

## Repellency and Toxicity of Azadirachtin and Neem Concentrates to Three Stored-Product Beetles

Y. S. XIE, P. G. FIELDS,<sup>1</sup> AND M. B. ISMAN<sup>2</sup>

Winnipeg Research Centre, Agriculture and Agri-Food Canada, 195 Dafoe Road, Winnipeg, MB R3T 2M9 Canada

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**ABSTRACT** Repellency and toxicity of azadirachtin (98% AZA, which contains 98% azadirachtin) and 3 neem extracts (48, 23, and 7% AZA) to 3 stored-product insects, the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens), the rice weevil, *Sitophilus oryzae* (L.), and the red flour beetle, *Tribolium castaneum* (Herbst), were investigated in the laboratory. Each test material repelled all 3 species in a standard repellency test using a food preference apparatus. Significant negative correlations were found between insect settling response and extract concentrations. *T. castaneum* was more sensitive to the repellent action of neem than the other 2 species. The test materials were also toxic to the 3 pest species, with *C. ferrugineus* being the most susceptible. Six-week LC<sub>50</sub> values for 48, 23, and 7% AZA for *C. ferrugineus* were 18.8, 37.0, and 127.3 ppm, respectively. The F<sub>1</sub> adults of all 3 insect species in almost all treatments were significantly reduced compared with controls. This reduction was significantly dose dependent. The relationship between bioactivity of neem materials and their azadirachtin content was established and is discussed. We confirmed that azadirachtin was largely responsible for both repellent (behavioral) and toxic (physiological) actions of neem on stored-product insects. However, the neem extracts are slightly more active than pure azadirachtin when applied at equivalent azadirachtin concentrations, indicating that azadirachtin is not the only active compound in neem.

**KEY WORDS** *Cryptolestes*, *Sitophilus*, *Tribolium*, natural products

ANNUAL POSTHARVEST LOSSES caused by insect damage, microbial deterioration, and other factors are estimated to be in the order of 10–25% worldwide (Matthews 1993). Synthetic pesticides are currently the method of choice to protect grain from insect damage, however, their widespread use has led to the development of pest strains resistant to insecticides (Champ and Dyte 1976, Zettler and Cuperus 1990, White 1995). Also, there is a demand for safer insecticides because of concerns about insecticide residues on grain and health hazards to grain handlers. Alternatives for stored-product protection to replace synthetic chemical insecticides are highly desirable.

Plants are a rich source of compounds that have insecticidal activity (Arnason et al. 1989). The effectiveness of many plant derivatives against stored-product insects has already been demonstrated (Su 1977, 1990; Malik and Naqvi 1984; Delobel and Malonga 1987; Weaver et al. 1991; Khaire et al. 1992; Hu 1993; Xie et al. 1995). Among these, extracts of the neem tree, *Azadirachta indica* A. Juss, have received the most attention (Jacobson 1983, Saxena et al. 1988). It has been an age-old practice in rural India to mix dried neem

leaves with stored grain to control stored-product insects. Data are available describing the bioactivity of neem against a broad spectrum of insects, including stored-product insects (Saxena et al. 1988, Koul et al. 1990, Schmutterer 1990, Mordue [Luntz] and Blackwell 1993).

The major active constituent in neem is the limonoid, azadirachtin. Azadirachtin is well known for its antifeedant, toxic, and growth regulating effects on insects (reviewed in Saxena 1989, Schmutterer 1990, Mordue [Luntz] and Blackwell 1993). Despite considerable efforts to synthesize azadirachtin (Ley et al. 1987, 1993; Simmonds et al. 1990), its extreme structural complexity makes synthesis on a commercial scale uneconomical and unlikely in the near future. A variety of preparations based on neem extracts have been tested against stored-product insects. A mixture of 1–2% neem seed powder with wheat kernels provided 9–12 mo protection against an external grain feeder, the khapra beetle, *Trogoderma granarium* Everts, and 2 internal grain feeders, the rice weevil, *Sitophilus oryzae* (L.), and the lesser grain borer, *Rhyzopertha dominica* (F.) (Jotwani and Sircar 1965). Paper strips dipped in 30% neem seed extract solutions were highly repellent to the larvae of the Mediterranean flour moth, *Anagasta kuhniella* (Zeller) (Roomi and Atiquiddin 1977). The application of crude neem oil and Margosan-O (W. R. Grace, Co-

<sup>1</sup>To whom correspondence should be addressed.

<sup>2</sup>Department of Plant Science, University of British Columbia, Vancouver, BC V6T 1Z4.

lumbia, MD), a commercial neem-based insecticide, on filter paper strips at 200, 400, or 800  $\mu\text{g}/\text{cm}^2$  reduced adult feeding of the red flour beetle, *Tribolium castaneum* (Herbst), and the lesser grain borer (Jilani et al. 1988, Jilani and Saxena 1990). A significant reduction in survival of parent and  $F_1$  generations of *S. oryzae* was observed when insects were exposed to wheat treated with Margosan-O (Dunkel et al. 1990).

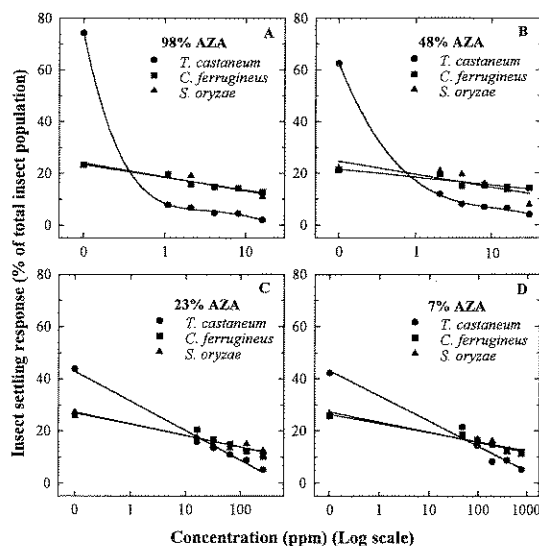
However, with the exception of Margosan-O, the azadirachtin content of neem materials used by previous researchers is unknown, making it extremely difficult to compare results. For stored-product insects, there is a lack of direct evidence showing that azadirachtin is the principal active constituent in neem. The objectives of this study were to evaluate repellent, toxic, and reproduction-inhibiting effects of azadirachtin-rich neem powders and pure azadirachtin against 3 stored-product insect pests, and to determine the efficacy of neem materials and their relationship to azadirachtin content.

### Materials and Methods

**Insects.** Three species of insects, rusty grain beetle, *Cryptolestes ferrugineus* (Stephens), rice weevil, *S. oryzae*, and red flour beetle, *T. castaneum*, were used in this study. All adults were obtained from laboratory cultures maintained at  $30 \pm 1^\circ\text{C}$  and  $70 \pm 5\%$  RH. *C. ferrugineus* was reared on Canada Western Hard Red Spring Wheat ('Katepwa') at 16% moisture content, mixed with 5% cracked wheat (wt:wt). The 2 other species were reared on the same medium at 14% moisture content. All species were kept in laboratory culture for >3 yr.

**Test Materials.** Dry neem concentrates were obtained from ITC, Hyderabad, India (48% AZA, which contains 48% azadirachtin), and from Neemoil Australia, Lismore, Australia (23 and 7% AZA). Azadirachtin contents were measured by high-performance liquid chromatography (HPLC) (Isman et al. 1990). Azadirachtin (98% AZA) (purity based on FAB-mass spectroscopy) was provided by J. T. Arnason, University of Ottawa, Ottawa, Canada.

**Repellency.** All neem extracts and azadirachtin were dissolved in methanol (MeOH) for stock solutions, and diluted with distilled water to provide the required concentrations as follows: 1–16 ppm for 98% AZA, 2–32 ppm for 48% AZA, 16–256 ppm for 23% AZA, and 48–768 ppm for 7% AZA. An appropriate amount of MeOH was used as a control, and 6 concentrations (including control) were prepared for each test material. Triton X-100 (Sigma, St. Louis, MO) served as an emulsifier at a concentration of 0.1%. Sixteen millilitres of aqueous solution were applied with a small atomizer (68011 Spra-Tool Power Pak, Crown, Whitby, ON) to 800 g of wheat kernels spread on a tray. The treated wheat was rotated on a roller (Chemical



**Fig. 1.** Settling response of adult *C. ferrugineus*, *S. oryzae*, and *T. castaneum* on wheat grains treated with azadirachtin (98% AZA) (A) and neem concentrates: 48% AZA (B), 23% AZA (C), and 7% AZA (D). Standard errors for all mean values are <5%.

Process Products, Akron, OH) for 30 min to obtain a uniform distribution of the compounds. Initial moisture content of the wheat was 13%, and the final moisture content was 15%.

Food preference chambers (Loschiavo 1952) were used to conduct a multiple choice bioassay to test the repellency of neem materials to the insects. The brass chamber was cylindrical (6 cm height, 30 cm diameter), with a raised arena (2 cm height, 10 cm diameter) in the center of the chamber. It was divided into 6 equal sections by brass partitions. Six 200-g portions of treated wheat kernels with different concentrations of neem were filled randomly into each section. Two hundred unsexed adult beetles (1–2 wk old) were introduced into the center of arena, confined by a brass ring (2.5 cm height, 5 cm diameter), and a circular black-painted plate was used to cover the top of the chamber. After 1 h of confinement to allow beetles to return to normal activity, the beetles were released by raising the brass ring. The chamber was kept at  $30 \pm 1^\circ\text{C}$  and  $70 \pm 5\%$  RH for 24 h. At the end of 24 h, which is sufficient time for beetle numbers to stabilize (Y.S.X. and P.G.F., unpublished data), the contents of each sector were vacuumed and the number of insects in each was determined. The entire experiment was repeated 4 times.

**Toxicity.** Wheat kernels used in the repellency tests were held at  $-35^\circ\text{C}$  for at least 3 h to kill any eggs laid during the multiple choice tests. Two sets of 5 glass vials (7 cm height, 2.7 cm diameter), 20 g of wheat per vial, were prepared for each treatment. Ten unsexed adult beetles (1–2 wk old) were

**Table 1. Relationship between insect settling response and concentration of neem materials**

% AZA	<i>C. ferrugineus</i>					<i>S. oryzae</i>					<i>T. castaneum</i>				
	Intercept	Slope	r <sup>2</sup>	P	n	Intercept	Slope	r <sup>2</sup>	P	n	Intercept	Slope	r <sup>2</sup>	P	n
98	18.44	-4.99	0.95	0.0009	6	18.57	-5.34	0.91	0.0033	6		<sup>a</sup>	0.98	0.0002	6
48	18.54	-3.13	0.82	0.0126	6	19.66	-5.04	0.70	0.0388	6		<sup>b</sup>	0.99	0.0001	6
23	22.69	-4.47	0.90	0.0040	6	22.52	-4.36	0.96	0.0007	6	31.52	-11.39	0.99	0.0001	6
7	22.77	-3.51	0.96	0.0006	6	23.36	-3.89	0.95	0.0010	6	33.41	-9.64	0.97	0.0004	6

Linear regression was performed between log<sub>10</sub>[concentration + 0.1] and insect settling response (percentage of total insect population), except 98 and 48% AZA versus *T. castaneum*, for which polynomial regression was performed (SigmaStat 1994).  
<sup>a</sup> Polynomial regression equation:  $y = 14.51 - 35.59x + 23.23x^2$ , where  $y$  = insect settling response (% of total population),  $x = \log_{10}[\text{concentration} + 0.1]$ .  
<sup>b</sup> Polynomial regression equation:  $y = 21.52 - 28.63x + 12.07x^2$ , where  $y$  = insect settling response (% of total population),  $x = \log_{10}[\text{concentration} + 0.1]$ .

introduced into each vial for the 1st set of vials (5 replicates). After 2 wk at 30°C and 70% RH, adult beetles were removed and placed in the 2nd set of vials with the wheat treated at the same concentration. Mortality of adult beetles was determined after 2, 4, and 6 wk. The 1st set of vials was held in the incubator for an additional 5 wk before counting F<sub>1</sub> adults.

**Data Analysis.** Linear regression was conducted to define all dose-response relationships when correlations between dose and test parameters (settling response, mortality, and number of offspring) were found to be significant (Anonymous 1991). A logarithmic transformation [ $\log_{10}(x + 0.1)$ , where  $x$  = concentration in ppm] was performed before regression analysis. Analysis of covariance (ANCOVA) (Zar 1984) and analysis of variance (ANOVA) (Anonymous 1991) was performed to test equality of regression coefficients and differences of means in Fig. 1. The EC<sub>50</sub> values (effective concentrations of neem materials required to reduce F<sub>1</sub> adults by 50% relative to controls) (Table 2) were determined by regression-prediction analysis (Anonymous 1991). Probit analysis (Finney 1971) was applied to determine the lethal concentration

of neem concentrates causing 50% mortality (LC<sub>50</sub>) for *C. ferrugineus* (Table 3). Abbott's formula (Abbott 1925) was used to correct for control mortality (which was <14%.) before probit analysis.

**Results and Discussion**

**Repellency.** All test materials repelled the 3 insect species in a dose-dependent manner (Fig. 1). Settling response of all 3 insect species was significantly ( $P < 0.05$ ) negatively correlated with concentration for all test materials (Fig. 1) (Table 1). Regression slopes and intercepts of all 4 test materials for *C. ferrugineus* and *S. oryzae* were not significantly different (ANCOVA for slopes, 98% AZA:  $t = 0.02$ ,  $df = 8$ ,  $P > 0.5$ ; 48% AZA:  $t = 0.11$ ,  $df = 8$ ,  $P > 0.5$ ; 23% AZA:  $t = 0.01$ ,  $df = 8$ ,  $P > 0.5$ ; 7% AZA:  $t = 0.03$ ,  $df = 8$ ,  $P > 0.5$ ), suggesting that both *C. ferrugineus* and *S. oryzae* responded to the repellent action of neem materials in a very similar fashion. In contrast, *T. castaneum* responded to the repellent action of all test materials differently compared with *C. ferrugineus* and *S. oryzae*. With 98 and 48% AZA, significant polynomial regressions were found between set-

**Table 2. Number of F<sub>1</sub> adults developing in wheat kernels treated with azadirachtin and neem concentrates**

Concn, ppm (azadirachtin equivalent)	98% AZA			48% AZA			23% AZA			7% AZA					
	No. offspring			No. offspring			No. offspring			No. offspring					
	<i>C. ferrugineus</i>	<i>S. oryzae</i>	<i>T. castaneum</i>	<i>C. ferrugineus</i>	<i>S. oryzae</i>	<i>T. castaneum</i>	<i>C. ferrugineus</i>	<i>S. oryzae</i>	<i>T. castaneum</i>	<i>C. ferrugineus</i>	<i>S. oryzae</i>	<i>T. castaneum</i>			
0 (0)	9.8	134.2	32.8	0 (0)	7.6	105.2	42.8	0 (0)	11.4	209.8	46.2	0 (0)	10.6	209.8	30.0
1 (0.98)	8.2	122.0	23.6	2 (0.96)	2.0	80.6	22.4	16 (3.68)	5.6	162.8	18.8	48 (3.36)	2.6	192.0	14.6
2 (1.96)	4.4	121.4	15.4	4 (1.92)	1.8	73.2	17.6	32 (7.36)	1.2	125.4	8.4	96 (6.72)	1.6	157.2	10.0
4 (3.92)	2.4	78.8	13.4	8 (3.84)	1.2	82.6	12.8	64 (14.72)	0.8	93.8	8.2	192 (13.44)	1.2	159.0	3.0
8 (7.84)	1.8	104.2	13.0	16 (7.68)	1.8	66.0	7.0	128 (29.44)	0.6	99.8	1.8	384 (26.88)	0.4	107.6	0.2
16 (15.68)	1.6	88.6	3.6	32 (15.36)	1.0	46.0	2.2	256 (58.88)	0.4	95.4	0.2	768 (53.76)	0.2	85.8	0.0

Regression was performed between log<sub>10</sub>[concentration + 0.1] and number of F<sub>1</sub> adults (see Table 3).

Table 3. Regression parameters for number of F<sub>1</sub> adults developing in wheat kernels treated with azadirachtin and neem concentrates, data from Table 2

	98% AZA				48% AZA				23% AZA				7% AZA			
	No. offspring		T. cast-aneum		No. offspring		T. cast-aneum		No. offspring		T. cast-aneum		No. offspring		T. cast-aneum	
	<i>S. oryzae</i>	<i>C. ferrugineus</i>	<i>S. oryzae</i>	<i>C. ferrugineus</i>	<i>S. oryzae</i>	<i>C. ferrugineus</i>	<i>S. oryzae</i>	<i>C. ferrugineus</i>	<i>S. oryzae</i>	<i>C. ferrugineus</i>	<i>S. oryzae</i>	<i>C. ferrugineus</i>	<i>S. oryzae</i>	<i>C. ferrugineus</i>	<i>S. oryzae</i>	<i>C. ferrugineus</i>
<i>n</i>	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
Intercept	115.8	21.3	87.6	27.1	6	6	6	6	179.4	32.5	6	6	199.1	32.5	6	6
Slope	-21.9	-12.5	-20.2	-16.2	-3.5	-3.5	-3.5	-3.5	-36	-13.9	-36	-36	-27.2	-13.9	-36	-36
R <sup>2</sup> -value	0.63	0.94	0.83	0.99	0.93	0.93	0.93	0.93	0.89	0.98	0.89	0.89	0.64	0.98	0.64	0.64
P-value	0.0055	0.0012	0.0085	0.0001	0.0021	0.0021	0.0021	0.0021	0.0047	0.0001	0.0047	0.0047	0.0055	0.0001	0.0055	0.0055
EC <sub>50</sub> (ppm) <sup>a</sup>	34.0	2.5	34.7	2.2	5.1	5.1	5.1	5.1	97.5	4.9	97.5	97.5	704.7	4.9	704.7	704.7
Azadirachtin equivalent	33.32	2.45	16.66	1.06	1.17	1.17	1.17	1.17	22.43	1.13	22.43	22.43	49.33	1.13	49.33	49.33

Regression was performed between log<sub>10</sub>[concentration + 0.1] and a number of F<sub>1</sub> adults.  
<sup>a</sup> EC<sub>50</sub> represents effective concentration resulting in 50% reduction of F<sub>1</sub> adults relative to controls.

ting response of *T. castaneum* and concentration (Table 1), and >60% of the total insect populations were found in the control section alone, significantly more than in any other sections treated with the neem materials (ANOVA, 98% AZA:  $F = 398.56$ ;  $df = 5, 18$ ;  $P \leq 0.0001$ ; 48% AZA:  $F = 115.30$ ;  $df = 5, 18$ ;  $P \leq 0.0001$ ) (Fig. 1). With 23% AZA and 7% AZA, significant linear relationships were also found between insect settling response and concentration (Table 1). However, ANCOVA indicated that regression slopes and for this species were significantly greater than that for the other 2 species (23% AZA:  $t = 4.23$ ,  $df = 8$ ,  $P < 0.001$ ; 7% AZA:  $t = 3.86$ ,  $df = 8$ ,  $P < 0.001$ ), suggesting that *T. castaneum* adults were more sensitive to the repellent action of neem than the other 2 species.

An insect repellent has been defined as a chemical substance that causes the insect to make oriented movements away from the source (Dethier et al. 1960). The strong repellency of azadirachtin and neem concentrates in this study was reflected by reduced numbers of insects on treated wheat (Fig. 1). This reduction is presumably caused by chemosensory effects of neem, either olfactory or gustatory. Numerous studies have been conducted to determine the repellent activity of neem materials against stored-product insects (Saxena et al. 1988, Mordue [Luntz] and Blackwell 1993), but most studies either lacked standardized techniques or used only 1 or 2 concentrations, which do not verify a dose-dependent response. Our study, using a standard technique, demonstrated that insects could detect small differences when food was treated with neem ( $\leq 1$  ppm azadirachtin), and respond to the repellent action of neem in a dose-dependent manner.

**Toxicity.** All 4 test materials were toxic to the 3 species, although the toxic action was slow and the efficacy varied among species. *C. ferrugineus* was the most susceptible (Fig. 2), followed by *S. oryzae* (Fig. 3), with *T. castaneum* being the least susceptible (Fig. 4). Good control (>95% mortality) was obtained only for *C. ferrugineus* after 6 wk at 256 ppm of 23% AZA and 768 ppm of 7% AZA (Fig. 2). In general, mortality for *S. oryzae* and *T. castaneum* was below 40% (Figs. 3 and 4). Both *C. ferrugineus* and *S. oryzae* had increased mortality with time (Figs. 2 and 3), but *T. castaneum* did not (Fig. 4).

Dunkel et al. (1990) observed >70% mortality for adult *S. oryzae* exposed for 2 wk to wheat treated with the neem-based insecticide Margosan-O at a concentration of 0.2% (6 ppm azadirachtin). In contrast, our study showed that *S. oryzae* was far less sensitive to neem (Fig. 3). We conducted preliminary tests with Margosan-O at a similar concentration to Dunkel et al. (1990), and obtained only 6% mortality with *S. oryzae* (Y.S.X., R.P. Bodnaryk, and P.G.F., unpublished data). Some possible reasons for these differences are insect strains, insect ages (0–3 days versus 7–14 d), test conditions (27°C and 65% RH versus 30°C and 65%

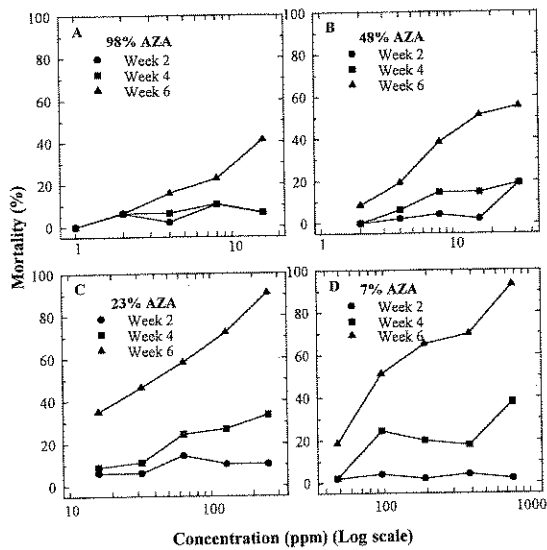


Fig. 2. Mortality of adult *C. ferrugineus* held on wheat grains treated with azadirachtin (98% AZA) (A) and neem concentrates: 48% AZA (B), 23% AZA (C), and 7% AZA (D). Standard errors for all mean values are <10%.

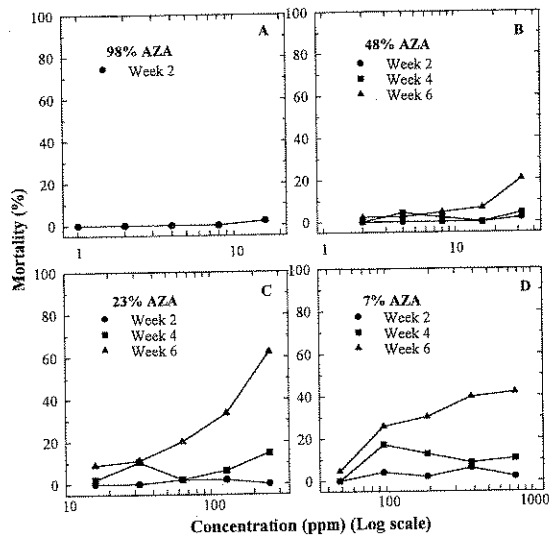


Fig. 3. Mortality of adult *S. oryzae* held on wheat grains treated with azadirachtin (98% AZA) (A) and neem concentrates: 48% AZA (B), 23% AZA (C), and 7% AZA (D). Standard errors for all mean values are <10%.

RH), or test material (Margosan-O). A comparative evaluation of repellency and toxicity of several commercial neem-based insecticides against stored-product insects under identical test conditions is underway.

Antifeedant effects of neem on stored-product insects have been extensively investigated (Saxena et al. 1988). Treating stored grain can disrupt insect feeding by making the treated materials unattractive or unpalatable, and as a consequence, insect growth, survival, and reproduction are adversely affected (Norris 1986, Saxena 1987). It has been shown that azadirachtin is toxic to the Mexican bean beetle, *Epilachna varivestis*, only if applied at concentrations of >1,000 ppm (Rembold 1989). In our study, the observed mortality is likely a consequence of antifeedant (starvation) effect, rather than toxic action. Topical application of azadirachtin would have clearly distinguished toxicity from secondary mortality resulting from starvation. Specific bioassays are needed to separate these effects.

**Reduction of Offspring.** There was a great reduction of offspring for all 3 insect species in almost all treatments compared with the controls and this reduction was significantly dose dependent ( $P < 0.05$ ) (Tables 2 and 3). Both *C. ferrugineus* and *T. castaneum* showed a similar reduction in offspring due to neem extracts, with  $EC_{50}$  values ranging between 2.0 and 12.7 ppm for the various test materials (Table 3). *S. oryzae* was much less sensitive to the test materials with  $EC_{50}$  values between 34.7 and 704.7 ppm.

There are a number of factors that could explain the differences in  $F_1$  progeny production of the 3

species. *S. oryzae* eggs and larvae are only found inside the seeds, and hence, are not exposed to neem extracts. In contrast, *C. ferrugineus* and *T. castaneum* eggs can be laid on the surface of grain kernels, and larvae move freely through the grain mass. The eggs and newly hatched larvae of these insects are directly exposed to neem. Also, neem is known to have adverse effects on ovarian devel-

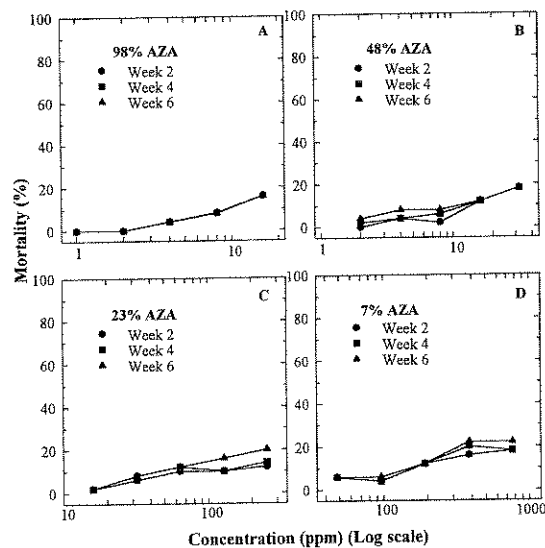


Fig. 4. Mortality of adult *T. castaneum* held on wheat grains treated with azadirachtin (98% AZA) (A) and neem concentrates: 48% AZA (B), 23% AZA (C), and 7% AZA (D). Standard errors for all mean values are <10%.

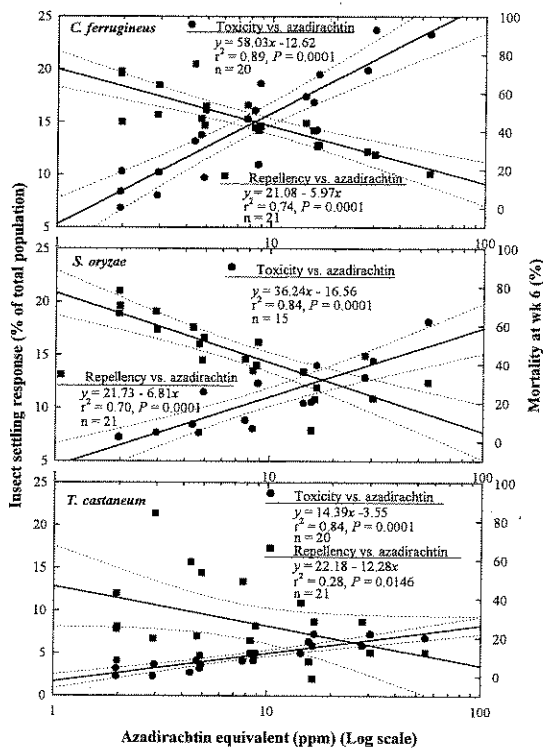


Fig. 5. Relationship between bioactivity (repellency and toxicity, data from Figs. 1–4) of neem materials and their azadirachtin contents. Dotted lines represent 95% CI.

opment, fecundity, and fertility (reviewed by Karnavar 1987), hence the effects on F<sub>1</sub> progeny we observed could be caused by inhibition of egg laying. Adult mortality during the 1st 2 wk could not explain the differences in F<sub>1</sub> progeny as *T. castaneum* had the lowest adult mortality, yet the F<sub>1</sub> progeny was affected more than *S. oryzae*. As unsexed adults were used, there is a slight possibility that the differences could be explained by differences in the number of females placed in the various treatments. Further tests are needed to differentiate these possible modes of action.

**Relationship Between Bioactivity and Azadirachtin Content.** Isman et al. (1990) found that 72–90% of the variation in bioactivity of neem oils against the variegated cutworm, *Peridroma saucia* Hübner, can be accounted for by azadirachtin con-

tent, and concluded that azadirachtin is the major active ingredient against *P. saucia* in neem seed oils. In this study, we have demonstrated and confirmed that azadirachtin is largely responsible for both repellent (behavioral) and toxic (physiological) activities of neem materials against 3 stored-product insect pests. The basis for this conclusion is the strong correlations between bioactivity (repellency and toxicity) of neem materials and their azadirachtin concentrations (Fig. 5). More than 70% of the variation in repellency and over 80% in toxicity of the neem materials tested (except repellency versus *T. castaneum*) could be accounted for by variation in azadirachtin content (Fig. 5). This close relationship was also reflected by similar LC<sub>50</sub> values (9.0, 8.5, and 8.9 ppm in terms of azadirachtin concentrations) for 48, 23, and 7% AZA for *C. ferrugineus* (Table 4).

However, at similar azadirachtin concentrations (7–8 ppm), refined neem concentrates were significantly more toxic than pure azadirachtin (ANOVA,  $F = 8.77$ ;  $df = 3, 16$ ;  $P = 0.0011$ ) (*C. ferrugineus* mortality at week 6: 51, 47, and 51% for 7, 23, and 48% AZA, respectively, versus 23% for 98% AZA). There are several other chemicals in neem extracts that have been shown toxic to insects (Ley et al. 1993, Mordue [Luntz] and Blackwell 1993), which could cause this additional mortality. The use of neem extracts as an insecticide is an advantage because the isolation of azadirachtin is difficult.

The chemical complexity of azadirachtin and the diverse structural requirements for insect bioactivity restrict the synthesis of this molecule, and, therefore, commercial neem products will depend on neem seed extracts. However, azadirachtin content in neem extracts shows considerable variability, depending on their geographic origin (Ermel et al. 1987). Azadirachtin content is highly correlated with both behavioral and physiological effects of neem extracts on lepidopterans (Isman et al. 1990) and on the stored-product coleopterans we tested. Isman et al. (1990) suggested that azadirachtin content should be used as a quality-control criterion for the formulation of neem-based botanical insecticides. Our results suggest that neem treatments containing  $\geq 50$  ppm azadirachtin should provide good control of *C. ferrugineus* by 6 wk, but would not be sufficient to control adult *S. oryzae* and *T. castaneum*. However, offspring of *C. ferrugineus* and *T. castaneum* could be com-

Table 4. Toxicity of neem concentrates to *C. ferrugineus*

% AZA	n	LC <sub>50</sub> <sup>a</sup> (95% CI) (ppm)		Slope ± SEM	Intercept	$\chi^2$	P
		Actual material	Azadirachtin equivalents				
48	250	18.8 (10.0–35.5)	9.0 (4.8–16.9)	1.24 ± 0.21	3.42	2.49	<0.01
23	250	37.0 (21.8–62.6)	8.5 (5.0–14.4)	1.30 ± 0.21	2.96	1.77	<0.01
7	250	127.3 (84.8–191.2)	8.9 (5.9–13.4)	1.65 ± 0.22	1.54	5.94	<0.05

<sup>a</sup> LC<sub>50</sub> represents lethal concentration for 50% mortality at week 6.

pletely inhibited and, thus, populations could be significantly reduced at this level.

Finally, azadirachtin, at levels as low as 10 ppm, repelled the 3 species studied. Grain treated with neem extracts could have lower insect populations because of reduced immigration. This needs to be verified under field conditions.

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