The Indole Alkaloid Tryptamine Impairs Reproduction in *Drosophila melanogaster*

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ABSTRACT The plant-produced indole alkaloid tryptamine is one of a large array of neuroactive substances that may affect insect behavior, development, and physiology. We tested the role of tryptamine on insect reproduction using the fruit fly, *Drosophila melanogaster* (Meigen), as a model system. Measurements were made of reproductive success, oviposition rate, and preadult survival of insects on artificial diets containing tryptamine, its precursor tryptophan, as well as glycine and serotonin (5-hydroxytryptamine). *Drosophila* reproduction was reduced to 15% of controls when adult insects mated and the young were allowed to develop on medium containing 75 mM tryptamine. Tryptamine-induced depression in reproductive success was due to decreased oviposition rate and preadult survival. Serotonin, but not tryptophan or glycine, also reduced oviposition rate. Preference tests indicated that tryptamine may act as an antiattractant or antifeedant in this species. The accumulation of the indole alkaloid tryptamine in plants may provide a mechanism for reducing insect reproduction, which is potentially useful in protecting crop plants.

KEY WORDS neuroactive compounds, alleochemicals, antifeedant, fruit flies

INSECT SELECTION OF a host plant for feeding and reproduction begins with the sensing of the plant structure with peripheral chemoreceptors on the legs and labellum (Städler and Roessingh 1990). Some of these receptors are involved in sugar and other substance sensing, leading to subsequent plant acceptance or rejection (Dethier et al. 1960). Tactile, chemo- and hygroreceptors on the exposed ovipositor are thought important in the process of oviposition site selection (Thomas 1965). Besides chemosensory mechanisms, postingestive and behavioral factors can also be important for host plant acceptance/rejection (Jackson et al. 1986 Glendinning 1996). All available information about the potential host plant is integrated, and if found acceptable, insect reproduction is successful.

Alkaloids are one of several classes of plant-produced substances known to influence insect host selection. In monocotyledonous plants, levels of the alkaloid gramine are inversely related to the extent of aphid abundance (Zuñiga et al. 1988). Furthermore, some polyphagous aphids colonize only low alkaloid-producing plants, whereas aphids with restricted host specificity prefer high alkaloid producers, using the ingested alkaloids in their own defense (Niemeyer 1990).

In several plant species, the indole alkaloid tryptamine is thought to protect young seedlings against insects, particularly in newly emergent seedling stems and cotyledons (McKenna et al. 1984, Aerts et al. 1991, Bracher and Kutchan 1992). In addition, techniques are now available to add or elevate tryptamine levels in higher plants. Introduction of tryptamine biosynthesis into transgenic plants has been shown to depress *Bemisia tabaci* Genn. (whitefly) reproduction (Thomas et al. 1995). To estimate the potential of this technology, an adequate data base of information about the effects of tryptamine on insect behavior, reproduction, and development must be collected.

To assess insect responses directly to tryptamine, we selected the fruit fly, *Drosophila melanogaster* (Meigen), as a model insect for investigating the role of tryptamine on insect vitality. This insect has many advantages including: established methods to map genes involved in insecticide sensitivity/resistance, existing mutant populations, transformation/transposon systems to manipulate gene expression, and established procedures for studying gene expression at the molecular level (Wilson 1988). Furthermore, the genetics of developmental biology in *D. melanogaster* is very similar to that observed in other, perhaps more agronomically significant, insects (Thomas et al. 1984). Responses of *D. melanogaster* to tryptamine could form the basis of additional studies concerning the biochemical mechanisms involved in the effects of tryptamine and related indole alkaloids on agronomically important insect species.

Reported here, reproductive success, oviposition rate, and preadult survival of *D. melanogaster* were all measured on insects placed on artificial diets containing 14-75 mM tryptamine (0.3-1.5 mg/g dry weight of medium). Tryptamine concentrations tested here were similar to those found in tryptamine-accumulating plants (5 mg/g, plant T-148-1 in Songstad et al.

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1990), assuming plant dry weight is 20% of plant fresh weight. *D. melanogaster* tryptamine treatments (14–75 mM) significantly reduced insect reproduction when compared with untreated controls. Our results underline the diverse and significant effects of the indole alkaloid tryptamine on *D. melanogaster* and suggest that further examination of this compound on insect pest reproduction is needed.

Materials and Methods

***Drosophila* Reproduction Success.** "Oregon red" *D. melanogaster* stock flies were maintained by routine transfer on an artificial diet (Formula 4-24, Carolina Biological, Burlington, NC) and reared at 25°C ± 4°C under Philips CW/HO type fluorescent lights (150–200 μmol m^{-2} s^{-1} photon flux density).

For reproductive success and other tests, tryptamine HCl (Sigma, St. Louis, MO) was dissolved in water and added to the diet at final concentrations of 14–75 mM. At these concentrations the pH was maintained at 7.11 ± 0.2 for all tests. Two females and 3 males were placed in a vial with Formula 4-24 (20 ml) containing 0–75 mM tryptamine. After 3 d, the adults were removed, and the subsequent progeny was scored 18 d after the adults were removed. For each experiment, duplicate vials of each tryptamine concentration were tested. Experiments were repeated 7 times.

Preadult Survival Tests. Gravid females (4–5) were placed on grape juice-agar in a petri dish and eggs were deposited for 6 h. Using a fine paintbrush, 15 eggs were carefully placed on Formula 4-24 artificial diet containing either 0, 14, 28, 38, or 55 mM tryptamine. Eggs were allowed to develop and flies counted after 14 d. Each concentration of tryptamine was tested 5 times in duplicate.

Oviposition Rate. Effects on egg laying were estimated by allowing females to mate in Formula 4-24. Swollen females (3 per 16 by 100 mm petri dish) were then placed on 2% Bactoagar (Difco, Detroit, MI) made in grape juice (Libby's Juicy-Juice) with 0, 38, or 75 mM tryptamine. Eggs were easily discerned using this method and the egg numbers determined for 3 d. Each concentration was tested in 5 experiments with triplicate treatments per experiment. To verify that grape juice agar (without tryptamine) did not affect fecundity, some eggs were left on this medium. In 2 separate experiments, 25 of 27 and 24 of 29 eggs developed into fertile animals on grape juice agar, as on Formula 4-24.

To investigate the potential interaction of water availability and tryptamine on egg laying, 3 gravid females were placed on grape juice agar with additional water added to each plate. Egg numbers were then counted. For each experiment, duplicate vials of each tryptamine concentration were tested. Experiments were repeated 4 times.

Preference Tests. A Y-shaped olfactometer chamber was constructed with glass blowing (Rodrigues and Siddiqi 1978). The lower entry chamber (2.5 cm in diameter) was bifurcated to lead to 2 upper chambers of 2.5 cm in diameter. The instrument was ≈30 cm long. Clean white foam stoppers were placed in each upper chamber. One milliliter of 1% sucrose was added to 1 upper-chamber foam plug, while 1 ml of 1% sucrose containing 38 or 75 mM tryptamine was added to the foam plug of the 2nd chamber. One hundred *Drosophila* were placed in the lower entry chamber of the device and allowed to migrate for 1 or 16 h in a dark room at 20°C. The top portion of each chamber was illuminated with a single 25-W microscope illuminator. Insects in each chamber were counted after the incubation time. Each tryptamine concentration was tested 6 times. Mean data were converted to a ratio of the number of insects in the chamber with sucrose + tryptamine per number of insects in the both upper chambers (Rodrigues and Siddiqi 1978). Because of the extended times used (16 h), volatility or evaporation of tryptamine, and possible substance contact by insects, tryptamine tests as described were considered to be preference tests and not distinct olfactory measurements.

Statistical Analysis. Data were analyzed statistically using a 1-way analysis of variance (ANOVA) followed by mean separation using the Dunnett multiple comparison test, *t*-tests, or other tests within the InStat Statistical Program (GraphPad Software, 1992).

Results

The net effect of increasing tryptamine on *Drosophila* reproduction and adult reproduction (reproductive success) was examined by placing 2 females and 3 males in a Formula 4-24 artificial *Drosophila* diet with or without tryptamine. Results from 7 experiments (duplicate samples) suggested tryptamine was deleterious toward insect reproduction. One-way ANOVA ($F = 17.37$, df = 34, $P < 0.0001$) indicated that the difference between controls and tryptamine tests were extremely significant. Dunnett Multiple Comparison Test found that all treatments were significantly different than controls ($P < 0.05$ for 0 versus 20, 38, 55, or 75 mM) (Fig. 1). No significant differences were obtained among pupae and adult fly counts on tryptamine treatments (data not shown), suggesting low pupae to adult mortality.

To access better the developmental stage of tryptamine action, the effect of tryptamine on preadult survival was tested. Eggs were collected (from control females) and placed on media containing increasing concentrations of tryptamine (15 eggs per culture). A significant depression in insect reproduction was observed in 28, 38, or 55 mM tryptamine treatments versus the no-tryptamine control (Fig. 2), ($F = 45.79$, df = 54, $P < 0.0001$). Dunnett multiple comparison test found all treatments (except 14 mM) were significantly different than control ($P < 0.01$). Results were derived from 5 experiments (duplicate samples). Again, no differences were obtained between pupae and adult counts (unpublished data).

To test oviposition deterrent per stimulant activity, 3 gravid females were placed on 20 ml of grape juice containing 1% Bacto Difco agar with 0–75 mM

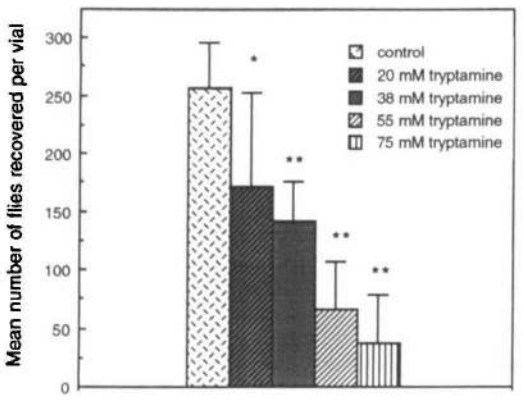

Fig. 1. Number of flies emerging in vials containing various concentrations of tryptamine, 18 d after oviposition. Bars indicate SEM number of eclosed adults. Experiments were repeated in duplicate 7 times. *($P < 0.05$) or **($P < 0.001$) indicates significant difference from the no-tryptamine control.

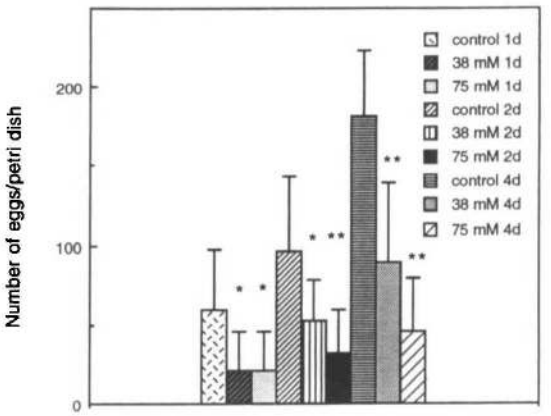

Fig. 3. Negative effect of increasing tryptamine on *Drosophila* oviposition in a no-choice artificial medium. In triplicate, 3 gravid females were placed on 20 ml of grape juice containing 1% Bacto Difco agar with increasing tryptamine concentrations. Animals were allowed to deposit eggs for 4 d and egg counts were recorded daily. For each day, controls were significantly different from the tryptamine treatments (Bonferroni multiple comparison: *$P < 0.05$ or **$P < 0.001$.

tryptamine. Animals were allowed to deposit eggs, and egg counts were recorded. A significant decrease in the number of eggs laid on tryptamine was observed (Fig 3). Using a Bonferroni multiple comparison test, controls were significantly different from the tryptamine treatments ($P < 0.05$) for day 1, 38 and 75 mM and day 2, 38 mM. For the remainder of the treatments differences from controls were more significant, $P < 0.001$.

The antioviposition activity of 5-hydroxytryptamine (serotonin), tryptophan, and glycine was also measured (Fig. 4). For tryptophan (the precursor of tryptamine), control was not significantly different from either 38 mM ($t = 0.946$, df = 8, $P = 0.372$) or 75 mM ($t = 0.16$, df = 8, $P = 0.877$). Similarly, the controls were not significantly different from 38 mM ($t = 0.928$, df = 8, $P = 0.380$) or 75 mM ($t = 0.344$, df = 8, $P = 0.740$) glycine. Unlike the previous 2 amino acids, 75 mM serotonin was significantly different from untreated controls ($t = 3.993$, df = 8, $P = 0.004$), whereas 38 mM serotonin was not significant ($t = 1.979$, df = 8, $P = 0.083$) compared with controls.

One physiological consequence of tryptamine exposure is the alteration of water excretion from the insect (Maddrell 1962, Just and Walz 1996). For this reason, grape juice agar supplemented with additional water was examined. Additional water was not able to reverse the tryptamine effect on the oviposition rate (Fig. 5) ($F = 1.06$, df = 35, $P = 0.38$). Thus, simple desiccation of the insect does not explain tryptamine inhibition reproduction in *Drosophila*.

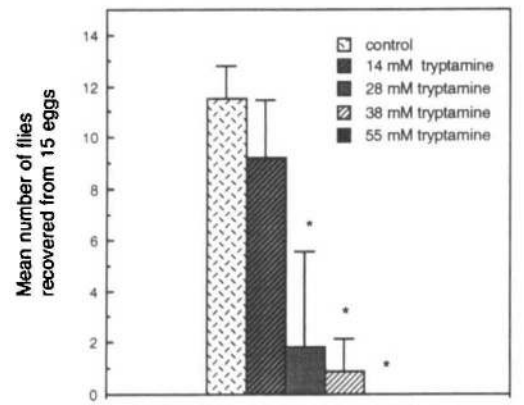

Fig. 2. Preadult survival of *D. melanogaster* is restricted by increasing tryptamine levels. Data represents the mean of insects obtained after 14 d, bars represent the SD. Each concentration of tryptamine was tested 5 times in duplicate. Dunnett multiple comparison indicated that all treatments except the 14 mM were significantly different from control, $P < 0.01$ (*).

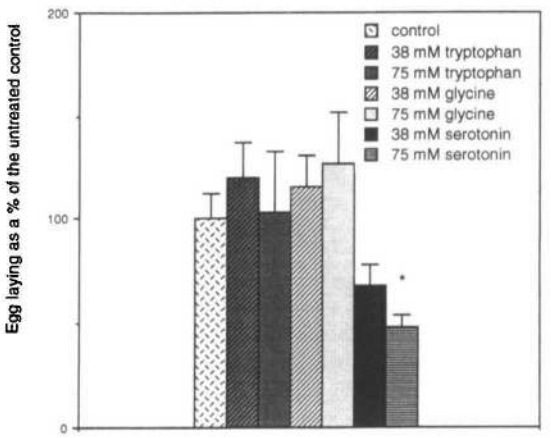

Fig. 4. Serotonin (75 mM), but not tryptophan or glycine, inhibited *Drosophila* oviposition. Egg counts were performed on day 2 and data expressed as the percentage of the no-treatment control. Bars indicate the SEM *, significant difference between treatment and control.

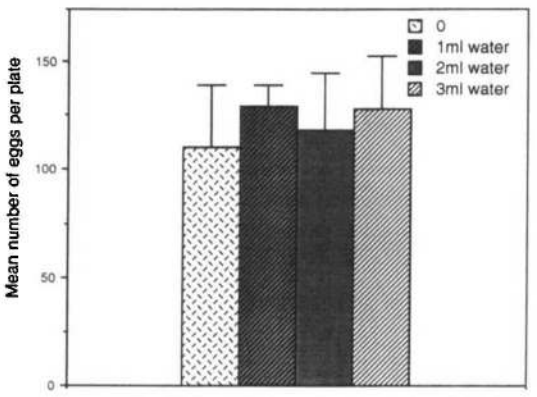

Fig. 5. Additional water did not affect tryptamine inhibition of oviposition. Egg counts were performed on day 2 and data expressed as number of eggs per plate. Bars indicate the mean and SE of experiments done in triplicate 3 times.

To test tryptamine as a possible antiattractant and/or antifeedant, an olfactometer (described in Rodrigues and Siddiqi 1978) was used in preference tests. Within 1 h, *D. melanogaster* were attracted more strongly to a 1% sucrose solution than a similar sucrose solution containing 75 mM tryptamine (Fig. 6). At the lower level of tryptamine (38 mM), this indole alkaloid was less effective as an "antiattractant" compared to the higher tryptamine level. However, when the sucrose versus sucrose + 38 mM tryptamine experiments were allowed to go overnight, ≈75% of the *Drosophila* population was found associated with the sucrose-alone region of the testing track (Fig. 6). The 75 mM (1 h) and 38 mM (16 h) tryptamine treatment

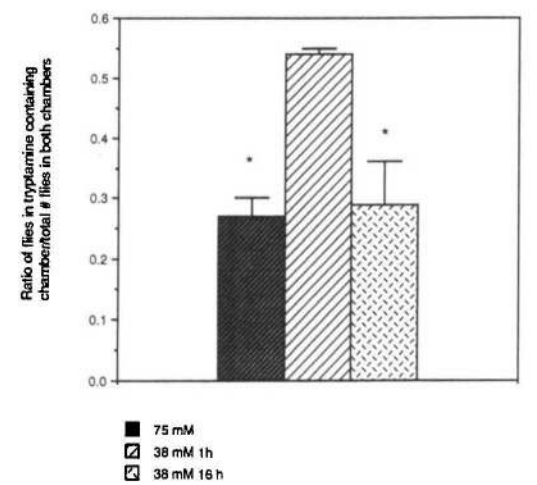

Fig. 6. Tryptamine may function as an antiattractant. Mean data (from 6 independent experiments) are presented as the ratio of flies in the tryptamine chamber divided by the total number of flies. The number of flies in the tryptamine chamber of both the 75 mM (1 h) and 38 mM (16 h) tryptamine treatments were significantly different (*) compared to the chamber without tryptamine ($t = 3.053$, df = 5, $P = 0.0283$ and $t = 5.547$ df = 5, $P = 0.005$ respectively). Bars indicate standard deviations.

was found significantly different from the control values ($t = 3.053$, df = 5, $P = 0.0283$ and $t = 5.547$, df = 5, $P = 0.005$ respectively). The difference between the 38 mM (1 h) and the controls was not significantly different ($t = 1.00$, df = 5, $P = 0.373$).

Discussion

***Drosophila melanogaster* Model System.** Because this model is based on only 1 insect, extrapolation of any result from this species to numerous field-borne insect pests must be viewed with caution. Not all behavioral aspects of plant–insect interactions in situ can be reproduced exactly during artificial feeding experiments. However, *D. melanogaster* is ideal for these experiments because it has been reared successfully on artificial diets for decades. In addition, many insects, both pest and non-pest, share elements of neural and developmental biology (Dethier 1960; Thomas et al. 1984).

Tryptamine Activity and Oviposition Rate. Insect oviposition is regulated by numerous insect and plant-produced chemicals. For example, a chemical signal from male *Drosophila* can promote female *Drosophila* oviposition (Jaenike et al. 1992). Diverse plant surface chemicals interact with insect chemoreceptors, assisting in oviposition site selection or rejection (Renwick 1989; Städler and Roessingh 1990; Hattori et al. 1992). In 1 example, use of tarsal sensilla and tarsal chemoreceptors during plant drumming behavior in butterflies is thought important to oviposition (Calvert and Hanson, 1983). Iridoid glycosides found in *Plantage lanceolata* are active oviposition stimulators in the specialist *Junonia coenia* (Pereyra and Bowers, 1988), whereas glucosinolates in the Brassicaceae promote insect oviposition (Reed et al. 1989, Renwick et al. 1992). This study verifies that tryptamine, like eugenol, can depress oviposition (Hattori et al. 1992).

Malpighian Tubules, Ion Homeostasis, and Signal Transduction. Tryptamine may affect oviposition by changes in hormones, secondary messengers, or electrical potential in 1 potential target tissue, malpighian tubules. Measurements of electrical potentials in *Drosophila melanogaster* malpighian tubules suggest that at least 2 separate signaling mechanisms affect oviposition, perturbation of intracellular Ca^{2+}, and activation of a cation-transporting V-ATPase (O'Connell et al. 1996). The latter protein is also present in malpighian tubules of pests such as *Heliothis virescens* (Pietrantonio and Gill 1995).

Tryptamine-mediated water loss may also help explain this alkaloid's effects on insect reproduction. Tryptamine has been shown to stimulate greatly malpighian tubule secretion from 0.1–0.5 nl/m to 300 nl/m (Maddrell 1962). Subsequently, tryptamine (and 5-hydroxytryptamine) lead to plasticization of the abdominal cuticle of larvae of *Rhodnius prolixus*, the bloodsucking bug (Maddrell et al. 1971). Similar to tryptamine, the diuretic hormone (DH) of *Manduca sexta* increased diuretic activity 100-fold when injected into *Bombyx mori* (Maeda 1989). In *Carausis morosus* and *R. prolixus*, analogous results have been

recorded with tryptamine and 5-hydroxytryptamine, suggesting that alkaloids such as tryptamine mimic diuretic hormone and are deleterious toward insect water/ionic homeostasis (Reynolds 1980). Liquid secretion in other tissues is also stimulated by tryptamine and its analogs. In isolated salivary glands of *Periplaneta americana* (cockroach), serotonin stimulated salivation containing both fluid and protein originating in the central cells (Just and Walz 1996). Tryptamine and tryptamine analogs may act specifically against water-ion homeostasis because tryptophan (the tryptamine and 5-hydroxytryptamine precursor) was inactive as a diuretic and plasticization inducer and antioviposition substance (Maddrell et al. 1971; Fig. 4).

Tryptamine, A Neurotransmitter Analog. Tryptamine can be a precursor of the neurotransmitter serotonin (5-hydroxytryptamine). Structurally related to serotonin, tryptamine likely binds the same site(s) (Mousseau and Butterworth 1994). Both amines blocked the neuromuscular transmitter glutamate on the postsynaptic membrane (Piek 1985). Serotonin induced excitatory effects on many visceral insect muscles and was a powerful agonist of salivary secretion and diuresis (Maddrell 1962; Just and Walz 1996). Ca^{2+}-dependent efflux of serotonin may link serotonin to other predigestive responses including salivation (Ali and Orchard 1996, Just and Walz 1996). Excess tryptamine may also affect aceylation of dopamine and serotonin by arylalkylamine *N*-acetyltransferase, the rate-limiting reaction in melatonin formation, likewise playing a role in neurotransmitter catabolism in *Drosophila melanogaster* (Hintermann et al. 1995). Thus, tryptamine may in some way mimic or alter neurological processes caused by serotonin.

Psychedelic Tryptamines. The levels of insect-ingested amines entering the blood may be further modified, leading to noxious activity in insects. Many studies on the nervous system of animals from worms to mammals have demonstrated that tryptamine can inhibit the breakdown of serotonin in the brain by monoamine oxidase (MAO) (Jones 1982, Csaba 1993). If MAO is blocked, serotonin will be converted by endogenous methyltransferase enzymes into known psychedelic tryptamines such as 5-MeO-tryptamine and 5-MeO-dimethyl tryptamine (McKenna et al. 1984). Endogenous insect methyltransferase may also produce the above-mentioned deleterious amines from ingested tryptamine, contributing to depressed insect reproduction and slow development (Csaba 1993).

In the experiments presented here, tryptamine was effective in depressing oviposition rate and preadult survival. Both oviposition rate and preadult survival have been shown important in resistance to *Bemisia tabaci* (whitefly) grown on several crop plants (Byrne and Draeger 1989). Results with the preference tests are consistent with the insects being able to smell tryptamine at 75 mM. However, at the lower concentration (38 mM), a combined taste and smell response is likely as the animals required an overnight incubation to reject tryptamine in lieu of sucrose alone (Fig. 6). An additional factor for consideration is that evaporation during the 16-h portion of the experiment may have increased the tryptamine concentration in the chamber. Clearly more study is needed to determine the exact mechanism of tryptamine action on insect reproduction.

High concentrations of tryptamine can be produced in some plant species (Songstad et al. 1990, Thomas et al. 1995). Furthermore, tryptamine is a small molecule and likely finds its way into the phloem, the primary feeding site of aphids and whiteflies. In previous studies (Thomas et al. 1995) there was some correlation between tryptamine accumulation in tobacco leaves and the amount of whitefly emergence ($r^2 = 0.65$) (unpublished data). However, this relationship may be oversimplified because the analysis of tryptamine was done on whole leaves whereas whiteflies primarily ingest phloem contents. Our data suggest tryptamine does accumulate in phloem, albeit lower in concentration than leaves (Thomas et al. 1995). Similarly, accumulation of tryptamine in transgenic *Brassica* resulted in decreased glucosinolate levels, 4- to 5-fold lower than nontransformed controls (Chavadej et al. 1994). Glucosinolates (produced from methionine, phenylalanine and tryptophan) are known to influence greatly insect feeding behavior (Niemeyer 1990). Because small differences in feeding rates can translate into major differences in survivorship under field conditions (Eigenbrode et al. 1990), we believe that modifying crops to produce tryptamine has great potential for profound negative developmental and behavioral effects on pest insects (Huang et al. 1993).

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