

Behavioral Responses of *Schistocerca americana* (Orthoptera: Acrididae) to Azadirax (Neem)-Treated Host Plants

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ABSTRACT Azadirax (azadirachtin and other biologically active extracts from neem trees) has been shown to have considerable potential to be used in integrated pest management systems based on its growth regulator/insecticide properties. Less well known are the antifeedant properties. The feeding-deterrent properties of a commercial azadirax formulation (Azatrol EC) were evaluated using both no-choice and choice tests, the American grasshopper, *Schistocerca americana* (Drury), and four host plants [savoy cabbage, *Brassica oleracea* variety *capitata* L.; cos (romaine) lettuce, *Lactuca sativa* variety *longifolia* Lam.; sweet orange, *Citrus sinensis* variety *Hamlin* L.; and peregrina, *Jatropha integerrima* Jacq.]. These studies demonstrated that azadirax application can significantly affect the feeding behavior of grasshoppers. Some degree of protection can be afforded to plants that differ markedly in their innate attractiveness to the insect, although the level of protection varies among hosts. The tendency of grasshoppers to sometimes feed on azadirax-treated foliage suggests that it will be difficult to prevent damage from occurring at all times, on all hosts. No evidence of rapid habituation to azadirax was detected. Rapid loss of efficacy was observed under field conditions, suggesting that daily retreatment might be necessary to maintain protection of plants from feeding.

KEY WORDS azadirachtin, feeding deterrent, antifeedant, grasshopper, locust

The neem tree, *Azadirachta indica* A. Juss, produces numerous allelochemical compounds, the best known of which are tetranortriterpenoids, the azadirachtins. Physiologically, azadirachtins function primarily as insect growth regulators, but they also interfere with chemoreception, and cause damage to insect tissues such as muscle, fat body, and the gut. The azadirachtins (and precursors) also affect insect behavior, functioning as antifeedants and oviposition and mating deterrents (Koul 2004). Treatment of the locust *Schistocerca gregaria* (Forskål) with neem also inhibits flight (Wilps et al. 1992) and reduces gregarization, including development of the gregarious phase (Langewald and Schmutterer 1992). The allelochemicals conferring toxic properties are not necessarily the same as those conferring antifeedant properties (Aerts and Mordue 1997). Thus, less purified plant extracts are often more efficacious than single compounds because they are mixtures of compounds. The term “azadirax” has been proposed to express the insecticidally active extract of neem seeds containing azadirachtin and related biologically active compounds (Morgan 2004).

Azadirax affects >400 species of insects from many orders, including both hemimetabolous and holometabolous taxa (Koul 2004). Other invertebrates such as mites, nematodes, and snails as well as some

fungi also are affected. Despite this wide range of toxic effects, vertebrates are notably unaffected. Neem is widely used as animal fodder in the tropics and as a component of cosmetics and medicines. For example, this medicinal plant is used to treat intestinal disorders, skin diseases, inflammations, diabetes, bacterial infections, and gum diseases.

This interesting blend of biologically active properties has generated considerable interest in azadirax as an insecticide, and registration has been obtained, or applied for, in many countries (Koul 2004). It is not without some problems, of course, including the poorly defined chemical nature of some products, low stability under certain circumstances, and high cost. Also, not all insects are affected; for example, Lowery and Isman (1993) found that only three of the six aphid species they tested were deterred from feeding, and plant virus transmission by aphids was not interrupted. Its use as a feeding deterrent is particularly intriguing, because although there are many efficacious insecticides available commercially, there are few antifeedants. As noted by Koul (2005), azadirax has considerable potential to be used as an antifeedant in integrated pest management systems. Here, we report behavioral responses of the American grasshopper, *Schistocerca americana* (Drury), to plant foliage treated with an azadirax product. The American grasshopper is one of the two most important grasshopper species in the southeastern United States; the other

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species is the eastern lubber grasshopper, *Romalea guttata* (Houttuyn) [= *R. microptera* (Beauvois)]. Both grasshoppers occasionally attain high numbers, at least locally, and feed on vegetables, flowers, and ornamental shrubs. In Florida, they sometimes cause serious damage to citrus crops.

Methods

Interaction of Host Plant and Azadirex. The effect of host plant on the behavioral response of American grasshoppers to an azadirex product was assessed using choice and no-choice tests. Both types of tests were conducted in transparent plastic containers, 18 cm in diameter and 8 cm in height. Each container received a moist paper towel in the bottom to maintain high humidity and to keep the foliage fresh, and the test foliage was placed atop the towel. A single fifth instar was added to each container and allowed to feed for 24 h at 32°C. Instars were determined according to the methods of Capinera (1993a). In no-choice tests, the grasshoppers were offered either two 2-cm-diameter disks of azadirex-treated foliage or untreated (control) foliage. In choice tests, the grasshoppers were simultaneously offered one 2-cm-diameter disk of treated foliage and another of untreated foliage. Foliage samples were randomly selected from three host plants. Twenty grasshopper nymphs were used in each of the assessments: choice, no-choice treated, and no-choice control. Treatment disks were sprayed on both upper and lower surfaces with 0.32% Azatrol EC insecticide (3% azadirachtin; PBI/Gordon Corp., Kansas City, MO) freshly mixed the day of application. Applications were made with compressed air sprayer to runoff and allowed to dry before testing. Dryness was assessed visually and normally required <30 min. Foliage disks were cut pretreatment for laboratory studies, minimizing any handling effects. For field application, disks were cut from the foliage postapplication of azadirex. The rate of azadirex application is within the range recommended for grasshopper control. Control disks received water only. The same grasshoppers were tested in an identical manner with new foliage disks for a second 24-h feeding period immediately after the first to assess habituation to the treatment. Consumption data from grasshoppers that died or molted during evaluation, or within 24 h posttreatment, were discarded, and new replicates were initiated to attain a total of 20 grasshopper evaluations. The treatments were applied to four different plant hosts, chosen to reflect a range in innate grasshopper preference: savoy cabbage, *Brassica oleracea* variety *capitata*; cos (romaine) lettuce, *Lactuca sativa* variety *longifolia*; sweet orange, *Citrus sinensis* variety *Hamlin*; and peregrina, *Jatropha integerrima*. Percentage of consumption was estimated visually, and consumption values were transformed to square root ($\% + 0.5$) to normalize the data. Student's *t*-test was used to test for significant differences ($P = 0.05$) in consumption levels of treatment and control disks, for both choice and no-choice tests and for each host plant. Paired *t*-tests were used for choice tests (Horton 1995). One-way analysis of variance (ANOVA) was used to compare consumption levels of azadirex-treated and untreated foliage disks,

among the four host plants, for both days of the testing protocol. Significant means were separated using Tukey-Kramer's mean separation test ($P = 0.05$). InStat Biostatistics software (GraphPad Software Inc., San Diego, CA) was used for data analysis.

Nymphal and adult *S. americana* have very similar feeding behavior (Capinera 1993b), but to verify that adults also were affected by azadirex, a modification of the aforementioned protocol was used. We evaluated the response of 20 adults in a no-choice test by using a single azadirex-treated or untreated orange foliage disk. Data were collected and analyzed in the aforementioned manner.

Host Plant Preference. The relative preference of grasshoppers for the host plant species was assessed using the protocol described under Interaction of Host Plant and Azadirex, except for the manner of plant presentation and data analysis. In one set of host preference tests, three 2-cm-diameter disks of foliage for each prospective host plant were presented simultaneously (12 disks per assay arena), and the average percentage of consumption of the three disks recorded; 20 fifth instars were tested individually. In the other host preference test, single intact leaves of orange and peregrina, and equivalent sections of cabbage and lettuce, were presented simultaneously; 13 fifth and sixth instars were tested. Leaf area was ≈ 30 cm² for each plant type, and foliage was collected randomly from a citrus planting, peregrina hedge, or from store-purchased heads of lettuce and cabbage.

The difference between the two preference tests was the leaf area presented, but this is potentially relevant because the larger leaf areas of the latter limit the tendency of insects to move to less desirable plants after exhaustion of the most preferred host. The consumption data within each of these preference tests were subjected to one-way ANOVA and a Tukey-Kramer mean separation test ($P = 0.05$). The percentage of consumption data from the two preference tests were compared with Pearson's linear correlation analysis.

Some characteristics of the host plant foliage were assessed by determining wet and dry weight and by calculating percentage of moisture for each host type. Wet weight was determined by weighing 10 disks of fresh foliage of each plant type immediately after disks were punched with a cork borer. Dry weight of the disks was determined by drying the 10 disks in a drying oven to a constant weight. The mean weights of the wet and dry disks were used to calculate the average moisture content for each host plant. Differences in wet and dry weights among hosts were assessed with a one-way ANOVA, and means were separated with Tukey-Kramer multiple comparison test ($P = 0.05$). Data were not transformed for these analyses. Mean wet weight per disk, mean dry weight per disk, and mean percentage of moisture content were correlated with leaf disk consumption from the leaf disk preference study to determine whether there was a significant relationship between food weight, moisture variables, and amount of food consumed. Pearson's linear correlation analysis was used to calculate the correlation coefficients. InStat Bio-

statistics software (GraphPad Software Inc., San Diego, CA) was used for data analysis.

Duration of Feeding Deterrence under Field Conditions. Azatrol EC was diluted to 0.32% and applied with a compressed air sprayed to runoff on adaxial and abaxial surfaces. Applications were made to an orange tree to runoff on two dates: 27 June and 6 July 2005, at ≈ 9 a.m. Individual leaves were collected from the east side of the tree after 4 and 8 h of sunlight exposure on the day of application, and the next day after an additional 4 h of sunlight (hereafter considered to be 12 h of sunlight exposure), with each leaf sample considered a replicate for bioassay purposes. Environmental conditions after the treatment were a maximum temperature of $\approx 32^\circ\text{C}$ and a minimum of 24°C on the June dates, and 34 and 25°C on the July dates, with no precipitation. Disks (2 cm in diameter) were cut from the treated foliage after 4, 8, and 12 h of exposure to sunlight. Eighteen replicate leaf samples consisting of two leaf disks, from each posttreatment interval, were provided separately to individual fifth instars. Percentage of consumption was determined after 24 h. Ranked consumption data were analyzed with one-way ANOVA (SAS Institute 2002), and means were separated with Tukey's test ($P = 0.05$). Ranked data were used to accommodate the many zero values.

Similarly, a comparison of the 0.32% treatment rate and a 2X rate (0.64%) was made on 1 August 2005 by using the same methods. Maximum and minimum temperatures during this application were 32 and 23°C , respectively, with no precipitation. Application of azadirex, collection of foliage samples, and insect bioassays were conducted in the aforementioned manner except that foliage samples taken at the time of application (0 h) also were included in the bioassay. Consumption data were ranked and analyzed with ANOVA, PROC-GLM (SAS Institute 2002).

Grasshopper and Plant Sources. The grasshoppers used in this study were from a laboratory colony that has been maintained for ≈ 10 yr. The insects are fed a dry diet consisting of whole wheat flour, soy flour, and wheat bran, supplemented with romaine lettuce. The nymphs and adults are maintained at $\approx 30^\circ\text{C}$, but they had access to light bulbs, so they could attain a warmer temperature if desired. The lettuce and cabbage used in this study were purchased from a grocery store; the orange and peregrina foliage were field harvested and consisted of mature leaves from sunny locations. Before testing, the grasshoppers were deprived of vegetation for ≈ 24 h.

Results

When presented with azadirex-treated or untreated (control) foliage in choice tests, grasshoppers consistently consumed significantly more untreated foliage (Table 1). Likewise, in no-choice tests, azadirex proved to be a statistically significant feeding deterrent. Inhibition of feeding occurred on both days of the testing, indicating no immediate habituation after exposure to neem treatment.

Table 1. Comparative consumption (percentage) of four host plants by *S. americana* nymphs over a 2-d period when presented azadirex-treated and untreated foliage in choice and no-choice tests

Day	Test	df	Cabbage		Lettuce		Peregrina		Orange	
			Azadirex	Control	Azadirex	Control	Azadirex	Control	Azadirex	Control
1	Choice	19	0.0a	87.5a	5.0a	99.8a	11.3a	95.0a	100.0a	4.2/ <0.001
	No-choice	38	0.0a	75.1b	2.0a	98.5a	6.0a	98.0a	99.5a	12.7/ <0.001
2	Choice	19	0.0a	72.5b	6.5a	96.5a	9.3ab	96.5a	98.7a	98.7/ <0.001
	No-choice	38	0.0a	64.5b	0.6a	100.1a	1.5a	92.5a	99.5a	99.5/ <0.001

Significance of comparison between treated and untreated for each pair of means within a plant type is indicated by the t-value and P after each pair of values. Means within rows followed by the same letter indicate that there is no significant difference between means when comparing among treated plants, or comparing among untreated plants.

Table 2. Comparative consumption of four host plants by *S. americana* nymphs when presented in small foliage disks or larger, relatively intact leaves, and characteristics of the leaf disks

Plant host	Consumption (% ± SE)		Disk characteristics (mg ± SE)	
	Leaf disk	Leaf	Wet wt	Dry wt
Cabbage	12.3a ± 2.6	8.1a ± 3.1	158.1a ± 2.9	25.8a ± 0.9
Lettuce	64.1b ± 5.4	53.5b ± 10.1	127.7a ± 10.0	7.8b ± 0.4
Peregrina	72.6b ± 4.9	61.2b ± 8.9	75.2b ± 2.4	19.5ab ± 0.5
Orange	49.5b ± 5.7	22.7ab ± 6.1	68.8b ± 2.1	27.9a ± 0.7

Means followed by the same letter are not significantly different ($P > 0.05$).

ANOVA conducted to compare the level of consumption in azadirax-treated disks among the four host diets in choice tests showed that there was a significant difference on both days, with significantly higher levels of consumption of azadirax-treated orange foliage than cabbage, lettuce, and peregrina on day 1 ($F = 14.97$; $df = 3, 76$; $P < 0.001$) or cabbage and lettuce on day 2 ($F = 5.99$; $df = 3, 76$; $P < 0.001$) (Table 1). This was not the case in no-choice tests, where azadirax treatment consistently reduced feeding on all hosts to an equivalent low level ($F = 1.65$; $df = 3, 76$; $P > 0.18$ on day 1 and $F = 0.37$; $df = 3, 76$; $P = 0.77$ on day 2). In all tests, there was a tendency for cabbage to be consumed less, whether it was treated with azadirax.

Comparison of grasshopper consumption of control (untreated) foliage in choice tests (Table 1) demonstrated that there were no significant differences in consumption on day one ($F = 1.65$; $df = 3, 76$; $P = 0.19$), but cabbage consumption was lower on day two ($F = 7.39$; $df = 3, 76$; $P < 0.001$). In no-choice tests, less cabbage was consumed when compared with the other three hosts on both day one ($F = 9.43$; $df = 3, 76$; $P < 0.001$) and day two ($F = 8.54$; $df = 3, 76$; $P < 0.001$).

Adult grasshoppers were also deterred from feeding on orange foliage. In 24 h no-choice tests, the grasshoppers consumed (mean ± SE) 11.9 ± 5.2% of the azadirax-treated foliage, but significantly more (53.5 ± 9.3%) of the untreated (water control) foliage ($F = 3.12$; $df = 18$; $P = 0.006$).

In foliage preference studies, both tests indicated a mean preference ranking of peregrina > lettuce > orange > cabbage (Table 2). In the leaf disk preference analysis, cabbage was significantly less consumed than the other three plants ($F = 14.75$; $df = 3, 76$; $P < 0.001$). In the whole leaf test, cabbage was less preferred than lettuce and peregrina; orange was intermediate ($F = 11.04$; $df = 3, 48$; $P < 0.001$). Comparison of the two preference tests showed a significant degree of correlation ($r = 0.959$, $P = 0.04$).

The moisture characteristics of the host plants varied significantly (Table 2). The annual vegetable crops (cabbage and lettuce) had higher wet weights than the perennial woody crops ($F = 61.5$; $df = 3, 36$; $P < 0.001$). This pattern was not evident in the dry weight measurements, where orange and cabbage had the highest dry weights, lettuce had the lowest dry weight, and peregrina was intermediate ($F = 186.5$; $df = 3, 36$; $P < 0.001$). There was not a significant correlation between preference (per-

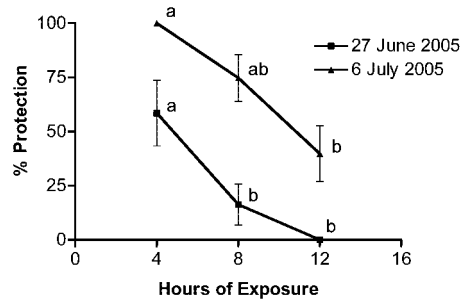


Fig. 1. Level of protection (proportion of leaf area remaining, mean ± SE) achieved by treatment of orange trees with azadirax (Azatrol) on two dates when foliage was harvested at three time intervals (4, 8, and 12 h) posttreatment and provided to American grasshopper nymphs in a no-choice test. Mean levels of protection, on each date, followed by the same letter are not significantly different.

centage of consumption) and wet weight of the disks ($r = -0.68$, $P = 0.318$) or dry weight of the disks ($r = -0.53$, $P = 0.284$). Percentage of moisture was calculated as 83.7, 93.9, 74.0, and 59.6% for cabbage, lettuce, peregrina, and orange, respectively. The correlation between disk preference and percentage of moisture was not significant ($r = -0.06$; $P = 0.938$).

Persistence of azadirax under field conditions was limited. The results of two trials are shown in Fig. 1 and typify the results obtained in preliminary assessments as well: a rapid decrease in efficacy and considerable variability from trial to trial. In the 27 June test of azadirax persistence, there was a statistically significant decrease in leaf protection between 4 h (58.5 ± 15.1%, mean ± SE) of the foliage protected from herbivory, and the 8 h (16.2 ± 9.5% protection) and 12 h (no protection, complete foliage consumption) of exposure to sunlight ($F = 12.79$; $df = 2, 29$; $P < 0.001$). A similar result was attained in the 6 July study ($F = 11.43$; $df = 2, 29$; $P < 0.001$), when a significant decrease was noted between 4 h (complete protection) and 12 h (39.7 ± 12.8% protection) post-treatment; the 8-h treatment was intermediate (74.7 ± 10.8% protection). We also conducted one trial with a 1X (0.32%) and 2X rate (0.64%) of azadirax. Comparison of the two treatment levels by ANOVA indicated a non-significant treatment rate effect ($F = 0.63$, $df = 1$, $P = 0.41$) but a significant sampling time effect ($F = 13.77$, $df = 1$, $P < 0.001$). The application rate and sampling time interaction was not significant ($F = 2.61$, $df = 1$, $P = 0.11$). However, the 0.64% application rate averaged higher levels of leaf protection throughout the bioassay period except for the initial period (time 0), as would be expected. The high degree of variability among replicates may have masked the benefits of the higher rate of application.

Discussion

Feeding behavior of the polyphagous North American grasshopper *S. americana* was affected by treatment of its food source with a commercial formulation of azadirax. Isman (2004) reported that North American grasshop-

pers could "eat neem-treated plants with impunity, though they subsequently suffer from the physiological effects of azadirachtin." Mulkern and Mongolkiti (1975) and Champagne et al. (1989) found that purified azadirachtin was not an effective feeding deterrent for 10 different North American grasshoppers, including two species of *Schistocerca*. This relationship does not apply to all azadirachtin-containing formulations, because *S. americana*, like its African relative *S. gregaria*, proved sensitive to neem extracts. We suspect that the use of purified azadirachtin formulations by previous investigators explains the discrepancies. The title of the Mulkern and Mongolkiti (1975) article, "Desert locust feeding deterrent ineffective against North American grasshoppers," is particularly unfortunate and potentially misleading because antifeedant reports published by other investigators elsewhere in the world typically involve evaluation of crude, complex neem extracts, not just azadirachtin. Thus, we recommend that azadirex, not azadirachtin, be assessed for insect antifeedant activity.

The usefulness of azadirex for grasshopper management would be greatly enhanced if it effectively deterred feeding. Many grasshopper problems develop not within the crop, but in weedy areas near or distant from the crop, with crop damage occurring at crop margins as immature or adult grasshoppers disperse from depleted or senescing vegetation. The growth regulator component of azadirex toxicity is of limited value in such circumstances because considerable damage occurs before insect death. Many insecticides, particularly pyrethroids, provide rapid control, but even in these cases injury to crop margins can occur. If the crop is of high value or crop appearance is critical, as is the case with many ornamental shrubs, any detectable insect feeding may be unacceptable, and a feeding deterrent would be a particular asset.

The level of feeding inhibition induced by application of azadirex varied among host plants. On cabbage, the least preferred plant, azadirex completely inhibited feeding. On the more preferred hosts, some feeding occurred even on the azadirex-treated foliage. Raffa (1987) reported less feeding inhibition by fall armyworm, *Spodoptera frugiperda* (J.E. Smith), on lima beans (a more preferred host) than on cotton (a less preferred host) treated with azadirachtin (purity undefined). He deduced that the interplay of stimulants and deterrents influenced acceptance of the host plants and noted that even though feeding deterrence was reduced on lima bean, the level of larval mortality was high, thus achieving crop protection.

It was surprising to see the relatively high level of consumption of azadirex-treated orange foliage, and to a lesser extent peregrina, in choice tests, and we initially attributed this high level to the effects of treating highly preferred hosts. However, we determined that the level of acceptance of treated hosts was not directly related to foliage preference. Lettuce, which seems to be highly preferred, was effectively protected from consumption by azadirex in both choice and no-choice tests. Also, orange seems to be less preferred, or at least no more preferred than lettuce, but azadirex-treated orange leaves were con-

sumed at a proportionally higher level than lettuce in choice tests.

The use of leaf area for assessment of consumption could be criticized when comparing among different plant types because it does not account for differences in initial mass. However, other metrics suffer from complications as well. In this case, the initial mass (wet weight) of cabbage and lettuce disks was not significantly different, so we concluded (based on leaf area consumed) that cabbage was less preferred by grasshoppers. Similarly, if dry weight is taken as a more relevant metric for consumption, we see that cabbage did not differ significantly from peregrina and orange, yet the consumption of cabbage (based on leaf area consumed) by the grasshoppers was significantly less. Wet weight and dry weight of orange did not differ significantly from peregrina, so we also concluded that orange was less protected from herbivory by *S. americana*.

Although the stimulants or other characteristics associated with some hosts may offset the antifeedant properties of azadirex, the nature of the insect-plant-feeding deterrent interaction seems to be complex, and the resultant feeding behavior not entirely predictable. Thus, although azadirex significantly reduced feeding in no-choice tests, it seems unlikely that azadirex would provide complete protection from feeding, at least on all host plants.

No evidence of habituation was evident; grasshoppers behaved very similarly on both days of the testing in both choice and no-choice tests. Bomford and Isman (1996) reported rapid desensitization of *Spodoptera litura* (F.) larvae to pure azadirachtin in choice tests, although they similarly found that the insects did not habituate over a 4-d test period to the more complex chemical mixture found in azadirex. Held et al. (2001) found some evidence of habituation by Japanese beetle, *Popillia japonica* Newman, to azadirex-treated foliage after 3 d of exposure. A 3- or 4-d test period is not really relevant in this case due to the short residual nature of the tested product (see below) unless repeated applications were made.

Poor persistence under field conditions is a recurring problem that limits effective use of azadirex, particularly as an antifeedant. The persistence data reported above, and data from other preliminary tests designed to determine optimal sampling times (data not shown), were fairly consistent in their poor level of persistence and high degree of variability among trials. This discouraged us from further field tests. The short period of activity is in line with other studies. Carboni et al. (2002) studied field degradation and determined that the half-life was only 0.8 d, too short to provide good efficacy on olives. Carboni et al. (2002) attributed the loss of efficacy to photodegradation rather than evaporation, thermodegradation, or the chemistry of the plant surface. Interestingly, a commercial formulation of azadirachtin was less photostable than purified azadirachtin, suggesting that formulation needed significant improvement. Johnson and Dureja (2002) also found that some surfactants promoted photodegradation but that they could dou-

ble the photostability of azadirachtin by careful selection of surfactant. Similarly, Sundaram et al. (1997) found a half-life of ≈ 27 h in needles of white spruce, *Picea glauca* (Moench) Voss. Such studies usually report results of work with purified azadirachtin, and consider only chemistry, not insect behavior, so considerable work remains to be accomplished before use of azadirex can be fully optimized. Assessment of soil-applied azadirex, which can be translocated to various plant tissues, suggests enhanced persistence: maximum concentrations in foliage 10 d posttreatment in aspen, *Populus tremuloides* Michx. (Sundaram et al. 1995). Unfortunately, the cost of application is high with soil applications, so the number of practical uses may be limited.

These studies demonstrated that azadirex application can reduce herbivory of plants by grasshoppers. Azadirex can provide antifeedant protection to plants that differ markedly in their innate attractiveness to the insect, although complete protection was not demonstrated. We detected no evidence of rapid habituation to azadirex. The rapid loss of efficacy under field conditions precludes the concern about rapid habituation, but it indicates that daily retreatment would be necessary to maintain complete protection of plants from feeding, and it leaves open the question about habituation after multiple applications over a protracted period. Daily treatment of crops would likely be cost-prohibitive, so UV screens or other materials that reduce azadirex photodegradation should receive further investigation. In some cases, systemic application may provide longer term stability of the active properties of azadirex.

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References Cited

- Aerts, R. J., and A. J. Mordue (Luntz). 1997. Feeding deterrence and toxicity of neem triterpenoids. *J. Chem. Ecol.* 23: 2117–2132.
- Bomford, M. K., and M. B. Isman. 1996. Desensitization of fifth instar *Spodoptera litura* to azadirachtin and neem. *Entomol. Exp. Appl.* 81: 307–313.
- Capinera, J. L. 1993a. Differentiation of nymphal instars in *Schistocerca americana* (Orthoptera: Acrididae). *Fla. Entomol.* 76: 175–179.
- Capinera, J. L. 1993b. Host-plant selection by *Schistocerca americana* (Orthoptera: Acrididae). *Environ. Entomol.* 22: 127–133.
- Carboni, P., M. Cabras, A. Angioni, M. Russo, and P. Cabras. 2002. Persistence of azadirachtin residues on olives after field treatment. *J. Agric. Food Chem.* 50: 3491–3494.
- Champagne, D. E., M. B. Isman, and G. H. N. Towers. 1989. Insecticidal activity of phytochemicals and extracts of the Meliaceae, pp. 95–109. *In* J. T. Arnason, B.J.R. Philogene, and P. Morand [eds.], *Insecticides of plant origin*. Am. Chem. Soc. Symp. 387.
- Held, D. W., T. Eaton, and D. A. Potter. 2001. Potential for habituation to a neem-based feeding deterrent in Japanese beetles, *Popillia japonica*. *Entomol. Exp. Appl.* 101: 25–32.
- Horton, D. R. 1995. Statistical considerations in the design and analysis of paired-choice assays. *Environ. Entomol.* 24: 179–192.
- Isman, M. D. 2004. Factors limiting commercial success of neem insecticide in North America and western Europe, pp. 33–41. *In* O. Koul and S. Wahab [eds.], *Neem: today and in the new millennium*. Kluwer Academic Publishers, Dordrecht, The Netherlands.
- Johnson, S., and P. Dureja. 2002. Effect of surfactants on persistence of azadirachtin-A (neem-based pesticide). *J. Environ. Sci. Health B* 37: 75–80.
- Koul, O. 2004. Neem: a global perspective, pp. 1–19. *In* O. Koul and S. Wahab [eds.], *Neem: today and in the new millennium*. Kluwer Academic Publishers, Dordrecht, The Netherlands.
- Koul, O. 2005. Insect antifeedants. CRC, Boca Raton, FL.
- Langewald, J., and H. Schmutterer. 1992. Effects of neem oil treatment on the phase status of desert locust, *Schistocerca gregaria*, pp. 142–154. *In* C. J. Lomer and C. Prior [eds.], *Biological control of locusts and grasshoppers*. CAB International, Wallingford, United Kingdom.
- Lowery, D. T., and M. B. Isman. 1993. Antifeedant activity of extracts from neem, *Azadirachta indica*, to strawberry aphid, *Chaetosiphon fragaefolii*. *J. Chem. Ecol.* 19: 1761–1773.
- Morgan, E. D. 2004. The place of neem among modern natural pesticides, pp. 21–32. *In* O. Koul and S. Wahab [eds.], *Neem: today and in the new millennium*. Kluwer Academic Publishers, Dordrecht, The Netherlands.
- Mulkern, G. B., and S. Mongolkiti. 1975. Desert locust feeding deterrent ineffective against North American grasshoppers (Orthoptera Acrididae). *Acrida* 4: 95–103.
- Raffa, K. F. 1987. Influence of host plant on deterrence by azadirachtin of feeding by fall armyworm larvae (Lepidoptera: Noctuidae). *J. Econ. Entomol.* 80: 384–387.
- SAS Institute. 2002. SAS release 9.1. SAS Institute, Cary, NC.
- Sundaram, K. M. S., R. Campbell, L. Sloane, and J. Studens. 1995. Uptake, translocation, persistence and fate of azadirachtin in aspen plants (*Populus tremuloides* Michx.) and its effect on pestiferous two-spotted spider mite (*Tetranychus urticae* Koch). *Crop Prot.* 14: 415–421.
- Sundaram, K. M. S., A. Sundaram, J. Curry, and L. Sloane. 1997. Formulation, selection and investigation of azadirachtin—a persistence in some terrestrial and aquatic components of a forest environment. *Pestic. Sci.* 51: 74–90.
- Wilps, H., O. Nasseh, and S. Krall. 1992. The effect of various neem products on the survival and flight activity of adult *Schistocerca gregaria*, pp. 337–346. *In* C. J. Lomer and C. Prior [eds.], *Biological control of locusts and grasshoppers*. CAB International, Wallingford, United Kingdom.

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