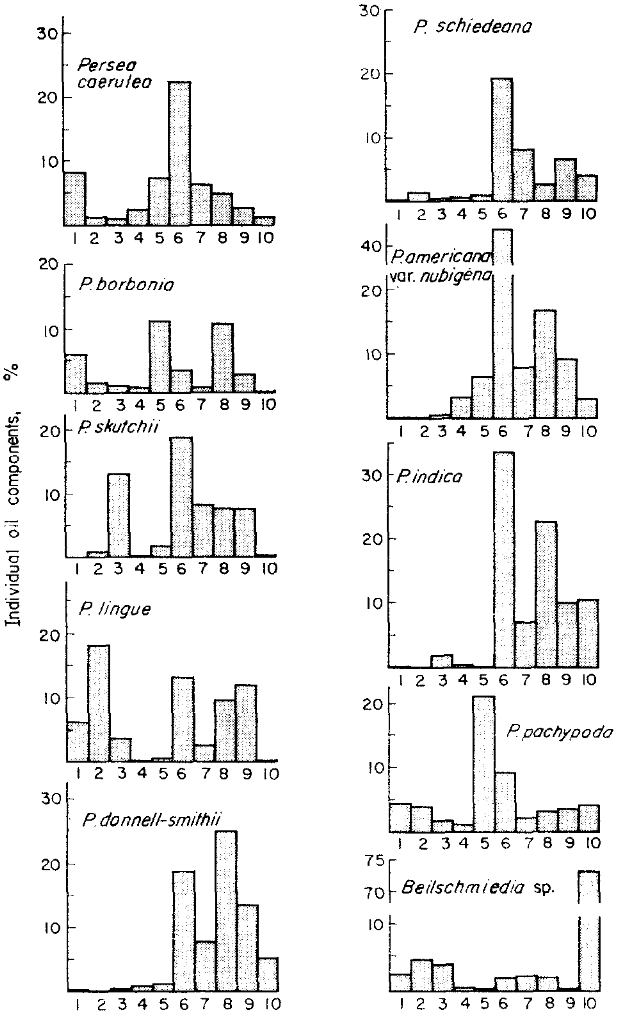

FIG. 1. PERCENTAGE OF AVOCADO LEAF OIL COMPONENTS IN STEAM-DISTILLED OIL.

Bar 1 represents *sabinene*; bar 2 *β-myrcene*; bar 3 *β-ocimene*; bar 4 *octanal*; bar 5 *farnesol*; bar 6 *caryophyllene*; bar 7 *α-humulene*, bar 8 $C_{15}H_{24}$; bar 9 *ϵ-cadinene* and bar 10 an isomeric *myristicin*.

The two most similar terpene profiles are those of *Persea americana* var. *nubigena* and *P. indica*: in both cases about 90% of the total oil consists of the ten major components; the largest fraction is caryophyllene (bar 6), with α-humulene, $C_{15}H_{24}$ and ε-cadinene (bars 7–10) providing most of the remainder. Apart from the fact that over half of its volatile oil consists of numerous minor constituents not represented in Fig. 1, *P. schiedeana* is rather similar to the first two taxa except for a reduced amount of the sesquiterpene (bar 8). *P. caerulea* falls in between *P. americana* var. *nubigena* and *P. schiedeana*. A large amount of β-ocimene (bar 3) is unique for *P. skutchii*. *P. donnell-smithii* differs in another direction: the bulk of the oil consists of caryophyllene (6), α-humulene (7), $C_{15}H_{24}$ (8), and ε-cadinene (9), with the proportion of (8) greater than caryophyllene. *P. lingue* is the only species that has β-myrcene (bar 2) as the largest fraction. *P. borbonia* and *P. pachypoda* both contain large amounts of farnesol (bar 5) and sabinene (bar 1). *Beilschmiedia miersii* differs markedly from any of the preceding in that nearly 75% of its oil consists of an isomer of myristicin (bar 10).

In summary, the distributions of ten selected terpenes would suggest a rather close relationship between *P. americana* var. *nubigena* and *P. indica*. The relationship of *P. schiedeana*, *P. caerulea*, *P. skutchii* and *P. donnell-smithii* is a little more removed from the first two as well as from each other. Finally, *Beilschmiedia* is quite distinct.

A 3 factor computer analysis utilizing all essential oil components was constructed to provide a geometrical description of the relationships between the tested species. Species closely related had a high correlation in their total leaf oil components. Each of the three dimensions represents a discrimination of one group of species from the others. The three dimensions are left, right and vertical (Fig. 2).

Two species groupings are here discernible. The first comprises those numbered 4–7 and the second numbers 1, 2, 3 and 8. The two groups have about the same average left dimension, but the first group is farther to the right and shorter vertically. The two remaining taxa do not fit into either group; they are intermediate in terms of the right dimension and 9 (*P. pachypoda*) is intermediate in the vertical dimension. They are at opposite left-dimension extremes and, considering the three dimensions jointly, both of these are obviously distinct from the other nine as well as each other.

DISCUSSION

Kopp[1] concluded that a considerable number of previously accepted species names in *Persea* were synonyms, described several new species, corrected the epithets of certain others and accepted a total of 81 (New World) species. While hybridization experiments at the University of California (unpublished data) have shown that some of these supposedly good morphologically distinct *Persea* species are completely cross compatible with no sterility apparent in the F_1 and subsequent generations, this is not true of the 10 taxa studied here.

Kopp rejected the four *Persea* sub-genera of Mez's[7] revision in favour of the two sub-genera as proposed by Nees von Esenbeck:[8] Eriodaphne, and Persea (originally Gnesiopersea). This agrees with graft compatibility experiments at the University of California,[9] and with our hybridization attempts, in which all species so far tested have fitted into one or other of these two distinct groups.

---

[7] C. Mez, *Jahrb. Botan. Gart. Berlin* **5**, 1 (1889).

[8] C. G. D. Nees von Esenbeck, *Berolin, Veitii et Sociorum* (1836).

[9] E. F. Frohlich, C. A. Schroeder and G. A. Zentmyer, *Calif. Avoc. Soc. Yrbks.* **42**, 102 (1958).

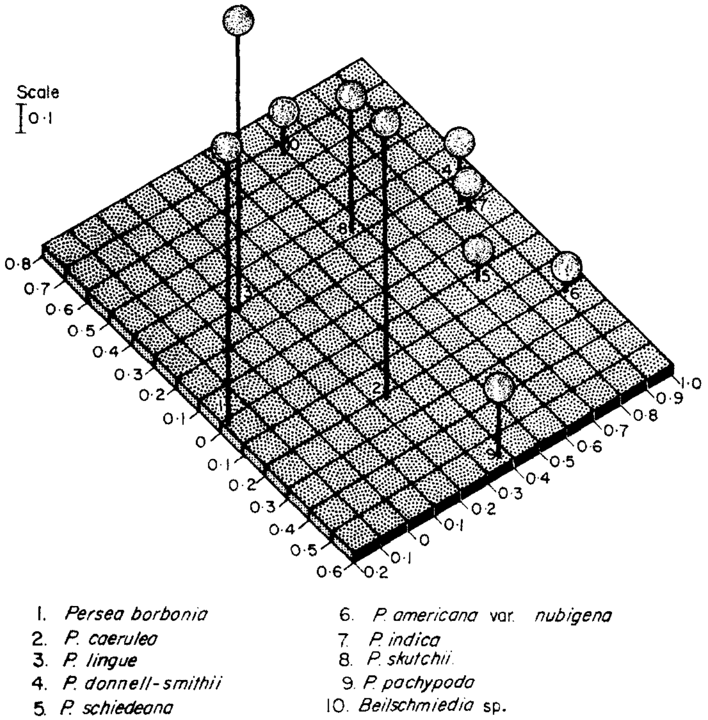

FIG. 2. 3-D REPRESENTATION OF NINE *Persea* AND ONE *Beilschmiedia* SPECIES.

In the *Persea* sub-genus Eriodaphne, we analyzed *P. caerulea*, *P. borbonia*, *P. donnell-smithii* and *P. lingue*, all in the section Eriodaphne; also *P. skutchii*, which Kopp[1] treated as a synonym of *P. caerulea*; and *P. indica*, indigenous to the Canary and nearby islands (not dealt with by Kopp), but indicated by our compatibility tests to be in the Eriodaphne sub-genus. Of this group, *P. caerulea* and *P. skutchii* should then be closely related and *P. borbonia* morphologically quite similar. *P. donnell-smithii* and *P. lingue* appear to be phylogenetically more distant. *P. indica* is probably even more distant, since it is an Old World species.

The two species of sub-genus Persea, *P. schiedeana* and *P. americana* var. *nubigena*, are thought to be closely related to each other and comparatively distinct from members of sub-genus Eriodaphne. Since *P. pachypoda* is morphologically so different that it is sometimes placed in another genus, it would be expected to differ markedly from the other *Persea* species.

The profiles of the bar histograms (Fig. 1) do not agree with the assumed relationships given by Kopp. Thus, they indicate that *P. americana* var. *nubigena* and *P. indica* are closely related even though they have been assigned by Kopp to different sub-genera.

The morphological similarities of *P. skutchii*, *P. caerulea* and *P. borbonia* are not reflected in their leaf oil profiles; in fact, *P. borbonia* is more like the dubious "*P. pachypoda*" than it is like the other two species. It is difficult to see any clear relationship between morphology and the leaf oil profiles, except that the single species in a different genus, *Beilschmiedia*, is very different in having a high level of the isomer of myristicin (bar 10).

The 3-D computer analysis taking all of the thirty mono- and sesquiterpenes into account gave much better agreement (Fig. 2). *Beilschmiedia miersii* and *Persea pachypoda* were both clearly separate from all other species. The eight species remaining divided visually into two groups, the first of which contains the sub-genus Eriodaphne (*P. lingue*, *P. borbonia*, *P. caerulea* and *P. skutchii*). The second group contains the two analyzed species from sub-genus Persea and also the Eriodaphne species *P. donnell-smithii* and *P. indica*.

The computer diagram based on the total oil analysis, indicates a definite association between morphology and volatile leaf oils. A major complicating factor should however be noted. Most of the essential oil components are identical in all of the species studied. However, there are a few exceptions. α- and β-Pinene, camphene, sabinene, β-myrcene and β-ocimene occur as such in all species. In *P. skutchii* and *P. borbonia* there is a sizeable peak in the gas chromatogram that immediately follows β-ocimene; in *P. skutchii* this peak is allo-ocimene but in *P. borbonia*, it is 1,8-cineole. Similarly, farnesene, eluted immediately after caryophyllene, is replaced by α-guaine in *P. schiedeana*. Although caryophyllene (bar 6) is universal to the genus, in *P. pachypoda* it is mixed with another, as yet unidentified, substance. α-Humulene (bar 7) is replaced by another (unidentified) substance in *P. schiedeana* only. The sesquiterpene $C_{15}H_{24}$ (bar 8) is also replaced by a different terpene in *P. indica* and *P. borbonia*. The component reported as bar 9 is for *P. skutchii* not ϵ-cadinene. These unfortunate coincidences show that an independent means must be used for identifying each component separately in each taxon.

EXPERIMENTAL

Healthy mature leaves were macerated in a Waring Blendor and steam-distilled. The crude oils were separated in a Varian gaschromatograph M1520 at a temperature range from 50 to 182°. The column used was 10 ft long, stainless steel and ¼ in. in diameter. The liquid phase was LAC 446 on 80–100 mesh Chromosorb W. The areas under the G.C. peaks were counted with a Varian digital integrator M475 and expressed as percentage of the total oil sample injected.

Factor analysis was used to provide a three-dimensional representation of the correlations between the various species as determined by essential leaf oil components. Common logarithms were taken to reduce replicate variation and give more emphasis to small peaks; then averages were calculated for each species for each peak. The matrix of correlations between species (the peaks were considered as the observations on each species) was factor analyzed on program BMDO3M[10] which performed a principal component solution and an orthogonal rotation of the factor matrix. The three dimensions accounted for 72% for the avocado species. The individual oil fractions were caught in capillary tubes and these were mounted in a Kontes micro NMR sample holder. Low resolution NMR spectra were obtained on a Varian model T-60 NMR spectrometer. The liquid then was transferred with CCl_4 to a 0·05 mm micro i.r. cell. Normal and expanded i.r. spectra were obtained on a Beckman IR-12 spectrometer for identification.

Acknowledgement—Appreciation is expressed to G. A. Zentmyer, Department of Plant Pathology on this campus, for making available material of all of the species of *Persea* and *Beilschmiedia* used in this study. The original materials were collected by Zentmyer in Mexico, Central and South America over the past 18 years and are incorporated in his field plantings at the University's South Coast Field Station and on the Riverside campus.

[10] W. J. Dixon, *Biomedical Computer Progress*, pp. 196–284, Univ. Calif. Press, California (1967).