Effect of α-Tomatine and Tomatidine on the Growth and Development of the Colorado Potato Beetle (Coleoptera: Chrysomelidae): Studies Using Synthetic Diets[1]

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Abstract Glycoalkaloids are found throughout the genera *Solanum* (potato) and *Lycopersicon* (tomato). Certain glycoalkaloids, i.e., α-tomatine, solanocardenine, and leptine, have been implicated as resistance factors to the Colorado potato beetle, *Leptinotarsa decemlineata* Say. The allelochemical properties of these glycoalkaloids have primarily been demonstrated by studies *in planta,* correlating Colorado potato beetle resistance with high levels of foliar glycoalkaloids: solanocardenine in *S. neocardenasii,* α-tomatine in *S. pinnatisectum,* and leptine in *S. chacoense.* Although the evidence that these glycoalkaloids mediate resistance is compelling, controlled analyses of Colorado potato beetle response to purified glycoalkaloids, fed to insects in synthetic diets, are necessary to characterize the allelochemic nature of these compounds. In this study, Colorado potato beetle reared on a meridic, synthetic diet supplemented with increasing concentrations of α-tomatine exhibit retarded growth and delayed development. These effects were evident throughout the insects' development, from egg to prepupal stage. Tomatidine (the aglycone of α-tomatine) has no effect on Colorado potato beetle, suggesting that the tetrasaccharide moiety of the glycoalkaloid is essential for insecticidal activity, consistent with a membrane-lytic mechanism of action.

Key Words Glycoalkaloids, *Solanum tuberosum, Lycopersicon esculentum,* synthetic diet, host-plant resistance, allelochemical

The Colorado potato beetle, *Leptinotarsa decemlineata* Say, is the most serious insect pest of the cultivated potato, *Solanum tuberosum* L., in eastern North America, and a major pest worldwide (Hare 1990). The economic impact of uncontrolled infestations can be substantial, with entire fields being defoliated. The Colorado potato beetle is highly adaptable, rapidly evolving in response to changing biotic and abiotic conditions (Hare 1990). It has experienced a steady host expansion since the mid-Nineteenth Century, from its original host plant, buffalo burr (*S. rostratum* Dun.) to *S. tuberosum* and eggplant (*S. melongena* L.), as well as approximately 18 other species (Jacques 1991). The tomato, *Lycopersicon esculentum* Mill., is a marginal host for the Colorado potato beetle, and there is considerable variability in the ability of Colorado potato beetle to utilize it as a suitable food source; whereas, some regional populations seem to be well-adapted to tomato, while others perform very poorly.

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However, there is evidence that relatively non-adapted populations have the genetic potential to develop the ability to thrive on tomato (Lu et al. 1997).

Glycoalkaloids are a class of steroid glycosides found throughout the genera *Solanum* and *Lycopersicon* (Tingey 1984). The glycoalkaloids found in *S. rostratum* (the native host plant of the Colorado potato beetle) are solasonine and solamargine (Schreiber 1968). The major glycoalkaloids found in the cultivated potato are solanine and chaconine (Sanford et al. 1994). Fraenkel (1959) noted that neither solasonine, solamargine, solanine nor chaconine had inhibitory effects against the Colorado potato beetle. These observations were subsequently repeated (Flanders et al. 1992) and recently confirmed (Yencho et al. 1996, 1998, Kowalski et al. 1997).

In the cultivated tomato and in some species of wild potato, e.g., *S. pinnatisectum* Dunl., *S. neocardenasii* Hawkes, and *S. demissum* Lindl., α-tomatine (Fig. 1) is the predominant glycoalkaloid (Schreiber 1968, Sinden et al. 1991). Sinden et al. (1991) proposed α-tomatine as a resistance factor to the Colorado potato beetle in *S. pinnatisectum* and *S. neocardenassi.* Studies conducted *in planta* (Sinden et al. 1978, Flanders et al. 1992) have implicated α-tomatine as active against the Colorado potato beetle in the cultivated tomato (*L. esculentum*), the wild tomato species (*L. hirsutum* Humb.), and several species of potato. However, in another study, differences in dietetic indices for Colorado potato beetle larvae fed on *L. esculentum* cv. 'Walter' or *L. hirsutum* f. *glabrutum* could not be attributed to variation in foliar α-tomatine concentrations (Barbour and Kennedy 1991). Studies wherein Colorado potato beetle were fed potato leaf discs infiltrated with α-tomatine, at concentrations occurring in tomato foliage (e.g., 68 to 520 mg per 100 gram fresh weight) have implicated

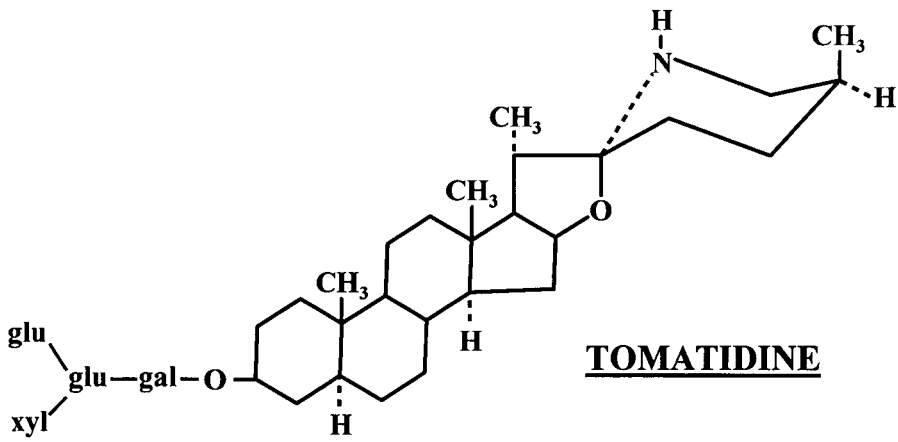

Fig. 1. Structure of the glycoalkaloid α-tomatine. The aglycone portion of the molecule is the steroidal alkaloid tomatidine. The lycotetraose polysaccharide moiety is made up of four sugar residues: gal = D-galactose, glu = D-glucose, xyl = D-xylose.

α-tomatine as active against the Colorado potato beetle (Buhr et al. 1958, Roddick 1974, Sinden et al. 1978, Sinden et al. 1991). Similarly, fourth-instar Colorado potato beetle larvae fed artificial diets supplemented with α-tomatine, at concentrations approximating those occurring in tomato foliage, exhibited significant reduction in fitness, as compared to larvae fed control diets (Hsiao and Fraenkel 1968, Hare 1987).

Although most of the research cited above has strongly suggested that α-tomatine is active against the Colorado potato beetle, there has not yet been a systematic study to examine the effect of α-tomatine as a single factor against the Colorado potato beetle. Therefore, to determine the precise effect which α-tomatine has on the Colorado potato beetle, it would be informative to conduct controlled, dose-response experiments, *ex planta,* throughout the entire development cycle of the insect. In this study, a meridic diet for the Colorado potato beetle was used to analyze the response of the Colorado potato beetle (from egg to pupating fourth stadium) to the glycoalkaloid α-tomatine and the aglycone tomatidine. Results presented herein provide a greater understanding of their impact on Colorado potato beetle development and biology.

Materials and Methods

Chemicals and compounds. Meridic diets were prepared by the method of Domek et al. (1997). Colorado potato beetle diet constituents were obtained from commercial sources and were always obtained from the same sources to ensure reproducibly consistent preparations of the Colorado potato beetle diet. The glycoalkaloid α-tomatine and the aglycone tomatidine were purchased from Sigma Chemical Co. (St. Louis, MO). α-Tomatine and tomatidine were mixed into the diet, to an even suspension, using an IKA ultra-turrax dispersing tool (Janke and Kunkel Gmbh and Co. KG, Staufen, Germany). The concentrations of α-tomatine used in this study, i.e., 1.0, 3.0 and 5.0 mM, can also be represented as 100, 300 and 500 mg/100 gram diet, respectively.

Colorado potato beetle colony. The colony was maintained at 23°C, with 16 h day and 8 h night schedules, in a Normco-Cascade controlled environment chamber. *Solanum tuberosum,* var. 'Kennebec,' was the food source for the Colorado potato beetle colony. Plants were changed weekly, watered and fertilized as needed. In order to maintain vigor and genetic heterozygosity within the colony, Colorado potato beetle were collected from the field and introduced into the colony during the spring and summer months. Egg masses (approximately 40 eggs per mass) were collected weekly and stored for 3 to 5 d at 10°C.

Colorado potato beetle diet study/experimental design. Colorado potato beetle eggs (from 4, 40-egg, egg masses) were surface sterilized by rinsing in 2% v/v bleach in de-ionized water and placed on pre-cut cubes (approximately 0.5 cm^3) of diet in sterile, 96-well plates. As the larvae grew and progressed through their developmental stadia, they were transferred to 24-well plates (second and third instars), and then to 12-well plates (fourth instars) with correspondingly greater portions of diet. The experiment was conducted under aseptic conditions. Each experimental treatment contained 24 eggs, consisting of four sets of six eggs derived from four egg masses, oviposited by four female Colorado potato beetles. Eggs from each female were divided equally among all treatments to account for variability in progeny among female Colorado potato beetles. Therefore, for example, in an experiment with five treatments, e.g., control, three concentrations of α-tomatine and one concentration of

tomatidine, four, 40-egg egg masses were equally divided among the treatments, such that each treatment had a uniform array of 24 eggs, with 6 eggs contributed from each of the four females, i.e., 4 (females) × 6 (eggs from each individual female) = 24 (eggs per each treatment). Development, from egg to prepupal stage, typically took 15 d in the control treatments. Diet was changed daily. Weights of individual Colorado potato beetle larvae were recorded every 2 d, starting on the sixth day after egg hatch. Observations of molting and survivorship were recorded daily.

Statistical analyses. Analysis of the effect of α-tomatine, at various concentrations (Table 1) on Colorado potato beetle growth and development was performed using PROC MIXED (SAS Institute 1996). Data were analyzed as a two-factor general, linear-repeated measures model. Treatment and day were fixed effects with the dependent variable measured over day. Means were compared using pair-wise contrasts. Statistical significance was tested at the 0.5 probability level. Please note that data presented in Tables 1 and 2 are from the same experiment. This was a single experiment, starting with 24 eggs per treatment.

Analysis comparing the effects of tomatidine and α-tomatine on Colorado potato beetle growth and development (Table 3) was performed using PROC MIXED (SAS Institute 1996). Data were analyzed as a two-factor, general linear-repeated measures mixed model with the weight being measured over day. Experiment was the random factor. The natural log (ln) transformation was used to correct for variance heterogeneity. Means were compared using pairwise contrasts. Statistical significance was tested at the 0.05 probability level. Data from two separate, replicated, experiments were combined in the analysis; that is, each of the two experiments was started with 24 eggs per treatment. Please note, data presented in Tables 3 and 4 are from these two combined data sets.

Results

Effect of α-tomatine on Colorado potato beetle. Colorado potato beetle larvae exhibited a dose-dependent response to increasing concentrations of α-tomatine in the artificial diet. As the concentration of α-tomatine increased, mean larval weights

Table 1. Daily mean weights (in mg) of Colorado potato beetle larvae reared on artificial diet supplemented with increasing concentrations of the glycoalkaloid α-tomatine

Days after eclosion	Concentration of α-tomatine in diet (mM)			
	0	1.0	3.0	5.0
6	18a	16a	9b	6b
8	45a	41a	15b	11b
10	108a	91a	26b	17b
12	148a	132a	49b	28c
14	146a	137a	71b	46c

Treatment within day means (compared using pair-wise contrasts) with different letters are different at the $P \leq 0.05$ significance level.

Table 2. Effect of α-tomatine on the development of Colorado potato beetle larvae: percent of individuals in a treatment which have molted into designated instars (second, third and fourth), as a function of [α-tomatine] and time

Days after eclosion	Control diet (no α-tomatine)		1 mM α-tomatine		3 mM α-tomatine		5 mM α-tomatine	
6	2nd instar	17%	2nd instar	30%	2nd instar	71%	2nd instar	75%
	3rd instar	83%	3rd instar	70%	3rd instar	29%	3rd instar	25%
	4th instar	0%	4th instar	0%	4th instar	0%	4th instar	0%
	*n = 23		n = 23		n = 24		n = 24	
8	2nd instar	0%	2nd instar	0%	2nd instar	21%	2nd instar	33%
	3rd instar	39%	3rd instar	57%	3rd instar	58%	3rd instar	67%
	4th instar	61%	4th instar	43%	4th instar	21%	4th instar	0%
	n = 23		n = 23		n = 24		n = 24	
10	2nd instar	0%	2nd instar	0%	2nd instar	17%	2nd instar	22%
	3rd instar	0%	3rd instar	4%	3rd instar	48%	3rd instar	56%
	4th instar	100%	4th instar	96%	4th instar	35%	4th instar	22%
	n = 23		n = 23		n = 23		n = 23	
12	2nd instar	0%	2nd instar	0%	2nd instar	13%	2nd instar	4%
	3rd instar	0%	3rd instar	0%	3rd instar	30%	3rd instar	53%
	4th instar	100%	4th instar	100%	4th instar	57%	4th instar	43%
	n = 23		n = 23		n = 23		n = 23	
14	2nd instar	0%	2nd instar	0%	2nd instar	9%	2nd instar	5%
	3rd instar	0%	3rd instar	0%	3rd instar	9%	3rd instar	27%
	4th instar	100%	4th instar	100%	4th instar	82%	4th instar	68%
	n = 23		n = 23		n = 22		n = 22	

* Note: the designation "n" indicates the number of individuals in an experimental treatment which were still alive.

decreased (Tables 1, 3). This trend was evident at all three concentrations tested, and was statistically significant at the 3.0 and 5.0 mM concentrations (Table 1). Larval development also was delayed, but not arrested, as the concentration of α-tomatine increased (Table 2). Even at the highest concentration of α-tomatine used in this study (5.0 mM), there was not a decrease in survivorship of the Colorado potato beetle (Table 2). However, it should be noted that the experiment was not specifically designed to screen for mortality beyond 16 d post-eclosion, and, therefore, must be considered as highly right-censored.

Effect of tomatidine on Colorado potato beetle. Colorado potato beetle larvae reared on diet supplemented with tomatidine (the aglycone of α-tomatine), at 5.0 mM concentration, performed as well as Colorado potato beetle on control diet; mean

Table 3. Daily mean weights (mg) of Colorado potato beetles larvae reared on artificial diet supplemented with increasing concentrations of the glycoalkaloid α-tomatine and the aglycone tomatidine

Days after eclosion	Concentration of α-tomatine in diet (mM)				Tomatidine 5.0 mM
	0	1.0	3.0	5.0	
6	14a	13a	9b	6c	12a
8	33a	27a	18b	10c	36a
10	76a	52b	33c	14d	72a
12	105a	86a	50b	21c	112a
14	101a	87a	62b	26c	117a

Treatment within day means (compared using pair-wise contrasts) with different letters are different at the $P \leq 0.05$ significance level.

larval weights between the two treatments were not statistically different (Table 3). Additionally, as in the control diet, Colorado potato beetle reared on diet supplemented with tomatidine displayed significantly better performance, i.e., weight gain, than Colorado potato beetle reared on diet supplemented with α-tomatine (Table 3). Larval development also was neither delayed nor arrested in the treatment supplemented with tomatidine (Table 4).

Discussion

In the present study, α-tomatine had a negative, dose-dependent effect on growth and development of Colorado potato beetle, from egg to adult (Tables 1, 3). α-Tomatine did not, however, affect Colorado potato beetle survivorship (Table 2). The delay in development may affect overall fitness, however. Concentrations of α-tomatine used were close to those found in tomato and several wild species of potato, and are therefore physiologically relevant (Bloem et al. 1989, Sinden et al. 1991, Felton et al. 1992).

The mode of action of α-tomatine against the Colorado potato beetle has been the focus of several investigations. The Colorado potato beetle has not evolved a receptor specific for α-tomatine (Mitchell and Harrison 1985). However, α-tomatine will elicit bursting activity in chemosensilla of adult Colorado potato beetle, rendering amino acid receptors (but not sucrose receptors) unresponsive (Mitchell 1987). α-Tomatine may, therefore, depolarize nerve membranes (Mitchell and Harrison 1985). Although this might be expected to produce an antixenotic effect, it apparently does not. Harrison (1987) noted that "at ecologically high levels, alkaloids from *S. tuberosum* and *L. esculentum* do not influence the acceptance of food plants by adult beetles." The effect of α-tomatine on the Colorado potato beetle may be long term (chronic) due to a "postingestive accumulation of tomatine affecting general activity" (Harrison and Mitchell 1988).

Several possible mechanisms could explain the effect of α-tomatine against the Colorado potato beetle. α-Tomatine has been shown to act as a noncompetitive inhibitor of acetylcholinesterase purified from the Colorado potato beetle. However, it

Table 4. Effect of the aglycone tomatidine on the development of Colorado potato beetle larvae: percent of individuals in a treatment which have molted into designated instars (second, third and fourth), as a function of treatment and time

Days after eclosion	Control		5.0 mM tomatidine	
6	2nd instar	19%	2nd instar	31%
	3rd instar	81%	3rd instar	69%
	4th instar	0%	4th instar	0%
	*n = 37		n = 39	
8	2nd instar	3%	2nd instar	0%
	3rd instar	36%	3rd instar	42%
	4th instar	61%	4th instar	58%
	n = 36		n = 38	
10	2nd instar	0%	2nd instar	0%
	3rd instar	9%	3rd instar	0%
	4th instar	91%	4th instar	100%
	n = 34		n = 38	
12	2nd instar	0%	2nd instar	0%
	3rd instar	3%	3rd instar	0%
	4th instar	97%	4th instar	100%
	n = 33		n = 38	
14	2nd instar	0%	2nd instar	0%
	3rd instar	0%	3rd instar	0%
	4th instar	100%	4th instar	100%
	n = 33		n = 38	

* Note: the designation "n" indicates the number of individuals in an experimental treatment which were still alive.

is a relatively poor inhibitor, with an inhibition constant (Ki) of 4.9×10^{-4} (Zhu and Clark 1995, Zhu et al. 1996). α-Tomatine will form insoluble and biologically-inactive complexes with some β-sterols, thereby rendering these essential nutrients unavailable to the insect (Dhillon 1986). α-Tomatine's complexing with membrane sterols, e.g., cholesterol, is a possible mechanism of toxicity (Roddick 1974). The strong interaction of α-tomatine with cholesterol in membranes, combined with its surfactant properties, will induce a general disruption of membrane integrity, viz. permeabilization and lysis (Keukens et al. 1995). The polysaccharide moiety of α-tomatine is essential for membrane disruption to occur.

In this study, tomatidine (the aglycone of α-tomatine) did not have a significant effect on Colorado potato beetle growth and development (Tables 3,4). Hence, the

glycoalkaloid α-tomatine, and not the aglycone tomatidine, appears to be the biologically active moiety against the Colorado potato beetle, suggesting that the tetrasaccharide component of α-tomatine is essential for biological activity. Therefore, the mechanism of action of α-tomatine against the Colorado potato beetle could be membrane lysis (Keukens et al. 1995). This is consistent with α-tomatine (tomatidine linked to tetrasaccharide) exhibiting greater inhibitory action against the Colorado potato beetle than β-tomatine (tomatidine linked to trisaccharide) (Fraenkel 1959). Similarly α-tomatine, but not tomatidine, inhibits growth of the closely-related "false Colorado potato beetle", *Leptinotarsa juncta* Germar (Kearns 1985), the red flour beetle, *Tribolium castaneum* Herbst (Weissenberg et al. 1998), and the potato leafhopper, *Empoasca fabae* Harris (Dahlman and Hibbs 1967). Sanford et al. (1996) reported that α-tomatine elicited significant mortality to the potato leafhopper when fed in artificial diets. α-Tomatine has also been shown to be toxic to other insects, e.g., *Helicoverpa zea* Boddie (Bloem et al. 1989, Juvik and Stevens 1982, Stamp and Yang 1996), *Spodoptera exigua* Hübner (Bloem et al. 1989), and *S. littoralis* Boisd. (Dhillon 1986).

α-Tomatine fed to the Colorado potato beetle will most likely be absorbed as the intact glycoalkaloid, and not a hydrolysis product (tomatidine). Although acid will hydrolyze α-tomatine, to yield the aglycone tomatidine and the monosaccharides D-glucose, D-galactose and D-xylose (Glasby 1976), hydrolysis occurs quantitatively at relatively low pH levels (pH 1-2), elevated temperatures (65 degrees C), and in concentrated methanolic solutions (95-98%) (Friedman and McDonald 1995). The pH of the midgut of the Colorado potato beetle is between 5.5 and 6.6 (Felton et al. 1992), and the digestive tract and hemolymph of the Colorado potato beetle is an aqueous-based environment. At this pH, in an aqueous-based solution, and at temperatures which are conducive to Colorado potato beetle growth and development, i.e., below 40° C, little, if any, α-tomatine ingested by the Colorado potato beetle will be hydrolyzed to tomatidine.

Whereas, Sinden et al. (1978) had correlated increasing α-tomatine concentrations with decreased performance of the Colorado potato beetle, Barbour and Kennedy (1991) could not detect a significant relationship between α-tomatine concentrations and Colorado potato beetle performance. These findings, however, are not necessarily contradictory. Variability in the complex array of compounds within plants, and their potential interactions, could partially explain this. Additionally, variability in Colorado potato beetle populations is another, at least partial, explanation (see Lu et al. 1997). α-Tomatine, even at high concentrations, did not affect survivorship in the Colorado potato beetle (Table 2). Also, α-tomatine's effects may vary with different stages of insect development, e.g., we observed that neonates feeding on diet supplemented with high concentrations of α-tomatine displayed agitated, restless behavior, and did not readily settle onto the diet. However, later instars settled and fed on the high α-tomatine diet, without obvious agitation. Potato leafhopper nymphs exhibit similar agitation and restlessness when reared on diet supplemented with high concentrations of α-tomatine; this behavior may be caused by the effect of α-tomatine on the insect's nervous system (Dahlman and Hibbs 1967). Digitonin, a compound which will disrupt membranes, produces a response similar to α-tomatine when applied to chemosensilla of Colorado potato beetle (Mitchell and Harrison 1985). α-Tomatine may, therefore, cause a generalized disruption of membranes and depolarization of neurons.

In planta studies correlating levels of α-tomatine with Colorado potato beetle

growth, development and performance, whether with whole plants, leaf-disk assays or α-tomatine-infiltrated potato leaf disks, albeit compelling, always contain the variable of other unidentified secondary compounds, of unknown concentration and interactive effect (Berenbaum 1985, Ananthakrishnan 1997). Temperature (Stamp and Yang 1996) and the nutritional status of the plant (Hare 1987) can also ameliorate α-tomatine's effect on insect performance (Panda and Khush 1995). Although previous *ex planta* diet studies had shown α-tomatine to inhibit Colorado potato beetle growth (Hare 1983, Hare 1987, Hsiao 1974, Hsiao and Fraenkel 1968), these were conducted solely with fourth-instar larvae and, therefore, are not representative of the insect's cycle of development; fourth-instar larvae are voracious feeders, and will readily accept foods rejected by neonates (Kearns 1985). The meridic Colorado potato beetle diet developed by Domek et al. (1997) is a powerful tool; studies (heretofore impossible) can now be routinely performed to determine how plant secondary compounds affect the Colorado potato beetle. The potential arsenal of allelochemicals in a plant can now be dissected and reassembled to determine both individual and synergistic effects on the growth, development (from egg to prepupal stage) and behavior of the Colorado potato beetle.

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