Root exudates of *Banksia* species from different habitats – a genus-wide comparison

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Abstract

The genus *Banksia* is a uniquely Australian plant group. *Banksias* dominate the physiognomy and ecology of several Australian plant communities. Flowers and fruits of several species are successful export products. The physiology of nutrient uptake is of great importance for this genus, particularly since the soils on which *Banksias* occur are extremely low in nutrients.

All *Banksias* possess proteoid (cluster) roots that exude a range of carboxylates into the rhizosphere. Carboxylates act to enhance the availability of nutrients, particularly phosphorus, but the efficiency of different carboxylates varies with soil type. We examined the hypothesis that *Banksia* species with different soil preferences differ in the amount and composition of rhizosphere carboxylates.

Our data show that, when grown in a standardised substrate, the 57 *Banksia* species studied, exude roughly similar carboxylates into their rhizosphere, predominantly citrate. We found no evidence for phylogenetically determined differences, or correlations with species' soil preferences. This may indicate that the conditions in the topsoil and litter layer in all *Banksia* habitats are sufficiently similar for these carboxylates to be effective. Alternatively, species differences were not expressed in the single substrate that was used. Ongoing research explores the ability of *Banksias* to adjust exudation patterns to contrasting soils, and the impact on growth and nutrient uptake.

Keywords

Banksia – root exudates - carboxylates – rhizosphere – soils - phylogeny

Introduction

Roots of several native and cultivated species exude large amounts of carboxylates, particularly when growing in soils with low concentrations of available phosphorus. Very high exudate concentrations are found in the rhizosphere of proteoid (cluster) roots, for example in the genus *Banksia* (Grierson 1992, Roelofs et al. 2001).

Carboxylates enhance phosphorus acquisition by mobilising adsorbed and complexed forms of this essential nutrient, but there is important variation in the effectiveness of different carboxylates (Jones 1998).. At the same time, there are biochemical and physiological implications of exuding one or another carboxylate, and it is therefore likely that the costs and benefits of different exudation spectra depend on growing conditions. The current paper explores the possibility that, when all are grown in the same medium, different species of *Banksia* have intrinsically different exudation spectra, depending on characteristics of the soil types they are adapted to. Knowledge of root exudates and their effect on plant nutrition are essential for an understanding of *Banksia* distribution patterns (e.g., to support habitat conservation), and for optimising nutrition of plantation-grown *Banksias* for floriculture.

Banksia is a genus that is particularly species-rich in the south-west of Australia where it occurs on a range of soil types. Within this group there is great diversity in growth form (trees to prostrate shrubs), distribution range (widespread to rare), climate preference, fire-tolerance and other ecological traits (Lamont & Connell 1996; Lamont & Markey 1995; Richardson et al. 1995). We sampled seeds of 57 species from natural populations, grew plants of all species in the same conditions for three years, and sampled rhizosphere carboxylates. The current paper explores whether variation in carboxylate spectra is correlated with species' habitat preferences or, alternatively, defined by phylogenetic affinities.

Methods

Sampling and growing of plants

This study used a total of 57 *Banksia* species (Appendix A). For each species we used a single population of a representative habitat of the species. At the sampling location we obtained mature seed cones from three plants. Samples were also taken, for each plant, of green leaves, recently shed leaves, the litter layer and topsoil. Seeds were extracted from cones and germinated in potting mix. Five plants per species were planted individually into 3-L pots filled with river sand. Plants were kept in the greenhouse, watered frequently and provided with all essential nutrients except phosphorus. After two to three years in the greenhouse, plants were transferred outdoors after repotting in 5-L pots. Repotting was done without disturbing the root system, by placing a potting mix as a layer around the river sand.

Sampling and extraction of cluster roots

Cluster roots were sampled from the potting mix layer. Selection of clusters, three per plant, was based on their developmental stage, judged from branching, colour and presence of root hairs. Clusters (roots plus the soil they bind) were cut from the root system and loose soil was shaken off, minimising tissue damage. Single clusters (on average 5.5 g fresh weight) were extracted in 10-15 mL 0.2 mM $CaCl_2$ solution by gentle shaking. The pH of the extract was determined, and a 1 ml subsample was filtered, acidified and frozen for carboxylate analysis.

Carboxylate analysis

Carboxylates were analysed by HPLC (method described in detail in Cawthray 2003).

Data analysis

Results of carboxylate analyses are expressed in molar concentrations per unit cluster fresh weight (roots plus adhering soil, as sampled). Despite careful selection of clusters based on developmental traits, exudation activity of clusters was found to be highly variable due to the very short duration of the "exudation burst" (Shane et al. 2004). The data shown in this report are based on three samples per species, being the three highest total rhizosphere carboxylate concentrations from three different plants.

Results

Rhizospheres of all *Banksia* species contained carboxylates, in particular citrate, iso-citrate, malate and cis- and trans-aconitate. Trace amounts of acetate, lactate and maleate were found very infrequently and may have been contaminations. Succinate and malonate were never found. Oxalate could not be quantified by our HPLC method, but gas chromatography on selected samples indicated that it was present in at least some *Banksia* rhizospheres.

Concentrations of carboxylates in the rhizosphere extracts were variable. Observations during sampling suggest that this variability may be largely attributed to the range of developmental stages of the cluster roots. Despite our efforts to standardise sampling, our samples probably included cluster roots that were not fully mature, or already senescent. Interestingly, the rhizosphere extracts also varied widely in pH, roughly between pH 2 and pH 6, with bulk soil at pH 5.4. This variation was linked with variation in carboxylates. Figure 1 shows that high concentrations of citrate coincided with low pH, whereas aconitate (sum of cis- and trans-aconitate) was more abundant in samples of higher pH. Since iso-citrate and malate behaved in a similar way as citrate, the highest total carboxylate concentrations were associated with low pH. We interpret these samples as belonging to clusters with peak exudation activity, and the following species comparisons are based on those samples (n=3 per species).

Rhizosphere chemistry was quite similar among the different *Banksia* species. All species showed all major carboxylates, as well as lowered rhizosphere pH. When arranged by phylogenetic group (following the classification of Thiele & Ladiges 1996), differences in rhizosphere chemistry were relatively small and without any obvious patterns (Figure 2). Similarly, eastern Australian species were not different from western Australian species (Figure 3).

There were no obvious differences in rhizosphere carboxylate composition between species with contrasting soil preferences. Accordingly, there was no justification for comprehensive soil analyses. The absence of a clear effect of native soil on rhizosphere chemistry is illustrated with three very closely-related species from the south-west of Western Australia (Figure 4). The soil preferences for these species are: sandy soils (seasonally wet or around lakes, swamps) for *B. littoralis* and *B. occidentalis*; heavy loamy gravelly soils for *B. seminuda*; granite outcrop sandy/rocky soil for *B. verticillata* (Taylor & Hopper 1988). Carboxylate spectra of these three species were not greatly different. Although the figure suggests that aconitate was absent from *B. littoralis* and *B. occidentalis*, this is due to the strict selection of samples

based on highest total amount of carboxylates. Other samples of these species did contain aconitate.

Carboxylate concentrations in rhizosphere extracts appeared to be somewhat lower, on average, for *Banksia* species from regions with low rainfall, compared to those from regions with higher rainfall. The comparison in Figure 5 is based on four high-rainfall species from the south and southwest of Western Australia, *B. praemorsa, B. quercifolia, B. seminuda* and *B verticillata*, and eight low-rainfall species from the lower-rainfall regions north and east of the high-rainfall zone, *B. ashbyi, B. benthamiana, B. elderiana, B. epica, B. laevigata, B. lindleyana, B sceptrum* and *B victoriae*.

Discussion

Our study demonstrates that contrasting soil preferences among species of the genus *Banksia* are not necessarily associated with differences in the amount and composition of exuded carboxylates. The similarity in rhizosphere chemistry within the genus is far more striking than small differences between species groups based on phylogeny, geography, soil or climatic factors (Figures 2-5). This finding suggests that the ability to exude carboxylates is a valuable trait for all *Banksias* in any of the soils where they occur. The tendency for high-rainfall species to exude more carboxylates (Figure 5) may be related to potentially greater leaching rates under high rainfall, requiring greater exudate production to maintain effective concentrations in the rhizosphere. Alternatively, high-rainfall species have inherently higher growth rates and metabolic activities, and faster resource uptake, associated with greater root exudation.

Our results do not exclude the possibility that important differences in rhizosphere chemistry do occur in the field. It must be noted that all plants in our experiment were grown in the same substrate. We conclude that all species behaved similarly on this standardised substrate, but we have not challenged species by growing them on a range of different substrates. Perhaps different soil preferences of species are expressed as different abilities to adjust carboxylate exudation rate and composition to specific needs on each soil type. Early results with *Banksia grandis* indicate that root exudation patterns do change in response to soil factors, e.g., levels of phosphate, or the presence of Al or Fe ions (Lambers et al. 2002).

The possibility exists that even in the field carboxylate exudation varies little across species. The cluster roots of all *Banksia* species are most prevalent in the topmost centimeters of soils, where they are in contact with decomposing organic matter. This micro-environment may be relatively similar across soils with quite contrasting mineralogy, texture and chemistry. Further studies are needed of rhizosphere chemistry of plants grown in different soils, to describe possibly adaptive responses to different conditions, in terms of exudation patterns and their effect on plant nutrient uptake.

In this study, where plants were not challenged by adverse soil conditions or competing plants, root exudation of all *Banksia* species was similar. For practical purposes, like floriculture and use of *Banksias* in gardens and parks, this probably indicates that rhizosphere chemistry does not impose limitations for any *Banksia* species

to be used when growing conditions are favourable. For effective conservation in native habitats, the interaction between roots and native soils is potentially much more critical.

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Appendix A

species		location	
aculeata	A.S.George	Cranbrook	WA
aemula	R.Br.	Stradbroke Island	QLD
ashbyi	Baker	Shark Bay	WA
attenuata	R.Br.	Swan	WA
baueri	R.Br.	Jerramungup	WA
baxteri	R.Br.	Albany	WA
benthamiana	C.A.Gardner	Dalwallinu	WA
brownii	R.Br.	Albany	WA
burdettii	Baker	Moora	WA
caleyi	R.Br.	Jerramungup	WA
candolleana	Meisn.	Dandaragan	WA
chamaephyton	A.S.George	Coorow	WA
coccinea	R.Br.	Cranbrook	WA
dryandroides	Sweet	Albany	WA
elderiana	F.Muell.&Tate	Ravensthorpe	WA
epica	A.S.George	Dundas	WA
gardneri var. gardneri	A.S.George	Cranbrook	WA
goodii	R.Br.	Albany	WA
grandis	Willd.	Murray	WA
grossa	A.S.George	Dandaragan	WA
hookeriana	Meisn.	Carnamah	WA
ilicifolia	R.Br.	Swan	WA
incana	A.S.George	Dandaragan	WA
integrifolia var. integrifolia	L.f.	Coogee	NSW
laevigata ssp. fuscolutea	Meisn.	Ravensthorpe	WA
lanata	A.S.George	Coorow	WA
laricina	C.A.Gardner	Gingin	WA
lemanniana	Meisn.	Jerramungup	WA
leptophylla	A.S.George	Dandaragan	WA
lindleyana	Meisn.	Shark Bay	WA
littoralis	R.Br.	Rockingham	WA
media	R.Br.	Jerramungup	WA
menziesii	R.Br.	Swan	WA
micrantha	A.S.George	Dandaragan	WA
nutans var. cernuella	R.Br.	Cranbrook	WA
oblongifolia	Cav.	Stradbroke Island	QLD
occidentalis	R.Br.	Esperance	WA
oligantha	A.S.George	Kojonup	WA
oreophila	A.S.George	Cranbrook	WA
petiolaris	F.Muell.	Esperance	WA
pilostylis	C.A.Gardner	Esperance	WA
praemorsa	Andrews	Albany	WA
prionotes	Lindl.	Dandaragan	WA
pulchella	R.Br.	Esperance	WA
quercifolia	R.Br.	Albany	WA

repens	Labill.	Cranbrook	WA
sceptrum	Meisn.	Northampton	WA
seminuda	(A.S.George)Rye	Waroona	WA
solandri	R.Br.	Cranbrook	WA
speciosa	R.Br.	Esperance	WA
sphaerocarpa var. sphaerocarpa	R.Br.	Dandaragan	WA
spinulosa var. collina	Sm.	Wallangarra	QLD
telmatiaea	A.S.George	Gosnells	WA
tricuspis	Meisn.	Dandaragan	WA
verticillata	R.Br.	Albany	WA
victoriae	Meisn.	Northampton	WA
violacea	C.A.Gardner	Jerramungup	WA

Figure legends

Figure 1.
Covariation of pH and the concentration of citrate and aconitate in the rhizosphere of cluster roots of 57 *Banksia* species. Data are for three cluster roots per plant, five plants per species. Total aconitate represents, on average, 95% trans-aconitate and 5% cis-aconitate.

Figure 2.
Mean rhizosphere carboxylate concentrations for seven phylogenetic species groups within the *Banksia* genus. Groups are series as defined in Thiele & Ladiges (1996; Aust Syst Bot 9: 661–773), and contain 2-12 species.

Figure 3.
Mean rhizosphere carboxylate concentrations for western and eastern Australian species of the *Banksia* genus.

Figure 4.
Rhizosphere carboxylate concentrations of four closely related *Banksia* species from south-western Australia with contrasting soil preferences.

Figure 5.
Mean rhizosphere carboxylate concentrations for selected species of the *Banksia* genus from high- and low-rainfall regions of south-western Australia.

Figure 1.

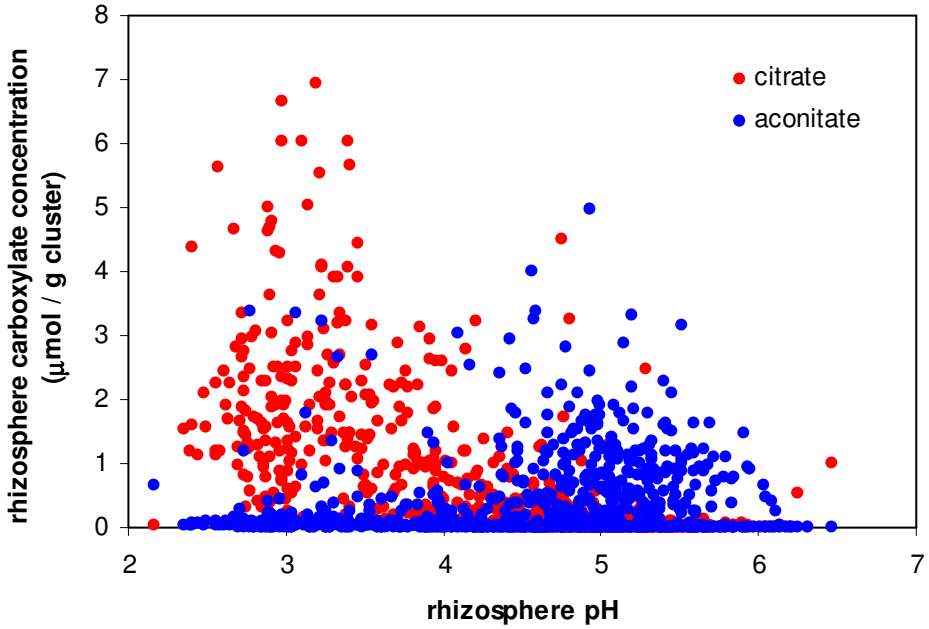
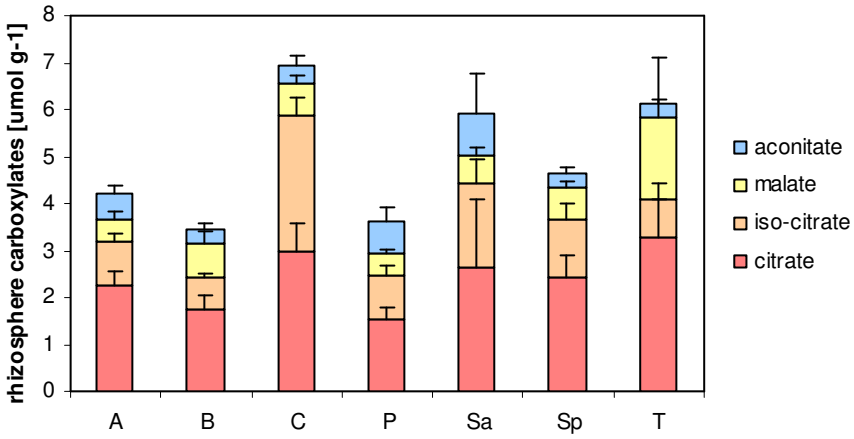

rhizosphere carboxylate concentration (µmol / g cluster)
rhizosphere pH
citrate
aconitate
0
1
2
3
4
5
6
7
8
2
3
4
5
6
7

Figure 2.



A	needle-leaved species
B	*prionotes* group
C	*media* group
P	prostrate species
Sa	*marginata* group
Sp	*littoralis* group
T	*caleyi* group

Figure 3.

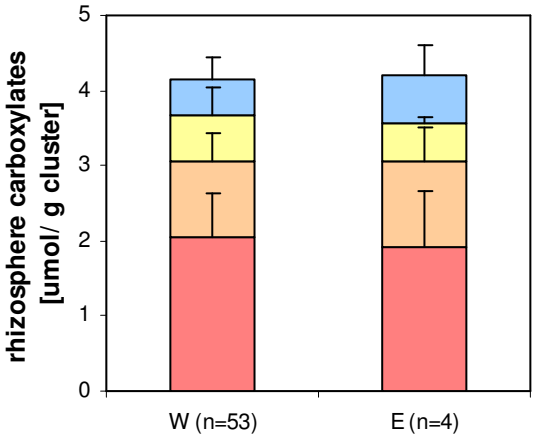


Figure 4.

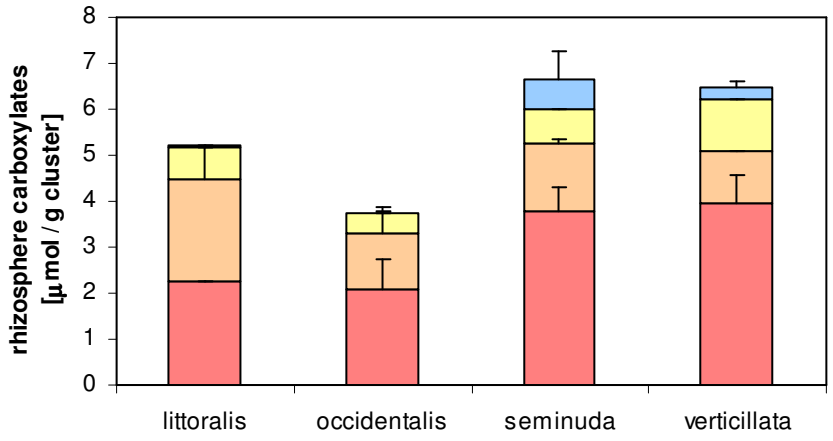

rhizosphere carboxylates
[µmol / g cluster]
8
7
6
5
4
3
2
1
0
littoralis
occidentalis
seminuda
verticillata

Figure 5.