Variation in Bud Exudate Composition of *Populus nigra* Assessed by Gas Chromatography-Mass Spectrometry

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Bud exudates from twelve clones of *Populus nigra* originating from seven countries were analyzed by gas chromatography-mass spectrometry and the principal compounds identified. Specimens of *P. nigra* var *betulifolia* differed in bud exudate composition from the other specimens analyzed, and were more similar in exudate composition to *P. deltoides* than were the other specimens. The range of bud exudate composition was greater than that which would be expected to occur within a single coherent species.

Introduction

Populus nigra L. (Section Aigeiros), the black poplar, has a distribution which includes much of Europe, North Africa and locations in Asia [1, 2]. The species varies considerably in morphology throughout its range [3] but there are two broadly accepted groupings, *P. nigra* var *betulifolia* Torrey, the English or pubescent black poplar, and *P. nigra* var *typica* Schneider, the continental or glabrous black poplar [4].

P. nigra var *betulifolia* is restricted in distribution to France and the U.K. [4], although in England it is now rare, the total population being estimated at about one thousand trees in 1975 [5]. *P. nigra* var *typica* is absent from the U.K. but occurs throughout the rest of the range of *P. nigra*. The variation in this variety is so great that it is frequently subdivided into other varieties or even species [3].

The identification of *P. nigra* is complicated by the occurrence of naturalized hybrids with the North American *P. deltoides* Marsh (Section Aigeiros), the eastern cottonwood, some of which closely resemble *P. nigra*. *P. deltoides* was introduced into France from America about 1700 [6] and was established in both France and England by 1730 [7]. At that time the regular flooding of lowland rivers provided the muddy sites necessary for the successful germination of seed and the establishment of seedlings of both *P. nigra* and of its frequent hybrids with the introduced *P. deltoides* [2, 8]. These hybrids, now collectively named *P.* × *euramericana* (Dode) Guinier [1], display greater vigour than either parental species and are better timber trees. They were therefore selected and planted in preference to the native *P. nigra*. Since the 18th century many clones of *P.* × *euramericana* have been propagated [1] and it is the presence of these clones, together with their backcrosses with *P. nigra*, which renders the identification of *P. nigra* difficult [8].

Poplars of the Section Aigeiros produce from their buds a characteristic sticky exudate which contains a variety of phenolic compounds. The composition of this exudate is characteristic of the clone of poplar [9] and hybridization between poplars can be established by analysis of bud exudate [10, 11]. We here report the analysis by GC-MS of bud exudate from specimens of *P. nigra* from several countries to assess the variation in the bud exudate and discuss whether its composition provides an indication of hybridity with *P. deltoides*.

Materials and Methods

Reagents and sample preparation

Were as previously described [12].

Plant material

Bud exudate was collected from twelve poplars originating from seven countries (Table I). All the specimens were considered to be typical of *P. nigra* at the original locality, and not hybrids. Of the specimens from the U.K., those from Cheshire ori-

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Table I. Origins of *Populus nigra* specimens analyzed.

Specimen	Original location	Site of propagation[1]	Reference number[2]
A	Cambridgeshire, U.K.	Westonbirt	420294
B	Cheshire, U.K.	Westonbirt	410616
C	Cheshire, U.K.	Westonbirt	410434
D	Cheshire, U.K.	Westonbirt	420293
E	Stalingrad Province, U.S.S.R.	Alice Holt	S1
F	Kasakhstan Province, U.S.S.R.	Alice Holt	S2
G	Xinjiang Province, P.R.C.	Shan Xi	BEIO3
H	Naples, Italy	Alice Holt	N
I	Modena, Italy	Alice Holt	M
J	Rhone Valley, France	Geraardsbergen	88.033
K	Terwolde, Netherlands	Geraardsbergen	88.045.1
L	Papignies, Belgium	Geraardsbergen	88.043

[1] Alice Holt = Alice Holt Lodge, Forestry Commission, Farnham, U.K.
Geraardsbergen = Rijksstation voor Populierenteelt, Geraardsbergen, Belgium.
Shan Xi = Experimental Bureau of High Yield Poplar Forest of Shan Xi, Datong, Shan Xi Province, P.R.C.
Westonbirt = Westonbirt Arboretum, Forestry Commission, Tetbury, Gloucs., U.K.
[2] The number given is the clone reference number at the site of propagation.

ginated from a seed collection made by E. Milne Redhead and that from Cambridgeshire was collected as a cutting by J. White from the "Hobsons Brook" tree. The U.K. specimens were considered typical of *P. nigra* var *betulifolia*, the English black poplar [4]. Specimens propagated at Alice Holt were confirmed as *P. nigra* by J. Jobling and those propagated at Geraardsbergen were collected and identified by Ir. V. Steenackers.

Gas chromatography-mass spectrometry (GC-MS)

Was as previously described [12].

Principal component analysis

Principal component analysis is a multivariate exploratory statistical method for presenting several experimental measurements in two dimensions so that any intrinsic clusters that the total data possesses will become apparent; at the same time any data which is deviant will be emphasized [13]. The analysis was performed by correlation matrix using Minitab version 7.2 (Minitab Inc., PA, U.S.A.) on a Data General MV 4000 minicomputer.

Results

The composition of bud exudate from the twelve specimens analyzed is listed in Table II. Only compounds which exceed 2% of the total ion current (TIC) in any one of the specimens are listed in Table II. The percentage compositions of the major groups of constituents are summarized in Table III; these data were subjected to principal component analysis (Fig. 1).

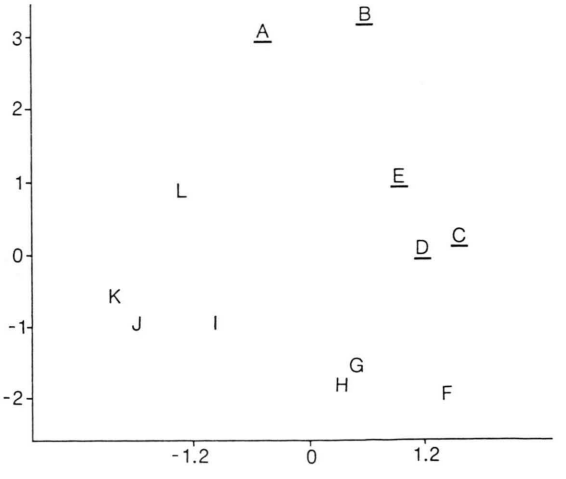

Fig. 1. Principal component analysis of eight major groups of bud exudate components, shown in Table III, from twelve specimens of *Populus nigra*. The origins of the specimens are shown in Table I. The analysis shows no clear clusters, although a broad division is apparent between specimens A, B, C, D and E (representing "var *betulifolia*") and the other specimens (representing "var *typica*").

Table II. Composition of bud exudate of *Populus nigra* assessed by GC-MS.

Peak	Compound	% Total ion current[1] Specimen[2] A	B	C	D	E	F	G	H	I	J	K	L
1	succinic acid	–	–	–	–[3]	T[3]	2	–	1	3	4	T	T
2	cinnamylidene acetic acid	–	–	–	–	–	–	–	–	T	T	1	4
3	2′,6′-dihydroxy-4′-methoxypentanophenone	–	–	–	–	–	–	5	–	–	–	–	–
4	dimethoxycinnamic acid	1	2	4	4	3	2	3	2	T	T	2	1
5	isoferulic acid	1	6	7	7	5	10	10	6	2	1	1	2
6	caffeic acid	5	5	8	5	2	3	5	1	T	1	1	3
7	octadecenoic acid	–	T	–	–	1	22	1	7	2	–	–	–
8	3-methyl-3-butenyl-caffeate	5	4	6	7	8	8	5	12	18	10	5	3
9	2′,6′-dihydroxy-4′-methoxydihydrochalcone	T	T	T	T	T	–	T	1	T	2	1	1
10	2-methyl-2-butenyl caffeate	1	T	1	1	2	3	3	T	T	T	1	T
11	3-methyl-2-butenyl caffeate	12	8	11	13	12	13	8	15	15	14	9	5
12	2′,6′-dihydroxy-4′-methoxychalcone (pinostrobin chalcone)	11	8	5	8	5	5	15	6	21	14	23	13
13	C25 hydrocarbon	1	2	3	3	1	1	T	T	1	1	1	2
14	pinocembrin (5,7-dihydroxyflavanone)	10	10	16	21	5	3	4	T	5	3	1	7
15	pinocembrin chalcone	6	2	2	4	2	1	6	3	8	6	9	10
16	pinobanksin (3,5,7-trihydroxyflavanone)	3	5	T	T	2	–	T	–	T	1	1	–
17	pinobanksin-3-acetate	14	10	T	1	9	–	2	T	–	6	3	–
18	C27 hydrocarbon	5	2	4	3	14	5	2	13	3	3	9	11
19	chrysin (5,7-dihydroxyflavone)	8	7	12	9	4	2	5	1	6	1	1	13
20	galangin methyl ether	–	4	–	–	–	–	–	–	–	T	–	–
21	galangin (3,5,7-trihydroxyflavone)	5	7	1	T	5	–	1	T	T	3	2	T
22	pinobanksin-3-butanoate[4]	–	2	–	–	–	–	–	–	–	–	–	–
23	phenylethyl caffeate	1	2	3	3	3	15	8	2	4	13	3	7
24	C29 hydrocarbon	1	1	1	T	1	T	T	6	T	1	5	6
25	sugar	1	1	T	–	1	T	1	3	1	5	1	1

[1] The ion current generated depends on the characteristics of the compound concerned and is not a true quantitation (see ref. 17).
[2] See Table I for the origins of specimens A – L.
[3] T (trace) indicates amounts of between 0.1% and 0.5%. Compounds marked – comprised either < 0.1% of the TIC or were not detected.
[4] We do not know whether the substituent at the 3 position is linear or branched.

Table III. Summary of the major constituents of bud exudates of *Populus nigra*. This table includes all relevant components of the bud exudate, whether listed in Table II or not.

		% Total ion current[1] Specimen[2] A	B	C	D	E	F	G	H	I	J	K	L
1	Cinnamic and coumaric acid and their esters	2	3	6	7	4	3	7	4	3	4	4	2
2	Caffeic and isoferulic acids and their esters	24	26	37	38	33	54	44	38	40	40	25	22
3	Chalcones	19	13	10	15	8	6	22	10	31	21	33	24
4	Dihydrochalcones	1	T[3]	T	1	T	–[3]	1	1	T	4	1	1
5	Total flavanones	27	28	19	24	16	4	13	1	6	10	6	8
6	Flavanones methylated or esterified in the 3 position	14	12	T	1	9	–	2	–	–	6	3	–
7	Flavones	13	18	13	10	10	2	5	1	7	5	4	14
8	Terpenoids	–	–	–	–	–	–	–	–	–	–	–	–

[1] The ion current generated depends on the characteristics of the compound and is not a true quantitation (see ref. 17).
[2] See Table I for the origins of specimens A – L.
[3] T (trace) indicates amounts of between 0.1% and 0.5%. Compounds marked – comprised either < 0.1% of the TIC or were not detected.

All specimens have low levels of cinnamic and coumaric acids and their esters (2–7%) and high levels of caffeic and isoferulic acids and their esters (22–54%), with 3-methyl-3-butenyl caffeate[8]* (3–18%) and 3-methyl-2-butenyl (prenyl) caffeate[11] (5–15%) being the major components. Several other esters of caffeic and isoferulic acids, which occur in amounts too low to be listed in Table II, are described elsewhere [14, 15].

The chalcones are principally represented by pinocembrin chalcone[15] (1–10%) and pinostrobin chalcone[12] (5–23%). Pinostrobin chalcone is accompanied by only traces of its corresponding flavanone (pinostrobin = 5-hydroxy-7-methoxyflavanone), although we cannot be sure whether this is due to naturally low levels of the flavanone or to its facile conversion to the chalcone during sample preparation. The major dihydrochalcone present, 2′,6′-dihydroxy-4′-methoxydihydrochalcone[9], is probably derived from the *in vivo* reduction of pinostrobin chalcone.

The principal flavanones present were pinocembrin[14] (1–21%) and pinobanksin[16] (0–5%) together with pinobanksin acetate[17] (0–14%). The specimens from the U.K. (A–D) contained greater quantities of flavanones (19–28%) than did the specimens (E–L) originating from mainland Europe (1–16%).

The flavones chrysin[19] (1–13%) and galangin[21] (0–7%) were the principal flavones detected. Again the U.K. specimens (A–D) contained a higher level of flavones (10–18%) than did the majority of the mainland Europe specimens.

Other flavonoids, such as 7-methyl-pinocembrin and 7-methyl-chrysin were present, but did not exceed 2% of TIC in any specimen, and are not therefore listed in Table II. The larger molecular weight flavonoids do not transmit well through GC columns, and we do not detect some larger flavonoids, such as 7-methyl-quercetin, which have been previously reported from *P. nigra* bud exudate [16].

A few compounds occur in quantity in isolated specimens but are absent from the majority. Thus octadecenoic acid[7] comprises 22% of exudate from specimen F and 7% of exudate from specimen H, but it is absent from most other specimens. Similarly the phenone[3] present (5%) in specimen G is absent from all other specimens. This phenone (methylene unit retention time 19.16) was recently identified in a hybrid poplar (clone 1c) close to *P. angustifolia* James, although it was absent from other related poplars [11]. It appears to be a compound of erratic occurrence.

A principal component analysis of the eight major classes of compounds present indicates that the twelve specimens analyzed fall broadly into two main groups (Fig. 1). The first includes all the U.K. specimens (A–D) plus a single specimen from Russia (E). The second group includes all other mainland Europe specimens (F–L). It is clear from the pattern of peaks recorded by GC-MS that there is considerable variation in bud exudate composition between the specimens. A comparison of the TIC recorded for specimens B, F and J, indicated by principal component analysis to be the extremes of the specimens analyzed, clearly demonstrates this (Fig. 2).

Discussion

All twelve specimens of *P. nigra* analyzed have high levels of caffeic and isoferulic acids and their esters but low levels of cinnamic and coumaric acids and their esters. This, together with the low levels of dihydrochalcones and absence of terpenoids, is typical of Section Aigeiros poplars.

The *P. nigra* var *betulifolia* specimens (A–D) together with specimen E, which we assume belongs to this group, differ in bud exudate composition from the other specimens analyzed in having higher levels of the flavanones pinocembrin and pinobanksin and of the flavones chrysin and galangin. Two of the specimens (A, B) also contain higher levels of pinobanksin esterified with short chain acids (*e.g.* pinobanksin-3-acetate). In these respects the bud exudate compositions of *P. nigra* var *betulifolia* more closely resemble that of *P. deltoides* [10] than do those of the other specimens analyzed. This suggests a connection between *P. nigra* var *betulifolia* and *P. deltoides*. The typical range of the former species (*i.e.* France and England) does indeed coincide with the areas into which *P. deltoides* was initially introduced. Late 17th century herbarium records exist of plants typical of *P. nigra* var *betulifolia* and trees of this variety are recorded which were probably planted in

* Superscripts refer throughout to peak numbers in Table II.

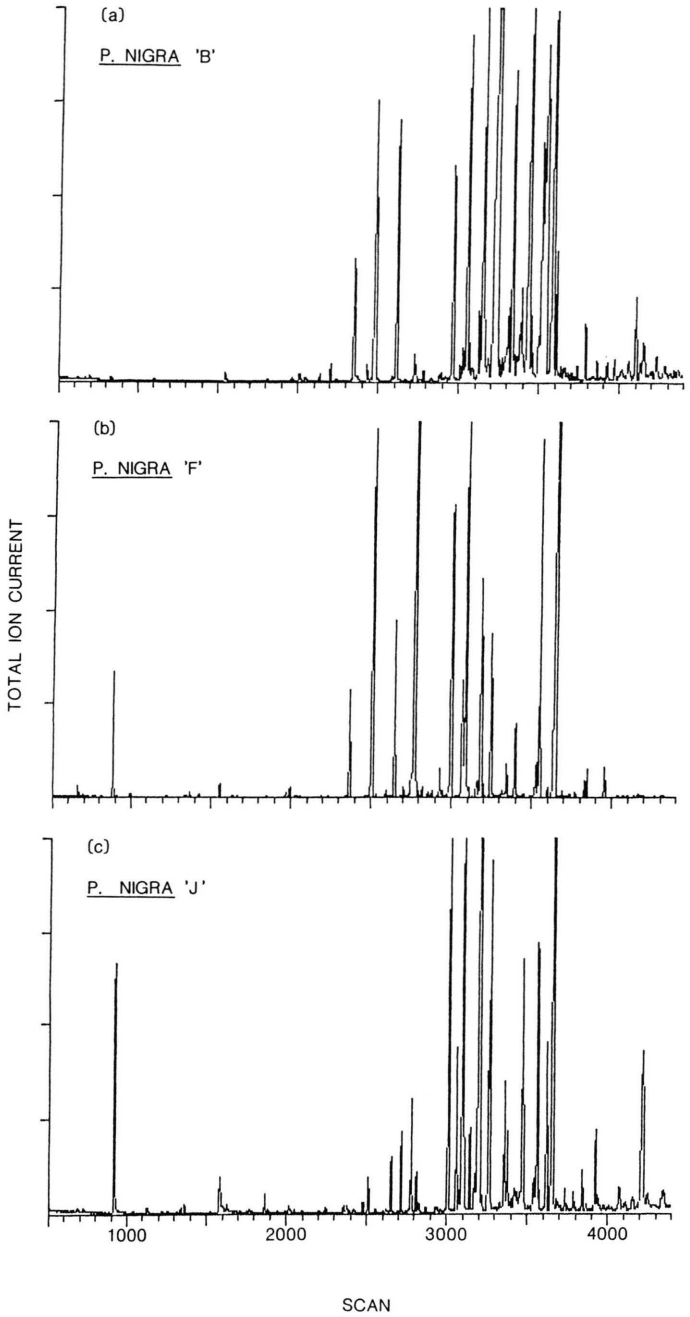

Fig. 2. Total ion chromatograms (TIC) of *Populus nigra* (a) specimen B (Cheshire, U.K.), (b) specimen F (Kasakhstan, U.S.S.R.) and (c) specimen J (Rhone Valley, France). The principal component analysis (Fig. 1) indicates these specimens to be the extremes of those analyzed for bud exudate composition. The TIC "fingerprints" are clearly different; this would not be expected if the specimens belonged to a single coherent species.

the late 17th century [4]. If *P. nigra* var *betulifolia* is the result of some degree of hybridization with *P. deltoides,* then the initial hybridization must date from late in the 17th century, close to the time when *P. deltoides* was first introduced from North America into France.

The remaining seven specimens, which represent the European *P. nigra* var *typica,* show variations particularly in percentages of chalcones and flavones (Table III) and principal component analysis implies that they are not a coherent group.

Comparison of the TIC "fingerprints" of specimens B, F and J, which represent the extremes of the twelve specimens studied (Fig. 1), show them to be clearly different (Fig. 2). Previous studies have demonstrated that poplars of the same species with similar morphology have bud exudate patterns that are recognizably similar [11]. The very variable TIC fingerprints recorded here suggest that the specimens of *P. nigra* studied do not represent members of a single coherent species but rather represent a complex of trees which are at least as diverse as those embraced by the *P. deltoides*/*P. fremontii*/*P. sargentii*/*P. wislizeni* complex of North America.

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