Laboratory Effects of Two Organically-Certified Insecticides on *Trichopoda pennipes* (Diptera: Tachinidae)[1]

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Abstract The objective of this laboratory study was to determine the effects of two organically-certified insecticides, azadirachtin and spinosad, on the stink bug parasitoid *Trichopoda pennipes* (F.) (Diptera: Tachinidae) in residual, topical, and oral toxicity tests. The insecticide lambda-cyhalothrin was used as a conventional crop production standard for control of lepidopterous pests. Azadirachtin was the only insecticide in which *T. pennipes* adults survived after exposure to dried residues, topical applications, and insecticide-treated food. Spinosad was as highly toxic to this parasitoid as lambda-cyhalothrin in all 3 tests even though spinosad was slower acting than lambda-cyhalothrin. These results suggest that azadirachtin would probably be safer to *T. pennipes* adults than spinosad in organically-grown crops.

Key Words azadirachtin, spinosad, residual toxicity, topical toxicity, oral toxicity

Demand for organically-grown products in the U. S. has increased at approximately 20% per year for the last 10 yrs (Dimitri and Greene 2002). This indicates that organic agriculture is one of the fastest growing sectors of the U. S. agricultural economy. Azadirachtin and spinosad are 2 of the few insecticides currently certified for suppression of insect pests in organic crops.

Azadirachtin is a compound found in seeds and leaves of the neem tree, *Azadirachta indica* A. Juss. It has shown good biological activity against insects of different orders (Schmutterer 1990, Ascher 1993, Mordue and Blackwell 1993), and it has been suggested for use in IPM programs due to its selectivity against natural enemies (Schmutterer 1995, Simmonds et al. 2002, Santolamazza-Carbone and Fernández de Ana-Magán 2004). Azadirachtin is the predominant active ingredient in neem. It acts as a repellent or antifeedant on phytophagous insects and also affects reproduction and development of pest insects by inhibiting oviposition and interfering with larval molts (Schmutterer 1990, Mordue and Blackwell 1993).

Spinosad is a fermentation product produced by the soil actinomycete *Saccharopolyspora spinosa* Mertz & Yao. It belongs to the naturalyte class of control compounds and acts by depolarizing insect neurons by activating nicotinic acetylcholine receptors (Salgado 1997). It has been used for control of lepidopterous pests in cotton due to its high activity at low rates and the tolerance by natural enemies of different orders (Nolting et al. 1997, Peterson et al. 1997).

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Trichopoda pennipes (F.), a tachinid endoparasitoid of *Nezara viridula* (L.) nymphs and adults, is one of the most effective natural enemies of this pest (Jones 1988). With the increase in organic production of agricultural crops, it is vital to understand the impact that azadirachtin and spinosad have on the survival of this natural enemy. Currently, no studies have been conducted on the effects of these 2 biopesticides on *T. pennipes*. Consequently, the goal for this research was to determine the effects of 2 organically-certified insecticides, azadirachtin and spinosad, on *T. pennipes* adults in residual, topical, and oral toxicity tests in the laboratory.

Materials and Methods

Insects. To obtain the *T. pennipes* adults necessary for the laboratory experiments, *N. viridula* adults which appeared to be parasitized by this parasitoid were collected from sorghum in Irwin Co., GA, in 2007 and held for emergence of parasitoids as described by Tillman (2006a). Newly-emerged *T. pennipes* adults were fed sugar water (300 g granulated sugar and 5 g ascorbic acid in 3.8 L of distilled water), and 2- to 3-d-old adults were used in toxicity tests. Voucher specimens of *T. pennipes* are held in the USDA-ARS, Crop Protection & Management Research Laboratory in Tifton, GA.

Insecticides. The residual, topical, and oral toxicity tests included the following 3 treatments and rates: (1) azadirachtin (Aza-Direct™ [222 μg/ml], Gowan Co., Yuma, AZ), (2) lambda-cyhalothrin (Karate™ [478 μg/ml], Syngenta Crop Protection, Inc., Greensboro, NC), and (3) spinosad (Tracer™ [1026 μg/ml], Dow AgroSciences LLC, Indianapolis, IN). In the feeding toxicity tests, all insecticides were mixed in the sugar water solution described above. The pyrethroid lambda-cyhalothrin was used as a conventional crop production standard for control of lepidopterous pests. Doses of each insecticide used in these experiments simulated the concentrations of field-use rates based on applications at a total volume of 93.5 L/ha.

Residual toxicity. In residual tests, an insecticide treatment was sprayed on the top and bottom of a plastic Petri dish (100 × 15 mm) using a Preval™ sprayer (Precision Valve Corp., Yonkers, NY). Water was used as the control. After the compounds dried for 1 h, *T. pennipes* were placed singly in the Petri dishes. Preliminary tests revealed that residues of lambda-cyhalothrin, the conventional crop production standard, killed *T. pennipes* adults within 1 h. In addition, under field conditions *T. pennipes* adults would probably not be exposed to residues of insecticide continuously for long periods of time because they can fly. Therefore, insects were kept in treated Petri dishes for only 1 h. Insects were not fed during the exposure period to avoid the possibility of the insects feeding on contaminated food. After the exposure period, insects were moved to clean Petri dishes and provided sugar water. A randomized complete block design was used with 2 pairs (each pair with 1 male and 1 female) of insects per block (day) for 4 blocks (16 insects per treatment). Mortality was recorded 1, 24, and 48 h after exposure to the insecticide treatments.

Topical toxicity. To keep a *T. pennipes* adult from flying away while an insecticide treatment was being topically applied, the insect was cooled down in the refrigerator for 1-2 min. As soon as the cooled insect was placed in a plastic Petri dish (100 × 15 mm), the insecticide treatment was applied topically to the dorsal surface of the insect using the Preval sprayer. Water was used as the control. The height of the sprayer above the insect was ~30 cm. After the treatment was applied, the insect was transferred to a clean Petri dish and provided sugar water. A randomized complete

block design was used with 2 pairs (each pair with 1 male and 1 female) of insects per block (day) for 4 blocks (16 insects per treatment). Mortality was recorded 1, 24, and 48 h after exposure to the insecticide treatments.

Oral toxicity. In the field, *T. pennipes* adults drink free water and feed on extrafloral nectaries. Thus, oral toxicity was tested by allowing *T. pennipes* adults to feed on insecticide-treated sugar water in feeding wells as described by Tillman (2006a). Sugar water was used as the control. Individual feeding wells were placed in Petri dishes (60 × 15 mm). Then, *T. pennipes* adults that had been starved 24 h before the test were placed singly into these feeding arenas. During the test, insects were allowed to feed once, and amount of time to begin feeding and total feeding time were recorded. After the insects fed on treated food, they were placed individually in clean Petri dishes and given sugar water. A randomized complete block design was used with 2 pairs (each pair with 1 male and 1 female) of insects per block (day) for 4 blocks (16 insects per treatment). Where applicable, the amount of time it took an insect to die after feeding was recorded. Mortality also was recorded at 24 and 48 h after the insects fed on insecticide treatments.

Statistical analysis. Because percentage mortality for *T. pennipes* in residual, topical, and oral toxicity tests was either 0 or 100, an estimate of variance for the mean could not be obtained. Therefore, these mortality data were analyzed using PROC FREQ (SAS Institute 2003). Percentage mortality data were the same for 24 and 48 h after treatment of all insecticide treatments so only the 1 and 24 h after treatment data were analyzed. In oral toxicity tests, preliminary analyses revealed that there was no significant sex effect for time to begin feeding, feeding time, and time to die after feeding. Therefore, the 4 insects (2 males and 2 females) in a block were considered to be 4 random samples of adults. The 3 groups of feeding data were analyzed using PROC MIXED (SAS Institute 2003). The fixed effect was insecticide treatment. Random effects were block, block by insecticide, block by sex, insecticide treatment by sex, and residual error. Least squares means were separated by least significant difference (LSD) (SAS Institute 2003) where appropriate.

Results

Residual toxicity. Prolonged tarsal contact with dried residues of lambda-cyhalothrin and spinosad resulted in 100% mortality for *T. pennipes* adults even though it took longer for flies to die when exposed to spinosad compared with lambda-cyhalothrin (Table 1). In contrast, exposure to residues of azadirachtin was nontoxic to adults of this parasitoid.

Topical toxicity. Topical application of lambda-cyhalothrin and spinosad also resulted in 100% mortality for *T. pennipes* adults (Table 2). Again, spinosad was slower acting than lambda-cyhalothrin. Topical applications of azadirachtin, though, were nontoxic to this tachinid.

Oral toxicity. Feeding on sugar water treated with lambda-cyhalothrin and spinosad resulted in 100% mortality for *T. pennipes* (Table 3). Ingesting azadirachtin-treated sugar water had no impact on survival of *T. pennipes*. For lambda-cyhalothrin and spinosad, factorial analysis revealed that there was a significant insecticide treatment effect for amount of time to die (F = 213.55; df = 1, 6; P = 0.0001) when individuals fed on insecticide-treated sugar water (Table 3). Adults died slower when they ingested sugar water treated with spinosad than when they fed on food containing lambda-cyhalothrin.

Table 1. Percentage mortality 1 and 24 h after treatment of *T. pennipes* adults walking on residues of azadirachtin, lambda-cyhalothrin, and spinosad

Insecticide	μg/ml	% Mortality	
		1 h after treatment	24 h after treatment
Azadirachtin (1)	222	0	0
Lambda-cyhalothrin (2)	478	100	100
Spinosad (3)	1026	0	100
Control (4)		0	0
Insecticide group comparisons		χ^2, df, *P*	χ^2, df, *P*
1-4		64.0, 3, 0.0001	64.0, 3, 0.0001
1, 4 and 2, 3		64.0, 1, 0.0001	64.0, 1, 0.0001

Table 2. Percentage mortality 1 and 24 h after treatment of *T. pennipes* adults topically sprayed with azadirachtin, lambda-cyhalothrin, and spinosad

Insecticide	μg/ml	% Mortality	
		1 h after treatment	24 h after treatment
Azadirachtin (1)	222	0	0
Lambda-cyhalothrin (2)	478	100	100
Spinosad (3)	1026	0	100
Control (4)		0	0
Insecticide group comparisons		χ^2, df, *P*	χ^2, df, *P*
1-4		64.0, 3, 0.0001	64.0, 3, 0.0001
1, 4 and 2, 3		64.0, 1, 0.0001	64.0, 1, 0.0001

Insecticide treatment significantly affected the amount of time for *T. pennipes* adults to begin feeding ($F = 3.65$; df = 3, 5.26; $P = 0.0943$) (Table 4). It took significantly more time for *T. pennipes* adults to start feeding on food treated with lambda-cyhalothrin than on food treated with azadirachtin or control food. Thus, the flies were more reluctant to feed on food containing lambda-cyhalothrin than on insecticide-free sugar water. These results strongly indicate that lambda-cyhalothrin acted as a repellent to *T. pennipes* adults.

Factorial analysis revealed that there was no significant insecticide treatment effect for feeding time ($F = 1.14$; df = 3, 4.85; $P = 0.4191$) for *T. pennipes* adults presented insecticide-treated sugar water (Table 4). The amount of time for adults to feed on food was not significantly different among insecticide treatments. The flies readily fed as long on insecticide-treated food as they did on insecticide-free food. Therefore, for at least the first feeding event, no insecticides in the study exhibited antifeedant activity.

Table 3. Percentage mortality 24 h after treatment and total time to die for *T. pennipes* adults feeding on sugar water treated with azadirachtin, lambda-cyhalothrin, and spinosad

Insecticide	μg/ml	% Mortality 24 h after treatment	Time to die (min)
Azadirachtin (1)	222	0	—
Lambda-cyhalothrin (2)	478	100	9.02b
Spinosad (3)	1026	100	66.89a
Control (4)		0	—
Insecticide group comparisons		χ^2, df, *P*	
1-4		64.0, 3, 0.0001	
1, 4 and 2, 3		64.0, 1, 0.0001	

Least squares means within a column followed by the same lowercase letter are not significantly different between insecticide treatments for the time for adults to die after feeding (PROC MIXED, LSD, $P > 0.05$, $n = 16$, SE = 2.8, df = 6).

Table 4. Least squares means for the time for adult *T. pennipes* to begin feeding and for the total amount of time male and female *T. pennipes* fed on sugar water treated with azadirachtin, lambda-cyhalothrin, and spinosad

Insecticide	μg/m	Time to begin feeding (min)	Feeding time (sec)
Azadirachtin	222	2.31b	37.12a
Lambda-cyhalothrin	478	17.69a	34.23a
Spinosad	1026	12.93ab	22.41a
Control		1.41b	43.73a

Least squares means within a column followed by the same lowercase letter are not significantly different between insecticide treatments for the time for adults to begin feeding (PROC MIXED, LSD, $P > 0.05$, $n = 16$, SE = 4.2, df = 5.26). Least squares means within a column followed by the same lowercase letter are not significantly different between insecticide treatments for the time adults fed (PROC MIXED, LSD, $P > 0.05$, $n = 16$, SE = 8.35, df = 4.9).

Discussion

Walking on residues of azadirachtin, being sprayed topically with azadirachtin, and ingesting azadirachtin-treated sugar water had no impact on survival of *T. pennipes.* In contrast, spinosad and lambda-cyhalothrin negatively affected this parasitoid in residual, topical, and oral toxicity bioassays. When exposed to lambda-cyhalothrin, adults were immobilized and quickly died within 1 h of treatment. In residual, topical, and feeding tests with spinosad, adults initially appeared to be unaffected, even feeding on clean sugar water, but they continuously excreted fluids onto the bottom

of the Petri dish. By 24 h after treatment with spinosad, adult *T. pennipes* became incapacitated, lying on their backs, shaking uncontrollably, kicking their legs, and unable to right themselves to walk. These adults were considered to be dead. By 48 h after treatment, these adults were motionless. A similar response to spinosad has been observed with other natural enemies of pest insects (Schneider et al. 2003, Myers et al. 2006).

An earlier study provided the first information on the susceptibility of *T. pennipes* to insecticides commonly used in conventional crop production (Tillman 2006a). Cyfluthrin, dicrotophos, oxamyl, and thiamethoxam were highly toxic, acetamiprid was moderately toxic, and indoxacarb was nontoxic to *T. pennipes* after exposure to residues of these chemicals. In oral toxicity tests using insecticide-treated sugar water, all 6 of these insecticides were highly toxic to *T. pennipes*. Dicrotophos apparently is also highly toxic to immatures of this parasitoid in the field (Tillman 2006b). For all insecticides in the earlier and current studies, azadirachtin along with indoxacarb are the only 2 compounds that have no residual activity, and azadirachtin is the only insecticide without feeding activity against *T. pennipes* adults. Thus, for all 9 insecticides which have been tested, only azadirachtin is safe to the parasitoid via both methods of exposure.

Generally, spinosad is less toxic to natural enemies than broad-spectrum insecticides such as organophosphates and second-generation pyrethroids. Also, in general, field applications of spinosad seem to be less detrimental to natural enemies than laboratory treatments. In the laboratory, spinosad was moderately toxic to adults of the predator *Geocoris punctipes* (Say), but less toxic than the organophosphate methyl parathion (Boyd and Boethel 1998). In topical, residual, and field assays, lambda-cyhalothrin generally exhibited greater toxicity to adults of 3 parasitoid species, 2 lady beetle predators, and *G. punctipes* than spinosad (Tillman and Mulrooney 2000). This compound was highly toxic to each parasitoid species and *G. punctipes* in topical toxicity tests, but in the field, spinosad generally did not affect the density of *G. punctipes* and the 2 lady beetle species. In other field trials, spinosad was less toxic to 2 lady beetle species and the predator *Orius insidiosus* (Say) than to lambda-cyhalothrin (Musser and Shelton 2003). In the current study, spinosad was equally as highly toxic to *T. pennipes* adults as lambda-cyhalothrin in direct toxicity tests in the laboratory indicating that this parasitoid is more susceptible to spinosad than some species of natural enemies.

Azadirachtin also is generally less toxic to natural enemies than broad-spectrum insecticides. In residual tests, neem was not toxic to *Encarsia sophia* (Girault and Dodd); whereas, the 3 synthetic insecticides, endosulfan, chlorpyriphos, and triazophos, were highly toxic to this parasitoid (Aggarwal and Brar 2006). Akol et al. (2002) reported that topical and residual contact with 2 formulations of neem did not cause acute toxicity or affect longevity in adults of the parasitoid *Diadegma mollipla* (Holmgren). In contrast, lambda-cyhalothrin was acutely toxic to this parasitoid which is consistent with the results obtained in this study. Azadirachtin certainly has been proven to be safer than synthetic insecticides on *T. pennipes* and other parasitoids and predators.

In previous studies, azadirachtin was less toxic, equally toxic, or equally nontoxic to natural enemies compared with spinosad. In laboratory bioassays, survival of *G. punctipes* adults following exposure to insecticide residues was very high for azadirachtin, but lower for spinosad (Myers et al. 2006). In contrast, in both residual contact assays and ingestion treatments, both azadirachtin and spinosad were harmful to

adults of the parasitoid *Psyttalia* (=*Opius*) *concolor* Szèpl. (Viñuela et al. 2000, 2001). However, Medina et al. (2001) reported that topical application of azadirachtin and spinosad was not toxic to eggs and pupae of *Chrysoperla carnea* (Stephens). In contact toxicity tests, mortality of adults of the parasitoid *Cotesia plutellae* (Kurdjumov) was low for azadiractin-based insecticides, moderately high for spinosad, and very high for lambda-cyhalothrin (Haseeb et al. 2004). The results of the laboratory test for this parasitoid are consistent with the results of the current study.

In the current study, azadirachtin did not exhibit any direct toxicity to *T. pennipes* adults during prolonged tarsal contact to residues of the insecticide, through feeding on insecticide-treated food or from topical applications of the insecticide. Similarly, azadirachtin had no adverse effect on the egg parasitoid *Anaphes nitens* Girault in the field and in residual studies in the laboratory (Santolamazza-Carbone and Fernández de Ana-Magán 2004). Also, neem seed kernel extracts did not cause mortality of adults of the larval parasitoid *Bracon hebetor* Say through feeding and contact routes of exposure (Raguraman and Singh 1998). In contrast, neem seed oil was mildly toxic adults of the egg parasitoid *Trichogramma chilonis* Ishii in feeding and contact toxicity tests (Raguraman and Singh 1999).

Azadirachtin can act as a repellent inhibiting feeding by predators and parasitoids and oviposition by parasitoids. Neem seed kernel extracts inhibited feeding of *B. hebetor* for a limited period (Raguraman and Singh 1998). Both feeding and oviposition by the egg parasitoid *Trichogramma chilonis* Ishii were reduced when honey or host eggs were treated with neem seed oil (Raguraman and Singh 1999). In addition, Simmonds et al. (2002) reported that a high concentration of neem deterred the parasitoid *Encarsia formosa* Gahan from stabbing neem-treated hosts. In the current feeding study, *T. pennipes* adults exhibited repellency only to lambda-cyhalothrin, but not to the formulation of azadirachtin used or to spinosad. In an earlier study, *T. pennipes* adults exhibited repellency to oxamyl, cyfluthrin, indoxacarb, and acetamiprid, but they were unaffected by dicrotophos and thiamethoxam (Tillman 2006c). Except for azadirachtin which does not have any direct toxicity to the parasitoid, the repellency behavior of the flies to insecticides may be their only means of survival in the field. Spinosad is highly toxic to *T. pennipes* adults and has little or no repellent activity, and so it may be deleterious to this parasitoid in organic fields.

Azadirachtin also can act as an antifeedant stopping the feeding process. Feeding by newly-emerged adults of the scelionid, *Gryon fulviventre* (Crawford), was reduced when their food source was mixed with aqueous neem suspension (Mitchell et al. 2004). The authors suggested that the neem acted as a repellent, but it appears to have been acting as an antifeedant. In the current feeding study, *T. pennipes* spent about the same amount of time feeding during the single feeding event regardless of insecticide treatment. If feeding had been assessed over time, antifeedant activity may have been observed: in an earlier study, *T. pennipes* adults exhibited diminution of feeding when they fed over a 60-min time interval on sugar water containing any of the insecticides (Tillman 2006c). It is important, though, to know that only one feeding event resulted in the death of an individual *T. pennipes* adult feeding on spinosad or lambda-cyhalothrin because it suggests that mortality was not based on feeding time, but on occurrence of feeding. The parasitoids only have to feed once on these insecticides to be harmed by the toxicants so they may have a very negative impact on these natural enemies in organic fields.

Many of the older insecticides were highly toxic to most insects in residual and topical tests and in the field, thus, the effect of these insecticides on natural enemies

was relatively easy to assess and feeding activity was not considered. Many of the newer compounds, however, have feeding activity along with residual and/or topical activity. Originally, compounds with feeding activity were considered to be harmless to natural enemies because, unlike plant pests, the main food source of natural enemies was not plant tissue. Natural enemies, though, can ingest insecticides through a variety of mechanisms. For example, the predator *G. punctipes* plant feeds and is highly susceptible to indoxacarb when feeding on plants with residues of this insecticide (Tillman et al. 2001). In the current feeding study, a feeding well was designed so that the insect only fed on the chemical, separating feeding and residual exposure to the insecticide. Insect feeding behavior was subsequently observed and recorded. Both mode of action of an insecticide and behavior of the insect should be considered carefully in developing the design of the test and drawing conclusions from the test when conducting an experiment to assess the impact of an insecticide on a natural enemy.

In summary, azadirachtin was the only insecticide in which *T. pennipes* adults survived after walking on the insecticide, feeding on insecticide-treated food, and being sprayed topically with the compound. On the other hand, spinosad was as highly toxic to adults of this parasitoid as lambda-cyhalothrin in residual, topical, and oral toxicity tests. These results suggest that azadirachtin would probably be safer to *T. pennipes* adults than spinosad in organically-grown crops.

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