

Short Communications / Kort Mededelings

Relative attractiveness of seeds of myrmecochorous Australian and South African plants to ants, and the chemical basis of this attraction

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We compared the responses of an indigenous and an exotic (South American) ant to seeds from exotic (Australian) and indigenous Cape myrmecochorous plants. Non-South African ants were more attracted to seeds of myrmecochorous species, than to non-myrmecochorous species from other continents. However, the chemical basis of this attraction was not due to oleic acid derivatives, as previously reported in the literature. This suggests that there is a broad spectrum of compounds that are attractive to ants. Our results indicate convergence (seeds from myrmecochorous plants are attractive to non-indigenous ants), but that there is no highly specific chemical convergence as a basis for this attraction.

Keywords: Convergence, fynbos, myrmecochory.

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Both Australian and South African mediterranean shrublands have high levels of myrmecochory (Milewski & Bond 1982), although this does not appear to be the case with other mediterranean shrublands (Le Maitre & Midgley 1992). We were interested in determining whether ant-dispersed plant species from one continent were equally attractive to ants from another continent; in other words, whether there was convergence in the chemistry of attractants. There has been no comparative investigation into this aspect of convergence.

Bond & Slingsby (1984) noted that the exotic South American ant, *Iridomyrmex humilis*, which has invaded some areas of the Cape fynbos, is attracted to seeds of indigenous myrmecochorous species. Also, Holmes (1990) concluded that seeds of Australian *Acacia* spp., including bird-dispersed species, are removed by ants in the Cape. Furthermore, Breytenbach (1988) observed that certain ants from the Karoo (a system largely without myrmecochorous plants), were attracted to seeds from myrmecochorous fynbos plants. Therefore, there is some qualitative evidence to suggest that convergence may exist; ants appear to respond to elaiosome-bearing seeds from many plant species. This may be because the chemical nature of ant attractants is similar, or that there is a large pool of chemical attractants found in elaiosomes generally.

There is a large amount of literature on the chemical basis of attraction (Brew *et al.* 1989; Handel & Beattie 1990; O'Dowd & Gill 1986; Kusmenoglu *et al.* 1989) and there is general acceptance that polar lipids derived from oleic acid, or those of ricinoleic acid, are most important in this regard (Brew *et al.* 1989, Marshall *et al.* 1979). Hughes & Westoby (1992) suggested that strong convergence must exist in the elaiosome chemistry of Australian myrmecochores, because they detected few differences in ant responses to a variety of elaiosomes from many

plant species. Beattie (1991) considered the chemical basis of ant attraction to be an outstanding problem in ant–plant research.

We asked: (i) do indigenous fynbos ants respond equally, in both a quantitative and behavioural way, to seeds with elaiosomes, from indigenous and non-indigenous species, (ii) do indigenous fynbos ants respond to oleic acid derivatives and (iii) is the exotic ant *Iridomyrmex* more strongly attracted to myrmecochorous seeds than non-myrmecochorous seeds but which nevertheless have a fleshy nutritious aril.

Experiments were conducted in fynbos vegetation in the Jonkershoek Valley near Stellenbosch (33°57'S, 18°55'E) and at Silvermine (34°5'S, 18°26'E) near Cape Town. We used the indigenous ant, *Anoplolepis custodiens*, because this is the dominant disperser of seeds in the SW Cape, and *Iridomyrmex humilis* as a local example of an exotic ant.

Various Australian *Acacia* species were used as examples of non-indigenous species with and without elaiosomes. Dispersal has been studied intensively in this genus and species have been assigned to various dispersal agents by O'Dowd & Gill (1986). They considered *A. cyclops* to be bird-dispersed, whereas *A. saligna* and *A. terminalis* were seen as ant dispersed. These three species now occur in the Cape and fresh seeds of these species were available. Our previous work has shown that *Anoplolepis* is most attracted to seeds from the Proteaceae genera of *Mimetes* and *Leucospermum* than to seeds from a range of other local species. Since *Leucospermum conocarpodendron* was seeding abundantly at both study sites at the time of this work, it was used as the local example of a highly attractive seed. Fresh weights of seeds and elaiosomes/arils respectively are as follows; *L. conocarpodendron* 143 mg and 23.6 mg, *A. cyclops* 32.3 mg and 12.3 mg, *A. saligna* 19.7 mg and 1.3 mg and *A. terminalis* 25.6 mg and 2.9 mg. *A. cyclops*, therefore, has the greatest relative investment in elaiosome/aril to seed weight of the study species.

Generally, we noted removal rates of seeds from mixed-species piles of seeds which were placed about 10–20 cm from the entrances of *Anoplolepis* or from the trails of *Iridomyrmex*. At the same time, we made more detailed observations on the recruiting behaviour of ants at Silvermine. For these observations we allocated behaviour of ants towards seeds to four classes: ignore (ant finds seed but walks on), antennate (ants 'feel' seeds with antennae for at least 5 sec), move (seed is moved > 5 cm by up to four ants) and recruit (seed is also moved > 5 cm but with the help of from five to 15 recruits). The last category was used in this study because it appeared that in some cases an ant, after discovering a seed, especially a large seed, left it temporarily to recruit assistants.

For trials with *Anoplolepis*, we considered a seed to be removed if it was taken more than 5 cm from the original pile during the 30-min observation period. For *Iridomyrmex*, a species only capable of transporting small seeds, we merely counted numbers of ants attracted to the various seeds over the 30-min study period.

We obtained oleic acid and some oleic acid derivatives (from Sigma Chemical Co., U.S.A.) and used seeds of a local non-myrmecochorous legume (*Virgilia divaricata*) for experimental manipulations. Seeds of this species are small enough (length 3.5 mm) to be transported by *Anoplolepis*. Removal rates of seeds of this species that had been soaked in oleic acid derivatives were compared with removal rates of untreated control seeds.

Finally, we removed polar lipids (the supposed attractants), as well as non-polar lipids and sugars/amino acids (relatively less attractive), from fresh seeds of *Leucospermum conocarpodendron* by leaving them in methanol, cyclohexane or water, respectively, for 12 hours (Brew *et al.* 1989). After soaking, seeds were patted dry on absorbent paper and allowed to dry fully in a cool

Table 1 Mean removal rates (s.d.) of seeds from five piles of five seeds of each species with each pile placed near a separate nest entrance of *Anoplolepis custodiens* at Silvermine and Jonkershoek. Asterisk indicates where a species was not used

	Jonkershoek	Silvermine
<i>L. conocarpodendron</i>	3.9 (0.5)	4.7 (0.6)
<i>A. cyclops</i>	1.6 (0.8)	2.0 (1.8)
<i>A. terminalis</i>	4.6 (0.5)	*
<i>A. saligna</i>	*	3.4 (1.6)

ventilated room for one hour. The response of ants to these seeds was compared with untreated controls.

Anoplolepis was strongly attracted to myrmecochorous Australian *Acacia* species, especially *A. terminalis*, but also to *A. saligna* (Tables 1 & 2). At both sites, significantly fewer seeds of *A. cyclops* were removed than of the more attractive myrmecochore (*t*-test, $P < 0.05$). *Iridomyrmex* was more strongly attracted to seeds of myrmecochorous plants (*L. conocarpodendron*) than to the non-myrmecochorous species *Acacia cyclops* (Table 3; *t*-test, $P < 0.01$). Also, there appeared to be differences in the behaviour of ants towards seeds of different species. In particular it appeared that *Anoplolepis* was recruited to seeds of *L. conocarpodendron* in relatively large numbers (Table 2). Dipping seeds of *Virgilia divaricata* in oleic acid and its derivatives did not enhance attractiveness (Table 4) relative to controls. Ants which encountered seeds soaked in various chemicals, did not respond in a negative (sudden retreat) or positive (move) fashion.

Soaking seeds in cyclohexane or methanol resulted in a loss of attraction in the ants when compared with controls (Table 5; *t*-test, $P < 0.01$), whereas soaking in water did not cause a reduction in removal rates.

The fleshy elaiosome or aril is far larger and has a greater lipid concentration in *A. cyclops* than in either *A. terminalis* or *A. saligna* (O'Dowd & Gill 1986). That the latter two species are more attractive to ants suggests that the ants are primarily responding to chemical attractants, rather than food value, of elaiosomes. Three lines of evidence suggest oleic acid derivatives are not the important chemical attractants in this system.

Firstly, *A. terminalis* is more attractive than *A. cyclops* (Table 2), despite the fact that levels of oleic acid derivatives are similar in these two species (O'Dowd & Gill 1986). Secondly, *L. cono-*

Table 2 Behaviour of *Anoplolepis custodiens* at seeds provided at Silvermine. Five piles of five seeds of each species were used, each pile was placed at a separate *Anoplolepis* nest entrance. Observations were made every 5 min over 30 min, trials were repeated five times and totals summed. Numbers are numbers of observations per category (Chi-square goodness of fit $P < 0.001$ for the myrmecochorous *L. conocarpodendron* and *A. saligna*)

Plant species	Ignore	Antennate	Move	Recruit
<i>L. conocarpodendron</i>	9	4	25	119
<i>A. saligna</i>	51	33	70	2
<i>A. cyclops</i>	66	43	33	28

Table 3 Mean numbers of ants attracted to seeds placed out at Jonkershoek. Five piles, each with five seeds of each species were placed out near the trails of *Iridomyrmex humilis*. Numbers are mean (s.d.) numbers of ants per species

<i>L. conocarpodendron</i>	4.3 (3.2)
<i>A. cyclops</i>	0.3 (0.2)

Table 4 Mean removal rates (s.d.) of treated and untreated seeds of *Virgilia divaricata* placed into five piles of five seeds, each pile placed around a separate nest entrance of *Anoplolepis* at Jonkershoek

Oleic acid	0.6 (1.2)
Ricinoleic acid	0 (0)
Triolein	1.0 (2.0)
Palmitic acid	0 (0)
Control (untreated)	1.0 (1.1)

carpodendron seeds lose their attractiveness when dipped in both cyclohexane and methanol (but not water). Only cyclohexane removes the polar-lipids, which are the postulated attractants to ants. However, because soaking seeds in both cyclohexane and methanol resulted in a decline in attraction in ants, it is possible that both polar and non-polar lipids are involved. Thirdly, ants did not respond to oleic acid, ricinoleic acid or triolein (Table 1), although these are some of the chemicals previously considered to be attractive to ants (Brew *et al.* 1989). The greater incidence of 'recruiting' behaviour of local ants to *L. conocarpodendron* seeds is probably a response to a strong chemical stimulus, rather than to the relatively large seed size, because a single *Anoplolepis* individual is capable of dragging these seeds (pers. obs.).

Nevertheless, our results indicate a degree of convergence in the attractiveness of myrmecochorous seeds to ants. This could be because the same, or similar compounds, occur in elaiosomes of myrmecochorous seeds, even those from different continents. Alternately, there may be a large group of compounds which ants find attractive. The convergence would be more precise in the former case, although the latter explanation appears more likely. This is because oleic acid derivatives have only been shown to be important in myrmecochory elsewhere. In conclusion, we predict that seeds of Cape myrmecochores, such as *L. conocarpoden-*

Table 5 Mean removal rates (s.d.) of seeds from five piles of five seeds of each treatment, each pile placed at a separate nest entrance of *Anoplolepis* at Jonkershoek. *Leucospermum conocarpodendron* seeds were used and were soaked in the following solvents for 12 hours

Cyclohexane	0.2 (0.4)
Methanol	0.2 (0.4)
Water	4.6 (0.5)
Control (untreated)	4.8 (0.4)

dron, will be very attractive to appropriate Australian ants but that the basis of attraction will not be oleic acid derivatives.

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Smithiozyma gen. nov. (Lipomycetaceae)

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The yeast species described as *Lipomyces japonicus* van der Walt, Smith, Yamada et Nakase differs significantly from the type species, *Lipomyces starkeyi* Lodder et Kreger-van Rij, in terms of ascospore topography, lipid composition and rRNA base sequence substitution. The species is consequently reclassified in the new, unispecific genus *Smithiozyma* as *Smithiozyma japonica*.

Die gisspesie wat as *Lipomyces japonicus* van der Walt, Smith, Yamada et Nakase beskryf is, toon beduidende verskille met die tipespesie, *Lipomyces starkeyi* Lodder et Kreger-van Rij, ten opsigte van askosporotopografie, lipiedsamestelling en rRNS basissekwensie-substitusie. Die spesie is gevolglik in die nuwe, monotipiese genus *Smithiozyma* as *Smithiozyma japonica*, herklassifiseer.

Keywords: Lipomycetaceae, *Smithiozyma japonica*

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Smith *et al.* (1995), by transferring *Lipomyces anomalus* Babjeva et Gorin (1975) to the unispecific genus *Babjevia* van der Walt et Smith, restricted the terricolous yeast genus *Lipomyces* Lodder et Kreger-van Rij (1952) to the type species *L. starkeyi* Lodder et Kreger-van Rij (1952), *L. lipofer* Lodder et Kreger-van Rij ex Slooff (1970), *L. kononenkoae* Nieuwoudorp, Bos et Slooff (1974), *L. tetrasporus* Nieuwoudorp, Bos et Slooff (1974) and *L. japonicus* van der Walt, Smith, Yamada et Nakase (1989). In terms of its phenotypic and genetic characters, *L. japonicus*, nevertheless, takes an isolated position within the remodelled genus.

Unlike the type species and all other members of the genus, *L. japonicus* is ultrastructurally typified by unusual alveolate to reticulate ascospores which are individually enclosed by a thin, electron-dense, exospore membrane (van der Walt *et al.* 1989). This ascospore topography and presence of an exospore membrane suggest an unusual ascosporeogenesis that sets *L. japonicus* apart from all known members of the Lipomycetaceae Novák et Zsolt emend. van der Walt *et al.* (1987).

The singular position of *L. japonicus* within the genus is also manifested genetically. Yamada and Nogawa (1990), probing the molecular phylogeny of the budding lipomycetaceous genera by partial ribosomal ribonucleic acid (rRNA) base sequence analy-

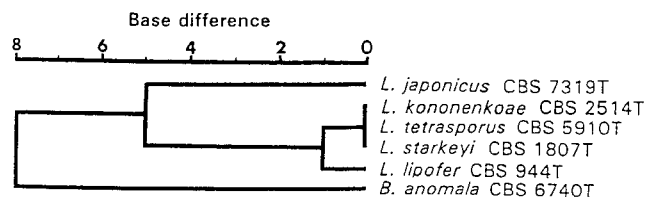


Figure 1 Dendrogram based on the calculated number of base differences in the partial base sequences of the 18S rRNA (positions 1451–1618; 168 bases) of the type strains of the genera *Lipomyces* and *Babjevia*. The dendrogram was drawn by the simple linkage method. (Data presented by Yamada & Nogawa 1990.)