2010

Tritrophic Effects of Host Plants on an Herbivore-Pathogen Interaction

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Abstract
Tritrophic interactions may include directly harmful effects of host plants on herbivores, and directly or indirectly harmful effects of host plants on the natural enemies of herbivores. Tritrophic interactions involving parasitoids and predators have received considerable attention but less is known about how host plants affect entomopathogens. We compared growth and resistance to entomopathogenic nematodes for "woolly bear" caterpillars, *Grammia incorrupta*(=*geneura*) (Hy. Edwards) (Lepidoptera: Arctiidae) fed lettuce, *Lactuca sativa* L. (Asteraceae), versus threadleaf groundsel, *Senecio longilobus* Benth. (Asteraceae). Both plants are members of the Asteraceae, but only *S. longilobus* contains pyrrolizidine alkaloids. Caterpillars gained more mass when fed *L. sativa* compared with *S. longilobus;* yet, in one of four cases studied, resistance to nematodes was higher when caterpillars ate *S. longilobus.*Caterpillar resistance to nematodes did not differ between host plants in the other cases. In addition, nematode reproduction was higher in cadavers of *G. incorrupta* that had been fed *L.sativa* instead of *S. longilobus,* suggesting that *S. longilobus* had indirectly detrimental effects on entomopathogenic nematodes. Our results illustrate how trade-offs may arise in tritrophic interactions involving entomopathogens, as the cost of decreased growth imposed by *S.longilobus* was accompanied by the benefit of greater resistance to entomopathogenic nematodes.

Keywords
alkaloids, entomopathogenic nematodes, pathogen reproduction, polyphagy, tradeoff

Disciplines
Agronomy and Crop Sciences | Entomology | Systems Biology

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Author(s): Aaron J. Gassmann, S. Patricia Stock, Bruce E. Tabashnik, and Michael S. Singer
Source: Annals of the Entomological Society of America, 103(3):371-378. 2010.
Published By: Entomological Society of America
DOI: http://dx.doi.org/10.1603/AN09130
URL: http://www.bioone.org/doi/full/10.1603/AN09130

Tritrophic Effects of Host Plants on an Herbivore–Pathogen Interaction

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Ann. Entomol. Soc. Am. 103(3): 371–378 (2010); DOI: 10.1603/AN09130

ABSTRACT Tritrophic interactions may include directly harmful effects of host plants on herbivores, and directly or indirectly harmful effects of host plants on the natural enemies of herbivores. Tritrophic interactions involving parasitoids and predators have received considerable attention but less is known about how host plants affect entomopathogens. We compared growth and resistance to entomopathogenic nematodes for "woolly bear" caterpillars, *Grammia incorrupta* (=*geneura*) (Hy. Edwards) (Lepidoptera: Arctiidae) fed lettuce, *Lactuca sativa* L. (Asteraceae), versus threadleaf groundsel, *Senecio longilobus* Benth. (Asteraceae). Both plants are members of the Asteraceae, but only *S. longilobus* contains pyrrolizidine alkaloids. Caterpillars gained more mass when fed *L. sativa* compared with *S. longilobus*; yet, in one of four cases studied, resistance to nematodes was higher when caterpillars ate *S. longilobus*. Caterpillar resistance to nematodes did not differ between host plants in the other cases. In addition, nematode reproduction was higher in cadavers of *G. incorrupta* that had been fed *L. sativa* instead of *S. longilobus*, suggesting that *S. longilobus* had indirectly detrimental effects on entomopathogenic nematodes. Our results illustrate how trade-offs may arise in tritrophic interactions involving entomopathogens, as the cost of decreased growth imposed by *S. longilobus* was accompanied by the benefit of greater resistance to entomopathogenic nematodes.

KEY WORDS alkaloids, entomopathogenic nematodes, pathogen reproduction, polyphagy, trade-off

Tritrophic interactions may include direct effects of host plants on herbivores and direct or indirect effects of host plants on the natural enemies of herbivores. Host plants may reduce the fitness of herbivores through the presence toxic allelochemicals or physical defenses (Rosenthal and Berenbaum 1991). However, these harmful effects may be offset by benefits if the plant defenses are detrimental to the natural enemies of herbivores and increase survival of herbivores when challenged with natural enemies (Price et al. 1980, Jeffries and Lawton 1984). Greater resistance to natural enemies may result from direct effects of plant defenses on natural enemies, such as a plant's trichomes reducing access of natural enemies to herbivores (e.g., Gassmann and Hare 2005) and through indirect effects if consumption of plant allelochemicals by herbivores increases their resistance to parasitoids (e.g., Barbosa et al., 1991) or deters predators from feeding (e.g., Dyer, 1995).

Several examples exist of such trade-offs arising in tritrophic interactions with parasitoids and predators (Kennedy 2003, Ode 2006). A natural tritrophic interaction that has received considerable study includes the polyphagous "woolly bear" caterpillar *Grammia incorrupta* (=*geneura*) (Hy. Edwards) (Lepidoptera: Arctiidae), several of its host plants, and parasitoids (Hymenoptera and Diptera) (Singer 2007). In this system, inclusion of plants with pyrrolizidine alkaloids (PAs) in the herbivore's diet imposed the cost of reduced growth efficiency but also conferred the benefit of higher survival when caterpillars were challenged with hymenopteran and dipteran parasitoids (Singer et al. 2004b). However, it is unknown whether these tritrophic effects might extend to entomopathogenic organisms, which are an important group of natural enemies (Roy et al. 2009). Moreover, compared with parasitoids and predators, far less is known about plant-mediated interactions between herbivorous insects and pathogens, and the extent to which greater resistance to entomopathogens may offset detrimental effects of host plant defenses on herbivores (Cory and Hoover 2006).

In the current study, we consider interactions among entomopathogenic nematodes, host plants with and without PAs, and *G. incorrupta.* Because entomopathogenic nematodes differ in their biology from other natural enemies of insects, effects observed with entomopathogens could differ considerably from those observed between herbivores and predators or parasitoids. Unlike other natural enemies, nematodes

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kill their hosts through the action of symbiotic bacteria, which the nematodes harbor (Burnell and Stock 2000, Dowds and Peters 2002, Park and Stanley 2006). In addition, infecting nematodes must survive in the host hemocoel until they release their symbiotic bacteria (Li et al. 2007). Consequently, indirect effects of host plants on infecting nematodes or their symbiotic bacteria could influence the susceptibility of insects to nematodes.

We tested whether a host plant with PAs affected growth and resistance to entomopathogenic nematodes for *G. incorrupta* and whether tritrophic effects extended to reproduction of entomopathogenic nematodes in their phytophagous host. Because resistance to entomopathogens is influenced by larval instar (Novotny 1991, James and Lighthart 1992), we tested both sixth and seventh instar caterpillars. We report that feeding on a host plant with PAs decreased herbivore growth and reduced pathogen reproduction. However, for seventh instar caterpillars, consumption of a host plant with PAs conferred the benefit of greater resistance to nematodes.

Species Studied. The two host plants studied were threadleaf groundsel *Senecio longilobus* Benth. (Asteraceae) and red leaf lettuce *Lactuca sativa* L. (Asteraceae). Both plants are members of the same family, Asteraceae, but they differ in their defensive chemistry. *S. longilobus* contains PAs (Singer et al. 2004b) that are toxic to a wide range of taxa, including many species of insects and vertebrates (Hartmann 1991). By contrast, *L. sativa* is defended primarily by terpenoids and phenolics (Cole 1984, Sessa et al. 2000) and is readily consumed by numerous insect species (Huang et al. 2003, Mou and Liu 2003, Sethi et al. 2006).

The highly polyphagous *G. incorrupta* larvae occur at 1,200–1,800-m elevation in arid grasslands of the southwestern United States (Ferguson and Opler 2006, Singer 2007). *G. incorrupta* has two generations per year and an average of seven larval instars (Ferguson and Opler 2006, Singer 2007). The natural host range of *G. incorrupta* spans at least 30 families and >80 species of plants (Singer and Stireman 2001). Although *L. sativa* is not a natural host of *G. incorrupta*, it does naturally feed on many members of the Asteraceae, including *S. longilobus* (Singer and Stireman 2001). In addition, *G. incorrupta* can be readily reared in the laboratory on synthetic, wheat-germ based diet (Singer 2001).

The nematodes *Steinernema riobrave* (ML29 strain) (Nematoda: Steinernematidae) and *Heterorhabditis sonorensis* (CH35 strain) (Nematoda: Heterorhabditidae) are soil-borne pathogens of lepidopteran larvae found in the southwestern United States at 1,400–2,000 m (Stock and Gress 2006, Stock et al. 2009). During the free-living infective juvenile stage, these nematodes enter the hemocoel of their living insect host and release symbiotic bacteria that produce insecticidal compounds, which kill the host (Boemare 2002, Dowds and Peters 2002, Park and Stanley 2006). Nematodes then feed, mature, and reproduce inside the host cadaver, with a new generation of infective juveniles subsequently dispersing (Kaya and Gaugler 1993). Both nematode species occur in the top 20 cm of soil (Stock and Gress 2006), although their vertical distribution will probably be affected by soil moisture. For example Gouge et al. (2000) found that *S. riobrave* remained within the top 10 cm of the soil under moist conditions but migrated deeper into the soil when confronted with dryer conditions. These nematode species probably differ in their foraging tactics. Whereas *S. riobrave* exhibits an intermediate foraging behavior using both active foraging and sit-and-wait tactics, members of the genus *Heterorhabditis*, such as *H. sonorensis*, are typically active foragers (Grewal et al. 1994, Lewis et al. 2006).

Both nematode species occupy the same habitat as *G. incorrupta* caterpillars, but it is not known whether these nematode species infect *G. incorrupta* in the field. However, during all instars caterpillars move along the ground when foraging and typically feed on several host plants per day (Singer et al. 2002), suggesting that they may contact entomopathogenic nematodes. Nevertheless, we view these plant–herbivore–pathogen interactions as a model system rather than a natural set of interactions.

Materials and Methods

The experiment was a fully crossed design that included three factors: host plant (*S. longilobus* versus *L. sativa*), nematode species (*S. riobrave* versus *H. sonorensis*), and larval instar (sixth versus seventh). *G. incorrupta* were offspring of insects collected in south central Arizona during 2006 and 2007. All caterpillars were raised to the sixth instar on a wheat germ-based, standard laboratory rearing medium that did not contain PAs (Adkinsson et al. 1960), which is referred to hereafter as artificial diet. This was done to standardize nutritional history as much as possible among treatments, although it necessarily excludes effects of consuming PAs by earlier instars. From the first through third instar, caterpillars were reared in 165-ml cups with 15 g of artificial diet. Approximately, 20 caterpillars were placed in each cup. When caterpillars reached third instar, as assessed by body size (≈9 d of age), they were placed individually in 165-ml cups with diet. Caterpillars were checked every other day for molting to the fourth instar. Fourth instars of *G. incorrupta* are easily distinguished from earlier instars because of the marked increase in the density of cuticular hairs (M.S.S., personal observations). Upon initiation of the fourth instar, caterpillars were checked daily for molting to the fifth and sixth instar, as determined by the presence of an exuvium.

Newly molted sixth instar caterpillars were fed either *S. longilobus* or *L. sativa*. *S. longilobus* was collected from the University of Arizona's Santa Rita Experimental Range in southern Arizona, and *L. sativa* (organic red leaf lettuce) was purchased from Food Conspiracy Co-op (Tucson, AZ). While feeding on plants, caterpillars were held individually in 165-ml plastic cups lined with a filter paper disk (Whatman no. 1 qualitative), and fresh leaf tissue was provided

every second day (photoperiod of 16:8 [L:D] h at 25°C).

After feeding on host plants, caterpillars were exposed to nematodes as either sixth or seventh instars. In all cases, caterpillars were weighed immediately before exposure to nematodes. For the caterpillars exposed to nematodes as sixth instars (duration of sixth instar, 7.2 d ± 1.9 d [mean ± SD]), caterpillars were fed plants for the first 3 d of the sixth instar and then artificial diet for 1 d, after which they were immediately exposed to nematodes. For caterpillars exposed to nematodes as seventh instars, caterpillars were fed host plants for the first four days of their sixth instar and then artificial diet for the remainder of the sixth instar; after molting into seventh instar, caterpillars were fed artificial diet for one additional day and then exposed to nematodes. Caterpillars were fed artificial diet before expose to nematodes to remove plant material from the gut and minimize direct effects of plant tissue on the nematodes. After exposure to nematodes, caterpillars were held singly in 165-ml cups and fed artificial diet ad libitum.

We chose these two methods for rearing caterpillars because susceptibility of insects to natural enemies can vary with larval instar. Testing two larval instars allowed us to assess some of the potential variation that may arise in host plant-meditated resistance to entomopathogens.

Nematodes were cultured in *Galleria mellonella* L. following Kaya and Stock (1997). Caterpillars were exposed singly to infective juvenile nematodes for 24 h in petri dishes (diameter, 3.5 cm) lined with 3 g of sterile sand followingGassmann et al. (2008). Numbers of infective juvenile nematodes per dish were as follows: 4, 7, 10, 15, 20, and 30 of *S. riobrave* (ML29 strain) and 5, 10, 20, 40, 60, and 80 for *H. sonorensis* (CH35 strain). We selected these concentrations based on preliminary studies, which found that *S. riobrave* (ML29 strain) was more pathogenic to *G. incorrupta* than was *H. sonorensis* (CH35 strain). These concentrations were selected to achieve average mortality of roughly 50% based on preliminary results with both nematodes.

In total, we fed 430 sixth instar caterpillars host plants. Of these, 344 were exposed to nematodes and 86 were used as experimental controls. Experimental controls were not exposed to nematodes but otherwise experienced the same conditions as caterpillars exposed to nematodes. Any caterpillars that died within 14 d of exposure to nematodes were scored as killed by nematodes. Because nematodes typically kill their hosts within 2 d of infection (Kaya and Gaugler 1993), the 14-d scoring period virtually assured that we observed all nematode-imposed mortality. Dead caterpillars (cadavers) were placed on White traps (Kaya and Stock 1997) to collect nematode progeny produced inside cadavers. Infective juvenile nematodes (progeny) that emerged from cadavers were collected and counted using a microscope and hemocytometer.

This experiment was repeated three times over a 6-month period from April to September, 2007, with each replication lasting ≈2 mo. Although host plant quality may have varied over this period, it was not our intention to test whether intraspecific variation in host plants affected this tritrophic interaction, but rather whether host plants containing or lacking PAs might influence caterpillar growth and survival in the presence of nematodes. An average of 43 ± 2.2 (mean ± SD) caterpillars were exposed to nematodes in each of the eight experimental treatments of two nematode species by two larval instars by two host plants. Caterpillars were distributed equally and randomly among the various nematode concentrations. For each combination of host plant by larval instar, an average of 21.5 ± 3 control caterpillars were not exposed to nematodes. Caterpillars serving as experimental controls were run with each replication of the experiment.

Data Analysis. Mass of caterpillars before nematode exposure was analyzed with an analysis of variance (ANOVA) using a general linear model that assumed a normal distribution (PROC GLM in SAS; SAS Institute 1999). The model included the factors of host plant (*S. longilobus* or *L. sativa*), larval instar (sixth or seventh), and their interaction.

Logistic regression was used to analyze survival of caterpillars after exposure to nematodes. The analysis employed a general linear model and assumed a binomial (i.e., discrete) distribution for the response variable (PROC GENMOD in SAS). The response variable was the number of caterpillars surviving at each nematode concentration during each replication of the experiment. On average, 2.6 ± 0.5 (mean ± SD) caterpillars were exposed to each nematode concentration during each of the three replicates. As such, the number of caterpillars surviving per concentration per replicate was a discrete variable composed of values such as 0, 1, 2, and 3. Because no control caterpillars died (see Results), mortality from nematodes did not need to be corrected for control mortality.

The logistic regression included the categorical variables of instar, host plant, and their interaction, and the continuous covariate of nematode concentration. Data were analyzed separately for each nematode species, because regression slopes for survival as a function of nematode concentration differed between nematode species ($\chi^2 = 16.30$, df = 7, $P = 0.02$). However, slopes for survival as a function of nematode concentration were homogenous among host plant and instar combinations for *S. riobrave* ($\chi^2 = 1.68$, df = 3, $P = 0.64$) and *H. sonorensis* ($\chi^2 = 3.81$, df = 3, $P = 0.28$). Thus, the data satisfied the assumption of homogenous regression slopes for the covariate (nematode concentration) among treatments (Sokal and Rohlf 1995). A significant interaction was found between host plant and instar for caterpillars treated with *H. sonorensis* (see Results); consequently, we used linear contrasts to compare survival of caterpillars fed *L. sativa* versus *S. longilobus* at the sixth and seventh instars (CONTRAST statement in PROC GENMOD). Mean survival and corresponding standard errors were calculated based on survival at each nematode concentration for each replicate of the experiment.

Table 1. Analysis of variance for mass of *G. incorrupta* immediately before nematode assays

Source	df	*F*	*P*
Host plant	1	5.71	0.02
Instar	1	185.89	<0.0001
Host plant × instar	1	0.93	0.33
Error	419		

Because mass of caterpillars differed between instars and between host plant treatments (see Results), an analysis was conducted that included the additional covariate of mass. Mass of caterpillars was transformed with the inverse function (1/x) in ensure homogeneity of regression slopes. Slopes for survival as a function of mass were homogenous among host plant and instar combinations for *S. riobrave* ($\chi^2 = 4.97$, df = 3, $P = 0.17$) and *H. sonorensis* ($\chi^2 = 5.26$, df = 3, $P = 0.15$).

The number of cadavers from which nematode progeny emerged, which indicates successful reproduction by nematodes, was analyzed with a test of independence (PROC CATMOD in SAS). No *S. riobrave* emerged from cadavers of caterpillars fed *S. longilobus.* To allow for analysis of data, we assumed emergence of *S. riobrave* from one cadaver for sixth and seventh instar caterpillars that were fed *S. longilobus*, which made our analysis more conservative. The test of independence included the factors of host plant, instar, and nematode species. The proportion of cadavers that yielded progeny was calculated based on all cadavers across for the entire experiment, and error bars are the stand error of the proportion as described in Sokal and Rohlf (1995).

In contrast to *S. riobrave*, progeny of *H. sonorensis* emerged from cadavers of *G. incorrupta* fed *L. sativa* and *S. longilobus.* The number of progeny emerging per cadaver was compared between host plants with a one-way ANOVA (PROC GLM). Because progeny emerged from only one cadaver of seventh instar caterpillars fed *S. longilobus*, instars were pooled in the analysis. To test whether differences in progeny production between caterpillars fed *L. sativa* versus *S. longilobus* were due to differences in size of caterpillars or their suitability for nematode reproduction, data also were analyzed with a one-way analysis of covariance that included the covariate of caterpillar mass and the factor of host plant.

Results

G. incorrupta caterpillars gained significantly more mass when fed *L. sativa* (lacks PAs) than *S. longilobus* (contains PAs) (Table 1; Fig. 1). Immediately before exposure to nematodes, caterpillars fed *L. sativa* weighed more than those fed *S. longilobus*, and this difference occurred for both sixth and seventh instar caterpillars.

Mortality was 49% for caterpillars exposed to nematodes and 0% for caterpillars serving as experimental controls. Effects of nematode-imposed mortality on *G. incorrupta* caterpillars differed between nematode species. For *H. sonorensis*, a significant interaction

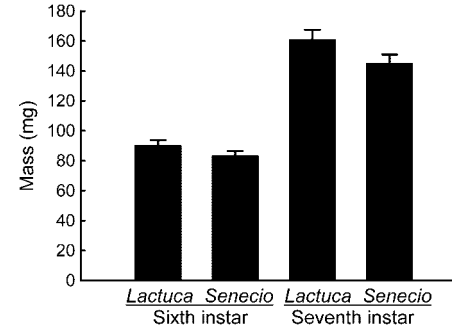

Fig. 1. Mass of caterpillars before exposure to nematodes. Bar heights are sample means and error bars are the standard error of the mean. The x-axis describes the host plant caterpillars were fed, *S. longilobus* (contains PAs) and *L. sativa* (lacks PAs), and the instar of caterpillars. Sample size per treatment was 108 ± 7 caterpillars (mean ± SD).

occurred between host plant and instar of caterpillars (Table 2, Survival). This interaction remained significant after survival data were corrected for differences in mass of caterpillars among treatments (Table 2, Survival adjusted for mass). For sixth instar caterpillars, there was no significant difference in survival between individuals fed *L. sativa* or *S. longilobus* ($\chi^2 = 2.20$, df = 1, $P = 0.14$). By contrast, for seventh instar caterpillars, survival was significantly higher for caterpillars fed *S. longilobus* (contains PAs) than for those fed *L. sativa* (lacks PAs) ($\chi^2 = 4.48$, df = 1, $P = 0.03$) (Fig. 2a).

For the *S. riobrave* treatment, there was a marginally significant effect of instar on survival, with seventh instar caterpillars displaying greater survival than sixth instars (Table 3, Survival; Fig. 2b). When mass of caterpillars was included in the analysis, larval instar was no longer significant, but mass did explain a significant amount of the variation in survival (Table 3, Survival adjusted for mass).

The number of cadavers yielding progeny differed significantly between nematode species and host plants (Table 4). Across all combinations of host plant and instar, more cadavers yielded progeny of *H. sonorensis* than *S. riobrave* (Fig. 3). In addition, more cadavers of caterpillars fed *L. sativa* yielded progeny than did cadavers of caterpillars fed *S. longilobus*, and this pattern was present for both *S. riobrave* and *H.*

Table 2. Logistic regression for the proportion of *G. incorrupta* surviving exposure to the entomopathogenic nematode *H. sonorensis*

Source	df	Survival		Survival adjusted for mass	
		χ^2	*P*	χ^2	*P*
Host plant	1	0.43	0.51	0.48	0.49
Instar	1	5.32	0.02	5.66	0.02
Host plant × instar	1	6.61	0.01	6.54	0.01
Nematode concn (covariate)	1	11.71	0.0006	11.98	0.0005
Mass of caterpillars (covariate)	1			0.71	0.40

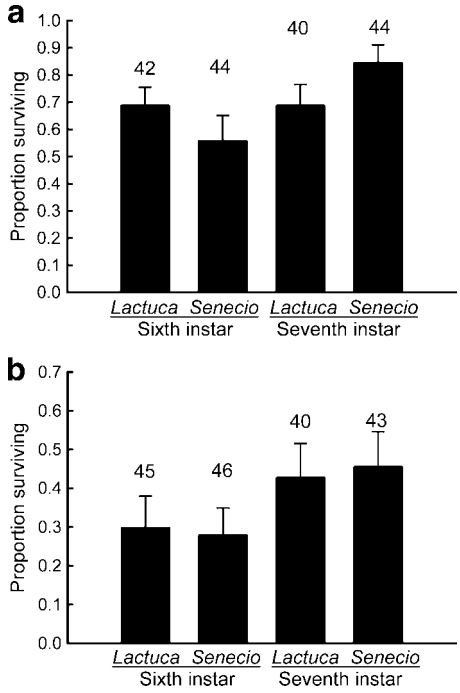

Fig. 2. Proportion of caterpillars surviving exposure to the entomopathogenic nematodes for (a) *H. sonorensis* and (b) *S. riobrave*. The x-axis describes the host plant caterpillars were fed, *S. longilobus* (contains PAs) and *L. sativa* (lacks PAs), and the instar of caterpillars. Numbers above bars give the total sample sizes, excluding controls, for each treatment. For caterpillars exposed to *H. sonorensis*, survival did not differ between host plants for the sixth instar ($\chi^2 = 2.20$, df = 1, $P = 0.14$), but survival was significantly greater for caterpillars fed *S. longilobus* than *L. sativa* for the seventh instar ($\chi^2 = 4.48$, df = 1, $P = 0.03$). Bars heights are average survival per replicate at each nematode concentration, and error bars are the standard error of the mean calculated per nematode concentration for each replicate.

sonorensis (Fig. 3). No cadavers of caterpillars fed *S. longilobus* yielded nematode progeny *S. riobrave*.

The number of progeny of *H. sonorensis* emerging per cadaver was significantly greater for cadavers of caterpillars fed *L. sativa* ($n = 9$; 1,370 ± 412) than those fed *S. longilobus* ($n = 5$; 410 ± 552) (mean ± SE) ($F = 7.35$; df = 1, 12; $P = 0.02$). The number of progeny produced per cadaver was not significantly affected by

Table 3. Logistic regression for the proportion of *G. incorrupta* surviving exposure to the entomopathogenic nematode *S. riobrave*

Source	df	Survival		Survival adjusted for mass	
		χ^2	P	χ^2	P
Host plant	1	0.01	0.93	0.01	0.92
Instar	1	3.51	0.06	0.01	0.93
Host plant × instar	1	0.19	0.67	0.44	0.51
Nematode concn (covariate)	1	20.91	<0.01	22.44	<0.01
Mass of caterpillars (covariate)	1			6.07	0.01

Table 4. Test of independence for the likelihood of emergence of nematodes from cadavers

Source	df	χ^2	P
Nematode species	1	7.25	0.007
Host plant	1	4.65	0.03
Instar	1	0.32	0.57
Nematode species × host plant	1	0.46	0.50
Nematode species × instar	1	0.33	0.56
Host plant × instar	1	0.41	0.52
Nematode species × host plant × instar	1	0.01	0.92

mass of caterpillars ($F = 1.35$; df = 1,11; $P = 0.49$) indicating that differences in host suitability, but not host size, were responsible for differences in nematode reproduction.

Discussion

Feeding by *G. incorrupta* caterpillars on *S. longilobus*, a host plant containing PAs, decreased larval growth and nematode reproduction, and in one of four cases evaluated, increased resistance of caterpillars to entomopathogenic nematodes. Both sixth and seventh instar caterpillars displayed decreased growth when fed *S. longilobus* (Table 1; Fig. 1). However, this harmful effect on caterpillars was countered by the benefit

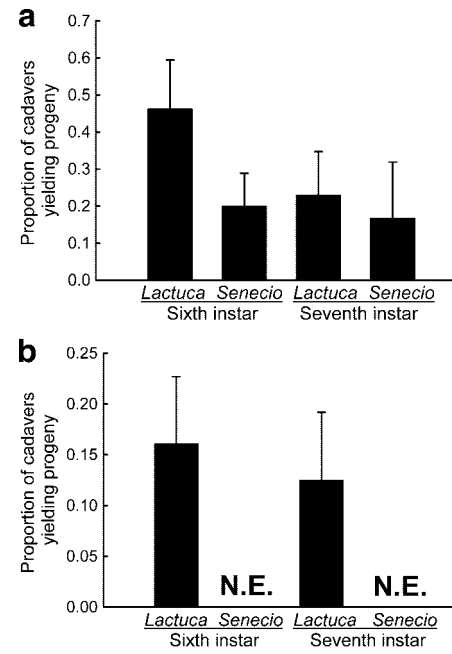

Fig. 3. Proportion of cadavers yielding nematode progeny for (a) *H. sonorensis* and (b) *S. riobrave*. For *S. riobrave*, N.E. means that there was no emergence of nematodes for cadavers of caterpillars that ate *S. longilobus*. The x-axis describes the host plant caterpillars were fed, *S. longilobus* (contains PAs) and *L. sativa* (lacks PAs), and the instar of caterpillars. Bar heights represent the proportion of cadavers that yielded progeny for all cadavers across from the entire experiment. Error bars are the stand error of the proportion as described in Sokal and Rohlf (1995).

of greater resistance to the entomopathogenic nematode *H. sonorensis* by seventh instar caterpillars (Table 2; Fig. 2a). No significant differences in mortality were present between host plant treatments for sixth instar caterpillars exposed to *H. sonorensis* or when caterpillars were exposed to *S. riobrave* (Table 3; Fig. 2).

Past research on *G. incorrupta* has found that caterpillars consuming PAs either as part of a host plant or incorporated into artificial diet will have greater resistance to parasitoids, and that caterpillars will respond to parasitism by ingesting greater quantities of PAs compared with unparasitized individuals (Singer et al. 2004b, Singer et al. 2009). Together, these results and the results reported here suggest that resistance to a diverse set of natural enemies may favor feeding on PA-containing host plants, such as *S. longilobus*, even though *G. incorrupta* caterpillars suffer decreased growth when feeding on this host.

In general, nematodes and other entomopathogens may be affected by the plant secondary metabolites and host plant species consumed by their insect hosts. The southern corn rootworm, *Diabrotica undecimpunctata howardi* Barber (Coleoptera: Chrysomelidae), displayed differing levels of susceptibility to entomopathogenic nematodes depending on which host plant it consumed; moreover, host plant effects extended to nematode reproduction (Barbercheck 1993, Barbercheck et al. 1995). Similarly, susceptibility to nematodes was lower for larvae of fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), that consumed grass infected with the alkaloid-producing fungus *Neotyphodium lolii* compared with larvae that consumed uninfected grass (Richmond et al. 2004). More broadly, host plants also may influence susceptibility of insects to entomopathogenic bacteria, fungi, and viruses (Cory and Hoover 2006). These results suggest that theory developed to describe tritrophic interactions among plants, herbivores, and arthropod natural enemies (Price et al. 1980, Jeffries and Lawton 1984, Singer and Stireman 2005) also may be applicable to tritrophic interactions involving entomopathogens.

Factors contributing to the reduced growth of *G. incorrupta* fed *S. longilobus* versus *L. sativa* may include the presence of PAs and other differences between host plants in allelochemicals and nutrients. Past research on *G. incorrupta* and on another generalist, *Estigmene acrea* Drury (Lepidoptera: Arctiidae), found that caterpillars fed *S. longilobus*, either alone or in combination with other plants, displayed decreased growth compared with caterpillars fed a host plant diet that lacked *S. longilobus* (Singer et al. 2004b, Singer et al. 2004a). Additionally, studies with artificial diet provide evidence that alkaloids reduced larval growth of insects (Gunasena et al. 1990, Harvey et al. 2007). The growth of herbivorous insects also may be limited by protein availability and quality, and the availability of other primary plant metabolites (Berenbaum 1995, Felton 1996, Barbehenn et al. 1999). Differences in secondary chemistry, primary metabolites, or both may have contributed to the effects on growth observed in this study.

The increased resistance to *H. sonorensis* by *G. incorrupta* that consumed *S. longilobus*, which contains PAs, seems to be due to indirect effects of host plants on nematodes, because caterpillars were only fed host plants before exposure to nematodes. However, the mechanistic basis of this increased resistance is unknown. *Grammia incorrupta* can sequester PAs in the hemolymph and integument, and greater concentrations of sequestered PAs increases resistance to parasitoids (Singer et al. 2004b). Greater resistance to nematodes for *G. incorrupta* that consumed *S. longilobus* was only present in one of four cases (seventh instar caterpillars challenged with *H. sonorensis*), suggesting that host plant effects on entomopathogenic nematodes in nature may be variable. PAs can be toxic to nematodes (Thoden et al. 2007) and their associated symbiotic bacteria (Kunkel et al. 2004), which is consistent with the indirect effect of *S. longilobus* on this class of natural enemy. However, differences in immune response, which can be affected by host plant quality (Ojala et al. 2005, Lee et al. 2006, Klemola et al. 2007), also may have contributed to the observed effects on resistance to nematodes.

The decreased reproduction of both nematode species in cadavers of *G. incorrupta* that ate *S. longilobus* supports the hypothesis that indirect effects of *S. longilobus* on infecting nematodes were present. Once a nematode infects its host and releases symbiotic bacteria, which eventually kill the host, the nematode must survive in the hemocoel until the host dies (Li et al. 2007). Even then, successful reproduction is not assured as the nematodes must feed within the hemocoel and develop to adulthood (Kaya and Gaugler 1993). We hypothesize that sequestered PAs may decrease the likelihood of nematodes maturing to adulthood or of their progeny developing into infective juveniles within the hemocoel.

Although none of the treatments tested yielded successful nematode reproduction in all cadavers, *G. incorrupta* is probably a viable host for these nematodes. Nematodes do not reproduce successfully in all infected hosts (Koppenhöfer and Kaya 1999, Koppenhöfer et al. 2003, Gassmann et al. 2006). There are several reasons why this may occur, for example, nematodes in the genus *Steinernema* have obligate sexually reproduction during their first generation within an infected host (Burnell and Stock 2000). Failure of male and female nematodes to infect the same host prevents successful reproduction. In addition, because several essential steps must occur between infection and reproduction, an infection causing host mortality does not ensure production of progeny.

The results presented here raise questions concerning whether effects of host plants on nematodes would be observed in the field and the extent to which entomopathogens may impose natural selection on *G. incorrupta* to consume plants with PAs. Many field-collected *G. incorrupta* contain PAs in their hemolymph (Singer et al. 2004b) and foraging caterpillars in the field consume hosts with PAs (Singer and Stireman, 2001; Hartmann et al., 2004), suggesting that indirect effects of host plants such as *S. longilobus* on

entomopathogenic nematodes may arise in nature. Studies of entomopathogens as natural sources of mortality for *G. incorrupta* would provide valuable information on the potential evolutionary significance of this interaction. Natural selection may act to structure ecological interactions in either a pairwise or diffuse manner, with adaptation arising in response to one or many selective agents, respectively (Thompson 1994). It may be the case that use of host plants with PAs by *G. incorrupta* is an example of diffuse selection, with caterpillars displaying enhanced resistance to diverse natural enemies. However, a necessary step in answering this question is quantifying the intensity of natural selection by entomopathogenic nematodes in the field.

Acknowledgments

We thank Ana Romo, Gopalan Unnithan, and Alex Yelich for assistance. Funding was provided by the Center for Insect Science at the University of Arizona and by the U.S. Department of Agriculture through grants 2006-35302-17365 and 2007-35302-18415.

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Received 30 August 2009; accepted 1 January 2010.