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SHORT COMMUNICATION

FUNGISTATIC FLAVONES IN THE LEAVES OF CITRUS SPECIES RESISTANT AND SUSCEPTIBLE TO *DEUTEROPHOMA TRACHEIPHILA**

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Abstract—The fungistatic flavones nobiletin, tangeritin and 5,4'-dihydroxy-6,7,8,3'-tetramethoxyflavone have been estimated quantitatively in the leaves of a number of citrus species and varieties. No simple correlation appears to exist between the concentrations of these compounds and the resistance of the different taxa of citrus to the pathogenic fungus *Deuterophoma tracheiphila*.

INTRODUCTION

THE FUNGUS *Deuterophoma tracheiphila* Petri is responsible for the highly destructive citrus disease known as Mal-secco.[1] This disease, which is widespread throughout the Mediterranean region, induces loss of foliage followed by progressive drying of branches and finally death of the trees. Although all the citrus cultivars can be attacked by the fungus, their resistance to the disease appears to be widely variable.[2]

Antifungal activity was reported[3] for water extracts of bark of the highly resistant variety Cleopatra Mandarin. More recently,[4] nobiletin (5,6,7,8,3',4'-hexamethoxyflavone) and tangeritin (5,6,7,8,4'-pentamethoxyflavone) were isolated from fruit peel of resistant varieties of tangerines, and the former was assumed to be the main compound imparting resistance to *D. tracheiphila* in some varieties of citrus. In 1968, Pinkas *et al.*[5] described the isolation of two more fungistatic flavones (5,4'-dihydroxy-6,7,8-trimethoxyflavone and 5,4'-dihydroxy-6,7,8,3'-tetramethoxyflavone) from leaves of the variety Avana Mandarin. Nobiletin and 5,4'-dihydroxy-6,7,8,3'-tetramethoxyflavone were found to be much more active against *D. tracheiphila in vitro* than tangeritin and 5,4'-dihydroxy-6,7,8-trimethoxyflavone.[5]

Since nothing is known of the concentration of these flavones in citrus plants, a number of citrus species having different susceptibility to Mal-secco was examined to try to establish whether the resistance to the disease is related to the content of the fungistatic flavones.

RESULTS AND DISCUSSION

Dried leaves of citrus trees were extracted with chloroform and the crude extract column chromatographed on silica gel. The appropriate fractions were pooled and the fungistatic

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[1] L. PETRI, *Boll. Real. Staz. Pat. Veg. Firenze* **10,** 63 (1930); L. PETRI, *Boll. Real. Staz. Pat. Veg. Firenze* **10,** 191 (1930).
[2] G. RUGGIERI, *Ann. Sper. Agrar. Roma New Ser.* **2,** 255 (1948).
[3] A. BEN-AZIZ, M. CHORIN, S. P. MONSELISE and I. REICHERT, *Science* **135,** 1066 (1962).
[4] A. BEN-AZIZ, *Science* **155,** 1026 (1967).
[5] J. PINKAS, D. LAVIE and M. CHORIN, *Phytochem.* **7,** 169 (1968).

TABLE 1. AVERAGE AMOUNTS OF SOME FLAVONE CONSTITUENTS IN THE LEAVES OF CITRUS SPECIES RESISTANT AND SUSCEPTIBLE TO *Deuterophoma tracheiphila*

Species investigated	Concentration (μg/g Dry weight)			
	Nobiletin	Tangeritin	5,4′-Dihydroxy-6,7,8,3′-tetramethoxy-flavone	5-Hydroxy-6,7,8,3′,4′-pentamethoxy-flavone
Citrus reticulata Blanco "Avana"	1110	420	41	1100
"Cleopatra"	2650	1900	0	3410
C. sinensis (L.) Osbeck "Tarocco"	115	30	0	75
C. volkameriana Pasquale	220	90	0	95
C. paradisi Macf.	30	7	0	0
C. aurantium L.	88	56	0	55
C. aurantium var. *myrtifolia* Ker-.Gawl.	90	52	0	10
C. medica L. "Diamante"	0	12	0	0
C. aurantifolia (Christm). Swingle	0	0	0	0
C. bergamia Risso et Poiteau	0	0	0	0
C. limon (L.) Burm. "Monachello"	0	0	0	0
"Interdonato"	0	0	0	0
"Femminello" healthy	0	0	0	0
"Femminello" *D. tracheiphila*-infected	0	0	0	0
"Santa Teresa"	0	0	0	0
"Santa Tecla"	0	0	0	0
Poncirus trifoliata (L.) Raf.	0	20	0	0
Fortunella japonica (Thumberg) Swingle	0	0	0	0

flavones (and also the biologically inactive but closely related 5-hydroxy-6,7,8,3′,4′-pentamethoxyflavone), after further purification by TLC, were determined spectrophotometrically. 5,4′-Dihydroxy-6,7,8-trimethoxyflavone, which is reported to occur in the leaves of the variety Avana Mandarin,[5] could not be detected in any of the species investigated.

The results, summarized in Table 1, only in part agree with the view that resistance to *D. tracheiphila* in citrus species can be ascribed to the presence of nobiletin and other fungistatic flavones. It is true that these compounds are absent, or present in negligible amounts, in the citron (*C. medica*), in the lime (*C. aurantifolia*), and in the trifoliate orange (*Poncirus trifoliata*) which are reported to be highly susceptible,[6-8] and present in high concentrations in species such as mandarin (*C. reticulata*), sweet orange (*C. sinensis*) and volkamerian lemon (*C. volkameriana*) known to be resistant to Mal-secco.[2,6,9] However, the fact that in the very susceptible sour orange (*C. aurantium*) and myrtle leaf orange (*C. aurantium* var. *myrtifolia*)[2] the content of fungistatic flavones is almost as high as in the tolerant sweet orange shows that the presence of these compounds is not sufficient to determine the resistance to *D. tracheiphila*. Furthermore, from the results reported in the present paper it is readily apparent that other factors than the investigated flavones must be involved in determining both the high resistance to Mal-secco of the bergamot (*C. bergamia*)

[6] H. CHAPOT, *Al Awamia* **9**, 89 (1963).
[7] G. RUGGIERI, *Boll. Staz. Pat. Veg. Roma* **22**, 63 (1942).
[8] L. PETRI, *Boll. Real. Staz. Pat. Veg. Firenze* **10**, 353 (1930).
[9] F. RUSSO, *Riv. Agrum. (Acireale)* **1**, 207 (1956).

and the different susceptibility of the lemon cultivars, which ranges from very low ("Monachello" and "Santa Teresa") to high ("Femminello").[2,10]

In conclusion, fungistatic flavones are perhaps one of the causes of the resistance to *D. tracheiphila* observed in certain citrus varieties, but their presence appears to be neither necessary nor sufficient to protect the plant from the pathogenic fungus.

EXPERIMENTAL

Plant material. Six-month-old leaves collected from healthy trees (unless otherwise stated) were used in this investigation.

Extraction of flavonoids. Dried leaves (5 g) were crushed and continuously extracted (Soxhlet) with $CHCl_3$ for 24 hr. The solvent was removed and the residue, dissolved in benzene, column chromatographed on acid-washed silica gel. The column was eluted with benzene followed by increasing concentrations of ethyl acetate in benzene and the eluate collected in 20-ml fractions. The separation was monitored by TLC over silica gel using *n*-BuOH–hexane (3:17, v/v) and benzene–MeOH–tetrachloromethane (20:3:7, v/v) as solvents. The spots were located with I_2 vapour followed by spraying with 2% $NaHCO_3$.[5] The appropriate fractions were pooled and the flavones further purified by TLC. Nobiletin and tangeritin were eluted from the scraped bands with Et_2O, 5,4′-dihydroxy-6,7,8,3′-tetramethoxyflavone and 5-hydroxy-6,7,8,3′4′-penthamethoxyflavone were eluted with MeOH containing a small amount of HOAc.

Estimation of flavones. The individual flavones were quantitized spectrophotometrically, using the following ϵ (λ_{max}) values for pure compounds [tangeritin: 30900 (323 nm); nobiletin: 27020 (333 nm); 5-hydroxy-6,7,8,3′,4′-pentamethoxyflavone: 16900 (341 nm); 5,4′-dihydroxy-6,7,8,3′-tetramethoxyflavone: 20800 (341 nm)].

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[10] G. RUGGIERI, *Riv. Agrum. (Acireale)* **1**, 201 (1956).