ANTIFEEDANT ACTIVITY OF EXTRACTS FROM NEEM, Azadirachta indica, TO STRAWBERRY APHID, Chaetosiphon fragaefolii

D. THOMAS LOWERY¹ and MURRAY B. ISMAN*

Department of Plant Science University of British Columbia Vancouver, British Columbia, Canada V6T 1Z4

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Abstract—Leaf disk choice test bioassays demonstrated that formulated neem seed oil (NSO) was equally deterrent to first- and third-instar nymphs and adult strawberry aphids, *Chaetosiphon fragaefolii* (Cockerell). Concentrations of NSO resulting in 50% feeding deterrence were approximately 1.1% for this species. The rapid disruption of aphid feeding (<1 hr) was not related to the presence of the limonoid azadirachtin, and deterrence likely results from the combined activity of several compounds. Activity to *C. fragaefolii* disappeared within 12–24 hr following application to strawberry in the greenhouse. NSO was deterrent to only half of the six aphid species tested. The antifeedant properties of neem do not appear to contribute significantly to the control of aphids and the viruses they transmit.

Key Words—Antifeedant, *Chaetosiphon fragaefolii*, aphids, Homoptera, Aphididae, azadirachtin, *Azadirachta indica*, deterrent, neem.

INTRODUCTION

Antifeedants offer a novel approach to vector and disease management by rendering plants unattractive or unacceptable to pest insects (Saxena and Khan, 1987). Neem has almost legendary insect repellent and antifeedant properties from its long historical use as a crop protectant in many countries of Asia and Africa (Saxena, 1986). A bioassay based on the antifeedant activity of neem to

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^{*}To whom correspondence should be addressed.

¹Present address: Agriculture Canada Research Station, Summerland, British Columbia, Canada V0H 1Z0

desert locust, Schistocerca gregaria Forsk., led to the isolation and determination of the most active ingredient, the limonoid azadirachtin (AZA) (Butterworth and Morgan, 1968, 1971). Subsequently, the antifeedant activity of neem seed extracts or AZA has been reported for numerous insect pests (Warthen, 1989), exemplified by the variegated cutworm, Peridroma saucia Hübner (Isman et al., 1990); striped, Acalymma vittatum (F.); and spotted, Diabrotica undecimpunctata Barber, cucumber beetles (Reed et al., 1982); green rice leafhopper, Nephotettix virescens (Distant) (Saxena and Khan, 1985); and brown rice planthopper, Nilaparvata lugens (Stäl) (Saxena et al., 1981). Concentrations necessary to significantly deter feeding vary markedly between species, however, and feeding of some insect pests does not appear to be influenced by neem extracts or AZA. For example, AZA offered on sucrose-impregnated filter paper to desert locusts completely inhibited feeding at rates as low as 0.01-0.04 ppm (Butterworth and Morgan, 1968, 1971), whereas the migratory grasshopper, Melanoplus sanguinipes (F.), readily consumed leaf disks of cabbage treated with AZA at rates as high as 500 ppm (Champagne et al., 1989).

The antifeedant activity of neem could have contributed to the control of aphids reported from previous laboratory and field studies (Siddig, 1987; Lowery et al., 1993) and might help reduce the spread of aphid-transmitted plant diseases. Previous studies of the antifeedant activity of neem extracts or AZA to aphids have produced contradictory results. A neem-based product, RD-Repelin, was highly repellent to the pea aphid, *Acyrthosiphon pisum* Harris, at concentrations of 1–10% (Hunter and Ullman, 1992), but extracts of neem seeds failed to deter settling and probing of the green peach aphid, *Myzus persicae* (Sulzer) (Griffiths et al., 1989). In order to effectively protect crops, deterrency of neem to aphids should be consistently achieved at concentrations appropriate for use in the field, i.e., approximately 1% neem seed oil (NSO) (Lowery et al., 1993).

The purpose of the present investigation was to evaluate the antifeedant activity of neem to the strawberry aphid, *Chaetosiphon fragaefolii* (Cockerell) and compare the response of this aphid to that of other species. Studies involving various instars and aphid species should help clarify earlier contradictory reports. In order to clarify which component(s) of neem is responsible for the antifeedant activity to *C. fragaefolii*, deterrency of NSO, AZA, and the major volatile component of neem, di-*n*-propyl disulfide (DNPD), were assessed in leaf disk choice bioassays. Furthermore, to be of practical use, the deterrent activity of neem should persist for several days. Persistence of the deterrent activity of neem to *C. fragaefolii* was therefore evaluated under laboratory and greenhouse conditions.

METHODS AND MATERIALS

Plant Material. Strawberry, *Fragaria X ananassa* Duch., cv Totem; head lettuce, *Lactuca sativa* L., cv Ithaca; sweet pepper, *Capsicum annuum* L., cv California Wonder; broadbean, *Vicia faba* L., cv Windsor long pod; and mus-

tard cabbage, *Brassica chinensis* L., cv Pakchoi, were grown in plastic pots (10 cm diameter) containing a mixture of sandy loam soil and peatmoss (4:1). Pots were placed in a greenhouse with supplemental lighting supplied by sodium vapor lamps (≈ 1500 ft-c), fertilized biweekly with a water-soluble nutrient mix (20:20:20 N:P:K), and irrigated as required. Leaf disks were removed from young, fully expanded leaves of 6-to 8-week-old plants, except for strawberry, which was maintained in permanent plantings.

Leaf Disk Choice Bioassays. Test conditions for the bioassays were adapted from a method for rearing individual apterous *M. persicae* on leaf disks of potato (Lowery and Sears, 1986). Aphids were placed two per disk on four leaf disks (20 mm diameter) cut from leaves with a cork borer and placed in a Petri dish (9 × 50 mm) having 10 small holes in the tight-fitting lids. Dishes were placed upside down on several layers of moist Kimwipe lining the bottom of clear plastic containers, which were held in a growth chamber ($17 \pm 2^{\circ}$ C) under constant, indirect fluorescent light.

For the choice bioassays, two leaf disks treated with the test material and two disks treated with emulsifier only (1.25 ml/liter Triton X-100, BDH Chem-icals, Toronto, Ontario) as a control were allowed to dry and then arranged alternately in each dish with their edges barely touching. Because aphids are very sensitive to the condition of their host plant (van Emden, 1972), the four disks in each dish were excised from the same leaf or leaflet. At concentrations of 0%, all four disks were dipped in emulsifier only, and one pair of opposite disks was randomly assigned to the control treatment. Deterrency of test materials was determined by the proportion of aphids on the treated disks relative to the total number of aphids on treated and untreated disks in each dish.

Deterrent Activity of NSO. To evaluate how rapidly neem affected settling of C. fragaefolii, deterrency of a 40% emulsifiable concentrate (EC) of NSO to adults was assessed for bioassays lasting 1, 3, 6, 24, and 48 hr. Deterrency of NSO was tested at five concentrations (0.0, 0.375, 0.75, 1.5, and 3.0%), and control disks were dipped in emulsifier (Mazon BSF19, Mazer Chemicals, Inc., Gurnee, Illinois) at a concentration equivalent to that in the corresponding NSO treatment. Six replicates (dishes) with eight aphids per dish were used for each time interval and concentration.

Deterrency of neem to first- and third-instar and adult *C. fragaefolii* on strawberry was determined in the manner outlined above, except for the following changes. NSO containing approximately 4000 ppm AZA was tested at rates ranging from 0 to 2% (0.0, 0.25, 0.5, 1.0, 2.0%) with the position of the aphids assessed after 24 hr. The entire experiment was replicated twice, resulting in 12 dishes for each concentration of NSO. Bioassays were also conducted with adult *Fimbriaphis fimbriata* Richards and an unidentified *Chaetosiphon* species [most likely *C. thomasi* (Hille Ris Lambers)] on strawberry, *A. pisum* on broad-

bean, *M. persicae* on pepper, and the lettuce aphid, *Nasonovia ribisnigri* (Mosley), on lettuce.

Determination of Active Principle. The constituent of NSO responsible for the deterrent activity was investigated in 24-hr bioassays with adult *C. fragaefolii* feeding on leaf disks treated with NSO containing variable concentrations of AZA (0.0, 0.25, 0.5, 1.0, and 2.0%), pure AZA (0.0, 62.5, 125.0, 250.0, and 500.0 ppm), or DNPD (Pfaltz and Bauer, Inc., Waterbury, Connecticut) (0, 10, 100, 1000, and 10,000 ppm). According to Balandrin et al. (1988), DNPD is the major volatile component of NSO (76% by weight of headspace volatiles). Six replicates were used for each concentration of AZA and DNPD and 12 for each concentration of every oil.

Persistence of Activity. Persistence of the deterrent effect of NSO (\approx 4,000 ppm AZA) to adult *C. fragaefolii* was determined for bioassays with treated leaf disks maintained in the laboratory and for disks removed from leaflets of strawberry treated in the greenhouse. For the first experiment, leaf disks were treated with NSO (0.0, 0.25, 0.5, 1.0, and 2.0%) and held in Petri dishes under test conditions. Bioassays (24 hr) were then conducted daily for four days using the treated leaf material. On each day, each rate was replicated 12 times.

For the second experiment, one leaflet of an intact strawberry leaf was dipped in NSO (1.0 or 2.0%), while one of the remaining two leaflets was dipped in emulsifier only as a control. Pairs of treated and control leaf disks from an individual leaf were then used for bioassays (24 hr) beginning 0 to 48 hr after the leaf material had dried. Treatments were replicated 10 times in a single trial.

Determination of AZA Concentration. The AZA content of the NSOs (provided by Safer Ltd., Victoria, British Columbia) was determined using reversephase gradient high-performance liquid chromatography (HPLC) (Isman et al., 1990). The HPLC system consisted of a Waters model 840 chromatograph (Millipore Canada Ltd., Waters Chromatography Div., Mississauga, Ontario) with a model 490 multiwave UV detector. A comparative standard of pure AZA (>95%) was supplied by J.T. Arnason (University of Ottawa, Ottawa, Ontario).

Statistical Analysis. Proportions of aphids on treated disks were transformed by $\operatorname{arcsin} \sqrt{x}$ to normalize the variances (Neter et al., 1985). Transformed values were subjected to analysis of variance (ANOVA) (Wilkinson, 1990) and linear regression analysis. Inverse prediction (Neter et al., 1985) was used to determine the effective concentration required to deter 50% of the aphids (EC₅₀) (i.e., when 25% of the aphids remained on the treated disks). The coefficient of determination (R^2) was partitioned, with the values shown being equivalent to those from regression based on the transformed treatment means. Treatment concentrations were transformed, $\ln_e(x + 1)$, as required, to improve linearity; concentrations in experiments with rates exceeding 100 ppm were scaled to improve precision. For the final bioassay to evaluate the persistence of NSO applied to intact plants in the greenhouse, following ANOVA, Fisher's least significant difference test was used to determine differences between treatment means (Wilkinson, 1990).

RESULTS AND DISCUSSION

Deterrent Activity of NSO. Deterrency of NSO (40% EC) to adult C. fragaefolii did not differ for bioassays lasting from 1 to 48 hr, indicating that the response of aphids occurred rapidly, within the first hour (Table 1). EC₅₀ values decreased slightly as the duration of the tests increased from 1 to 24 hr, but the slopes of the regression equations did not differ significantly (P > 0.05) for any of the bioassays. Multiple regression analysis involving both oil concentration and time also demonstrated that duration of the bioassay (time) was not significant (P = 0.968). These results indicate that bioassays lasting 24 hr were sufficiently long to accurately measure the deterrent activity of neem.

In a previous study, AZA applied to wheat seedlings at concentrations of 250 or 500 ppm reduced probing and increased locomotory activity of English grain aphid, *Sitobion avenae* (F.), and bird cherry-oat aphid, *Rhopalosiphum padi* (L.), during the first 25 min of feeding (West and Mordue, 1992). Antifeedant activity of neem seed extract to desert locust occurred after the insects had examined test filter papers impregnated with neem solutions (Butterworth and Morgan, 1971). Although neem deterred feeding in a very rapid manner, it was not repellent, as the insects crawled upon and tasted the treated papers. On the other hand, fewer adult sweet-potato whitefly, *Bemisia tabaci* (Gennadius), landed on cotton treated with neem seed extract (Coudriet et al., 1985), demonstrating that neem was repellent to this insect.

Time (hr)	EC ₅₀ (%NSO)	Slope \pm SE ^{<i>a</i>}	R^2	P (reg.)
1	3.3	-0.187 ± 0.024	0.951	0.027
3	3.1	-0.173 ± 0.066	0.694	0.028
6	2.8	-0.183 ± 0.062	0.743	0.015
24	2.0	-0.280 ± 0.063	0.869	0.004
48	4.8	-0.158 ± 0.036	0.864	0.049

TABLE 1. EFFECTIVE CONCENTRATIONS OF NEEM SEED OIL (NSO) RESULTING IN 50% DETERRENCY (EC₅₀) TO SECOND-INSTAR STRAWBERRY APHID, *Chaetosiphon fragaefolii*, FOR BIOASSAYS LASTING 1–48 h

^aSlopes of regression equations for proportions of aphids on NSO-treated leaf disks vs. \ln_e [NSO] are not significantly different (P > 0.05), based on 95% confidence intervals.

Results of the current bioassays demonstrate that NSO deterred C. fragaefolii in less than 1 hr, but they do not distinguish between repellent and antifeedant activity. Careful observation of M. persicae, N. ribisnigri, and C. fragaefolii on NSO-treated leaf disks, with the aid of a $10 \times$ magnifying lens, indicated that NSO was not repellent to these aphids. During the 20 min of observation, all aphids attempted to probe the treated leaf material at least once (data not shown).

Responsiveness of aphids to volatile substances is generally weak and variable, and most studies have concluded that aphids discriminate between suitable hosts following ingestion of plant fluids (Papaj and Rausher, 1983). Aphids invariably attempt to probe the surface of any substrate they land on (Gibson and Plumb, 1977), and it is during these short test probes that a small amount of fluid is ingested and "tasted" with the precibarial chemosensillae (Backus, 1988).

First- and third-instar and adult *C. fragaefolii* were equally deterred by NSO (Table 2). EC₅₀ values were almost identical, around 1.1% NSO, and slopes of the regression equations were not significantly different (P > 0.05). However, aphid species were not equally deterred by NSO over the range of concentrations tested (Table 2). In addition to *C. fragaefolii*, NSO deterred *A. pisum* (EC₅₀ = 1.7% NSO), and *Chaetosiphon* sp. (EC₅₀ = 2.1% NSO), but it was ineffective against *F. fimbriata*, *M. persicae*, and *N. ribisnigri* (regression P > 0.05).

Previous studies of the deterrent activity of neem to aphids have produced

Instar, species, ^a host plant	EC ₅₀ (%NSO)	Slope \pm SE ^b	<i>R</i> ²	P (reg.)
First, C. fragaefolii, strawberry	1.1	-0.234 + 0.041	0.916	< 0.001
Third, C. fragaefolii, strawberry	1.2	-0.186 ± 0.023	0.957	< 0.001
Adult, C. fragaefolii, strawberry	1.2	-0.206 ± 0.027	0.950	< 0.001
Adult, A. pisum, broad bean	1.7	-0.153 ± 0.037	0.848	0.003
Adult, Chaetosiphon sp., strawberry	2.1	-0.126 ± 0.015	0.961	0.026
Adult, F. fimbriata, strawberry	NS ^c		0.006	0.801
Adult, M. persicae, pepper	NS		0.560	0.128
Adult, N. ribisnigri, lettuce	NS		0.099	0.888

TABLE 2. CONCENTRATIONS OF NEEM SEED OIL (NSO) RESULTING IN 50% DETERRENCY (EC₅₀) TO SIX SPECIES OF APHIDS IN 24-h LEAF DISK CHOICE BIOASSAYS

^a Strawberry aphid, C. fragaefolii; pea aphid, A. pisum; unidentified Chaetosiphon species; green peach aphid, M. persicae; lettuce aphid, N. ribisnigri.

^b Slopes of the regression equations for proportions of aphids on NSO-treated leaf disks vs. [NSO] are not significantly different (P > 0.05), based on 95% confidence intervals.

^cRegression nonsignificant (P > 0.05).

contradictory results. RD-Repelin at concentrations of 1–10% repelled *A. pisum* (Hunter and Ullman, 1992), and AZA at concentrations of 500 ppm deterred settling and probing of *R. padi* and *S. avenae* (West and Mordue, 1992). Choice bioassays demonstrated that NSO containing unknown amounts of AZA applied to broad bean leaves at a concentration of 2.5% deterred 42.4% of adult *A. pisum* compared to controls (Wilkins et al., 1990). Contrary to these findings, Griffiths et al. (1989) showed that extracts of neem seeds failed to deter settling or probing of *M. persicae*. Electronic monitoring of alate *M. persicae* feeding on iceburg lettuce treated with the neem-based insecticide Margosan-O revealed a slight reduction in the total amount of time aphids probed treated plants, but there was no difference in the amount of time salivating, walking, or ingesting phloem compared to controls (Braker et al., 1991). Based on the current findings, these opposing results might be explained by differences in behavioral response between the aphid species. In our trials *A. pisum* was deterred by NSO, whereas *M. persicae* was not.

A high degree of variation in the behavioral responses of insects to neem extracts and AZA has been documented previously. Concentrations of AZA necessary to reduce feeding on leaf disks by 70% ranged from 1 ppm for thirdinstar fall armyworm, Spodoptera frugiperda Smith, on lima bean to 250 ppm for second-instar Colorado potato beetle, Leptinotarsa decemlineata L., on potato (Wood, 1990). Similarly, a 0.01% hexane extract of neem seeds was deterrent to California red scale, Aonidiella aurantii (Maskell), while concentrations of 0.1% and 1.0% were required to deter yellow scale, Aonidiella citrina (Coquillet), and citrus mealybug, Planococcus citri (Risso), respectively (Jacobson et al., 1978). Significantly fewer female brown rice planthoppers and white-backed planthoppers, Sogatella furcifera (Horvath), arrived on rice plants treated with ultra-low volumes of NSO at concentrations of 5-50%, but NSO was not repellent to female green rice leafhoppers (Heyde et al., 1984). Feeding by all three species of homopterans decreased with increasing concentrations of NSO, however, suggesting that the antifeedant and repellent activities of neem are independent.

Determination of Active Principle. Our results show that deterrency of NSO to adult *C. fragaefolii* was not related to the concentrations of AZA. EC₅₀ values for oils with AZA contents ranging from < 50 to 6877 ppm were very similar, and slopes of the regression lines did not differ significantly (P > 0.05) (Table 3), indicating that some other component(s) of NSO contributed to the observed activity.

AZA applied to leaf disks of cabbage was not deterrent to *M. persicae* at rates up to 100 ppm ($R^2 = 0.296$, regression P = 0.198), but it was deterrent to *C. fragaefolii* on strawberry ($R^2 = 0.986$, regression P = 0.001) with an estimated EC₅₀ of 119.5 ppm. At a concentration of 100 ppm, 27% ($\pm 10.5\%$) of *C. fragaefolii* had settled on AZA-treated leaf disks after 24 hr, compared to

AZA, (ppm)	EC ₅₀ (%NSO)	Est. EC ₅₀ (AZA ppm)	Slope \pm SE ^{<i>a</i>}	R^2	Р
< 50	0.96	()	-0.413 ± 0.122	0.791	< 0.001
1084	0.89	(9.6)	-0.341 ± 0.015	0.994	< 0.001
2500	0.80	(20.0)	-0.406 ± 0.025	0.989	< 0.001
4000	0.98	(39.2)	-0.370 ± 0.073	0.895	< 0.001
4700	1.04	(48.9)	-0.353 ± 0.035	0.972	< 0.001
6877	0.86	(59.1)	-0.459 + 0.067	0.939	< 0.001

TABLE 3. CONCENTRATIONS OF NEEM SEED OILS CONTAINING VARIABLE AMOUNTS OF
Azadirachtin (AZA) Resulting in 50% Deterrency (EC ₅₀) to Adult
STRAWBERRY APHID, Chaetosiphon fragaefolii

"Slopes of regression equations for proportions of aphids on NSO-treated leaf disks vs. \ln_e [NSO] are not significantly different (P > 0.05, based on 95% confidence intervals).

49% (\pm 9.9%) at 0 ppm AZA (data not shown). Although AZA may be responsible for some of the deterrency of NSO to aphids, other components of neem likely make a greater contribution to the deterrent activity.

DNPD applied to leaf disks of strawberry at concentrations up to 10,000 ppm did not deter *C. fragaefolii*, and no dose-dependent effect was evident ($R^2 = 0.075$, P = 0.108) (data not shown). According to Balandrin et al. (1988), the presence of volatile organosulfur compounds might partially explain the insect repellent effect of neem leaves and seeds, but purified DNPD, which comprises approximately 76% of the volatile components of neem, does not account for the deterrency of NSO to *C. fragaefolii*.

Choice bioassays with various neem seed extract fractions demonstrated that several components were responsible for the antifeedant activity to the California red scale, yellow scale, citrus mealybug, and woolly whitefly, *Aleurothrixus floccosus* (Maskell) (Jacobson et al., 1978). Schwinger et al. (1984) showed that the neem components salannin and 3-deacetylsalannin were as effective as AZA for the prevention of feeding by Mexican bean beetles, *Epilachna varivestis* Mulsant, with 100% inhibition of feeding occurring at concentrations around 0.01% for all three compounds. In the same study, azadiron, azadiradion, 14-epoxyazadiradion, gedunin, nimbinen, 6-deacetylnimbinien, and melianone were effective deterrents at concentrations 10–100 times higher. Saxena and Rembold (1984) determined that the volatile component of neem seeds repelled adult female cotton bollworms, *Heliothis armigera* (Hübner), prior to contact, whereas neem seed oil was not repellent but did inhibit oviposition on contact. AZA neither repelled female moths nor deterred oviposition.

In light of the large number and types of triterpenoids isolated from neem (e.g., Jones et al., 1989), deterrency of NSO likely results from the combined

activities of several compounds. It is likely that the numerous volatile and nonvolatile components of neem work in concert, producing several behavioral responses that differ in magnitude between insect species.

Persistence of Activity. Deterrency of NSO to C. fragaefolii persisted for at least four days when treated leaf disks of strawberry were held in a growth chamber under low light conditions (Table 4). Although the regression line was not significant for bioassays conducted three days after treatment (P = 0.292), slopes of the remaining regression lines did not differ significantly for tests conducted on days 0-4 (P > 0.05). Estimated EC₅₀ values ranged from 1.2% NSO (day 0) to 1.9% (day 1). Under greenhouse conditions, deterrency of NSO 1% and NSO 2% disappeared after 12 and 24 hr, respectively (Table 5). At 24

TABLE 4. DETERRENCY OF NEEM SEED OIL (NSO) TO ADULT STRAWBERRY APHID, Chaetosiphon fragaefolii, FOR CHOICE BIOASSAYS BEGINNING 0-4 DAYS AFTER TREATMENT OF LEAF DISKS

Day	EC ₅₀ (%NSO)	Slope \pm SE ^a	R^2	Р
0	1.2	-0.206 ± 0.027	0.950	0.001
1	1.9	-0.123 ± 0.033	0.821	0.003
2	1.7	-0.134 ± 0.010	0.983	0.001
3	NS^b		0.084	0.292
4	1.6	-0.112 ± 0.094	0.320	0.020

"Slopes of regression equations for proportions of aphids on NSO-treated leaf disks vs. [NSO] are not significantly different (P > 0.05, based on 95% confidence intervals).

^bRegression nonsignificant, P > 0.05.

 TABLE 5. DETERRENCE OF NEEM SEED OIL (NSO) TO ADULT STRAWBERRY APHID,

 Chaetosiphon fragaefolii, FOR LEAF DISK CHOICE BIOASSAYS BEGINNING 1–48 hr

 AFTER TREATMENT OF STRAWBERRY IN GREENHOUSE^a

Treatment	0 hr	3 hr	6 hr	12 hr	24 hr	48 hr
NSO 0.0%	0.47a	0.40a	0.42a	0.45a	0.47a	0.49a
	(0.09)	(0.19)	(0.07)	(0.10)	(0.11)	(0.11)
NSO 1.0%	0.30b	0.12b	0.18b	0.34ab	0.33a	0.47a
	(0.16)	(0.11)	(0.16)	(0.12)	(0.17)	(0.15)
NSO 2.0%	0.06c	0.21b	0.11b	0.27b	0.34a	0.45a
	(0.04)	(0.13)	(0.06)	(0.18)	(0.21)	(0.09)

"For each time interval, means followed by the same letter are not significantly different (P > 0.05, Fisher's least significant difference test).

hr after treatment, fewer adult *C. fragaefolii* had settled on disks treated with NSO 1% or 2% compared with disks treated with emulsifier only, but the differences were not significant (P > 0.05).

The elevated light intensity and warmer, drier conditions in the greenhouse likely accounts for the rapid loss of deterrency. The rapid decline in activity suggests that neem would have limited value for the prevention of aphid-transmitted plant viruses. Deterrency would likely decline even more rapidly under field conditions.

As with many natural plant products, neem materials readily degrade in the environment (Barnby et al., 1989; Walter and Knauss, 1990). For example, feeding of fall armyworm on corn plants sprayed with 20–100 ppm AZA was significantly reduced for up to seven days when plants were held in the laboratory. After 72 hr in the field, however, AZA residues were no longer deterrent even at application rates as high as 600 ppm (Wood, 1990).

CONCLUSION

Neem extracts have been shown to repel and/or deter several homopteran pests, including leafhoppers and planthoppers on rice (Heyde et al., 1984; Saxena and Khan, 1985), sweet-potato whitefly on cotton (Coudriet et al., 1985), Asiatic citrus psyllid, *Diaphorina citri*, on citrus (Chiu, 1984), and *A. pisum* on broadbean (Hunter and Ullman, 1992).

Systemic application of AZA reduced feeding of *M. persicae* on *Nicotiana clevelandii* Gray, but only at rates of 500–1000 ppm (Woodford et al., 1991). Similarly, West and Mordue (1992) showed that AZA applied to barley at concentrations exceeding 250 ppm reduced probing by *R. padi* and *S. avenae* for up to four days. However, Margosan-O contains approximately 3000 ppm AZA (Larson, 1989), and rates of 8–33% would be required to effectively deter feeding of these species. Margosan-O is recommended at a rate of approximately 1%, or 30 ppm AZA (Larson, 1989; Lindquist et al., 1990).

According to Wood (1990), the commercial significance of the antifeedant activity of neem may be limited. Sensitivity of insects to AZA as an antifeedant appears to vary considerably between species; high concentrations are required to deter feeding by many species; and activity may be short-lived under field conditions. AZA does not appear to be a general inhibitor of insect feeding (Butterworth and Morgan, 1971). Results from our trials with several species of aphids support these statements. At concentrations useful for the control of aphids in the field ($\approx 1.0\%$ NSO) (Lowery et al., 1993), NSO deterred only half the species tested and activity was rapidly lost following applications to plants in the greenhouse.

ANTIFEEDANT ACTIVITY OF EXTRACTS

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REFERENCES

- BACKUS, E.A. 1988. Sensory systems and behaviours which mediate hemipteran plant-feeding: A taxonomic overview. J. Insect Physiol. 34:151-165.
- BALANDRIN, M.F., LEE, S.M., and KLOCKE, J.A. 1988. Biologically active volatile organosulphur compounds from seeds of the neem tree, *Azadirachta indica* (Meliaceae). J. Agric. Food Chem. 36:1048–1054.
- BARNBY, M.A., YAMASAKI, R.B., and KLOCKE, J.A. 1989. Biological activity of azadirachtin, three derivatives, and their ultraviolet radiation degradation products against tobacco budworm (Lepidoptera: Noctuidae) larvae. J. Econ. Entomol. 82:58–63.
- BRAKER, E., TOSCANO, N., REUTER, L., and PERRING, T. 1991. Botanically derived compounds as modifiers of aphid feeding behavior. Poster presentation. Entomological Society of America annual general meeting, Reno, Nevada.
- BUTTERWORTH, J.H., and MORGAN, E.D. 1968. Isolation of a substance that suppresses feeding in locusts. *Chem. Commun.* 23-24.
- BUTTERWORTH, J.H., and MORGAN, E.D. 1971. Investigation of the locust feeding inhibition of the seeds of the neem tree, Azadirachta indica. J. Insect Physiol. 17:969–977.
- CHAMPAGNE, D.E., ISMAN, M.B., and TOWERS, G.H.N. 1989. Insecticidal activity of phyto-chemicals and extracts of the Meliaceae, pp. 95-109, *in* J.T. Arnason, B.J.R. Philogène, and P. Morand (eds.). Insecticides of Plant Origin. ACS Symposium Series No. 387. American Chemical Society, Washington, D.C.
- CHIU, S.-F. 1984. The active principles and insecticidal properties of some Chinese plants, with special reference to Meliaceae, pp. 255-262, in H. Schmutterer and K.R.S. Ascher (eds.). Natural Pesticides from the Neem Tree (*Azadirachta indica* A. Juss.) and other Tropical Plants. Proceedings, 2nd International Neem Conference, Rauischholzhausen, Germany, 1983.
- COUDRIET, D.L., PRABHAKER, N., and MEYERDIRK, D.E. 1985. Sweetpotato whitefly (Homoptera: Aleyrodidae): Effects of neem-seed extract on oviposition and immature stages. *Environ. Ento*mol. 14:776–779.
- GIBSON, R.W., and PLUMB, R.T. 1977. Breeding plants for resistance to aphid infestation, pp. 473– 499, in K.F. Harris and K. Maramorosch (eds.). Aphids as Virus Vectors. Academic Press, New York.
- GRIFFITHS, D.C., PICKETT, J.A., SMART, L.E., and WOODCOCK, C.M. 1989. Use of insect antifeedants against aphid vectors of plant virus disease. *Pestic. Sci.* 27:269-276.
- HEYDE, J. V.D., SAXENA, R.C., and SCHMUTTERER, H. 1984. Neem oil and neem extracts as potential insecticides for control of hemipterous rice pests, pp. 377-390, in H. Schmutterer and K.R.S. Ascher (eds.). Natural Pesticides from the Neem Tree (*Azadirachta indica* A. Juss.) and other Tropical Plants. Proceedings, 2nd International Neem Conference, Rauischholzhausen, Germany, 1983.
- HUNTER, W.B., and ULLMAN, D.E. 1992. Effects of the neem product, RD-Repelin, on settling

behaviour and transmission of zucchini yellow mosaic virus by the pea aphid, Acyrthosiphon pisum (Harris) (Homoptera: Aphididae). Ann. Appl. Biol. 120:9-15.

- ISMAN, M.B., KOUL, O., LUCZYNSKI, A., and KAMINSKY, J. 1990. Insecticidal and antifeedant bioactivities of neem oils and their relationship to azadirachtin content. J. Agric. Food Chem. 38:1406-1411.
- JACOBSON, M., REED, D.K., CRYSTAL, M.M., MORENO, D.S., and SODERSTROM, E.L. 1978. Chemistry and biological activity of insect feeding deterrents from certain weed and crop plants. *Entomol. Exp. Appl.* 24:248–257.
- JONES, P.J., LEY, S.V., MORGAN, E.D., and SANTAFIANOS, D. 1989. The chemistry of the neem tree, pp. 19-46, in M. Jacobson (ed.). Focus on Phytochemical Pesticides, Vol. I. The Neem Tree. CRC Press, Bacon Raton, Florida.
- LARSON, R.O. 1989. The commercialization of neem, pp. 155–168, in M. Jacobson (ed.). Focus on Phytochemical Pesticides, Vol. I. The Neem Tree. CRC Press, Bacon Raton, Florida.
- LINDQUIST, R.K., ADAMS, A.J., HALL, F.R., and ADAMS, I.H.H. 1990. Laboratory and greenhouse evaluations of Margosan-O against bifenthrin-resistant and -susceptible greenhouse whiteflies, *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae), pp. 91–99, *in* J.C. Locke and R.H. Lawson (eds.). Neem's Potential in Pest Management Programs, Proceedings, USDA Neem Workshop. USDA ARS-86.
- LOWERY, D.T., and SEARS, M.K. 1986. Effect of exposure to the insecticide azinphosmethyl on reproduction of green peach aphid (Homoptera: Aphididae). J. Econ. Entomol. 79:1534–1538.
- LOWERY, D.T., ISMAN, M.B., and BRARD, N.L. 1993. Laboratory and field evaluation of neem for the control of aphids (Homoptera: Aphididae). J. Econ. Entomol. 86:864–870.
- NETER, J., WASSERMAN, W., and KUTNER, M.H. 1985. Applied Linear Statistical Models: Regression, Analysis of Variance, and Experimental Designs, 2nd ed. Richard D. Irwin, Inc., Homewood, Illinois, pp. 172–174.
- PAPAJ, D.R., and RAUSHER, M.D. 1983. Individual variation in host location by phytophagous insects, pp. 77-117, in S. Ahmad (ed.). Herbivorous Insects, Host Seeking Behaviour and Mechanisms. Academic Press, New York.
- REED, D.K., WARTHEN, J.D., UEDEL, E.C., and REED, G.L. 1982. Effects of two triterpenoids from neem on feeding by cucumber beetles (Coleoptera: Chrysomelidae). J. Econ. Entomol. 75:1109-1113.
- SAXENA, K.N. and REMBOLD, H. 1984. Orientation and ovipositional response of *Heliothis armigera* to certain neem constituents, pp. 199–210, in H. Schmutterer and K.R.S. Ascher (eds.). Natural Pesticides from the Neem Tree (*Azadirachta indica* A. Juss.) and other Tropical Plants. Proceedings, 2nd International Neem Conference, Rauischholzhausen, Germany, 1983.
- SAXENA, R.C. 1986. Antifeedants in tropical pest management. Insect Sci. Applic. 8:731-736.
- SAXENA, R.C., and KHAN, Z.R. 1985. Electronically recorded disturbances in feeding behavior of Nephotettix virescens (Homoptera: Cicadellidae) on neem oil-treated rice plants. J. Econ. Entomol. 78:222-226.
- SAXENA, R.C., and KHAN, Z.R. 1987. New bioactive products: Growth regulators, antifeedants, pheromones and other attractans. *Mem. Sci. Fisiche Natur. Ser. V.* 11(2):303–317.
- SAXENA, R.C., LIQUIDO, N.J., and JUSTO, H.D. 1981. Neem seed oil, a potential antifeedant for the control of the rice brown planthopper, *Nilaparvata lugens*, pp. 171–188, *in* H. Schmutterer, K.R.S. Ascher, and H. Rembold (eds.). Natural Pesticides from the Neem Tree (*Azadirachta indica* A. Juss.). Proceedings, 1st International Neem Conference, Rottach-Egern, Germany, 1980.
- SCHWINGER, M., EHHAMMER, B., and KRAUS, W. 1984. Methodology of the Epilachna varivestis bioassay of antifeedants demonstrated with some compounds from Azadirachta indica and Melia azedarach, pp. 181–198, in H. Schmutterer and K.R.S. Ascher (eds.). Natural Pesticides

from the Neem Tree (*Azadirachta indica* A. Juss.) and other Tropical Plants. Proceedings, 2nd International Neem Conference, Rauischholzhausen, Germany, 1983.

- SIDDIG, S.A. 1987. A proposed pest management program including neem treatments for combating potato pests in the Sudan, pp. 449-459, *in* H. Schmutterer (ed.). Natural Pesticides from the Neem Tree (*Azadirachta indica* A. Juss) and other Tropical Plants. Proceedings, 3rd International Neem Conference, Nairobi, Kenya.
- VAN EMDEN, H.F. 1972. Aphids as phytochemists, pp. 25–44, in J.B. Harborne (ed.). Phytochemical Ecology. Academic Press, London.
- WALTER, J.F., and KNAUSS, J.F. 1990. Developing a neem-based pest management product, pp. 29-31, in J.C. Locke and R.H. Lawson (eds.). Neem's Potential in Pest Management Programs. Proceedings, USDA Neem Workshop. USDA ARS-86.
- WARTHEN, J.D., JR. 1989. Neem [Azadirachta indica (A. Juss)]: Organisms affected and reference list update. Proc. Entomol. Soc. Wash. 91:367–388.
- WEST, A.J., and MORDUE, A.J. 1992. The influence of azadirachtin on the feeding behaviour of cereal aphids and slugs. *Entomol. Exp. Appl.* 62:75-79.
- WILKINS, R.M., OKELLO-EKOCHU, E., JEEVARATNAM, K., and WECHAKIT, D. 1990. Effects of some feeding inhibitors on two homopteran insect pests *Nilaparvata lugens* and *Acyrthosiphon pisum*, pp. 329–341, *in* J.E. Cassida (ed.). Pesticides and Alternatives: Innovative Chemical and Biological Approaches to Pest Control. Proceedings, International Conference, Orthodox Academy of Crete. Elsevier Press, New York.
- WILKINSON, L. 1990. Systat: The System for Statistics. Systat Inc., Evanston, Illinois, 677 pp.
- WOOD, T. 1990. Efficacy of neem extracts and neem derivatives against several agricultural insect pests, pp. 76-84, *in* J.C. Locke and R.H. Lawson (eds.). Neem's Potential in Pest Management Programs. Proceedings, USDA Neem Workshop. USDA ARS-86.
- WOODFORD, J.A.T., NISBET, A.J., STRANG, R.H.C., and CONNOLLY, J.D. 1991. Systemic antifeedant effects of azadirachtin on aphids, p. 305, *in* D.C. Peters, J.A. Webster, and C.S. Chlouber (eds.). Aphid–Plant Interactions: Population to Molecules. USDA/ARS and Oklahoma State University, Stillwater, Oklahoma.