

PLANT ALLELOCHEMICALS AND INSECT
PARASITOIDS Effects of Nicotine on *Cotesia congregata*
(Say) (Hymenoptera: Braconidae) and *Hyposoter annulipes*
(Cresson) (Hymenoptera: Ichneumonidae)^{1,2}

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Abstract—Parasitoids developing within tobacco hornworms or fall armyworms exhibit significant differences in development and survival depending on whether their hosts fed on nicotine-free or nicotine-containing diets. The effects of nicotine were more severe on the relatively less adapted parasitoid, *H. annulipes* than the specialist parasitoid, *Cotesia congregata*. Labeled alkaloid originally placed in herbivore diet was incorporated in several parasitoid tissues. These results suggest that interactions between plant allelochemicals and parasitoids should be considered in the development of theory on insect herbivory and plant defense.

Key Words—Plant allelochemical, parasitoids, three trophic level interactions, nicotine, *Manduca sexta*, Lepidoptera, Sphingidae, *Spodoptera frugiperda*, Noctuidae, *Cotesia congregata*, Hymenoptera, Braconidae, *Hyposoter annulipes*, Ichneumonidae.

INTRODUCTION

The role of plant nutrients and allelochemicals on the ecology of insect herbivores has been the subject of a great deal of research. Relatively little emphasis

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²Mention of a commercial product does not constitute an endorsement.

has been placed on the role of plant allelochemicals, within the tissues of herbivores, on the survival and ecology of secondary consumers such as insect parasitoids. Several researchers have speculated on the nature of these three trophic level interactions. Their conclusions were generally based on correlations between low levels of parasitism and the consumption by herbivores of plants with allelochemicals known to be toxic, the demonstration of the presence of an allelochemical within a parasitoid without an evaluation of its effects, or some other form of indirect or incomplete evidence (Narayanan and Subba Rao, 1955; Smith, 1957; Jones et al., 1962; Reichstein et al., 1968; Altahtawy et al., 1976; Rothschild et al., 1977; Smith 1978; Benn et al., 1979). Although many of the conclusions and speculations made in these studies are intuitively appealing, they remain speculations and assumptions to be fully and critically tested.

Morgan (1910) and Gilmore (1938a,b) suggested that nicotine might influence levels of parasitism of *Manduca sexta* (L.) (the tobacco hornworm) parasitized by *Cotesia congregata* (Say) (= *Apanteles congregatus*). Thurston and Fox (1972) found a significantly lower larval parasitoid emergence from hosts treated with nicotine. Development time of parasitoid larvae from nicotine-treated hornworms was longer, but the difference was not significant. Campbell and Duffey (1979, 1981) provided the most detailed and extensive investigations of the role of an allelochemical, within host tissues, on parasitoid survival and fitness. Campbell and Duffey (1979) showed that tomatine, a major alkaloid of tomato, caused prolonged larval parasitoid development, reduced pupal eclosion, reduced adult size and longevity, and caused morphological abnormalities in *Hyposoter exiguae* (Viereck), a parasitoid of *Heliothis zea* (Boddie) (corn earworm). Campbell and Duffey (1981) suggested that the detrimental influences of tomatine resulted from the possible disruption of sterol metabolism.

Our study attempted to confirm and extend the results of Thurston and Fox (1972). In addition to documenting other biological effects of nicotine, the study was designed to determine if nicotine was present at all three trophic levels and thus confirm the role of nicotine as the causal agent. A comparison was made of the outcome of interactions among a plant allelochemical, an herbivore, and its parasitoid using two herbivores, *Manduca sexta* and *Spodoptera frugiperda* (Smith) (the fall armyworm). The parasitoids of the hornworm and fall armyworm which were evaluated were *Cotesia congregata* and *Hyposoter annulipes*, respectively.

METHODS AND MATERIALS

M. sexta and *S. frugiperda* were reared on synthetic diets (Bio-Serv. Inc., Frenchtown, N.J.) which were either nicotine free or had nicotine incorporated. The concentration of nicotine in the fall armyworm diet (0.025% wet weight)

was $\frac{1}{4}$ that of the tobacco hornworm diet (0.1% wet weight) due to the higher sensitivity of the fall armyworm to nicotine toxicity. These concentrations are at the low end of the range of concentrations normally found in tobacco (Sisson and Saunders, 1983) and cause no significant mortality of host herbivores. To follow the low levels of nicotine incorporated into insect tissues, both diets were supplemented with 100 μCi /liter of generally labeled tritiated nicotine-*d*-bitartrate with specific activity of 1.27 $\mu\text{Ci}/\text{mmol}$ (Amersham Corp Arlington Heights, Illinois).

Newly enclosed tobacco hornworm and fall armyworm larvae, because of their sensitivity to manipulation, were left on nicotine-free diet before being used in experiments. Seven-day-old hornworm larvae (usually second instars) were individually parasitized and placed in 227-cm³ (8-oz.) paper cups. Larvae of this age are the host stage preferred by *C. congregata*. Half the parasitized larvae were placed on nicotine-free diet and the other half on nicotine-containing diet. For *S. frugiperda*, half of a cohort of 3-day-old larvae were transferred to nicotine-containing diet while half were placed on nicotine-free diet. All larvae were parasitized on day 4, which is the preferred host stage of *H. annulipes*. Larvae were placed in 10-cm-diam. plastic Petri dishes with diet in batches of 18/dish and exposed to parasitoid females.

C. congregata is gregarious, and larvae emerge from their hosts and spin cocoons in which they pupate and from which adults emerge. After larval parasitoid emergence, all host larvae were dissected and unemerged parasitoids counted. *H. annulipes*, a solitary parasitoid which consumes all host tissues except for its integument, emerges as a larva and spins a cocoon from which the adult emerges.

Samples of synthetic diet, parasitoid adults, cocoons, and meconia were taken to isolate, identify, and quantify the labeled alkaloid. Samples were frozen, weighed, lyophilized, reweighed, and ground in a mortar and pestle with 40% (v/v) methanol containing 0.1% (v/v) 1 N HCl. The homogenate was centrifuged at 10,000 *g* for 10 min and the supernatant filtered through 0.45 μ nylon 66 membrane filters. The samples were applied to an HPLC chromatographic system with a Waters automatic injector and a UV absorbance monitor with a Waters Model 440 detector. The specific radioactivity of each of the peaks in the eluant was monitored with a Radiomatic Model Flo-one HS radioisotope detector using Flow Scin II cocktail. Nicotine was separated and quantitated on a 10- μ C₁₈ reverse-phase column using a mobile phase at 0.6 ml/min 60% (v/v) methanol containing 0.1% (v/v) phosphoric acid buffered to pH 7.0 with triethylamine. Total radioactivity in each sample was checked by measuring the level of radioactivity in an aliquot of the extract with a Packard Tricarb scintillation counter as described previously (Saunders et al., 1977). Specific quench curves for each type of biological sample were used to quantitate the concentration of labeled compound in tissues.

RESULTS AND DISCUSSION

Nicotine in the tissues of an herbivore can effect the development and survival of its parasitoids. In tobacco hornworms reared on nicotine-containing diets, the proportion of parasitoid larvae that failed to emerge from their hosts was significantly greater than those failing to emerge from hornworms on nicotine-free diets (Table 1). These data confirm the results of Thurston and Fox (1972). Of the parasitoid larvae which emerged from their hosts, a greater proportion failed to form cocoons if they had emerged from nicotine-fed hornworms. No statistically significant differences were demonstrated in pupal mortality, average number of larvae emerging, or the average total number of adults resulting per host (Table 1). Larval and pupal development as well as the size of adult *C. congregata* were unaffected by nicotine (Table 2).

The effects of nicotine on *H. annulipes* were similar to those on *C. congregata* but somewhat more severe on survival and development (Table 3). Of the fall armyworms reared on nicotine-containing diet and exposed to parasitism, only about 46% produced parasitoid larvae compared to almost 76% of the hosts reared on nicotine-free diet. These results are, at least in part, comparable but in sharp contrast to the lack of significant differences in the total number of *C. congregata* produced. Clearly, some of the difference may have been due to fewer nicotine-fed armyworm being parasitized. The extremely small size of 4-day-old larvae and the large size of *H. annulipes* relative to its host made accurate determination of individual parasitization, rather than random contact, impossible. As with *C. congregata*, the ability of *H. annulipes* to

TABLE 1. INFLUENCE OF NICOTINE ON SURVIVAL OF *Cotesia congregata* (= *Apanteles congregatus*) A PARASITOID OF *Manduca sexta*

Treatment	Survival ^a					
	Total No. larvae produced	Larvae failing to emerge (%) ^b	Larvae failing to form cocoons (%) ^b	Pupal Mortality (%) ^b	Total No. larvae emerging	Total No. adults emerging
Nicotine (0.1%)	93.0a	23.3a	19.9a	18.5a	67.7a	42.6a
Control	99.7a	8.1b	6.1b	15.5a	79.6a	54.6a

^a All figures are least-square estimates of means of 54 nicotine-diet and 34 control-diet reared hornworms. Values in columns followed by different letters are significantly different at $P < 0.05$ (SAS, Proc GLM, Type III SS).

^b All percentages were transformed to $\arcsin \sqrt{\%}$ prior to analysis. The values reported are the back-transformed mean percentages.

TABLE 2. INFLUENCE OF NICTONE ON DEVELOPMENT OF *Cotesia congregata* (= *Apanteles congregatus*) A PARASITOID OF *Manduca sexta*

Treatment	Development			Size (dry wt, mg/individual) ^a
	Larval (days) ^b	Pupal (days) ^c	Total (days)	
Nictone (0.1%)	11.9a	6.7a	17.7a	0.224a
Control	11.7a	6.8a	17.5a	0.244a

^aAll figures are least-squares estimates of mean of 54 nicotine-diet and 34 control-diet reared hornworms. Values in columns followed by the same letter are not significantly different (SAS, Proc GLM, Type III SS).

^bTime period between oviposition and cocoon formation.

^cTime period between cocoon formation and adult emergence.

form cocoons is influenced by nicotine. Another important difference between parasitoid species is that while larval development in *C. congregata* is unaffected by nicotine, nicotine exposure prolongs larval development in *H. annulipes*.

Perhaps most important were differences in adult size. *H. annulipes* adults from hosts reared on nicotine-containing diet were significantly smaller than those produced from hosts free of nicotine. These results are in marked contrast to those with *C. congregata* where the slight numerical differences between nicotine and nicotine-free adults were not statistically significant. The decline

TABLE 3. INFLUENCE OF NICOTINE ON SURVIVAL AND DEVELOPMENT OF *Hyposoter annulipes*, A PARASITOID OF *Spodoptera frugiperda*^a

Treatment	Survival			Development			
	Parasitism (%)	Larval failing to form cocoons (%)	% Adults emerging (%)	Larval	Pupal	Size (dry wt, mg/individual)	Sex ratio (% females)
Nicotine (0.025%)	46.4a	12.8a	74.2a	10.4a	6.3a	0.643a	48.4a
Control	75.8b	3.7b	81.3a	8.9b	6.2a	0.787b	41.3a

^aValues in columns with different letters are significantly different at $P < 0.05$.

in adult parasitoid weight associated with the presence of nicotine in hosts may have important negative consequences for *H. annulipes* fecundity and overall fitness. Other developmental parameters were not affected by nicotine. Essentially identical results have been obtained in field experiments using low and high nicotine cultivars in which parallel observations were made (Thorpe and Barbosa, 1986, 1319–1328).

Determination of the presence and quantity of the labeled compound in parasitoid tissues confirms the transfer of a plant allelochemical from the diet of an herbivore to the herbivore and subsequently to its parasitoid (Tables 4 and 5). Campbell and Duffey's (1979) study was the first and this is the second to detect directly a plant allelochemical in the tissues of a parasitoid and simultaneously to demonstrate its deleterious effects.

Tables 4 and 5 show that the parasitoids of both the tobacco hornworm and the fall armyworm may deal with accumulated concentrations of a plant allelochemical by shunting the material to cocoon silk and meconium (waste

TABLE 4. AMOUNT OF LABELED ALKALOID IN TISSUES AND EXCRETORY PRODUCTS OF *Cotesia congregata* EMERGING FROM TOBACCO HORNWORMS (*Manduca sexta*) REARED ON 0.1% NICOTINE DIET^a

	Adult	Cocoon	Meconium
No. of samples	37	37	35
Mean dry wt (mg)/sample	16.72	17.94	3.07
Mean μ g labeled alkaloid/g dry wt	8.18a	126.5c	82.0b

^a Values in rows followed by the same letter are not significantly different. Wilcoxon rank-sum test, $\alpha = 0.048$ (Bonferroni's adjustment for multiple a-posteriori pairwise tests).

TABLE 5. AMOUNT OF LABELED ALKALOID IN TISSUES AND EXCRETORY PRODUCTS OF *Hyposoter annulipes* EMERGING FROM ARMYWORMS (*Spodoptera frugiperda*) REARED ON 0.025% NICOTINE DIET^a

	Adults	Cocoons	Meconia
No. of samples	6	6	7
Mean dry wt (mg)/sample	8.50	2.77	1.67
Mean μ g labeled alkaloid/g dry wt	3.8a	43.5b	36.3b

^a Values in row followed by the same letter are not significantly different. Wilcoxon rank-sum test, $\alpha = 0.048$ (Bonferroni's adjustment for multiple a-posteriori pairwise tests).

products remaining in the cocoon after adult emergence). The highest concentrations of radioactive alkaloid per gram dry weight were observed in the cocoon silk and meconium. Relatively little radioactivity remained in adult tissues. In *C. congregata*, a significantly greater concentration of alkaloid is shunted to the cocoon silk rather than into the meconium. This is an efficient mechanism since cocoon biomass is six times greater than that of meconia. No such efficiency is exhibited in *H. annulipes*. Nicotine may (1) be directly toxic to parasitoid larvae, (2) interfere with parasitoid nutrition, or (3) act indirectly by reducing host size beyond that caused only by parasitism. Host size and nutrition can also affect both parasitoid growth and development (Beckage and Riddiford, 1978, 1983). Regardless of the specific mechanism, the presence of nicotine in parasitoid tissues supports the role of nicotine as the causal factor in the biological changes observed.

In *C. congregata* the only aspects of development which are detrimentally affected are those changes occurring just prior to and just after larval parasitoid emergence. The fact that any detrimental changes were noted is somewhat unexpected since the parasitoid was exposed to very little of the ingested nicotine. Indeed the concentration of nicotine in the diet of the hornworm causes no significant hornworm mortality. Hemolymph is the principal food source of parasitoid larvae. Of the small amount of nicotine that remains in hornworm tissues, the lowest concentrations are found in the hemolymph. Self et al., (1964) demonstrated that the percent recovery of ^{14}C -labeled nicotine in hornworm larvae 4, 15, and 24 hr after ingestion was 5.2%, 4.1%, and 0%, respectively, while after 24 hr, 92.4% of ingested nicotine is recovered in the feces (Self et al., 1964). Both data sets suggest the rapid egestion of nicotine. Six hours after ingestion, the ratio of recovered nicotine in hemolymph, larva, and feces was 1:6:182. Although one might speculate that parasitoids may concentrate blood alkaloids, there is no evidence indicating that parasitoids possess such a capacity, and thus it is most reasonable to assume that the food consumed (blood) contains relatively little alkaloid. A second reason why, a priori, one would not expect any major effect due to nicotine is that the evolution of *C. congregata* is closely associated with that of *M. sexta* and thus closely associated with nicotine. Although *C. congregata* is recorded from other Sphingidae, the relative abundance of other hosts is scarcely comparable to that of *M. sexta*. In addition, whether or not the parasitoids emerging from these other sphingid hosts are actually *C. congregata* is a subject of debate (Krombein et al., 1979).

Hyposoter annulipes, on the other hand, is not routinely exposed to nicotine. It attacks a variety of noctuids which feed on a large number of plant families (i.e., a wide variety of plant allelochemicals). Tobacco (and thus nicotine) is not a prime or frequent host plant of any *H. annulipes* hosts. In the Maryland area, *S. frugiperda* attacks tobacco only when its populations reach relatively high densities on other crops like corn. These factors may, at least in

part, explain the more severe effects of nicotine on this parasitoid compared to those on *C. congregata*. However, since little is known of the metabolism of nicotine by the fall armyworm, intrinsic changes (i.e., metabolic alteration of nicotine) may be responsible for the modification of survival and development in *H. annulipes*.

Finally, data like these may have a significant effect on theories describing insect-plant interactions. Although the role of plant allelochemicals in host-habitat and host-finding has been extensively reviewed (Vinson 1975, 1976, 1977, 1981; Price, 1981; etc.), there has been less emphasis on the effects of plant allelochemicals within the tissues of herbivores on their natural enemies. This study, Campbell and Duffy's (1979, 1981), and others (see Introduction) suggest that the development of ecological theories on plant-insect interactions should consider the role of natural enemies. Issues that might be affected by such consideration include the role of insect herbivory in the evolution of certain forms of plant defense, the relationship between type of defense and growth forms of plants, the protection of "valuable" tissues at the potential expense of less important tissues, etc. We suggest that parasitoids (and other natural enemies) are critical participants in three trophic level interactions and may affect the evolution of plant and herbivore. Thus, theoretical predictions that include parasitoids (or other third-trophic-level organisms) may be quite distinct from those that exclude them from consideration and may be more parsimonious with ecological and evolutionary patterns (see Barbosa and Saunders, 1985; Schultz, 1983).

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