Effects of the Allelochemical, α-tomatine, on the Soybean Looper (Lepidoptera: Noctuidae)[1]

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ABSTRACT To determine the impact of the allelochemical, α-tomatine, on the soybean looper, *Pseudoplusia includens* (Walker), various concentrations (0.001 to 0.10% wet weight of diet [wwt]) were added to an artificial diet. Neonate larvae were fed on the diet to determine effects on larval weight, developmental time, mortality, and pupal weight. Longer development times were observed at the higher concentrations (0.05 and 0.10% wwt). Larval mortality at 0.10% α-tomatine was signficantly greater ($P \leq 0.05$) than all other concentrations. Larval weight was decreased by concentrations of α-tomatine above 0.005% wwt. A linear regression ($Y = 217.8 - 2089.5\ X$, $r^2 = 0.90$) described the relationship between larval growth and α-tomatine concentrations. The effective dose to reduce larval weight by 50% was calculated ($ED_{50} = 0.048\%$ wwt) and then validated in a subsequent experiment. In that study, a significant reduction ($P \leq 0.05$) in mean body weight of 10- and 12-day-old larvae was obtained when larvae were fed diet containing the ED_{50} dose of α-tomatine. Percent growth relative to controls ranged from 56.1 to 52.1% at 6 and 12 days, respectively.

KEY WORDS *Pseudoplusia includens, Lycopersicon esculentum,* tomato, α-tomatine, allelochemical, antibiosis, soybean looper.

The soybean looper (SBL), *Pseudoplusia includens* (Walker) is a common defoliating pest of soybean, *Glycine max* L. (Merr.) in much of North, Central, and South America (Turnipseed and Kogan 1976). The SBL attacks a wide range of agricultural crops and noncultivated plants. The host list contains members of 28 plant families, including Solanaceae (Herzog 1980). In that family, the tomato (*Lycoperscion esculentum* Mill.) is listed as a host for the SBL. In a survey done by Canerday and Arant (1967) among common agricultural crops in southern United States, 85% of SBL larvae collected were found on soybeans while 0.4% were collected on tomato. Also, Martin et al. (1976) demonstrated that under intercropping systems that included several crops, the SBL preferred soybeans over tomato.

The fact that the SBL is not commonly found on tomato plants may be partly attributed to detrimental effects of plant allelochemicals on larval growth and development. One such allelochemical, α-tomatine, is a steroid glycoalkaloid that is found in all parts of *Solanum* and *Lycopersicon* plants except in dormant seeds and red fruits (Roddick 1974; Schlosser 1975).

Previous studies (Duffey et al. 1986; Elliger et al. 1981) showed that α-tomatine inhibited the growth of *Heliothis zea* (Boddie) and *Spodoptera exigua* (Hübner)

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larvae. Also, α-tomatine is a feeding deterrent to the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), (Sinden et al. 1978) and a feeding inhibitor of the potato leafhopper, *Empoasca fabae* (Harris), (Dahlman and Hibbs 1967).

Those studies suggest that α-tomatine is an important component in the defense of tomato plants against certain insect pests. This led us to investigate the effect of α-tomatine on the larval growth and development of the SBL.

Materials and Methods

α-Tomatine was obtained from United States Biochemical Corp. (Cleveland, Ohio) and was incorporated into a pinto bean-based diet (Burton 1969) at concentrations ranging from 0.001 to 0.10% wet weight of diet (wwt). The α-tomatine was added to the diet while the agar was blended with the nutrient mix. Diet was dispensed into individual 30-ml plastic cups and allowed to cool to room temperature. Neonate larvae of the SBL obtained from laboratory-reared colonies (USDA-ARS, Stoneville, Mississippi) were placed individually into the cups and maintained at $27 \pm 1°$ C, $50 \pm 10\%$ RH and LD 14 : 10.

Ten replicates per concentration were used with ten neonates per replicate. Control diets contained no α-tomatine. Data on larval weight at 10 days after eclosion, larval developmental time, pupal weight, and larval percent mortality were recorded.

Tests for normality of the data were performed. In cases where the data did not follow a normal distribution, the data were subjected to log transformation prior to analysis. Data on percentages were subjected to arc-sin transformation for performing the analysis. If the ANOVA was significant, Duncan's Multiple Range test (SAS Institute 1985) was used to discriminate significant differences ($\alpha = 0.05$) among treatment means.

Larval weight at 10 days after egg hatch was subjected to linear and non-linear regression analysis to determine the best-fitting equation to describe the relationship between larval weight and α-tomatine concentration. The effective dose to decrease larval weight by 50% (ED_{50}) relative to controls was calculated from the regression equation.

A second experiment was conducted to determine the validity of the ED_{50} previously calculated. The same procedures used in the first experiment were followed. Four replicates per treatment were used with 20 larvae per replicate. A control (devoid of α-tomatine) and the ED_{50} dose (0.048% wwt) treatment were included. Data on larval weight at 6, 8, 10 and 12 days after egg hatch, larval developmental time, larval mortality and pupal weight were recorded. Body weight gain expressed as percent relative growth (RG) (% Relative growth = [α-tomatine weight/control weight]*100) was calculated for the ED_{50} concentration. Data were tested for normality and if the data did not follow a normal distribution, transformations were performed as described earlier. The repeated measures analysis of variance of the general linear model (SAS Institute 1985) was used to analyze the larval weight data. Paired t-tests were used to identify significant differences ($\alpha = 0.05$) in larval weights between the α-tomatine and control treatment for different days. Also, larval weights on different days were subjected to linear regression analysis (SAS Institute 1985) to compare the linear equations obtained for the α-tomatine (ED_{50}) and the control treatment. Data on larval developmental time, larval mortality, pupal weight and relative growth were analyzed by the general linear model (SAS Institute 1985), and paired t-test was used to discriminate significant differences ($\alpha = 0.05$) among treatment means.

Results and Discussion

Mean body weight of 10-day-old larvae fed diet containing 0.005% wwt or more of α-tomatine differed significantly ($F = 106.31$; df = 5, 462; $P \leq 0.05$) from that of control larvae (Table 1). In addition to effects on larval growth and development, α-tomatine at 0.10% wwt caused a significant ($F = 6.75$; df = 5, 48; $P \leq 0.05$) increase in larval mortality (Table 1).

Larval developmental times in the 0.05 and 0.10% wwt treatments were significantly ($F = 29.91$; df = 5, 224; $P \leq 0.05$) increased when compared with the control (Table 1). Presumably, this increase in development time (2.1 and 4.1 day extension compared with the control in the 0.5 and 0.10% treatments, respectively) allowed larvae to attain weight which resulted in pupal weights similar to that of the control (Table 1). Also, in the 0.10% treatment, significantly higher ($F = 8.49$; df = 5, 54; $P \leq 0.05$) mortality (48%) of the larvae occurred between day 10 and pupation, leaving only the healthy or larger larvae to pupate. However, pupal weight obtained with 0.05% wwt of α-tomatine was significantly higher ($F = 6.66$; df = 5, 339; $P \leq 0.05$) when compared with the control (Table 1).

Mean larval weights in the α-tomatine treatments ranged from 28.7 to 214.5 mg. (Table 1). Regression analyses indicated a linear relationship ($r^2 = 0.90$) between larval weight and concentration of α-tomatine. The general linear equation obtained was: $Y = 217.8 - 2089.5\ X$, from which the ED_{50} (0.048 % α-tomatine) was calculated (Fig. 1).

In the validation experiment, the interaction of day*treatment was significant ($F = 14.25$; df = 2, 149; $P \leq 0.05$) and the day effect in larval weight was highly significant ($F = 198.66$; df = 2, 149; $P \leq 0.01$), indicating that through time the ED_{50} dose of α-tomatine was decreasing the larval weight compared with the control. A comparison of the linear equation obtained for the α-tomatine ED_{50} and the control treatment indicated that the ED_{50} dose decreased the growth rate of the larvae by 50% (Fig. 2). There was a significant reduction ($P \leq 0.05$) in mean body weight in 10- and 12-day-old larvae fed diet containing α-tomatine when compared with larvae fed the control diet (Table 2). Also, a significant increase ($F = 28.40$; df = 1, 6; $P \leq 0.05$) in total larval mortality was observed in the α-tomatine (45%) treatment when compared with the control (5%). No significant differences ($P > 0.05$) were observed in larval weights between the treatment and control at 6 and 8 days (Table 2). Also, no significant ($P > 0.05$) differences were obtained in larval developmental time and pupal weight between the a-tomatine treatment and control. No significant differences ($P > 0.05$) were obtained in the percent relative growth observed on the different evaluation days. However, percent RG in the α-tomatine treatment ranged from 56.1 to 52.1% at 6 and 12 days, respectively.

The ED_{50} of α-tomatine reduced the SBL larval weight by approximately 50% compared to the control. In addition, it was demonstrated that α-tomatine, when added to artificial diet at 0.05 and 0.10% (0.5 and 1 μmoles/g wwt, respectively), resulted in detrimental effects on the growth and development of the SBL. Also, α-tomatine at 0.05 and 0.10% increased the mortality of the larvae. The average of α-tomatine level in tomato foliage is 1 μmole/g wwt, and it ranges from 0.5 to 10

Table 1. Effects of α-tomatine on growth, development, and larval mortality of *Pseudoplusia includens*.

	Mean weight (mg) †		Larval development	
α-tomatine*	10-d-old larvae	pupae	time (days) †	Mortality (%) ‡
control	234.2 ± 5.4 a	221.7 ± 3.1 b	12.6 ± 0.2 c	8.8 ± 2.0 b
0.001	214.5 ± 8.1 a b	225.0 ± 2.9 b	12.9 ± 0.2 c	8.8 ± 2.6 b
0.005	192.7 ± 8.8 b	223.6 ± 3.2 b	13.3 ± 0.2 c	17.7 ± 2.2 b
0.010	210.1 ± 8.2 b	230.6 ± 3.0 b	13.0 ± 0.2 c	15.5 ± 4.1 b
0.050	115.4 ± 6.1 c	246.3 ± 4.0 a	14.7 ± 0.2 b	14.4 ± 2.4 b
0.100	28.7 ± 8.8 d	230.7 ± 5.5 b	16.7 ± 0.3 a	32.2 ± 5.2 a

* Concentration, % wet weight of diet.

† Means ± SE followed by same letter within columns are not significantly different ($P > 0.05$, Duncan's Multiple Range test [SAS Institute, 1985]).

‡ Larval mortality at 10 days. Means ± SE followed by the same letter are not significantly different ($P > 0.05$, Duncan's Multiple Range test [SAS Institute, 1985]).

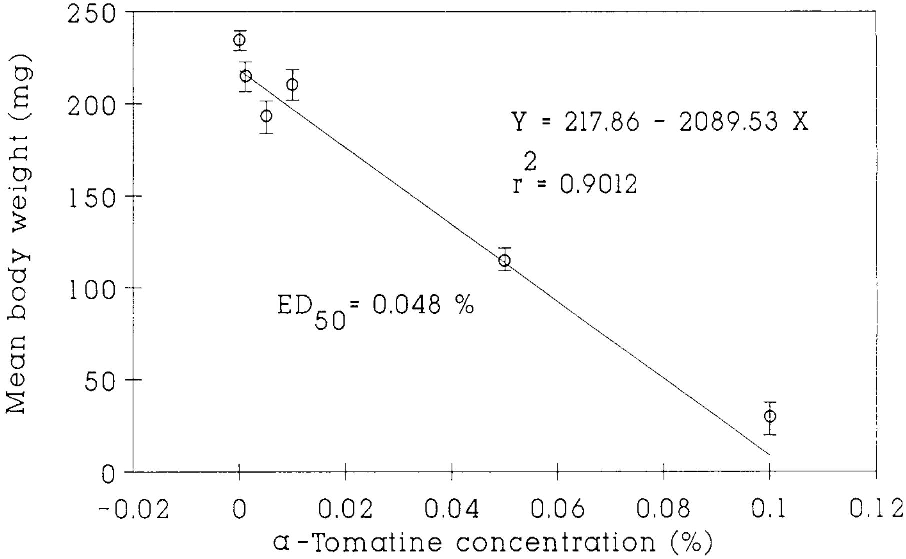

Fig. 1. Linear regression for α-tomatine concentration effects on *Pseudoplusia includens* larvae (10-day-old) weight. Vertical lines represent ± SEM.

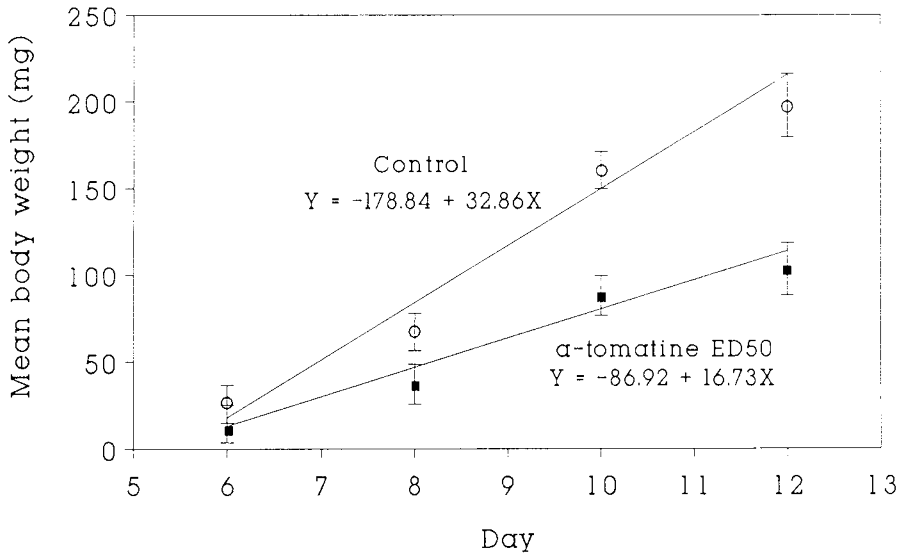

Fig. 2. Linear regression for the effects of α-tomatine ED_{50} dose and control on *Pseudoplusia includens* larvae weight at different days after egg hatch. Vertical lines represent ± SEM.

Table 2. Effects of α-tomatine ED_{50} dose on *Pseudoplusia includens* larval weight at different days.

Larval Age	Mean weight (mg)*		Relative
days †	α-tomatine	control	Growth ‡
6	14.6 ± 10.8 a	26.0 ± 10.8 a	0.56 a
8	37.3 ± 11.4 a	67.2 ± 10.8 a	0.56 a
10	88.0 ± 11.4 b	160.5 ± 10.8 a	0.55 a
12	103.2 ± 15.2 b	197.8 ± 18.2 a	0.52 a
Pupae	160.6 ± 16.2 a	180.4 ± 10.5 a	

* Means ± SE within rows followed by the same letter are not significantly different ($P > 0.05$, Paired t-test [SAS Institute, 1985]).

† After egg hatch.

‡ Larval Relative Growth = α-tomatine weight/control weight.

μmoles/g wwt (Duffey et al. 1986). Thus, these results suggest that under natural allelochemical concentrations the SBL is able to feed on tomato plants but further development would be affected. The latter may be one reason why the SBL is not commonly found on tomatoes.

Antibiosis is often manifested in decreased body weight, total weight gain, pupal weight, and larval survival with a concomitant increase in developmental period (Kogan 1975). With the exception of decreased pupal weights, all these conditions were observed during this bioassay. Because α-tomatine is an effective growth reducer in artificial diet at levels commensurate with that in plants, its role as a defense against the SBL warrants further investigation. Also, α-tomatine occurs in tomato plants along with other compounds that may antagonize or synergize its toxic properties to the SBL. Because of the latter, further studies are needed to understand the effects of other tomato plant allelochemicals such as chlorogenic acid and rutin (Isman and Duffey 1982) on the SBL.

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