Oecologia (1989) 80:490–497

Oecologia

Seed dispersal by ants: behaviour-releasing compounds in elaiosomes

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Summary. In a study of the biochemical basis of seed dispersal by ants, elaiosomes of *Acacia myrtifolia* and *Tetratheca stenocarpa* induced seed collection: intact diaspores and elaiosomes were taken rapidly by ants while most seeds remained on the forest floor. Extracts of elaiosomes (non-polar lipids, polar lipids, and aqueous fractions) were differentially collected by ants. Small pieces of pith impregnated with the polar lipid fraction from elaiosomes of either species elicited a removal rate by ants equivalent to that of intact elaiosomes and significantly higher than that of untreated pith. The non-polar lipid fraction, highest in concentration in elaiosomes of both species, elicited removal that did not differ from that of untreated pith. In *T. stenocarpa*, however, the aqueous fraction also induced removal equivalent to the polar lipid fraction. 1,2-Diglycerides with unsaturated groups are present in the active polar fractions of both species and unsaturated oleate is the major acid group of the glycerides in elaiosomes. Most oleate-containing compounds tested were taken more rapidly by ants than saturated compounds, and oleic acid, associated with corpse-carrying in ants, induced rapid removal. 1,2-Diolein, but not 1,3-diolein, was taken suggesting that the specific configuration of fatty acid moieties influences collection by ants. We hypothesize that a small suite of oleyl-containing compounds in elaiosomes elicit a stereotyped carrying response by a variety of ants. While the nutrient composition of elaiosomes may provide the underlying selective advantage for ants in seed dispersal, specific compounds may manipulate their behaviour and maximize seed dispersal.

Key words: Ants – Elaiosome – Lipids – Mutualism – Seed dispersal

Ants are important dispersal agents of seeds and can have major consequences for their survival and establishment (Culver and Beattie 1978; Handel 1978; O'Dowd and Hay 1980; Hanzawa et al. 1988). Seed dispersal by ants occurs in over 80 plant families and involves members from 4 of the 7 subfamilies of ants (Beattie 1985). Regionally and locally, it can represent one of the primary dispersal modes in communities (Berg 1975; Rice and Westoby 1981; Beattie and Culver 1980; Bond and Slingsby 1982).

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Understanding the biochemical basis of seed dispersal by ants may provide clues to the factors that influence the probability of seed removal and the degree of reciprocal specialization in these plant-ant mutualisms. The focus of the interaction is a food body (the elaiosome) that induces a variety of ants to collect the diaspore (i.e., the seed plus elaiosome). While lipid, protein, and sugar are all present in elaiosomes (Bresinsky 1963), lipid usually constitutes the major class of compounds and is the only fraction known to induce collection of diaspores by ants (Bresinsky 1963; Marshall et al. 1979). Working with elaiosomes of *Viola odorata*, both Bresinsky (1963) and Marshall et al. (1979) concluded that a specific active component within the lipid fraction promotes seed collection by ants.

We examine here seed dispersal by ants in *Acacia myrtifolia* (Sm.) Willd. (Mimosaceae) and *Tetratheca stenocarpa* J.H. Willis (Tremandraceae) in southeastern Australia. We consider three alternative (but not mutually exclusive) hypotheses as a basis of seed dispersal: (1) ants respond to the general nutrient composition of the elaiosome; (2) specific essential nutrients in the elaiosome elicit collection by ants; and, (3) ants are induced to collect diaspores by specific compounds that release carrying behaviour.

Materials and methods

Study site and species

Field studies were conducted on a 14 × 16 m plot divided into 56 grid locations spaced at 2 m intervals in moist sclerophyll forest at Gembrook State Forest, Victoria (37°56′S, 145°36′E; 700 m elevation). We chose to examine *Acacia myrtifolia* and *Tetratheca stenocarpa* because they are abundant on the site (6% and 10% of total plant cover, respectively) and are not closely related. *Acacia myrtifolia* is a woody, short-lived shrub widely distributed in southeastern Australia, while *T. stenocarpa* is a sprawling herbaceous shrub that is restricted to small pockets of forest east of Melbourne, Victoria.

The role of the elaiosome in seed dispersal

To determine whether the elaiosome is responsible for seed dispersal by ants, we conducted field studies on the removal of (1) intact diaspores (seeds plus elaiosomes), (2) seeds with elaiosomes removed, and (3) detached elaiosomes of both species. Diaspores for experiments were collected from

plants adjacent to the study site. Ten units of a given treatment (1, 2, or 3) and species were placed on a Perspex tray (0.5 × 6 × 6 cm, with a 2 mm deep circular depression to prevent units from rolling off the tray) at each of 6 randomly-selected grid locations. To exclude potential removal by small mammals and birds, we placed wire cages (15 × 15 × 10 cm; 1.2 cm mesh) over the trays. A polyethylene cover was fastened on top of each cage to prevent scattering of the units by rain. Leaf litter was cleared around the trays to a 30 cm radius to facilitate observations. We conducted the experiment twice for seven days each, during summer when seedfall of these species occurs. The number of units remaining in each tray was counted frequently during the first 24 h (hourly for the first 8 h, and then every 2 h), and subsequently twice daily.

Ants observed removing units were identified. Ant abundance and species composition at each grid location were determined by pitfall trapping. Polyvinyl chloride tubes were placed in the ground adjacent (< 30 cm) to each grid location into which 2 cm diameter test tubes containing 10 ml of 70% ethanol were inserted (Majer 1978).

Extraction and identification of the elaiosome constituents

Prior to extraction, seed and elaiosome mass, and the mass of the elaiosome as a percentage of the diaspore mass (reward-to-bulk carried ratio- Herrera 1981) of 20 diaspores of each species were determined. To avoid possible contamination, we used latex gloves and forceps when detaching elaiosomes from seeds. Elaiosomes were stored under nitrogen at −20° C until sufficient quantities were available for extraction. They were then ground with a mortar and pestle, weighed, and placed into an extraction thimble. Three serial extractions of 5 h each were made using a Soxhlet apparatus: (1) non-polar lipids were removed with cyclohexane in which waxes, triglycerides and steroids are soluble; (2) polar lipids such as free fatty acids, mono- and diglycerides were extracted with methanol; and, (3) amino acids and sugars were removed with distilled water. The mass of each extracted fraction was determined gravimetrically after evaporation of the solvent. All extracts were stored under nitrogen at −20° C.

We investigated the composition of the polar and non-polar lipid fractions of elaiosomes of both species using nuclear magnetic resonance spectroscopy (NMR). Amino acids in the aqueous fractions were analyzed using a HPLC Millipore Waters system.

We determined the fatty acid (FA) composition of elaiosomes of *A. myrtifolia* by transmethylation of FA without prior extraction (Welch 1977). FA were analyzed using a Varian Aerograph 204B gas chromatograph equipped with a hydrogen flame ionization detector and columns of Silar 10C on Chrom Q. Peak areas were calculated with reference to standard mixtures (Applied Sciences).

Collection responses of ants to the elaiosome fractions

To determine the response of ants to the elaiosome fractions of both species, 100 µg of extract in solvent was absorbed onto pieces of polyporous pith (cleaned bracket fungi – Sydney Entomological Supplies) that approximated the size and shape of an elaiosome (3 × 1 × 1 mm, average mass of 0.15 mg). Each artificial "elaiosome" was then allowed to dry and stored under nitrogen at −20° C to prevent oxidation of the constituents. Untreated polyporous pith and intact elaiosomes were used as controls. We conducted the experiment the day following preparation of pith and repeated it three times in July–August (winter); 5 replicates (5 units per tray) of each treatment (untreated pith, aqueous, polar lipid and non-polar lipid fractions of elaiosomes of both plant species) were placed at random grid locations. Observations were made at 15 min intervals for 6 h, then hourly for 4 h, and followed by a final census at 18 h. Ant species observed removing units from trays were identified.

We assayed specific compounds for their effect on collection response of ants as for elaiosome fractions. To compare saturated and unsaturated lipids, the unsaturated mono- and triglycerides of oleic acid (monolein and triolein) and the saturated fatty acid, palmitic acid, and its associated diglycerides, 1,2-palmitin and 1,3-palmitin (Sigma Chemical Company, St. Louis), were assayed for ant-collecting response in December (summer). These fatty acid groups (oleate and palmitate) were used because they are the major moieties present in elaiosomes of *A. myrtifolia*. To determine whether oleic acid induces carrying behaviour (Gordon 1983), pith impregnated with oleic acid was compared to pith treated with the polar and non-polar lipid fractions of elaiosomes of *A. myrtifolia* in May (autumn). To assay whether diglycerides derived from oleic acid induce seed dispersal (Marshall et al. 1979), removal of pith treated with 1,2-diolein and 1,3-diolein was compared in September (spring). Untreated pith and intact elaiosomes of *Acacia myrtifolia* were used as controls in all experiments except that involving oleic acid, where diaspores (seed plus elaiosome) were used. Although some assays were conducted outside of summer when seedfall occurs, removal responses by ants to controls (untreated pith and elaiosomes) in each experiment were consistent.

Statistical analysis

For all experiments, time-specific removal rates for each treatment were calculated by adopting a method that uses age-specific death rates in populations (Finch 1983). For tests involving the elaiosome, removal rates of intact diaspores, seeds, and elaiosomes were compared. For elaiosome fractions and specific compounds, we compared removal rates of treated pith to control pith and elaiosome "standards". For each treatment, the percentage of units collected during each time interval is calculated from the number remaining at the end of the previous interval. Only intervals where at least one unit was collected were considered; hence, the degrees of freedom vary among comparisons. Time-specific ratios (r_i) are calculated

$$r_i = (c_i^1 - c_i^2)/(c_i^1 + c_i^2) \quad (1)$$

where c_i is the percentage of units collected in time interval i in treatments 1 and 2. Since

$$c_i^1/c_i^2 = (1 + r_i)/(1 - r_i), \quad (2)$$

the removal rates of the two treatments compared will be the same when all r_i are near zero. To determine whether r_i values for a comparison differed from zero, the mean of the r_i values is referred to the t-distribution with n-1 degrees of freedom by calculating

$$\tau = n^{1/2}\mu/\sigma \quad (3)$$

where n is the number of time intervals, and μ and σ are the mean and standard deviation of the ratios determined in equation (1).

Results

General characteristics of the elaiosomes

The diaspores of both *A. myrtifolia* and *T. stenocarpa* have features consistent with seed dispersal by ants, but they differ in mass and general morphology. The testa of the diaspore of *A. myrtifolia* is smooth and the elaiosome clavate, whereas the seedcoat of the diaspore of *T. stenocarpa* is covered with fine hairs and the elaiosome is spherical. Seeds and elaiosomes of *A. myrtifolia* weigh over twice as much as those of *T. stenocarpa* (on a wet mass basis, 8.90 ± 0.30 (SE) mg and 3.65 ± 0.19 mg for seeds and 0.92 ± 0.03 mg and 0.28 ± 0.02 mg for elaiosomes, respectively). Water content of the elaiosomes averages 8.7 ± 0.9 and $14.0 \pm 1.5\%$ for *A. myrtifolia* and *T. stenocarpa*, respectively. The reward-to-bulk carried is similar for both species ($9.5 \pm 0.1\%$ for *A. myrtifolia* and $7.1 \pm 0.2\%$ for *T. stenocarpa*). The elaiosomes of *A. myrtifolia* contained $47.5 \pm 2.4\%$ lipid on a wet-mass basis while those of *T. stenocarpa* averaged $20.7 \pm 1.1\%$ lipid.

Induction of seed removal by the elaiosome

Removal rates of diaspores and elaiosomes of both species far exceeded that of seeds alone. All intact diaspores and elaiosomes had been removed after 8 h while 89% and 80% of seeds of *A. myrtifolia* and *T. stenocarpa*, respectively, still remained on trays after 7 days (Fig. 1). In all experiments, diaspores and elaiosomes were removed significantly faster than seeds alone (Table 1). The only obvious difference between the experiments was the significantly faster removal of elaiosomes over diaspores of *A. myrtifolia* in one case.

Ten ant species (43% of the total ant species collected on the site) were observed removing diaspores; of these, *Rhytidoponera victoriae* and *Notoncus ectatommoides* were the most frequent collectors of diaspores of both plant species (accounting for 94% and 66% of the observed removals of diaspores of *A. myrtifolia* and *T. stenocarpa*, respectively) and comprised 82% of the total ants trapped (Table 2). For each potential seed disperser, its relative importance in diaspore removal was proportional to its distribution over the site. The percent of the total traps in which an ant species was caught was correlated with the percent of total diaspores removed by each species ($r = 0.98$ and 0.89, $n = 10$, $P < 0.001$, for *A. myrtifolia* and *T. stenocarpa*, respectively).

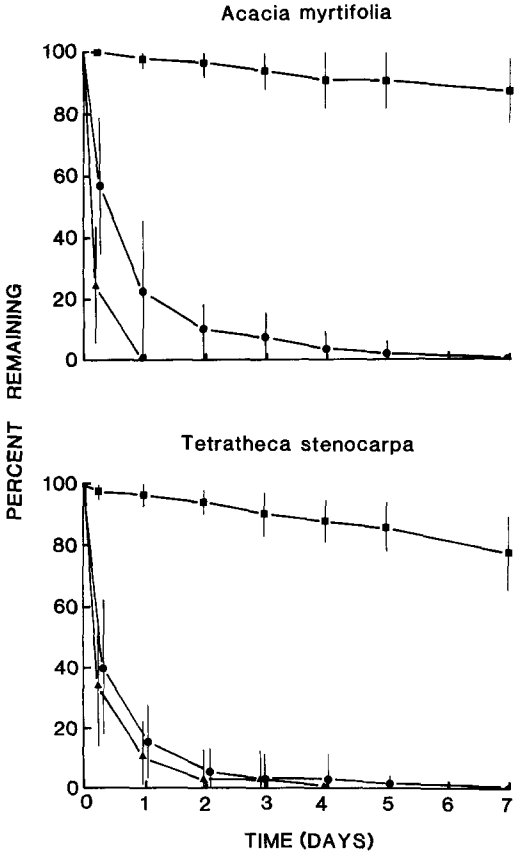

Fig. 1. Pooled results from the removal experiments showing the average percent of intact diaspores (*circles*), seeds with elaiosomes removed (*squares*), and elaiosomes alone (*triangles*) of *Acacia myrtifolia* and *Tetratheca stenocarpa* remaining on trays over 7 days. Bars are ± 1 SE. Six replicates were used for each treatment

Table 1. Comparisons of removal rates of intact diaspores, seeds, and elaiosomes of *Acacia myrtifolia* and *Tetratheca stenocarpa* by ants at Gembrook State Forest, Victoria. Table should be read from row variable to column variable where sign indicates comparison ('−' is significantly less than, '=' is equivalent to, and '+' is significantly greater than), e.g., intact diaspores of *Acacia myrtifolia* are removed at a significantly faster rate than seeds with elaiosomes removed. Superscripts indicate significance levels: ns = not significant, *** = $P < 0.001$

	Experiment	Seed only	Elaiosome only
Acacia myrtifolia			
Diaspore	1	$+^{***}$	$=^{ns}$
	2	$+^{***}$	$-^{***}$
Seed only	1		$-^{***}$
	2		$-^{***}$
Tetratheca stenocarpa			
Diaspore	1	$+^{***}$	$=^{ns}$
	2	$+^{***}$	$=^{ns}$
Seed only	1		$-^{***}$
	2		$-^{***}$

Table 2. Ant abundance (percent of total ants caught in traps), distribution (percent of total traps in which a species was caught), and percent of total diaspore removals observed for each ant species at Gembrook State Forest, Victoria. N = total number of ants trapped, total number of traps, and total number of observations of diaspore removals for *Acacia myrtifolia* (*AM*) and *Tetratheca stenocarpa* (*TS*) by ants

Ant species	% of total trapped	% of traps occupied	% removal	
			AM	*TS*
Seed dispersers				
Rhytidoponera victoriae	70.0	95.8	63.0	55.0
Notoncus ectatommoides	11.9	46.5	31.0	11.0
Prolasius nitidissima	4.4	15.5	1.5	2.7
Crematogaster sp.	2.9	11.1	1.5	20.5
Camponotus innexus	0.3	2.8	0.0	6.9
Others ($n = 5$)	5.5	6.0	3.0	3.9
All others ($n = 13$)	4.9	23.9	–	–
N	654	71	68	73

Table 3. Soluble components of elaiosomes of *Acacia myrtifolia* and *Tetratheca stenocarpa* expressed as percent of elaiosome wet mass for individual fractions (non-polar lipid, polar lipid, aqueous, and total fractions) and as percent concentration of total extractable material. N = number of extractions. Standard error is in parentheses

Species	Fraction			Total
	Non-polar	Polar	Aqueous	
Acacia myrtifolia				
% of total mass	39.5 (2.3)	7.1 (0.2)	9.0 (2.0)	55.6 (3.0)
% concentration	71.1 (2.4)	12.6 (1.0)	15.9 (2.9)	
N	5	5	5	5
Tetratheca stenocarpa				
% of total mass	11.1 (2.2)	9.6 (1.1)	2.5 (0.6)	23.1 (1.4)
% concentration	47.8 (6.6)	41.3 (7.1)	10.8 (2.3)	
N	4	4	4	4

Table 4. Chemical shifts of hydrogen nuclei (attached to the three carbon centres of glycerol-positions 1, 2, and 3) for the NMR spectra of the polar and non-polar lipid fractions of elaiosomes for *Acacia myrtifolia* and *Tetratheca stenocarpa*. Glyceride standards, including glycerol, monoglyceride, 1,2- and 1,3-diglyceride, monounsaturated triglyceride and polyunsaturated triglyceride, indicate the types of compounds in the elaiosome extracts. To identify any of the standards in the elaiosome fractions, the set of hydrogen chemical shifts must closely correspond (see underlined chemical shifts of monoglyceride, 1,2-diglyceride, and monounsaturated triglyceride in relation to fractions)

	Hydrogen position*		
	1	2	3
Elaiosome fractions			
A. myrtifolia			
polar lipid	4.25	5.25; 5.10	3.75; 3.65
non-polar lipid	4.25	5.25	4.25
T. stenocarpa			
polar lipid	4.18; 4.25	4.00; 5.10	3.75; 3.65
non-polar lipid	4.25	5.25	4.25
Standards			
glycerol	3.68	3.75	3.68
monoglyceride	4.18	3.93	3.68
1,2-diglyceride	4.28	5.10	3.72
1,3-diglyceride	4.23	4.18	4.23
monounsaturated triglyceride	4.23	5.25	4.23
polyunsaturated triglyceride	4.23	5.30	4.23

* (1) (2) (3)
$CH_2-CH-CH_2$
| | |
O O O

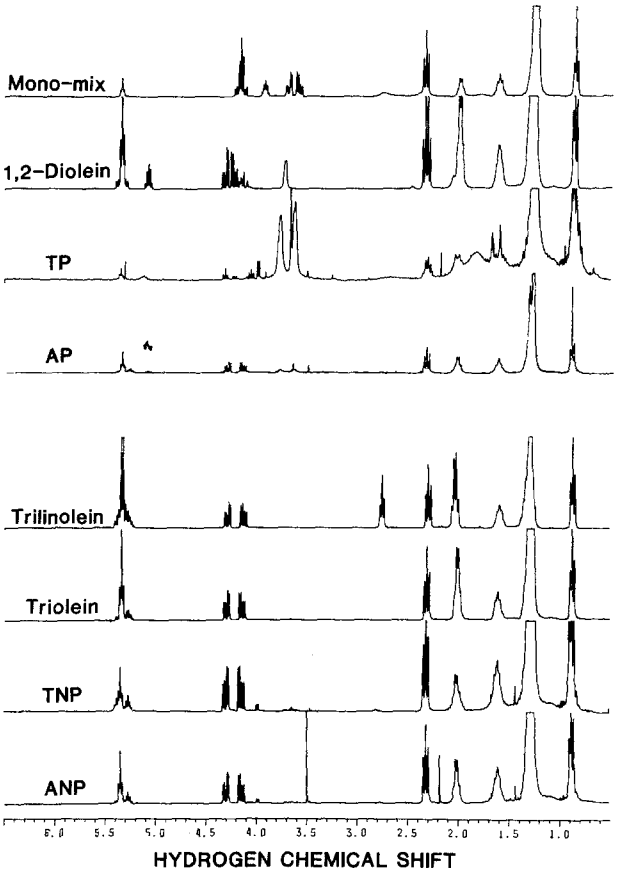

Fig. 2. NMR spectra of the lipid fractions of *Acacia myrtifolia* (non-polar lipid = ANP; polar lipid = AP) and *Tetratheca stenocarpa* (non-polar lipid = TNP; polar lipid = TP); Mono-mix (monoglyceride standard, predominantly monolein with some monostearin and monopalmitin), diglyceride standard (1,2-diolein), monounsaturated triglyceride standard (triolein) and polyunsaturated triglyceride (trilinolein). The horizontal axis of the spectra indicates the chemical shift of the hydrogens within the molecules with respect to the reference standard tetramethylsilane (TMS). For the spectra of AP, the signal at 5.1 is enlarged 6-fold. Signals common to all compounds correspond to the hydrogen nuclei in bold; 0.9, $-\mathbf{CH_3}$; 1.3, $(\mathbf{CH_2})_n$; 1.6, $\mathbf{CH_2}-CH_3$; 2.0, $\mathbf{CH_2}-CH=CH-\mathbf{CH_2}$; 2.3, $\mathbf{CH_2}-CHO$; 4.2–4.3, $\mathbf{CH_2}-O-CO-$; 5.3, $-\mathbf{CH}=\mathbf{CH}-$. For trilinolein: 2.8, $-CH=CH-\mathbf{CH_2}-CH=CH-$. For 1,2-diolein and polar lipids: 3.7–3.8, $\mathbf{CH_2}-\mathbf{OH}$. Refer to Table 4 for further details

Elaiosome constituents

A large fraction of elaiosome mass of both species was extractable with the three solvents: significantly more (56%) of the total mass of elaiosomes of *A. myrtifolia* was extracted compared to just under one-quarter (23%) for that of *T. stenocarpa* ($t=3.69$; $n=5$, $P<0.01$, Student's t-test) (Table 3). This was largely a consequence of the significantly higher concentration of lipids in elaiosomes of *A. myrtifolia*. Of the lipid fraction, non-polar lipids represented the bulk of extractable compounds for both species (Table 3). Polar lipid comprised only 13% of extractable mass in *A. myrtifolia* but over 41% in *T. stenocarpa*. The aqueous fractions accounted for a minor fraction of the extractable material in elaiosomes of both species (Table 3).

The NMR spectra indicate the types of glycerides present in the solvent extracts of elaiosomes (Table 4, Fig. 2). We identified the position of the acyl groups and the acid moieties on the glycerol backbone by comparing the hydrogen chemical shifts of glyceride standards with those of the lipid fractions. The chemical shifts refer to the signals of the spectra that arise following application of a strong magnetic field. The relationship between the chemical shift and the chemical bonding associated with each hydrogen allows identification of the types of compounds present (Dyke et al. 1978).

Table 5. Diversity and concentration (μg/mg) of quantifiable amino acids in the aqueous fraction of the elaiosomes of *Acacia myrtifolia* and *Tetratheca stenocarpa*. For unquantifiable unknown amino acids, + = present; + + + = large amount present. tr = trace. For total number of amino acids, number in parentheses includes trace amino acids

Amino acid	*Acacia myrtifolia*	*Tetratheca stenocarpa*
cysteic	0.0	tr
unknown 1	0.0	+ + +
aspartic acid	7.7	15.5
threonine	1.1	7.1
serine	11.5	7.7
glutamic acid	105.9	37.3
proline	0.0	17.9
glycine	14.7	11.1
alanine	tr	21.6
valine	tr	7.7
isoleucine	tr	5.9
leucine	tr	9.3
phenylalanine	tr	12.7
unknown 2	0.0	+
histidine	15.4	32.5
lysine	tr	tr
arginine	tr	tr
AA Concentration (μg/mg)	156.3	186.3
Number of AA	6 (13)	14 (17)

NMR spectra of the non-polar lipid fractions of both species showed that triglycerides are the main component (Table 4, Fig. 2). More unsaturated chains occur in the lipids of *A. myrtifolia* than in those of *T. stenocarpa*, judging from the integrated areas of spectra of the non-polar lipids of both species.

For the polar lipid fraction of elaiosomes, the spectrum for *A. myrtifolia* indicated the presence of both 1,2-diglycerides and triglycerides (Table 4, Fig. 2), but not 1,3-diglycerides (Table 4). The areas of peaks at 5.30 (triglycerides) and 5.10 (diglycerides) were in the ratio 3:1, which indicates that triglycerides are three times more abundant than diglycerides (Table 4, Fig. 2). Signals with chemical shifts corresponding to those of monoglycerides were not apparent (Table 4, Fig. 2). For *T. stenocarpa*, the polar lipid fraction contains unsaturated monoglycerides and 1,2-diglycerides, but not triglycerides (Table 4, Fig. 2). Other, unidentified peaks were also present.

In all spectra, the signal at 5.35 is associated with unsaturated bonds (Fig. 2), and at least for *A. myrtifolia*, corresponds mainly with oleate. Over 72% of the fatty acids (FA) in elaiosomes of *A. myrtifolia* are unsaturated (24% and 4.0% is saturated palmitate and stearate, respectively), and 95% of that is oleate (5% was linoleate, a polyunsaturated FA). The signal specific to linoleate (2.80) is nearly undetectable in all fractions (Fig. 2). While the fatty acid composition of elaiosomes of *T. stenocarpa* is not known, the spectra indicate that monounsaturated oleate is the likely moiety (Fig. 2).

Signals associated with free FA could not be detected in any of the spectra. If present, however, most of their signals would be covered by those of their esters.

The aqueous extracts of the elaiosomes of *A. myrtifolia* and *T. stenocarpa* contained 13 and 17 amino acids, respectively (Table 5), although some of these were present only in trace amounts. Neglecting these traces, the numbers reduce to 6 and 14, respectively. For the quantifiable amino

Table 6. Differences in removal rates of fractions extracted from elaiosomes of *Acacia myrtifolia* (*AM*) and *Tetratheca stenocarpa* (*TS*) and specific compounds in relation to control pith and elaiosomes. '−' is less than, '=' is equivalent to, and '+' is greater than either control or elaiosomes. Superscripts indicate significance levels: * = $P<0.05$, ** = $P<0.01$, *** = $P<0.001$, and ns = not significant

Experiment	Control			Elaiosome			Effect
	1	2	3	1	2	3	
Fractions							
Non-polar							
AM	$=^{ns}$	$+^{**}$	$=^{ns}$	$-^{**}$	$=^{ns}$	$-^{**}$	Unattractive
TS	$=^{ns}$	$=^{ns}$	$=^{ns}$	$-^{***}$	$-^{**}$	$-^{***}$	Unattractive
Polar							
AM	$+^{***}$	$+^{***}$	$+^{***}$	$=^{ns}$	$=^{ns}$	$=^{ns}$	Attractive
TS	$+^{***}$	$+^{***}$	$+^{**}$	$=^{ns}$	$=^{ns}$	$=^{ns}$	Attractive
Aqueous							
AM	$=^{ns}$	$=^{ns}$	$=^{ns}$	$-^{*}$	$-^{*}$	$-^{*}$	Unattractive
TS	$=^{ns}$	$+^{**}$	$+^{***}$	$=^{ns}$	$=^{ns}$	$=^{ns}$	Attractive
Compounds							
Oleyl-containing							
Oleic acid		$+^{***}$			$=^{ns}$		Attractive
Monolein		$=^{ns}$			$-^{*}$		Unattractive
1,2-diolein		$+^{***}$			$=^{ns}$		Attractive
1,3-diolein		$=^{ns}$			$-^{***}$		Unattractive
Triolein		$+^{***}$			$=^{ns}$		Attractive
Palmitoyl-containing							
Palmitic acid		$=^{ns}$			$-^{*}$		Unattractive
1,2-palmitin		$=^{ns}$			$-^{***}$		Unattractive
1,3-palmitin		$=^{ns}$			$-^{***}$		Unattractive

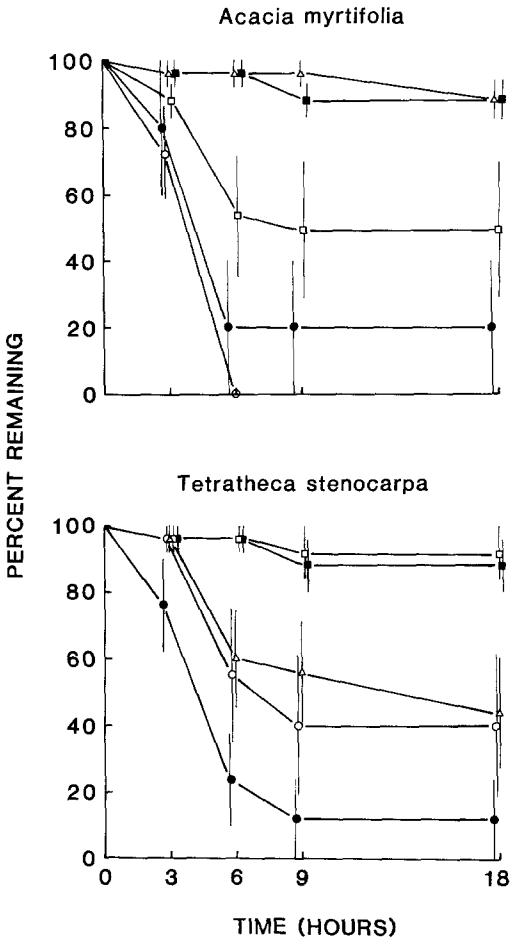

Fig. 3. Percent of pith treated with different fractions (*open symbols* – non-polar lipid (*squares*), polar lipid (*circles*), and aqueous (*triangles*)) from elaiosomes of *Acacia myrtifolia* and *Tetratheca stenocarpa* remaining on trays over 18 h in the second experiment (results for experiments 1 and 3 were similar except as noted in Table 6). Elaoisomes (*solid circles*) and untreated pith (*solid squares*) were used as controls. Bars are ±1 SE. Five replicates were used for each treatment

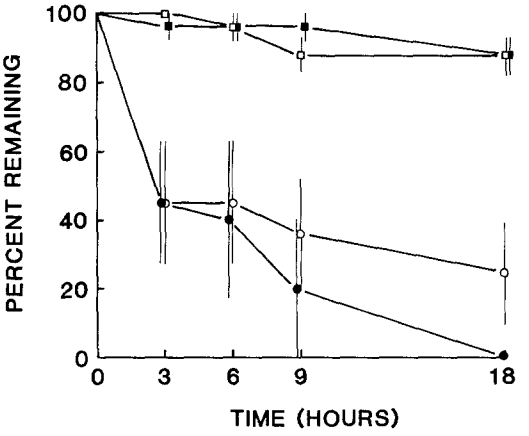

Fig. 4. Percent of pith treated with the dioleyl esters, 1,2-diolein (*open circles*) and 1,3-diolein (*open squares*), remaining on trays over 18 h. Elaiosomes (*solid circles*) and untreated pith (*solid squares*) were used as controls. Bars are ±1 SE. Five replicates were used for each treatment

acids, a total of 156.3 and 186.3 μg/mg were present in the aqueous fractions of *A. myrtifolia* and *T. stenocarpa*. For *T. stenocarpa*, this represented a minimum concentration because a large but unquantifiable amount of an unknown amino acid (8.34 min retention time) was present.

Response of ants to the elaiosome fractions

Ants removed treated pith and of 166 observations (35% of total removals), *Notoncus ectatommoides* was responsible for 76% and *Rhytidoponera victoriae* 22%. Removal of untreated pith was negligible, and intact elaiosomes of both species were always removed at a significantly faster rate than untreated pith. The elaiosome fraction absorbed onto pith strongly affected removal rates by ants and was consistent across the three experiments (Table 6, Fig. 3). The polar lipid fraction of both species was collected at rates indistinguishable from those of elaiosomes but significantly faster than that of the pith control (Table 6). Pith treated with non-polar lipids was collected significantly slower than elaiosomes and at a rate equivalent to that of the pith control (in 5 of the 6 cases).

Several differences in removal occurred between the same fractions of elaiosomes in each species. First, in contrast to the polar lipid, the aqueous fraction of *A. myrtifolia* was removed at rates significantly slower than that of elaiosomes and indistinguishable from that of the pith control (Table 6). For *T. stenocarpa*, however, the aqueous fraction was taken at rates indistinguishable from those of elaiosomes and significantly faster than that of control pith (in 2 of 3 cases). Second, in 2 of 3 cases, the non-polar lipid fraction of *A. myrtifolia* was collected significantly faster than the non-polar lipid of *T. stenocarpa* (for experiment 2, $\tau = 3.12$, df = 8, $P < 0.05$; for experiment 3, $\tau = 34.7$, df = 4, $P < 0.001$).

Response to specific compounds

Ants responded differentially to specific lipid compounds (Table 6). Of those tested, only pith treated with the unsaturated oleyl-based lipids (oleic acid, 1,2-diolein, and triolein) were removed at rates comparable to those of intact elaiosomes; pith treated with other oleyl esters (monolein and 1,3-diolein) and saturated palmitin-based lipids (1,2- and 1,3-palmitin, and palmitic acid) were all collected by ants at rates indistinguishable from those of untreated controls. 1,2-Diolein, which differs from 1,3-diolein only by the position of one oleyl chain, was collected at a significantly faster rate (Fig. 4).

Discussion

Elaiosomes provide the basis for seed dispersal by ants and induce removal of diaspores of both *Acacia myrtifolia* and *Tetratheca stenocarpa*. Diaspore characteristics (e.g., mass, reward-to-bulk carried, lipid concentration of elaiosomes) are comparable to those of other species known to be dispersed by ants (Bresinsky 1963; O'Dowd and Gill 1986).

Three hypotheses may explain the collecting responses of ants to elaiosomes. First, elaiosomes may represent a general nutrient source for ants. If so, then ants should collect the most abundant fraction in elaiosomes or all fractions equally. Our results show that this is not so, since the non-polar lipid fractions of elaiosomes of both species

elicited the lowest removal responses by ants yet represent over 70% and 45% of the extractable material. Furthermore, for the elaiosomes of *A. myrtifolia*, the highest collection response was recorded for the polar lipid fraction which represented less than 13% of the extractable material. Equivalent collection responses to the aqueous and polar fractions of *T. stenocarpa* occurred, and these fractions represented 11% and 41% of the extractable material from elaiosomes, respectively. This suggests that the less abundant but highly attractive fractions (the polar lipids in *A. myrtifolia*, and the polar and aqueous fractions of *T. stenocarpa*) play important roles in maximizing the collection response of ants to diaspores. A general nutrient hypothesis alone is insufficient to explain the patterns of removal.

Second, the active fractions may contain specific nutrients essential to ants. We found lower collection responses to the saturated fatty acid and its esters than to the monounsaturated fatty acid and its esters, suggesting that unsaturated lipids are more attractive to ants. This observation is consistent with responses by ants in wider screenings of saturated and unsaturated compounds (Vinson et al. 1967; Marshall et al. 1979). However, insects can synthesize both saturated and monounsaturated fatty acids, so it is unlikely that the collection response of ants is related to requirements for specific nutrients. Although polyunsaturated fatty acids are required by insects in their diet (Dadd 1973), only minimal amounts occur in the elaiosomes. Diglycerides, intermediates in the glycerol-3-phosphate pathway of triglyceride synthesis (Kennedy 1961), have been hypothesized to be specific nutrients (Marshall et al. 1979). This hypothesis, however, cannot account for the differential collection of 1,2-diolein in relation to 1,3-diolein, 1,2-palmitin, and 1,3-palmitin.

Third, the active fractions may release behaviour in ants that is cued by chemical signals. A response may occur to a single compound (Bresinsky 1963; Marshall et al. 1979) or to a small group of compounds of similar structure. In our study, oleic acid was collected at a rate comparable to that of diaspores of *A. myrtifolia*. It is reported to elicit corpse-carrying behaviour in a wide variety of ant species (*Pogonomyrmex badius*, Wilson et al. 1958; Gordon 1983; *Solenopsis saevissima*, Wilson et al. 1958; Vinson et al. 1967; *Myrmecia vindex*, Haskins and Haskins 1974) and Gordon (1983) showed that this collection response depended upon the social activities of the ant colony; ants involved in nest maintenance removed objects to the midden but foragers returned them to the nest. The oleate moiety present in either its free form or in a diester (e.g., 1,2-diolein) may be acting in the active polar lipid fractions of both *A. myrtifolia* and *T. stenocarpa* as a behaviour-releasing agent. Since ants responded to 1,2-diolein just as they did to elaiosomes, but not to monolein or 1,3-diolein, this suggests that the specific configuration of oleyl groups is important in inducing carrying behaviour by ants.

Triolein also induced collection by ants. Since the main triglyceride fraction of the elaiosomes (non-polar lipid) was the least attractive, removal of this triglyceride seems contradictory, although triglycerides are also present in the polar lipid fraction. Further work considering the activity of this and other triglycerides in the polar fraction is required.

Our results also suggest that compounds additional to lipids may be important in seed dispersal. The aqueous fraction of *T. stenocarpa* induced collection comparable to the polar lipid fraction and may contain specific nutrients or some behaviour-releasing compound(s). Both the concentration and diversity of amino acids were higher in the aqueous fraction of *T. stenocarpa* than in that of *A. myrtifolia*, and a large peak for an unknown amino acid occurred in *T. stenocarpa*. Other studies have shown that ants prefer specific amino acids (Lanza and Krauss 1984), and artificial nectaries with amino acids over those containing only sugars (Lanza 1988).

Thus, overall evidence is inconsistent with a specific compound (1,2-diolein) alone or a larger class of compounds (all oleyl-containing lipids) acting as a cue for carrying behaviour. A small suite of oleyl-containing compounds (1,2-diolein, triolein, and perhaps other untested oleyl-containing di- and triglycerides) in the lipid faction of elaiosomes may potentially induce seed dispersal by ants. In some cases, non-lipid compounds, perhaps amino acids, may also be important in inducing seed dispersal by ants.

Parallel structures for seed dispersal by ants have evolved independently in taxonomically diverse and geographically isolated plant taxa (Bond and Slingsby 1982; Beattie 1983). Our findings (plus those of Marshall et al. 1979) suggest that similarities extend beyond general morphology, mass, and nutrient composition to the presence of specific compounds or classes of compounds. These similarities emerge despite the fact that the studies used different techniques (removal responses to compounds in the field vs. behavioural responses to compounds in the laboratory) and involved distantly-related sets of ants and plants on different continents.

Ant-seed mutualisms are diffuse, perhaps as a consequence of the low probability of the association of any pairwise combination of ant and plant species (O'Dowd and Hay 1980). It would not be surprising if many plant species have converged upon similar cues for seed dispersal that capitalize on a generalized carrying response that is widespread among ants and related to nest-cleaning activities and the detection of suitable prey by foragers (Wilson et al. 1958; Gordon 1983). The activity of specific compounds in eliciting seed dispersal may be related to their recognition as collecting cues within the social context of the ant colony. While the general nutrient composition of elaiosomes may provide the underlying selective advantage for ants in seed dispersal, specific compounds may manipulate their behaviour and maximize the probability and fidelity of seed dispersal in an uncertain world.

Acknowledgements. The Department of Conservation, Forests and Lands gave permission to work in Gembrook State Forest. J.A. Edgar, J. Weigold, and G. Wilson helped in analysis and interpretation of elaiosome chemistry. P. Finch suggested the statistical analysis of removal rates and provided other statistical advice. I. Sablovs, T. Raadik, and J. Wright helped in the field. R.W. Taylor confirmed the ant identifications. M.F. Willson and A.J. Beattie improved the manuscript.

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Received April 10, 1989