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Composition of bud Exudate of *Populus × interamericana* Clones as a Guide to Clonal Identification

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Abstract

Capillary column gas chromatographic analysis of bud exudate enabled the *Populus × interamericana* VAN BROEKHUIZEN clones 'Barn', 'Beaupré', 'Boelare', 'Donk', 'Hunnegem', 'Raspalje', 'Rap' and 'Unal' to be distinguished.

Key words: *Populus × interamericana*, Clonal identification, bud exudate, GC/MS.

Zusammenfassung

Kapillar-Gaschromatographie/Massenspektroskopie der Knospen-Exkrete von *Populus × interamericana* erlaubt die Unterscheidung der Klone 'Barn', 'Beaupré', 'Boelare', 'Donk', 'Hunnegem', 'Raspalje', 'Rap' und 'Unal'.

Introduction

Chemotaxonomy has been used to assist in differentiating poplar species and identifying poplar hybrids by a number of workers. ANDERSON and BOWMAN (1979) investigated chemical composition of leaves of 17 pure species and 14 hybrids of poplar and used leaf polyphenols to verify the origin and hybridity of various clones. They also concluded that polyacrylamide gel disc electrophoretic banding patterns of peroxidase and esterase might well permit clonal identification. KIM and CHUNG (1974) had similarly concluded that isoperoxidase patterns would allow clones of *P. × euramericana* (DODE) GUINIER to be distinguished, and GUZINA (1974) also suggested that isoperoxidase patterns could be a valuable chemotaxonomic criterion for *Populus* spp. Clones have also been identified by means of serological reactions by MOTTI and STERBA (1973) using immunoelectrophoresis. Other workers have assessed the use of compounds (primarily phenolic glucosides) extracted from leaves of *Populus heterophylla* L. as a taxonomic guide (PEARL and DARLING, 1977). Phenolic glycosides of poplar and willow bark (JULKUNEN-TIHTO, 1985; 1986; RONALD *et al.*, 1973; RONALD and STEELE, 1974; STEELE and RONALD, 1973; STEELE *et al.*, 1973) and flavonoid aglycones of poplar leaves and bud exudate (BOCCONE, 1975; CRAWFORD, 1974; JONES and SEIGLER, 1975; WOLLENWEBER, 1975) have also been investigated as chemotaxonomic criteria. From detailed studies of flavonoid aglycones of poplar bud exudates WOLLENWEBER (1975) established that analysis of flavonoid aglycones of poplar bud exudate allowed species of the sections *Aigeiros* and *Tacamahaca*, together with some of their hybrids, to be separated, although analysis of flavonoid aglycones

alone did not permit differentiation between different clones of a species or hybrid.

Previous work has established that the bud exudate of poplars consists of a complex mixture including aliphatic acids, substituted benzoic and phenolic acids and their esters, terpenoids and flavonoid aglycones (CHADENSON *et al.*, 1971; GREENAWAY *et al.*, 1987; NAGY *et al.*, 1986; PAPAY *et al.*, 1986; WOLLENWEBER, 1975; WOLLENWEBER and EGGER, 1971; WOLLENWEBER and WEBER, 1973). We have separated this complex mixture of compounds from buds of *P. × euramericana* 'Robusta' in a capillary gas-chromatographic column to produce a complex chromatogram (GREENAWAY *et al.*, 1987), which can be regarded as a 'fingerprint'. Preliminary work (unpublished) indicated that these 'fingerprints' could be used to differentiate between poplar species. We here report the apparent stability of bud exudate composition within a poplar clone and the use of bud exudate composition to differentiate between morphologically similar clones of *P. × interamericana* VAN BROEKHUIZEN.

Materials and Methods

Plant material

Bud exudate was obtained from 25-year-old trees of a single clone of *P. × euramericana* 'Robusta' at Buckland, Oxon., U.K. and from nursery stock of the *P. × interamericana* clones 'Barn', 'Beaupré', 'Boelare', 'Donk', 'Hunnegem', 'Raspalje', 'Rap' and 'Unal' at Alice Holt Lodge, Farnham, U.K. *P. × euramericana* 'Robusta' is a male cultivar which arose spontaneously in a nursery near Metz in the north east of France in about 1890. The seed parent

Table 1. — Parental plants and origin of *P. × interamericana* clones.

clone	female parent	male parent	source
'Barn'	<i>P. deltoides</i>	<i>P. trichocarpa</i>	Dorschkamp ²
'Beaupré'	<i>P. trichocarpa</i> 'Fritzi Pauley'	<i>P. deltoides</i> ¹	Geraardsbergen ³
'Boelare'	<i>P. trichocarpa</i> 'Fritzi Pauley'	<i>P. deltoides</i> ¹	Geraardsbergen
'Donk'	<i>P. deltoides</i>	<i>P. trichocarpa</i>	Dorschkamp
'Hunnegem'	<i>P. trichocarpa</i> 'Fritzi Pauley'	<i>P. deltoides</i> ¹	Geraardsbergen
'Raspalje'	<i>P. trichocarpa</i> 'Fritzi Pauley'	<i>P. deltoides</i> ¹	Geraardsbergen
'Rap'	<i>P. trichocarpa</i>	<i>P. deltoides</i>	Dorschkamp
'Unal'	<i>P. trichocarpa</i> 'Fritzi Pauley'	<i>P. deltoides</i> ¹	Geraardsbergen

¹) This was an intraspecific hybrid bred from Iowa and Missouri provenances.

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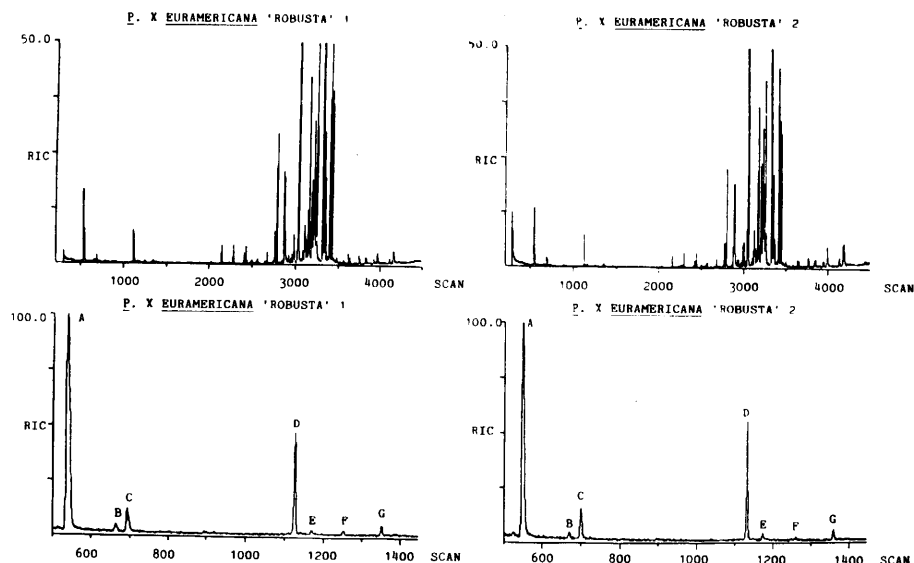


Figure 1. — Reconstructed ion chromatograms (RIC) of bud exudate of two trees of the same clone of *P. × euramericana* 'Robusta'. The upper chromatograms show complete runs, 300 to 4500 scans (retention time in methylene units 11 to 35.5). Methylene units (MU) are defined by DALGLIESH *et al.* (1966). The lower chromatograms, 500 to 1450 scans (MU 12 to MU 16.5) represent an area which is helpful in distinguishing *P. × interamericana* clones. The y axis is in relative intensity units. Peaks numbered are identified as (A) benzoic acid; (B) glycerol; (C) succinic acid; (D) malic acid; (E) trans cinnamic acid; (F) terpenoid resembling caryophyllene epoxide; (G) protocatechuic acid.

was *P. deltoides* MARSH ssp. *angulata*; the pollen parent is unknown but may be *P. nigra* L. 'Plantierensis'. The origins of the *P. × interamericana* clones are shown in table 1. Hereafter cultivar names only will be used.

Chemicals

Bis-(trimethylsilyl)trifluoroacetamide (BSTFA) including 1% trimethylchlorosilane (TMCS) was obtained from Sigma Chemical Co. Ltd., Dorset, U.K.

Sample preparation

Exudate from buds was obtained by dipping 2–4 buds in 1 ml freshly distilled ether in a 5 ml screw-top conical glass tube for 10 seconds. The ether was evaporated under a stream of N₂ and the residual material briefly freeze dried (5 min) to remove residual water. After addition of 50 μl pyridine and 100 μl BSTFA (inc. 1% TCMS) the tube was sealed and heated for 30 min at 100°C to produce trimethylsilyl (TMS) derivatives for gas chromatography.

Gas chromatography/mass spectrometry

The derivatized samples were separated and analysed in a Finnigan 1020 automated gas chromatograph/mass spectrometer (GC/MS) system (incorporating a Data General Nova 3 computer); the GC system was fitted with a 50 m, 0.3 mm internal diameter Thames Chromatography silica column, coated with 0.5 micron bonded phase OV1, and a splitless injector with a flush 30 seconds after sample injection to remove residual gases. The end of the column was introduced directly into the mass spectrometer analyser chamber. The system was operated under the following conditions: helium pressure 20 lbs/in²; injector temperature 310°C; GC temperature 85°C to 310°C at 3°C per min. The mass spectrometer was set to scan 40–650 atomic mass units (AMU) per nominal second with an ionizing voltage of 70 eV. The filament was switched on 250 seconds after the injection of the sample (0.3 μl to 0.5 μl) into the GC.

Results and Discussion

Exudate from buds of five 25-year-old trees of 'Robusta' were analysed by GC/MS. The total ion mass chromatograms were very similar (Fig. 1). There was little difference between axillary and apical buds or between buds from fast growing young shoots (morphologically typical of 'Robusta') and slower growing older shoots (morphologically typical of *P. nigra*). KLIMCZAK *et al.* (1972) noted that phenolic acid composition of poplar buds did not change during the vegetative period and we also found bud exudate composition to be stable during this period.

Bud exudate from the morphologically similar clones 'Barn', 'Beaupré', 'Boelare', 'Donk', 'Hunnegeg', 'Raspalje', 'Rap' and 'Unal' were then analysed and noticeable differences in the composition of the bud exudate were found (Fig. 2). Subsequent tests demonstrated that unnamed clonal material of *P. × interamericana* could be correctly identified by GC analysis of bud exudate alone. These tests were conducted at a single site, Alice Holt Lodge, and we have not yet assessed the variation in bud exudate composition which may result from growth at different sites.

Variations in some peaks occur due to the relative instability on a GC column of the TMS derivatives of the compounds they represent. In particular the polyhydroxy flavonoid aglycones are likely to be unstable as TMS derivatives (GREENAWAY *et al.*, 1987). Comparison of a portion of the chromatogram which does not contain these relatively unstable compounds may well provide the most reliable identification of the clones and preliminary analyses suggest the region of 500 to 1400 scans (12 MU - 16 MU: DALGLIESH *et al.*, 1966) could be a diagnostic region (Fig. 3).

The chromatograms (ie. 'fingerprints') which are produced will be affected by the quality of the chromatographic equipment, and especially by the condition of the GC column. However we would expect chromatograms

produced on one instrument to be comparable with others produced either on the same instrument or on other instruments fitted with a similar quality GC column.

Our results indicate that gas-chromatographic 'fingerprinting' of bud exudate will be sufficient to distinguish reliably between clones. In cases where GC 'fingerprinting' alone does not provide a clear answer, this technique combined with electrophoretic analysis of enzyme patterns (ANDERSON and BOWMAN, 1979; KIM and CHUNG, 1974; GUZINA, 1974) should provide an unambiguous identification of clonal material.

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In memoriam

JOHN JOBLING died suddenly whilst this paper was in preparation. We shall sadly miss his bubbling enthusiasm and kind guidance in all matters concerning poplars.

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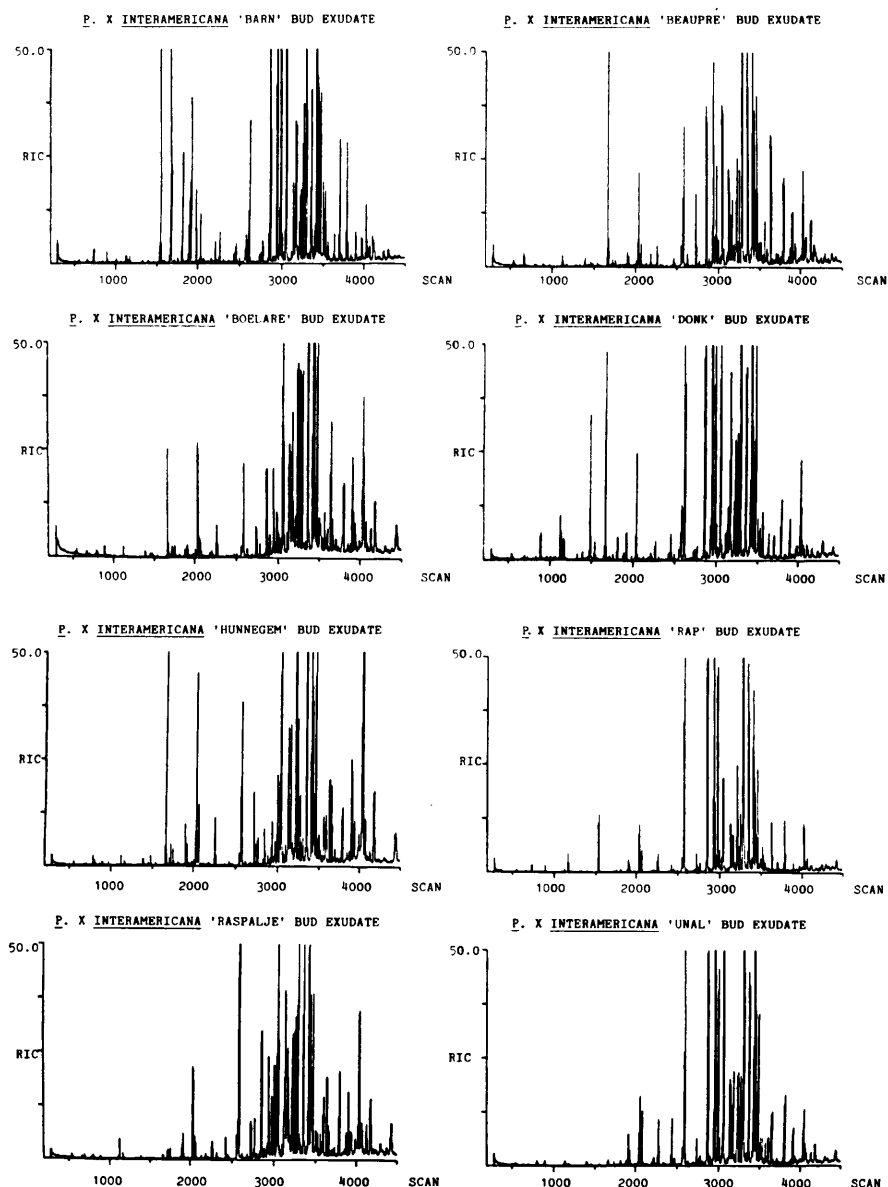


Figure 2. — Reconstructed ion chromatograms, 300 to 4500 scans (MU 11 to MU 35.5) of morphologically similar clones of *P. × interamericana*.

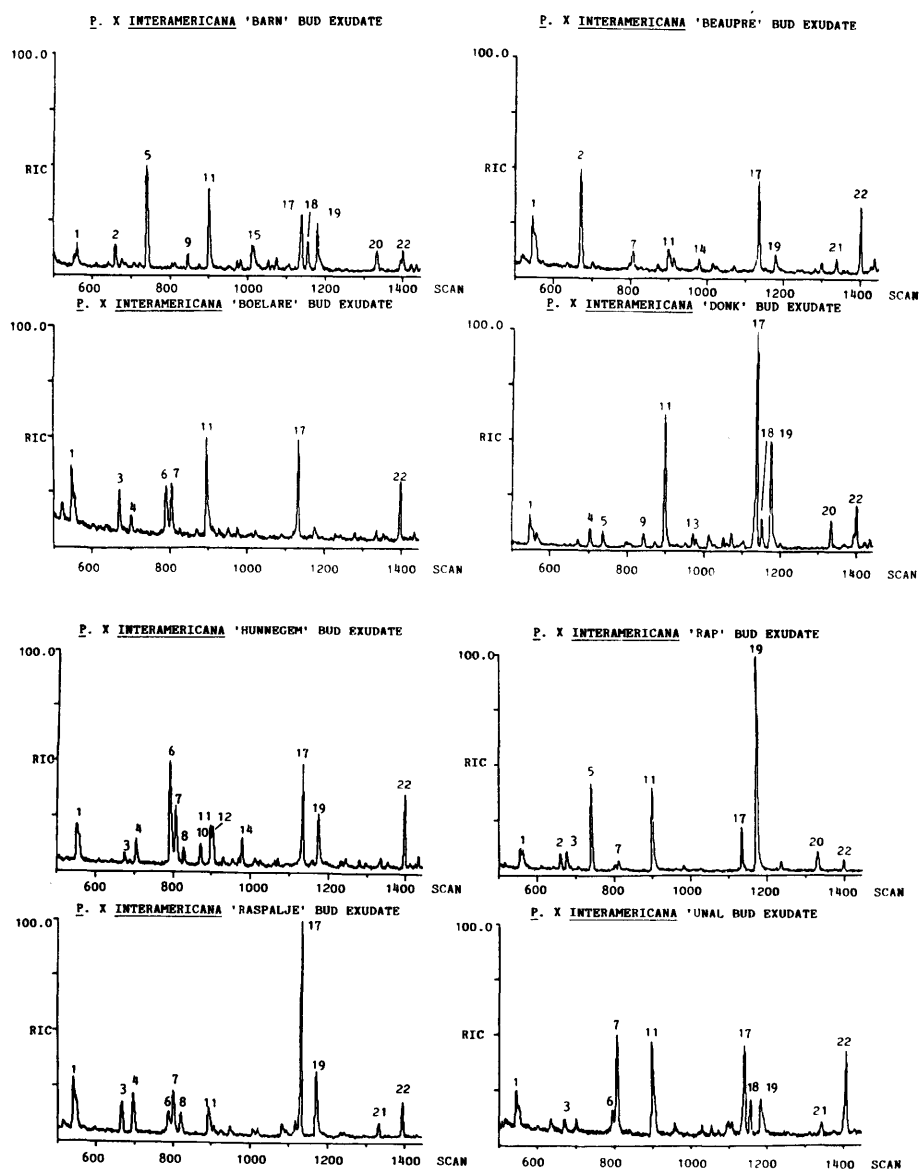


Figure 3. — Reconstructed ion chromatograms, 500 to 1450 scans (MU 12 to MU 16.5) of morphologically similar *P. × interamericana* clones: Peaks are identified as: (1) benzoic acid; (2) unknown; (3) glycerol; (4) succinic acid; (5) α -terpineol; (6) p-hydroxybenzaldehyde; (7) nonanoic acid; (8) threonic acid lactone (from threonic acid); (9) copaene; (10) bergamottene; (11) hydrocinnamic acid; (12) hydroquinone; (13) bergamottene; (14) resembles β -farnesene; (15) p-hydroxyacetophenone; (16) terpenoid resembling farnesene; (17) malic acid; (18) δ -cadinene; (19) trans cinnamic acid; (20) unknown; (21) threonic acid (probably from ascorbic acid); (22) p-hydroxybenzoic acid: Terpenoids are identified by mass spectra and MU retention time but have not been confirmed by cochromatography with authentic standards nor by chromatography on GC columns of different polarity.

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Reduction in Levels of Inbreeding in a Seed Orchard of *Eucalyptus regnans* F. Muell. compared with natural Populations

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Summary

The 1983 seed crop of a *Eucalyptus regnans* seed orchard was assayed for allozyme genotypes at 10 loci to determine multilocus estimates of the mating system parameters. Outcrossing was high with an overall estimate for the whole orchard of 91% at the germinated seedling stage. However, both for the overall seed orchard and the individual blocks, estimates showed significant departure from random mating ($t = 1.0$). There was also significant variation in outcrossing between the 29 open pollinated families sampled. The level of outcrossing was significantly higher in the seed orchard ($t = 0.91$) compared to a nearby natural population ($t = 0.74$). This increase in outcrossing could have been due to a reduction in neighbourhood inbreeding, or differences in the size and density of trees and hence fecundity. This reduction in inbreeding with the use of seed orchards in eucalypt breeding programs could result in significant genetic gains for characters such as growth rate.

Key words: Eucalypts, seed orchards, mating system, outcrossing rates.

Introduction

In natural populations of eucalypts a significant fraction of the viable seed originates from inbreeding. A mixed mating system, predominantly outcrossing but with significant inbreeding, has been shown to be common to a number of species (MORAN and BELL, 1983). For instance, in a population of *E. delegatensis* R. T. BAK. the level of outcrossing was 77% with a significant amount of inbreeding (23%) (MORAN and BROWN, 1980). This inbreeding may be largely due to mating between relatives in neighbourhoods within the population. The markedly detrimental effects of selfing on the growth, seed set and other characters in eucalyptus have been well documented and currently the evidence suggests that the mixed mating system is maintained under natural conditions by selection against inbreds through self-thinning of the population (HODGSON, 1976; ELDRIDGE and GRIFFIN, 1983; GRIFFIN *et al.*, 1987).

In Australia, eucalypt breeding programs are at an early stage of development. Current strategy calls for the commercial production of improved seed for plantations from selected superior families in seedling seed orchards. A similar strategy in conifers appears to be soundly based because the levels of selfing in both seed orchards and natural populations has been found to be less than 10% (MORAN *et al.*, 1980; SHAW and ALLARD, 1982; FREIDMANN and

ADAMS, 1985; RIIFLAND and EL-KASSABY, 1985; NEALE and ADAMS, 1985; MUONA and HARJU, 1989). In effect, in conifer orchards, the seed crops can potentially reflect the genetic superiority of the orchard families to non-selected stock. In comparison, such gains may not be realised in eucalypts if the outcrossing rates in seed orchards mirror that in natural populations and significant selfing occurs. However, differences in outcrossing rates between seed orchards and natural populations could occur if the assumptions underlying the mixed mating model (CLEGG, 1980), on which the estimation procedure is based, are met in one but not both places. For instance, one of the assumptions of the model is that pollen gene frequencies are uniform over maternal plants within a population (BROWN *et al.*, 1985). In particular, the pollen involved in each outcrossing event is assumed to come randomly from a homogenous pollen pool encompassing the whole population. In fact spatial heterogeneity in the pollen pool could be expected if neighbourhoods of related individuals occur in a natural population. As a result estimates of the outcrossing rate may be lower since both selfing and neighbourhood inbreeding could occur.

There are currently seed orchards of 5 eucalypt species in Australia. For one of these, *E. regnans* F. MUELL., which is one of the three major timber species in southeastern Australia, genetic improvement commenced in Victoria in 1970 (ELDRIDGE, 1971). In this paper we report estimates of the mating system parameters in a 13 year old seedling seed orchard of this species and compare them with those from a nearby natural population.

Materials and Methods

An open-pollinated seedling seed orchard was established at Silver Creek near Thorpdale in Gippsland, Victoria in 1970 by APM Forests Pty Ltd (CAMERON and KUBE, 1983). Seed was collected in 1969 from plus trees selected in natural populations in the Traralgon Creek area of southern Gippsland. The orchard contained 40 families laid out in a square of 4×4 (= 16) randomised complete blocks over an area of 2.4 ha. Initially each family was represented within each block by a plot of five trees in a closely spaced line with 0.6 m between trees. These family plots, spaced at $6 \text{ m} \times 6 \text{ m}$ centres, were culled to one vigorous tree during the first 3 years.

In April 1983 seed was collected from all trees that had seed in the four central blocks of the orchard. Since