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Unexpected reactions of a generalist predator towards defensive devices of cassidine larvae (Coleoptera, Chrysomelidae)

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Abstract Studies of multitrophic interactions show that insect faeces may act as a defensive device against predators, as kairomone source for attraction of antagonists and as a significant energy source for microorganisms. In the present study, we investigated effects of larval faeces from leaf beetles of the subfamiliy Cassidinae towards a generalist predator, the ant *Myrmica rubra*. Most cassidine larvae collect their faeces together with exuviae as so-called abdominal defensive shields on two movable spines at the posterior tip. The effects of these abdominal shields towards *M. rubra* were studied in three cassidine species, which feed mono- or oligophagously upon tansy (*Chrysanthemum vulgare*): *Cassida denticollis* and *C. sanguinosa* which possess such faecal shields and, for comparison, *C. stigmatica* with shields made of exuviae only (= skin shield). Bioassays revealed that larvae with both faecal and skin shields were attacked by the ant *M. rubra* more often than larvae whose shields had been removed. This attractiveness of shields towards ants contrasts with other studies, which found that abdominal shields of chrysomelid larvae act as defensive mechanisms against generalist predators like ants. To characterize the attractive cues of the shields, we studied possible chemical and physical stimuli. Olfactometer bioassays with *M. rubra* and chemical analyses revealed that plant-derived volatiles from faecal shields of *C. denticollis* attracted the ant, whereas odour from skin shields of *C. stigmatica* did not. Skin shields also emitted volatiles which derived from tansy, but in much lower quantities. Exclusion of contact to surface chemicals of a faecal shield reduced the ants' aggressive behaviour, whereas a change in the moisture content of a faecal shield had no influence. Visual stimuli cannot be ruled out as enhancing the ants' reaction towards faecal shields with their attractive volatiles, and are suggested to play a major role in the ants' response towards skin shields. This novel attractive effect of the abdominal shields of cassidine larvae is discussed, especially with respect to host plant chemistry and possible functions of the shields that might outweigh the negative consequences of the attraction of the predator *M. rubra*.

Key words *Cassida* spp · Faeces · Kairomone · Larval shield · *Myrmica rubra*

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Introduction

Faeces of phytophagous insects may play a role in numerous multitrophic interactions, for example through nutritional recycling, influencing microbial activity (Stadler and Müller 1996) or by mediating communication between organisms. Signals from insect faeces may be used as pheromones (e.g. Byers 1989) or as defensive devices against enemies (Whitman et al. 1990). On the other hand, cues from faeces of phytophagous insects may be used as kairomones by predators and parasitoids to locate their prey (e.g. Jones and Finch 1987; Nordlund et al. 1988; Grewal et al. 1993; Olmstead 1994; Köpf et al. 1997; Steidle and Schöller 1997).

Several Chrysomelidae are known to use physical (stickiness, toughness) features and chemicals of faeces for defensive purposes (Pasteels et al. 1988a). *Pyrrhalta viburni*, for example, covers its eggs with faeces which contain high concentrations of bitter-tasting toxic host plant triterpenes (Hilker 1992). Some chrysomelid species even have distinct morphological features to form and collect their faeces. For example, females of *Clytra* species construct egg cases from tiny and flat plates of excrements, which are compressed by chitinous structures of the rectum, soaked by a secretion and then glued to eggs (Erber 1968). Cassidine larvae have two movable abdominal spines onto which they collect faeces and/or exuviae with each defecation and moult. The function

of the abdominal shield of cassidine larvae has been addressed in several studies (Engel 1936; Olmstead and Denno 1993; Gómez 1997) and found to be a defence against predators (Eisner et al. 1967; Vencl and Morton 1998).

In the present study, we investigated the effects of larval shields of *Cassida* spp. that feed upon tansy towards the ant *Myrmica rubra*, a generalist predator. Tansy (*Chrysanthemum vulgare*, Asteraceae) is well known to contain numerous bioactive components, among them insect repellents (Panasiuk 1984; Schearer 1984; Teuscher and Lindequist 1994) which might be discharged by tansy-feeding herbivores with their faeces for defensive purposes. Several herbivorous insects protect themselves by sequestering compounds of the food plant, which have a feeding deterrent or repellent effect on predators (Duffey 1980; Pasteels et al. 1988b; Harborne 1993; Dettner et al. 1997). While *Cassida stigmatica* feeds only on tansy, *Cassida denticollis* and *Cassida sanguinosa* use tansy and other Asteraceae of the tribe Anthemideae (Koch 1992). These latter two oligophagous larvae build faecal shields, which consist of faeces and exuviae. *C. stigmatica* carries a skin shield, made of exuviae only without added faeces. The effects of such skin shields towards a natural enemy were examined for the first time in this study. Because *M. rubra*, one of the most abundant ant species of the genus in Europe (Seifert 1996), is frequently found on ruderal sites with tansy, where, along with other ants, it attends tansy-feeding aphids like *Metopeurum fuscoviride* (Völkl 1997), we used this ant in our study as one potential predator of *Cassida* spp.

In contact bioassays, we investigated whether ants differentiate between *Cassida* larvae with shields and larvae whose shields had been removed. Additionally, we compared the ants' reactions towards *Cassida* larvae with faecal shields and those with skin shields. We also examined whether surface chemicals and moisture content of the faecal shields are crucial factors for the ants' reactions. Olfactometer bioassays were made to study the ants' response towards volatiles of the shields of larvae of *C. denticollis* (faecal shield) and *C. stigmatica* (skin shield). Finally, we analysed volatiles from shields by coupled gas chromatography-mass spectrometry (GC-MS). To investigate whether volatiles from the shield are derived from the host plant, tansy leaf volatiles were also analysed by GC-MS.

Materials and methods

Insects

Adults of tansy-feeding *C. stigmatica* Suffr., *C. denticollis* Suffr. and *C. sanguinosa* Suffr. (Coleoptera, Chrysomelidae) (determination after Steinhausen 1950) were collected in Berlin-Adlershof in May 1997 and were kept at 20°C, 75% relative humidity (r.h.), and a photoperiod of L16:D8. A colony of *M. rubra* L. was maintained in the laboratory at 25°C, 75% r.h., and a photoperiod of L16:D8.

Plants

Whole tansy plants (*C. vulgare* Bernh., Asteraceae) were collected in May, before blooming, from a field site in Berlin. Fresh leaves were cut off in the laboratory and used immediately.

Bioassays

All bioassays were conducted at 20°C under a central spotlight (1200 lux) in a dark room.

Video-taped contact bioassays

These bioassays were conducted with *C. denticollis*, *C. sanguinosa* and *C. stigmatica*. Reactions of *M. rubra* towards *Cassida* larvae (4th–5th instar) with intact abdominal shields and towards larvae whose shields had been experimentally removed were video-taped for a period of 5 min. One larva of each *Cassida* species with its shield and one larva without shield were offered simultaneously to 20 foraging *M. rubra* ants within a Petri dish (diameter 9 cm). We recorded: (a) the number of contacts between ant (mandibles or antennae) and larva; (b) the duration of contacts; (c) the number of bites; (d) the number of actions where a larva was dragged through the Petri dish by an ant ('dragging actions'). Data from 10 replicates were statistically evaluated by the Wilcoxon signed-rank test for paired samples.

The following parameters were tested in the *Cassida* species mentioned using the same bioassay described above.

Skin shield and faecal shield. To compare the reactions of ants to shields made of cast skins only with their reactions towards shields made of faeces, dummies were built to exclude different influences from the larval bodies. A skin shield of *C. stigmatica* (5th instar) and a faecal shield of *C. sanguinosa* (2nd instar) were attached to the abdomen of larvae of *Galleria mellonella* L. (2nd instar) with water-soluble glue. A skin shield of a 5th-instar larva of *C. stigmatica* is about of the same size as a faecal shield of a 2nd-instar larva of *C. sanguinosa*. One dummy with a skin shield and one with a faecal shield were offered simultaneously to the ants.

Moisture content. To study the effect of moisture content on the ants' contacts and bitings, *M. rubra* were offered simultaneously one larva of *C. denticollis* (4th–5th instar) with an untreated shield and one larva with a wet faecal shield, which had been submerged in distilled water for 1 min.

Chemical stimuli. To elucidate the importance of surface chemicals of the shields for the reaction of *M. rubra* towards the tested *Cassida* larvae, contact to surface chemicals was excluded by "wrapping" the shield in paraffin. Larvae of *C. denticollis* (4th–5th instar) were held with forceps, and the shield was dipped for a few seconds in heated paraffin. This paraffin treatment also diminished evaporation of volatiles from the shields (see below, Chemical analyses). The reactions of *M. rubra* towards larvae with paraffin-treated shields were compared with their reaction towards (1) larvae whose shields had been removed and whose three last abdominal segments were dipped into paraffin (control for the ants' reaction to paraffin) and (2) larvae with untreated shields.

Olfaction bioassays

The reactions of *M. rubra* towards volatiles from intact faecal shields of *C. denticollis* (5th instar) and from skin shields of *C. stigmatica* (5th instar) were tested in a T-shaped olfactometer without airflow, as described by Hilker (1989). The olfactometer was placed upon a gauze (mesh: 0.5 mm) and samples were offered 0.3 cm below the gauze to prevent ants from making contact with them. Either two intact faecal shields (total amount 28 mg) or two skin shields (total amount 0.16 mg) were offered as test samples below one T-arm about 1–2 cm away from the crossing of the T-arms. As control, a dark spot was supplied below the opposite T-arm, mimicking the size and colour of the test sample.

Ants were released one by one at the base of the T-olfactometer, and their decisions for either T-arm were recorded. After 2 min, the test was stopped. Three samples of faecal shields and three samples of skin shields were tested. Each sample was offered alternately on the left or right side of the T-arm (change after 10 tested ants) to exclude a possible influence of ants' trail pheromones. Numbers of ants tested were 49 (sample 1), 45 (sample 2), and 46 (sample 3) for the faecal shield, and 50 for each skin shield sample. Data were statistically evaluated by the sign test of Dixon and Mood (1946).

Chemical analyses of volatiles

To examine whether volatiles from faecal shields of *C. denticollis* larvae are derived from the host plant, extracts of both the shield and the plant were chemically analysed. Freshly removed faecal shields (30 mg) of seven *C. denticollis* larvae (3rd and 5th instar) and 30 mg of a particular tansy leaf on which the larvae had fed were macerated in 120 µl hexane. After ultrasound treatment for 15 min and centrifugation at 10,000 rpm for 5 min, the clear supernatants of shield and tansy extract were each kept in an airtight vial (3.5 ml). Volatiles were trapped for 1 h from the headspace of the extract with a solid-phase micro extraction (SPME) holder (manual) equipped with dimethyl silicon and injected splitless into a gas chromatograph (Fisons Instruments, GC 8165, injector temperature 280°C). A 30 m × 0.32 mm db5 column was used, which was programmed from 40 to 280°C at 3°C/min, isothermic for the first 3 min (carrier: 10 kPa helium). The gas chromatograph was coupled to a quadrupole mass spectrometer (Fisons Instruments, MD 800; EI mass spectra: 70 eV). The structures of the detected compounds were confirmed by comparison with synthetic reference samples or by comparison with proposals of the National Institute of Standards and Technology (NIST) library.

To elucidate whether the paraffin treatment diminishes or excludes evaporation of volatiles from the shields, one freshly removed faecal shield of *C. denticollis* (5th instar) was kept in an airtight vial (3.5 ml), and its volatiles were trapped for 1 h by SPME. After injection of volatiles into the GC-MS system (conditions as described in above), the same shield was completely dipped into paraffin as described in the section Chemical stimuli, its volatiles were collected for 1 h and analysed by GC-MS. The relative amounts of volatiles evaporating from an intact and a paraffin-treated shield were estimated by comparing the peak areas of the chromatograms. Quantities and qualities of volatiles evaporating from an untreated faecal shield did not differ when collecting volatiles 0–1 h or 1–2 h after removal from the larva.

Volatiles from 20 (1.6 mg) intact skin shields of *C. stigmatica* (3rd–5th instar) kept in an airtight vial (3.5 ml) were collected by the SPME-technique for 1 h and analysed by GC-MS as described above. Quantities of volatiles evaporating from a skin shield within 1 h were compared to those from an untreated faecal shield by comparing the peak areas of the chromatograms (the peak areas of volatiles from a single skin shield were calculated by dividing by 20).

Results

Video-taped contact bioassays

When offering larvae of *Cassida* with and without shields simultanously to foragers of *M. rubra*, the number and duration of contacts between ants and larvae with intact shields were significantly higher than with larvae whose shields had been removed. Larvae of *C. denticollis* and *C. stigmatica* with intact shields were bitten and dragged significantly more often by the ants than larvae without shields, while no such significant differences were observed with *C. sanguinosa* larvae (Fig. 1).

Dummies with faecal shields were contacted for as long as dummies with skin shields, but from a higher number of ants. Dummies with skin shields were bitten and dragged with a similar frequency as dummies with faecal shields (Fig. 2A).

The ants' reactions towards untreated shields of *C. denticollis* larvae did not differ from those to water-soaked shields (Fig. 2B).

To examine the role of shield chemicals, reactions of *M. rubra* towards *C. denticollis* larvae with shields dipped into paraffin were compared with their reactions towards (1) larvae without shields and (2) larvae with untreated shields. Dipping shields into paraffin excluded effects of surface chemicals and strongly diminished evaporation of volatiles (Table 1). Larvae with shields treated with paraffin were contacted by significantly more ants and for a longer period than larvae without shields, but the number of the ant bites and dragging actions were not significantly higher in larvae with paraffin-treated shields (Fig. 3A). When comparing larvae with paraffin-treated shields to larvae with untreated shields, the ants were more interested in larvae with untreated shields (significantly higher number or duration of contact, bites, dragging actions; Fig. 3B).

Olfaction bioassays

Volatiles of all three samples of faecal shields of *C. denticollis* showed a significant attractive effect to the ants, while volatiles of skin shields of *C. stigmatica* did not evoke a response (Table 2).

Chemical analyses

The identified volatile compounds evaporating from an extract of tansy leaves (Fig. 4A) and from an extract of crushed faecal shields of *C. denticollis* (Fig. 4B) are listed in Table 3. The following compounds were detected in both samples: α-pinene (peak 1 in Fig. 4A,B), camphene (peak 2), α-phellandrene (peak 3), 1,8-cineole (peak 4), α-thujone (peak 7), β-thujone (peak 8), acetic acid thujyl ester (peak 9), camphor (peak 10), borneol (peak 13), germacrene D (peak 20), two sesquiterpene derivatives (peaks 21, 22) and the unidentified compounds of peaks 5, 6, 14, and 16 (Fig. 4A,B). 4-Isopropyltoluene (peak 23) and the compounds of peaks 24, 25 and 26 evaporated only from the shield, but not from the plant.

When comparing the volatiles evaporating from an untreated and a paraffin-treated faecal shield, most compounds (α-pinene, camphene, α-thujone, camphor, borneol and several unidentified compounds) were totally excluded by the paraffin treatment, while phellandrene, 1,8-cineole, β-thujone and 4-isopropyltoluene were diminished to 7–12% of the amount of volatiles

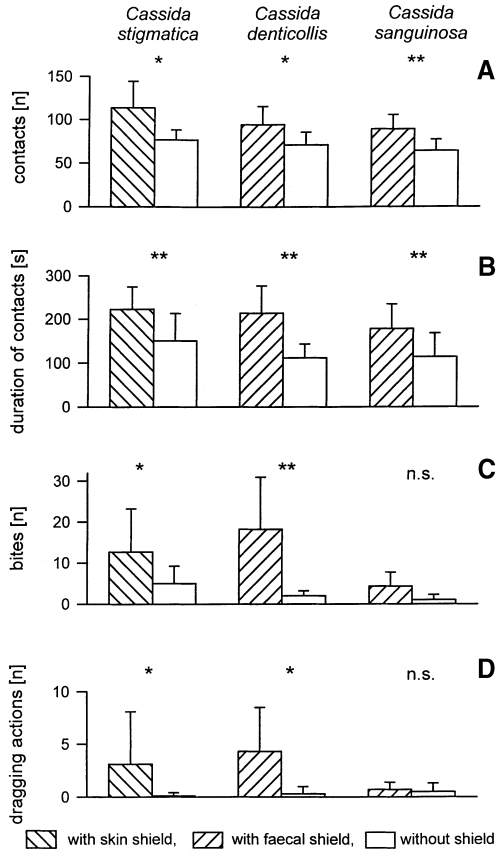

Fig. 1A–D Reactions of 20 individuals of the ant *Myrmica rubra* towards a *Cassida* larva with and a larva without shield. Shown are the means ± SD of ten replicates of reactions observed (video-taping) for a period of 5 min (*n.s.* not significant, $*P < 0.05$, $**P < 0.01$; Wilcoxon signed-rank test for matched pairs)

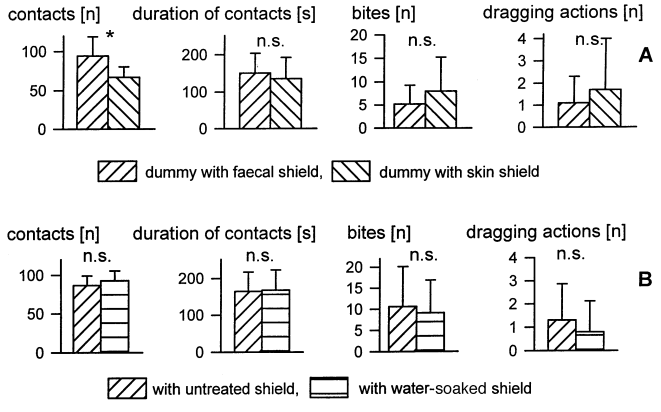

Fig. 2 Comparison of reactions of 20 individuals of the ant *Myrmica rubra* towards a larva of *Galleria mellonella* (= dummy) with a faecal shield of *Cassida sanguinosa* and a dummy with a skin shield of *C. stigmatica*m (**A**), and towards a larva of *C. denticollis* with an untreated, but dry shield and a larva whose shield was soaked with water (**B**). Shown are the means ± SD of ten replicates of reactions observed (video-taping) for a period of 5 min (*n.s.* not significant, $*P < 0.05$; Wilcoxon signed-rank test for matched pairs)

evaporating from an untreated shield (Table 1). One unidentified compound was detectable in an untreated faecal shield, but not in the hexane extract of a faecal shield (compound a in Table 1).

Volatiles evaporating from untreated faecal shields of *C. denticollis* were also detected in the headspace of skin shields of *C. stigmatica*, but quantities of volatiles from skin shields were much lower than those from untreated or even paraffin-treated faecal shields (Table 1).

Discussion

The present study shows for the first time that abdominal shields of cassidine larvae feeding upon tansy attract, rather than repel, a generalist predator. The ant *M. rubra* attacked larvae of *C. stigmatica*, *C. denticollis* and *C. sanguinosa* with shields more often than larvae whose shields were removed (Fig. 1). The ants responded similarily to faecal and skin shields attached to a dummy (Fig. 2A).

Chemical analyses of the volatiles from tansy leaves and the faecal shield of *C. denticollis* revealed that this cassidine larva discharges with its faeces numerous terpenoid components of the host plant tansy qualitatively unaltered and stores them in the shield, such as α-pinene, camphene, α-phellandrene, 1,8-cineole, α- and β-thujone, camphor, borneol and several sesquiterpenes and their derivatives. The skin shields of *C. stigmatica* which do not contain any faeces, but only exuviae, also emit several plant-derived terpenes. It is not clear whether *C. stigmatica* sequesters these host plant components into the integument or whether the integument/exuviae absorb plant and faecal volatiles. However, volatiles from skin shields are only detectable in much smaller amounts (less than 1%) than from the faecal shield of *C. denticollis*.

When investigating the stimuli responsible for the ants' reactions towards these abdominal shields in olfactometer bioassays, we found that volatiles from skin shields do not attract the ants (Table 2). We suggest that the higher frequency of attacks of larvae with skin shields in contact bioassays is caused by visual cues (Fig. 1). But volatiles evaporating from the faecal shields of *C. denticollis* larvae act as kairomones towards *M. rubra*, as demonstrated by olfactometer bioassays (Table 2) and by the contact bioassays comparing the ants' reaction towards larvae with untreated and paraffin-treated shields (Fig. 3B). Surface chemicals of faecal shields were shown to play no major role in attracting *M. rubra*, since exclusion of the possibility of contact with surface chemicals by paraffin treatment hardly reduced the attractiveness of the shields (Fig. 3A). The higher attractiveness of larvae with paraffin-treated faecal shields to those larvae whose shields had been removed may have two causes: (a) the low amounts of volatiles still evaporating from the shield despite the treatment (Table 1) are attractive, and (b) visual cues attract the ants and stimulate attacks, as was

Table 1 Volatile compounds, collected by solid-phase micro-extraction from the headspace of one intact faecal shield, one paraffin-treated shield of *Cassida denticollis*, and 20 intact skin shields of *C. stigmatica*. Numbers (*No.*) refer to peak numbers in Fig. 4B (*a* compound found only in the headspace of an intact faecal shield, but not in the headspace of a hexane extract of the shield or of tansy leaves; compare Table 3, and Fig. 4A,B). The amounts of compounds evaporating from one paraffin-treated faecal shield and the calculated amounts of volatiles from one skin shield are compared to those from an untreated faecal shield (= 100%) (+ compound present, – compound not present)

No.	Compound	Untreated faecal shield	Treated faecal shield	Skin shield
1	α-Pinene	+	–	–
2	Camphene	+	–	–
3	α-Phellandrene	+	+ (10.5%)	+ (0.4%)
4	1,8-Cineole	+	+ (7.2%)	+ (0.6%)
7	α-Thujone	+	–	+ (0.2%)
8	β-Thujone	+	+ (11.7%)	+ (0.3%)
10	Camphor	+	–	–
13	Borneol	+	–	–
16	Unidentified	+	–	+ (4.1%)
20	Germacrene D	+	–	+ (0.7%)
23	4-Iso-propyltoluene	+	+ (8.3%)	–
24	Unidentified	+	–	–
25	Unidentified	+	–	–
a	Unidentified	+	–	–

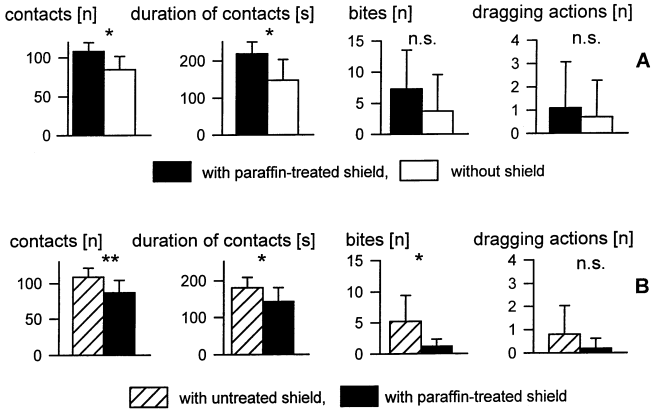

Fig. 3 Reactions of 20 individuals of the ant *Myrmica rubra* towards a larva of *Cassida denticollis* with a faecal shield dipped into paraffin and a larva whose shield was removed (**A**) and with a faecal shield dipped into paraffin and a larva with an untreated shield (**B**). Shown are the means ± SD of ten replicates of reactions observed (videotaping) for a period of 5 min (*n.s.* not significant, $*P < 0.05$, $**P < 0.01$; Wilcoxon signed-rank test for matched pairs)

suggested above for the attractiveness of skin shields. Visual cues like size and movement are often used by generalist invertebrate predators (Richerson and DeLoach 1972; Dippel and Hilker 1998).

We cannot yet decide which of the volatiles detected from the headspace of the untreated faecal shield of *C. denticollis* are essential for shield attractiveness to *M. rubra*. The appearance of essential oils is qualitatively and quantitatively very variable in tansy (Schantz and Järvi 1966; Tétényi et al. 1975; Holopainen et al. 1987). Thus, the volatile pattern of the shields of tansy-feeding

Table 2 Olfactory reactions of individuals of the ant *Myrmica rubra* in a T-shaped tube towards faecal shields of *Cassida denticollis* or skin shields of *C. stigmatica*, and controls. Percentages of ants leaving the T-tube on test and control sides are given (*n* number of specimens tested; *n.s.* not significant, $**P \leq 0.01$, $***P \leq 0.001$, sign test)

	Shields	Control	*n*	Significance
C. denticollis (faecal shields)				
Sample 1	73.5	26.5	49	**
Sample 2	73.3	26.7	45	**
Sample 3	84.8	15.2	46	***
All samples	77.1	22.9	140	***
C. stigmatica (skin shields)				
Sample 1	48	52	50	n.s.
Sample 2	52	48	50	n.s.
Sample 3	48	52	50	n.s.
All samples	49.3	50.7	150	n.s.

cassidine larvae may vary with the chemical composition of the host plant. Collection of volatile and non-volatile host plant components in faecal shields has also been shown in other chrysomelid larvae. Gómez (1997) examined the larval faeces of the cassidine *Eurypedus* which fed upon leaves of *Cordia* spp. (Boraginaceae) of different chemotypes. The terpenoids of the faecal shields reflected the terpenoid pattern of the particular chemotype foliage on which the larvae had fed. Faecal shields of larvae of the flea beetle *Blepharida rhois* (Chrysomelidae, Alticinae) were shown to contain plant-derived substances which are mainly of very low volatility (Vencl and Morton 1998). Further studies of the effects of shields of cassidine larvae feeding upon tansy will be necessary to elucidate whether the attractive effects towards *M. rubra* found in this study are dependent on the tansy chemotype or even on the host plant species.

If *M. rubra* responds to volatiles that are specific to certain insect species, such a response would not fit into the current concepts of foraging behaviour of generalist antagonists. Bradshaw and Howse (1984) hypothesize that generalist ants do not use specific host allelochemicals in foraging behaviour. Vet and Dicke (1992) predict that the specifity of information used by generalist predators or parasitoids decreases as the diet breadth increases. We can exclude the possibility that *M. rubra* had learned to locate offered cassidine prey by recognizing shield volatiles because all bioassays were conducted with naive ants. The adaptive value to *M. rubra* of tansy-specific volatiles from shields of cassidine larvae is unclear, since *M. rubra* is known to attack numerous insect species with a wide range of host plants. Several volatiles detected from the faecal shield of *C. denticollis* feeding upon tansy are very common in other host plant species, for example the monoterpenes α-pinene, camphene and cineole (Dev et al. 1982; Langenheim 1994). Such generally occurring volatiles might provide reliable cues for a generalist predator to locate its herbivore prey.

The attractiveness of larval shields towards a generalist predator, as found in the present study, contrasts

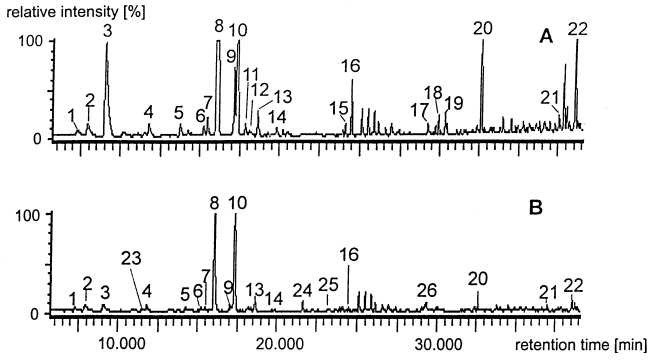

Fig. 4A, B Total ion current chromatograms of volatiles collected from the headspace of samples by solid-phase micro-extraction. Numbers refer to compounds listed in Tables 1 and 3. **A** Hexane extract of tansy leaves. **B** Hexane extract of faecal shields of *Cassida denticollis*

Table 3 Volatile compounds, collected by solid-phase micro-extraction from the headspace of a hexane extract of leaves of *Chrysanthemum vulgare* and an extract of faecal shields from *Cassida denticollis*. The structures of the compounds were confirmed by comparison of the EI spectra and retention times with those of synthetic reference samples (*Synth.*) or with the proposals of the National Institute of Standards and Technology (NIST) library. Numbers (No.) refer to peak numbers in Fig. 4A,B. Interpretations of mass spectra of peaks no. 13, 18, 20, 21 and 22 suggest the class of compound (+ compound present, – compound not present)

No.	Compound	Tansy	Faecal shield	Comparison with
1	α-Pinene	+	+	Synth.
2	Camphene	+	+	Synth.
3	α-Phellandrene	+	+	NIST
4	1,8-Cineole	+	+	Synth.
7	α-Thujone	+	+	Synth.
8	β-Thujone	+	+	Synth.
9	Acetic acid thujyl ester	+	+	NIST
10	Camphor	+	+	Synth.
13	Borneol	+	+	Synth.
15	Bornyl acetate	+	–	NIST
18	Sesquiterpene	+	–	
20	Germacrene D	+	+	NIST
21	Sesquiterpene derivative	+	+	
22	Sesquiterpene derivative	+	+	
23	4-Isopropyltoluene	–	+	Synth.

with results of laboratory bioassays of other Cassidinae. Shield-carrying larvae of the cassidine *Coptocycla leprosa* (host plant: *Cordia alliodora*, Boraginaceae) were better protected against the ant *Azteca* sp. than larvae whose shields had been removed (Gómez 1997). The faecal shields of larvae of *Cassida rubiginosa* (host plant: *Cirsium arvense*, Asteraceae) repel the ant *Formica exsectoides* (Eisner et al. 1967). The role of volatiles in the repellent activity of these shields has not yet been evaluated. Eisner et al. (1967) observed that moisture of the shield of *C. rubiginosa* adds to its defensive potential. In this study, we found no effect of high moisture content on the attractiveness of the shield of *C. denticollis*. When comparing the ants' reaction to water-soaked shields of *C. denticollis* with their responses to untreated shields, no significant behavioural differences were detected (Fig. 2B). In contrast, the moisture content of faeces of a phytophagous insect was shown to be a crucial factor in host-finding behaviour of the parasitoid wasp *Cotesia rubecula*, which preferred volatiles from wet to those from normal faeces of its host *Pieris rapae* in wind tunnel experiments (Agelopoulos et al. 1995).

While this study demonstrated the attractiveness of the abdominal shield of tansy-feeding cassidine larvae towards the ant *M. rubra*, the reaction of other ants and generalist predators towards these larvae and their shields is as yet unknown. Other typical enemies of cassidine larvae, like arachnids, predatory Heteroptera, ant species other than *M. rubra*, and parasitoids (Windsor 1987; Gómez 1997) need to be studied for their reactions towards tansy-feeding *Cassida* spp. Field studies of the survival of tansy-feeding cassidine larvae with and without shields may elucidate the selective advantages of collecting faeces at the abdominal tip in these species. The abdominal shield of cassidine larvae has also been suggested to function as a parasol or as protection against desiccation (Eisner et al. 1967; Olmstead and Denno 1992). We do not know whether the cassidine species investigated in the present study use their shields for these purposes. Future laboratory and field studies of these cassidine larvae are needed to provide information on both a possible microclimatic function of the shields and more detailed knowledge of the response of specialist and generalist predators towards the shields.

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