

Influence of dietary nicotine on the fall armyworm, *Spodoptera frugiperda* and its parasitoid, the ichneumonid wasp *Hyposoter annulipes*

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Abstract

Experiments were conducted to determine the influence of nicotine (at a range of concentrations) in the food of an herbivorous host on the development, size and survival of its parasitoid. Fall armyworms, *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) were reared on diets of 0, 0.025, 0.050 and 0.075% nicotine and exposed to parasitism by *Hyposoter annulipes* (Hymenoptera: Ichneumonidae). As nicotine concentration increased parasitoid mortality and development time increased and adult weight decreased. Development time, pupal weight and survival were recorded for unparasitized armyworms. Unparasitized fall armyworms showed lengthened development and higher mortality but pupal weights were greatest at intermediate nicotine concentrations.

Introduction

Price *et al.* (1980) urged that theories attempting to explain the ecological and evolutionary bases of insect herbivory and plant defense should include the role of natural enemies. If allelochemicals can move up the food chain and affect parasitoids, pathogens and predators, then the ability of these enemies to influence herbivore fitness and population growth may in turn be altered. Various aspects of the development and survival of parasitoids can be detrimentally influenced by the presence of alkaloids, like tomatine and nicotine, in the food of their herbivore hosts (Thurston & Fox, 1972; Campbell & Duffey, 1979; Barbosa & Saunders, 1985; Barbosa *et al.*, 1986; and Thorpe & Barbosa, 1986). These and other authors (Bergman & Tingey, 1979) have suggested that biological control strategies, in-

volving the release of parasitoids, may be incompatible with the use of resistant cultivars that contain high levels of toxic allelochemicals.

Previous studies have demonstrated the effects of a single concentration of nicotine, in host diet, on two parasitoid species, *Cotesia congregata* and *Hyposoter annulipes* (Cresson) (Barbosa *et al.*, 1986; Thorpe & Barbosa, 1986). However, no data are available on trends in development and survival of parasitoids, exposed to a range of concentrations of dietary nicotine (i.e., levels found in tobacco plants) or on the influence of these concentrations across generations of the parasitoid. Parasitoid survival and developmental rate may respond non-linearly across a range of concentrations and thus evaluation of only one or two concentrations is insufficient. This study assesses the effects of nicotine, in the diet of the fall armyworm, *Spodoptera frugiperda*

(Smith), on the development and survival of the solitary endoparasitoid, *H. annulipes*, over three generations. The effects of nicotine on the development and survival of unparasitized fall armyworms also were evaluated to compare the effects of nicotine on herbivore hosts with the effects on their parasitoids.

Materials and methods

Effects on unparasitized fall armyworms. Adult fall armyworms were kept in cylindrical screen cages covered with moistened white paper towelling for oviposition. Collected egg sheets and fall armyworm larvae were kept at $27^{\circ}\text{C} \pm 2^{\circ}\text{C}$, a 16:8 h (L:D) regime and $50\% \pm 5\%$ R.H. Neonate larvae were placed singly in plastic 22 ml cups filled with fall armyworm synthetic diet (BioServ., Inc.), enhanced with one of the following concentrations of nicotine: 0.0%, 0.025%, 0.050%, or 0.075%. The nicotine concentrations selected were based on data from preliminary studies and represented concentrations which allowed substantial survival and which were within the typical concentration range found in tobacco. Development time of larvae and pupae were determined by checking individuals daily. Fresh weights of pupae also were measured.

Effects on *Hyposoter annulipes*. Adult wasps were maintained in plastic $25 \times 30 \times 17.5$ cm boxes and kept in a rearing chamber ($22^{\circ}\text{C} \pm 2^{\circ}\text{C}$, 15:9 h (L:D) and $45\% \pm 5\%$ R.H.). Honey was provided as food. Three to five day old fall armyworm larvae (the preferred host stage) were placed in petri dishes on cubes of treated or control diet and then exposed to parasitoid adults within the plastic box. Parasitized larvae were transferred individually to 22 ml plastic cups containing one of four diets. Parasitized larvae were kept at $27^{\circ}\text{C} \pm 2^{\circ}\text{C}$, a 16:8 h (L:D) regime and $50\% \pm 5\%$ R.H. Insects were checked daily and development and survival were recorded to the nearest day. We recorded parasitoid mortality during three developmental stages: (1) 'Larval mortality' was assumed if successful ovipositions were observed but host larvae completed development; (2) 'Prepupal mortality' occurred when parasitoid larvae successfully emerged from the host but failed to form co-

coons; and (3) 'Pupal mortality' was defined as failure to successfully emerge as an adult from the cocoon. When host larvae died during larval development they were excluded from calculations. For each nicotine concentration, emerging parasitoids were used to parasitize fall armyworm larvae reared at the same concentration, thus producing another generation of parasitoids. Adults were kept separate and later, dried and weighed. Data were evaluated by stepwise multiple regression and analysis of covariance (Statistical Analysis System). Percentage data were arcsine transformed before regression analyses.

Results and discussion

Nicotine effects on unparasitized fall armyworms. Nicotine caused slowed development of unparasitized fall armyworms (Fig. 1). ANCOVA's showed no significant sex or sex by nicotine concentration interactions. That is, sex of the armyworm affected neither development time nor the influence of nicotine on development time. Each regression line was therefore drawn to show an average sex effect. For all three dependent variables (Fig. 1) there was a highly significant linear effect of nicotine concentration ($P < 0.0001$). There was also a significant quadratic (i.e., nicotine concentration squared) effect of nicotine on larval and total development times ($P = < 0.0001$, for both larval and total) suggesting that the effects of nicotine on development time levels off at higher nicotine concentrations.

The influence of increasing nicotine concentration on pupal weight is illustrated in Fig. 2. Lines for both females and males are presented because the nicotine by sex interaction was nearly significant ($P = 0.0925$). The model predicting the relationship is highly significant ($P = 0.0038$, $R^2 = 0.18$, $N = 80$) as are the linear ($P = 0.0013$) and quadratic ($P = 0.0050$) terms. Contrary to expectations, maximum pupal weights of this generalist herbivore occurred at moderate rather than at the lowest nicotine concentrations.

One explanation for this relationship (Fig. 2) may be that prolongation of larval development results in greater pupal weights. If, for instance, nicotine disrupts hormone levels so that the onset of pupation

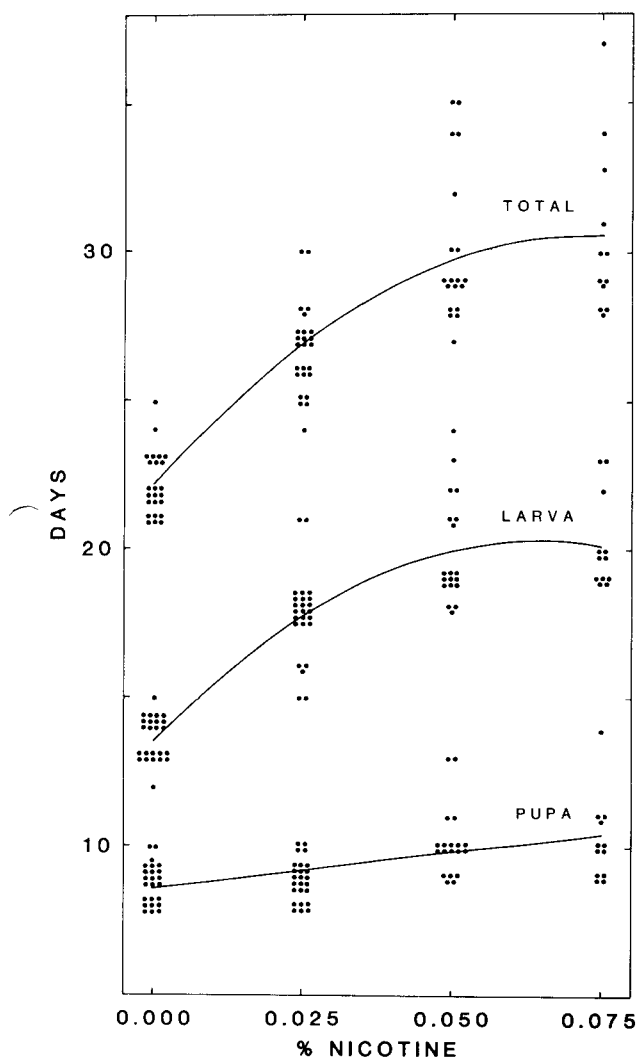


Fig. 1. Relationships between level of dietary nicotine and development times of unparasitized fall armyworms. Total development time (larval + pupal) = $22.15 + 231.24$ (nicotine concentration) - 1578.23 (nicotine concentration)², $N = 80$, $R^2 = 0.76$. Larval development time = $13.53 + 208.90$ (nicotine concentration) - 1605.81 (nicotine concentration)², $N = 80$, $R^2 = 0.81$. Pupal development time = $8.60 + 24.26$ (nicotine concentration), $N = 80$, $R^2 = 0.31$. Intercepts represent an average for the two sexes (see text).

is delayed by a few days, then fall armyworm larvae will be larger at pupation. We examined the influence of larval development time on pupal weight with multiple regression (SAS General Linear Models). In the final model there was a significant nicotine concentration by larval development time interaction ($P = 0.0313$). (Other terms in the model

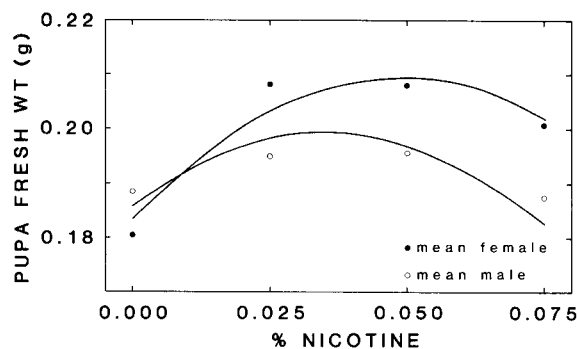


Fig. 2. Relationship of unparasitized fall armyworm pupal weight to level of dietary nicotine ($N = 80$, $R^2 = 0.18$). Male pupal weight = $0.1859 + 0.7569$ (nicotine concentration) - 10.6970 (nicotine concentration)². Female pupal weight = $0.1833 + 1.0564$ (nicotine concentration) - 10.6970 (nicotine concentration)².

were: nicotine concentration ($P = 0.0478$), sex ($P = 0.1962$) and larval development time ($P = 0.0048$). The quadratic effect of nicotine concentration ($P = 0.9817$) had been dropped). This suggests that extended larval development, caused by nicotine in the diet, increases pupal weight more strongly at moderate nicotine concentrations than at higher concentrations. At 0.075% nicotine, toxic effects may outweigh the effects of extended development or nicotine may act as a feeding deterrent.

A second possibility is that pupal weights were greater at moderate nicotine concentrations, not because of increased development time but rather due to direct phagostimulation by intermediate nicotine concentrations. The pattern of maximum consumption at intermediate concentrations was also noted in *Schistocerca gregaria* feeding on diets with one of several non-nutrient chemicals (e.g., non-protein amino acids, sesquiterpenes, phenolics, fatty acids, alkaloids, etc.), at increasing concentrations (Bernays & Chapman, 1978). One of the four patterns presented, which described the relationship between consumption and increasing concentration, was one in which maximal consumption occurred at intermediate concentrations (e.g., with tomatine, an alkaloid also found in the Solanaceae). Bernays & Chapman (1978) also noted that besides alkaloids, many glycosides and non-protein amino acids elicit this type of feeding pattern. Thus, the pattern noted in this study parallels patterns in feeding behavior in

other species and may thus reflect changes in the fall armyworm feeding behavior rather than prolonged development. High concentrations of nicotine may lose the phagostimulatory effect or may be inhibitory. Other herbivores have been shown to respond differentially to a number of allelochemicals depending on their concentration. For example, the dimeric sesquiterpenoid, gossypol, stimulates feeding by the boll weevil. However, at high concentrations it inhibits growth and development (Maxwell *et al.*, 1967).

Table 1 illustrates the influence of increasing nicotine concentration on fall armyworm survival. There is little difference in larval and pupal mortality among larvae reared on control or 0.025% diets. However, mortality increases sharply at higher nicotine concentrations.

Influence on Hyposoter annulipes. The greater the concentration of nicotine in the diet of the fall armyworm hosts, the greater the mortality of *H. annulipes* larvae (Fig. 3). In regression analyses, larval and total (i.e., egg to adult) mortality were positively and linearly related to nicotine concentration ($P = 0.0087$ and 0.0279 , $R^2 = 0.78$ and 0.71 for the full models, respectively, $N = 12$ cohorts). Larval and total mortality were also greater in later generations ($P = 0.0012$ and 0.0039 , respectively), but the nicotine by generation interactions were nonsignificant. Trends in pupal mortality differed in different generations ($p = 0.03$ for generation by percent nicotine and $p = 0.02$ for generation by (percent nicotine)² interactions).

Table 1. Influence of dietary nicotine on stage specific mortality of *Spodoptera frugiperda*, the fall armyworm.

Nicotine concentration (% wet wt)	Mortality(%)			
	Larval	Pre Pupal	Pupal	Total
0.0	0.0	3.7	7.7	1.2
0.025	0.0	3.7	3.8	7.4
0.050	3.7	3.7	24.0	29.6
0.075	37.0	0.0	29.4	55.6

¹ Each mortality represents (the number dying during that stage divided by the number that entered that stage) \times times 100.

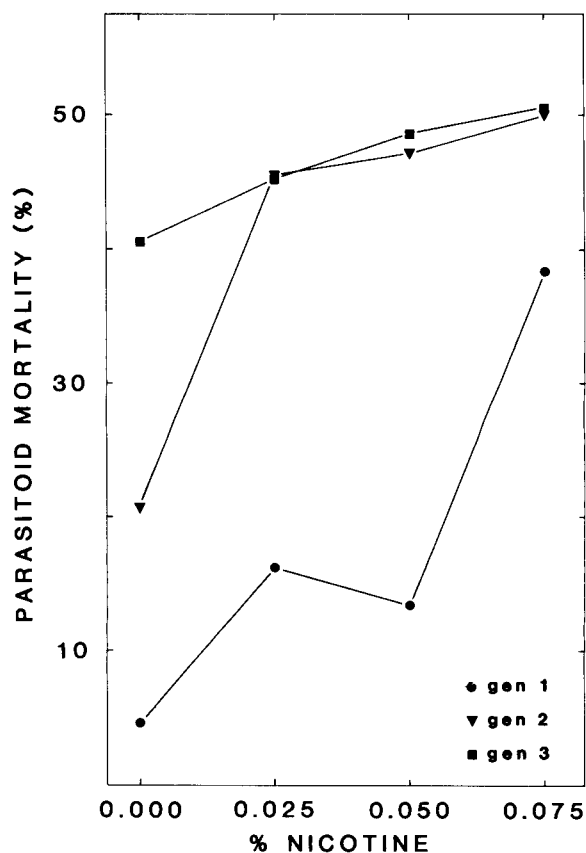


Fig. 3. Influence of nicotine in fall armyworm diet on mortality of *H. annulipes* larvae. Each data point is based on a cohort of 58–105 larvae.

The largest increase in development time of *H. annulipes* was between 0.0 and 0.025% nicotine (Fig. 4). It is of interest that maximal prolongation was roughly similar to that which occurred in unparasitized hosts. In generation one, when both unparasitized fall armyworms and *H. annulipes* were kept under identical conditions, larval development times increased by about 48% and 38%, respectively. Thus, prolongation of development in these parasitoid larvae may have been caused by that of their hosts. Parasitoid size was also adversely affected by nicotine. As dietary nicotine increased, the dry weight of both male and female *H. annulipes* dropped significantly ($P < 0.0001$, $R^2 = 0.50$, $N = 322$, Fig. 5).

Parasitoids of the second and the third generations had greater mortality, longer development and smaller size even among the controls. This may have

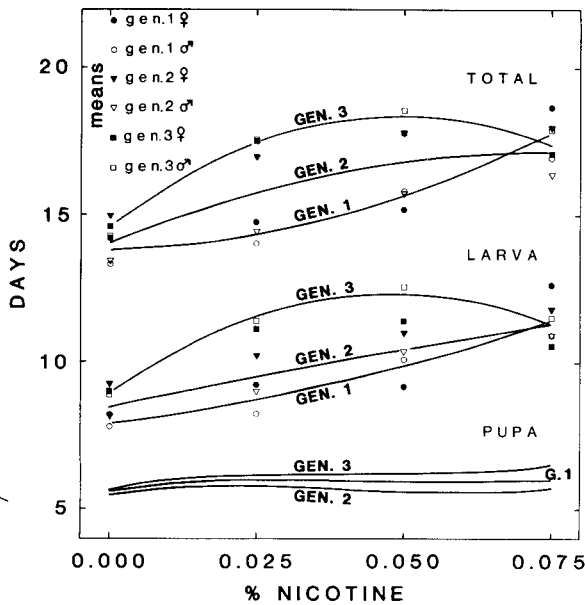


Fig. 4. Relationships between nicotine concentration in fall armyworm diet and development times of *H. annulipes* in three generations. Each regression line is an average for the two sexes. For each dependent variable, the overall regression model included nicotine concentration, generation, sex, and significant two- and three-way interactions. For each of the three models, $N = 450$ and $P < 0.01$. R^2 values were 0.45, 0.41, and 0.19 for total, larval, and pupal development times, respectively.

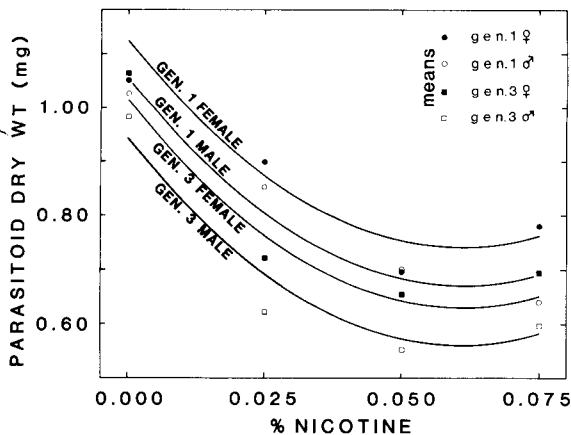


Fig. 5. Regression of percent nicotine in fall armyworm diet on dry weight of adult *H. annulipes* in generations one and three. In the overall regression model ($P < 0.01$), the following terms were significant: sex ($P < 0.01$), generation ($P < 0.01$), and $(\% \text{ nicotine})^2$ ($P < 0.01$). All other two- and three-way interactions were nonsignificant.

been caused by lower nutritional quality of the batches of diet used for generations 2 and 3. It was not possible to use the same batch of diet for all three generations. Even if it had been possible, storage of diet for such prolonged periods, even under refrigeration, would result in severe oxidation and other changes in the diet. Campbell & Duffey (1981), Moore (1983) and Duffey *et al.* (1986) have demonstrated that the effects of toxins can be more severe as the quality of food or the type and quantity of protein changes. Such an interaction between food quality and nicotine may explain the disproportionate toxicity of nicotine in the last two generations.

In summary, increasing nicotine concentration had a consistent negative effect on *H. annulipes* fitness. In general, mortality and development time were greater and adult weight was lower as nicotine concentration increased. Similarly, survival and development of the unparasitized host, the fall armyworm, were detrimentally influenced by increasing concentrations of dietary nicotine.

The concentrations evaluated in our study were far below those known to be detrimental to the relative specialist, *Manduca sexta* (Barbosa, P., P. Grass & J. Kempen, unpubl. data) or its parasitoid, *C. congregata* (Barbosa *et al.*, 1986). This is not surprising since the fall armyworm is a generalist, feeding on several species in the Poaceae and Fabaceae (Hill, 1983) and *H. annulipes* similarly attacks 15 herbivore host species in 12 genera (Krombein *et al.*, 1979). Thus, nicotine is only one of many plant allelochemicals to which these species are exposed.

An unresolved question is whether the effects of nicotine on *H. annulipes* are direct or indirect, i.e., are the deleterious effects observed in this study due to the direct action of nicotine on the parasitoid or due to a decline in host vigor, development, size, etc., brought about by nicotine in the herbivore diet? The overall similarity the shapes of, e.g., the larval development time curves for the fall armyworm and *H. annulipes* suggests (compare Figs. 1 and 4) that changes in the parasitoid simply reflect changes in the host. However, the dissimilarity in weight-gain response between the host and parasitoid (compare Figs. 2 and 5) strongly suggests that the effect of nicotine on this parasitoid is direct.

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Résumé

Influence de la nicotine dans l'alimentation de Spodoptera frugiperda (Noctuidae) et de son parasitoïde, Hyposoter annulipes (Ichneumonidae)

Des quantités croissantes de nicotine dans l'alimentation ont prolongé la durée du développement des chenilles saines, bien que l'effet ait chuté aux concentrations les plus élevées. Le sexe de la noctuelle n'a pas eu d'effet sur la durée du développement ou l'action de la nicotine. L'influence de la nicotine sur le poids des chrysalides est inhabituel, en ce sens que les chrysalides les plus lourdes ont été obtenues aux concentrations moyennes. Des hypothèses sont proposés sur l'origine de ce phénomène. La mortalité et la durée de développement de *H. annulipes* ont augmenté et le poids des adultes a diminué quand la concentration de l'aliment de l'hôte en nicotine s'est élevée. L'effet différent de la nicotine sur des générations successives a pu provenir de modifications de la toxicité de la nicotine en fonction de changements dans la qualité de l'aliment. Quoi qu'il en soit, l'augmentation de la concentration de la nicotine dans l'alimentation de l'hôte a eu un effet négatif cohérent sur la valeur adaptative de *H. annulipes*.

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