Effects of azadirachtin-based insecticides on the egg parasitoid *Trichogramma minutum* (Hymenoptera: Trichogrammatidae)

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Abstract—Effects of neem formulations on the reproduction and survival of the egg parasitoid *Trichogramma minutum* Riley were examined to assess the compatibility of the two control strategies in integrated pest management programs. A laboratory bioassay was developed for this purpose, which could be used as a model ecotoxicological system. Eggs of the Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), which had been treated with an acetone solution containing an azadirachtin-based formulation, were presented to individual *T. minutum* females. These eggs were held until parasitoids completed development and emerged from the eggs. Survival of *T. minutum* females 1 day after treatment, number of Mediterranean flour moth eggs parasitized, proportion of parasitized eggs from which adults emerged, and sex ratios of emerging adult parasitoids were determined. Two formulations of neem-seed extracts containing azadirachtin and a purified azadirachtin standard were tested at an operational dose and at 10 times the operational dose. At 50 g azadirachtin/ha (operational dose), no significant effects were observed on survival of parasitoid females. At 500 g azadirachtin/ha, female survival after 1 day was significantly reduced by Azatin EC and Neem EC. No reduction was evident with the 100% azadirachtin treatment, suggesting that other components of the formulations were in part responsible for the toxicity to females. Likewise, at 500 g azadirachtin/ha, the number of eggs parasitized was greatly reduced by Azatin EC and slightly reduced by Neem EC but was not reduced by an azadirachtin standard. These reductions in egg parasitism were probably due to the observed effects on female survival. At 500 g azadirachtin/ha, parasitoid developmental success was reduced by all treatments including the azadirachtin standard. Neem EC and Azatin EC at the lower dose also had a small but significant effect on developmental success. Sex ratio of emerging adults was not affected. These results indicate that azadirachtin is compatible with *T. minutum* during egg parasitism at operational dosages.

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Résumé—L'examen des effets de préparations de neem sur la reproduction et la survie du parasite des oeufs, *Trichogramma minutum* Riley, a permis d'évaluer la compatibilité de deux stratégies dans des programmes de lutte intégrée. Nous avons mis au point dans ce but un test biologique en laboratoire qui peut être utilisé comme modèle de système écotoxicologique. Des oeufs de la pyrale méditerranéenne de la farine, *Ephestia kuehniella* Zeller (Lepidoptera : Pyralidae), traités à une solution d'acétone contenant une préparation à base d'azadirachtine, sont offerts à des femelles isolées de *T. minutum*. Les oeufs sont gardés jusqu'à ce que le parasitoïde complète son développement et émerge des oeufs. Nous avons déterminé la survie des femelles de *T. minutum* un jour après le traitement, le nombre d'oeufs parasités de la pyrale méditerranéenne de la farine, le pourcentage d'oeufs parasités d'où ont émergé des adultes et le rapport mâles:femelles des parasitoïdes adultes à l'émergence. Nous avons testé deux préparations d'extraits de graines de neem contenant de l'azadirachtine, ainsi qu'une solution standard d'azadirachtine purifiée à la dose opérationnelle et à dix fois la dose opérationnelle. À 50 g d'azadirachtine/ha (dose opérationnelle), il n'y a pas d'effet significatif sur la survie des parasitoïdes femelles. À 500 g d'azadirachtine/ha, la survie des femelles après une journée est significativement réduite par l'Azatin EC et le Neem EC. Il n'y a pas de réduction décelable lors du traitement à l'azadirachtine 100 %, ce qui fait croire que ce sont d'autres composantes des préparations qui sont en partie responsables de la toxicité pour les femelles. De même, à 500 g d'azadirachtine/ha, le nombre d'oeufs parasités est fortement réduit par l'Azatin EC et légèrement réduit par le Neem EC, mais il n'y a pas de réduction par la solution standard d'azadirachtine. Ces réductions du parasitisme des oeufs sont probablement dus aux effets observés sur la survie des femelles. À 500 g d'azadirachtine/ha, le succès du développement du parasitoïde diminue avec tous les traitements, y compris celui à la solution standard d'azadirachtine. Le Neem EC et l'Azatin EC à la dose la plus faible ont aussi un effet faible, mais significatif, sur le succès du développement. Le rapport mâles:femelles des adultes à l'émergence n'est pas affecté. Ces résultats indiquent que le traitement à l'azadirachtine à des doses opérationnelles est compatible avec *T. minutum* durant le parasitisme des oeufs.

[Traduit par la Rédaction]

Introduction

Azadirachtin, a tetranotriterpenoid, is the most active phytochemical principle of the neem tree, *Azadirachta indica* A. Juss. (Meliaceae), on insect populations. Effects of azadirachtin on insects include feeding and oviposition deterrence, growth inhibition, and fecundity and fitness reductions (Schmutterer 1990). Several other insecticidally active compounds are also present in neem extracts but in lower concentrations (Schmutterer 1990). Insecticides containing azadirachtin are effective against a broad-spectrum of phytophagous insects (Schmutterer 1990); these include a number of defoliating forest pests (Helson 1992) such as gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae) (Shapiro *et al.* 1994), spruce budworm, *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae) (Wanner and Kostyk 1995), introduced pine sawfly, *Diprion similis* (Hartig) (Hymenoptera: Diprionidae), and cedar leaf miners, *Argyresthia thuiella* (Packard) and *Argyresthia aureoargentella* Brower (Lepidoptera: Argyresthiidae) (Helson *et al.* 2001).

There have been a number of recent investigations into the potential effects of neem-based insecticides on hymenopterous parasitoids used in biological control programs (Schmutterer 1997). Larval and pupal parasitoids studied have included predominately braconids (*e.g.*, Raguraman and Singh 1998, Goudegnon *et al.* 2000), but ichneumonids (McCloskey *et al.* 1993) and encyrtids (Villanueva-Jiménez *et al.* 2000) have also been investigated. Egg parasitoids are represented by trichogrammatids (*e.g.*,

Klemm and Schmutterer 1993, Raguraman and Singh 1999, Thakur and Pawar 2000) and eulophids (Deepak and Choudhary 1998). Reported effects on parasitoids have been insignificant (Goudegnon *et al.* 2000) or have included adult mortality (Raguraman and Singh 1999), larval mortality (Raguraman and Singh 1998), egg mortality (Srivastava *et al.* 1997), reduction in adult eclosion (Lakshmi *et al.* 1997), reduction in adult longevity (Feldhege and Schmutterer 1993), reduction in parasitism rate (Raguraman and Singh 1999), increased duration of stages (Osman and Bradley 1993), feeding deterrence (Raguraman and Singh 1998, 1999), reduction in F_1 progeny (Stark *et al.* 1992), and disruption of ecdysis (Osman and Bradley 1993). Field applications of neem products are often less detrimental than laboratory treatments (Lowery and Isman 1995).

Some investigators have tested neem-seed extracts of unknown (Raguraman and Singh 1998, 1999) or known (Goudegnon *et al.* 2000) azadirachtin content. Others have evaluated commercial formulations (Lakshmi *et al.* 1997) that may or may not have specified azadirachtin content. Unless the concentration of the active ingredient is known, experiments would be difficult to replicate and results would be variable. All these products also contain other potentially active compounds that would confound the interpretation. Commercial formulations contain adjuvants that might enhance or mitigate the activity of azadirachtin. Few investigators (McCloskey *et al.* 1993) have tested the impact of purified azadirachtin on entomophagous parasitoids.

The inundative release of egg parasitoids is a biological control strategy that is used in a number of agricultural situations and has shown considerable promise in forestry (Smith *et al.* 1990; Smith 1996). The dominant group of interest for these studies has been *Trichogramma* spp. (Smith 1996). Previous investigations documenting the interaction of *Trichogramma* spp. and neem-based insecticides have had variable results, probably as a consequence of using products of unknown or variable azadirachtin content.

For suppression of forest insects with potentially extended oviposition and larval emergence periods, such as the pine false webworm, *Acantholyda erythrocephala* (L.) (Hymenoptera: Pamphiliidae) (Lyons 1994), *T. minutum* applications for egg suppression can overlap with azadirachtin sprays for larval control. Our objective in this study was to examine and compare the effects of pure azadirachtin and neem-based insecticides containing azadirachtin on *T. minutum.* Neem-based insecticides (Lyons *et al.* 1996, 1998) and *Trichogramma* spp. (Bourchier *et al.* 2000) have both been tested for control of the pine false webworm in Ontario. Specifically, we tested the effect of these products on the survival of ovipositing parasitoids, fecundity of *T. minutum* females, and host suitability for parasitoid development.

Materials and methods

Trichogramma minutum rearing

Trichogramma minutum females used in the bioassays were from a colony reared on eggs of the Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). These factitious host eggs, which had been killed by gamma-radiation treatment (Laing and Eden 1990), were obtained fresh weekly from Bio-Logicals (Ciba-Geigy Canada Ltd), Guelph, Ontario. Gamma radiation does not reduce the suitability of the eggs as hosts for the parasitoids but prevents the emergence of flour moth larvae. The colony of *T. minutum* had been initiated from adults reared from spruce budworm eggs collected at Quetico, Ontario (48°07′N, 91°01′W), in July 1988 and had been in culture for over 200 generations. Subcolonies were maintained in 25 mm diameter × 95 mm high glass vials stored in an environmental chamber set at 25°C, 50% RH, and 16L:8D.

New factitious host eggs, glued to the surface of strips of file card, were placed in the culture vials on the day prior to emergence of adults of the new generation.

Azadirachtin formulations

Azadirachtin was tested in three formulations, all of which were applied in acetone. The use of acetone guaranteed the solubility of the formulations and enhanced their even diffusion over the egg surfaces. The high volatility of acetone also meant that the solvent evaporated quickly from the test strips and left the strips dry for introduction of the parasitoids. The first formulation, Azatin® EC, was a proprietary product of AgriDyne Technologies Inc (Salt Lake City, Utah) containing 3.0% azadirachtin; the second formulation was Neem EC (experimental formulation; Phero Tech, Delta, British Columbia), which contained 4.6% azadirachtin; and the final product was a 100% azadirachtin standard (provided by JT Arnason, University of Ottawa, Ottawa, Ontario). In addition, a Neem EC formulation blank (provided by M Isman, University of British Columbia, Vancouver, British Columbia) and a naphthalene control were compared with treated controls. Naphthalene is an ingredient of the AgriDyne formulation and was tested at 5%. The Neem blank was tested at the equivalent of the highest dose used. Applied concentrations of active ingredient (azadirachtin) were 0.00375% (50 g azadirachtin/ha) and 0.0375% (500 g azadirachtin/ha). An azadirachtin-based insecticide (Neemix 4.5, azadirachtin 4.5%; Thermo Trilogy Corporation, Columbia, Maryland) is registered in Canada for use against conifer-feeding sawflies at application rates of up to 50 g azadirachtin/ha (registration No. 26548, Pest Control Products Act). This application rate is comparable to the lowest concentration (*i.e.*, 0.00375%) of the three formulations used in our bioassays. The highest concentration (*i.e.*, 0.0375%) tested was an order of magnitude greater than the recommended dose. Five separate experiments were conducted, each repeated once. The replicates were pooled for analysis. Each experiment compared three to five of the treatments.

Bioassays

To determine the effects of azadirachtin formulations on *T. minutum*, females were exposed to treated flour moth eggs stuck on strips of Post-it® notes. Post-it® notes were cut into 25 × 6 mm strips, with a 6 × 6 mm sticky portion on which flour moth eggs could adhere (Corrigan and Laing 1991). The strips were dipped into a vial of fresh flour moth eggs on the day of treatment (approximately 200 eggs sticking to the strip). Each strip was suspended on a pin over filter paper for chemical treatment. A test formulation of 20 μL was applied to the entire surface of the egg-covered strips using an Eppendorf pipet, which completely covered the eggs without runoff. Each treated egg strip was placed in an individual 12 mm diameter × 35 mm high shell vial with a single female parasitoid and the vial was plugged with a cork. All experiments were set up in the morning, just after adult eclosion, to minimize the amount of time females were active without hosts. Preliminary investigations had indicated that females often died after becoming stuck in honey droplets, which was provided for nourishment to increase longevity. The majority of ovipositions by the females takes place on the first day after introduction to the host eggs, and it was concluded that the benefits of adding honey did not outweigh its detrimental effects. Thus, females were not fed.

Only three to five treatments were compared in each experiment because excessive set-up time reduced the reproductive potential of females that did not have access to new host eggs. Each treatment consisted of 30 strips and each experiment was repeated once. Treatment vials were placed in an environmental chamber at 25°C, 50% RH, and 16L:8D.

Female mortality was determined 1 day after treatment. Replicated experiments were pooled for analysis. Numbers of surviving and dead females were compared between treatments within an experiment using a *G* test ($\alpha = 0.05$) (Sokal and Rolf 1981). Seven days after treated eggs had been exposed to females, the number of melanized eggs in each treatment vial was recorded. Melanization indicated that a parasitoid was developing within the host egg. Mean numbers of melanized eggs were determined for each treatment. Following emergence of the new generation, the vials were placed in a freezer to prevent development and emergence of subsequent generations. Numbers of males and females present in the vials were counted at a later date. The total number of emerging adults in each vial was divided by the number of melanized eggs to determine parasitoid survival within the host egg. The means of these proportions, for each treatment within an experiment, were calculated. The proportion of females eclosing from treated eggs in each vial was also determined and the means were computed for each treatment. Distributions for which means were determined were compared using the Kruskal–Wallis one-way ANOVA on ranks followed by Dunn's test for differences in ranked means ($\alpha = 0.05$) in SigmaStat 2.0 (Jandel Corporation 1995).

Results

Mortality of *T. minutum* females in the solvent-treated control insects for all experiments ranged from 3.3 to 27.6% (Table 1). Except for the 45% mortality value for the neem blank in Experiment 3, there were no differences in mortality between the untreated, naphthalene-treated, neem-blank-treated, and solvent-treated controls. Successful parasitism in the former treatment (Table 2) suggested that mortality occurred late in the experimental period. Similarly, for the three formulations of azadirachtin at the lower concentration (*i.e.*, 0.00375% azadirachtin), mortality was not different from the controls. For the higher application rate (0.0375% azadirachtin) of Azatin EC, mortality was always higher than for the controls. In all experiments except Experiment 5, mortality was higher for the higher application rate of Neem EC than in the controls. In Experiment 5, control mortality was considerably higher than in Experiments 1–4 and this resulted in the lack of significant difference between control and treated. Mortality for *T. minutum* females exposed to the higher application rate of the azadirachtin standard (0.0375%) (Exp. 2) was not different from that of controls.

Mean numbers of melanized eggs (Table 2) of the Mediterranean flour moth, indicating successful parasitism, in the solvent-treated controls ranged from 45.0 in Experiment 5 to 67.4 in Experiment 2. Mean numbers of melanized eggs in the untreated, naphthalene-treated, and neem blank-treated controls were not different than mean number of melanized eggs for the solvent-treated controls. Similarly, mean numbers of parasitized eggs for the lowest concentrations of the three azadirachtin formulations were never different than their corresponding solvent-treated controls. The number of parasitized eggs for the azadirachtin standard treatment at the highest concentration was also not different from the controls. At the highest concentration of the Azatin EC formulation, there were large differences in parasitism between the treated eggs and the controls. For eggs treated with the higher concentration of the Neem EC formulation, mean numbers of melanized eggs were always lower than for treated control eggs but higher than the Azatin EC treated eggs at the same concentration of active ingredient.

Mean proportions of melanized eggs, from which *T. minutum* adults eclosed, varied from 0.35 to 0.94 (Table 3). When compared with the solvent-treated control eggs, eclosion rates for the untreated, naphthalene-treated, and neem-blank-treated control eggs were not different except in Experiment 1 where the untreated control had a higher proportion. At the lower concentration of Azatin EC, the proportion of eggs from which

TABLE 1. Mortality (%) of *Trichogramma minutum* females after 1-day exposure to *Ephestia kuehniella* eggs that were treated with different concentrations and formulations of azadirachtin-based insecticides.

	Control				Azatin EC*		Azadirachtin standard*		Neem EC*			
Experiment	Untreated	Solvent-treated	Naphthalene	Neem blank	0.00375%	0.0375%	0.00375%	0.0375%	0.00375%	0.0375%	*G*	*P*
1	15.0*a*	5.0*a*	—	—	8.5*a*	76.7*c*	—	—	—	44.1*b*	108.45	<0.005
2	3.4*a*	3.3*a*	—	—	—	53.3*b*	—	1.7*a*	—	46.7*b*	103.56	<0.005
3	—	13.3*a*	10.0*a*	45.0*b*	—	76.7*b*	—	—	—	52.5*b*	85.59	<0.005
4	—	6.7*a*	—	15.0*a*	11.7*a*	—	11.7*a*	—	6.7*a*	—	3.44	>0.1
5	—	27.6*a*	—	—	—	66.7*b*	—	—	—	46.6*ab*	18.58	<0.005

NOTE: Percentages (n = 58–60 females/treatment) in the same row followed by the same letter are not different (G test, $P > 0.05$; Sokal and Rohlf 1980).

* Percent azadirachtin.

TABLE 2. Mean (±SE) number of azadirachtin-treated eggs of *Ephestia kuehniella* that were parasitized by *Trichogramma minutum*.

	Control				Azatin EC*		Azadirachtin standard*		Neem EC*				
Experiment	Untreated	Solvent-treated	Naphthalene	Neem blank	0.00375%	0.0375%	0.00375%	0.0375%	0.00375%	0.0375%	df	*H*	*P*
1	58.1±2.8*a*	54.7±2.7*ab*	—	—	51.0±2.8*ab*	1.3±0.5*c*	—	—	—	43.9±2.4*b*	4	138.21	<0.001
2	68.7±1.8*a*	67.4±1.9*a*	—	—	—	7.8±1.2*c*	—	67.5±1.9*a*	—	43.7±1.9*b*	4	193.45	<0.001
3	—	57.6±2.9*a*	52.3±2.7*a*	50.7±2.0*ab*	—	11.0±2.1*c*	—	—	—	43.8±2.2*b*	4	122.84	<0.001
4	—	58.9±2.3*a*	—	50.5±3.1*a*	52.1±2.8*a*	—	53.2±2.9*a*	—	55.0±2.2*a*	—	4	7.32	0.120
5	—	45.0±3.8*a*	—	—	—	4.7±0.9*c*	—	—	—	28.8±2.9*b*	2	14.36	0.0001

NOTE: Means (n = 58–60 strips of about 200 eggs/treatment) within a row followed by the same letter are from distributions that were not different (Kruskal–Wallis one-way ANOVA on ranks followed by Dunn's test for differences in ranked means, $P > 0.05$) (Jandel Corporation 1995).

* Percent azadirachtin.

TABLE 3. Mean (±SE) proportion of azadirachtin-treated and parasitized eggs of *Ephestia kuehniella* from which *Trichogramma minutum* adults emerged.

	Control				Azatin EC*		Azadirachtin standard*		Neem EC*				
Experiment	Untreated	Solvent-treated	Naphthalene	Neem blank	0.00375%	0.0375%	0.00375%	0.0375%	0.00375%	0.0375%	df	*H*	*P*
1	0.94±0.01*a*	0.84±0.02*b*	—	—	0.67±0.02*c*	0.08±0.04*d*	—	—	—	0.35±0.02*d*	4	182.35	<0.001
2	0.92±0.01*a*	0.93±0.01*a*	—	—	—	0.17±0.02*c*	—	0.58±0.01*b*	—	0.51±0.01*b*	4	215.33	<0.001
3	—	0.93±0.01*a*	0.91±0.02*a*	0.89±0.02*a*	—	0.23±0.03*b*	—	—	—	0.62±0.02*b*	4	152.18	<0.001
4	—	0.93±0.01*a*	—	0.93±0.01*a*	0.77±0.01*b*	—	0.90±0.01*a*	—	0.84±0.01*b*	—	4	133.54	<0.001
5	—	0.93±0.01*a*	—	—	—	0.24±0.03*c*	—	—	—	0.52±0.02*b*	2	88.08	<0.001

NOTE: Means (n = 41–60 strips of about 200 eggs/treatment, except for Azatin at 0.0375% where n = 8–24) within a row followed by the same letter are from distributions that were not different (Kruskal–Wallis one-way ANOVA on ranks followed by Dunn's test for differences in ranked means, $P > 0.05$) (Jandel Corporation 1995).

* Percent azadirachtin.

TABLE 4. Mean (±SE) proportion of *Trichogramma minutum* adults emerging from treated eggs of *Ephestia kuehniella* that were females.

	Control				Azatin EC*		Azadirachtin standard*		Neem EC*				
Experiment	Untreated	Solvent-treated	Naphthalene	Neem blank	0.00375%	0.0375%	0.00375%	0.0375%	0.00375%	0.0375%	df	*H*	*P*
1	0.80±0.01*a*	0.76±0.02*a*	—	—	0.77±0.01*a*	1.00±0.00*b*	—	—	—	0.71±0.03*a*	4	18.80	<0.001
2	0.74±0.02*a*	0.73±0.02*a*	—	—	—	0.75±0.06*a*	—	0.71±0.02*a*	—	0.75±0.02*a*	4	8.49	0.075
3	—	0.77±0.01*a*	0.75±0.02*a*	0.79±0.02*a*	—	0.73±0.06*a*	—	—	—	0.77±0.02*a*	4	5.91	0.206
4	—	0.75±0.01*a*	—	0.73±0.02*a*	0.71±0.02*a*	—	0.74±0.01*a*	—	0.70±0.02*a*	—	4	7.08	0.132
5	—	0.67±0.02*a*	—	—	—	0.72±0.07*a*	—	—	—	0.69±0.03*a*	2	3.62	0.164

NOTE: Means (n = 41–60 strips of about 200 eggs/treatment, except for Azatin at 0.0375% where n = 4–21) within a row followed by the same letter are from distributions that were not different (Kruskal–Wallis one-way ANOVA on ranks followed by Dunn's test for differences in ranked means, $P > 0.05$) (Jandel Corporation 1995).

* Percent azadirachtin.

adults eclosed was lower than the proportion of eggs in the solvent-treated control. At the higher concentration of this formulation, eclosion was reduced for the few eggs that were melanized. At the lower concentration of the azadirachtin standard, the proportion of emerging adults was similar to those in the solvent-treated control. Mean proportion of emerging adults was lower for Neem EC at the lower concentration. At the highest concentrations of these formulations, the proportion of emerging adults was much lower than in the solvent-treated control.

Sex ratios of emerging adults (Table 4) for all treatments were similar with the exception of Azatin EC at the higher concentration in Experiment 1; however, the sample size in this treatment was only four because of the high female mortality.

Discussion

At recommended operational doses, azadirachtin-based insecticides in our experiments had no direct effects on female survival. At 10 times the operational dose, increased mortality of ovipositing females was observed for the two formulated insecticides but not for the azadirachtin standard. None of the formulation adjuvants that were tested separately had any effect on female mortality (except perhaps the Neem EC formulation blank in one experiment), host acceptance, or parasite development. One explanation for this contradiction is that an adjuvant, or a combination of adjuvants, in the formulated products synergizes the effects of azadirachtin. Another possible explanation is that other compounds in the neem extract contributed to this mortality. Although mortality of females was variable, this is not the critical variable measured in these experiments. *Trichogramma minutum* females not presented with food (*i.e.*, honey) do not live much beyond 1 day (Laetemia *et al.* 1995); however, during this day of life they deposit the majority of their potential eggs. The critical measure of a parasitoid's success is its ability to parasitize hosts and produce offspring. These insecticides did not act as an oviposition deterrent at operational doses. At higher doses of the formulated products, reduction in parasitism was observed, but this probably reflected the reduced survival of ovipositing females. Azatin EC at the higher dose had the greatest impact on survival and parasitism rates. At the highest doses of all three products, fecundity was adversely affected. Azatin EC and Neem EC also slightly affected fecundity and (or) host acceptance at the operational doses.

Trichogramma spp. have also been used as inoculative and inundative agents in biological control programs against insect pests of forests (Smith 1996). Consequently, the two control techniques could potentially be used in concert during integrated control programs. Therefore, it is imperative that we understand any adverse effects neem-based insecticides might have on populations of *T. minutum*, a species that has been used inundatively in forestry situations (Smith *et al.* 1990). *Trichogramma minutum* performs best when attacking exposed host eggs, ones that would receive the greatest deposit of azadirachtin-based insecticides during spray programs. Females would be exposed to these chemicals as they walk over egg masses and oviposit in host eggs. Disappearance of azadirachtin residues from sprayed conifer and deciduous foliage has been shown to be rapid with 50% loss occurring after 17–22 h (Sundaram and Curry 1994). This short residual life of azadirachtin is a disadvantage from the perspective of extending the window for foliage protection; however, it is a significant advantage from the point of view of integrating botanical insecticides with biological control programs. Application of test compounds in acetone during these experiments suggests that the results may not be directly applicable to field situations where the compounds would be applied in water. Azadirachtin-A, however, has been shown to be even more stable in acetone than in water (Dureja *et al.* 1999).

Trichogramma minutum exhibits a female-biased sex ratio. Populations are typically arrhenotokous with 50–75% female offspring (Wang and Smith 1996). Sex ratios in all experiments were remarkably similar, suggesting that treatments had little differential effects on males or females. The only significant difference in sex ratios was in a treatment where the total number of progeny was greatly reduced. The incidence of female-only offspring was rare in the experiments and did not appear to be affected by azadirachtin.

The bioassay system described here is a valuable tool for assessing the impact of insecticides on egg parasitoids and could be incorporated into evaluation strategies for many control products. *Trichogramma minutum* is relatively easy to rear in the laboratory on a number of host species and the bioassay technique requires minimal space to accommodate an adequate sample size. The techniques allow for the sequential evaluation of the impact of the tested material on several population parameters including adult mortality, oviposition success, realized fecundity, developmental performance, and resulting sex ratios.

The minimal impact of the operational concentration (0.00375%) of products containing azadirachtin on the survival of ovipositing *T. minutum*, fecundity of female parasitoids, or host suitability for parasitoid development in our experiments indicates that the use of these insecticides should be compatible with *T. minutum* activity. Thus, control methodologies incorporating both neem treatments and the use of inundative releases of *T. minutum* might be integrated into forest pest management strategies without adverse interactions. Neem-based insecticides should also have minimal impact on endemic *T. minutum*, which attack eggs of forest pests. This will have to be verified under field conditions.

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