Chemoecology 16:45–50 (2006)
0937–7409/06/010045–6

DOI 10.1007/s00049-005-0327-3

CHEMOECOLOGY

Furanocoumarins and their detoxification in a tri-trophic interaction

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Summary. The parsnip webworm, *Depressaria pastinacella*, specializes on wild parsnip, *Pastinaca sativa*, and several species of *Heracleum*, hostplants rich in toxic furanocoumarins. Rates of furanocoumarin metabolism in this species are among the highest known for any insect. Within its native range in Europe, webworms are heavily parasitized by the polyembryonic parasitoid wasp *Copidosoma sosares*. In this study, we determined whether these parasitoids are exposed to furanocoumarins in host hemolymph, whether they can metabolize furanocoumarins, and whether parasitism influences the ability of webworms to detoxify furanocoumarins. Hemolymph of webworms fed artificial diet containing 0.3 % fresh weight xanthotoxin, a furanocoumarin prevalent in wild parsnip hosts, contained trace amounts of this toxin; as well, hemolymph of webworms consuming *P. sativa* flowers and fruits contained trace amounts of six of seven furanocoumarins present in the hostplant. Thus, parasitoids likely encounter furanocoumarins in host hemolymph. Assays of xanthotoxin metabolism in *C. sosares* failed to show any ability to metabolize this compound. Parasitized webworms, collected from populations of *Heracleum sphondylium* in the Netherlands in 2004, were on average 55 % larger by weight than unparasitized individuals. This weight is inclusive of host and parasitoid masses. Absolute rates of detoxification (nmoles min^{-1}) of five different furanocoumarins were indistinguishable between parasitized and unparasitized ultimate instars, suggesting that the intrinsic rates of metabolism are fixed. Thus, although parasitized larvae are larger, detoxification rates are not commensurate with size; rates in parasitized larvae expressed per gram of larval mass were 25 % lower than in unparasitized larvae.

Key words. *Copidosoma sosares* – *Depressaria pastinacella* – *Pastinaca sativa* – furanocoumarins – tri-trophic – detoxification – hemolymph – cytochrome P450

Introduction

The use of parasitoids in integrated pest management programs has led to increased interest in ascertaining third trophic level effects of chemically based hostplant resistance (Campbell & Duffey 1979; Ode 2006). Most research on parasitoid resistance to toxins has involved insecticides (e.g., Keeratikasikorn & Hooper 1981; Spollen *et al*. 1995; Zhu *et al.* 1999; Willrich & Boethel 2001; Xu *et al*. 2001; Liu *et al.* 2003); in only a small number of these studies has pesticide resistance been linked to rates of metabolic detoxification by parasitoids (e.g., Perez-Mendoza *et al.* 2000; Wu *et al*. 2004; Wu & Miyata 2005). Even fewer studies have examined the effects of hostplant toxins on parasitoids. Among hostplant allelochemicals documented to have adverse effects on parasitoids are nicotine, rutin, alpha-tomatine, DIMBOA (reviewed by Ode 2006) and pyrrolizidine alkaloids (Singer *et al*. 2004a, 2004b); in contrast, iridoid glycosides appear to have little, if any, effect on parasitoids and gossypol may have a positive effect (Ode 2006).

Parasitoids that show no response to allelochemicals ingested by their hosts may be protected against exposure by efficient metabolic transformation and excretion of toxins by their hosts. Alternatively, parasitoids themselves may possess some of the same mechanisms employed by herbivores for coping with plant allelochemicals, including target site insensitivity and metabolic detoxification (Strauss & Zangerl 2002). Despite its potential importance in integrated pest management programs using both biological control and hostplant resistance, virtually nothing is known about the ability of parasitoids to influence the capacity of their hosts to detoxify or excrete plant allelochemicals or to detoxify those allelochemicals directly.

Furanocoumarins are broadly biocidal allelochemicals, particularly abundant in Rutaceae and Apiaceae, that have been implicated as plant resistance factors against a broad variety of herbivorous insects (reviewed in Berenbaum & Zangerl 1996). That furanocoumarins may act tritrophically as well and influence parasitoid success was demonstrated in two laboratory studies. The dipteran parasite *Archytas marmoratus* experienced higher mortality when its host *Spodoptera exigua* was fed artificial diet containing increasing concentrations of a mixture of three furanocoumarins—bergapten, psoralen, and xanthotoxin (Reitz & Trumble 1997). Similarly, mortality of *Copidosoma floridanum* increased in *Trichoplusia ni* fed the same furanocoumarins (Reitz & Trumble 1996).

The parsnip webworm, *Depressaria pastinacella*, feeds exclusively on species in the genera *Pastinaca* and *Heracleum*, which are well-endowed with furanocoumarins. Webworms feed on reproductive structures of their host plants and thus encounter furanocoumarins in concentrations exceeding

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1 % of the dry mass ingested; rates of detoxification of furanocoumarins in this species are the highest known for any insect (Zangerl & Berenbaum 1999). Despite their extraordinary ability to metabolize furanocoumarins, webworms are differentially sensitive to particular combinations of furanocoumarins in their principal midwestern U.S. host, *Pastinaca sativa* (Zangerl & Berenbaum 1993, Zangerl & Berenbaum 2003). Rates of parasitism of *D. pastinacella* in the midwestern United States rarely if ever exceed rates of 3 % and these parasitoids tend to be opportunistic generalists (Gorder & Mertins 1984; Berenbaum 1990). In contrast, in Europe, within its area of indigeneity, parsnip webworms are heavily parasitized by the specialist wasp *Copidosoma sosares* (Ode *et al.* 2004; Guerrieri & Noyes 2005). Like other polyembryonic encyrtids (Strand *et al*. 1991), *C. sosares* oviposits in the eggs of its host; clonal proliferation of morulae takes place, giving rise to anywhere from 10 to 400 genetically identical larvae that complete development during the host's ultimate instar (Ode *et al*. 2004). Success of *Copidosoma sosares* in parasitizing parsnip webworms in the Netherlands has been linked to the furanocoumarin composition of its hosts (Ode *et al.* 2004); concentrations of isopimpinellin were negatively correlated with the likelihood that a given webworm would be successfully parasitized and xanthotoxin concentration was negatively correlated with both within-brood survivorship and clutch size.

Given the apparent tritrophic impact of furanocoumarins on *C. sosares* in the field, we set out to determine whether unmetabolized, and hence potentially toxic, furanocoumarins are present in the hemolymph of webworms and thus act directly to reduce survivorship and clutch size of *C. sosares*. We also investigated whether the parasitoid itself has some capacity for cytochrome P450-mediated detoxification of furanocoumarins and whether its presence can alter its host's capacity to metabolize these compounds.

Methods and Materials

Materials

Xanthotoxin and bergapten were purchased from Sigma (St. Louis); isopimpinellin and angelicin were obtained from Indofine (Belle Mead, NJ); sphondin was provided by Dr. R. Mumma; reagents for cytochrome P450 assays were obtained from Sigma.

Furanocoumarin metabolism by parsnip webworms. Parasitized and unparasitized fifth instar webworms were collected in July 2004 from two host plant species, *Heracleum sphondylium* and *Pastinaca sativa* in the Netherlands from four populations in or near (within 2 km E or S of the town center) Heteren and four populations in Zwolle (near NW, SW, and SE corners of the ring road). Webworms were placed on plant-based artificial diet in 30-ml plastic creamer containers for transport back to the United States. This diet, modified from Nitao and Berenbaum (1988), substitutes lyophilized wild parsnip reproductive parts for half the amount of casein, sucrose, and Wesson's salt mix. Sixth instars were weighed and then dissected to remove their midguts. Midguts were rinsed in ice-cold dissection buffer [0.1 M sodium phosphate (pH 7.8), 0.25 M sucrose, 1 % w/v polyvinylpyrrolidone (PVP), 1.1 mM ethylenediaminetetraacetic acid (EDTA), and 2 mM phenylmethylsulfonyl fluoride (PMSF) (Crankshaw *et al*. 1979)] to remove all food particles. Because the food contains furanocoumarins, presence of food in the gut could compromise accurate assessment of metabolic activity in the disappearance assay we use (see below). Parasitoid larvae within the body cavity were dislodged during the dissection of parasitized webworms and were collected using a 1000 µL micropipette. Individual midguts were homogenized in 50 µL of grinding buffer per gut (dissection buffer which contained additional leupeptin and glycerol) and flash-frozen in liquid nitrogen for storage at –80 ºC. Parasitoids were flash-frozen without homogenization and stored at –80 ºC.

The metabolism reaction was initiated by adding 750 µL of reaction buffer [0.1 M sodium phosphate (pH 7.8), 0.3 mM nicotinamide adenine dinucleotide phosphate (NADP), 3 mM glucose-6-phosphate dehydrogenase (G-6-PDH)] and 10 µL of a substrate mixture containing imperatorin, bergapten, isopimpinellin, xanthotoxin, and sphondin in methanol. This mixture was representative of relative concentrations encountered in the wild parsnip host and was obtained by column purification of an extract of wild parsnip seed and calibrated against a mixture of authentic standards (Berenbaum & Zangerl 1998). The metabolism mixture was then incubated at 30 ºC for 30 minutes with mild agitation followed immediately with a five-minute period in 100 ºC water to stop the reaction. Nineteen time-zero controls containing no protein were prepared as described using 50 µL of grinding buffer in place of homogenate.

Unmetabolized furanocoumarins were extracted by adding 350 µL ethyl acetate to each reaction, followed by 30-second vortexing and one-minute centrifugation. A 15-µL aliquot of the organic phase of each sample was analyzed using high pressure liquid chromatography (HPLC) (Waters Associates) with a 150-mm normal phase silica column (Absorbosphere, 4.6 mm internal diameter, 5 micron particle size, Alltech, Deerfield, IL). The mobile phase consisted of 55 % cyclohexane, 42 % isopropyl ether, and 3 % n-butanol pumped at a rate of 1.5 mL/minute, and detection was at 254 nm. To determine the amount of metabolized furanocoumarins, the peak area on the chromatogram associated with each furanocoumarin remaining was subtracted from the average of the time-zero areas. Raw metabolism rates were expressed as nmoles/min/larva, and mass-adjusted rates were expressed as nmoles/min/g larva. Differences in metabolism rates per individual (nmoles/min/larva) and larval mass were analyzed by t-test, with degrees of freedom adjusted for the test of mass due to unequal variances (SPSS 9.0, Chicago, IL). An analysis of covariance was performed with parasitism as the main effect, metabolism rates per individual as the dependent variable, and larval mass as the covariate. The assumption of homogeneity of slopes was satisfied in the latter analyses, as there were no significant interactions between the covariate and treatment ($Ps \geq 0.45$).

Webworm hemolymph analysis. Fifth (penultimate) instar webworms reared on diet containing no added furanocoumarins were placed on artificial diet containing 0.3 % fresh weight xanthotoxin and allowed to feed for three days. On the fourth day after the molt to sixth instar, the cuticle of each of three larvae was punctured with a 27-gauge hypodermic needle, and hemolymph was drawn up into a 10-µL hematocrit microcapillary tube. Volumes of 3.75 µL, 4.06 µL, and 4.38 µL were obtained from the first, second, and third larva, respectively. The samples were dried and extracted with 5 µL ethyl acetate, and 2 µL of this extract was analyzed using gas chromatography - mass spectrometry (GC-MS), HP-5973 mass spectrometer interfaced with a HP 6890 gas chromatograph, using a DB-5 capillary column (30 m × 0.32 mm × 0.25 µm film thickness) in splitless mode with helium as the carrier gas and temperature program with initial temperature of 50 ºC held for 1 minute and then increased by 15 ºC until the final temperature of 300 ºC was reached and held for two minutes.

Additionally, in order to determine whether components of the furanocoumarin mixture present naturally in plant tissue are differentially transferred into hemolymph, sixth instar webworms that had been laboratory-reared on wild parsnip growing in the greenhouse (30 ºC with 16L : 8D photoperiod) were also analyzed as described. Volumes of 0.72 µL, 1.01 µL, 1.05 µL, 1.07 µL, and 2.32 µL were obtained from five larvae, individually.

Xanthotoxin metabolism by C. sosares. Xanthotoxin, which has been linked to mortality of *C. sosares* (Ode *et al*. 2004), was used as the substrate for assaying furanocoumarin metabolism by parasitoids. Preliminary assays revealed that furanocoumarin metabolism by larval polyembryonic (and hence genetically identical) parasitoids within a single webworm larva was below the detection threshold (data not shown). Consequently, larval *C. sosares* from all 48 parasitized webworms were pooled and

Table 1 Analysis of covariance of furanocoumarin metabolism rate in *Depressaria pastinacella* as a function of parasitism by *Copidosoma sosares*

Furanocoumarin	Source	d.f.	M.S.	F	P
imperatorin	parasitism	1	0.106	2.268	0.137
	mass	1	0.277	5.951	0.017
	error	69	0.046		
bergapten	parasitism	1	1.301	5.847	0.018
	mass	1	3.324	14.94	< 0.001
	error	69	0.223		
isopimpinellin	parasitism	1	0.0324	6.097	0.016
	mass	1	0.0943	17.72	< 0.001
	error	69	0.0053		
xanthotoxin	parasitism	1	3.874	6.758	0.011
	mass	1	9.345	16.303	< 0.001
	error	69	0.573		
sphondin	parasitism	1	0.0032	5.332	0.024
	mass	1	0.0064	10.588	0.002
	error	69	0.0006		

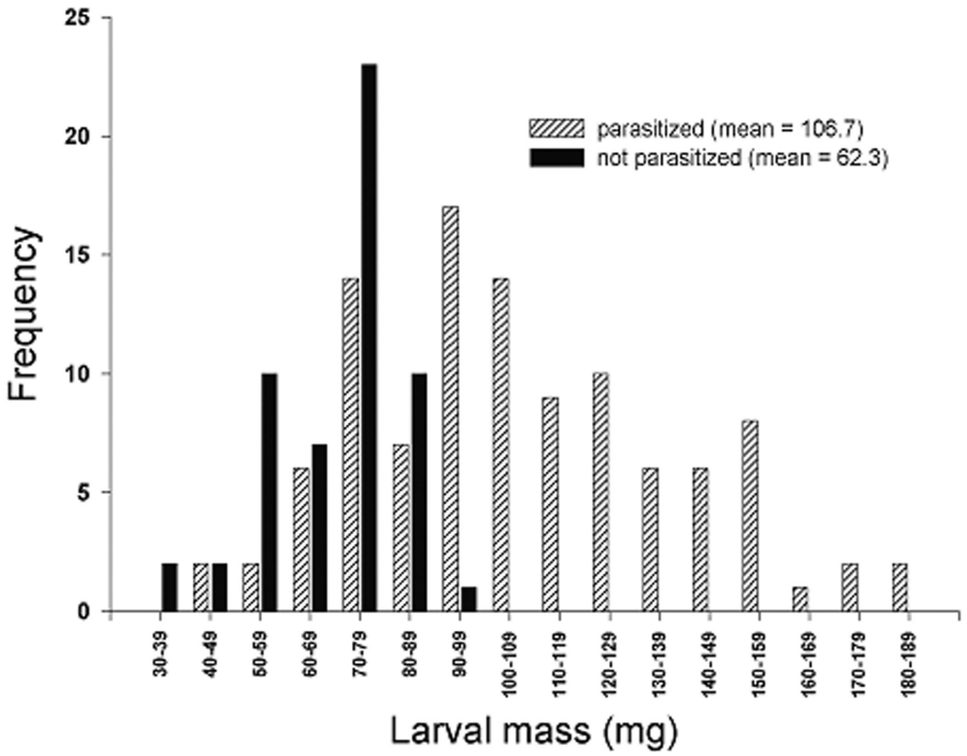

Fig. 1 Effect of parasitism by *Copidosoma sosares* on *Depressaria pastinacella* larval mass. Means were significantly different by t-test ($t = 10.43$, $p < 0.001$, d.f. = 156.5, adjusted for unequal variances). Sample sizes were 108 and 52 for parasitized and unparasitized larvae, respectively

collected. A single larval host typically contains 100 to 200 parasitoid larvae. Parasitoid larvae were grouped into three replicate groups and homogenized in one mL of grinding buffer. The metabolism reaction was initiated by adding 3.5 mL of reaction buffer and 35 µL xanthotoxin (1 mg/mL in methanol). Aliquots from each reaction were removed at 0, 10, 20, and 30 minutes after incubation at 30 °C with mild agitation. The reaction in each aliquot was stopped by placing it in a 100 °C bath for 5 minutes. Unmetabolized xanthotoxin was extracted by adding one mL ethyl acetate to each aliquot, followed by a 30-second vortexing and one-minute centrifugation. A 15-µL aliquot of each sample was analyzed by HPLC as described earlier. Total soluble protein in each reaction was quantified spectrophometrically by the Bradford method employing bovine serum albumen as the standard (BioRad, Hercules, CA). The amount of xanthotoxin metabolized was regressed against time (minutes).

Results

Furanocoumarin metabolism by parsnip webworm. Webworms that had been parasitized by *C. sosares* were on average 55 % larger (mean = 106.7 mg) than unparasitized webworms (mean = 69.3 mg) (Fig. 1). Absolute metabolic

Table 2 Furanocoumarins detected in hemolymph samples from five webworms (A-E) reared on *Pastinaca sativa* by GC-MS. Four of the five samples contained six of the furanocoumarins detectable by GC – MS (imperatorin, which is also present in wild parsnip, is not readily detectable by GC-MS at low concentrations), the fifth sample lacked only psoralen. A furanocoumarin was judged to be present (+) if its retention time (R.T.) matched that of a standard and it exhibited both major spectral fragments characteristic of the furanocoumarin.

Furanocoumarin	R.T.	m/z	Presence of identifying fragments in five hemolymph samples (µl)				
			A(0.72)	B(1.01)	C(1.05)	D(1.07)	E(2.32)
Angelicin	12.37	186,158	+	+	+	+	+
Psoralen	12.71	186,158		+	+	+	+
Xanthotoxin	14.12	216,173	+	+	+	+	+
Bergapten	14.24	216,173	+	+	+	+	+
Sphondin	14.28	216,173	+	+	+	+	+
Isopimpinellin	15.33	246,231	+	+	+	+	+

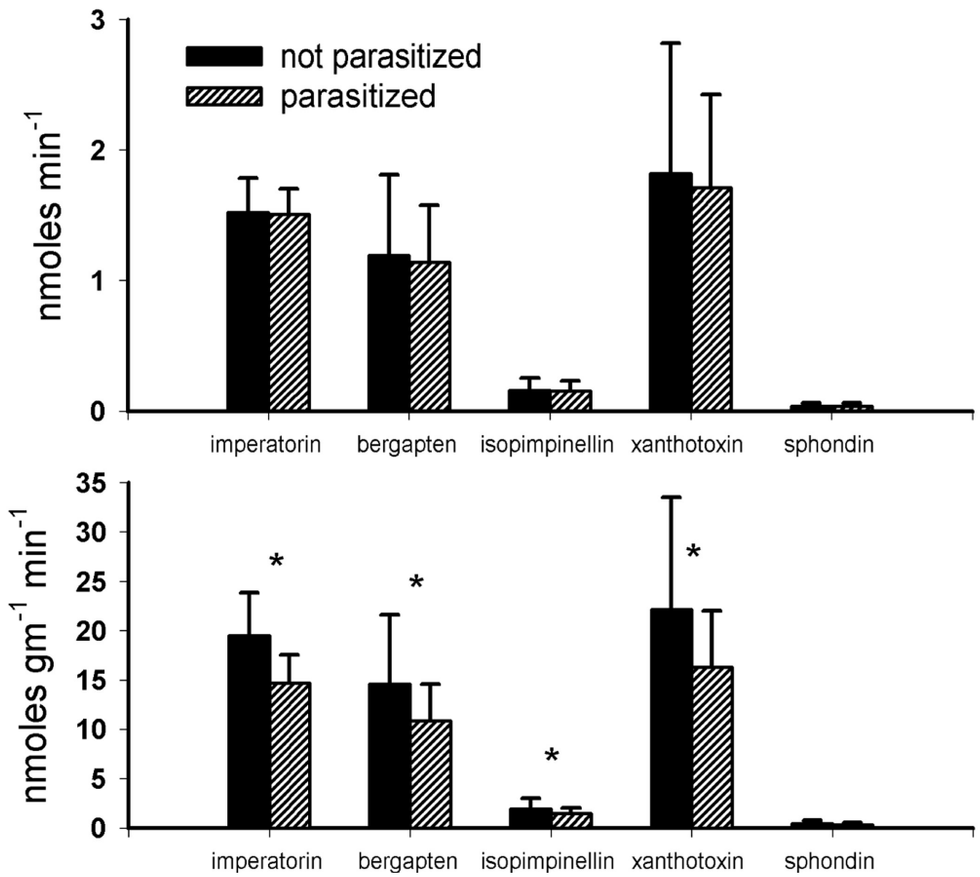

Fig. 2 Effect of parasitism by *Copidosoma sosares* on furanocoumarin metabolism by *Depressaria pastinacella*. Top panel shows absolute rates of metabolism per larva. No significant differences were seen in the five furanocoumarins substrates tested (all *P* values from t-tests > 0.6; sample sizes were 36 and 30 for parasitized and unparasitized larvae, respectively). Bottom panel shows rates of metabolism relative to webworm larval mass. Significant differences (denoted by asterisks) between parasitized and unparasitized larvae were found by t-test for imperatorin ($P < 0.0001$), bergapten ($P = 0.012$), isopimpinellin ($P = 0.042$), and xanthotoxin ($P = 0.014$) but not sphondin ($P = 0.114$)

rates (nmoles min^{-1}) were not significantly different between parasitized and unparasitized individuals for the five furanocoumarins tested (Fig. 2). When metabolic rates were examined in relation to body mass in an analysis of covariance, however, detoxification rates were approximately 25 % lower in parasitized individuals (Table 1, Fig. 2), and differences were significant for all furanocoumarins except imperatorin (Table 1).

Webworm hemolymph analysis. The hemolymph samples of all three webworms fed 0.3 % xanthotoxin diet contained xanthotoxin, as indicated by GC-MS. The sample spectrum included a base peak at 216 m/z, the molecular weight of xanthotoxin and a fragment at 173, which is characteristic of methoxylated linear furanocoumarins. Additionally, the retention time matched that of xanthotoxin. Based on mass spectral fragments characteristic of

furanocoumarins and retention matching with standards, the hemolymph samples of four of the five webworms reared on plant material contained furanocoumarins commonly found in parsnip, including angelicin, psoralen, xanthotoxin, bergapten, sphondin and isopimpinellin (Table 2), while the fifth sample contained all but psoralen.

Xanthotoxin metabolism by C. sosares. No metabolic detoxification of xanthotoxin by *C. sosares* larvae was detectable. All three regressions of xanthotoxin substrate remaining against time were not significant and no trend was present, despite the assay of thousands of individuals contributing large amounts of protein to the assays–total soluble protein in the three reactions, respectively, was 506 µg, 704 µg, and 569 µg.

Discussion

In a study of fitness of *C. sosares* in webworm hosts feeding on three plant species with contrasting furanocoumarin composition, Ode *et al*. (2004) found that two furanocoumarins reduced parasitoid success. Webworms feeding on hostplants high in isopimpinellin were less likely to elicit oviposition by *C. sosares* and survivorship of broods was significantly reduced in webworms feeding on high-xanthotoxin plants. It is clear from our results that the specialist parasitoid *C. sosares* is likely to encounter a range of furanocoumarins in the hemolymph of its host; thus, the negative correlations between parasitoid fitness and furanocoumarin content of webworm host plants (Ode *et al.* 2004) are likely manifestations of this encounter. That this parasitoid uses *D. pastinacella* exclusively as a host, however, suggests that some degree of defense against furanocoumarins exists. There appears to be no intrinsic ability on the part of the larval parasitoid to metabolize furanocoumarins in hemolymph; it stands to reason, then, that parasitoid success is mediated by the ability of webworm hosts to metabolize these compounds. It seems unlikely that ovipositing female wasps can assess the ability of an individual webworm to metabolize furanocoumarins while it is still in the egg stage; in at least one other furanocoumarin-feeding lepidopteran (*Papilio polyxenes*), there is no metabolic detoxification detectable in the egg stage (Harrison *et al*. 2001). Females thus have no way of ascertaining the detoxification capacity of a particular host individual once it develops beyond the egg stage. Determining brood size and sex ratio (both of which may be influenced by furanocoumarins) may thus be decisions better left to the offspring than to the ovipositing female. Craig *et al*. (1997) suggest that polyembryony may be particularly advantageous in circumstances in which offspring are better able to assess environmental conditions and adjust clutch size than ovipositing females; such appears to be the case for *C. sosares*.

Although detoxification rates in the parsnip webworm vary with many other environmental factors, including age (Cianfrogna *et al*. 2001) and geographic origin (Berenbaum & Zangerl 1998), parasitism per se does not appear to have a significant effect on absolute detoxification rates. However, parasitism does affect caterpillar mass; in this study, parasitized larvae were 55 % larger than unparasitized larvae. In some other caterpillar species, especially gregarious koinobionts, parasitism can lead to ingestion of larger quantities of food (Slansky 1992; Coleman *et al*. 1999; van der Meijden & Klinkhamer 2000) and consequently larger mass consisting of both host mass and parasitoid mass. If the increased size of parasitized webworms is the result of increased food consumption, then parasitism may lead to increased rates of furanocoumarin ingestion. Expressed on a per-weight basis, parasitized larvae have a reduced capacity to metabolize furanocoumarins; thus, the hemolymph of these larvae may contain even higher concentrations of unmetabolized furanocoumarins than does the hemolymph of unparasitized larvae.

Koinobiont parasitoids face many metabolic challenges integrating their development with that of their host; for *C. sosare*s, an apparent specialist on *D. pastinacella* (Guerrieri & Noyes 2005), coping with the toxin-containing hemolymph of its host is one such challenge. This species has recently been reported in the western US on *D. pastinacella*, apparently as an accidental introduction (Carroll 2004); its major hostplant throughout this region is the indigenous cowparsnip *Heracleum lanatum.* In terms of its fur nocoumarin chemistry, *H. lanatum* differs not only from *P. sativa* but also from European species of *Heracleum* (Ode *et al*. 2004; Zangerl and Berenbaum 2003). Whether *C. sosares* can establish and expand its range may depend both on variation in rates of P450-mediated furanocoumarin metabolism in North American *D. pastinacella*, which differ from those of *D. pastinacella* in the area of indigeneity (Zangerl & Berenbaum in preparation), and on variation in its toxicological response to *H. lanatum* furanocoumarins in its host hemolymph.

Acknowledgments

This research was supported by NSF grant 0235773 to MRB and ARZ and NSF grant 0321028 to PJO and MRB. We extend our appreciation and gratitude to Felix Wäckers at The Netherlands Institute of Ecology (NIOO-KNAW) for his assistance in navigating the area and to the The Netherlands Institute of Ecology for providing facilities.

References

Barbosa P, Saunders JA, Kemper J, Trumbule R, Olechno J, Martinat P (1986) Plant allelochemicals and insect parasitoids: Effects of nicotine on *Cotesia congregata* (Say) (Hymenoptera, Braconidae) and *Hyposoter annulipes* (Cresson) (Hymenoptera, Ichneumonidae). J Chem Ecol 12: 1319–1328

Berenbaum MR, Zangerl AR (1996) Phytochemical diversity: Adaptation or random variation? Pp 1–24 in Romeo JT, Saunders JA, Barbosa P (eds) Phytochemical Diversity and Redundancy in Ecological Interactions. Elsevier Press, NY

Berenbaum MR, Zangerl AR (1998) Chemical phenotype matching between a plant and its insect herbivore. Proc Natl Acad Sci 95: 13743–13748

Berenbaum MR, Zangerl AR (1999) Genetic variation in cytochrome P450-based resistance to plant allelochemicals and insecticides. Pp 55–84 in Olff H, Brown VK, Drent RH (eds) Herbivores: Between Plants and Predators. Blackwell Science, Oxford

Campbell BC, Duffy SC (1979) Tomatine and parasitic wasps: Potential incompatibility of plant antibiosis with biological control. Science 205: 700–702

Carroll M (2004) Effects of dietary lutein on the parsnip webworm, *Depressaria pastinacella* (Lepidoptera: Elachistidae). PhD dissertation. University of Illinois, Urbana-Champaign, Illinois

Cianfrogna JA, Zangerl AR, Berenbaum MR (2002) Effects of furanocoumarins on feeding behavior of parsnip webworms, *Depressaria pastinacella*. J Chem Ecol 28: 1365–1375

Coleman RA, Barker AM, Fenner M (1999) Parasitism of the herbivore *Pieris brassicae* L. (Lep., Pieridae) by *Cotesia glomerata* L. (Hym., Braconidae) does not benefit the host plant by reduction of herbivory. J Appl Entomol 123: 171–77

Craig SF, Slobodkin LB, Wray GA, Biermann CH (1997) The 'paradox' of polyembryony: a review of the cases and a hypothesis for its evolution. Evol Ecol 11: 127–143

Crankshaw DL, Hetnarski HK, Wilkinson CF (1979) Purification and characterization of NADPH-cytochrome C reductase from the midgut of the southern armyworm (*Spodoptera eridania*). Biochem J 181: 593–605

Guerrieri E, Noyes J (2005) Revision of the European species of *Copidosoma* Ratzeburg (Hymenoptera: Encyrtidae), parasitoids of caterpillars (Lepidoptera). Syst Entom 30: 97–174

Harrison T, Zangerl AR, Schuler MA, Berenbaum MR (2001) Developmental variation in cytochrome P450 expression in *Papilio polyxenes*. Arch Insect Biochem Physiol 48: 179–189

Keeratikasikorn M, Hooper GHS (1981) The comparative toxicity of some insecticides to the potato moth *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) and two of its parasites *Orgilus lepidus* Muesebeck and *Copidosoma desantisi* Annecke and Mynhardt Australia, includes effects on biological control. J Aust Entomol Soc 20: 309–311

Liu SS, Li YX, Tang ZH (2003) Host resistance to an insecticide favors selection of resistance in the parasitoid, *Cotesia plutella* (Hymenoptera: Braconidae). Biol Cont 28: 137–143

van der Meijden E, Klinkhamer PGL (2000) Conflicting interests of plants and the natural enemies of herbivores. Oikos 89: 202–8

Murray RDH, Mendez J, Brown SA (1982) The Natural Coumarins. John Wiley and Sons. Chichester

Nitao JK, Berenbaum MR (1988) Laboratory rearing of the parsnip webworm, *Depressaria pastinacella* (Lepidoptera: Oecophoridae). Ann Ent Soc Amer 81: 485-487

Ode PJ (2006) Plant chemistry and natural enemy fitness: effects on herbivore and natural enemy interactions. Ann Rev Entom 51: 163–185

Ode PJ, Berenbaum MR, Zangerl AR, Hardy CW (2004) Host plant, host plant chemistry and the polyembryonic parasitoid *Copidosoma sosares*: indirect effects in a tritrophic interaction. Oikos 104: 388–400

Perez-Mendoza J, Fabrick JA, Zhu KY, Baker JE (2000) Alterations in esterases are associated with malathion resistance in *Habrobracon hebetor* (Hymenoptera: Braconidae). J Econ Ent 93: 31–37

Reitz SR, Trumble JT (1996) Tritrophic interactions among linear furanocoumarins, the herbivore *Trichoplusia ni* (Lepidoptera: Noctuidae), and the polyembryonic parasitoid *Copidosoma floridanum* (Hymenoptera, Encyrtidae). Environ Ent 25: 1391–1397

Reitz SR, Trumble JT (1997) Effects of linear furanocoumarins on the herbivore *Spodoptera exigua* and the parasitoid *Archytas marmoratus*: host quality and parasitoid success. Entomol Exp Appl 84: 9–16

Singer MS, Rodrigues D, Stireman JO, Carriere Y (2004a) Roles of food quality and enemy-free space in host use by a generalist insect herbivore. Ecology 85: 2747–2753

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

Spollen KM, Johnson MW, Tabashnik BE (1995) Stability of fenvalerate resistance in the leafminer parasitoid *Diglyphus begini* (Hymenoptera, Eulophidae). J Econ Ent 88: 192–197

Slansky F, Jr (1992) Allelochemical-nutrient interactions in herbivore nutritional ecology, Chapter 4, Pp. 135–174 in Rosenthal GA, Berenbaum MR (eds.) Herbivores: Their Interaction with Secondary Plant Metabolites, Vol. II Ecological and Evolutionary Processes. Academic Press, San Diego. 2nd ed

Strand MR, Goodman WG, Baehrecke EH (1991) The juvenile hormone titer of *Trichoplusia ni* and its potential role in embryogenesis of the polyembryonic wasp *Copidosoma floridanum*. Insect Biochem 21: 205–214

Strauss SY, Zangerl AR (2002) Invertebrate-plant Interactions. Pp 77–106 in Herrera C, Pellmyr O (eds), Plant-Animal Interactions. Blackwell Science, Oxford

Thorpe KW, Barbosa P (1986) Effects of consumption of high and low nicotine tobacco by *Manduca sexta* (Lepidoptera, Sphingidae) on survival of gregarious endoparasitoid *Cotesia congregata* (Hymenoptera, Braconidae). J Chem Ecol 12: 1329–1337

Timmermann SE, Zangerl AR, Berenbaum MR (1999) Ascorbic and uric acid responses to xanthotoxin ingestion in a generalist and a specialist caterpillar. Arch Insect Biochem Physiol 42: 26–36

Willrich MM, Boethel DJ (2001) Effects of diflubenzuron on *Pseudoplusia includens* (Lepidoptera: Noctuidae) and its parasitoid *Copidosoma floridanum* (Hymenoptera: Encyrtidae). Environ Entomol 30: 794–797

Wu G, Miyata T (2005) Susceptibilities to methamidophos and enzymatic characteristics in 18 species of pest insects and their natural enemies in crucifer vegetable crops. Pest Bioch Phys 82: 79–93

Wu G, Jiang SR, Miyata T (2004) Effects of synergists on toxicity of six insecticides in parasitoid *Diaeretiella rapae* (Hymenoptera: Aphidiidae). J Econ Ent 97: 2057–2066

Xu JX, Shelton AM, Cheng XN (2001) Variation in susceptibility of *Diadegma insulare* (Hymenoptera: Ichneumonidae) to permethrin. J Econ Ent 94: 541–546

Zangerl AR, Berenbaum MR (1993) Plant chemistry, insect adaptations to plant chemistry, and host plant utilization patterns. Ecology 74: 47–54

Zangerl AR, Berenbaum MR (2003) Phenotype matching in wild parsnip and parsnip webworms: Causes and consequences. Evolution 57: 806–815

Zhu YC, Dowdy AK, Baker JE (1999) Detection of single-base substitution in an esterase gene and its linkage to malathion resistance in the parasitoid *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae). Pest Sci 55: 398–404

Received 23 June 2005; accepted 12 October 2005