Hybrid Origin of *Populus* × *jackii* Confirmed by Gas Chromatography-Mass Spectrometry Analysis of Its Bud Exudate

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Populus balsamifera, Populus deltoides, Populus × *jackii,* Poplar Bud Exudate, GC/MS

Bud exudate of *Populus balsamifera* contains an array of characteristic compounds. Bud exudate of *P. deltoides* contains a different array of characteristic compounds. The exudate of *P.* × *jackii* contains compounds characteristic of both *P. balsamifera* and *P. deltoides,* indicating its hybrid origin.

Introduction

Most *Populus* spp. produce a bud exudate which is a complex mixture of chemicals, including many phenolic compounds. The composition of this mixture is characteristic of a species [1], and even morphologically similar clones can be distinguished by analysis of their bud exudate [2].

The exudate of *P. balsamifera* L. (Section Tacamahaca) and *P. deltoides* Marsh (Section Aigeiros) differ markedly [3, 4]. Exudate of *P. balsamifera* contains high levels of dihydrochalcones and terpenoids, which are not present in exudate of *P. deltoides,* whereas exudate of *P. deltoides* contains a high percentage of pinocembrin (5,7-dihydroxyflavanone) and pinocembrin derivatives, which are not present in *P. balsamifera* exudate [3, 4].

Natural hybridization between *P. balsamifera* and *P. deltoides* has been suggested as the origin of both *P. candicans* Ait. (*P. gileadensis* Rouleau) and *P.* × *jackii* Sarg. [5–8]. We have already demonstrated that the bud exudate of *P. candicans* contains only compounds characteristic of *P. balsamifera,* lacking all those compounds we regard as characteristic of *P. deltoides* [9]. From these results we concluded that *P. candicans* was unlikely to be the result of a cross involving *P. deltoides.*

On the contrary the bud exudate of *P.* × *jackii* contains compounds characteristic of both *P. balsamifera* and *P. deltoides* and this confirms its identity as a cross between these two species.

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Materials and Methods

Reagents

Bis-(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) was obtained from Sigma (Poole, U.K.).

Plant material

Bud exudate was collected in Canada from a specimen of *P. balsamifera* (ref. B 56) at the North Burgess Sector of the Ministry of Natural Resources, Ontario, and from the following specimens at the Agriculture Canada Morden Arboretum, Morden, Manitoba: *P. deltoides* (ref. 2493-71) originating from Ramseyville, Ontario; *P.* × *jackii* (ref. 67-1116) originating from Helena, Montana, U.S.A., and *P.* × *jackii* (ref. 70-1802) originating from Delta Beach, Manitoba.

Sample preparation

Exudate was collected by dipping 2–4 buds in 3 ml ethyl acetate in a screw-top conical glass tube for 10 s. The ethyl acetate was evaporated under a stream of N_2 and the extract freeze-dried for 10 min to remove residual water. After addition of 50 µl pyridine and 100 µl BSTFA, containing 1% TMCS, the tube was sealed and heated for 30 min at 100 °C to produce trimethylsilyl (TMS) derivatives for gas chromatography.

Gas chromatography-mass spectrometry

As previously described [10] excepting that a 25 m × 0.32 mm ID Thames Chromatography (Maidenhead, U.K.) silica column coated with

0.5 μm of immobilized dimethylsiloxane was used, with a helium pressure of 76 kN/m^2.

Identification of compounds

Compounds in bud exudates were identified as described previously [10]. Flavonoid standards were either purchased from Apin Chemicals (Abingdon, U.K.) or from Plantech U.K. (Reading, U.K.), or provided as a gift by Professor E. Wollenweber (Darmstadt, F.R.G.). Other reference compounds were synthesized as described previously [11].

Results

The GC/MS total ion chromatograms (T.I.C.) of bud exudate of *P. balsamifera, P. deltoides* and *P.* × *jackii* differ primarily in the region of scans 1700–3700 (17–28 MU). This is the region in which trimethylsilyl derivatives of sesquiterpenols and flavonoid aglycones chromatograph. The T.I.C. (17–28 MU) recorded from bud exudate of the three species are shown in Fig. 1, the principal compounds which differ between the species are identified in Table I and a summary of the major groups of compounds present is shown in Table II.

The T.I.C. obtained for *P. balsamifera* B 56 was similar to those previously recorded [3] and that of *P. deltoides* 2493-71 was within the range previously reported for the species [4].

Dihydrochalcones formed 70% of the T.I.C. and sesquiterpenols formed 11% of the T.I.C. recorded for bud exudate of *P. balsamifera.* Both these classes of compound were absent from bud exudate of *P. deltoides.* However bud exudate of *P.* × *jackii* contained 14–15% dihydrochalcones and 2–3% terpenoids (Table II). Flavanones formed the principal components of the T.I.C. of *P. deltoides* bud exudate (65%), were a lesser component of *P.* × *jackii* bud exudates (42% or 53%) and a minor component of *P. balsamifera* bud exudate (2%). The flavanones pinobanksin-3-methyl ether and pinobanksin-3-acetate, major components of *P. deltoides* bud exudate, were absent

Table I. Dihydrochalcones, flavanones and sesquiterpenols which distinguish *P. balsamifera* from *P. deltoides* and which indicate the hybrid status of *P.* × *jackii.* Gas chromatographic retention times in methylene units (MU)[1] are given to two decimal places to indicate the elution sequence of peaks which chromatograph closely. Factors such as concentration of the compound concerned and concentration of adjacent compounds, together with the characteristics of a particular GC column are liable to affect the chromatography, and for general purposes the MU figures are probably applicable only to a single decimal place.

Peak No.	Compound	No. TMS groups	Retention time MU	% Total ion current[2] *Populus balsamifera* B 56	*Populus* × *jackii* 67-1116	*Populus* × *jackii* 70-1802	*Populus deltoides* 2493-71
	Terpenoids						
1	sesquiterpenol	1	17.19	1	T[3]	1	–
2	sesquiterpenol	1	17.39	6	1	T	–
3	sesquiterpenol	1	19.29	1	T	1	–
	Dihydrochalcones						
4	2′6′-dihydroxy-4′-methoxydihydrochalcone	2	23.78	8	1	1	–
5	2′4′6′-trihydroxydihydrochalcone	3	24.41	28	6	4	–
8	2′,6′-dihydroxy-4′-4-dimethoxydihydrochalcone	2	26.10	3	1	4	–
11	2′4′6′-trihydroxy-4-methoxydihydrochalcone	3	26.65	24	2	2	–
12	2′6′4-trihydroxy-4′-methoxydihydrochalcone	3	27.12	7	4	4	–
	Flavanones						
6	5,7-dihydroxyflavanone	2	24.92	2	22	38	21
7	3,5,7-trihydroxyflavanone	3	25.77	1	6	2	13
9	5,7-dihydroxy-3-methoxyflavanone	2	26.16	–	4	1	12
10	5,7-dihydroxy-3-acetoxyflavanone	2	26.34	–	7	2	18

[1] Methylene units are defined by Dalgliesh *et al.* [13].

[2] The ion current generated depends on the characteristics of the compound concerned and is not a true quantitation [see 11]. Where peaks are mixed the total ion current for the compound quoted is assessed by measuring ions characteristic for the compound concerned.

[3] T (trace) indicates amounts between 0.1% and 1%. Compounds marked – contain either <0.1% or were not detected.

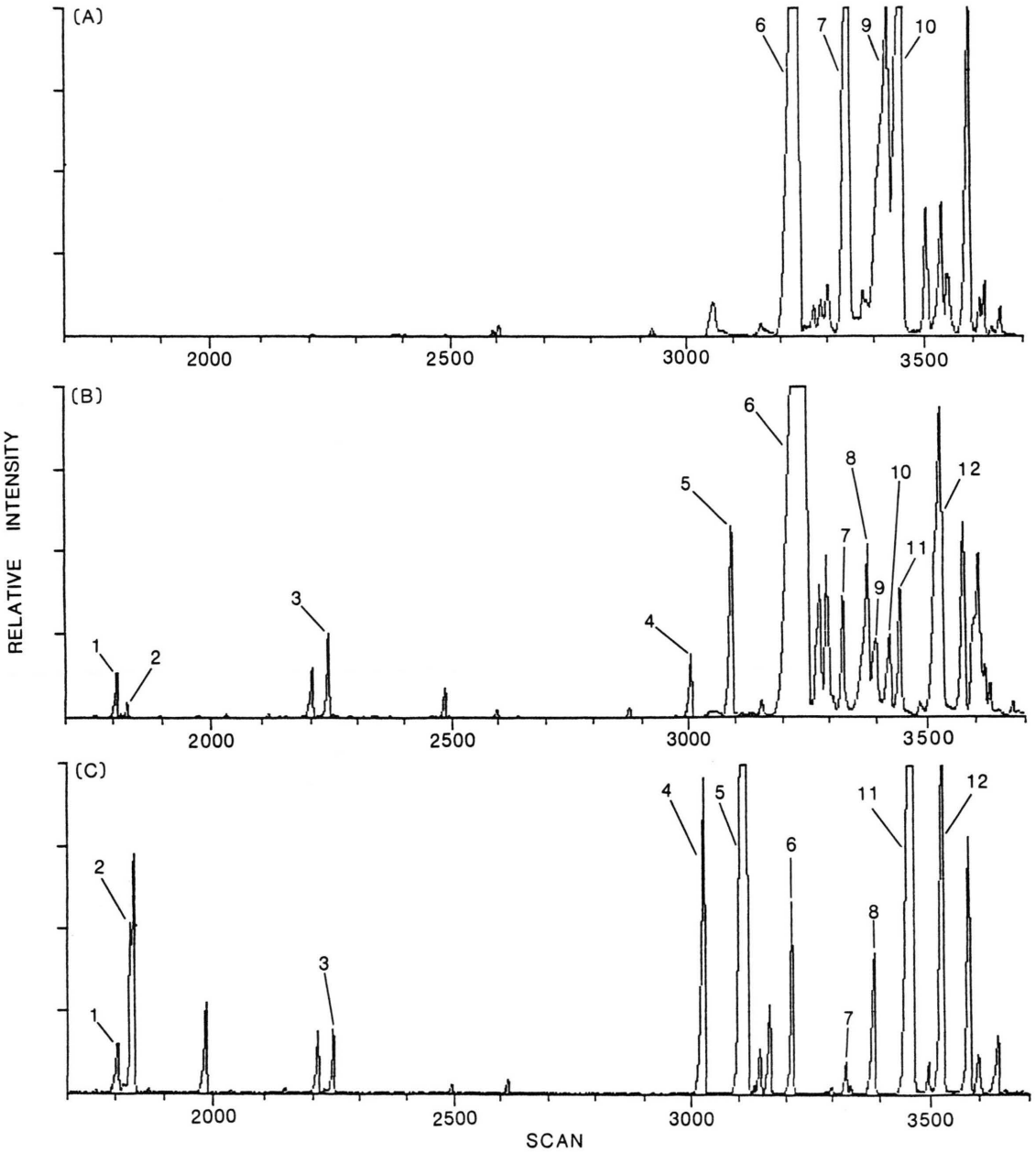

Fig. 1. Total ion chromatograms of bud exudate from *P. deltoides* (A), *P.* × *jackii* (B) and *P. balsamifera* (C), scans 1700–3700 (17–28 MU). Terpenoids (1, 2, 3) and dihydrochalcones (4, 5, 8, 11, 12) are present in *P. balsamifera* and *P.* × *jackii* but absent from *P. deltoides*. Pinocembrin = 5,7-dihydroxyflavanone (6), and pinobanksin = 3,5,7-trihydroxyflavanone (7) are primarily present in *P. deltoides* and *P.* × *jackii* whereas pinobanksin-3-methyl ether (9) and pinobanksin-3-acetate (10) are present only in these species. For detailed peak identification see Table I.

Table II. Summary of the major constituents of *Populus balsamifera, P. deltoides* and *P.* × *jackii* bud exudate.

	% Total ion current[1]			
	Populus balsamifera B56	*Populus* × *jackii* 67-1116	*Populus* × *jackii* 70-1802	*Populus deltoides* 2493-71
Cinnamic and coumaric acids and their esters	5	4	5	T[2]
Caffeic and ferulic acids and their esters	–	–	–	5
Chalcones	4	4	4	7
Dihydrochalcones	70	14	15	–
Total flavanones	2	30	49	35
Flavanones methylated or esterified in the 3 position[3]	–	12	4	30
Flavones	6	28	13	13
Terpenoids[4]	11	2	3	–

[1] The ion current generated depends on the characteristics of the compound concerned and is not a true quantitation [11].
[2] T (trace) indicates amounts of between 0.1% and 1%. Components marked – comprised either < 0.1% of the total ion current or were not detected.
[3] This category also includes minor amounts of pinobanksin esterified in the 3 position with propanoic, butanoic, pentanoic and hexanoic acids [14].
[4] In addition to the main sesquiterpenols referred to in Table I, there are many (20–30) minor terpenoid compounds present. Positive identification of these compounds is very difficult as they are very similar in structure and in M/S fragmentation patterns. Furthermore reference standards are not available.

from *P. balsamifera* bud exudate, although both were present in *P.* × *jackii* bud exudates (Table I).

Caffeic and ferulic acids and their esters were present in bud exudate of *P. deltoides* but absent from bud exudates of *P. balsamifera* and *P.* × *jackii,* whereas cinnamic and coumaric acids and their esters, present in bud exudate of both *P. balsamifera* and *P.* × *jackii,* were virtually absent from bud exudate of *P. deltoides.*

Discussion

Our results indicate that the bud exudate of typical North American species of the Section Tacamahaca, such as *P. balsamifera* [3] and *P. trichocarpa* Torr. and Gray (unpublished data) contain a high percentage of dihydrochalcones and terpenoids which are absent from typical species of the Section Aigeiros, such as *P. deltoides* [4] and its allied species *P. fremontii* S. Wats., *P. sargentii* Dode and *P. wislizeni* Sarg. (unpublished data). In contrast the Section Aigeiros species contain a high percentage of flavanones which are virtually absent from *P. balsamifera* and *P. trichocarpa* of the Section Tacamahaca, and in particular contain pinobanksin (3,5,7-trihydroxyflavanone) derivatives methylated or esterified in the 3 position, which are entirely lacking from the species of the Section Tacamahaca.

The species of the Section Tacamahaca studied typically contain cinnamic acid (3-phenyl-2-propenoic acid) and coumaric acid (3(4-hydroxyphenyl)-2-propenoic acid), whereas species of the Section Aigeiros studied typically contain the 3-substituted phenylpropenoic acids caffeic (3(3,4-dihydroxyphenyl)-2-propenoic acid) and ferulic (3(3-methoxy-4-hydroxyphenyl)-2-propenoic acid). It therefore seems probable that North America species of the Section Tacamahaca typically lack enzymes capable of methylating or esterifying at the 3 position of the phenyl or fla-

vonoid ring systems, whereas species of the Section Aigeiros possess them.

As a number of enzymes are involved inheritance of the complex of phenolics characteristic of poplar species will be multigenic and putative hybrids will show the bud exudate chemicals characteristic of both parents in varying degrees. Our results with *P.* × *jackii* clearly show that it has inherited both the ability to methylate or esterify phenyl and flavonoid rings in the 3 position from *P. deltoides* and the tendency to synthesize dihydrochalcones from *P. balsamifera*. Presumably the bud exudate of other crosses which are intermediate between the two parents will resemble the bud exudate of *P.* × *jackii* which we have analyzed.

Ronald [7, 8] found that in areas where *P. deltoides* and *P. balsamifera* overlap in distribution natural hybrids may occur. More recently Rood [12], while studying *P. deltoides, P. balsamifera* and *P. angustifolia* James in southern Alberta found that all three interbred quite freely producing a single, dissectional trispecific hybrid swarm. In these locations *P.* × *jackii* represents a morphologically recognizable hybrid.

Acknowledgements

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