Effects of Neem Seed Derivatives on Behavioral and Physiological Responses of the *Cosmopolites sordidus* (Coleoptera: Curculionidae)

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ABSTRACT Both in a choice and multi-choice laboratory tests, fewer adults of the banana root borer, *Cosmopolites sordidus* (Germar), settled under the corms of the susceptible banana "Nakyetengu" treated with 5% aqueous extract of neem seed powder or cake or 2.5 and 5% emulsified neem oil than on water-treated corms. Feeding damage by larvae on banana pseudostem discs treated with 5% extract of powdered neem seed, kernel, or cake, or 5% emulsified neem oil was significantly less than on untreated discs. The larvae took much longer to locate feeding sites, initiate feeding and bore into pseudostem discs treated with extract of powdered neem seed or kernel. Few larvae survived when confined for 14 d on neem-treated banana pseudostems; the survivors weighed two to four times less than the larvae developing on untreated pseudostems. Females deposited up to 75% fewer eggs on neem-treated corms. In addition, egg hatching was reduced on neem-treated corms. The higher the concentration of neem materials the more severe the effect.

KEY WORDS *Cosmopolites sordidus*, banana, neem, *Azadirachta indica*

THE BANANA CORM BORER, *Cosmopolites sordidus* (Germar), is the most destructive insect of the banana plant (*Musa* spp.); the fruit of which is the most widely consumed worldwide after citrus (Hallam 1995). Although *C. sordidus* originated in Southeast Asia (Neuenschwander 1988), it is now distributed in all banana producing areas (Ostmark 1974). The volatiles emanating from the banana plant attract beetles to banana corms (Ndiege et al. 1991), which constitute the preferred egg laying sites and food for the larvae (Treverrow et al. 1992). The latter bore extensive tunnels in the corm and occasionally in the pseudostem (Vilardebó 1973), causing severe yield losses ranging from 20 to 100% (Koppenhöfer 1993, Peña et al. 1993).

Although organophosphate and carbamate insecticides have been used successfully for the control of *C. sordidus* (Vilardebó et al. 1974, Mitchell 1980), these synthetic pesticides are expensive, hazardous to human health and to the environment. *C. sordidus* has also developed resistance to several synthetic insecticides (Georghiou and Lagunes-Tejada 1991).

To date, there are few studies on sustainable *C. sordidus* management, using inexpensive plant derivatives that could serve as substitutes for toxic insecticides. The neem tree, *Azadirachta indica* A. Juss., has an array of complex chemicals, "limonoids," that cause diverse biological effects on insects. In general, extracts of neem fruit, seeds, seed kernels, and bark possess insect repellent, antifeedant, growth inhibitor, and other insecticidal properties (Jacobson 1989, Saxena 1989, Schmutterer 1990). More than 100 highly bioactive compounds have been isolated from various parts of the tree (Kraus 1995). We therefore studied the effects of neem seed derivatives on the *C. sordidus* adult orientation and settling, oviposition and egg hatching, larval feeding, growth, and development. Our hypothesis was that neem derivatives would adversely affect the physiology of the insect and hence reduce the pest population and damage.

Materials and Methods

Studies were conducted in a laboratory at the International Center of Insect Physiology and Ecology (ICIPE), Mbita Point Field station, Kenya, under a temperature range of 20–28°C, 55–65% RH, and a photoperiod of 12:12 (L:D) h.

Cosmopolites sordidus. *C. sordidus* adults were trapped in the banana fields using longitudinally split pieces of the banana pseudostems (Vilardebó 1973). They were brought to the laboratory and reared in 10-liter plastic buckets, each containing a ≈2 kg of fresh corm of 'Nakyetengu' (AAA-EA), an eastern African highland cooking banana cultivar, highly susceptible to *C. sordidus.* The buckets were covered with mosquito netting to prevent *C. sordidus* from escaping. Corms in buckets were kept moist by watering them as needed.

Neem Materials. Because seeds are rich in azadirachtin (Kraus 1995), we used neem seed powder, neem kernel powder, neem cake, and neem oil as test materials. Fresh seeds, collected from ripe fruits from

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coastal Kenya, were washed and dried in the shade for 3–4 d to ≈13% moisture content. Neem seed powder and neem kernel powder were prepared at the field station by pounding whole seeds and dehulled seeds, respectively. Neem cake and neem oil were obtained by crushing neem seeds in a single-screw vegetable oil expeller with a three-phase motor (IBG Monforts, Mönchengladbach, Germany). The azadirachtin-A content (4,000, 5,500, 5,800, and 850 ppm in neem seed powder, neem kernel powder, neem cake, and neem oil, respectively) was determined by high-performance liquid chromatography at ICIPE's Natural Products Chemistry Laboratory, Nairobi. Neem materials were used as aqueous emulsions or extracts. To prepare neem seed powder, neem kernel powder, and neem cake extracts at different concentrations, the known quantities of test materials were soaked in water in a beaker and stirred overnight using a magnetic stirrer. The suspensions were strained through fine cheesecloth. Emulsions of neem oil of varying concentrations were prepared in distilled water (vol:vol) by adding 1% of a liquid detergent.

Adult Settling Response. To test the deterrent effect of neem, adult *C. sordidus* were given a choice of neem-treated and water-treated pieces of banana corms arranged in a galvanized iron tray (66 cm diameter). Freshly cut 200-g pieces of Nakyetengu corm were dipped in aqueous extracts of 1, 2.5, and 5% neem seed powder and neem cake; or in 1, 2.5, and 5% emulsified neem oil; control corms were dipped in water. After 15 min, the corms were removed and excess liquids drained. Three neem-treated and three control corms were arranged alternatively equidistant from the periphery of the trays. Fifty adult *C. sordidus*, starved for 24 h, were released at 1800 hours at the center of each tray, which was covered with a black cloth to reduce light and to prevent *C. sordidus* from escaping. *C. sordidus* could move freely in the tray and settle on/under the preferred corms. At 48 h after release, adults that settled on various treatments were counted. Percentages of weevil settled on or under treated and control corms were calculated as 100T/(T+C); where T and C were the number of weevil settled on treated and untreated corms, respectively. In a multi-choice test, *C. sordidus* were likewise given a free choice of five corms, each treated with extract of 5% neem seed powder or 5% neem cake or 2.5 or 5% emulsified oil or water. *C. sordidus*' settling response was calculated as described above. Both the one-choice and multi-choice tests were repeated 12 times.

Larval Feeding. Banana pseudostem discs (8 cm diameter) were dipped for 5 min in 5% aqueous extracts of neem seed powder, neem kernel powder, and neem cake or 5% emulsified neem oil and placed singly in 9-cm-diameter petri dishes; control discs were similarly dipped in water. Laboratory-reared, third instars were then released singly on each pseudostem disc. Dishes for each treatment were arranged randomly in four replicates on a table in the laboratory; each dish constituted a replicate. The time that each larva spent searching for the feeding site, initiating feeding on the disc and penetrating the disc was recorded. Larval feeding-damage based on the percentage coefficient of infestation (PCI), during a 72-h-period of confinement of a larva to treated or control discs, was scored on a 0–100 scale (where 0 = no galleries, 5 = traces of galleries, 20 = galleries on an area of ¼ of the corm, 40 = galleries on an area of ½ of the corm, 60 = galleries on an area of 3/4 of the corm and 100 = galleries on the entire corm) (Vilardebó 1973).

Table 1. Percentage of *C. sordidus* adults settled on banana corms treated with aqueous extract of neem seed powder (NSP), neem cake (NC) or emulsified neem oil (NO) at different concentration, 48 h after release in mluti-choice tests

Treatment	Weevils settled, (%)
NSP extract 5%	8a
NC extract 5%	22ab
NO 2.5%	11a
NO 5%	6a
Water (control)	53b

Means followed by the same letter are not significantly different ($P < 0.05$) by Tukey's test. Average of 12 replicates. Each replicate had 50 weevils.

Larval Growth and Development. Freshly cut pieces of banana pseudostems (4 by 15 cm) were dipped in water (control) or in neem extracts or emulsified neem oil, as described above and placed in glass jars (6 by 18 cm). Ten second instars, obtained from the insect culture, were released into each jar containing a treated or control pseudostem, and then the jar was closed with a screened screw-type lid. Each treatment was replicated four times; each jar with 10 larvae constituted a replicate. Fourteen days after release of the larvae into jars, respective pseudostems were dissected and for each treatment, the surviving larvae, pupae, and adults, if any, were counted and individually weighed using a Mettler balance.

Oviposition and Egg Hatching. For oviposition tests, fresh banana corms, free from weevil attack, were pared to a depth of 3–5 cm to remove any *C. sordidus* eggs previously laid. Freshly pared pieces of banana corms (≈500 g each) were dipped for 15 min each in neem seed powder, neem kernel powder, neem cake extracts, emulsified neem oil, and water as described above. Then, each corm was placed in 10-liter plastic buckets into which five males and five females laboratory-reared *C. sordidus* were released. Weevils were sexed based on the distribution of punctuation on the rostrum (Longoria 1968) and by observing the angle of inclination of the ninth abdominal segment (Mestre 1995). In males, the distal end of the abdomen truncates sharply, whereas in females the ovipositor truncates gently. Each bucket was covered with mosquito net and then arranged randomly in a dark room. Each treatment was replicated four times; each bucket constituted a replicate. After 1 wk, the corms were removed from buckets and the number of eggs laid on each corm counted.

To determine the effect of neem materials on egg hatchability, 1-d-old eggs in batches of 10 each were collected from the laboratory culture and inserted

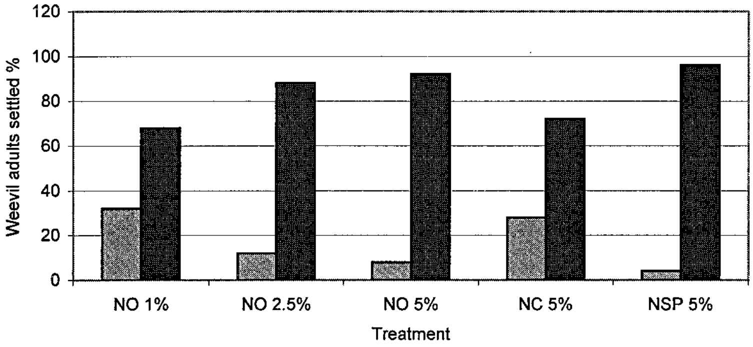

Fig. 1. Settling response of *C. sordidus* adults when given a choice of pieces of a banana corm treated with emulsified neem oil (NO), aqueous neem seed powder (NSP), or neem cake (NC) and water 48 h after their release in a galvanized iron tray.

singly in slits (1–2 mm deep) made in a slice of neem- or water-treated corms kept in petri dishes (9 cm diameter) lined with moist filter paper. The dishes containing corm slices were arranged randomly on a table in the laboratory. Each treatment was replicated four times; each dish constituted a replicate. Starting from the third day after the egg insertion, the corm slices were examined daily for 1 wk to record the number of hatched eggs. Percentage of hatched eggs was calculated then for each treatment.

Data Analysis. Data were analyzed using analysis of variance (ANOVA) or general linear model procedures of SAS/STAT (SAS Institute 1987). To stabilize the variance, the data were transformed using either square root or logarithm transformation. The treatment means were separated by the Student–Neuman–Keuls or Tukey test.

Results

Settling Response. When given a choice between pieces of neem-treated or water-treated corms, 48 h after release, <11% of the *C. sordidus* settled on banana corms treated with neem seed powder extract or with emulsified neem oil (Table 1). Likewise, in the multi-choice test, fewer *C. sordidus* settled on neem seed powder- or neem oil-treated corm pieces than on untreated corms (Fig. 1). The repellent effect was dose dependent; the higher the concentration of neem materials, the stronger the repellency.

Feeding Response. Although larvae were deterred from feeding on neem-treated discs, the degree of deterrence differed among neem materials. On neem kernel powder-treated discs, larvae spent 106.2 ± 5.6 min locating the feeding site, spent another 24.2 ± 2.2 min initiating feeding, and then took 22 ± 3.1 min partially boring into the disc, but after that they retracted and stopped feeding. A similar feeding pattern was observed when larvae were confined to neem oil- or neem cake-treated discs. In contrast, larvae readily started feeding on control discs and in 4 min bored deep into the discs. Within 72 h, feeding damage caused by weevil larvae to control discs was ≈75%, whereas little damage (5–22%) was done to neem-treated discs (Table 2).

Growth and Development. Significantly more larvae died (40–60%) when confined for 14 d to neem-treated pseudostem pieces than when confined to untreated pseudostems (Table 3). Larvae that survived in the neem-treated corms were smaller in body size and weighed significantly less (four to six times) than those that fed on the control corms (Table 3). Within 2 wk, some adults were recovered in the control; but none of larvae on neem-treated pseudostems even reached the pupal stage.

Oviposition and Egg Hatching. Compared with the untreated control, 3–10 times significantly fewer eggs were laid in neem-treated corms; the fewest eggs were laid in corms treated with 5% neem kernel powder extract (Fig. 2). Egg hatching was low, ranging from

Table 2. Feeding response of third-instars *C. sordidus* when confined to banana pseudostem discs, each treated with extract of neem seed powder (NSP), neem kernel powder (NKP), neem cake (NC), or emulsified neem oil (NO) or water

Treatment	Time spent, min			Corm damage PCI, %
	Locating feeding site	Initiating feeding	Boring into disc	
NSP 5%	29.7 ± 2.1b	14.0 ± 2.2b	11.7 ± 0.9b	19 ± 1.6b
NKP 5%	106.2 ± 5.6a	24.2 ± 2.2a	22.0 ± 3.1a	5 ± 1.2b
NC 5%	10.2 ± 2.4c	7.2 ± 0.8c	7.7 ± 1.2bc	22 ± 0.8b
NO 5%	19.0 ± 0.9c	7.5 ± 1.0c	7.2 ± 0.5bc	7 ± 1.1b
Water (control)	9.6 ± 0.5c	4.7 ± 0.9c	3.7 ± 0.9c	75 ± 2.0a

Within columns, means followed by the same letter are not significantly different ($P < 0.05$) by Student–Neuman–Keuls's test, average of four replicates.

Table 3. Effect of neem on larval development when second-instar *C. sordidus* were confined to banana pseudostem pieces treated with extract of neem seed powder (NSP), neem kernel powder (NKP), neem cake (NC), or emulsified neem oil (NO) after 14 d

Treatment	Mortality after 14 d, %	Weight (mg)/surviving larva	Adult, %
NSP 5%	57 ± 3.3b	64 ± 7.8c	0.0 ± 0.0b
NKP 5%	60 ± 10.9b	61 ± 9.2c	0.0 ± 0.0b
NC 5%	40 ± 6.7b	188 ± 27.7b	0.0 ± 0.0b
NO 1%	43 ± 13.5b	61 ± 17.3c	0.0 ± 0.0b
NO 2.5%	57 ± 22.0b	81 ± 2.1c	0.0 ± 0.0b
NO 5%	50 ± 6.7b	68 ± 9.3c	0.0 ± 0.0b
Control	17 ± 3.3a	297 ± 14.52a	10. ± 3.3a

Within columns, means followed by the same letter are not significantly different ($P < 0.05$) by Student–Neuman–Keuls's test; average of four replicates; each replicate had 10 larvae.

2 to 13% in the various neem treatments, whereas it was 41% in the untreated-control. Oviposition and egg hatching deterrence was greater with higher concentrations.

Discussion

The most important response categories for the insect invasion and utilization of plants are orientation and settling, feeding, metabolism of ingested food, growth, survival, fecundity, oviposition (Saxena 1969) and egg hatching for those insects that deposit eggs inside the plant tissue (Khan and Saxena 1985). Disruption of one or more of these responses should reduce insect establishment and consequent damage.

Natural bioactive products, which repel, reduce feeding, or inhibit growth and development of insects have some potential in the pest management (Saxena 1989, Klocke et al. 1989). The insect repellent and antifeedant properties of neem are well known. For centuries, farmers in India protected crops using dried neem leaves and seed cake (Saxena 1989). Recently, the growth inhibitory and ovipositional deterrent properties of neem materials, which upon contact affect the pest's overall fitness, have come to light (Schmutterer 1990). Various neem materials have been tested against ≈79 species of Coleoptera (Schmutterer 1995), except for *C. sordidus.*

Our results show that drenching the banana corm or pseudostem with aqueous neem seed powder, neem kernel powder, or neem cake extract or with emulsified neem oil strongly affected *C. sordidus* settling response, egg laying, and larval feeding on treated corms, indicating the strong deterrent and antifeedant effect of neem. However, the deterrence of larval feeding on neem-treated banana pseudostems and corms not only reduced the feeding damage but also adversely affected larval growth and development, resulting in smaller size and body weight of larvae, increased mortality, and failure of larvae to reach the adult stage. In addition to reduced food intake, some of these growth inhibitor effects, including reduced egg hatching, could also be attributed to the action of some of neem's bioactive constituents, such as azadirachtins, which are known to interfere with neuroendocrine regulation of juvenile and molting hormone titers during insect development (Rembold 1989, Mordue (Luntz) and Blackwell 1993).

Earlier studies have also reported the repellent and feeding deterrent effects of neem materials against other coleopteran insects (Jilani et al. 1988, Karel 1989, Schmutterer 1995) and on a wide range of insect and mite pests (Gujar and Mehrotra 1984, Dimetry et al. 1993, Sundaram and Sloane 1995). Also, Rembold (1989) observed that immature insects exposed to neem-treated materials often develop into adults with physical abnormalities. Dorn (1986) reported that not only longevity was reduced if azadirachtin was injected into newly emerged *Oncopellus fasciatus* adults but also its fecundity and egg hatching. These results concur with our results. In our study, neem seed derivatives reduced the weevil oviposition and egg hatching. The treated corms might be unfavorable for egg laying and consequently for larval feeding, growth, and development. Eggs deposited in neem-treated plants are often less fertile and more susceptible to fungal attack (Schmutterer 1987).

We conclude that neem materials adversely affect the behavior and physiology of *C. sordidus.* These results may have practical implications in providing

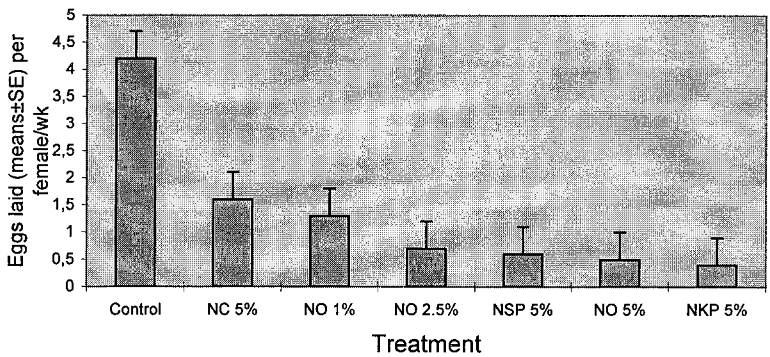

Fig. 2. Effect of neem treatments at different concentrations on the number of eggs laid by *C. sordidus* female on neem-treated corms and untreated corms.

long-term control of this banana pest in the field (Musabyimana 1999). In addition, because natural pesticides are safer than synthetic to the beneficial organisms and the environment (Schmutterer 1995), the application of neem materials will contribute toward preservation of biodiversity.

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