

## STUDY OF THE FLAVONOIDS AS CHEMOTAXONOMIC MARKERS IN POPULUS (SALICACEAE) OF SPAIN. PRELIMINARY RESULTS

DIEGO RIVERA, CONCEPCIÓN OBÓN,  
FRANCISCO TOMÁS-BARBERÁN & MARIA JOSÉ ARENAS

### Abstract

A preliminary screening and of phenolics in bud exudates of Spanish Betulaceae and Salicaceae species has been made. The seasonal variation in the presence of flavonoids has been verified in bud exudates of *Populus* (Sect. *Aigeiros*). The verification of the taxonomic value of the chromatograms as reported in the literature is currently in process.

### Introduction

Analysis of phenolics has been suggested as an acceptable system for determining the geographical and botanical origin of honey (FERRERES & al., 1992). These phenolics have been identified as being flavones, flavanones and flavonols which are major constituents of propolis. Propolis is present in commercial honey in a minor part, but it is enough to determine a characteristic pattern in it.

Pliny, 1st Century AD (Book 24, chapter 32), described the use of poplar exudates by bees for propolis production. It has been assumed since the Middle Ages that bees obtained propolis from the boughs, leaves and buds of the birch, ash, and balsam trees, but recent researches recognised as major sources of propolis in the Northern Hemisphere, poplar (*Populus* sp., Salicaceae) and alder (*Alnus* sp., Betulaceae) species (GHISALBERTI, 1979; CRANE, 1988).

The bud exudate of poplars is a complex mixture of compounds, including many phenolics. The bud exudate analysis has been used as a sort of "fingerprint" to identify individuals at the level of section, species or even clones.

Eckhardt WOLLENWEBER (1975a) proposed a chemosystematics of *Populus*, which was based on the compounds found in the bud lipophilic coat. This work was followed of monographs by GREENAWAY & al. (1987, 1990-92) which furnished an exhaustive framework for comparison (Table 3, Table 4).

Our research was focused on the Spanish species of *Populus*. The ethanolic extract of buds, was analysed for selected individuals of the several species considered, using HPLC techniques. In the cases of presence of flavonic exudates the analyses were prosecuted along a whole annual cycle. Samples were collected from selected specimens in Winter, Spring, Summer and Autumn. Differences in the flavonic pattern between samples collected from the same individual in different seasons have been investigated.

The seasonal evolution of exudates rich in phenolics has been investigated in order to determine changes in the propolis composition.

Species analysed	Origin	Date of collection	Presence of phenolics
Using chromatography by silica column			
SALICACEAE			
<i>Populus canescens</i> (Aiton) Sm.	Ulea (MU)	17-III-1993	+
<i>Populus deltoides</i> Marshall	Riopar (AB)	21-III-1993	+
<i>Populus nigra</i> L.	Isso (AB)	21-III-1993	+
<i>Populus nigra</i> L. cvar. <i>italica</i> ( <i>P. italica</i> (Duroi) Moench)	Mesones (AB)	21-III-1993	+
<i>Populus x canadensis</i> Moench	Riopar (AB)	21-III-1993	+
<i>Populus x canadensis</i> Moench	Archena	21-III-1993	+
<i>Salix atrocinerea</i> Brot. (female)	Mesones (AB)	17-III-1993	-
<i>Salix atrocinerea</i> Brot. (female)	Mesones (AB)	21-III-1993	-
<i>Salix atrocinerea</i> Brot. (male)	Mesones (AB)	21-III-1993	-
<i>Salix elaeagnos</i> Scop. (female)	Mesones (AB)	21-III-1993	-
<i>Salix fragilis</i> L.	Riopar (AB)	21-III-1993	-
<i>Salix neotricha</i> Görz	Mesones (AB)	21-III-1993	-
<i>Salix purpurea</i> L.	Isso (AB)	21-III-1993	-
<i>Salix purpurea</i> L.	Archena (MU)	21-III-1993	-
<i>Salix purpurea</i> L. (female)	Mesones (AB)	21-III-1993	-
Using HPLC techniques			
BETULACEAE			
<i>Alnus glutinosa</i> (L.) Gaertner	Catal. Pyrenees (LE)	5-VIII-1993	-
<i>Betula pendula</i> Roth.	San Mauricio (LE)	28-VII-1993	-
SALICACEAE			
<i>Populus alba</i> L.	Archena	10-VII-1993	-
<i>Populus canescens</i> (Aiton) Sm.	Ulea (MU)	21-III-1993	traces
<i>Populus simonii</i> Carrière	Teruel (TE)	5-VII-1993	+

Table 1. Screening of phenolics in bud exudates.

## Materials and methods

Identification of taxa was made using the following literature: *Populus* (VICIOSO, 1951; SORIANO, 1993), *Salix* (VICIOSO, 1951; BLANCO, 1993; DÍAZ & LLAMAS, 1987). Samples were collected from living specimens and let to dry in the laboratory at 20° approximately. A preliminary screening of phenolics in exudates of Betulaceae and Salicaceae species was made. Also a seasonal control of exudates of *Populus* (Sect. *Aigeiros*) was made along a whole year. Voucher specimens have been deposited in the Herbarium MUB.

HPLC analysis of poplar bud exudate. The basic methodology was followed as described by FERRERES & al. (1991, 1994) and GARCÍA VIGUERA & al. (1993). For

Species analysed	Origin	Presence of flavonoids			
		spring	summer	autumn	winter
<i>Populus deltoides</i> Marshall	Riopar (AB)	+	+	+	+
<i>Populus deltoides</i> Marshall	Murcia		+		+
<i>Populus nigra</i> L.	Isso (AB)	+	+	+	+
<i>Populus nigra</i> L. cvar. "italica" ( <i>P. italica</i> (Duroi) Moench)	Mesones (AB)	+	+	+	+
<i>Populus x canadensis</i> Moench	Riopar (AB)	+	+	+	+
<i>Populus x canadensis</i> Moench	Archena (MU)	+	+	+	+

Table 2. Study of seasonal variation of exudates, by HPLC techniques.

each sample approximately 20 poplar buds were extracted in ethanol for 24 h in bath. The extract was filtered for analysis in silica column and after for qualitative analysis by HPLC with a Lichrochart 100 RP-18 reverse-phase column (12.5 x 0.4 cm, particle size 5 µm) using as mobile phase water-formic acid (95:5, solvent A) and methanol (solvent B). Elution was performed at a flow rate of 1 ml/min using a linear gradient starting with 35% B for 25 min increasing to levels of 40% B at 35 min, 50% B at 40 min, 65% B at 50 min, 90 % B at 55 min and Ø0% B at 60 min to reequilibrate the column. Detection was achieved at 340 nm.

## Results

The screening of phenolics in exudates of Betulaceae and Salicaceae confirmed the data available in the literature concerning Betulaceae (lacking of most flavonoids, only flavonoid aglycones are found) (WOLLENWEBER, 1975b). It has been shown for the species of *Salix* analysed the lacking of phenolic exudates.

The presence of phenolic exudates in *Populus* sect. *Aigeiros* and sect. *Tacamahaca* has been verified for Spanish populations of wild and cultivated taxa (autoctonous and exotic) (Table 1). These phenolics, for their major part, are flavonoids. These are present along the whole year in the bud and bough exudates (Table 2). It has been demonstrated for *Populus nigra* L., *P. nigra* cvar. "italica", *P. x canadensis* Moench, and *P. deltoides* Marshall the regular presence of flavonoids in the exudate (Table 2).

## Discussion

We are currently studying the HPLC chromatograms obtained in order to determine seasonal variations of flavonoid composition. We are also comparing our results with the markers reported in the literature (Table 3 and Table 4).

Section	Substances
Sect. <i>Populus</i> (= Sect. <i>Leuce</i> Duby)	The major components are cinnamic acid derivatives and hydrocarbons. Lacking flavonoids (1,2)
Sect. <i>Tacamahaca</i> Spach	The major components are dihydrochalcones and sesquiterpenols (3,4). Lacking flavones, flavanones, flavanonols and pinobanksin (3,5,6).
Sect. <i>Aigeiros</i> Duby	The major components are flavones, flavanones, flavanonols and pinobanksin is a characteristic (3,5,6). Lacking dihydrochalcones (or traces) and sesquiterpenols (3,4).
Sect. <i>Turanga</i> Bunge	Lacking flavonoids (7).

Table 3. Chemotaxonomic markers for major sections in *Populus*. This table has been compiled from (1) WOLLENWEBER (1975a), (2) ENGLISH & al. (1992), (3) ENGLISH & al. (1991a), (4) GREENAWAY & WHATLEY (1991), (5) GREENAWAY & al. (1992), (6) ENGLISH & al. (1991), (7) GREENAWAY & Al. (1991).

Species	Substances
<i>Populus nigra</i> L.	Major components are caffeic and isoferulic acids and their esters, traces of dihydrochalcones, lacking terpenoids (1, 2)
<i>Populus x canadensis</i> Moench	Intermediate character between the parentals (4)
<i>Populus deltoides</i> Marshall	Major components are flavones and flavanones, lacking dihydrochalcones and terpenoids (1, 3)

Table 4. Chemotaxonomic markers for *Populus* species (Section *Aigeiros* Duby). This table has been compiled from (1) Greenaway & Whatley (1991), (2) Greenaway & al. (1990), (3) English & al. (1992), (4) Greenaway & al. (1987).

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## References

- BLANCO, P. (1993). *Salix*. S. CASTROVIEJO & al. (eds.), *Flora Iberica* 3: 477-517. Real Jardín Botánico. Madrid. 730 pp.
- CRANE, E. (1988). *Beekeeping: science, practice and world resources*. Heinemann. London.
- DÍAZ-GONZÁLEZ, T. E. & F. LLAMAS (1987). Aportaciones al conocimiento del género *Salix* L. (Salicaceas) en la provincia de León (NW España). *Acta Bot. Malacitana* 12: 111-150.
- ENGLISH, S., W. GREENAWAY & F. R. WHATLEY (1991a). Analysis of phenolics of *Populus trichocarpa* bud exudate by GC-MS. *Phytochemistry* 30: 531-533.
- , W. GREENAWAY & F. R. WHATLEY (1991b). Bud exudate composition of *Populus tremuloides*. *Canad. J. Bot.* 69: 2291-2295.
- , W. GREENAWAY & F. R. WHATLEY (1992). Analysis of phenolics in the bud exudates of *Populus deltoides*, *P. fremontii*, *P. sargentii* and *P. wislizenii*. by GC-MS. *Phytochemistry* 31: 1255-1260.
- FERRERES, F., F. A. TOMÁS-BARBERÁN, M. I. GIL & F. TOMÁS-LORENTE (1991) An HPLC Technique for Flavonoid Analysis in Honey. *J. Sci. Food Agric.* 56: 49-56.
- , A. ORTÍZ, C. SILVA, C. GARCÍA-VIGUERA, F. A. TOMÁS-BARBERÁN & F. TOMÁS-LORENTE (1992). Flavonoids from La Alcarria honey. A study of their botanical origin. *Z. Lebensm. Unters. Forsch.* 194: 139-143.
- , F. A. TOMÁS-BARBERÁN, C. SOLER, C. GARCÍA-VIGUERA, A. ORTÍZ, & F. TOMÁS-LORENTE (1994). A simple extractive technique for honey flavonoid HPLC analysis. *Apidologie* 25: 21-30.
- GARCÍA-VIGUERA, C., F. FERRERES & F. A. TOMÁS-BARBERÁN (1993). Study of Canadian propolis by GC-MS and HPLC. *Z. Naturforsch.* 84c:731-735.
- GHISALBERTI, E. L. (1979). Propolis. A review. *Bee World* 60: 59-84.
- GREENAWAY, W., T. SCAYSBROOK & F. R. WHATLEY (1987). The analysis of bud exudate of *Populus x euramericana*, and of propolis, by gas chromatography-mass spectrometry. *Proc. R. Soc. Lond., B* 232: 249-272.
- & F. R. WHATLEY (1991). Analysis of phenolics of bud exudate of *Populus ciliata* by GC-MS. *Phytochemistry* 30: 1887-1889.
- , I. GÜMÜSDERE & F. R. WHATLEY (1991). Analysis of phenolics of bud exudate of *Populus euphratica* by GC-MS. *Phytochemistry* 30: 1883-1885.
- , S. ENGLISH & F. R. WHATLEY (1990). Variations in bud exudate composition of *Populus nigra* assessed by GC-MS. *Z. Naturforsch.* 45: 931-936.
- , S. ENGLISH & F. R. WHATLEY (1992). Relationships of *Populus x acuminata* and *P. x generosa* with their parental species examined by GC-MS of bud exudates. *Canad. J. Bot.* 70: 212-221.
- SORIANO, C. (1993). *Populus*. In S. CASTROVIEJO & al. (eds.), *Flora Iberica* 3: 471-477. Real Jardín Botánico. Madrid.
- VICIOSO, C. (1951). *Salicáceas de España*. Instituto Forestal de Investigaciones y Experiencias. Madrid.
- WOLLENWEBER, E. (1975a). Flavonoidmuster als systematisches Merkmal in der Gattung *Populus*. *Biochem. Syst. Ecol.* 3: 35-45.
- (1975b). Flavonoidmuster im Knospensexkret der Betulaceen. *Biochem. Syst. Ecol.* 3: 47-52.

**Address of the authors:**

Drs. D. Rivera, C. Obón & M. J. Arenas, Departamento de Biología Vegetal, Facultad de Biología, Universidad de Murcia, 30100 Murcia, Spain; Dr. F. Tomás-Barberán, Laboratorio de Fitoquímica, CEBAS, Apdo. 4195, 30080 Murcia, Spain.