Relation of "Antimicrobial" Compounds Present in Poplar Bud Exudate to Disease Resistance by Poplars

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Bud exudates from 9 poplars showing a range of resistance to bacterial canker and poplar rust were analyzed by gas chromatography-mass spectrometry and the components of the bud exudate identified. Those compounds previously reported in the literature to have notable antimicrobial activity were listed and the quantities present in the bud exudates of the specimens analyzed were compared. No correlation was observed between the amounts of these compounds and the known resistance of the poplars to bacterial canker or rusts.

Introduction

The bud exudate of poplars is a complex mixture of compounds which contains many phenolic compounds. The composition is characteristic of the bud exudate of a species [1] and even of a clone [2]. This bud exudate is collected by bees and incorporated into propolis, a material used as a general purpose glue for their hives. Propolis has long attracted attention as a herbal medicine [3], as have the dried buds of poplars [4]. Several phenolic compounds in propolis have been particularly identified as having antimicrobial activities (see Table I) and these compounds are also present in the bud exudate of poplars. A number of serious pathogens of poplars invade the tree *via* the buds and leaves, and we here investigate whether the antimicrobial phenolic compounds identified in propolis and also present in bud exudates influence the ability of several of these pathogens to invade poplar trees.

The pathogens investigated were the fungi *Melampsora alii-populina* Kleb., *M. larici-populina* Kleb. and *M. medusae* Thüm, which cause leaf rust in poplars, and the bacteria *Xanthomonas populi* Ridé, which causes bacterial canker [5].

Current breeding programmes take several years to establish whether resistance to rust and bacterial canker is present in a poplar clone [6]. If there were a correlation between resistance to pathogens and antimicrobial phenolic compounds present in poplar bud exudate then analysis of the bud exudate for phenolic composition should provide a rapid means of identifying resistant clones.

We examined two sets of trees for which the degree of resistance to the pathogens concerned was already known to see whether a correlation does exist; if it does then it should be possible to predict the resistance pattern of clones by analysis of their bud exudate.

Materials and Methods

Poplar bud exudates

Two series of poplars which showed a range of resistance to rust and bacterial canker were selected to examine the relationship between antimicrobial compounds in the bud exudate and disease resistance. Exudates were obtained from specimens of the progeny from *P. interamericana* Van Brockhuizen cross 78.025 (*P. deltoides* Marsh × *P. trichocarpa* Torr. and Gray) and *P. euramericana* (Dode) Guinier cross 87.001 (*P. deltoides* × *P. nigra* L.) produced at the Poplar Research Station, Geraardsbergen, Belgium. Within the progeny resulting from the two crosses (78.025 and 87.001) there is a range of resistance to leaf rust and to bacterial canker. The specimens selected for analysis are shown in Table II.

Reagents

Bis-(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) was obtained from Sigma (Poole, U.K.). Pyridine (Aristar) and ethyl acetate (Analar) were obtained from BDH Chemicals Ltd. (Poole, U.K.).

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Sample preparation

Bud exudate was collected by dipping 5–10 buds into a 50 ml beaker containing 3 ml ethyl acetate for 10 sec, the ethyl acetate extract was transferred to a screw top conical tube and the solvent removed by evaporation under a stream of N_2. The resulting extract was freeze-dried for 10–15 min to remove residual water. To produce trimethylsilyl (TMS) derivatives for GC, 50 µl pyridine and 100 µl BSTFA, containing 1% TMCS, was added to the tube which was then sealed and heated for 1 h at 100 °C.

Gas chromatography-mass spectrometry

The derivatized samples were separated and analyzed using a Finnigan 1020 automated GC-MS (incorporating a Data General Nova 3 computer). The GC system was fitted with a 25 m × 0.32 mm ID Thames Chromatography (Maidenhead, U.K.) silica column coated internally with 0.5 µm of immobilized polydimethylsiloxane and a splitless injector with a flush 30 sec after sample introduction to remove residual gases. The end of the column was introduced directly into the analyzer chamber of the mass spectrometer.

The following operating conditions were used: He pressure 20 lb/in^2; injector temperature 310 °C; GC temperature 85–310 °C at 3 °C min^{-1}. The mass spectrometer was set to scan 40-650 AMU per nominal second with an ionizing voltage of 70 eV. The filament was switched on 250 sec after injection of the sample into the GC.

Identification of compounds

Was as previously described [7].

Results

Previous work with propolis has identified eight phenolic compounds, present in poplar exudates, which have notable antimicrobial activity (Table I). The percentage of the total ion current (TIC) recorded by GC-MS analysis for these compounds in the 9 specimens analyzed is listed in Table II.

Benzyl caffeate, one of the eight compounds listed in Table I, was not identified in any of the clones analyzed here (Table II) although it does occur in some other *P. euramericana* clones [7].

Table I.

Compound	Reference
Caffeic acid	8 13 15
Benzyl caffeate	9
Phenyl ethyl caffeate	9
Cinnamyl caffeate	9
Pinocembrin	10 11 12 16
Galangin	12 14
Ferulic acid	13
Pinobanksin-3-acetate	17

Table II. Resistance gradings to rust and bacterial canker and percentage of antimicrobial compounds.

	% total ion current[1]								
	P. interamericana crosses 78.025				*P. euramericana* crosses 87.001				
Reference no.[2]	249	367	421	414	3	53	44	58	167
Resistance to rust[3]	0	0	0	0	0	3	3	4	5
Resistance to bacterial canker[4]	0	2.5	3.5	4.5	2	2	2	3	2
Compound									
Caffeic acid	–	–	–	–	1.0	1.3	1.5	1.1	0.5
Ferulic acid	–	–	–	–	0.1	0.1	0.1	0.1	<0.1
Pinobanksin-3-acetate	2.3	8.7	4.1	1.5	6.4	16.2	11.1	7.1	3.7
Pinocembrin	<0.1	0.4	0.4	0.2	25.2	16.4	14.8	18.6	30.8
Benzyl caffeate	–	–	–	–	–	–	–	–	–
Phenylethyl caffeate	–	–	–	–	0.8	2.7	1.1	2.7	1.4
Cinnamyl caffeate	–	–	–	–	–	0.2	0.1	0.2	<0.1
Galangin	2.1	3.3	3.3	1.6	20.3	14.2	22.7	19.4	16.2

[1] The ion current generated depends on the characteristics of the compounds concerned and is not a true quantitation [7].
[2] The reference numbers of the clones are those used at Geraardsbergen.
[3] Rust: −0 to 5, where 0 = very resistant and 5 = very susceptible.
[4] Bacterial canker: −0 to 5, where 0 = very resistant and 5 = very susceptible, using a girdling index [18].

The *P. euramericana* clones analyzed here contain in their bud exudates four listed compounds, caffeic acid (0.5–1.5% TIC), ferulic acid (<0.1–0.1% TIC), benzyl caffeate (0.0–0.2%) and phenylethyl caffeate (0.8–2.7% TIC) which are absent from the *P. interamericana* clones (Table II). The remaining three compounds, pinobanksin-3-acetate (= 5,7-dihydroxy-3-acetyloxyflavanone), pinocembrin (= 5,7-dihydroxyflavanone) and galangin (= 3,5,7-trihydroxyflavone) are present both in the *P. euramericana* clones (3.7–16.2%; 14.8–30.8% and 14.2–22.7% TIC respectively) and in the *P. interamericana* clones (1.5–8.7%; <0.1–0.4% and 1.6–3.3% TIC respectively).

Whereas in Table II we list only those compounds previously identified as having notable antimicrobial activity (Table I) it is possible that these compounds are simply representatives of classes of compound which possess antimicrobial activity. In Table III we therefore list the total percentage TIC of all the phenylpropenoic acids and their esters identified (a total of 23 compounds), of all the flavones (a total of 12 compounds) and of all the flavanones (a total of 11 compounds). The phenylpropenoic acids and their esters were present in all the bud exudates of all four *P. interamericana* clones (3.4–7.7% TIC) and all five *P. euramericana* clones (6.5–19.1%). Within this class the *P. interamericana* clones 249, 367, 421 and 414 contained 1.2%, 2.8%, 2.4% and 3.1% TIC of *p*-coumaric acid respectively, whereas the *P. euramericana* only had traces of *p*-coumaric acid. The flavones and flavanones were present in all the *P. interamericana* clones (2.8–4.9% TIC) and (4.0–11.3% TIC) respectively and all the *P. euramericana* clones (26.3–36.2% TIC and 33.9–53.2% TIC) respectively.

Discussion

In the case of resistance to rust the four *P. interamericana* clones showed a similar high resistance to rust, whereas the five *P. euramericana* clones showed a complete range of resistance, from clone 3, which is very resistant, to clone 167, which is very susceptible (Table II and III). If the eight previously identified antimicrobial compounds (Table I) are involved in resistance then a correlation between the % TIC of the compound and the degree of resistance shown would be expected. We see no such correlation (Table II), nor is there a correlation with the total % TIC of the major classes of antimicrobial compounds (Table III). We therefore conclude that neither the compounds listed in Table I, nor indeed the major classes of phenolics are likely to be specifically involved in the resistance of poplars to the *Melampsora* fungi responsible for rusts.

In the case of resistance to bacterial canker, the *P. euramericana* clones show similar moderate resistance, whereas the *P. interamericana* clones show a range of resistance from clone 249, which is very resistant, to clone 414, which is very susceptible (Table II and III). If the compounds are indeed involved in resistance a correlation should be present between the % TIC of antimicrobial compounds and the degree of resistance. There is no such correlation with the eight antimicrobial com-

Table III. Summary of compound groups associated with antimicrobial activity (Table I).

	% total ion current[1]								
	P. interamericana crosses 78.025				*P. euramericana* crosses 87.001				
Reference no.[2]	249	367	421	414	3	53	44	58	167
Resistance to rust[3]	0	0	0	0	0	3	3	4	5
Resistance to bacterial canker[4]	0	2.5	3.5	4.5	2	2	2	3	2
Compound groups									
Phenylpropenoic acids and their esters	3.4	5.6	3.5	7.7	6.5	14.2	19.1	10.6	7.7
Flavones	3.4	4.9	3.9	2.8	36.2	28.2	31.2	34.3	26.3
Flavanones	4.6	11.3	10.1	4.0	47.8	43.5	35.9	38.8	53.2

[1] The ion current generated depends on the characteristics of the compounds concerned and is not a true quantitation [7].
[2] The reference numbers of the clones are those used at Geraardsbergen.
[3] Rust: –0 to 5, where 0 = very resistant and 5 = very susceptible.
[4] Bacterial canker: –0 to 5, where 0 = very resistant and 5 = very susceptible, using a girdling index [18].

pounds specifically investigated (Table II); nevertheless there appears to be a slight correlation with the total phenylpropenoic acids and their esters present in the bud exudate of the *P. interamericana* clones (Table III). It seems improbable that the relatively small concentration differences seen could materially affect disease resistance. We therefore conclude that these classes of compounds are unlikely to be directly involved in resistance to bacterial canker. We do however note one peculiarity; *p*-coumaric acid is present at a low level (1.2% TIC) in the very resistant clone 249, intermediate levels in clones 367 and 421 (2.8% and 2.4% TIC respectively) which are of intermediate resistance and at its highest level (3.1% TIC) in clone 414 which is the most susceptible one. We speculate that levels of *p*-coumaric acid may be reduced in resistant clones by its further metabolism to very active antimicrobial metabolites, such as phytoalexins.

It is tempting to assume that because compounds exist in poplar bud exudate which have known antimicrobial activity *in vitro,* then this activity will directly affect resistance to pathogens *in vivo*. Our results however indicate that this is not the case.

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[1] E. Wollenweber, Biochem. Syst. Ecol. **3,** 35–45 (1975).
[2] W. Greenaway, J. Jobling, and T. Scaysbrook, Silvae Genetica **38,** 28–32 (1989).
[3] R. Hill, Propolis, the natural antibiotic, Wellingborough, Thorsons Publishers, 1977.
[4] F. R. Whatley, W. Greenaway, and J. May, Z. Naturforsch. **44c,** 353–356 (1989).
[5] Breeding poplars for disease resistance, FAO Forestry Ser. **56,** (B. A. Thielges, ed.), Rome 1985.
[6] V. Steenackers, S. Strobl, and M. Steenackers, Forestry, Forestry Biomass and Biomass Conversion, Elsevier Applied Science Publishers, London and New York 1990.
[7] W. Greenaway, T. Scaysbrook, and F. R. Whatley, Proc. R. Soc. Lond. B **232,** 249–272 (1987).
[8] B. König and J. H. Dustmann, Naturwissenschaften **72,** 659–661 (1985).
[9] E. M. Schneidewind, A. Büge, H. Kala, J. Metzner, and A. Zschunke, Pharmazie **34,** 103–106 (1979).
[10] V. R. Villanueva, M. Barbier, M. Gonnet, and P. Lavie, Annals Int. Pasteur, Paris **118,** 84–87 (1970).
[11] L. Shain and J. B. Miller, Phytopathology **72,** 877–880 (1982).
[12] S. Pepeljnjak, I. Jalsenjak, and D. Maysinger, Pharmazie **40,** 122–123 (1985).
[13] H. Raun, C. Andary, G. Kowács, and P. Mølgaard, Biochemical Systematics and Ecology **17,** 175–184 (1989).
[14] V. R. Villanueva, D. Bogdanovsky, M. Barbier, M. Gonnet, and P. Lavie, Annals Inst. Pasteur, Paris **106,** 292–302.
[15] L. A. Schaal and G. Johnson, Phytopathology **45,** 627–628 (1955).
[16] M. Miyakado, T. Karto, N. Ohno, and T. Mabry, Phytochemistry **15,** 846 (1976).
[17] E. L. Ghisalberti, Bee World **60,** 59–84 (1979).
[18] M. Ridé, Pro. Mtg. of Disease Working Group, Casale Monferrato. FAO/IPC/MAL 23, 18–43 (1963).