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Effects of Azadirachtin on Mortality, Growth, and Immunological Function in the Wolf Spider, *Schizocosa episina* (Araneae: Lycosidae)

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Azadirachtin, a triterpenoid of the neem tree, *Azadirachta indica* A. Juss. (Meliaceae), has potent antifeedant and growth disrupting effects on insects (Mordue and Blackwell, 1993). Neem extracts containing azadirachtin and other related triterpene limonoids have been used with a great deal of success in the control of many phytophagous and stored product insects as well as mosquitos. Although there are numerous studies on the effects of azadirachtin on the physiology of molting and feeding behavior of economically injurious insects (see reviews by Schmutterer, 1990; Mordue and Blackwell, 1993), there is little information available on the effects of this compound on predatory insects and spiders. Mansour et al. (1986) showed a relatively low toxicity of neem seed kernel extract when applied topically to the spider, *Chiracanthium mildei.* However, in their study, spiders were exposed to neem extracts through bodily contact with the treated substrate. No attempt was made to assess the oral toxicity of azadirachtin on these spiders.

In view of the important role that spiders can play in the control of insect pests in agroecosystems (Riechert and Lockley, 1984), the present study was undertaken in order to evaluate the effects of azadirachtin on the wolf spider, *Schizocosa episina* (Chamberlain), a cursorial hunting spider commonly found in agroecosystems in the southeastern United States. I studied the effects of this compound on their growth, development, survivorship, and immunological competency. Immunological parameters were investigated because any impairment of immunological function will decrease the fitness of an organism even though their overall adverse effects on survival may take a considerable period of time to manifest themselves. Intermittent studies that focus on survivorship and population dynamics may suggest that a population is thriving while the more subtle physiological effects of a toxic compound go undetected. Arthropods respond to the invasion of micro-organisms and parasites by a variety of immunological reactions, including phagocytosis by selected hemocytes, nodule formation (encapsulation), and humoral mechanisms (Ratcliffe and Rowley, 1979).

MATERIALS AND METHODS

Commercially available neem seed extract containing azadirachtin (Neemix 4.5, 4.5% azadirachtin) was obtained from W.R. Grace and Co. (Columbia, Maryland).

Schizocosa episina is a medium-sized wolf spider commonly found in central Florida. The average adult female size is 48.7 (± 2.5 SE) mg. These lycosids are wandering spiders which actively pursue or ambush their prey. They are generalist predators and feed on a wide variety of insects and other arthropods. Adult female spiders (N = 554) containing egg sacs (N = 105) were collected in Hillsborough Co., Florida, during June and July, 1994, and brought back to the laboratory. Each spider and its egg case was housed separately in an aerated plastic container, and maintained in a Percival Model 80A environmental chamber (21°C, 70% RH, 12L : 12D photoperiod regime). Upon hatching, the spiderlings climb onto the female's abdomen and remain there for 6- 12 days, after which they climb off and begin to disperse. Upon dispersal, the spiderlings were collected and housed individually in plastic containers under the same environmental conditions described above. These adults and spiderlings provided the subjects used in all subsequent experiments.

Spiders were allowed to feed on prey that were injected with varying concentrations of azadirachtin (four treatment groups) immediately before they were presented to a spider. Injections were made on CO_2-anaesthetized prey using a 10-ul Hamilton syringe. Azadirachtin was dissolved in acetone prior to injection. The concentrations of azadirachtin used in these experiments were: 0 (controls), 0.1, 1.0, and 10 ppm as described by Barnby and Klocke (1987). These dosages represent levels commonly used in the treatment of agroecosystems. Control insects were injected only with acetone.

Because these spiders feed on a variety of prey under natural conditions, several different prey species were used throughout these experiments. An additional 200 adult female spiders (50 per treatment group) captured in the field were fed three times per week as follows: one mealworm larva *(Tenebrio molitor*) on Mon.; one juvenile cricket *(Acheta* sp.) on Wed.; followed by one grasshopper nymph *(Schistocerca americana)* on Fri. All prey items were approximately the same size (6.0 ± 0.3 mg). Third to last instar spiderlings (N = 400) were also used, using the same time schedule, on the following prey species (2.3 ± 0.4 mg) : adult fruitflies, *Drosophila pseudoobscura* and *D. virilis* (Carolina Biological Supply, Burlington, NC), and newly hatched crickets *(Acheta* sp.). Adults and spiderlings were maintained on this feeding schedule for a period of 30 days, after which they were fed on prey items that had not been injected with azadirachtin. Data on mortality rates were recorded every fifth day for adults and spiderlings. In addition, growth parameters were assessed

for spiderlings by measuring changes in mass (± 0.1 mg) and carapace width (± 0.1 mm), and the presence or absence of successful molts. These measurements were taken over a 60-day period or until the spiderling died. Only growth data on spiderlings that survived until they reached maturity (90 days) were used in statistical analyses (N = 224). Weight measurements were taken with a Sartorius electronic balance. Carapace width measurements were taken with a Unitron dissecting microscope fitted with an ocular micrometer.

Experiments were also conducted to assess the effects of azadirachtin treatment on hematological and immunological parameters. An additional 20 adult female spiders (experimental group, EG) were injected through the intersegmental membrane between the sternum and coxa of the first leg with a 1-uI suspension of *Bacillus popilliae.* Bacteria were obtained from a stock culture originally isolated from larvae of the Japanese beetle, *Popillia japonica,* and maintained in my laboratory on brain-heart infusion agar at 37°C. Pilot studies have indicated that spiders, like insects, exhibit nodule formation and varying degrees of phagocytosis when innoculated with this and other bacterial pathogens. Control group (CG) spiders (N = 20) were injected with 1.0 uI of sterile medium. The spiders were then allowed to feed on azadirachtin-treated prey (0, 0.1, 1.0, and 10 ppm) as described above for a period of three weeks. At the end of this period, hemolymph was collected from each spider with a 5-uI micropipette as described by Hoffmann (1970). For total hemocyte counts (THC), hemolymph samples were diluted in an anticoagulant solution (0.1 M EDTA, 0.1 M glucose, 0.060 M NaCl, 0.03 M trisodium citrate, 0.03 M citric acid, pH 4.7, 370 mOsmol / l) in the ratio of 1 part hemolymph :9 parts anticoagulant. The sample was then transferred directly to a hemocytometer (Fischer Scientific) for total hemocyte counts (THC) as described by Jones (1967). Differences in mean THC between the four treatment groups were analyzed for statistical significance using a one-way ANOVA and Scheffe F test (Sokal and Rohlf, 1981).

The hemocyte aggregation assay described by Gunnarsson and Lackie (1985) was used to determine whether azadirachtin blocks nodule formation *in vivo .* To summarize, hemolymph was diluted in the anticoagulant solution and strirred gently in a shaker bath to ensure an even distribution of hemocytes. The hemolymph was then examined in a hemocytometer using a Unitron phase-contrast microscope. Only hemocyte aggregates (nodules) > 30 um were scored for analysis.

RESULTS AND DISCUSSION

Ingesting prey injected with 1.0 and 10.0 ppm azadirachtin resulted in significant mortality over the 30-day test period (Table 1). A post-hoc Tukey's test for nonadditivity (Sokal and Rohlf, 1981) showed no significant difference in mortality ($p > 0.50$) between control spiders (0 ppm) and those ingesting prey injected with 0.1 ppm azadirachtin,

Table 1. Cumulative mortality (number) of *Schizocosa episina* spiderlings and adults over a 30-day period caused by the ingestion of prey injected with azadirachtin.

		Injected azadirachtin conc. (ppm)			
	n	0	0.1	1.0	10.0
Spiderlings	100	4	8	25 *	65 **
Adult females	50	2	3	7	18 **

* significantly different from controls (0 ppm) (* $p < 0.05$; ** $p < 0.01$)

regardless of developmental stage. A concentration of 1.0 ppm caused a significant increase in mortality ($p < 0.05$) among spiderlings. Ingestion of prey injected with 10 ppm azadirachtin resulted in significant mortality in both spiderlings and adult females ($p < 0.01$) (Table 1). Although significantly higher mortality rates (50 - 100%) have been reported for some insects reared on diets containing 1.0 - 10.0 ppm of azadirachtin (Arnason et al., 1985; Arpaia and van Loon, 1993), the results of this study indicate that azadirachtin can cause significant mortality in wolf spiders as well. However, available information suggests that there is a wide range of tolerance among spiders toward neem extracts. The wolf spider, *Lycosa pseudoannulata,* an important predator of rice pests, exhibited no mortality when exposed to 100 ug / spider of neem oil extract (Chiu, 1985). Additional studies should be conducted on various species of spiders so that we may better understand the potential impact of neem extracts on these arthropods.

Ingestion of prey treated with azadirachtin had a significant effect ($p < 0.01$, Scheffe F test, Sokal and Rohlf, 1981) on various growth parameters including mass of spiderlings and width of the cephalothorax. Control spiderlings exhibited a mean mass of 9.4 mg (± 0.3 SE) over a 60-day test period as compared to 5.3 (± 0.18 SE) mg and 3.3 (± 0.11 SE) mg for spiders feeding on prey injected with 1.0 and 10 ppm azadirachtin, respectively. Cephalothoracic width was 1.5 (± 0.10 SE) mm in control spiderlings as compared to 0.98 mm (feeding on prey injected with 1.0 ppm azadirachtin), and 0.80 mm (10 ppm) ($p < 0.05$). It has been shown that overall fitness in several species of arachnids is directly related to their adult size (Spiller, 1984; Polis and McCormick, 1987). The ingestion of prey treated with 1.0 and 10 ppm of azadirachtin results in a significant decrease in the size of *S. episina* spiderlings as

reflected by measurements of weight change and carapace width. Smaller spiders are more vulnerable to a wider range of predators and are restricted to the capture of a smaller range of prey items (Punzo, 1989, 1991).

It should be noted that 176 of the original 400 spiderlings died before reaching maturity (44% mortality). Some of these individuals died between molts and others were found dead with partially shed exoskeletons, unable to complete the molting process. Some had partially deformed appendages and palps. Although the ability of neem extracts to inhibit growth and molting in larval insects is well known (Mordue and Blackwell, 1993), its effects on molting in arachnids has not been studied. The pupae of azadirachtin-treated insects often exhibit deformities to the head and thoracic appendages. Future studies should focus on identifying the effects of azadirachtin on the physiology of ecdysis in arachnids.

The ingestion of azadirachtin-treated prey over a three week period caused a significant decrease in the THC of female spiders. Controls exhibited a mean THC count of 37.3×10^3 ($\pm 3.4 \times 10^3$ SE). In contrast, spiders exposed to 1.0 and 10 ppm azadirachtin exhibited a THC count of 27.2×10^3 (4.8×10^3 SE) ($p < 0.05$, Scheffe F test), and 10.7×10^3 ($+ 2.3 \times 10^3$ SE) ($p < 0.01$), respectively. There was no significant difference between control spiders and those feeding on prey injected with 0.1 ppm azadirachtin. When the hemolymph was examined microscopically, scattered nodules were observed floating freely in the hemolymph samples taken from control spiders and those exposed to 0.1 ppm azadirachtin. The mean number of nodules observed per uI of hemolymph for these spiders was 28.7 ± 3.8 SE and 26.9 ± 2.8 SE, respectively. Spiders exposed to higher concentrations of azadirachtin (1.0 and 10.0 ppm), exhibited a significant decrease ($p < 0.01$) in the number of nodules present (9.8 ± 1.6 SE and 4.5 ± 0.8 SE, respectively). Nodules represent groups of hemocytes packed with engulfed bacteria. Nodule formation (encapsulation) is an important part of the immune response to bacterial pathogens in arthropods. Clearly, azadirachtin has a significant adverse effect on the THC and the efficacy of the immune response in *S. episina* . This is the first demonstration of azadirachtin influenced immune reactivity for a spider.

This study indicates that azadirachtin can have deleterious effects on at least one species of beneficial arthropod that may serve as a biological control agent. In *S. episina ,* mortality increases and immunological competency is impaired. In addition, spiderlings are more sensitive to this insecticide than are adult spiders. As a result, spider populations may be depressed in fields treated with azadirachtin.

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REFERENCES

Arnasan JT, Philogene BJ, Donskov N, Hudson M, McDougall C, Fortier G, Morand P, Gardner D, Lambert J, Morris C, Nozzolillo C (1985) Antifeedant and insecticidal properties of azadirachtin to the European Corn Borer, *Ostrinia nubilalis.* Entomologia Exp Appl 38: 29-34

Arpaia S, van Loon JA (1993) Effects of azadirachtin after systemic uptake into *Brassica oleracea* on larvae of *Pieris brassicae.* Entomologia Exp Appl 66:39-45

Barnby MA, Klocke JA (1987) Effects of azadirachtin on the nutrition and development of the tobacco budworm, *Heliothis virescens* (Fabr.) Noctuidae. J Insect Physiol 33:69-75

Gunnarsson SGS, Lackie AM (1985) Hemocyte aggregation in *Schistocerca gregaria* and *Periplaneta americana* as a response to injected substances of microbial origin. J Invert Path 46:312-319

Hoffmann JA (1970) Endocrine regulation of the production and differentiation of hemocytes in an Orthopteran insect. Gen Comp Endocr 15:198-215

Jacobson M (1986) Focus on phytochemical pesticides. Vol. 1, The neem tree, CRC Press, Boca Raton, Florida

Jones JC (1967) Effects of repeated hemolymph withdrawals and of ligaturing the head on hemocyte counts of *Rhodnius prolixus.* J Insect Physiol 13:1351-1360

Mansour FA, Ascher KRS, Omari N (1986) Toxicity of neem seed kernel extracts prepared with different solvents on the spider, *Chiracanthum mildei.* Phytoparasitica 14:73-76

Mordue AJ, Blackwell A (1993) Azadirachtin : An update. J insect Physiol 39:903-924

Polis GA, McCormick SJ (1987) Intraguild predation and competition among desert scorpions. Ecology 68:332-343

Punzo F (1989) Effects of hunger on prey capture and ingestion in *Dugesiella echina* Chamberlain (Orthognatha, Theraphosidae). Bull Br Arachnol Soc 8:72-79

Punzo F (1991) Field and laboratory observations on prey items taken by *Lycosa lenta* (Araneae, Lycosidae) Bull Br Arachnol Soc 8: 261-264

Ratcliffe NA, Rowley AF (1979) Role of hemocytes in defense against biological agents. In: Insect hemocytes, development, form, function and techniques. Cambridge University Press, Massachusetts, pp. 57-89.

Riechert SE, Lockley T (1984) Spiders as biological control agents. Annu Rev Entomol 29:299-320
Schmutterer H (1990) Properties and potential of natural pesticides from the neem tree, *Azadirachta indica* . Annu Rev Entomol 35:271-297
Sokal RR, Rohlf FJ (1981) Biometry, 2nd ed. WH Freeman, New York
Spiller DA (1984) Competition between two spider species: Experimental field study. Ecology 65:909-919