Chemoecology (2013) 23:1–11
DOI 10.1007/s00049-012-0117-7

CHEMOECOLOGY

RESEARCH PAPER

Making scents of defense: do fecal shields and herbivore-caused volatiles match host plant chemical profiles?

Ken Keefover-Ring

Received: 18 March 2012 / Accepted: 26 September 2012 / Published online: 13 October 2012

Abstract Many plant families have aromatic species that produce volatile compounds which they release when damaged, particularly after suffering herbivory. *Monarda fistulosa* (Lamiaceae) makes and stores volatile essential oils in peltate glandular trichomes on leaf and floral surfaces. This study examined the larvae of a specialist tortoise beetle, *Physonota unipunctata*, which feed on two *M. fistulosa* chemotypes and incorporate host compounds into fecal shields, structures related to defense. Comparisons of shield and host leaf chemistry showed differences between chemotypes and structures (leaves vs. shields). Thymol chemotype leaves and shields contained more of all compounds that differed than did carvacrol chemotypes, except for carvacrol. Shields had lower levels of most of the more volatile chemicals than leaves, but more than twice the amounts of the phenolic monoterpenes thymol and carvacrol and greater totals. Additional experiments measured the volatiles emitted from *M. fistulosa* in the absence and presence of *P. unipunctata* larvae and compared the flower and foliage chemistry of plants from these experiments. Flowers contained lower or equal amounts of most compounds and half the total amount, compared to leaves. Plants subjected to herbivory emitted higher levels of most volatiles and 12 times the total amount, versus controls with no larvae, including proportionally more of the low boiling point chemicals. Thus, chemical profiles of shields and volatile emissions are influenced by the amounts and volatilities of compounds present in the host plant. The implications of these results are explored for the chemical ecology of both the plant and the insect.

K. Keefover-Ring (✉)
Department of Ecology and Evolutionary Biology, University of Colorado, N122 Ramaley, Boulder, CO 80309, USA
e-mail: kenneth.keefover-ring@plantphys.umu.se

Present Address:
K. Keefover-Ring
Umeå Plant Science Centre, Department of Plant Physiology, Umeå University, Umeå 90187, Sweden

Keywords Aromatic plants · Herbivore-caused volatiles · Larval fecal shields · *Monarda fistulosa* · *Physonota unipunctata* · Terpenoids

Introduction

Plants emit volatiles when fed upon by insects. The volatiles resulting from herbivory for many plant species consist of various mono- and sesquiterpenoids, heterocyclic compounds such as indole, and saturated and unsaturated six-carbon oxygenated compounds known as "green-leaf" volatiles (Pare and Tumlinson 1999). These chemicals often mediate interactions between the plants that emit them and other organisms, including the attraction of herbivore natural enemies (De Moraes et al. 1998), or the deterrence (Bernasconi et al. 1998; De Moraes et al. 2001; Zebelo et al. 2011) and attraction of additional herbivores (Kalberer et al. 2001; Heisswolf et al. 2007; Zebelo et al. 2011). Different herbivores elicit varying amounts and types of volatiles with the highest levels released by plants attacked by chewing insects, as opposed to much lower amounts from piercing-sucking herbivores (Turlings et al. 1998).

The volatiles emitted by aromatic plants during herbivory have received less attention (Degenhardt and Lincoln 2006; Kannaste et al. 2008; Zebelo et al. 2011). Yet, many plant families (Apiaceae, Pinaceae, Rutacae, Solanaceae, etc.) have aromatic species that produce a wide array of volatile compounds, usually made and stored in specialized structures. Herbivore damage to tissues containing these structures liberates large amounts of volatiles (Degenhardt and Lincoln 2006; Kannaste et al. 2008; Zebelo et al. 2011).

The Lamiaceae is a family especially well known for aromatic plants (e.g. thyme, lavender, peppermint). Most species in this family make mono- and sesquiterpenes, commonly referred to as essential oils, constitutively on the surface of leaves, reproductive parts, and stems, mostly in peltate glandular trichomes consisting of secretory cells mounted on a stalk which produce the terpenes and secrete them into an outer subcuticular sac for storage (Turner et al. 2000; Croteau 2001). Any damage, including herbivory, to plant parts containing these trichomes results in their rupture, content release, and subsequent volatilization. Once released mono- and sesquiterpenes often act as semiochemicals, an ideal role given their volatility and structural diversity (Gershenzon and Dudareva 2007).

Despite the relatively large amounts of secondary compounds found in aromatic plants, some specialist insects have evolved the ability to tolerate these chemicals. This has certainly been the case with the Chrysomelidae family, which contains many species that not only eat the foliage of plants containing high amounts of secondary chemicals, but often also use these compounds for their own defense (Pasteels et al. 1983; Morton and Vencl 1998; Vencl and Morton 1998; Becerra et al. 2001; Weiss 2006). The subfamily Cassidinae, known as tortoise beetles due to the shell-like appearance of the adults, comprise one group of Chrysomelids noted for using host chemistry for protective purposes. In many species larvae accumulate a fecal shield, consisting of frass sometimes combined with exuvia, and often rich in host plant secondary compounds, on a caudal fork (Gómez et al. 1999; Vencl et al. 1999; Vencl et al. 2005; Weiss 2006; Chaboo 2007). While cassidine fecal shields effectively defend larvae against natural enemies (Olmstead and Denno 1993; Gómez et al. 1999; Vencl et al. 1999; Eisner and Eisner 2000; Bacher and Luder 2005; Vencl et al. 2005, 2009), other evidence indicates that the volatiles from chrysomelid fecal shields also attract natural enemies (Müller and Hilker 1999; Schaffner and Müller 2001).

The tortoise beetle *Physonota unipunctata* uses *Monarda fistulosa* as its only host (Hamilton 1884; Criddle 1926; Sanderson 1948). Like most labiates, *M. fistulosa* produces essential oils in trichomes on leaves and reproductive parts (Heinrich 1973). Throughout much of its range, plants belong to one of two chemical phenotypes (chemotypes), producing either of the phenolic (six-membered aromatic ring with an hydroxyl side group) monoterpenes thymol or carvacrol as the dominant components of their essential oil (Fig. 1; Scora 1967; Weaver et al. 1995; Johnson et al. 1998; Keefover-Ring,

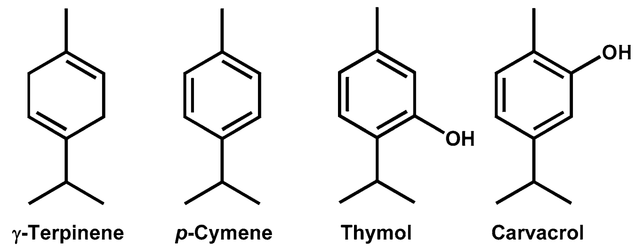

Fig. 1 The four major monoterpenes found in the essential oil of *Monarda fistulosa* thymol (*T*) and carvacrol (*C*) chemotypes

unpublished data). Both of these compounds deter a greater variety of pathogens and parasites than do other non-phenolic monoterpenes (Linhart and Thompson 1995, 1999), yet, *P. unipunctata* readily feeds on plants of both chemotypes (Keefover-Ring, unpublished data). Thus, these beetles may benefit defensively from host chemicals that they incorporate into their fecal shields, but the increased volatiles emitted during feeding may attract their natural enemies or additional competitive herbivores.

To begin to understand the chemically mediated interactions between *P. unipunctata* and its host plant, *M. fistulosa*, this study addresses two fundamental questions: (1) How does the chemical composition of larval fecal shields compare to that of the foliage of its host plant, and; (2) What quantitative and qualitative differences exist between the volatile profiles released from host plants with or without feeding by the larvae of this dietary specialist?

Methods and materials

Study organisms

Monarda fistulosa L. var. *menthifolia* (Graham) Fernald (Lamiaceae), commonly known as wild bergamot, bee balm, or horse mint, is a perennial labiate that occurs throughout much of the western half of North America (USDA 2012). Individual plants consist of multiple ramets, up to 1 m high, which arise directly from the ground. Each ramet has one to several terminal capitate inflorescences consisting of lavender-colored tubular flowers subtended by leaf-like bracts. Below the capitula the stem has alternating pairs of lanceolate leaves with serrated margins.

In Colorado, *M. fistulosa* has two main essential oil chemotypes, with individual plants producing either thymol (T) or carvacrol (C) as their most abundant terpenoid (Fig. 1; Keefover-Ring, unpublished data). In addition to their dominant monoterpene, both chemotypes contain numerous minor components, including relatively high amounts of γ-terpinene and *p*-cymene (Fig. 1; Scora 1967; Weaver et al. 1995; Johnson et al. 1998; Keefover-Ring, unpublished data). The aliphatic monoterpene γ-terpinene serves as a biosynthetic precursor for the aromatic *p*-cymene, which then undergoes hydroxylation to produce either thymol or carvacrol (Poulose and Croteau 1978a; b; R. Croteau, pers. comm.).

Monarda fistulosa has few reported herbivores (Davis et al. 1988; Wyckhuys et al. 2007). However, one specialist found on the species is the horsemint tortoise beetle, or one-spotted tortoise beetle, *Physonota unipunctata* (Say 1823, *Cassida unipunctata*; Coleoptera: Chrysomelidae), which is monophagous on *M. fistulosa* (Hamilton 1884; Criddle 1926; Sanderson 1948). Both larvae and adult *P. unipunctata* feed exclusively on *M. fistulosa* and can reduce seed set up to 88 % (Keefover-Ring, unpublished data). In Colorado, adult tortoise beetles lay a cluster of eggs covered by a filamentous film on the underside of a leaf in mid-May (Fig.. 2a, b). After hatching, larvae chew their way through to the top of the leaf and usually climb the plant stem, initially as a group, to feed on leaves or floral structures (Fig. 2c). Larvae protect themselves through gregarious feeding during early instars (5 instars total), often on the underside of leaves. In addition, like some *Physonota*, they accumulate fecal material on fork-like paired urogomphi located on the last segment of the abdomen using their muscular telescopic and highly protrusible anus (Fig. 2c, d; white arrow in c indicates caudal fork; C. Chaboo, pers. comm.). Larvae hold their shields above their bodies and wave them at an offender, if perturbed (Keefover-Ring, pers. obs.).

Larval fecal shield and host plant chemistry

To compare the essential oil chemistry of larval fecal shields with that of the leaves that larvae feed upon, I extracted essential oils from both leaves and shields and analyzed the resulting solutions with gas chromatography (GC) with a flame ionization detector (FID). I collected leaves and larval shields from both T ($N = 21$ shields and 20 leaves) and C ($N = 17$ shields and 16 leaves) chemotype host plants from two natural populations near Boulder and Broomfield, CO on June 23 and 25, 2005, respectively. I removed fecal shields from larvae with a stainless steel probe, cleaning it with ethanol between individuals, and placed the shield material in 2 ml microcentrifuge tubes together with 100 µl of an internal standard solution (*m*-xylene in pure ethanol). In addition, I clipped the individual leaves that each larva was feeding upon, placed them in the same size microcentrifuge tubes, and submersed them in 1 ml of the internal standard solution. Sometimes more than one larva fed upon the same host leaf and in these cases multiple shields per leaf were taken, but analyzed separately. After 30 s of sonication and brief vortex mixing, the samples soaked for one week at ambient temperature in the dark and then a small aliquot was removed for GC analysis (see "Chemical analysis" below). After GC testing, I removed shield material and leaves from the solvent and dried them to a constant weight at 60 °C.

Fig. 2 Life history of *Physonota unipunctata*: **a** Adult tortoise beetle on *Monarda fistulosa* leaf, **b** Ootheca on the abaxial leaf surface, **c** first instars just after hatching, chewing on the adaxial leaf surface, **d** last instars with fecal shields. Note the round, peltate glandular trichomes on the leaf surfaces in all photos, especially apparent in **c**. *White arrow* in **c** indicates caudal fork. *Scale bars* 5 mm

Volatile emissions in the absence and presence of herbivores

To measure the volatiles emitted without and with tortoise beetle larvae feeding on *M. fistulosa*, I collected air samples above plants using dynamic headspace methods and identified and quantified compounds by GC with mass spectrometry (MS) detection. Beginning on June 29, 2006, and on four subsequent sampling days (July 4, 6, 7, and 14), I collected fresh T chemotype plants for volatile capture each morning from a site in the foothills about 4 km west of the University of Colorado at Boulder campus. I clipped two flowering stems each from multiple individual plants at ground level, immediately placed them in fresh water, and returned them to a greenhouse at the University of Colorado. I cut the lowest two sets of leaves from each stem and slid the remaining plantlet, including the flowering head and several sets of leaves, stem-first into the top opening of 1,000 ml separatory funnels (stopcocks removed and holes covered), which served as headspace enclosures. I wrapped a cotton strip around the plant where it exited the funnel and placed the end of the stem in a floral watering pick. I randomly assigned one of the two stems from each plant to the herbivory treatment and using tweezers placed 4–5 late instar larvae, without fecal shields and collected from plants not used for volatile sampling, on the first pair of leaves below the capitula. I attached volatile chemical traps, with glass and PTFE two-way valves on both sides, to the top of each separatory funnel. A bypass tube connected to both two-way valves allowed the traps to be either inline or isolated while still maintaining air flow through the separatory funnels. The traps consisted of 65 mm long and 3 mm ID glass tubes packed with 20 mg of Super Q adsorbent (80/100 mesh size, DVB/ ethylvinylbenzene polymer; Alltech Associates Inc., Deerfield, IL, USA) held in place by glass wool plugs. I initially withdrew air from the enclosures for 1 h with the chemical traps bypassed to purge any volatiles released due to trichome breakage that might have occurred during handling (Owen 1998). After the purge, I placed the chemical traps inline and collected volatiles for 4 hr. Calibrated flow meters (Aalborg Instruments & Controls, Inc., Orangeburg, New York, USA) and an AirLite precision pump (SKC, Inc., Eighty Four, PA, USA) maintained the flow rate for each enclosure at 181 ml min^{-1} for both the purge and sample collections. To control for ambient volatiles, I ran a separate 1,000 ml separatory funnel without a plant stem on each sampling day. Partial shading of the entire setup in the greenhouse prevented overheating within the headspace enclosures. On each sampling day, I ran three pairs of stems and a control, except for the last day when only two pairs and the control were tested, for a total of 14 pairs. I carried out volatile sampling at approximately the same time each day, beginning the purge around 11:30 a.m. (±1 h). Most larvae fed during the entire sampling time, mostly on foliage and not on inflorescences. At the conclusion of each sampling period, I rinsed the Super Q traps with 0.6 ml *n*-hexane (GC^2, Honeywell Burdick & Jackson, Morristown, NJ, USA), then combined 110 µl of each sample with 5 µl of an internal standard solution (*m*-xylene in *n*-hexane) and injected them into a GC-MS for chemical analysis (see "Chemical analysis" below).

Chemistry of plants from herbivore volatile experiment

To assess the relative contribution of volatiles from different plant tissues, I removed the plant stems from the enclosures following volatile capture and separately soaked 7–8 single flowers, including all reproductive parts, and a single leaf from the first set of leaves below the capitula (from the June 29, and July 4, and 14 headspace samples only), in 1 ml of the ethanol internal solution for 1 week. I analyzed the resulting solutions by GC-FID (see "Chemical analysis" below), then removed the tissue from the solvent dried and weighed them, as above.

I also dried the entire plant stems from all volatile sampling days, using only the trichome-rich capitula and leaves to calculate emissions rates per plant biomass. The amounts of the various compounds in flowers and leaves were reported as mg g^{-1} DW and volatile emission rates as ng g^{-1} DW h^{-1}, using the specific gravities of liquid chemicals to convert where necessary.

Chemical analysis

I analyzed fecal shields and all flower and leaf samples on a Hewlett Packard 6890 GC equipped with a FID and fitted with a DB-Wax glass capillary column (15 m × 0.25 mm I.D., 0.25 µm film thickness; J&W Scientific, Folsom, CA, USA). Helium served as the carrier gas at a flow rate of 37 cm s^{-1} (1.3 ml min^{-1}) with a split flow ratio of 50:1. Injector temperature was set at 260 °C and detector at 250 °C. The oven profile for the 2005 fecal shield and leaf extracts consisted of an isothermal hold at 50 °C for 5 min followed by a ramp of 3 °C min^{-1} to 75 °C, a second ramp of 10 °C min^{-1} to 160 °C, and a final ramp of 25 °C min^{-1} to 240 °C. The 2006 flower and leaf extracts used a different oven profile with an initial temperature of 60 °C for 5 min followed by a ramp of 6 °C min^{-1} to 125 °C, a second ramp of 10 °C min^{-1} to 170 °C, and a final ramp of 25 °C min^{-1} to 240 °C. Three µl of both samples and standards were injected. Due to a solvent impurity, the amounts of α-pinene, α-thujene, and camphene could not be determined in the 2005 larval fecal shield and leaf samples. In addition, because of the different oven profile used for the 2006 flower and leaf samples the usually small terpinen-4-ol peak co-eluted with carvacrol methyl ether.

I analyzed the volatile collection samples using an Agilent 6890N GC coupled with an Agilent 5975C inert mass selective detector with an ion source of 70.0 eV at 230 °C, also using helium as the carrier gas at 36 cm s^{-1} (1.0 ml min^{-1}) with the injector temperature set at 260 °C. I injected 1 µl of each sample in the splitless mode onto an EC-Wax capillary column (30 m × 0.25 mm I.D., 0.25 µm film thickness; Alltech Associates Inc., Deerfield, IL, USA). Oven conditions included an isothermal hold at 60 °C for 5 min, followed by a ramp of 6 °C min^{-1} to 250 °C. Under this GC oven profile α-phellandrene co-eluted with methyl seneciote and thymol methyl ether with terpinen-4-ol, so the amounts of these pairs were calculated together.

I quantified individual compounds by comparing sample peak areas (GC-FID peak areas for all samples, except the volatile samples which used GC-MS peak areas) to standard curves of available authentic compounds diluted into internal standard solution (all standards from Sigma-Aldrich, St. Louis, MO, USA, except β-phellandrene, which was from Glidco Organics, Jacksonville, FL, USA; see Table 2). The amounts of compounds for which no standards were available were calculated by the peak area of the nearest structurally similar chemical. I identified compounds using retention times and mass spectra of available authentic standards, the NIST 2005 mass spectral library, and published mass spectra (Adams 2007; El-Sayed 2012).

Additional compound identification consisted of injection of a continuous series of *n*-alkanes (C_8–C_{24}; Sigma-Aldrich, St. Louis, MO, USA) to calculate compound linear retention indices on the same 15 m DB-Wax and 30 m EC-Wax columns used in the above analyses, and with an HP-5MS capillary column (30 m × 0.25 mm I.D., 0.25 µm film thickness; Agilent Technologies, Inc., Santa Clara, CA, USA) installed on the GC-MS. All GC conditions remained the same as above, except for the oven profile, which for all retention indices runs consisted of an initial temperature of 40 °C followed by an immediate ramp of 3 °C min^{-1} to 200 °C. I compared calculated retention indices of peaks from representative samples and standards to published values (Jennings and Shibamoto 1980; Davies 1990; Figueredo et al. 2006; Adams 2007; El-Sayed 2012). Owing to the more gradual oven ramp, all peaks that had previously co-eluted were resolved during retention indices' runs.

Statistical analysis

I used SAS version 9.1 (SAS Institute 2003) for all statistical analyses and to examine the distributions of all variables to insure they met assumptions of normality, applying transformations where necessary. Initially, I used the PROC GLM function with the MANOVA statement to perform a two-factor multivariate analysis testing for overall differences between the essential oil profiles of the 2005 fecal shield and host leaf extract samples, with chemotype (T or C) and structure (shields or leaves) as factors. I performed a similar MANOVA analysis on the 2006 extracts of T plants used in the headspace sampling with plant part (flowers or leaves) and herbivory (without and with) as factors. In all cases, the multiple variables consisted of all individual chemical amounts (mg g^{-1} DW). Any significant MANOVA analyses were followed with separate ANOVA analyses to look for differences in individual compounds and the total for all of the above factors.

Due to a much larger number of compounds (43) detected in the headspace collection experiment, I first performed a factor analysis to reduce the number of variables using the PROC FACTOR command with a Varimax rotation and accepted components with eigen values greater than one. I then analyzed the resulting factor scores (from seven factors) with MANOVA to test for overall chemical profile differences between the no herbivore and herbivore treatments. In light of a significant MANOVA result, I then ran one-way ANOVAs to look for individual compound and total differences in amounts emitted (ng g^{-1} DW h^{-1}) between the no herbivore and herbivore treatments.

Results

Larval fecal shield and host plant chemistry

GC-FID analysis detected 18 compounds (14 monoterpenes, 1 sesquiterpene, 1 secondary alcohol, and 2 unknowns; Fig. 3) in the 2005 fecal shield and host leaf extracts. Overlays of matching fecal shield and leaf chromatograms from both chemotypes showed only a few small extraneous peaks, indicating little change to host chemistry after larval digestion.

MANOVA analysis of the 2005 samples revealed that essential oil profiles differed as a function of both chemotype (T vs. C; Wilks' $\lambda = 0.02$, $F_{20,51} = 101.8$, $P < 0.001$) and structure (fecal shields vs. leaves; Wilks' $\lambda = 0.08$, $F_{20,51} = 29.9$, $P < 0.001$). Two-way (chemotype and structure) ANOVA results for single chemicals for the chemotype factor showed that T chemotype leaves and shields contained more of all compounds that differed than C chemotypes, except for carvacrol (Fig. 3; Table 1). The amounts of sabinene, δ-3-carene, β-phellandrene, terpinen-4-ol, and the total did not differ between chemotypes

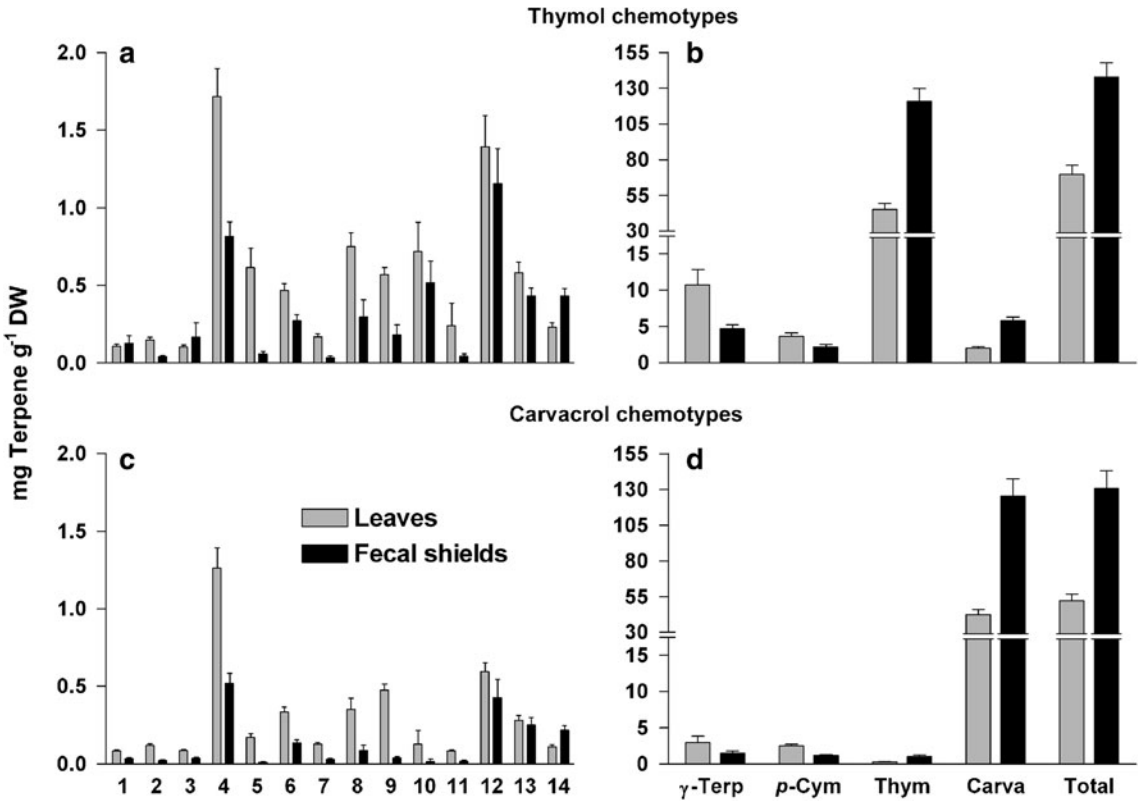

Fig. 3 Mean (±SE) amounts of compounds and the total [mg g^{-1} dry weight (DW)] extracted from *Monarda fistulosa* host leaves (*gray bars*) and *Physonota unipunctata* larval fecal shields (*black bars*) from T (*graphs* **a**, **b**) and C chemotype (*graphs* **c**, **d**) plants. *1* β-pinene, *2* sabinene, *3* δ-3-carene, *4* β-myrcene, *5* α-terpinene, *6* limonene, *7* β-phellandrene, *8* 1-octen-3-ol, *9* *cis*-sabinene hydrate, *10* carvacrol methyl ether, *11* terpinen-4-ol, *12* germacrene D, *13* unknown 4, *14* unknown 5, *γ-Terp* γ-terpinene, *p-Cym* *p*-cymene, *Thym* thymol, and *Carva* carvacrol. Note the differences in scale; see Table 1 for corresponding statistics

(Fig. 3a–d; Table 1). The comparison of leaves and fecal shields (the structure factor) showed that leaves had more of all of the chemicals that differed, except for thymol, carvacrol, and the total. Fecal shields contained more than twice the amount of these three variables (Fig. 3b, d; Table 1). Carvacrol methyl ether, terpinen-4-ol, and unknown 4 did not differ between leaves and fecal shields (Fig. 3; Table 1). The factors of chemotype and structure interacted for the variables α-terpinene, thymol, and carvacrol (Table 1). For α-terpinene, this was due to the higher amount in the leaves of T chemotype plants (Fig. 3a, c) and for thymol and carvacrol because of their big differences in the respective chemotypes (Fig. 3b, d).

Volatile emissions in the absence and presence of herbivores

Monarda fistulosa T chemotype emission samples, analyzed with the more sensitive GC-MS method, contained 43 different compounds (25 monoterpenes, 5 sesquiterpenes, 2 benzenoids, 2 green-leaf volatiles, 3 hydrocarbons, 1 secondary alcohol, and 5 unknowns; Table 2). The MANOVA test showed that the volatile profile differed substantially between the control and herbivory treatments (Wilk's $\lambda = 0.25$, $F_{7,20} = 8.8$, $P < 0.001$). ANOVA results of individual compounds and total amounts revealed that plants with herbivores emitted more of all volatiles measured compared to control plants, expect for methyl isovalerate, hexyl butanoate, bornyl acetate, isobornyl acetate, methyl benzoate, unknown 2, methyl salicylate, and unknown 3 (Table 2). As a result, plants with herbivores emitted an average of 12 times more total volatiles than those without (Table 2).

Chemistry of plants from herbivore volatile experiment

Multivariate comparison of essential oil profiles between flower and leaf extracts of T chemotype plants used in the volatile collection experiments showed a difference between the two types of plant tissue (Wilk's $\lambda = 0.03$, $F_{20,9} = 13.7$, $P < 0.001$; Fig. 4), but no difference when comparing the chemistry of plants without and with larval feeding (Wilk's $\lambda = 0.54$, $F_{20,9} = 0.4$, $P = 0.964$). ANOVA analyses of individual compounds between flowers and leaves found that leaves contained more of all terpenes that differed, except α-terpinene, and greater totals

Table 1 *F* and *P* values from a two-factor ANOVA comparing individual and total compounds from extracts of *Physonota unipunctata* larval fecal shields and *Monarda fistulosa* leaves between chemotype [thymol (T) or carvacrol (C) plants], and structure (shields or leaves), and their interaction

Compound	Chemotype		Structure		Chemotype by structure	
	F	*P*	*F*	*P*	*F*	*P*
β-Pinene	5.3	0.025	12.6	0.001	2.0	0.159
Sabinene	1.9	0.176	67.9	<0.001	0.2	0.668
δ-3-Carene	2.0	0.160	11.4	0.001	1.0	0.313
β-Myrcene	6.1	0.016	41.5	<0.001	0.1	0.762
α-Terpinene	16.4	<0.001	78.6	<0.001	4.9	0.030
Limonene	10.8	0.002	32.1	<0.001	0.4	0.515
β-Phellandrene	0.2	0.686	79.2	<0.001	0.9	0.344
γ-Terpinene	30.2	<0.001	7.8	0.007	1.4	0.239
p-Cymene	7.9	0.007	20.9	<0.001	0.1	0.803
1-Octen-3-ol	9.4	0.003	34.7	<0.001	1.0	0.320
cis-Sabinene hydrate	5.6	0.021	70.5	<0.001	0.2	0.625
Carvacrol methyl ether	25.9	<0.001	1.0	0.314	0.0	0.965
Terpinen-4-ol	1.4	0.246	2.8	0.100	0.7	0.409
Germacrene D	21.3	<0.001	4.2	0.044	0.0	0.998
Unknown 4	19.5	<0.001	2.7	0.106	1.2	0.287
Unknown 5	23.8	<0.001	20.6	<0.001	1.9	0.172
Thymol	725.7	<0.001	59.0	<0.001	42.5	<0.001
Carvacrol	453.2	<0.001	75.0	<0.001	32.4	<0.001
Total	3.2	0.079	66.1	<0.001	0.7	0.400

Degrees of freedom equals one for all tests; see Fig. 3 for corresponding data

(Fig. 4; Table 3). Camphene, *β*-pinene, sabinene, *p*-cymene, and the sesquiterpene germacrene D showed no differences between the two plant tissues.

Discussion

When *Physonota unipunctat*a beetle larvae feed upon *Monarda fistulosa* they incorporate and concentrate plant secondary chemicals into their fecal shields, and at the same time cause the release of large amounts of host-derived volatiles. Comparisons of host leaf with *P. unipunctata* fecal shield chemistry revealed that shields contained more than twice the amount of total essential oils, mainly driven by much higher amounts of the two phenolic monoterpenes thymol and carvacrol. The increased amounts of these compounds in fecal shields should enhance larval defense. From the plant perspective, both of these compounds appear to be important for defense against natural enemies. *Thymus vulgaris* T and C chemotypes deterred or inhibited a wider variety of organisms than did chemotypes with non-phenolic monoterpenes (Linhart and Thompson 1995, 1999).

Physonota unipunctata larval shields showed large quantitative differences compared to foliage. However, there was no evidence that larvae could induce facultative changes in host leaf chemistry. Overlays of paired fecal shield and leaf chromatograms revealed only a few very small extraneous peaks. Cassidinae larvae feeding on terpenoid containing hosts in other studies also did not appear to sequester (Morrow and Fox 1980; Gómez et al. 1999) or biotransform (Gómez et al. 1999) any of the secondary compounds they ingested. However, the chemical analyses used in these past studies and in the current work (GC, which only measures volatile chemicals) may underestimate the modification of host compounds by larvae. Using liquid chromatography, Vencl et al. (2009) demonstrated that fecal shields of the tortoise beetle *Chelymorpha alternans* contained high amounts of a non-volatile catabolite of chlorophyll, pheophorbide *a*, that effectively deterred predatory ants. Given the ubiquitous nature of chlorophyll, it seems likely that pheophorbide *a* occurs in the fecal shields of many other beetle larvae, including *P. unipunctata*.

This study demonstrated that tortoise beetle larvae feeding upon wild bergamot resulted in a large terpenoid volatile signature, which was much greater than the emissions released from undamaged leaves without herbivores. This finding likely has several significant ecological and evolutionary implications for the tortoise beetle–mint interaction. First, *M. fistulosa* plants may benefit from the increased volatiles which can attract parasitoids or predators to prey on the tortoise beetles. Compounds found in this study, such as (*Z*)-3-hexenyl acetate and (*Z*)-3-hexen-1-ol, can act as an indirect defense by attracting herbivore natural enemies (Pare and Tumlinson 1999), but their levels were quite low compared to the large background of terpenoids. Specific chemical emissions from fecal shields do seem important in larval parasitism. Schaffner and Müller (2001) showed that lily leaf beetle (*Lilioceris lilii*) fecal shields attracted specialist parasitoid wasps, either while on larvae or alone, indicating a chemical cue. Another study found that ants used terpenoid volatiles from tortoise beetle larval shields to locate their prey (Müller and Hilker 1999). In addition, volatiles emitted due to herbivory may influence colonization by other herbivores. Several studies have documented deterrence of herbivores by terpenoids, many of which *M. fistulosa* emitted, in a variety of plant species (Bernasconi et al. 1998; De Moraes et al. 2001; Zebelo et al. 2011). Conversely, these volatile signals could also be detrimental to *M. fistulosa* by attracting additional herbivores (Kalberer et al. 2001; Heisswolf et al. 2007; Bruce and Pickett 2011; Zebelo et al. 2011). Finally, it should be noted that some of the volatiles

Table 2 Linear retention indices on a polar and non-polar column, emission rates, identification parameters, and one-way ANOVA results for individual and total compounds emitted from thymol (T) chemotype *Monarda fistulosa* flowering stems in the absence and presence of *Physonota unipunctata* larval feeding

Compound	Linear retention indices		Emission rate [ng g^{-1} DW h^{-1} (±SE)]		Identification parameters			*F*	*P*
	30 m EC-Wax	30 m HP5-MS	No larvae	Larvae					
Methyl isovalerate[a]	1009	–	6.0 (2.4)	2.5 (1.1)	–	RI	MS	0.7	0.428
α-Pinene	1013	930	74.1 (10.1)	522.1 (64.2)	CO	RI	MS	86.3	<0.001
α-Thujene	1018	924	61.6 (8.7)	2,071.7 (283.2)	–	RI	MS	300.8	<0.001
Camphene	1052	944	97.3 (13.4)	150.0 (16.2)	CO	RI	MS	6.3	0.019
Bornylene[a]	1074	–	ND	51.3 (7.9)	–	RI	MS	42.3	<0.001
β-Pinene	1097	973	28.3 (4.3)	137.8 (16.4)	CO	RI	MS	66.2	<0.001
Sabinene	1111	976	13.8 (1.9)	164.1 (19.7)	–	RI	MS	57.8	<0.001
δ-3-Carene	1136	1008	6.1 (3.0)	88.8 (13.9)	CO	RI	MS	71.8	<0.001
α-Phellandrene	1152	1002	72.2 (24.0)	149.4 (19.6)	CO	RI	MS	6.2	0.019
Methyl seneciote	1157	840	Co-eluted with α-phellandrene		–	RI	MS		
β-Myrcene	1155	990	58.7 (12.3)	1,312.8 (167.45)	CO	RI	MS	102.0	<0.001
α-Terpinene	1167	1014	5.8 (2.5)	767.3 (187.5)	CO	RI	MS	54.0	<0.001
Limonene	1186	1026	29.0 (3.8)	354.0 (52.6)	CO	RI	MS	74.2	<0.001
1,8-Cineole	1195	1028	1.5 (1.1)	20.2 (4.4)	CO	RI	MS	17.4	<0.001
β-Phellandrene	1196	1026	5.2 (1.42)	141.5 (19.5)	CO	RI	MS	84.8	<0.001
γ-Terpinene	1233	1056	220.5 (36.1)	3,188.0 (453.5)	CO	RI	MS	99.5	<0.001
cis-*β*-Ocimene	1241	1037	0.9 (0.6)	13.7 (4.1)	CO	RI	MS	9.3	0.005
p-Cymene	1257	1022	436.1 (57.7)	3,230.6 (470.5)	CO	RI	MS	72.0	<0.001
(*Z*)-3-Hexenyl acetate	1309	1007	4.6 (2.4)	34.0 (9.6)	CO	RI	MS	16.0	<0.001
(*Z*)-3-Hexen-1-ol	1371	857	2.7 (1.7)	18.7 (2.9)	CO	RI	MS	31.7	<0.001
Hexyl butanoate	1416	1190	4.2 (2.2)	6.0 (2.1)	–	RI	MS	0.5	0.474
1-Octen-3-ol	1442	979	10.8 (2.8)	152.4 (49.7)	CO	RI	MS	37.1	<0.001
cis-Sabinene hydrate	1452	1097	11.8 (3.5)	105.1 (18.6)	CO	RI	MS	48.5	<0.001
α-Copaene	1473	1373	ND	12.8 (3.9)	–	RI	MS	29.1	<0.001
β-Bourbonene	1498	1382	ND	36.2 (6.3)	–	RI	MS	131.6	<0.001
Bornyl acetate	1560	1284	4.4 (2.4)	5.8 (2.1)	CO	RI	MS	0.4	0.513
Isobornyl acetate	1564	1284	15.8 (6.8)	32.8 (10.1)	CO	RI	MS	2.0	0.175
Thymol methyl ether	1578	1233	8.0 (4.9)	89.3 (19.9)	CO	RI	MS	42.7	<0.001
Terpinen-4-ol	1583	1175	Co-eluted with thymol methyl ether		CO	RI	MS		
Carvacrol methyl ether	1588	1243	33.5 (16.2)	1,025.6 (301.5)	CO	RI	MS	25.0	<0.001
Methyl benzoate	1596	1093	29.8 (7.8)	46.9 (9.6)	CO	RI	MS	2.9	0.102
Borneol	1680	1163	39.0 (7.6)	63.3 (9.1)	CO	RI	MS	5.8	0.023
Unknown 1	1681	–	1.7 (1.7)	47.2 (7.7)	–	RI	b	34.2	<0.001
Germacrene D	1682	1478	5.8 (3.5)	109.0 (27.9)	–	RI	MS	34.7	<0.001
Unknown 2	1724	–	14.4 (11.4)	20.2 (13.7)	–	RI	c	0.2	0.686
δ-Cadinene	1733	1522	ND	14.9 (5.2)	–	RI	MS	13.6	0.001
(*E*,*E*)-α-Farnesene	1737	1508	ND	5.0 (2.3)	–	RI	MS	4.8	0.038
Methyl salicylate	1743	1191	13.5 (3.5)	23.7 (3.9)	CO	RI	MS	2.7	0.113
Unknown 3	1798	–	12.5 (7.2)	20.1 (6.9)	–	RI	d	3.2	0.086
Unknown 4	1830	1122	18.3 (7.95)	291.2 (28.9)	–	RI	e	83.0	<0.001
Unknown 5	2054	1297	2.4 (1.0)	65.3 (5.8)	–	RI	f	112.6	<0.001
Thymol	2162	1292	406.0 (112.4)	6,445.4 (1,841.6)	CO	RI	MS	64.6	<0.001
Carvacrol	2187	1301	29.1 (7.3)	499.9 (156.1)	CO	RI	MS	39.8	<0.001

Table 2 continued

Compound	Linear retention indices		Emission rate [ng g^{-1} DW h^{-1} (±SE)]		Identification parameters	F	P
	30 m EC-Wax	30 m HP5-MS	No larvae	Larvae			
Total			1,786.4 (253.2)	21,671.2 (3,717.7)		113.5	<0.001

Degrees of freedom equals one for all tests: *CO* co-injected authentic standard, *RI* retention index calculated, *MS* mass spectra match, *a* tentative identification, *ND* not detected, *b* Unknown 1 m/z: 69, 41, 125, 55, 139, 81, 97, 107, 31, 168; *c* unknown 2 m/z: 93, 69, 41, 68, 121, 80, 136, 107, 53, 27, *d* unknown 3 m/z: 69, 81, 41, 79, 95, 53, 175, 107, 136, 222, *e* unknown 4 m/z: 43, 97, 69, 55, 82, 107, 125, 27, 139, 168, *f* unknown 5 m/z: 98, 55, 111, 83, 84, 43, 69, 53, 126, 27

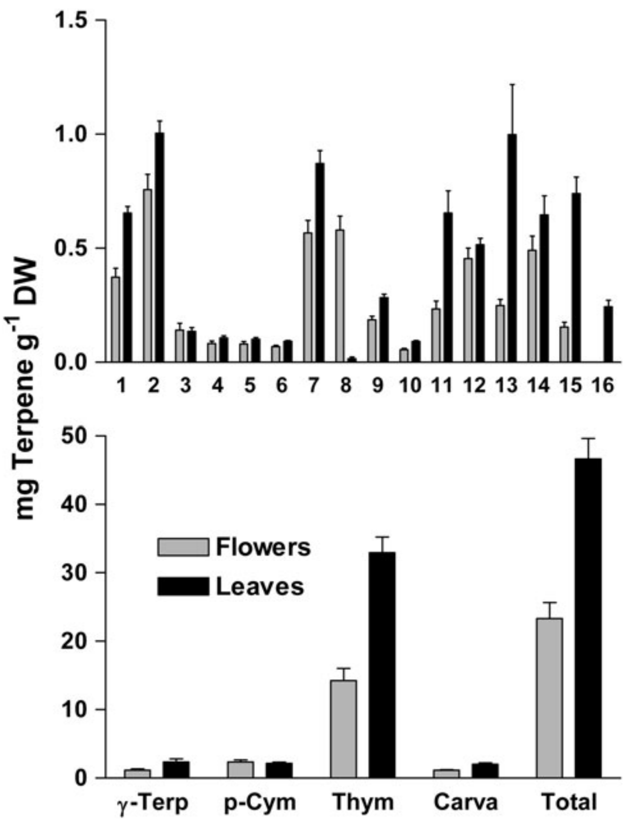

Fig. 4 Mean (±SE) amounts of compounds and the total [mg g^{-1} dry weight (DW)] extracted from flowers (*gray bars*) and leaves (*black bars*) of T chemotype *Monarda fistulosa* plants used in the volatile collection experiments. *1* α-pinene, *2* α-thujene, *3* camphene, *4* β-pinene, *5* sabinene, *6* δ-3-carene, *7* β-myrcene, *8* α-terpinene, *9* limonene, *10* β-phellandrene, *11* 1-octen-3-ol, *12* *cis*-sabinene hydrate, *13* carvacrol methyl ether, *14* germacrene D, *15* unknown 4, *16* unknown 5, *γ-Terp* γ-terpinene, *p-Cym* *p*-cymene, *Thym* thymol, and *Carva* carvacrol. Note the differences in scale; see Table 3 for corresponding statistics

Table 3 Linear retention indices on a 15 m DB-Wax column and one-way ANOVA results for differences of individual and total compounds between extracts of *Monarda fistulosa* flowers and leaves from plants used in volatile collection

Compound	Linear retention indices 15 m DB-Wax	F	P
α-Pinene	1018	33.8	<0.001
α-Thujene	1024	8.6	0.007
Camphene	1052	0.0	0.893
β-Pinene	1090	2.5	0.122
Sabinene	1107	3.0	0.092
δ-3-Carene	1130	11.2	0.002
β-Myrcene	1145	15.2	<0.001
α-Terpinene	1157	88.2	<0.001
Limonene	1177	20.7	<0.001
β-Phellandrene	1185	19.6	0.000
γ-Terpinene	1225	6.0	0.021
p-Cymene	1246	0.1	0.733
1-Octen-3-ol	1433	23.0	<0.001
cis-Sabinene hydrate	1445	1.2	0.279
Carvacrol methyl ether	1570	8.3	0.008
Germacrene D	1666	2.1	0.159
Unknown 4	1817	88.4	<0.001
Unknown 5	2058	92.0	<0.001
Thymol	2167	38.8	<0.001
Carvacrol	2191	14.2	<0.001
Total		36.4	<0.001

Degrees of freedom equals one for all tests; see Fig. 4 for corresponding data

emitted from both the control and herbivore treatment plants in this study may have resulted from the use of cut stems and the removal of lower leaves. While previous work with uncut *M. fistulosa* in the field found little difference in volatiles from the undamaged controls (without larvae) in this experiment (Keefover-Ring, unpublished data), increased volatile production after mechanical damage has long been recognized (Pare and Tumlinson 1997).

Disparities in the chemical profiles seen between *P. unipunctata* fecal shields, host leaves, and plant headspace samples most likely result from differences in the volatilities of the host's constituent terpenes. Specifically, both thymol and carvacrol have much higher boiling points (thymol, b.p. 231–232 °C and carvacrol, 236–237 °C at 760 mmHg) than most of the other compounds in this plant, especially compared to the relatively abundant γ-terpinene and *p*-cymene (b.p. 182 and 176–178 °C at 760 mmHg, respectively). The exposure of plant compounds, due to both the rupture of trichomes during larval feeding and the subsequent incorporation into fecal shields, would lead to differential loss of the more volatile essential oil components. This would account for the increased concentration of the two phenolic terpenes in shields compared to foliage, and the greater amounts of compounds with lower boiling points in the headspace samples. These volatility effects may help explain why other studies with tortoise beetle larvae exploiting terpenoid-containing plants also detected differences between fecal shield and host chemistry. Gómez et al. (1999) measured mono- and sesquiterpenes from host plant foliage and fecal shields of the tortoise beetle *Eurypedus nigrosignata* feeding on α-pinene or β-terpinene chemotypes of *Cordia curassavica.* They found that the shields of larvae feeding on α-pinene chemotypes contained less of this more volatile monoterpene than leaves, compared to similar amounts of β-terpinene in both structures (Table 1 in Gómez et al. 1999). They also saw a decline in both mono- and sesquiterpenes in fecal shields as larvae aged, another consequence of these compounds' inherent volatility. Finally, studies with other phenolic-producing labiates, such as *Origanum vulgare* plants with C chemotypes (Johnson et al. 2004) and with T and C chemotypes of *Thymus vulgaris* (Keefover-Ring, unpublished data), demonstrated increased amounts of γ-terpinene and *p*-cymene in headspace testing compared to their abundances relative to phenolic monoterpenes in foliage.

The use of host chemistry by tortoise beetle larvae may represent a trade-off between defense and the risk of emitting volatile signals that could possibly be exploited by an enemy or attract additional herbivores. Although several studies have characterized fecal shield chemistry and demonstrated the effectiveness of these structures as a defense (Olmstead and Denno 1993; Gómez et al. 1999; Vencl et al. 1999; Eisner and Eisner 2000; Vencl et al. 2005; Vencl et al. 2009), only limited work has shown that volatile emissions from chrysomelid shields may also play important roles in plant–animal interactions (Müller and Hilker 1999; Schaffner and Müller 2001). The fecal shield chemistry of *P. unipunctata* larvae, with its relatively high levels of the more toxic phenolics thymol and carvacrol, should deter predators and parasitoids more than other species that feed on hosts with less toxic secondary chemistry. In addition, the strong volatile signal produced from *M. fistulosa* as a result of damage by this specialist herbivore may influence the chemical ecology of both species.

Acknowledgments The suggestions of two anonymous reviewers greatly improved this paper. Deane Bowers, Russ Monson, Tom Ranker, Frank Stermitz, and especially Yan B. Linhart gave very helpful input on various versions of this manuscript. Lynn Riedel supplied permits to collect wild bergamot and tortoise beetle larvae on Boulder Open Space and Mountain Parks property. Rob Raguso shared his time and expertise explaining plant volatile collection techniques. Caroline Chaboo, Lech Borowiec, and Ed Riley helped with the taxonomy and natural history of *Physonota unipunctata*. Kailen Mooney, Harry Wu, and Chris Cole gave statistical advice. A Walker Van Riper grant from the University of Colorado Museum of Natural History partially supported this research.

References

Adams RP (2007) Identification of essential oil components by gas chromatography/mass spectroscopy, 4th edn. Allured Publishing Corp., Carol Stream

Bacher S, Luder S (2005) Picky predators and the function of the faecal shield of a cassidine larva. Funct Ecol 19(2):263–272

Becerra JX, Venable DL, Evans PH, Bowers WS (2001) Interactions between chemical and mechanical defenses in the plant genus *Bursera* and their implications for herbivores. Am Zool 41(4):865–876

Bernasconi ML, Turlings TCJ, Ambrosetti L, Bassetti P, Dorn S (1998) Herbivore-induced emissions of maize volatiles repel the corn leaf aphid *Rhopalosiphum maidis*. Entomol Exp Appl 87(2):133–142

Bruce TJA, Pickett JA (2011) Perception of plant volatile blends by herbivorous insects: finding the right mix. Phytochemistry 72(13):1605–1611

Chaboo CS (2007) Biology and phylogeny of the Cassidinae Gyllenhal *sensu lato* (tortoise and leaf-mining beetles) (Coleoptera: Chrysomelidae). Bull Am Mus Nat Hist 305:1–250

Criddle N (1926) A note on the synonymy of certain species of *Physonota*. Can Entomol 58:207–208

Croteau R (2001) Oil-filled glands on a leaf. Trends Plant Sci 6(9):439

Davies NW (1990) Gas chromatographic retention indexes of monoterpenes and sesquiterpenes on methyl silicone and Carbowax 20 M phases. J Chromatogr 503(1):1–24

Davis MA, Lemon M, Dybvig A (1988) The impact of prescribed burning and herbivorous insects on seed production and viability in two prairie forbs. Paper presented at The prairie: roots of our culture; foundation of our economy: proceedings of the Tenth North American Prairie Conference of Texas Women's University, Denton, Texas, June 22–26, 1986

De Moraes CM, Lewis WJ, Pare PW, Alborn HT, Tumlinson JH (1998) Herbivore-infested plants selectively attract parasitoids. Nature 393(6685):570–573

De Moraes CM, Mescher MC, Tumlinson JH (2001) Caterpillar-induced nocturnal plant volatiles repel conspecific females. Nature 410(6828):577–580

Degenhardt DC, Lincoln DE (2006) Volatile emissions from an odorous plant in response to herbivory and methyl jasmonate exposure. J Chem Ecol 32(4):725–743

Eisner T, Eisner M (2000) Defensive use of a fecal thatch by a beetle larva (*Hemisphaerota cyanea*). Proc Natl Acad Sci USA 97(6):2632–2636

El-Sayed AM (2012) The Pherobase: Database of Pheromones and Semiochemicals. http://www.pherobase.com/

Figueredo G, Cabassu P, Chalchat JC, Pasquier B (2006) Studies of Mediterranean oregano populations. VIII—Chemical composition of essential oils of oreganos of various origins. Flavour Frag J 21 (1):134–139

Gershenzon J, Dudareva N (2007) The function of terpene natural products in the natural world. Nat Chem Biol 3(7):408–414

Gómez NE (2004) Survivorship of immature stages of *Eurypedus nigrosignatus* Boheman (Chrysomelidae: Cassidinae: Physonotini) in Central Panama. Coleopt Bull 58(4):489–500
Gómez NE, Witte L, Hartmann T (1999) Chemical defense in larval tortoise beetles: essential oil composition of fecal shields of *Eurypedus nigrosignata* and foliage of its host plant *Cordia curassavica*. J Chem Ecol 25(5):1007–1027
Hamilton J (1884) On *Trogoderma ornata*, *Physonota unipunctata*, and *Tanysphyrus lemnae*. Can Entomol 16:133–135
Heinrich G (1973) Development, fine structure, and oil content of gland hairs of *Monarda fistulosa*. Plant Med 23(2):154–166
Heisswolf A, Gabler D, Obermaier E, Muller C (2007) Olfactory versus contact cues in host plant recognition of a monophagous chrysomelid beetle. J Insect Behav 20(2):247–266
Hoballah MEF, Tamo C, Turlings TCJ (2002) Differential attractiveness of induced odors emitted by eight maize varieties for the parasitoid *Cotesia marginiventris*: is quality or quantity important? J Chem Ecol 28(5):951–968
Jennings W, Shibamoto T (1980) Qualitative analysis of flavor and fragrance volatiles by glass capillary gas chromatography. Academic Press, San Francisco
Johnson CB, Kazantzis A, Skoula M, Mitteregger U, Novak J (2004) Seasonal, populational and ontogenic variation in the volatile oil content and composition of individuals of *Origanum vulgare* subsp. *hirtum*, assessed by GC headspace analysis and by SPME sampling of individual oil glands. Phytochem Anal 15(5): 286–292
Johnson HA, Rogers LL, Alkire ML, McCloud TG, NcLaughlin JL (1998) Bioactive monoterpenes from *Monarda fistulosa* (Lamiaceae). Nat Prod Lett 11(4):241–250
Kalberer NM, Turlings TCJ, Rahier M (2001) Attraction of a leaf beetle (*Oreina cacaliae*) to damaged host plants. J Chem Ecol 27(4):647–661
Kannaste A, Vongvanich N, Borg-Karlson A-K (2008) Infestation by a *Nalepella* species induces emissions of alpha- and beta-farnesenes, (-)-linalool and aromatic compounds in Norway spruce clones of different susceptibility to the large pine weevil. Arthropod Plant Intell 2(1):31–41
Linhart YB, Thompson JD (1995) Terpene-based selective herbivory by *Helix aspersa* (Mollusca) on *Thymus vulgaris* (Labiatae). Oecologia 102(1):126–132
Linhart YB, Thompson JD (1999) Thyme is of the essence: biochemical polymorphism and multi-species deterrence. Evol Ecol Res 1(2):151–171
Morrow PA, Fox LR (1980) Effects of variation in eucalyptus essential oil yield on insect growth and grazing damage. Oecologia 45(2):209–219
Morton TC, Vencl FV (1998) Larval beetles form a defense from recycled host-plant chemicals discharged as fecal wastes. J Chem Ecol 24(5):765–785
Müller C, Hilker M (1999) Unexpected reactions of a generalist predator towards defensive devices of cassidine larvae (Coleoptera, Chrysomelidae). Oecologia 118(2):166–172
Olmstead KL, Denno RF (1993) Effectiveness of tortoise beetle larval shields against different predator species. Ecology 74(5): 1394–1405
Owen SM (1998) Emissions of isoprene and monoterpenes from native Mediterranean vegetation. Lancaster University, Lancaster
Pare PW, Tumlinson JH (1997) De novo biosynthesis of volatiles induced by insect herbivory in cotton plants. Plant Physiol 114(4):1161–1167
Pare PW, Tumlinson JH (1999) Plant volatiles as a defense against insect herbivores. Plant Physiol 121(2):325–331
Pasteels JM, Rowellrahier M, Braekman JC, Dupont A (1983) Salicin from host plant as precursor of salicylaldehyde in defensive secretion of chrysomeline larvae. Physiol Entomol 8(3):307–314
Poulose AJ, Croteau R (1978a) Biosynthesis of aromatic monoterpenes: conversion of gamma-terpinene to para-cymene and thymol in *Thymus vulgaris* L. Arch Biochem Biophys 187(2):307–314
Poulose AJ, Croteau R (1978b) Gamma-terpinene synthetase: key enzyme in biosynthesis of aromatic monoterpenes. Arch Biochem Biophys 191(1):400–411
Sanderson MW (1948) Larval, pupal, and adult stages of North American *Physonota* (Chrysomelidae). Ann Entomol Soc Am 41(4):468–477
SAS Institute (2003) SAS version 9.1. SAS Institute, Cary, NC
Say T (1823) Descriptions of coleopterous insects collected in the late expedition to the Rocky Mountains, performed by order of Mr. Calhoun, Secretary of War, under the command of Major Long. By Thomas Say, zoologist to the expedition. J Acad Nat Sci Phila 3:403–462
Schaffner U, Müller C (2001) Exploitation of the fecal shield of the lily leaf beetle, *Lilioceris lilii* (Coleoptera: Chrysomelidae), by the specialist parasitoid *Lemophagus pulcher* (Hymenoptera: Ichneumonidae). J Insect Behav 14(6):739–757
Scora RW (1967) Study of the essential leaf oils of the genus *Monarda* (Labiatae). Am J Bot 54(4):446–452
Turlings TCJ, Bernasconi M, Bertossa R, Bigler F, Caloz G, Dorn S (1998) The induction of volatile emissions in maize by three herbivore species with different feeding habits: possible consequences for their natural enemies. Biol Control 11(2):122–129
Turner GW, Gershenzon J, Croteau RB (2000) Development of peltate glandular trichomes of peppermint. Plant Physiol 124(2):665–679
USDA (2008) National Resources Conservation Service. http://plants.usda.usda.gov/. Accessed 18 June 2012
Vencl FV, Morton TC (1998) The shield defense of the sumac flea beetle, *Blepharida rhois* (Chrysomelidae: Alticinae). Chemoecology 8(1):25–32
Vencl FV, Morton TC, Mumma RO, Schultz JC (1999) Shield defense of a larval tortoise beetle. J Chem Ecol 25(3):549–566
Vencl FV, Nogueira-de-Sa F, Allen BJ, Windsor DM, Futuyma DJ (2005) Dietary specialization influences the efficacy of larval tortoise beetle shield defenses. Oecologia 145(3):404–414
Vencl FV, Gómez NE, Ploss K, Boland W (2009) The chlorophyll catabolite, pheophorbide a, confers predation resistance in a larval tortoise beetle shield defense. J Chem Ecol 35(3):281–288
Weaver DK, Phillips TW, Dunkel FV, Weaver T, Grubb RT, Nance EL (1995) Dried leaves from Rocky Mountain plants decrease infestation by stored-product beetles. J Chem Ecol 21(2):127–142
Weiss MR (2006) Defecation behavior and ecology of insects. Annu Rev Entomol 51:635–661
Wyckhuys KAG, Koch RL, Heimpel GE (2007) Physical and ant-mediated refuges from parasitism: implications for non-target effects in biological control. Biol Control 40(3):306–313
Zebelo SA, Bertea CM, Bossi S, Occhipinti A, Gnavi G, Maffei ME (2011) *Chrysolina herbacea* modulates terpenoid biosynthesis of *Mentha aquatica* L. PloS One 6(3):e17195