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PLANT-ANIMAL INTERACTIONS - ORIGINAL PAPER

Complex effects of parasitoids on pharmacophagy and diet choice of a polyphagous caterpillar

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Abstract This study investigates complex effects of parasitoid infection on herbivore diet choice. Specifically, we examine how immunological resistance, parasitoid infection stage, and parasitoid taxonomic identity affect the pharmacophagous behavior of the polyphagous caterpillar, *Grammia incorrupta* (Arctiidae). Using a combination of lab and field experiments, we test the caterpillar's pharmacophagous response to pyrrolizidine alkaloids (PAs) over the course of parasitoid infection, as well as the effect of dietary PAs on the caterpillar's immunological response. Previous work from other systems gave the prediction that dietary PAs would be detrimental to the immune response and thus less acceptable to feeding early in the infection, when encapsulation of the parasitoid is most crucial. We found that the feeding acceptability of PAs was indeed low for caterpillars with early-stage parasitoid infections; however, this was not explained by PA interference with immune function. When allowed to choose among three host plant species, individuals harboring early-stage parasitoids increased their consumption of a nutritious plant containing antioxidants. This result was driven by wasp-parasitized caterpillars, whereas fly-parasitized caterpillars increased their consumption of plants containing iridoid glycosides. Individuals in the later time phase of infection exhibited an increase in PA intake that was consistent with previously reported self-medication behavior during late-stage parasitoid infection. This study reveals the depth of complexity and the dynamic nature of herbivore host plant choice, and underscores the importance of considering multitrophic interactions when studying insect diet choice.

Keywords Self-medication · Herbivory · Behavior · Tritrophic · Chemistry

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Introduction

Host plant selection by herbivorous insects is typically specialized and influenced by a multitude of ecological factors (Bernays and Chapman 1994; Schoonhoven 2005). The most well-studied factor, plant chemistry, influences host plant selection by providing oviposition and feeding cues (e.g., Da Costa and Jones 1971; Raybould and Moyes 2001; Macel and Vrieling 2003; Nieminen et al. 2003; Talsma et al. 2008), as well as deterring feeding and causing toxicity to the herbivore (e.g., Fraenkel 1953, 1959; Dethier 1954; Whittaker and Feeny 1971; Feeny 1975; Feeny et al. 1976; Rhoades and Cates 1976; Bowers and Puttick 1988; Camara 1997; Dyer et al. 2003). While the influence of plant chemistry is important, studying host plant selection in a multitrophic context captures important direct and indirect effects that can provide increased explanatory power (Price et al. 1980; Bernays and Graham 1988; Singer and Stireman 2005; Schmitz 2008). In particular, including natural enemies in the study of herbivore host plant selection has stimulated a body of research investigating how plant chemistry and natural enemies interact to influence host plant choice (Mueller 1983; Dyer 1995; Lill et al. 2002; Denno et al. 2003; Kursar et al. 2006; Barbosa and Caldas 2007; Singer et al. 2009). Given the influence of both trophic levels, a necessary goal for host plant selection studies is to elucidate the mechanisms

by which plant chemistry and natural enemies interact to determine host plant choice.

Parasitism by wasps and flies is an important factor determining host plant choice (Karban and English-Loeb 1997; Singer et al. 2009). For example, Karban and English-Loeb (1997) found that, when parasitized, individuals of the polyphagous arctiid caterpillar *Platyprepia virginalis* prefer to eat poison hemlock, resulting in a greater tolerance of parasitism. Similarly, host plant selection by another polyphagous arctiid caterpillar, *Grammia incorrupta*, is partly dependent upon whether it is parasitized. This caterpillar species exhibits pharmacophagy with respect to pyrrolizidine alkaloids (PAs) (Bernays et al. 2002). Pharmacophagy is a feeding behavior whereby herbivores preferentially ingest non-nutritive substances (Boppre 1984), typically consuming plant secondary metabolites from specific plants for defense, courtship, or both (e.g., Trigo et al. 1996; Tallamy et al. 2000; Wee et al. 2007). Despite numerous studies detailing the feeding plasticity of herbivorous insects with respect to plant nutrients and chemical defenses (reviewed in Behmer 2009; Mooney and Agrawal 2008), investigations of plasticity in pharmacophagy are rare. An exception concerns self-medication behavior by *G. incorrupta* caterpillars, which increase their intake of PAs when parasitized, a form of pharmacophagy that improves their chances of surviving parasitoid infection (Singer et al. 2009).

An important process that links plant chemistry to parasitoid success is the insect immune response. The immune response targets foreign objects inside the hemocoel and functions to defend against parasitoids, parasites, and pathogens. It is one of the most effective defenses that insects have against parasitic wasps and flies (Godfray 1994; Beckage 2008; Smilanich et al. 2009b). Several studies have investigated the effects of host plant chemistry on immune function in herbivorous insects (Benrey and Denno 1997; Turlings and Benrey 1998; Hunter 2003; Smilanich et al. 2009a), showing that the effects can be positive, negative, or neutral (Ojala et al. 2005; Kapari et al. 2006; Haviola et al. 2007; Klemola et al. 2007, 2008; Lee et al. 2008; Smilanich et al. 2009a; Bukovinszky et al. 2009). Nutrients (e.g., protein) can enhance encapsulation and lysozyme-like antibacterial activity, while other immune system components like phenoloxidase activity may not be affected (Lee et al. 2006, 2008; Povey et al. 2009; Srygley et al. 2009). Ingestion of certain plant secondary metabolites (e.g., iridoid glycosides) can diminish the immune response by directly interfering with melanization (Smilanich et al. 2009a), whereas other metabolites (e.g., hydrolyzable tannins) may act indirectly on immunity via reductions in herbivore performance (i.e., declines in host quality from the perspective of the parasitoid) (Haviola et al. 2007; Yang et al. 2008). Alternatively, antioxidant activity associated with carotenoids, phenolics, and synthetic chemicals can enhance insect immunity by putatively reducing harmful oxygen species (Ojala et al. 2005; Buyukguzel 2009).

The observation that host plant chemistry can affect the outcome of parasitism in diverse ways suggests that adaptive responses to parasitism may be as diverse. Given the nutritional and chemical diversity of plants, it seems likely that host switching herbivores can optimize fitness in the face of tritrophic interactions by regulating their intake of particular chemicals or nutrients based on subtle differences in their physiological condition. Grazing caterpillars that were shown to self-medicate against parasitoid infection exhibited a high degree of variation in the self-medication response (Singer et al. 2009). One explanation for the observed variation is that physiological differences exist among parasitized individuals which influence their feeding preference for PAs. In this study, we investigate how three sources of variation, the immunological resistance, the stage of parasitoid infection, and the type of parasitoid (wasp or fly) influence dietary choice in *G. incorrupta*. Based on evidence that high concentrations of plant secondary metabolites can compromise the immune system (Smilanich et al. 2009a), we expect caterpillars to avoid PAs early in the infection when the ability of the immune system to encapsulate the parasitoid larva is most crucial. Growth and survival costs associated with ingesting high concentrations of PAs (Singer et al. 2009) lead to the prediction that caterpillars will increase PA intake as an antiparasitoid defense only when the immune response fails.

Methods

Study system

Caterpillars. *Grammia incorrupta* (formerly *G. geneura*) (Lepidoptera: Arctiidae) caterpillars feed on over 80 species of plants from nearly 50 phylogenetically disparate plant families (Singer and Stireman 2001; Singer et al. 2002). These herbivores eat mainly forbs in grassland and savanna habitats of the southwestern USA and northwestern Mexico (Singer 2001). Unlike some generalists that can feed on a variety of plants, but whose maternal choice confines individuals to a single host plant for the duration of their larval stage, *G. incorrupta* is a grazing generalist, regularly moving between plants and mixing its diet (Singer et al. 2002). *Grammia incorrupta* larvae typically go through 6–8 instars. The frequency of mortality from parasitoids of field-collected *G. incorrupta* is highly variable (average = 15%), with the majority of these attacks coming from various species of tachinid flies, including

Exorista mella and several *Chetogena* species (Stireman and Singer 2002). The most numerically dominant parasitoid wasp of *G. incorrupa* is *Cotesia* nr. *phobetri*, an invalid species that has only been reared from *G. incorrupta* and thus appears to be host specific (Stireman and Singer 2002).

Parasitoids. *Chetogena edwardsi* and *C. tachinomoides* (Diptera: Tachinidae) were used for parasitism in laboratory experiments. Both species have broad host ranges on macrolepidopteran larvae (Stireman and Singer 2002). Flies were collected from natural populations of *G. incorrupta* caterpillars in April 2009. Previous tritrophic work in this system was done mostly with *Exorista mella* (Diptera: Tachinidae) (Singer and Stireman 2003; Singer et al. 2009). *Chetogena* and *E. mella* are closely related (Stireman 2002) and are likely to share many life history traits relevant to host parasitoid interactions. Like *E. mella*, *Chetogena* females oviposit macrotype eggs onto the caterpillar cuticle. Eggs hatch between 48 and 60 h after oviposition, at which time the tachinid larva burrows through the cuticle and into the caterpillar hemocoel. The development time of the *Chetogena* larvae averaged 10 ± 3 days under laboratory conditions.

Flies were kept in a plexiglass terrarium with water and sugar granules readily available. To maintain the colony, 3–5 seventh-instar *G. incorrupta* caterpillars were parasitized daily. Mating of adult flies was not controlled.

Experimental overview

We performed three laboratory experiments and two experiments with both a lab and a field component. Lab experiments were performed at Wesleyan University in the fall of 2008 and spring of 2009. These experiments addressed: (1) the feeding response to PAs of parasitized *G. incorrupta* at different time phases of the parasitoid infection, (2) the feeding response to PAs of *G. incorrupta* which were immune challenged with injected silica beads, and (3) the effects of PAs on the immune response of *G. incorrupta*. The immune response was measured using a standardized immune assay where the deposition of melanin on injected silica beads is quantified (Lavine and Beckage 1996; Smilanich et al. 2009b). The feeding response to PAs was measured using a no-choice feeding assay.

Field/lab experiments took place in and around Tucson, AZ, USA in the spring of 2007 and 2009. Caterpillars were collected from field sites in the area and brought back to the lab at the University of Arizona for manipulation and observation. Field experiments addressed (1) the influence of natural parasitism on PA consumption, and (2) the influence of natural parasitism on host plant choice between three chemically distinct host plants.

Laboratory experiments

Feeding response to PAs after parasitism

In this experiment, we measured the PA feeding response of *G. incorrupta* caterpillars at different time points following parasitism by tachinid flies. The specific PA used for all experiments described here was monocrotaline (Sigma–Aldrich). The PA monocrotaline was dissolved in 95% ethanol to produce a 0.1 mMol PA solution. This concentration is known from previous experiments to stimulate feeding (Bernays et al. 2002). The PA solution was pipetted onto 2.4 cm diameter glass fiber discs and allowed to completely dry. The discs were then weighed and pinned onto the inside wall of a plastic 167.2 ml plastic cup within reach of the caterpillars. Since we were specifically interested in whether feeding behavior changes over the course of the parasitoid infection, parasitized caterpillars were assigned to one of two groups: an early-stage infection group or a late-stage infection group. At the beginning of the seventh instar, individuals of *G. incorrupta* were parasitized by a single female tachinid fly. The number of eggs oviposited ranged between 1 and 3. Because eggs are oviposited singly onto the caterpillar's integument, we were able to count and manipulate the number of eggs each caterpillar received. A subset of caterpillars was assigned to the PA feeding assay 48 h after parasitoid oviposition to measure PA feeding of caterpillars at an early stage of the infection. Another subset of parasitized caterpillars was assigned to the PA feeding assay 96 h after parasitoid oviposition to measure the PA feeding response at a later stage of the infection. To act as a control group, nonparasitized caterpillars of the same developmental stage were paired with parasitized caterpillars in both early- and late-stage feeding assays. After 24 h, the mass of the PA-treated glass fiber disc consumed was recorded for each replicate. Data were analyzed in SAS (SAS Institute 2008) using two-way ANOVA (PROC GLM) with the mass of the glass fiber disc consumed as the response variable and treatment groups (parasitized vs. unparasitized) and time phase of infection (early vs. late) as the predictor variables. This same analysis was performed separately for individuals with one egg and two eggs. All caterpillars were monitored until parasitoids emerged. Individuals were excluded if no parasitoid emerged. Differences between means were identified using Tukey's multiple range test with $\alpha = 0.05$.

Immune assay

In the following experiments, we were interested in testing the strength of the immune response and the feeding behavior of immune-challenged caterpillars. By injecting

egg-sized foreign bodies into the caterpillars, we obtained a standardized measure of immunocompetence. Caterpillars were injected with 5–10 red silica beads as a proxy for parasitism (Lavine and Beckage 1996; Lovallo et al. 2002). This technique has been used widely and has been shown to accurately quantify immune capacity in insects (Gorman et al. 1996; Rantala and Roff 2007). The immune response was assessed by quantifying the caterpillar tissue melanization of silica beads injected into the caterpillars (Smilanich et al. 2009a, b).

Feeding response to PAs after immune challenge

A 24 h feeding assay using PA-treated glass fiber discs was used to measure the PA feeding response of immune-challenged *G. incorrupta* caterpillars. The PA feeding assay described above was used to measure PA feeding responses. Individuals at the second day of the seventh instar were taken from three different families and randomly assigned to one of three groups: bead injection, sham injection, or control. Caterpillars in the bead injection group were injected with silica beads. For the sham injection, individuals were injected with Ringer's solution without the beads in order to separate the response to the needle injury from the immune response to the beads. Control individuals were not injected. Twenty individuals from three different families were assigned to the bead-injected, sham-injected, and control groups. The total amount consumed was calculated by subtracting the initial dry mass of the disc from the final dry mass at the end of the 24 h feeding assay. The data were analyzed in SAS using ANOVA (PROC GLM), with the mass of the glass fiber disc consumed used as the response variable and treatment (bead, sham, control) used as the predictor variable. Caterpillar family was added to the model as a random interaction variable. Differences between means were determined using Tukey's multiple range test with $\alpha = 0.05$.

Effects of PAs on the immune response

For this experiment, we measured the immune response of individuals feeding on a standard rearing diet (Singer et al. 2009) with PAs added at either high (0.1% dry weight) or low (0.01% dry weight) concentrations. The concentrations of PAs reflect the natural range that individuals of *G. incorrupta* encounter in PA-containing plants in nature (Hartmann et al. 2004). At the beginning of their final instar (seventh), 90 individuals (30 per treatment) from three different families were randomly assigned to either a high, a low, or a control diet with no PAs added. Each treatment received ten individuals from each family such that the families were evenly dispersed across treatments. Caterpillars were reared individually in 162.7 ml plastic cups. At the second day of the seventh instar, individuals were injected with 5–10 glass beads and allowed to respond for 24 h before dissection (Smilanich et al. 2009a, b). To retrieve the beads, caterpillars were dissected in Ringer's solution and melanization was compared between treatments by photographing beads using a camera mounted on a dissection microscope (Carl Zeiss Discovery V.8, AxioCam Software). All photographs were taken at 80× magnification. Because the beads were dyed red before injecting them into the caterpillars, we were able to quantify the magnitude of melanization by measuring the red value (*r* value) of each bead. The *r* value is a numerical measure of the red value of an image on a scale ranging from 0 to 255, where 0 is pure gray and 255 is pure red. The lower the *r* value, the darker the bead, indicating increasing levels of melanization. Using Adobe Photoshop (version 6.0), the *r* value was obtained for each bead within a caterpillar, and these values were averaged to provide an *r* value score for each individual caterpillar. The mean *r* value was transformed into a percentage of melanization [1−(*r* value/maximum *r* value)] for ease of interpretation in graphs. Data were analyzed in SAS using ANOVA (PROC GLM), with *r* value used as the response variable and diet treatment (control, high PA, low PA) used as the predictor variable. Caterpillar family was also added to the model as a random interaction variable. Differences between means were determined using Tukey's multiple range test with $\alpha = 0.05$.

Field/lab experiments

Feeding response to PAs after natural parasitism

To measure the PA feeding response of naturally parasitized caterpillars, we subjected field-collected *G. incorrupta* caterpillars to a PA feeding assay in the laboratory and determined whether individuals were parasitized using post-assay dissections. First, we collected 120 late-instar *G. incorrupta* caterpillars from a natural population near Oracle, AZ, USA in April 2007. Because of the caterpillars' varied and unknown feeding histories prior to collection and the possibility that the PA feeding experience modifies subsequent feeding response to PAs (Bernays et al. 2003), we manipulated their feeding experience prior to the feeding assay by individually confining the caterpillars in 162.7 ml plastic cups, with half randomly assigned to *Senecio longilobus* (PA-containing plant), and the other 60 assigned to *Malva parviflora* (PA-free plant) for 24 h. Next, we placed all caterpillars that had fed on their assigned host plant ($N = 116$) individually into clean cups, each with an impaled PA-treated glass fiber disc (as described above), and allowed them to feed for 24 h. After

this PA-feeding assay period, the glass fiber discs were removed for drying and weighing (as described above), and the caterpillars were weighed, dissected, and examined under 50× magnification for the presence of endoparasitoids. Dissections mostly revealed caterpillars lacking any signs of parasitism ($N = 96$), a set of caterpillars containing a single early-instar tachinid larva surrounded by melanized tissue and often in conjunction with a single macrotype tachinid egg on the caterpillar's outer exoskeleton ($N = 7$), and a set of caterpillars harboring a single macrotype tachinid egg but no embedded tachinid larva ($N = 13$). In analyses comparing the PA feeding responses of parasitized and unparasitized caterpillars, the latter two categories were pooled to create a category of caterpillars assayed at early stages of parasitoid development. We analyzed the effects of parasitism (yes, no), pre-assay diet (*Senecio* or *Malva*), caterpillar mass, and their interactions with ANCOVA (JMP 7.0). The response variable was the log-transformed mass of glass fiber disc consumed.

Host plant selection after natural parasitism

To compare host plant selection of parasitized and unparasitized caterpillars, field collected *G. incorrupta* were given a choice between three chemically distinct host plant species. Fifty individuals were collected from a natural population near Oracle, AZ, USA in April 2009 and then brought back to the laboratory at the University of Arizona. At the lab, caterpillars were given a choice between excised leaves or uprooted individuals of three field-collected host plants: *Plantago patagonica* (Plantaginaceae), *Plagiobothrys arizonicus* (Boraginaceae), and *Malva parviflora* (Malvaceae). These plants are chemically distinct from each other. *Plantago patagonica* contains compounds of the monoterpene derived, iridoid glycosides (M.D. Bowers, personal communication). *Plagiobothrys arizonicus* contains PAs (Hartmann et al. 2004), and *M. parviflora* contains high levels of antioxidant flavonoids and phenolics (Wang et al. 2001; Afolayan et al. 2008). Previous studies with plants containing iridoid glycosides suggested that these compounds may confer resistance to parasitism for *G. incorrupta* (Singer and Stireman 2003). Resistance to parasitism has been shown for dietary PAs (Singer et al. 2009), and *M. parviflora*, which is known to have high protein and high antioxidant levels, has been shown to support superior growth and development of *G. incorrupta* (Singer 2001; Singer and Stireman 2003; Singer et al. 2004). Individuals were housed in plastic 167.2 ml cups with a weighed amount of each of the three plants. After 36 h, caterpillars were removed from their cups, and the dry mass of the remaining plant material from each cup was obtained. We calculated the dry mass consumed of each plant by estimating the initial plant dry mass using a wet-to-dry conversion curve. This was created by weighing a sample amount of each plant when harvested, and again after drying ($N = 20$). A subset of caterpillars ($N = 25$) was dissected in order to ascertain parasitism status during the feeding assay. From caterpillar dissections showing first-instar maggots and the number of days to adult fly eclosion among nondissected individuals, we determined that fly larvae were at a very early ontogenetic stage during these caterpillar feeding assays. This experiment therefore reflects caterpillar feeding preference at an early stage of parasitoid infection.

Because we were not able to meet the requirements of normality needed to analyze parametric statistics using the full dataset, due to frequent zeros (caterpillars eating only one or two of the three choices), we used three separate analyses to test for differences in the feeding responses of parasitized and unparasitized caterpillars. First, to test qualitatively for associations between parasitism status and the occurrence of feeding on the three different host plants, we categorized feeding data as presence or absence of consumption. Chi-square contingency tables were used to test for associations between the presence/absence of consumption on each plant and parasitism status (yes, no). Second, we tested for differences in the amount of plant material consumed once feeding was initiated, so data points with zeros (no feeding) were not included in this analysis. This subset of data met the requirements for normality following square root transformation of the consumption of *P. arizonicus* and *M. parviflora*. The mass of *P. patagonica* consumed could not be normalized and was not included in this analysis. Data were analyzed in SAS using ANOVA (PROC GLM), with the dry mass of each plant consumed used as the response variable and parasitism status (yes, no) used as the predictor variable. In the third analysis, we analyzed feeding data separately for each parasitoid taxon, since our parasitism data included both hymenopteran and dipteran parasitoids. Unlike the analysis of host plant consumption with all parasitoids included, feeding data for all plants met the requirements for normality when parasitoid species were analyzed separately. These data were analyzed in SAS using ANOVA (PROC GLM), with the dry mass of each plant consumed used as the response variable and parasitism status (yes, no) used as the predictor variable. Differences between means were identified using Tukey's multiple range test with $\alpha = 0.05$.

Results

Laboratory experiments

PA consumption by *G. incorrupta* was affected by the parasitism treatment ($F_{3,109} = 10.58, P < 0.0001, N = 113$),

and the interaction between parasitism and time ($F_{3,109} = 5.00$, $P = 0.0274$, $N = 113$, Fig. 1). Parasitized caterpillars at the later phase of the infection consumed greater than twice as much of the PA-treated fiber disc as the parasitized caterpillars in the early infection phase did. In addition, PA consumption differed between the late parasitized individuals and the late unparasitized individuals. This is in keeping with previous evidence for self-medication behavior (Singer et al. 2009), and demonstrates for the first time that increased PA ingestion induced by larval parasitoids begins at least 96 h after parasitoid oviposition. The Tukey test showed no significant difference in PA consumption between the early parasitized and early unparasitized groups (Fig. 1). When consumption was analyzed based upon the number of eggs oviposited, there were still significant differences in consumption between the early-infection and late-infection groups, regardless of egg number (one egg, $F_{3,33} = 27.05$, $P < 0.0001$, $N = 37$; two eggs, $F_{3,13} = 10.01$, $P < 0.0011$, $N = 17$). Interestingly, the effect size was larger for caterpillars with one egg versus two eggs (18.33 vs. 13.72).

When we analyzed the feeding response to PAs after caterpillars were given an immune challenge, we found that bead-injected caterpillars consumed significantly less PA fiber disc than sham-injected and control groups ($F_{8,47} = 6.02$, $P < 0.0001$, $N = 56$, Fig. 2). The Tukey test showed a significant difference in consumption among all three groups. We detected no effect of caterpillar family on PA consumption ($F_{8,47} = 1.20$, $P = 0.3088$, $N = 56$).

We detected no significant difference in bead melanization between individuals feeding on the high-PA (0.1%), low-PA (0.01%), and control diets ($F_{8,45} = 1.30$, $P > 0.2682$, $N = 54$, Fig. 3). Likewise, we detected no effect of caterpillar family on immune response ($F_{8,47} = 2.06$, $P = 0.1391$, $N = 54$).

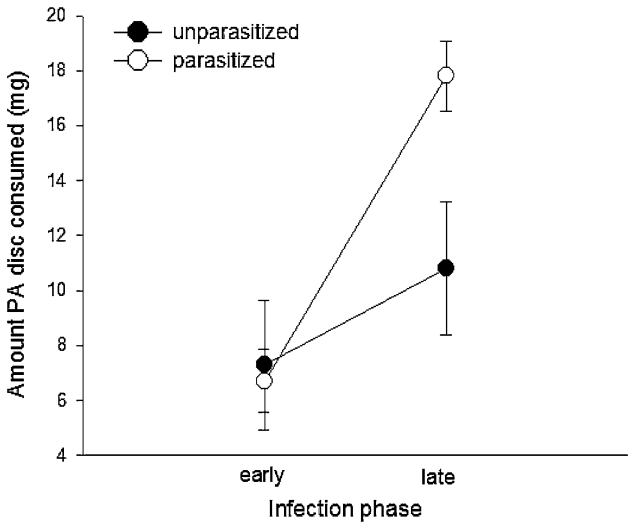

Fig. 1 Contrasting amounts of PA fiber disc consumed by *G. incorrupta* at early and late stages of parasitoid infection. There is a significant interaction between treatment (parasitized vs. unparasitized) and infection phase (early vs. late). *Error bars* represent the standard error of the mean

Fig. 2 Comparison of the mean amounts of PA fiber disc consumed by bead-injected, sham-injected (no beads) and control caterpillars. *Nonidentical letters* above *bars* indicate significant differences between treatment groups. *Error bars* represent the standard error of the mean

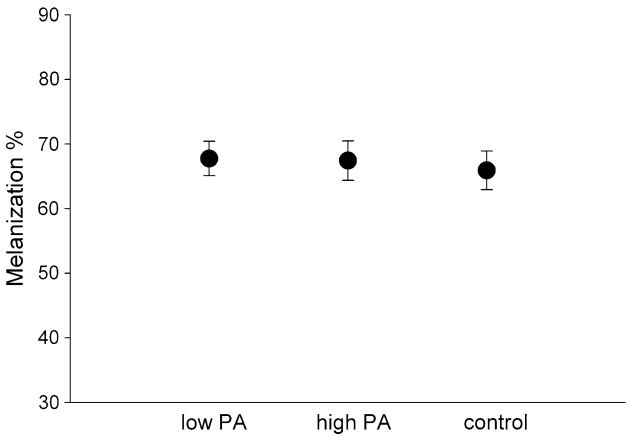

Fig. 3 Mean melanization scores for individuals of *G. incorrupta* consuming low (0.01%), high (0.1%), and control (0.0%) levels of PAs. *Error bars* represent one standard deviation of the mean

Field/lab experiments

Naturally parasitized caterpillars harboring early developmental stages of tachinid larvae consumed significantly less of the PA-treated fiber disc than did unparasitized caterpillars ($F_{7,108} = 5.35$, $P = 0.0221$, $N = 116$, Fig. 4). We failed to detect effects of host plant conditioning ($F_{7,108} = 2.42$, $P = 0.1228$, $N = 116$) and caterpillar mass ($F_{7,108} = 0.0043$, $P = 0.9476$, $N = 116$) on PA consumption. Finally, the interaction between caterpillar mass, host plant conditioning, and parasitism status did not affect PA consumption ($F_{7,108} = 0.8622$, $P = 0.3552$, $N = 116$).

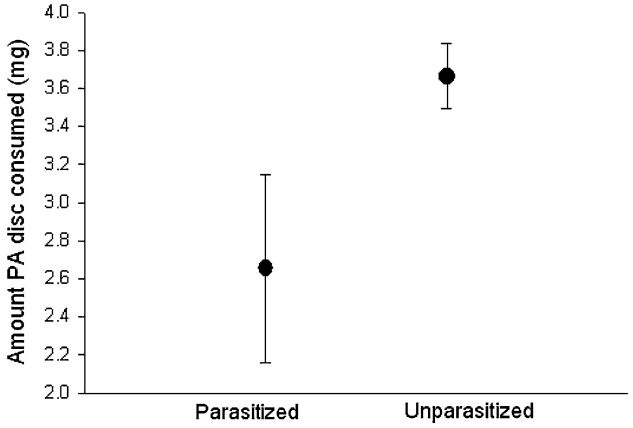

Fig. 4 Comparison of the amount of PA fiber disc consumed by early-stage parasitized and unparasitized caterpillars collected from the field. *Error bars* represent the standard error of the mean

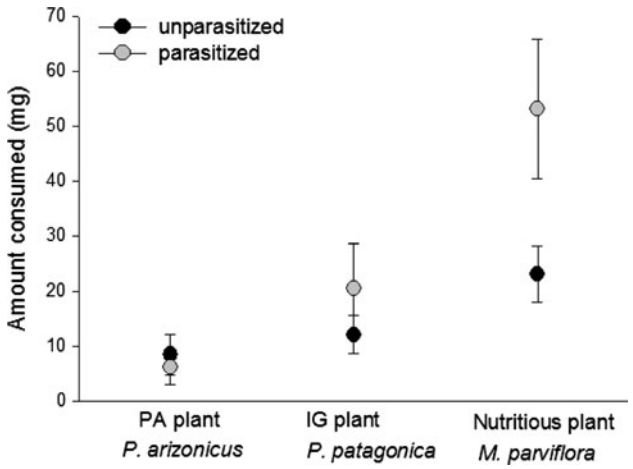

Fig. 5 Comparison of the amounts of three different plants consumed by early-stage parasitized and unparasitized caterpillars collected from the field. *Error bars* represent the standard error of the mean

In the second field experiment, we tested for associations between parasitism status and the presence of feeding on each of the three host plants. Two-dimensional contingency tables showed no association between parasitism status and the presence of feeding on each of the plants, *P. arizonicus* ($\chi^2 = 0.2110$, $df = 1$, $P = 0.6460$), *P. patagonica* ($\chi^2 = 0.2110$, $df = 1$, $P = 0.6460$), and *M. parviflora* ($\chi^2 = 0.7500$, $df = 1$, $P = 0.3865$). In the subset of data that included measurable host plant consumption, parasitized individuals consumed significantly more *M. parviflora* than unparasitized individuals did ($F_{1,30} = 4.96$, $P = 0.034$, $N = 32$, Fig. 5), but parasitized and unparasitized individuals did not differ in their consumptions of *P. arizonicus* ($F_{1,33} = 2.66$, $P = 0.113$, $N = 35$, Fig. 5). When caterpillars parasitized by wasps (*Cotesia* nr. *phobetri*) were analyzed separately from caterpillars parasitized by flies (*Chetogena* spp.), we found feeding differences specific to each parasitoid type. The wasp-parasitized caterpillars consumed significantly more *M. parviflora* than did unparasitized caterpillars ($F_{1,26} = 11.57$, $P = 0.002$, $N = 28$). However, there were no significant differences in consumption between wasp-parasitized and unparasitized caterpillars for *P. arizonicus* ($F_{1,9} = 0.68, P = 0.432, N = 11$) and *P. patagonica* ($F_{1,29} = 0.07$, $P = 0.794$, $N = 31$). In contrast, the fly-parasitized caterpillars consumed significantly more *P. patagonica* than did unparasitized caterpillars ($F_{1,28} = 5.37$, $P = 0.03$, $N = 30$), with no significant differences in the consumption of *M. parviflora* ($F_{1,23} = 0.42$, $P = 0.523$, $N = 25$) and *P. arizonicus* ($F_{1,9} = 0.70$, $P = 0.426, N = 11$).

Discussion

The results of the PA-feeding experiments show changes in pharmacophagous behavior over the course of parasitoid infection. Both parasitized and immune-challenged *G. incorrupta* caterpillars reduced their consumption of pyrrolizidine alkaloids during the early stages of the parasitoid infection. However, the increased ingestion of PAs by caterpillars at a later stage indicates that the onset of self-medication behavior occurs at least 96 h after parasitoid oviposition. Based on similar PA-feeding responses of caterpillars at early stages of infection (lab experiments) and those harboring parasitoids at early developmental stages (field/lab experiments), we suggest that this switch in feeding behavior is induced by a particular ontogenetic stage of the parasitoid larva, or by some correlate of parasitoid development. Because the number of fly eggs did not affect PA feeding in this study, we suggest that variation in parasitoid development within the host is the most likely source of variation in PA feeding seen in previous experiments (Singer et al. 2009).

Our results also provide some insight into the functional significance of avoiding PA consumption during early stages of parasitoid infection. Since previous work shows that PA consumption confers a higher survivorship to parasitized caterpillars (Singer et al. 2009), and increased PA consumption is associated with late-phase (this study) and late-stage infections (Singer et al. 2009), the reduced acceptability of PAs at the early infection stage suggests that *G. incorrupta* caterpillars avoid PAs either because of detrimental effects of the PAs, or because they seek alternative compounds that may be of greater benefit. Two results from the above experiments support the latter explanation. Firstly, injection assays showed that there was no detrimental effect of PAs on immune performance, and secondly, parasitized caterpillars in the host plant choice experiment showed increased consumption of alternative

host plants. Work in other systems, where the strength of the immune response has been shown to vary depending on the host plant, points to some possible immune-enhancing compounds of alternative host plants (Ojala et al. 2005; Yang et al. 2008; Smilanich et al. 2009a). For example, in the arctiid caterpillar *Parasemia plantaginis*, immunity is highest when feeding on plants that have high concentrations of antioxidants (Ojala et al. 2005). Since both *M. parviflora* and *P. patagonica* contain high levels of antioxidants, it is possible that choosing these hosts over PA plants during early stages of parasitoid infection aids the immune response by scouring destructive free radicals (Ojala et al. 2005; Buyukguzel 2009). However, it should also be noted that sustained PA consumption reduces the survival of unparasitized *G. incorrupta* caterpillars (Singer et al. 2009), so a direct physiological cost of PA ingestion cannot be ruled out as an explanation for the avoidance of PA feeding by newly infected, and possibly physiologically stressed, caterpillars.

Identifying the fitness effects of ingesting antioxidant-rich hosts may be particularly fruitful in studies integrating self-medication and immunity. *Malva parviflora* offers not only high-quality food to *G. incorrupta* (Singer and Stireman 2003), but also high concentrations of antioxidants, and antimicrobial activity in the leaves and seeds (Afolayan et al. 2008; Wang et al. 2001). In particular, *M. parviflora* has high concentrations of flavonoids, which exhibit free radical scavenging properties that are thought to contribute to the wound-healing properties in humans for which *M. parviflora* has traditionally been used (Afolayan et al. 2008; Shale et al. 1999). Parasitized individuals of *G. incorrupta* may benefit from these properties, since the melanization process produces free radicals which are harmful to parasitoid larvae but are also potentially harmful to *G. incorrupta*. In addition, during parasitism, the caterpillar's cuticle is punctured by the wasp ovipositor, or by the burrowing fly larva, leaving a wound on the caterpillar's body. The antimicrobial properties of *M. parviflora* may prevent the secondary infection of wounds by pathogens.

The increased consumption of the nutritious plant *M. parviflora* at the early infection stage was driven by the wasp-parasitized caterpillars and was not significantly higher for the fly-parasitized caterpillars. It may be that gregarious parasitoid wasps are especially taxing to the immune response, stimulating caterpillars to seek plants that can bolster it or ameliorate its harmful side-effects. A feeding strategy that aids the immune system may not be effective against tachinid parasitoids because of the ability of some species to form breathing tubes using the host's encapsulation response (Bailey and Zuk 2008). Fly-parasitized caterpillars showed a different feeding behavior, increasing their consumption of *P. patagonica*, a plant that contains iridoid glycosides. Like PAs, iridoid glycosides are not harmful to the immune response of *G. incorrupta* (Smilanich 2008); however, unlike the PA plant (*P. arizonicus*), the iridoid glycoside-containing *P. patagonica* was still eaten by parasitized caterpillars at the early infection stage. This preference for *P. patagonica* in the early infection stage is not understood, but may reflect self-medication similar to that shown for PAs. Previous work with *G. incorrupta* demonstrated a heightened gustatory response to iridoid glycosides in parasitized individuals, indicating a possible resistance response to consuming plants with these compounds (Bernays and Singer 2005).

It is also possible that *G. incorrupta* uses plant pigments to increase its melanization strength. Pigments such as carotenoids act to add coloration to the insect integument (Sandre et al. 2007), and a general association between dark integument and a reduced likelihood of mortality from parasitoids has been observed in a natural assemblage of tree-feeding caterpillars (Barbosa and Caldas 2007). In addition, Ojala et al. (2005) found that arctiid larvae consuming plant diets with high carotenoid concentrations had increased melanization responses. Thus, it is possible that caterpillars use plant pigments to enhance the immune response. Additionally, since *M. parviflora* is a high-quality host for *G. incorrupta*, any positive effects on the immune response may act indirectly by increasing overall physiological condition, such as body mass and growth rates (Srygley et al. 2009; Bukovinszky et al. 2009). Finally, the immune response is a resource-costly process (Schmid-Hempel and Ebert 2003), so parasitized caterpillars may select highly nutritious plants to recoup protein losses from mounting a response (Lee et al. 2006; Povey et al. 2009).

Since the effects of antioxidant and antimicrobial properties of *M. parviflora* on the immune response of *G. incorrupta* are untested, the possibility remains that the stimulus to consume more nutritious plants is induced by parasitoids. Manipulation of host behavior by parasites has been shown in many invertebrate systems (Medoc and Beisel 2009). In parasitoid–host interactions, if there is a high risk of secondary infection by pathogens, then inducing the host caterpillar to prefer a plant with high levels of antioxidant and antimicrobial activity would be advantageous to the developing parasitoid. Moreover, many studies have demonstrated a positive correlation between high herbivore performance on a particular host plant and parasitoid fitness (Vinson and Iwantsch 1980; Benrey and Denno 1997; Thompson and Redak 2008). Host manipulation seems most likely for highly specialized parasitoids, such as *Cotesia* nr. *phobetri*, which was indeed associated with feeding on high-quality *M. parviflora* plants in this study. In contrast, the fly parasitoids in this study are host generalists (Stireman and Singer 2002), and

are thus less likely to have specialized adaptations that are presumably needed for host manipulation.

In conclusion, these findings address several hypothesized relationships between diet choice and immune response. While previous studies of self-medication behavior by *Grammia incorrupta* caterpillars (Bernays and Singer 2005; Singer et al. 2009) implied that increased PA intake by parasitized caterpillars is mediated by changes in the taste system, they did not address the mechanism by which dietary PAs confer resistance against parasitoids. Because a variety of recent studies of other systems have highlighted varied effects of diet on the immune responses of insect herbivores (Ojala et al. 2005; Kapari et al. 2006; Haviola et al. 2007; Klemola et al. 2007, 2008; Lee et al. 2008; Smilanich et al. 2009a; Bukovinszky et al. 2009), our experiments addressed the role of immunological resistance in relation to PA self-medication. Behavioral and immunological assays reported here argue against the notion that the caterpillar immune system mediates antiparasitoid resistance conferred by dietary PAs. Additionally, there is no evidence that dietary PA interferes with immunological resistance in *G. incorrupta*. An alternative hypothesis, supported by our data, is that *G. incorrupta* uses two separate lines of defense against parasitoids. The first is the immune response, which begins immediately upon parasitoid infection, and does not involve increased ingestion of medicinal PAs. Rather, other plant chemicals, such as primary nutrients, antioxidants or pigments, might be employed to bolster immunity, as plants with such chemicals were especially acceptable to newly parasitized caterpillars. The second line of defense is self-medication via elevated ingestion of PAs, activated only after the early stages of parasitoid infection and presumably when the immune response is unsuccessful. Dietary PAs, which are sequestered in the hemolymph (Hartmann et al. 2004, 2005), most likely confer resistance through direct toxicity to hemolymph-feeding larval parasitoids. What is clear so far is that a polyphagous caterpillar exhibits both specificity and plasticity depending upon the taxon of parasitoid, the stage of infection, and host plant chemistry.

These findings reveal previously unexplored dimensions in the network of variables driving herbivore host plant choice. While a high level of complexity in ecological interactions is expected, empirical data demonstrating these interactions is generally lacking, especially in regard to foraging behavior (Abrams 2010). An important goal for future studies will be to investigate the effect of compounds such as flavonoids and antioxidants on the immune response of *G. incorrupta*, as well as to achieve a better understanding of the impetus for host switching over the course of parasitoid development. Further investigation is also needed to understand the function of PA avoidance and preference for alternative host plants or compounds in the early stages of parasitoid infection.

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References

Abrams PA (2010) Implications of flexible foraging for interspecific interactions: lessons from simple models. Funct Ecol 24:7–17

Afolayan AJ, Aboyade OM, Sofidiya MO (2008) Total phenolic content and free radical scavenging activity of *Malva parviflora* L. (Malvaceae). J Biol Sci 8:945–949

Bailey NW, Zuk M (2008) Changes in immune effort of male field crickets infested with mobile parasitoid larvae. J Insect Physiol 54:96–104

Barbosa P, Caldas A (2007) Do larvae of species in macrolepidopteran assemblages share traits that influence susceptibility to parasitism? Environ Entomol 36:329–336

Beckage NE (2008) Insect immunology. Academic, Oxford

Behmer ST (2009) Insect herbivore nutrient regulation. Annu Rev Entomol 54:165–187

Benrey B, Denno RF (1997) The slow-growth-high-mortality hypothesis: a test using the cabbage butterfly. Ecology 78:987–999

Bernays EA, Chapman RF (eds) (1994) Host plant selection by phytophagous insects. Chapman & Hall, New York

Bernays E, Graham M (1988) On the evolution of host specificity in phytophagous arthropods. Ecology 69:1153–1160

Bernays EA, Singer MS (2005) Taste alteration and endoparasites. Nature 436:476

Bernays EA, Chapman RF, Hartmann T (2002) A taste receptor neurone dedicated to the perception of pyrrolizidine alkaloids in the medial galeal sensillum of two polyphagous arctiid caterpillars. Physiol Entomol 27:312–321

Bernays EA et al (2003) Loss of gustatory responses to pyrrolizidine alkaloids after their extensive ingestion in the polyphagous caterpillar *Estigmene acrea*. J Exp Biol 206:4487–4496

Boppre M (1984) Redefining "pharmacophagy." J Chem Ecol 10:1151–1154

Bowers MD, Puttick GM (1988) The effect of qualitative variation in iridoid glycosides on generalist and specialist lepidopteran herbivores. J Chem Ecol 14:319–334

Bukovinszky T et al (2009) Consequences of constitutive and induced variation in plant nutritional quality for immune defence of a herbivore against parasitism. Oecologia 160:299–308

Buyukguzel E (2009) Evidence of oxidative and antioxidative responses by *Galleria mellonella* larvae to malathion. J Econ Entomol 102:152–159

Camara MD (1997) Predator responses to sequestered plant toxins in buckeye caterpillars: are tritrophic interactions locally variable? J Chem Ecol 23:2093–2106

Da Costa CP, Jones CM (1971) Cucumber beetle resistance and mite susceptibility controlled by the bitter gene in *Cucumis sativus* L. Science 172:1145–1146

Denno RF, Gratton C, Dobel H, Finke DL (2003) Predation risk affects relative strength of top-down and bottom-up impacts on insect herbivores. Ecology 84:1032–1044

Dethier VG (1954) Evolution of feeding preferences in phytophagous insects. Evolution 8:33–54
Dyer LA (1995) Tasty generalists and nasty specialists? A comparative study of antipredator mechanisms in tropical lepidopteran larvae. Ecology 76:1483–1496
Dyer LA et al (2003) Synergistic effects of three Piper amides on generalist and specialist herbivores. J Chem Ecol 29:2499–2514
Feeny P (1975) Biochemical coevolution between plants and their insect herbivores. In: Gilbert LE, Raven PH (eds) Coevolution of animals and plants, vol 1. University of Texas Press, Austin, pp 3–19
Feeny P, Wallace JW, Mansell RL (1976) Plant apparency and chemical defense. In: Biochemical interactions between plants and insects. Plenum, New York, pp 1–40
Fraenkel G (1953) The nutritional value of green plants for insects. Trans IX Int Congr Entomol 2:90–100
Fraenkel G (1959) The raison d'etre of secondary plant substances. Science 121:1466–1470
Godfray HCJ (1994) Parasitoids: behavioral and evolutionary ecology. Princeton University Press, Princeton
Gorman MJ, Cornel AJ, Collins FH, Paskewitz SM (1996) A shared genetic mechanism for melanotic encapsulation of CM-Sephadex beads and a malaria parasite, *Plasmodium cynomolgi* B, in the mosquito, *Anopheles gambiae*. Exp Parasitol 84:380–386
Hartmann T, Theuring C, Beuerle T, Ernst L, Singer MS, Bernays EA (2004) Acquired and partially de novo synthesized pyrrolizidine alkaloids in two polyphagous arctiids and the alkaloid profiles of their larval food-plants. J Chem Ecol 30(2)
Hartmann T, Theuring C, Beuerle T, Bernays EA, Singer MS (2005) Acquisition, transformation and maintenance of plant pyrrolizidine alkaloids by the polyphagous arctiid *Grammia geneura*. Insect Biochem Molec Biol 35:1083–1099
Haviola S, Kapari L, Ossipov V, Rantala MJ, Ruuhola T, Haukioja E (2007) Foliar phenolics are differently associated with *Epirrita autumnata* growth and immunocompetence. J Chem Ecol 33:1013–1023
Hunter MD (2003) Effects of plant quality on the population ecology of parasitoids. Agric For Entomol 5:1–8
Kapari L, Haukioja E, Rantala MJ, Ruuhola T (2006) Defoliating insect immune defense interacts with induced plant defense during a population outbreak. Ecology 87:291–296
Karban R, English-Loeb G (1997) Tachinid parasitoids affect host plant choice by caterpillars to increase caterpillar survival. Ecology 78:603–611
Klemola N, Klemola T, Rantala MJ, Ruuhola T (2007) Natural host plant quality affects immune defence of an insect herbivore. Entomol Exp Appl 123:167–176
Klemola N, Kapari L, Klemola T (2008) Host plant quality and defence against parasitoids: no relationship between levels of parasitism and a geometrid defoliator immunoassay. Oikos 117:926–934
Kursar TA, Wolfe BT, Epps MJ, Coley PD (2006) Food quality, competition, and parasitism influence feeding preference in a neotropical Lepidopteran. Ecology 87:3058–3069
Lavine MD, Beckage NE (1996) Temporal pattern of parasitism-induced immunosuppression in *Manduca sexta* larvae parasitized by *Cotesia congregata*. J Insect Physiol 42:41–51
Lee KP, Cory JS, Wilson K, Raubenheimer D, Simpson SJ (2006) Flexible diet choice offsets protein costs of pathogen resistance in a caterpillar. Proc R Soc B Biol Sci 273:823–829
Lee KP, Simpson SJ, Wilson K (2008) Dietary protein-quality influences melanization and immune function in an insect. Funct Ecol 22:1052–1061
Lill JT, Marquis RJ, Ricklefs RE (2002) Host plants influence parasitism of forest caterpillars. Nature 417:170–173
Lovallo N, McPheron BA, Cox-Foster DL (2002) Effects of the polydnavirus of *Cotesia congregata* on the immune system and development of non-habitual hosts of the parasitoid. J Insect Physiol 48:517–526
Macel M, Vrieling K (2003) Pyrrolizidine alkaloids as oviposition stimulants for the cinnabar moth, *Tyria jacobaeae*. J Chem Ecol 29:1435–1446
Medoc V, Beisel JN (2009) Field evidence for non-host predator avoidance in a manipulated amphipod. Naturwissenschaften 96:513–523
Mooney KA, Agrawal AA (2008) Phenotypic plasticity. In: Tilmon KJ (ed) Specialization, speciation, and radiation: the evolutionary biology of herbivorous insects. University of California Press, Berkeley, pp 43–57
Mueller TF (1983) The effect of plants on the host relations of a specialist parasitoid of *Heliothis* larvae. Entomol Exp Appl 34:78–84
Nieminen M, Suomi J, Van Nouhuys S, Sauri P, Riekkola ML (2003) Effect of iridoid glycoside content on oviposition host plant choice and parasitism in a specialist herbivore. J Chem Ecol 29:823–844
Ojala K, Julkunen-Tiito R, Lindstrom L, Mappes J (2005) Diet affects the immune defence and life-history traits of an Arctiid moth *Parasemia plantaginis*. Evol Ecol Res 7:1153–1170
Povey S, Cotter SC, Simpson SJ, Lee KP, Wilson K (2009) Can the protein costs of bacterial resistance be offset by altered feeding behaviour? J Anim Ecol 78:437–446
Price PW, Bouton EE, Gross P, McPheron BA, Thompson JN, Weiss AE (1980) Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. Annu Rev Ecol Syst 11:41–65
Rantala MJ, Roff DA (2007) Inbreeding and extreme outbreeding cause sex differences in immune defence and life history traits in *Epirrita autumnata*. Heredity 98:329–336
Raybould AF, Moyes CL (2001) The ecological genetics of aliphatic glucosinolates. Heredity 87:383–391
Rhoades D, Cates RG (1976) Toward a general theory of plant antiherbivore chemistry. In: Wallace J, Mansell R (eds) Biochemical interactions between plants and insects (Recent Advances in Phytochemistry, vol 10). Academic, New York, pp 155–204
Sandre SL, Tammaru T, Esperk T, Julkunen-Tiitto R, Mappes J (2007) Carotenoid-based colour polyphenism in a moth species: search for fitness correlates. Entomol Exp Appl 124:269–277
Schmid-Hempel P, Ebert D (2003) On the evolutionary ecology of specific immune defense. Trends Ecol Evol 18:27–33
Schmitz OJ (2008) Herbivory from individuals to ecosystems. Annu Rev Ecol Evol S 39:133–152
Schoonhoven LM (2005) Insect–plant relationships: the whole is more than the sum of its parts. Entomol Exp Appl 115:5–6
Shale TL, Stirk WA, van Staden J (1999) Screening of medicinal plants used in Lesotho for anti-bacterial and anti-inflammatory activity. J Ethnopharmacol 67:347–354
Singer MS (2001) Determinants of polyphagy by a woolly bear caterpillar: a test of the physiological efficiency hypothesis. Oikos 93:194–204
Singer MS, Stireman JO (2001) How foraging tactics determine host plant use by a polyphagous caterpillar. Oecologia 129:98–105
Singer MS, Stireman JO (2003) Does anti-parasitoid defense explain host plant selection by a polyphagous caterpillar? Oikos 100:554–562
Singer MS, Stireman JO (2005) The tri-trophic niche concept and adaptive radiation of phytophagous insects. Ecol Lett 8:1247–1255
Singer MS, Bernays EA, Carriere Y (2002) The interplay between nutrient balancing and toxin dilution in foraging by a generalist insect herbivore. Anim Behav 64:629–643

Singer MS, Carriere Y, Theuring C, Hartmann T (2004) Disentangling food quality from resistance against parasitoids: diet choice by a generalist caterpillar. Am Nat 164:423–429

Singer MS, Mace KC, Bernays EA (2009) Self-medication as adaptive plasticity: increased ingestion of plant toxins by parasitized caterpillars. Plos One 4(3):e4796. doi:10.1371/journal.pone.0004796

Smilanich AM (2008) Variation in plant chemical defenses and the physiological response of specialist and generalist herbivores (Ph.D. dissertation). Department of Ecology and Evolutionary, Tulane University, New Orleans

Smilanich AM, Dyer LA, Chambers JQ, Bowers MD (2009a) Immunological cost of chemical defence and the evolution of herbivore diet breadth. Ecol Lett 12:612–621

Smilanich AM, Dyer LA, Gentry GL (2009b) The insect immune response and other putative defenses as effective predictors of parasitism. Ecology 90:1434–1440

Srygley RB, Lorch PD, Simpson SJ, Sword GA (2009) Immediate protein dietary effects on movement and the generalised immunocompetence of migrating Mormon crickets *Anabrus simplex* (Orthoptera: Tettigoniidae). Ecol Entomol 34:663–668

Stireman JO (2002) Phylogenetic relationships of tachinid flies in subfamily Exoristinae (Tachinidae:Diptera) based on 28S rDNA and elongation factor-1 alpha. Syst Entomol 27:409–435

Stireman JO, Singer MS (2002) Spatial and temporal variation in the parasitoid assemblage of an exophytic polyphagous caterpillar. Ecol Entomol 27:588–600

Tallamy DW, Gorski PM, Burzon JK (2000) Fate of male-derived cucurbitacins in spotted cucumber beetle females. J Chem Ecol 26:413–427

Talsma JHR, Biere A, Harvey JA, van Nouhuys S (2008) Oviposition cues for a specialist butterfly—plant chemistry and size. J Chem Ecol 34:1202–1212

Thompson SN, Redak RA (2008) Parasitism of an insect *Manduca sexta* L. alters feeding behaviour and nutrient utilization to influence developmental success of a parasitoid. J Comp Physiol B 178:515–527

Trigo JR, Brown KS, Witte L, Hartmann T, Ernst L, Barata LES (1996) Pyrrolizidine alkaloids: different acquisition and use patterns in Apocynaceae and Solanaceae feeding ithomiine butterflies (Lepidoptera: Nymphalidae). Biol J Linn Soc 58:99–123

Turlings TCJ, Benrey B (1998) Effects of plant metabolites on the behavior and development of parasitic wasps. Ecoscience 5:321–333

Vinson SB, Iwantsch GF (1980) Host suitability for insect parasitoids. Annu Rev Entomol 25:397–419

Wang X, Bunkers GJ, Walters MR, Thoma RS (2001) Purification and characterization of three antifungal proteins from cheeseweed (*Malva parviflora*). Biochem Biophys Res Commun 282:1224–1228

Wee SL, Tan KH, Nishida R (2007) Pharmacophagy of methyl eugenol by males enhances sexual selection of *Bactrocera carambolae*. J Chem Ecol 33:1272–1282

Whittaker RH, Feeny PP (1971) Allelochemics: chemical interactions between species. Science 171:757–770

Yang S, Ruuhola T, Haviola S, Rantala MJ (2008) Effects of host plant shift on immune and other key life history traits of an eruptive Geometrid, *Epirrita autumnata* (Borkhausen). Ecol Entomol 33:510–516