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SHIELD DEFENSE OF A LARVAL TORTOISE BEETLE

FREDRIC V. VENCL,[1,*] TIMOTHY C. MORTON,[2]
RALPH O. MUMMA,[3] and JACK C. SCHULTZ[3]

[1]*Department of Neurobiology and Behavior*
State University of New York at Stony Brook
Stony Brook, New York 11794-5230
and The Smithsonian Tropical Research Institute
Balboa, Ancon, Republic of Panama

[2]*Department of Ecology and Evolution*
University of Chicago
Chicago, Illinois 60637

[3]*Department of Entomology*
Pennsylvania State University
University Park, Pennsylvania 16802

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Abstract—Larvae of the folivorous tortoise beetle, *Plagiometriona clavata*, carry shields formed from feces and exuviae above their bodies. We used an ecologically relevant predatory ant, *Formica subsericea*, in a bioassay to determine if shields functioned as simple barriers, as previous studies indicated, or whether they were chemical defenses. Shields were necessary for larval survival; shield removal rendered larvae vulnerable. Shields produced by larvae reared on a substitute diet failed to provide protection. Solvent-leached shields also failed to deter ants, indicating the shield had a host-derived chemical component likely located in the feces, not in the exuviae. *Solanum dulcamara*, the larval host plant, contained free phytol, steroidal glycoalkaloids, and saponins. Shields contained partially deglycosylated metabolites of host steroidal glycoalkaloids and saponins, a suite of fatty acids, and derivatives of phytol, which together formed a deterrent barrier against ant attack. We compared the mobile shield of *P. clavata* to the stationary shield of another *S. dulcamara*-feeding leaf beetle, *Lema trilinea*. Both larval shield defenses were formed from a very similar array of host-derived compounds with deterrent properties. We concluded that convergent patterns of limited chemical transformation and selective incorporation of particular deterrent metabolites in shield defenses of two unrelated taxa represented responses to selection from invertebrate predators.

Key Words—*Plagiometriona clavata*, *Lema trilinea*, *Solanum*, steroidal

*To whom correspondence should be addressed at Stony Brook.

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glycoalkaloid, phytol, saponin, fatty acid, fecal shield, larval defense, Chrysomelidae.

INTRODUCTION

Members of the phytophagous leaf beetle family Chrysomelidae exhibit a diverse array of morphological and behavioral attributes for defense against predation. Among these, glandular secretions, maternal care, thanatosis, crypsis, stridulation, and mimicry, to name a few, have been investigated in some detail (Pasteels et al., 1988, and references therein; Schmitt, 1994; Hilker, 1994; Windsor and Choe, 1995; Hespenheide, 1996). However, another putative defensive strategy, larval waste retention, remains poorly understood, although it is a remarkably widespread habit within the Chrysomelidae. For example, nearly 20% of the described chrysomelid species, most notably the members of the Camptosomata, Hispinae, and Criocerinae, have some form of fecal covering or feces-laced structure. Despite their ubiquity, the defensive efficacy of waste-based structures, or fecal shields, as these appurtenances are collectively termed, has yet to be demonstrated in the vast majority of cases (Blum, 1981, 1994; Pasteels et al., 1988; Olmstead, 1994). Available evidence from the few species studied in depth supports the hypothesis that shields, scatoshells, fecal cases, and other waste-based characters function primarily as nonchemical, barrier-type defenses, despite the fact that many species have diets rich in secondary chemicals (Eisner et al., 1967; Erber, 1988; Root and Messina, 1993; Olmstead and Denno, 1993). Fecal-based structures warrant further investigation because they may form more complex defenses than is presently appreciated, perhaps through the incorporation of host-derived or autogenously synthesized compounds.

Within the great variety of fecal attributes displayed by chrysomelid beetles, the shields of larval tortoise beetles, members of the cassidoid Hispinae (sensu Reid, 1995), are perhaps the most intriguing. Attached to a highly movable appendage (furca) emanating from the 8th abdominal segment, and held like a parasol above the larva, the shield is a composite structure made of accumulated feces and of exuviae or molted skins. A remarkable telescoping anus, formed by extrusion of the invaginated 9th and 10th abdominal segments, is capable of precisely depositing fecal material over the shield (Figure 1A). Long suspected to have a defensive function (Riley and Walsh, 1868), the shields of several species examined to date do provide protection by acting as mechanical barriers against some attacking enemies (Eisner et al., 1967; Olmstead and Denno, 1993; Olmstead, 1996).

We report here on the larval shield of the abundant and widespread tortoise beetle, *Plagiometriona clavata* (Fab.), which feeds on woody nightshade, *Solanum dulcamara* L. (Solanaceae), throughout North America. We suspected

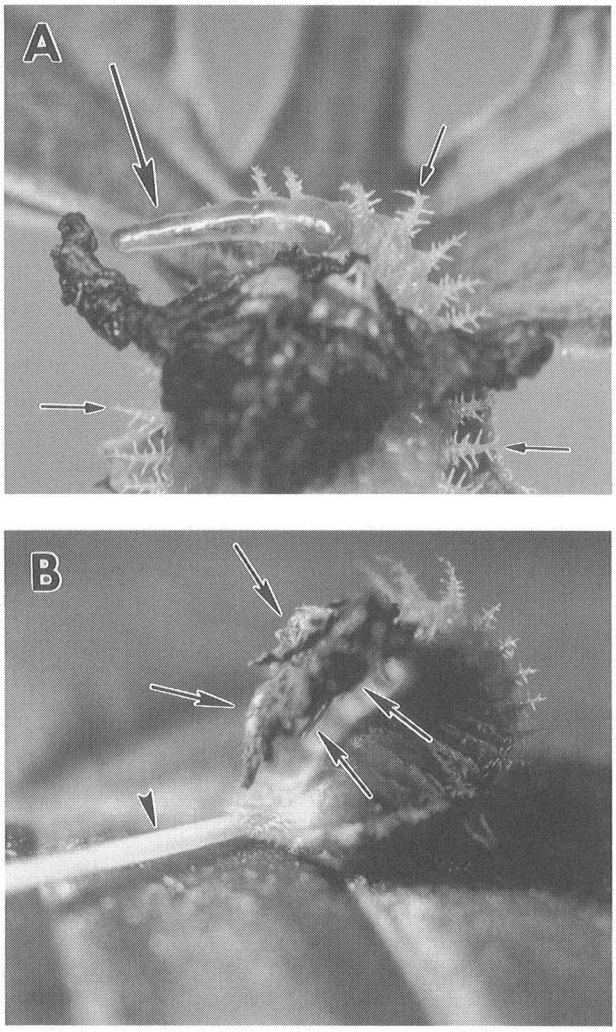


FIG. 1. (A) Larval *P. clavata* (dorsal aspect) with a fecal shield (dark mass) held aloft. Mechanosensitive scoli (small arrows) fringe the larva's flanks. The telescoping anus—the evaginated 9th and 10th abdominal segments—is visible as the translucent tube (arrow), extended to a length of 4.5 mm, and about to apply a bolus of feces to the shield's right projection. (B) Response to perturbation of a *P. clavata* larva with a solvent-leached, experimentally reattached shield. The abdomen is arched over the larva's back, and the chemically modified, reattached shield (bounded by arrows) is aimed forward and is rapidly flagged toward a tactile stimulus, in this instance, by a cat whisker (arrowhead) touching the larva's anterior scoli. This behavior is typical of larvae with unmanipulated shields.

shields might be chemical defenses because *S. dulcamara* is well known to contain many potentially toxic, complex secondary compounds, including steroidal glycoalkaloids (SGAs) and steroidal saponins (Mathé and Mathé, 1979; Tingey, 1984; Roddick, 1986; Hegnauer, 1992). Although a chemical component was suspected in one species (Eisner et al., 1967), the first evidence in support of the idea that shields were chemical defenses appeared only recently (Gómez, 1997; Morton, 1997). The possibility clearly existed that *P. clavata* larvae take advantage of the allelochemicals available in their diet.

Our objectives in this study were fourfold. Firstly, we wanted to know whether *P. clavata* shields provided effective protection from predators, as was indicated by preliminary behavioral observations in the field. Secondly, we wanted to know whether there was a chemical component to what evidently was, according to earlier studies, a simple barrier defense. Thirdly, if shields were found to be chemically deterrent or repellent, we wanted to determine to what extent they were dependent on host-derived compounds. We previously investigated another *Solanum*-feeding leaf beetle, *Lema trilinea* (Criocerinae), and found its shield to be a very effective chemical defense that contained derivatives of the noxious compounds found in the host. Fourthly, we hoped a comparison between *P. clavata*'s mobile, parasollike shield and *L. trilinea*'s static, fecal mound-type shield might reveal convergent metabolic mechanisms for handling inimical host allelochemicals—a comparison of unrelated species, occupying the same trophic niche, afforded us an opportunity to infer whether or not particular chemical or morphological shield features evolved as defensive adaptations or if they were fortuitous by-products of digestion and elimination.

METHODS AND MATERIALS

Collection and Rearing of Insects. Nine adults and a small number of eggs of *P. clavata* were collected from wild populations of *S. dulcamara* in Setauket, New York, on June 10, 1995. They were transported to Penn State University and were reared in cages in a walk-in growth chamber on *S. dulcamara* obtained from root stocks (V&J Seed Farms, Woodstock, IL) and grown in a greenhouse. Shields were collected with fine forceps by slowly teasing the complex of feces and exuviae off the urogomphi of third or fourth instars. This procedure did not harm the larvae (cf. Olmstead and Denno, 1993). Several 2-g samples were collected, immediately weighed, and stored until processed at 4°C in 20 ml of 80% methanol (MeOH). For comparison, we extracted 200 g of the *S. dulcamara* leaves used to feed *P. clavata*.

Bioassays of Larvae by Ant Attacks. Larvae (N = 135), collected as first instars from a host population growing in Crystal Brook Park, Mt. Sinai, New York, during June 1997, were divided into five groups for use in bioassays.

Larvae in all groups were reared under natural light (14L : 10D) and ambient temperature conditions to the third instar (mean length = 4 ± 0.3 mm and with three visible exuviae; N = 20) on 50-cm branches of *S. dulcamara* inside 2-gallon ZipLoc bags. The third instar was selected for assay because it was shown to suffer intermediate levels of predation in two other tortoise beetle species of (cf. Olmstead and Denno, 1993). Twenty-seven larvae had their shields removed with soft forceps 3 hr before being assayed. Shield removal did not harm larvae (Olmstead and Denno, 1993). Shields were removed from 10 additional larvae (not used in assays) and all survived to pupation. A second treatment group (N = 40), reared as above, underwent shield removal. Of these, 24 had their shields soaked in MeOH, while a third group (N = 16) had their shields soaked in water for 24 hr prior to assay. Shields were air-dried for 2 hr on filter paper before being reattached to the urogomphi with white, all-purpose Elmer's glue. Shields retained their wedge shape throughout this procedure. Larvae were checked prior to assay by gentle prodding to guarantee that they could move their shields directionally (Figure 1B). The fourth treatment group (N = 32) was reared on a diet of lettuce until the larvae reached the average size of third instars. Lettuce was previously shown to lack the compounds we found here to be of importance in shields (Morton and Vencl, 1998). Since their developmental rates were slow and some mortality occurred, 16 were assayed as a group when individuals reached the criterion length of ≥4.0 mm. As a control, third instars (N = 26) were assayed with intact, host-derived shields.

An assay trial consisted of the presentation of a single larva, randomized between shielded and treatment groups, in an arena consisting of a sheet of a 3 cm^2 waxed weighing paper pinned to the ground near a foraging trail running near the nest of the sympatric generalist predatory ant *Formica subsericea*. A trial lasted 5 min, or until a test larva was removed from the arena. Time to removal was recorded as was the number of ants contacting a larva. An assay was scored only if a larva was encountered at a rate of ≥2 ants/min.

Extraction and Fractionation. Procedures were the same as presented in Morton and Vencl (1998) and are summarized as follows. An 80% MeOH extract was reduced in volume under vacuum to remove MeOH. The aqueous solution was acidified and extracted with organic solvent (apolar fraction A), then made alkaline and again extracted with organic solvent (alkaline, alkaloidal fraction B). The remaining solution was taken to dryness and the residue was dissolved in methanol (polar fraction C). Fraction A was further purified to remove pigments to the acetonitrile phase by partitioning between hexane and acetonitrile. Fraction B was chromatographed on a silica gel solid phase extraction (SPE) column. The extract was loaded in chloroform ($CHCl_3$) solution, rinsed with ethyl acetate to remove pigments, and the components eluted with propanol. Fraction C was chromatographed on the same SPE silica column after removal of MeOH, loaded in 80 : 20 $CHCl_3$–MeOH and the components eluted with propanol.

Thin-Layer Chromatography (TLC). TLC was used to monitor purity of the B and C fractions through the extraction and fractionation processes. The TLC plates used were plastic-backed silica gel with fluorescent indicator. Alkaloid aglycones were resolved using a modified dichloromethane–acetone (4 : 1) solvent system (Hunter et al., 1976) with two drops of concentrated ammonium hydroxide (NH_4OH) was added to the chamber to improve separation. Alkaloid glycosides were resolved by a $CHCl_3$–EtOH–1% aqueous NH_4OH (2 : 2 : 1 bottom phase) system (Boll, 1962). Alkaloids were detected with Dragendorff's spray reagent (Sigma). Plates were also monitored by long- and short-wave UV.

Gas Chromatography (GC). GC was used to monitor fraction A. An HP 5890 system with a 60-m DB-5 column, FID detector, and Shimadzu CR-4A integrator was used. Run parameters included split injection mode; temperature programmed from 80 to 170°C at 20°C/min from 170 to 225°C at 3°C/min; from 225 to 285°C at 20°C/min with a 10-min final hold; He carrier gas flow rate 2.0 ml/min; injector temperature of 250°C; and a detector temperature of 280°C.

Gas Chromatography–Mass Spectrometry (GC-MS). Fraction A was run on an HP 5890 gas chromatograph equipped with a 30-m DB-5 column, and the column output was applied to an HP 5970 series mass selective detector (GC-MS). The GC operating parameters were as follows: splitless injection mode; temperature programmed from 40 to 250°C at 4°/min with 4-min initial and 20-min final holds; He carrier gas at 1.9 ml/min, with an injector temperature of 250°C. Spectra were statistically compared with the Wiley/NBS Mass Spectral Registry (McLafferty and Stauffer, 1989) by computer. GC-MS results were obtained by the injection of extract from 20 fecal shields.

Fast Atom Bombardment–Mass Spectrometry (FAB-MS). SPE purified fractions B and C were analyzed by FAB-MS. Samples were dissolved in a glycerol matrix and inserted via probe into a Kratos MS 50TC instrument (positive mode, Xenon) and analyzed with the MASSPEC data system.

Data Analysis. Assays of larvae, reported as frequencies (taken as prey or not taken as prey), were analyzed with the Mann-Whitney *U* test (Sokal and Rohlf, 1995).

RESULTS

Shield Bioassays. When *P. clavata* larvae were subjected to attacking ants, the presence of an intact, host-derived shield provided an effective defense against the natural generalist predator we used for an assay (Figure 2; solid bar). If shields were entirely removed, larvae readily fell prey to the ants (Figure 2; hatched bar). To be effective, however, shields had to be produced by larvae reared on a diet of *S. dulcamara*. Shields failed to provide protection if they were derived from a benign substitute diet of lettuce (Figure 2; open bar). Although

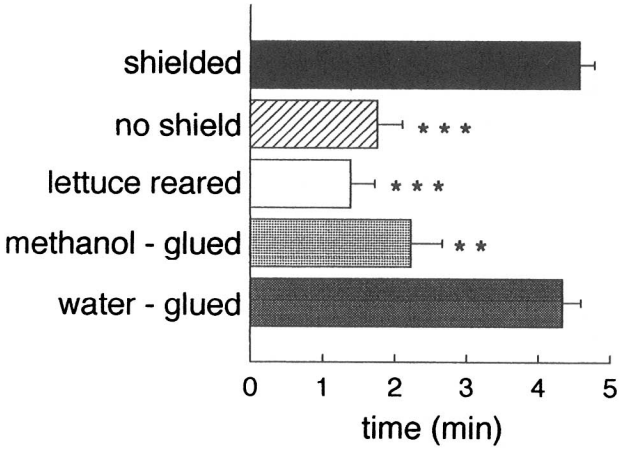


FIG. 2. Effectiveness of intact and modified *P. clavata* shields in a bioassay with the predatory ant, *Formica subsericea*. Solid bar: larvae with host-derived shields (control, $N = 26$); hatched bar: larvae without shields ($N = 27$); open bar: larvae reared on lettuce ($N = 16$); stippled bar: larvae with their shields removed, placed in methanol, and then reattached ($N = 24$); gray bar: larvae with shields removed, soaked in water, and reattached (controls, $N = 16$). Data are given as mean ± SE. Probabilities were calculated with the Mann-Whitney U test; significance levels: $^{**}P < 0.01$; $^{***}P < 0.001$.

able to fend off ants for almost 2 min, larvae with intact, mobile, but lettuce-based fecal shields eventually fell prey to predators. Assay results with micro-modified shields further implicated the importance of a chemical component. When mechanically removed, solvent-leached (MeOH), and reattached to the larval urogomphi, shields lost a significant amount of their effectiveness, despite the fact that micromanipulated, host-reared larvae could directionally maneuver their reattached shields as accurately as the untreated and water-soaked larvae could (Figure 2; stippled bar; Figure 1B). Host-derived shields maintained their protective effectiveness even after experimental removal, soaking in water and reattachment (Figure 2; gray bar). The active chemical component(s) was most likely contained in the methanol-soluble phase of host-derived shields.

Chemical Composition of Fraction A. Analysis by GC-MS of nonpolar fraction A resulted in 31 peaks with sufficient information for their partial or full characterization (Figure 3; Table 1). There were significant amounts of 17 fatty acids (FAs), ranging in length from 14 to 18 carbons. Although many were detected as methyl esters, the largest FA components, hexadecanoic (palmitic) acid (peak K) and 9-octadecenoic (oleic) acid (peak T), were present as free acids, which in combination, accounted for 31.6% of the total A fraction profile. FAs and their methyl esters comprised 48.8% of fraction A.

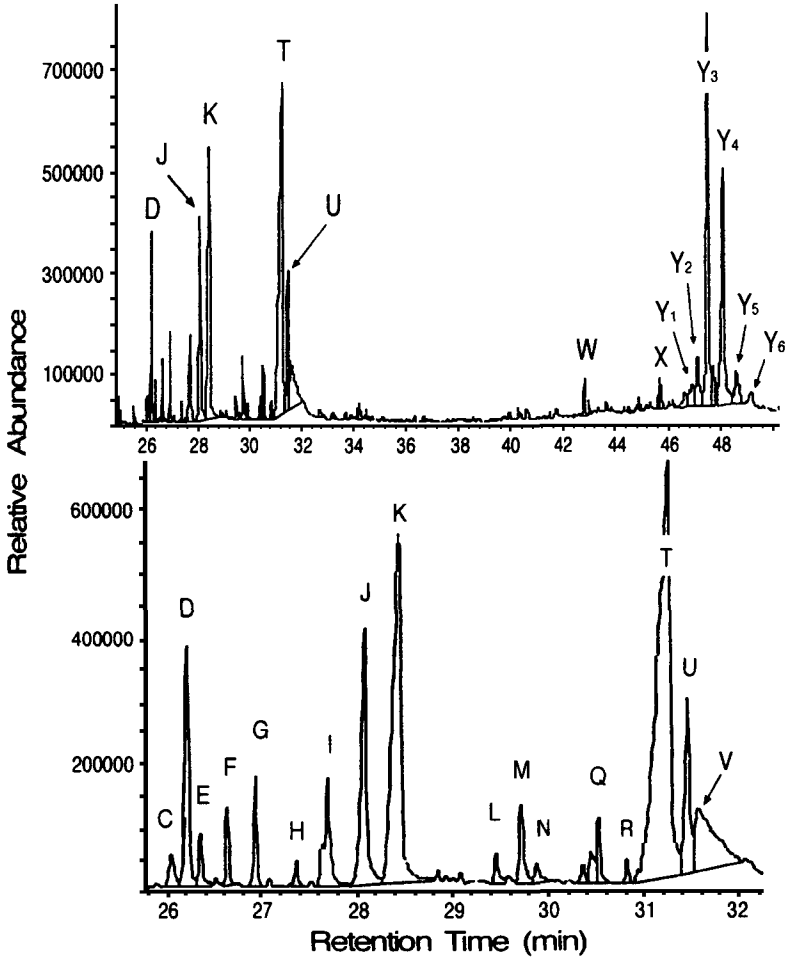


FIG. 3. Separation of compounds by GC-MS in the apolar fraction A of *P. clavata* shields. Top: entire total ion count spectrum. Bottom: expanded region of the congested area between 26 and 32 min from the upper trace. Lettered peaks are identified in Table 1.

Fraction A also included three uncharacterized phytol derivatives (Figure 3; peaks F, G, V). They likely represent degradation products of free phytol found in the plant and total about 11% of the A profile. The sapogenin, diosgenin (peak Y_1), and five related derivatives (peaks Y_2 to Y_6; Figure 3; Table 1) are metabolites of the host-plant steroidal saponin, dioscin (dioscin-chacotrios), that together accounted for 38% of fecal fraction A. The different retention times

TABLE 1. IDENTIFICATION OF GC-MS SPECTRUM FROM *P. clavata* SHIELD FRACTION A[a]

Peak	Retention time (min)	Compound	Mol. wt.	*Plagiometriona clavata*
A	24.96	tetradecanoic acid	228	0.7 (97)
B	25.53	tetradecanoic acid ethyl ester	256	0.3 (99)
C	26.05	pentadecanoic acid	242	0.6 (97)
D	26.22	phytol derivative	278	3.5
E	26.36	6,10,14-trimethylpentadecan-2-one	268	0.7 (91)
F	26.64	phytol derivative	278	0.9
G	26.94	phytol derivative	278	1.4
H	27.37	9-hexadecenoic acid methyl ester	268	0.4 (99)
I	27.70	hexadecanoic acid methyl ester	270	2.5 (98)
J	28.10	9-hexadecenoic acid	254	5.5 (94)
K	28.46	hexadecanoic acid	256	11.6 (99)
L	29.46	heptadecanoic acid methyl ester	284	0.4 (78)
M	29.73	heptadecanoic acid	270	1.3 (91)
N	30.37	9,12-octadecadienoic acid methyl ester	294	0.3 (99)
O	30.46	9-octadecenoic acid methyl ester	296	trace (91)
P	30.48	9,12,15-octadecatrienoic acid methyl ester	292	0.6 (76)
Q	30.54	11-octadecenoic acid methyl ester	296	0.9 (99)
R	30.83	octadecanoic acid methyl ester	298	0.3 (98)
S	31.04	9,12-octadecadienoic acid	280	trace (96)
T	31.27	9-octadecenoic acid	282	20.0 (99)
U	31.47	octadecanoic acid	284	3.4 (99)
V	31.58	phytol derivative	284	4.6
W	42.81	hexatriacontane	507	0.8 (94)
X	45.68	tigogenone	414	0.8 (94)
Y_1	46.65	diosgenin	414	0.9 (94)
Y_2	47.15	diosgenone	412	2.7
Y_3	47.59	diosgenone metabolite	412	18.0 (97)
Y_4	47.73	diosgenone minus 2-H	410	1.3
Y_5	48.16	diosgenone minus 2-H	410	12.7
Y_6	48.62	diosgenone minus 2-H	410	2.3

[a]Peak labels are those designated in Figure 3. Data for each peak include: retention time, identity, molecular weight, percentage of total integrated peak area, and confidence in the match with library standards (%).

of the diosgenin derivatives, with m/z 410, are possibly due to the addition of double bonds at various locations.

Chemical Composition of Fraction B. After partitioning of apolar compounds into fraction A, the fractionation scheme was designed to separate alkaloids into fraction B, while leaving polar substances in fraction C. However, due to their high polarity, the steroidal glycoalkaloids (SGAs) of *S. dulcamara* parti-

tioned between both fractions B and C. FAB-MS of the *P. clavata* fecal fraction B contained hydroxylated aglycones with differing numbers of double bonds (m/z 426, 428, and 430; Figure 4). By isolating and weighing the compounds from 2.0-g aliquots ($N = 2$) of feces, the concentration of aglycone metabolites in *P. clavata* feces was estimated to be approximately 0.65 mg/g fresh wt.

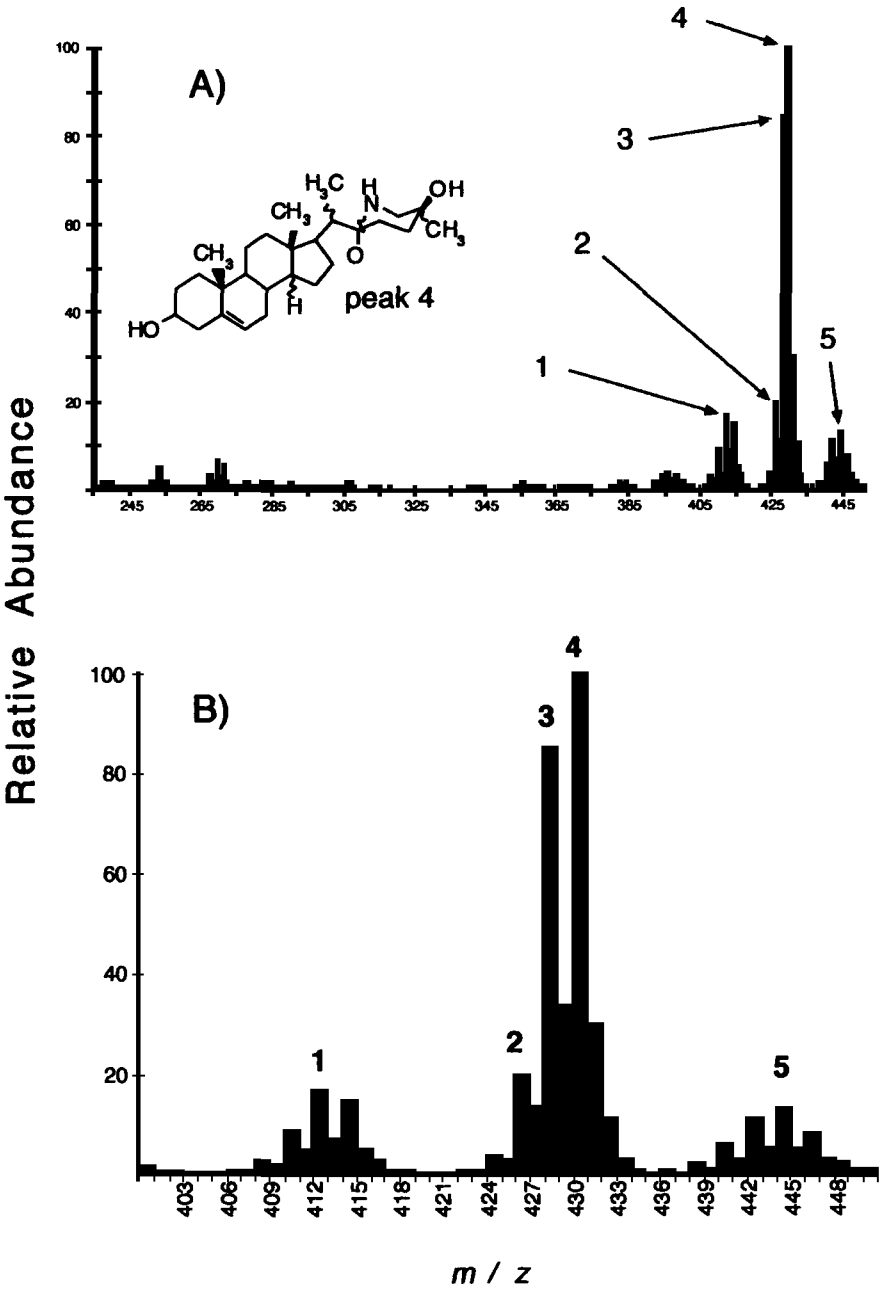
Comparison of Host Plant and Shield C Fractions. Figure 5 shows the FAB-MS fragmentation spectrum of SGA metabolites in the C fractions of host plant and of fecal shields. Structures were identified by comparison with fragmentation patterns of published spectra for *Solanum* SGAs (Price et al., 1985); each predicted fragment ion of the solasodine glycosides, α-solasonine and α-solamargine, were observed in the spectra from *S. dulcamara* (Figure 5A).

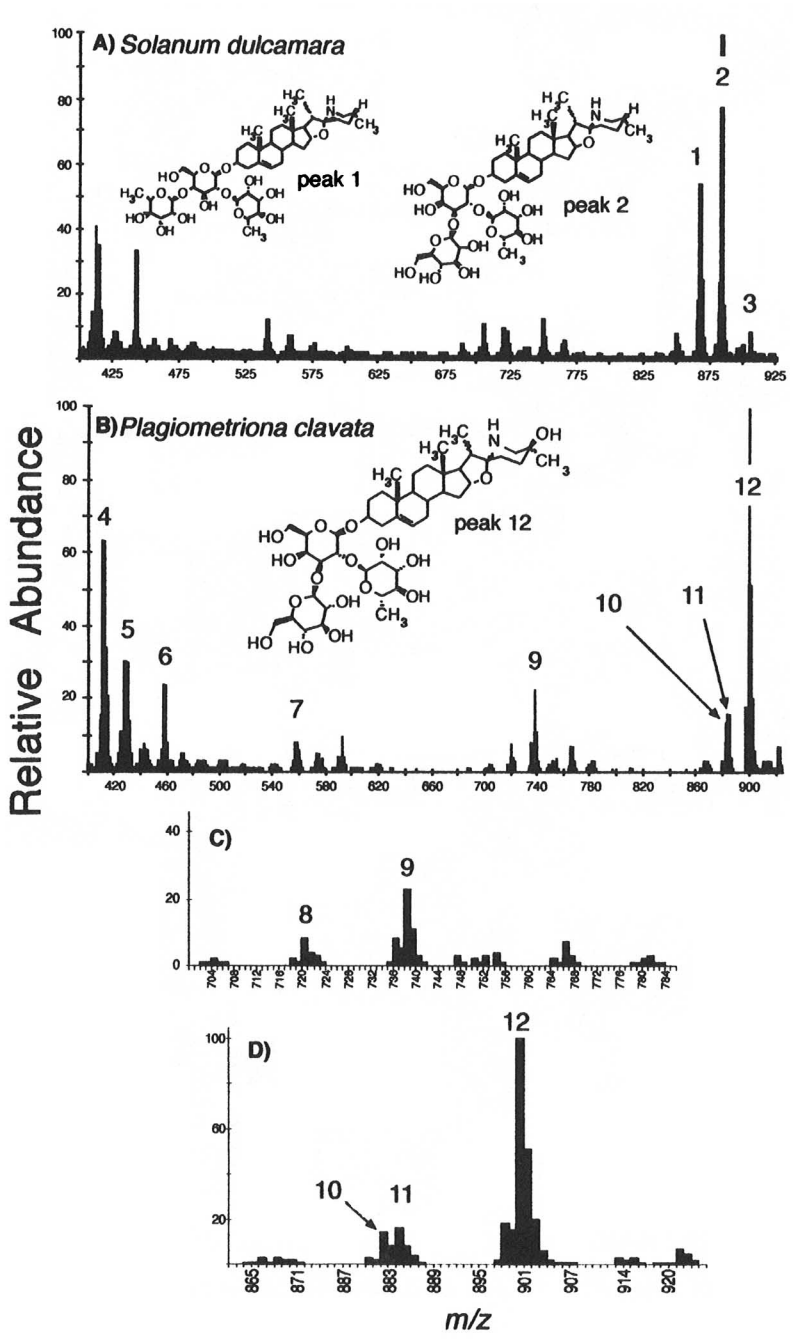
Plagiometriona clavata did not remove all the sugars from all the host plant's SGAs during transit through its gut. The fecal fraction C contained SGA metabolites with molecular ions and fragments 16 mass units higher than those for the host's SGAs, indicating that the insect can efficiently hydroxylate dietary SGAs but is evidently less efficient at deglycosylating them. We assume that this hydroxyl function is the same one observed on the aglycones at carbon 26 and that hydroxylation of the F ring represents the first step in SGA metabolism, which may prevent SGA membrane insertion, thereby preventing their toxic effects. The concentration of hydroxylated SGAs in *P. clavata* shields was estimated to be approximately 0.1 mg/g fresh wt.

DISCUSSION

Shield Bioassays. The bioassay clearly established that larval shields provided an effective defense against a natural predator of *Plagiometriona clavata*. Because of their parasol-like location above the larvae, *P. clavata*'s shields afforded a unique opportunity to analyze experimentally how they functioned as defenses without harming the larvae. Shields were necessary for larval survival, because without them, larvae fell prey to the ubiquitous generalist predatory ant that we used in the bioassay and which is found in *P. clavata*'s habi-

FIG. 4. (A) FAB-MS fragmentation spectrum of fraction B from *P. clavata*'s shield and the proposed structure of the most abundant metabolite. The main SGA metabolite, corresponding to peak 4, is an hydroxylated aglycone of the host-plant's SGA. (B) Expanded region, from m/z 400 to m/z 450, of fraction B from *P. clavata*'s shield. The cluster of molecular ions with $m/z+16$ mass units may represent hydroxylated forms of the main SGA metabolites surrounding peak 4. The cluster of ions with $m/z - 18$ mass units are fragments (minus water) of each of the ions within the main SGA cluster. The aglycone, solasodine (mol. wt. 413) is present as the small peak with m/z 414.


A)
B)
peak 4
Relative Abundance
m / z
1
2
3
4
5


A) Solanum dulcamara
peak 1
peak 2
1
2
3
B) Plagiometriona clavata
peak 12
4
5
6
7
9
10
11
12
Relative Abundance
C)
8
9
D)
10
11
12
m/z

tat. To determine whether shields function primarily as physical barriers or as chemical defenses, we modified shield chemical content, either by altering the larval diet or by treating them with solvents. In the diet-modification experiment, larvae without access to host compounds were defenseless. Not only did their shields lack the dietary compounds ordinarily obtained from the natural host, larvae reared on the substitute diet were unable to synthesize any defensive compounds of their own. The micromanipulation experiment, consisting of shield removal, solvent-soaking, and reattachment, demonstrated that the chemical component resided in a methanol-soluble phase. This implied that the mere presence of a shield, if devoid of host-derived compounds, was insufficient to protect its bearer. These observations lent additional support to the hypothesis that *P. clavata*'s shield is a chemical defense, rather than a physical barrier.

Larval chemical defenses have been hypothesized to contain volatile repellents that thwart invertebrate predators before they can contact and damage the soft larval cuticle (Eisner and Meinwald, 1966; Pasteels et al., 1983, 1984). By providing an effective defense against predation, the shields of *Eurypedus* and *Coptocycla* species, tropical tortoise beetles feeding on *Cordia* (Boraginaceae), were essential for larval survival (Gómez, 1997). The effectiveness of *E. nigrosignata*'s shield rests on volatile repellents (mono- and diterpene essential oils) acquired from the host plant and discharged unchanged in feces (Gómez, 1997). In contrast, our bioassay indicated that *P. clavata*'s shield owed its effectiveness to deterrents, rather than repellents; ants attacked larvae, but if they contacted shields, they retreated and groomed vigorously. This observation allowed us to focus our search on the nonvolatile constituents in *P. clavata*'s shield.

Origin, Identity, and Deterrence of Shield Compounds. Solanum species are well known to contain many complex and toxic secondary compounds, including steroidal glycoalkaloids and steroidal saponins (Roddick, 1986; Hegnauer, 1992). The leaves of *P. clavata*'s host, *S. dulcamara*, are also a rich source of

FIG. 5. Comparison of FAB-MS spectra from fractions C from *S. dulcamara* and from *P. clavata*'s shield. (A) Host plant. Structure at left, corresponding to peak 1, is α-solamargine (solasodine-chacotriose). Structure at right, corresponding to peak 2, is α-solasodine (solasodine-solatriose). Peak 3 is the sodium adduct (MNa^+) of peak 2 with m/z 906. (B) Fecal shield. The proposed structure of the SGA metabolite, corresponding to peak 12, is hydroxylated at carbon 26 of the F ring (peak 12, m/z 900). (C) The expanded region of *P. clavata*'s fraction C from m/z 700 to m/z 786. (D) The expanded region of *P. clavata*'s fraction C from m/z 801 to m/z 928. There is no evidence for the addition of double bonds prior to the removal of sugars, as there was for fraction B, which indicates that the first steps in SGA metabolism are hydroxylation followed by deglycosylation. The remaining ions are SGA fragments; each insect metabolite is 16 mass units greater than the equivalent SGA fragment.

free phytol, in addition to steroidal saponin, dioscin, and two glycosides of the SGA solasodine (Morton and Vencl, 1998). Data from host fraction C further confirmed the SGA component to be a mixture of two glycosides of solasodine that differed only in the configuration of their trisaccharide moieties.

Plagiometriona clavata's shield contained four major classes of compounds: (1) SGA derivatives, (2) a suite of dioscin metabolites, (3) FAa, and (4) phytol derivatives. With the possible exception of the FAs, which may have been liberated from plant triglycerides, all shield metabolites had host plant precursors. Representatives of the four compound classes, or their closest commercially available analogs, were previously demonstrated to be deterrent to ants (Morton and Vencl, 1998; Vencl and Morton, 1998), and we cite additional information justifying their functional designation as deterrent compounds.

The steroidal components of *P. clavata*'s shield were derived from the host's α-solasonine (solasonine-solatriose) and α-solamargine (solasodine-chacotriose), while the diosgenin derivatives were metabolites of the plant's saponin, dioscin (dioscin-chacotriose). The specifics of steroid metabolism will be discussed below. Steroidal alkaloids and saponins are well known to have significant toxic effects (Appelbaum and Birk, 1979; Harborne, 1986; Keukens et al., 1996; Mitchell and Harrison, 1985; Roddick, 1986; Roddick et al., 1990). However, with the exception of the leaf beetle shields discussed below, there are no other reports of insects either metabolizing or of using these steroids for defense (Brown, 1987); their presence in *P. clavata*'s shield is exceptional.

The third shield component, the FAs, accounted for nearly half (49%) of the total fraction A chemical profile. In contrast to the other shield components, there was a discrepancy between the ingested host FAs and the shield FA content. Neither the main shield FAs, octadecenoic (oleic) acid and hexadecenoic (palmitoleic) acid, nor a variety of minor FA methyl esters were detected in the host plant. Since the FA compositions of tortoise beetle shields and of leaf beetle body extracts are similar (Thompson, 1973), some shield FAs may have come from the larvae themselves. However, it is more likely that larvae liberated FAs from phospholipids and triglycerides of the plant. Since bound FAs do not extract into the 80% methanol solution, unbound FAs would not be detectable in the plant with the extraction and fractionation protocol employed here.

In contrast to SGAs and saponins, FAs are found in other invertebrate defenses. For example, unsaturated FAs, such as oleic and methyl palmitate, are also present in chrysomelid eggs and in the defensive secretions of wasps, where they act to deter ants (Howard and Tschinkel, 1975; Pasteels et al., 1986; Eisner et al., 1996; Dani et al., 1996). Several other FAs, including the ones identified in shields, evoke necrophoresis (undertaker) behavior (Blum, 1970; Haskins, 1970; Howard and Tschinkel, 1975; Hölldobler and Wilson, 1990) in members of the Formicidae.

Lastly, the defensive potential of unbound phytol and its derivatives has

been discussed elsewhere (Morton and Vencl, 1998). For example, 6,10,14-trimethylpentadecan-2-one, one of the phytol derivatives identified here, was found to be part of the defensive secretion in at least one species of formicine ant (Regnier and Wilson, 1968). Thus, the presence in shield defenses of compounds demonstrably capable of altering ant behavior, such as SGAs, diosgenin, FAs, and phytol derivatives, clearly seems of functional benefit to tortoise beetle larvae.

Adaptive Convergence of Solanum*-Derived Fecal Shields.* Whenever similar character states evolve independently in similar environments, one can ask if they were adaptations that benefit their bearers. Comparisons of convergent characters, especially if they are complex, can be useful for the identification of particular agents of contemporary selection that maintain them as functionally beneficial adaptations. We compared two unrelated fecal shield-bearing taxa occupying the same trophic niche.

The fecal shield of the shining leaf beetle, *Lema trilinea*, which also feeds on *S. dulcamara*, is an effective defense necessary for larval survival that is based on host-derived chemicals (Morton and Vencl, 1998). Besides the elaborate anatomical modifications for fecal deposition above the larvae, there were several striking chemical parallels between the shield systems of both species. For example, the profile of FAs, especially the large amount hexadecanoic acid, was similar. Although there is more oleic acid in the tortoise beetle's feces than occurred in *L. trilinea*'s, the suite of diosgenin derivatives is virtually identical for both species in both their amounts and their MS fragmentation patterns.

The most interesting similarity between shield systems is how the two beetle species transform and used the SGAs and saponins, potentially the most inimical allelochemicals they encounter. To compartmentalize and eliminate allelochemicals from the organism, metabolic detoxification systems typically increase allelochemical polarity (i.e., aqueous solubility) by using some combination of hydrolysis, oxidation, conjugation with a polar moiety, or reduction. (Dauterman and Hodgson, 1978; Duffey, 1980). As noted, SGAs and saponins are considered extremely toxic; they can bind with membrane-bound sterols, thereby altering membrane permeability or causing loss of cell integrity (Keukens et al., 1996; Mitchell and Harrison, 1985; Roddick et al., 1990). SGAs and saponins differ only by the substitution of nitrogen with oxygen in their respective F rings, and they produce their toxic effects by insertion of their apolar F-ring portions into the target membrane. Both beetles evidently use a three-step hydrolysis–reduction strategy to detoxify and then "sequester" SGAs in their shield defenses. They first hydroxylate, then deglycosylate, and finally add extra double bonds and additional hydroxyls to both SGA and diosgenin residues. Hydroxylation of the apolar portions of saponins and SGAs might well reduce their membrane binding tendency at the same time it increases their polarity, making them more readily confinable within the gut and facilitating their elimi-

nation in feces. SGA-laced feces, not accidentally we think, become strategically positioned on or above larvae, to act as deterrents in shield defenses.

CONCLUSIONS

Instead of being metabolized, erstwhile nutrients and some toxic secondary compounds formed part of the larval shield. The particular metabolites we identified were likely not incidental digestive by-products, but probably represented adaptations for defense. The presence of particular compounds in shields was most plausibly explained by their deterrence, which evidently outweighed their nutritional value. Before the sufficiency of this explanation was assumed, however, three sources of evidence provided by this study supported the idea that shields, and the specifics of their chemical makeup, are adaptations maintained as selective responses to predation: (1) shields are necessary for larval survival; (2) individual compounds have beneficial effects, i.e., they act as deterrents, and (3) shields convergently function as chemical defenses in species with the same ecology. In the limit, the inclusion of host-derived allelochemicals in shields may be due to the apparent inability of larvae to synthesize their own defensive compounds. Nonetheless, defense is perhaps the best explanation for the occurrence of particular compounds in shields.

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