

Ants and extrafloral nectaries: no evidence for plant protection in *Helichrysum* spp. – ant interactions

Dennis J. O'Dowd* and E.A. Catchpole

CSIRO, Division of Plant Industry, P.O. Box 1600, Canberra City, A.C.T. 2601, Australia

Department of Mathematics, Royal Military College, Duntroon, A.C.T. 2600, Australia

Summary. Characteristics of Australian endemic *Helichrysum bracteatum* and *H. viscosum* suggest that foraging ants act as “guards” of developing flowerheads, protecting capitula from seed predators: (1) extrafloral nectar is secreted from leaves subtending the capitula and from bracts encircling the floral disc during pre- to post-flowering periods; (2) capitula are attended by ants; and, (3) encounters between ants and other capitula visitors, including predispersal seed predators such as *Tephritis* sp. (Diptera), can be frequent. In experiments to test the ant-guard hypothesis, exclusion of ants from plants increased abundance of other insects on the developing capitula. The difference between ant-access and ant-exclusion treatments was related to ant abundance on the access plants. These effects were statistically significant in spite of the large variation in insect activity between sites and through the season.

The increased abundance of insects on capitula following ant-exclusion did not, however, result in significant increases in the number of adult seed predators observed on capitula, the number of immature seed predators in capitula, or capitula damage as estimated between ant-access and exclusion treatments of either *H. bracteatum* or *H. viscosum*. Further, the ant-exclusion treatment on *H. bracteatum* had no significant influence on pollination as measured by seed set or on the degree of parasitism of *Tephritis* sp. by *Megastigmus* sp. Site and season most strongly affected numbers of immature seed predators and damage to capitula.

We discuss these findings in relation to the ant-guard hypothesis and suggest that generalization of the protection hypothesis to all plants with extrafloral nectaries is premature.

for the ant-guard hypothesis and the biological significance of EFN comes from this quarter: (1) the classic field studies of von Wettstein (1889) who first demonstrated in ant-exclusion experiments from EFN of *Jurinea mollis* and *Serratula lycopifolia* that ants can depress capitula predators and resultant damage; and, (2) the contemporary work of Inouye and Taylor (1979) whose natural and ant-exclusion experiments from EFN of *Helianthella quinquenervis* showed that ants can decrease predispersal predation of seeds.

Tests of the ant-guard hypothesis are few and restricted to northern hemisphere temperate (e.g., Inouye and Taylor 1979) or tropical regions (e.g., Schemske 1980). In this study we examine Delpino's hypothesis in Southeast Australia using the relationship between *Helichrysum bracteatum* and *H. viscosum* (Asteraceae), their associated ants, capitula visitors and seed predators. The primary approach we use compares measures of insect abundance and damage by seed predators to *Helichrysum* capitula accessible to or excluded from ants. If ant-guard effects occur, we can generate a set of cascading predictions: (1) exclusion of ants releases insect activity from ant interference. Numbers of other insects and “spread” (proportion of capitula with insects) are greater on the exclusion treatment; (2) adult seed predators observed on capitula (or a subset of the total seed predators) follow suit and increase on the exclusion treatment; (3) effects observed on the capitula translate into increased numbers of seed predators (immatures) in capitula of the ant-exclusion treatment; and, (4) capitula damage from those seed predators is greater in the absence of ants.

Materials and methods

Organisms

Helichrysum is a large genus in the Asteraceae (tribe Inuleae) and comprises over 500 species. It is broadly distributed in the southern hemisphere, primarily in South Africa and Australia. The *Helichrysum bracteatum* “complex” which includes both *H. bracteatum* (Vent.) Andr. and *H. viscosum* Sieber ex Spreng. occurs throughout Australia and New Guinea (Morley 1978). In Southeast Australia both species are widely distributed, *H. bracteatum* generally east of the Great Dividing Range and *H. viscosum* to the western slopes. These species occur primarily in disturbed, open areas of sclerophyll forest. Both are herbaceous, *H. bractea-*

Introduction

Seed predators can operate as a strong selective force in the evolution of seed defense or escape (Janzen 1969). In the context of a general theory of ant defense of plants with extrafloral nectaries (EFN), Delpino (1886) suggested that EFN attract ant “guards” to developing capitula of several genera of Asteraceae, thereby interfering with seed predators. Indeed, some of the strongest empirical support

* Present address and offprint requests to: School of Biological Sciences, The Flinders University of South Australia, Bedford Park, South Australia, 5042, Australia

Table 1. Predispersal seed predators and their parasitoids observed in or on capitula of *Helichrysum bracteatum* and *H. viscosum*. Based on field observations and laboratory dissections of 1512 *H. bracteatum* capitula and 846 *H. viscosum* capitula

Species	Seed predators		Parasitoid ^a
<i>Helichrysum bracteatum</i>	Orthoptera	<i>Phaulacridium vittatum</i> ^c	— ^b
	Hemiptera	<i>Nysius vinitor</i> ^c	— ^b
	Lepidoptera	Phycitinae ^{c,d}	<i>Diadegma</i> sp. (Ichneumonidae) ^c
	Diptera	Cecidomyiidae ^{c,d}	Platygastrinae ^{c,d}
		<i>Tephritis</i> sp. ^c	<i>Brachymeria</i> sp. (Chalcididae)
<i>Helichrysum viscosum</i>	Hemiptera	<i>Nysius vinitor</i>	<i>Dimeromicrus</i> sp. (Torymidae)
		Cecidomyiidae ^d	<i>Megastigmus</i> sp. (Torymidae) ^c
	Diptera	<i>Tephritis</i> sp.	? <i>Platytelenomus</i> sp. (Scelionidae)
			<i>Pteromalus</i> sp. (Pteromalidae)
			— ^b
			Platygastrinae ^d
			<i>Megastigmus</i> sp. (Torymidae)

^a All parasitoids observed were order Hymenoptera

^b Not examined for parasitoids

^c Commonly observed seed predator or parasitoid

^d Undescribed species

tum being short-lived and monocarpic while *H. viscosum* is perennial and polycarpic. At the onset of reproduction, one to 10 flowering scapes may be produced by an individual of either species, arising from a basal rosette. Each flowerstalk bears a terminal flowerhead (hereafter referred to as a capitulum) and usually many axillary capitula. Many achenes (up to 440) are produced on each capitulum. Since achenes are single-seeded fruit, we refer to them hereafter as seeds.

Ants found foraging at the EFN of *H. bracteatum* and *H. viscosum* are generally the same species as encountered in the respective habitats of the plants at sugar solution baits. The predominant genus found foraging during daylight in all habitats and on capitula of both *Helichrysum* spp. was *Iridomyrmex* although species of other genera, including *Notoncus*, *Camponotus*, *Monomorium* and *Crematogaster*, were present on some *H. viscosum* plants.

Both *H. bracteatum* and *H. viscosum* are subject to predispersal seed predation (Table 1). Seed predators appear more important on *H. bracteatum* where some insects such as an undescribed phycitid moth destroy up to 100% of developing seed in a capitulum. Five different species of seed predators were observed on or dissected from capitula. With the exception of the external feeding grasshopper *Phaulacridium vittatum* and the hemipteran *Nysius vinitor*, each had associated parasitoids (Table 1). Seed predators appear less important in *H. viscosum* where total destruction of a capitulum was never observed, and only three types of predispersal seed predators were found in observations over two reproductive seasons.

The most abundant seed predators on both *H. viscosum* and *H. bracteatum* were an undescribed cecidomyiid fly, a tephritid fly (*Tephritis* sp.) and the Rutherglen Bug, *Nysius vinitor*. Cecidomyiid larvae are small (<2 mm in length) and damage few seeds per individual. They are occasionally abundant (>50) in capitula but are heavily parasitized by an undescribed polygastrid wasp. Adults of both *Tephritis* sp. and *N. vinitor* use capitula as mating sites and feed at EFN. The host range of *Tephritis* sp. is unknown but larvae of the genus as a whole are commonly found in flowerheads of Asteraceae (Colless and McAlpine 1970). While few *Tephritis* sp. ovipositions were observed, females insert the ovipositor between overlapping layers of

bracts and eggs are probably laid on inner bracts or the peripheral floret primordia. Seed destruction by *Tephritis* sp. is direct (or via damage to the receptacle) and seeds not damaged by feeding or pupation appear intact. *Nysius vinitor*, an extreme generalist, attacks seeds of a variety of species in SE Australia (Kehat and Wyndham 1972). Adults lay eggs among florets and nymphs feed on developing ovules and seeds.

Study sites

Observations and experiments with *H. viscosum* were made at two sites. In 1977–1978, we set up a preliminary experiment on Black Mountain, A.C.T. (35°17'S, 149°07'E; 640 m elevation) in a disturbed east-facing stand of *Eucalyptus rossii*. The site was relatively open and the understory was dominated by *Cassinia longifolia*, *Daviesia mimosoides* and *H. viscosum*. In the 1978–1979 reproductive season we continued studies on the same plants at Black Mountain and established another site on a southwest-facing aspect of Mt. Ainslie, A.C.T. (35°17'S, 149°10'E; 720 m) with a dense understory of *Cassinia longifolia* and *H. viscosum*. Studies on *Helichrysum bracteatum* were conducted in 1978–1979 at Tallaganda State Forest, N.S.W. (35°38'S, 149°30'E; 1200 m) about 50 km southeast of Canberra City, A.C.T. Plants were numerous in clearings in *Eucalyptus pauciflora* forest, particularly in moist saddles between hills. At Tallaganda State Forest, we conducted observations and experiments at three sites separated by approximately one km each.

Extrafloral nectar

We made qualitative observations of extrafloral nectar production on *H. viscosum* in late October 1977 by excluding ants from capitula at Black Mountain. Quantification of secretion using microcapillary tubes was unsuccessful because nectar was either too viscous or too thinly spread over leaf and bract surfaces to collect. To determine the pattern of nectar production, we segregated capitula into developmental classes and noted accumulation of extrafloral nectar on subtending leaves or bracts after 24 h. Developmental classes beyond the flowering stage were not present on the plants when ants were initially excluded.

Systematic observations of nectar accumulation on *H. bracteatum* capitula following ant exclusion were not made. Extrafloral nectar was occasionally noted when ants were excluded but accumulation was usually prevented when other insects collected nectar in the absence of ants. Composition of EFN for both *Helichrysum* spp. was assessed by analysis of nectar collected from leaves and bracts subtending the receptacle. Relative proportions of sugar and amino acid complements were determined by 2-D chromatography (I. Baker, personal communication).

Ant-exclusion experiments

Ant-exclusion and access treatments were set up as follows. Prior to initiation of capitula (or shortly thereafter for the preliminary experiments at Black Mountain in 1977), 10 pairs of plants were selected at each site. To ensure as far as possible that each pair member would be equally attractive to ants and other arthropods, each was matched in rosette size and was in close proximity to the other (<1 m apart). One member of each pair was selected at random from which ants were excluded. Following elongation of the flowerstalk a band of Tanglefoot resin (The Tanglefoot Company, Grand Rapids, MI, USA) was painted around each flowerstalk to prevent ants from reaching the developing capitula. Leaves 5 cm above and below the barrier were removed to prevent the formation of natural bridges over the exclusion band. The other plant of each pair (ant-access) was treated in an identical manner except Tanglefoot barriers were not painted on the flowerstalks. Vegetation around each plant was trimmed as necessary to a radius of 10 cm to prevent access by ants.

The Tanglefoot barrier did not attract seed predators or restrict their access to capitula. All observed adult seed predators were mobile and could easily fly from plant to plant. Further, vertebrates were not important seed predators. A total of nine capitula were browsed by vertebrates (kangaroos or wallabies) on ant-access plants at Mt. Ainslie during flowerstalk elongation. Otherwise, they had no observed impact on either *Helichrysum* spp. at these sites.

Abundance of ants and other arthropods on capitula

Abundance of arthropods on *Helichrysum* was assessed to estimate any ant interference with other insects. A diurnal and seasonal census was made for *H. viscosum* at Black Mountain in 1977–1978. We counted ants (and other capitula visitors) every three h over three 36 h periods at two week intervals, covering the pre- to post-flowering period of capitula development. Since abundance of arthropods (other than ants) was extremely low on *H. viscosum* capitula at Black Mountain and Mt. Ainslie in 1978–1979, we discontinued the census of non-ant insects and relied on weekly measures of immature seed predators and capitula damage to assess any treatment effects. Insects other than ants were abundant on *H. bracteatum* capitula, so a weekly census was made at approximately mid-day at each site over the period of capitula development (17 observations).

To determine whether ants alter the position of other insects on plants we determined the number of insects in two locations on flowerstalks of *H. bracteatum* at Tallaganda Forest – on capitula (or the nectar-secreting leaves encircling each capitulum) and on non-secreting leaves subtending each capitulum. This was done for both ant-access and exclusion plants when insect activity was at its peak

(week 2). For *H. viscosum*, activity of insects other than ants was never great enough to determine whether ants affected their position on flowerstalks.

Parallel to field observations, mature capitula were harvested weekly (biweekly in 1977–1978) at all sites prior to seed shedding, placed individually in sealed envelopes and returned to the laboratory. All capitula were dissected and immature seed predators were identified and tallied. The receptacle and achenes were then examined for damage by seed predators and assigned a damage rating ranging from 0 (no damage) to 10 (complete destruction of the capitulum). This was done on each of three visits during the preliminary experiment at Black Mountain in 1977–1978 and over the subsequent reproductive season at Black Mountain (10 visits), Mt. Ainslie (6 visits) and Tallaganda State Forest (three sites, 16 visits each). Number of intact seeds per capitulum was determined from a subsample at each site.

Ant effects on pollination and parasitism

Attraction of ants to capitula by EFN may represent a two-edged sword. First, ants may interfere with the natural enemies of seed predators. If ants interfere with parasitoids of seed predators (Table 1), percent parasitism should be higher in the ant-exclusion treatments. We determined parasitism of *Tephritis* sp. in *H. bracteatum* capitula by holding all puparia recovered in the laboratory until adult emergence.

Second, ants may interfere with pollinators. Since substantial seed set only occurs in open-pollinated capitula (D.J. O'Dowd, unpublished results), interference with pollinators may reduce seed set in the ant-access treatments. Alternatively, EFN may augment floral attraction of pollinators if ants can serve as pollinators. This possibility was examined by bagging pre-flowering capitula in glassine envelopes. These capitula were divided into two treatments, one with the bag open at the base allowing access to ants and the other with a Tanglefoot barrier which excluded ants. If ants play a positive role in pollination, we would expect greater seed set in the ant-access treatment.

Analysis of the ant-exclusion experiments

Two basic sets of data were collected in the ant-exclusion experiments: (1) field observations of ant and other insect activity on capitula, and (2) laboratory dissections based on immature seed predators within capitula. These two sets cannot be directly related because of the variable (and unknown) time lag in the development of capitula from predation to laboratory dissection. Thus, the two sets of data were analysed separately. Analysis of the ant-exclusion experiments for each *Helichrysum* spp. was complicated by two features of the data. First, the experiments were necessarily non-orthogonal in that the number of capitula to which each treatment was applied was variable among plants, between sites and through time. The simple difference between access and exclusion values does not give an unbiased estimate of any treatment effect. We allowed for this by fitting a linear model

$$y = \text{week} + \text{site} + \text{site} \cdot \text{pair} + \text{treatment} + \text{error} \quad (1)$$

where y is the dependent variable INSPC (number of non-ant insects per capitulum), NPRED (number of immature seed predators per capitulum) or DAMAGE (damage rating per capitulum). This enables an estimate of the average

Table 2. Accumulation of extrafloral nectar on capitula of *Helichrysum viscosum* following ant exclusion, Black Mountain A.C.T., 24–25 October 1977. Developmental classes of capitula are as follows: I=bracts <1 cm in length, II=bracts <1.5 cm, III=bracts >1.5 cm and deflexing and IV=bracts deflexed and flowering. Number of capitula is in parentheses. Values are proportions of capitula examined

	Developmental stage				Total
	I	II	III	IV	
Extrafloral nectar present	0.85 (23)	0.72 (18)	0.82 (23)	0.29 (2)	0.76 (66)
leaf	0.81 (22)	0.48 (12)	0.07 (2)	0.00 (0)	0.41 (36)
bract	0.00 (0)	0.08 (2)	0.71 (20)	0.29 (2)	0.28 (24)
both	0.04 (1)	0.16 (4)	0.04 (1)	0.00 (0)	0.07 (6)
Extrafloral nectar absent	0.15 (4)	0.28 (7)	0.18 (5)	0.71 (5)	0.24 (21)

treatment effect over each experiment to be made. For INSPC with *H. bracteatum*, a measure of ant abundance was also included in (1), allowing for the variable nature of the treatment (i.e., the uncontrolled “dose” of ants on access plants). This allowed us to test whether the size of any ant-exclusion effect on INSPC depended on the level of ant activity on capitula. It was not possible to do this with the laboratory observations because of the time-lag problem noted above. For *H. viscosum* at Black Mountain and Mt. Ainslie, sites were not compared because initiation and completion of capitula development were not parallel. While weekly visits to the sites overlapped, they did not coincide. The model (1) reduced to week, pair and treatment effects at each site. For *H. bracteatum*, we further determined the average effect of excluding ants from capitula on the dependent variable for each weekly set of observations using the model

Results

Secretion of extrafloral nectar

EFN attracted and maintained ants on the capitula of both *H. bracteatum* and *H. viscosum*. Secretion was rarely seen in the field unless ants were excluded from capitula. Seventy-six percent of *H. viscosum* capitula examined following ant-exclusion accumulated extrafloral nectar overnight, forming a viscous syrup on the leaves subtending the bracts or on the bracts themselves (Table 2). Capitula at early developmental stages generally produced most nectar from the subtending leaves. More fully developed capitula secreted nectar from the bract bases only. This shift in nectar production from leaf to bract was generally concurrent with bract deflexion and usually preceded flowering by one to two weeks. With *H. viscosum*, extrafloral secretion occurred on all developmental stages of inflorescences observed.

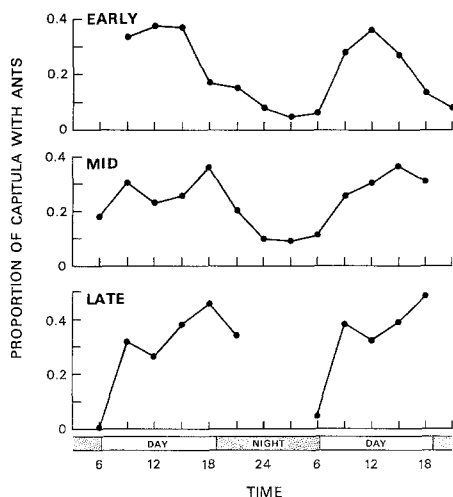


Fig. 1. The diurnal pattern of ant activity of *Helichrysum viscosum*, Black Mountain, A.C.T., 1977. Activity between 2100 and 600 h on 15–16 November was not recorded. $N=86$ capitula for “early” (18–19 October), 91 for “mid” (1–2 November) and 85 for “late” (15–16 November)

Number of ants on capitula varied diurnally, seasonally, among individual plants and between sites. Rhythmic diurnal shifts occurred in ants per capitulum on *H. viscosum* at Black Mountain, being highest during mid-day and reaching a low in the early morning (Fig. 1). Changes in the species composition of ants accompanied this shift in total ant activity. *Iridomyrmex purpureus*, the most abundant ant on *H. viscosum* capitula during daylight, retired to its nests at dusk. This corresponded to increased nocturnal foraging activity at capitula by other ant species, especially *Notoncus ectatommoides*.

Seasonal patterns in ant activity at capitula differed between the two plant species. Activity levels at Black Mountain were similar in early and midseason but increased later in the reproductive period (Table 3). Stronger seasonal shifts were observed on capitula of *H. bracteatum* but ant activity peaked early in the reproductive period (Fig. 2) and reached low levels as the proportion of mature capitula increased.

The number of ants per capitulum also varied between plants at a given site and between sites. Ant activity on capitula of *H. viscosum* was variable within a site, showing a twenty-fold difference among individual plants at Mt. Ainslie and a four-fold difference at Black Mountain. For *H. bracteatum* at Tallaganda Forest, ant activity varied strongly, both among plants within a site and among sites (Fig. 3).

Effects of ants on other arthropods

Ants were the most numerous arthropods observed on the capitula of both *Helichrysum* spp. They accounted for 95% of the arthropods associated with *H. viscosum* capitula, where other arthropods were never a major component of observed visitors on ant-access capitula (Table 3). On *H. bracteatum*, other arthropods (mostly Diptera and Coleoptera) were much more active on capitula and ants accounted for only 59% of the total arthropods observed on ant-access capitula.

Ants can significantly affect number of arthropods associated with capitula (Table 3, Fig. 4). On *H. viscosum* at

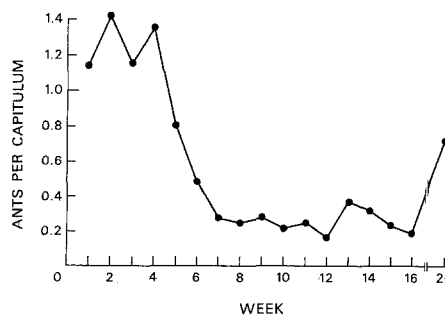


Fig. 2. Variation through time in ants per capitulum on *H. bracteatum*, Tallaganda State Forest, N.S.W., (January–May 1979). Data are pooled for the three sites

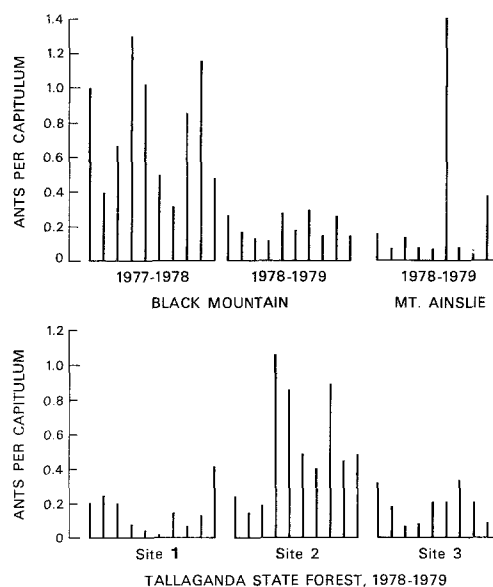


Fig. 3. Variation in ants per capitulum between plants for *H. viscosum* at Black Mountain (1977–1978 and 1978–1979), Mt Ainslie (1978–1979), Tallaganda State Forest (1978–1979). The average number of ants per capitulum per plant ($N=10$ plants at each site) is pooled over all weeks for each site

Black Mountain in 1977–1978, abundance of non-ant arthropods on capitula was very low until late in the reproductive season. Overall, significantly more non-ant arthropods were observed on ant-excluded capitula than on ant-access capitula ($G=102.6$, $df=1$, $P<0.001$, Goodness-of-fit test). Further, the effect of ants on other arthropods was dependent on season (for the 3-way interaction, $G=22.0$, $df=2$, $P<0.001$), the treatment effect being greatest later in the reproductive period when more ants and other arthropods were present. Ants also selectively reduced certain types of capitula visitors. Coleoptera were most strongly affected by ants (117 observations on exclusion vs. 9 on ant-access treatment) although reduction in numbers of Dipterans and Hymenopterans (primarily bees) was also marked (Table 3). On *H. bracteatum*, a significant increase in arthropod numbers also occurred on ant-exclusion capitula (Fig. 4a, b). There was a marked seasonal influence on exclusion which was most evident early in the season and corresponded to the highest levels of activity for both ants (Fig. 2) and other insects (Fig. 4). Later in the season when ant and other insect numbers were much

Table 3. Observations of arthropods on capitula of *Helichrysum viscosum* on ant-access and exclusion plants, Black Mountain, A.C.T., 1977. "Early" season, "mid" season and "late" season observations were made on 18–19 October, 1–2 November and 15–16 November, respectively

Treatment	Total observations of capitula	Number of arthropod observations		Predominant taxa on capitula (% of total arthropods observed)					
		ant	non-ant						
Ant-access									
“early”	1204	313	9	ants	(97)	flies	(2)	others	(1)
“mid”	1274	338	28	ants	(92)	flies	(4)	others	(4)
“late”	1020	450	21	ants	(96)	beetles	(2)	others	(2)
Total	3498	1110	58	ants	(95)	flies	(2)	others	(3)
Ant-exclusion									
“early”	1330	0	13	flies	(54)	spiders	(23)	others	(23)
“mid”	1414	0	63	flies	(40)	bees	(27)	others	(33)
“late”	1176	2	163	beetles	(71)	flies	(16)	others	(13)
Total	3920	2	239	beetles	(49)	flies	(22)	others	(29)

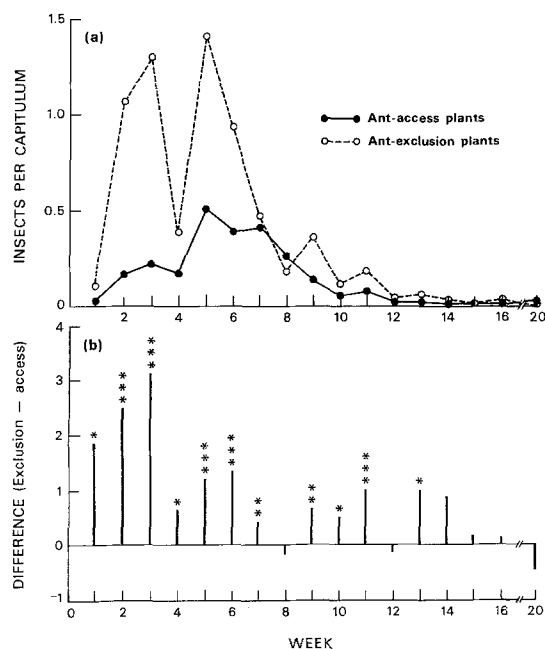


Fig. 4a, b. The effect of treatment (ant-access or exclusion) on other capitula visitors of *H. bracteatum* at Tallaganda State Forest, N.S.W., 1978–1979. **a** Number of non-ant insects per capitulum on ant-access and ant-exclusion plants. **b** Weekly effect of ants on other capitula visitors as determined by the logit model (3). Difference between the ant-exclusion and ant-access treatment is expressed as the log-odds difference and shows the proportional increase (or decrease) in odds-ratio from access to exclusion plants. Weekly effects of treatment of INSPC and $\log(\text{INSPC} + 1)$ estimated from (2) were similar and are not included here. Significance as follows: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. All other values are not significant

lower, relative differences between access and exclusion treatments were not as marked and absolute differences were much smaller. Overall, there was a significant effect of ant-exclusion on the number of other insects associated with *H. bracteatum* capitula (Table 4). The transformed variable $\log(\text{INSPC} + 1)$ and the arcsine transformation APWINS fit the data appreciably better than INSPC, indi-

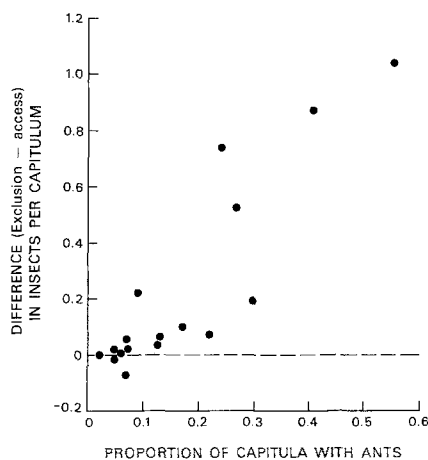


Fig. 5. Relationship between weekly ant abundance (proportion of capitula with ants) and treatment effects (ant-exclusion and ant-access) on number of non-ant capitula visitors of *H. bracteatum*, Tallaganda State Forest, N.S.W., (December 1978–May 1979). Treatment effects were estimated from model (2) with $y = \text{INSPC}$

Table 4. Summary of ANOVA fitting model (1) for effects of season, site, treatment (ant-access or exclusion) and ant abundance on measures of non-ant insect abundance on *H. bracteatum* capitula, Tallaganda Forest, N.S.W., 1978–1979. INSPC = non-ant insects per capitulum, APWINS = modified arcsine transformation of proportion of capitula with non-ant insects and APWANT = corresponding transformation for ants. NS = not significant, * $P < 0.05$, *** $P < 0.001$

Variable	df	F-values		
		INSPC	$\log(\text{INSPC} + 1)$	APWINS
week	16	23.72***	38.91***	45.82***
site	2	1.64 ^{NS}	1.36 ^{NS}	3.34*
site·pair	27	1.98***	2.67***	2.60***
treatment	1	36.37***	58.52***	61.49***
APWANT	1	33.44***	59.64***	52.78***
Variance explained		38%	51%	54%

Table 5. Summary of results of ant-exclusion experiments (ant-access and ant-exclusion treatments) on seed predators from capitula of *Helichrysum viscosum* (Black Mountain and Mt. Ainslie) and *H. bracteatum* (Tallaganda Forest). Sites at Tallaganda Forest are pooled. N given for intact seeds per capitulum is a subsample of total capitula in each treatment

	Black Mountain				Mt. Ainslie		Tallaganda Forest	
	1977–1978		1978–1979		1978–1979		1978–1979	
	Access	Exclusion	Access	Exclusion	Access	Exclusion	Access	Exclusion
% capitula with seed predators	15	11	30	26	38	31	61	56
Immature seed predators per capitulum (SD)	0.6 (2.6)	0.6 (2.1)	2.2 (5.8)	1.2 (3.0)	1.9 (4.3)	1.6 (3.9)	7.3 (15.5)	5.6 (10.5)
Damage rating per capitulum (SD)	0.3 (0.7)	0.4 (0.8)	0.6 (1.4)	0.4 (0.9)	0.4 (1.1)	0.4 (1.2)	1.6 (2.2)	1.5 (2.2)
Intact seeds per capitulum (SD)	237.6 (62.6)	252.3 (69.0)	275.2 (87.4)	221.0 (107.8)	208.7 (137.5)	206.3 (114.6)	71.3 (87.9)	68.6 (94.9)
N	19	20	21	21	19	22	331	374
Number of capitula examined	124	177	121	137	118	157	736	776

cating that ant effects on activity of other insects tend to be multiplicative rather than additive.

The number of non-ant insects on capitula of *H. bracteatum* was also significantly influenced by the abundance of ants at each weekly observation (Fig. 5, Table 4). When ant activity levels were high, the effect of ant exclusion on the abundance of other insects on the capitula was greater. The proportion of capitula with ants (or its arcsine transformation, APWANT) when used as a covariate accounted for a greater amount of variation than did the number of ants per capitulum. Thus, the spread of ants over the plant appears to be more effective than the actual number of ants per capitulum on the plant in depressing activity of other insects.

The ant-exclusion treatment also affected the positions of insects on the flowerstalks when both ants and other insects were abundant on *H. bracteatum*. During week 2 (Figs. 2 and 4), a seven-fold increase in insects occurred on ant-exclusion capitula (7 on access vs. 50 on exclusion treatments). Total numbers of other insects on the flowerstalks increased as well, but only two-fold (57 on access vs. 112 on exclusion treatments). Treatment significantly altered the position of insects on flowerstalks ($G=19.6$, $df=1$, $P<0.001$), where most insects on ant-access plants were relegated to leaves subtending the capitula. Thus, ants altered the distribution of other insects as well as decreased their absolute numbers on the flowerstalk.

Ants and seed predators

The most numerous adult seed predator, *Tephritis* sp., accounted for 11% of all non-ant insects on *H. bracteatum* (178 of 1592 observations) and 9% of insects observed on *H. viscosum* capitula (27 of 297). While the ant-exclusion treatment increased non-ant insect numbers two-fold on *H. bracteatum* and four-fold on *H. viscosum* overall, *Tephritis* adults were not so strongly affected. *Tephritis* numbers on ant-access capitula remained relatively high, and accounted for 45% and 41% of total observations of *Tephritis* on *H. bracteatum* and *H. viscosum* capitula, respectively.

On *H. bracteatum*, when ant and insect activity was high (week 2), position of adult *Tephritis* sp. on the flowerstalk was dependent on treatment. On plants with ant-access just 8% (two of 24 observations of *Tephritis*) occurred on capitula while on ant-exclusion plants, 58% (11 of 18) of the *Tephritis* were on capitula. Thus, treatment did not decrease the number of *Tephritis* associated with plants (24 observations on ant-access vs. 19 on ant-exclusion for week 2) *per se* but significantly altered their position from capitula to non-nectar secreting regions of the flowerstalk ($P\leq 0.001$, Fisher's exact test).

An overall summary of results from the ant-exclusion experiments based on laboratory dissections of capitula is presented in Table 5. Immature seed predators and their effects were much less evident on *H. viscosum* than on *H. bracteatum*, which corresponded to the infrequent observation of adult seed predators at Black Mountain and Mt. Ainslie. The effect of ant-exclusion on releasing arthropod numbers on capitula (Table 3, Fig. 4) did not lead to increased numbers of immature seed predators or higher damage levels to capitula (Table 5).

For *H. bracteatum*, ANOVA using the global model (1) indicated that there was no significant overall treatment effect on seed predators or on the associated damage rating (Table 6a). Variation from week to week and from site to site was very large. Separate analyses performed on the three most abundant seed predators in capitula (the undescribed cecidomyiid fly, *Tephritis* sp. and *Nysius vinitor*) produced results similar to NPRED.

Results for *H. viscosum* at Black Mountain and Mt. Ainslie generally concurred with those on *H. bracteatum* and indicated that week of observation accounted for most of the explained variation (Table 6b–d). No treatment effect was observed at Black Mountain in 1977–1978 or Mt. Ainslie in 1978–1979 but significant overall differences in NPRED and DAMAGE occurred at Black Mountain in 1978–1979. Number of seed predators per capitulum and damage levels to capitula were higher in the ant-access treatment, contrary to the predictions of the ant-guard hypothesis.

Table 6. Summary of ANOVA fitting model (1) for effects of season, site and treatment (ant access or exclusion) on seed predators and their effect on capitula of (a) *H. bracteatum*, Tallaganda Forest, N.S.W., 1978–1979 (b) *H. viscosum*, Black Mountain, A.C.T., 1977–1978 (c) *H. viscosum*, Black Mountain, A.C.T., 1978–1979 (d) *H. viscosum*, Mt. Ainslie, A.C.T., 1978–1979. NPRED = number of immature seed predators per capitulum, DAMAGE = capitula damage rating. NS = not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. The signs before the F-value for treatment indicate whether the data supported (+) or ran counter to (–) the ant-guard hypothesis

Variable	df	F-values		
		NPRED	log(NPRED + 1)	DAM-AGE
a) Tallaganda Forest 1978–1979				
week	15	49.5 ***	29.6 ***	26.1 ***
site	2	54.2 ***	85.2 ***	65.8 ***
site·pair	27	3.9 ***	7.5 ***	5.6 ***
treatment	1 (–)	0.9 ^{NS}	(–) 2.0 ^{NS}	(–) 3.4 ^{NS}
b) Black Mountain 1977–1978				
week	2	4.6 *	5.6 *	9.6 **
pair	9	2.4 ^{NS}	2.1 ^{NS}	1.6 ^{NS}
treatment	1 (+)	0.8 ^{NS}	(–) 0.1 ^{NS}	(–) 1.7 ^{NS}
c) Black Mountain 1978–1979				
week	9	9.1 ***	10.2 ***	8.2 ***
pair	9	1.7 ^{NS}	3.6 ***	2.5 *
treatment	1 (–)	11.7 ***	(–) 10.2 ***	(–) 8.7 **
d) Mt. Ainslie 1978–1979				
week	5	6.1 ***	5.3 ***	29.2 ***
pair	9	5.3 ***	4.1 ***	2.3 *
treatment	1 (–)	2.5 ^{NS}	(–) 2.5 ^{NS}	(+) 0.1 ^{NS}

There was no evident seasonal pattern in treatment effect on number of seed predators per capitulum or capitula damage for *H. bracteatum* (Figs. 6a and 7a). Estimated treatment effects from model (2) for weekly ANOVA showed the majority of weeks with negative regression coefficients which ran counter to the ant-guard hypothesis and indicated more seed predators on ant-access capitula (Fig. 6b). For NPRED, however, only a single significant difference (week 6) occurred. A similar picture was obtained with the log transformation of NPRED with values for weeks 5 and 6 being significantly different. For DAMAGE (Fig. 7b), weeks 12 and 16 supported the ant-guard hypothesis while week 9 ran counter to the hypothesis; no other week gave a statistically significant result.

Pollination and parasitism

Ants did not augment or interfere with pollination as measured by seed set per capitulum in ant-access and exclusion treatments (Table 5). No difference was found in seed set between capitula which were bagged but allowed ant access and those which were bagged and excluded ants ($U = 73$, $n_1 = 13$, $n_2 = 11$, $P > 0.05$, Mann-Whitney U-test).

Tephritis sp. at Tallaganda Forest was heavily parasitized by *Megastigmus* sp., increasing from 40% of puparia in early-maturing capitula (January) to 90% in capitula collected at the end of the season (May) (Fig. 8). The ant-exclusion treatment did not significantly influence the parasitoid load of *Tephritis* sp. ($G = 3.2$, $df = 1$, $P > 0.05$, Good-

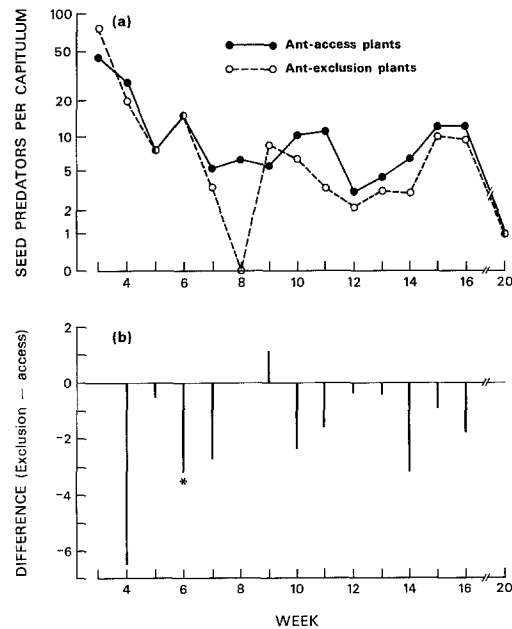


Fig. 6a, b. The relationship between treatment and the number of immature seed predators recovered from *H. bracteatum* capitula, Tallaganda State Forest, N.S.W., (January–May 1979). **a** Number of seed predators per capitulum on ant-access and ant-exclusion plants. Note that y-axis is log scale. **b** Weekly effects of ants on number of immature seed predators per capitulum estimated by model (2). For results to support the ant guard hypothesis weekly differences (exclusion – access) should be positive. No capitula were harvested in weeks one and two. Weeks 3 and 8 are omitted because of the small number of capitula harvested in these weeks. Significance as follows: * $P < 0.05$. All others are not significant

ness-of-fit test) but percent parasitism was significantly dependent on season ($G = 61.8$, $df = 4$, $P < 0.001$).

Discussion

Extrafloral nectar is a valuable food and the list of arthropods which collect it is long (Keeler 1977). It can increase the longevity and fecundity of insects which consume it (Maxwell et al. 1976) and in these *Helichrysum* spp., is attractive to ants, potential pollinators, seed predators and their parasitoids (Table 1). A significant overall increase in non-ant insects on capitula from which ants were excluded (Tables 3 and 4) suggests two possible effects on the plants: (1) increased damage to capitula, since seed predators are a subset of total arthropod observations; or, (2) since both *H. viscosum* and *H. bracteatum* are obligately outcrossed (O'Dowd, unpublished results), increased insect numbers on ant-excluded capitula could raise seed set. Neither of these outcomes was realized on capitula of either *Helichrysum* spp. Ants appear to have no positive effect on the outcome of overall capitula interactions (Tables 5, 6). These results are surprising, particularly when ants form the majority of capitula visitors (Table 3) and interfere with other arthropods (Table 4 and Fig. 5).

Several factors may contribute to the lack of any effect of ants on capitula events. First, an ant-guard effect is predicated on the coincidence of ants and seed predators. Our results show that ants are less than predictably associated with both *Helichrysum* spp., vary through time ((diurnally

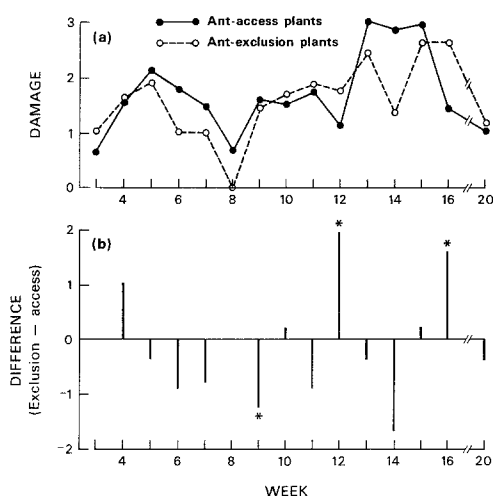


Fig. 7 a, b. The relationship between treatment and damage to capitula of *H. bracteatum* by seed predators, Tallaganda State Forest, N.S.W. (January–May 1979). **a** Damage ratings for capitula on ant-access and ant-exclusion plants. **b** Weekly treatment effects on the damage rating as estimated by model (2). Significance as follows: * $P < 0.05$. All others are not significant

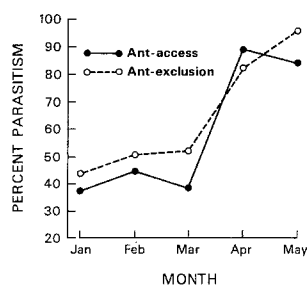


Fig. 8. Parasitism of *Tephritis* sp. by *Megastigmus* sp. in capitula of *H. bracteatum*, Tallaganda State Forest, N.S.W. (January–May 1979). Parasitism is expressed as percent *Megastigmus* emergences of total emergences from puparia. Treatments are ant-access and ant-exclusion. Number of total emergences for January = 50, February = 102, March = 101, April = 56 and May = 45

(Fig. 1) and seasonally (Fig. 2)), between sites (Fig. 3), and among plants within a site (Fig. 3). Since ants are not billeted on plants, intensity and fidelity of attendance of capitula relies on (1) the attractiveness of capitula as defined by extrafloral nectar secretion (Bentley 1977; O'Dowd 1979); (2) the availability of alternate food resources; (3) the location and density of ant nests relative to plants (Inouye and Taylor 1979); and, (4) environmental or circadian constraints on ant activity (O'Dowd 1979). This variability is further amplified by the diversity of ant species attending capitula on any one plant or at a site.

Likewise, seed predator abundance (both adults and immatures) varied seasonally (Fig. 6), between sites (Table 6), and among plants within a site (Table 6). For *H. viscosum* at Black Mountain and Mt. Ainslie, the density of adult seed predators may have been too low for ant-seed predator interactions to be detected. For *H. bracteatum*, however, the times of greatest abundance of ants and other insects coincided (Figs. 2 and 4) and numbers of immature seed predators in capitula (Table 5) approached those in the only other comparable system reported (Inouye and Taylor 1979).

Second, while ants altered the total number of other arthropods found on capitula, they did so selectively and some taxa were more strongly affected than others (Table 3). None of the seed predator taxa observed were negatively influenced by ants. Some seed predators may show activity patterns that coincide with low ant activity during night. The phycitid moth, for example, is nocturnally active and is presumably less likely to be encountered by ants when ovipositing. Additionally, some predators may be "immune" to ant interference. The grasshopper (*Phaulacridium vittatum*) was never observed to be attacked by ants perhaps because of its large size and the fact that it fed on the disc florets where it was infrequently encountered.

Third, ants may not have as strong an effect in removing insects from the flowerstalk as in altering their position on the flowerstalks. Some insects displaced from capitula by ants probably move to adjacent plants with ant-excluded capitula and account for the increased numbers of non-ant insects there (Fig. 4, Table 4). Others simply shift microposition, moving off the capitula to subtending leaves on the flowerstalk. This pool of "off-capitula" insects is dynamic and shifts strongly back to the capitula when ants depart. This same pattern is true for the seed predator, *Tephritis* sp., but our results indicate that this position effect of ants is insufficient to alter incidence of seed predation.

A trend intimating that ant-exclusion decreases numbers of seed predators and capitula damage is intriguing (Table 6), and, of course, runs counter to the ant-guard hypothesis. We thought initially that an unconscious biasing of plants in the initial pairing might cause this unexpected trend but pairing of plants at Tallaganda Forest showed no bias towards ant-access or exclusion treatments in number of capitula per plant. Pairing accounted for 83% of the total variation in capitula numbers. The only way we can explain this trend is if the presence of ants attracts more adult seed predators or increases survivorship of immatures in the capitula. Some plant species reduce nectar production or reabsorb nectar when it accumulates in flowers that are not visited (Corbet 1978; Koptur, in press). Exclusion of ants from capitula may slow flow rates of extrafloral nectar when it accumulates and evaporates leaving a viscous syrup over the nectary surface. Hence, access capitula may produce more extrafloral nectar and, overall, be more attractive than ant-exclusion capitula. If ants do not influence major seed predators then more oviposition may follow on the ant-access treatment.

Several limitations can be placed on the interpretation of our results: (1) EFN-mediated protection may still occur by organisms other than ants that were not influenced by the exclusion treatments. Both of the parasitoids, *Diadegma* sp. and *Megastigmus* sp., feed at EFN and may depress the numerical response of their hosts. A treatment effect on parasitism of *Tephritis* sp. by *Megastigmus* sp. did not occur (Fig. 8), so ants did not interfere with this parasitoid; (2) selection for EFN may not be continuous but variable through time. EFN may be maintained by infrequent periods of intense selection followed by long periods of relaxation. Short-term (2–3 year) studies of these interactions may examine "ordinary" years where the unusual conditions generating ant-guard effects are not present; and, (3) EFN may be relictual, vestiges from a time when ant-guard effects were important but have yet to be "selected away" (Darwin 1877).

Alternative hypotheses

While these results are contrary to the predictions of the ant-guard hypothesis, they do not resolve the biological significance of EFN on these *Helichrysum* spp. Alternative hypotheses follow: (1) EFN may be important in attraction of pollinators, augmenting visual attraction by the showy bracts. Certainly, many of the potential pollinators (e.g., syrphid flies, honeybees, butterflies) feed both at florets and EFN. Ants, however, are the predominant recipients of extrafloral nectar and they do not affect pollination; (2) EFN may distract ants from florets, thereby preventing nectar-thieving and interference with pollinators. Two features of these *Helichrysum* spp. argue against this hypothesis. First, floret structure prevents all but the smallest of ants (e.g., *Monomorium* sp.) from collecting floral nectar. Second, extrafloral nectar secretion occurs from early capitulum development through post-seed development, inconsistent with a brief role during flowering alone; and, (3) EFN may serve a physiological role, any ecological interaction being incidental. Many authors suggest that extrafloral nectar is an excretion of surplus carbohydrates, particularly in situations where nitrogen is limiting (Shuel 1954, Frey-Wyssling 1955). Generally, excretion is thought to occur as organs associated with EFN shift from a "sink" to a "source" of carbohydrates (Bentley 1977). In these *Helichrysum* spp., however, the developing capitulum never becomes a carbohydrate source yet nectaries remain active throughout development. Further, amino-acid diversity and concentration of the extrafloral nectar is great. If nectar is an excretion, the plant is dumping "excess" nitrogenous compounds along with carbohydrates.

A large number of temperate and tropical plant species secrete extrafloral nectar. K.H. Keeler (personal communication) estimates that 80 plant families have representatives with EFN. A general role of EFN as a deployment system for "ant-defenders" has sparked controversy for over a century (Delpino 1886; von Uxkull-Guldenbrandt 1907; Wheeler 1910). Although several studies experimentally demonstrate that ants protect plants (von Wettstein 1889; Inouye and Taylor 1979; Schemske 1980), our results indicate that it will be difficult to generalize the ant-guard hypothesis to all plants with EFN. The role of plant-ant interactions inferred from morphological and behavioral considerations alone or based on indirect evidence remains tentative without corresponding field experiments.

Acknowledgements. J. Green ably assisted both in field work and laboratory dissection of capitula. A.L. O'Dowd helped with seed counts. We thank the following taxonomists of CSIRO, Division of Entomology for identification of insects: D.H. Colless (Diptera), I. Common (Lepidoptera), J.F. Lawrence (Coleoptera), I. Naumann (non-formicine Hymenoptera), D. Rentz (Orthoptera), R.W. Taylor (Formicidae) and T. Weir (Hemiptera). H.G. and I. Baker graciously conducted sugar and amino acid analyses of nectar. The Department of Capital Territory, Agriculture and Conservation Branch granted permission for study in Black Mountain and Mt. Ainslie reserves. Statistical analysis of the data was begun in collaboration with J. Haslett. Comments by A.M. Gill, K.H. Keeler and A.D. Wilson improved the manuscript.

References

- Baker HG, Baker I (1977) Intraspecific constancy of floral nectar amino acids. *Bot Gaz* 138:183-191
- Baker RJ, Nelder JA (1978) The GLIM System 3 Release Manual. Oxford, Numerical Algorithms Group
- Bentley B (1977) Extrafloral nectaries and protection by pugnacious bodyguards. *Ann Rev Ecol Syst* 8:407-427
- Colless DH, McAlpine DK (1970) Diptera. In C.S.I.R.O. The Insects of Australia. Melbourne University Press. Victoria. pp 656-674
- Corbet SA (1978) Bee visits and the nectar of *Echium vulgare* L. and *Sinapis alba* L. *Ecol Ent* 3:25-38
- Darwin F (1877) On the glandular bodies of *Acacia sphaerocephala* and *Cecropia peltata* as food for ants, with an appendix on the nectar-glands of the common brake fern, *Pteris aquilina*. *Bot J Linn Soc (Lond)* 15:398-408
- Delpino F (1886) Funzione mirmecofila nel regno vegetale. *Memorie della R. Accademia delle Scienze dell'Istituto di Bologna, Serie Quarto Tomo VII*: pp 215-323
- Freeman MF, Tukey JW (1950) Transformations related to the angular and square root. *Ann Math Stat* 21:607-611
- Frey-Wyssling A (1955) The phloem supply to nectaries. *Acta Bot Neer* 4:358-369
- Inouye DW, Taylor OR (1979) A temperature plant-ant-seed predator system: consequences of extrafloral nectar secretion by *Helianthella quinque nervis*. *Ecology* 54:1-8
- Janzen DH (1969) Seed-eaters versus seed size, number, toxicity and dispersal. *Evolution* 23:1-27
- Keeler KH (1978) Insects feeding at extrafloral nectaries of *Ipomoea carnea* (Convolvulaceae). *Ent News* 89:163-168
- Kehat M, Wyndham M (1972) The influence of temperature on development, longevity, and fecundity in the Rutherglen bug, *Nysius vinitor* (Hemiptera: Lygaeidae). *Aust J Zool* 20:67-78
- Koptur S (1983) Flowering phenology and floral biology of *Inga* (Fabaceae: Mimosoideae). *Syst Bot*, in press
- Maxwell FG, Schuster MF, Meredith WR, Laster ML (1976) Influence of the nectariless character in cotton on harmful and beneficial insects. In Jermy T (ed) *The Host-Plant in Relation to Insect Behavior and Reproduction*. *Symp Bio Hung* 16:157-161
- Morley B (1978) *Helichrysum bracteatum* and allies. *J Roy Hort Soc* 103:489-491
- O'Dowd DJ (1979) Foliar nectar production and ant activity on a neotropical tree, *Ochroma pyramidale*. *Oecologia (Berlin)* 43:233-248
- Schemske DW (1980) The evolutionary significance of extrafloral nectar production by *Costus woodsonii* (Zingiberaceae): an experimental analysis of ant protection. *J Ecol* 68:959-967
- Shuel RW (1954) Nectar secretion in relation to nitrogen supply, nutritional status, and growth of the plant. *Can J Agric Sci* 35:124-138
- Uxkull-Guldenbrandt von, Nieuwenhuis M (1907) Extraflorale Zuckerausscheidungen und Ameisenschutz. *Annales du Jardin Botanique de Buitenzorg* 21:195-327
- Wettstein RR von (1889) Über die Composition der österreichisch-ungarischen Flora mit zuckerabscheidenden Hüllschuppen. *Österreichische Akademie der Wissenschaften. Sitzungsberichte. Abteilung I. Mineralogie, Krystallographie, Botanik* 97:570-589
- Wheeler WM (1910) *Ants, their Structure, Development and Behavior*. Columbia University Press, New York

Received March 14, 1983