

## Storage Temperature of Neem Kernel Extract: Differential Effects on Oviposition Deterrency and Larval Toxicity of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae)

DAVID A. JENKINS,<sup>1</sup> FLORENCE V. DUNKEL, AND KADIATOU TOURE GAMBY<sup>2</sup>

Department of Entomology, Montana State University, Bozeman, MT 59717

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**ABSTRACT** Neem has been used as an effective postharvest protectant for many crops. Neem is especially effective against the cowpea weevil, *Callosobruchus maculatus* (F.), a major pest of cowpeas. Although it is well known that azadirachtin A breaks down rapidly at high temperatures, the efficacy of neem kernel extract previously exposed to high temperatures for protecting stored pulses has not been conclusively investigated. Effectiveness of these materials would allow West African farmers to make a neem extract at their convenience and store it for later use. We found that neem kernel extract continued to reduce infestations of *C. maculatus*, after the neem kernel extract had been stored at high temperatures, including 2 wk at 50°C followed by up to 5 mo storage at 28°C. Neem kernel extract analyzed with high performance liquid chromatography revealed that azadirachtin A present in unheated or fresh neem kernel extract dissipated when stored at 50°C for 2 wk. Neem kernel extract heated to 28°C or above also lost effectiveness as an oviposition deterrent. However, the number of neem kernel extract-treated eggs that survive to become adults was significantly reduced even when the neem kernel extract was exposed to 50°C for 2 wk. We attribute the mortality we observed, which was maintained even when azadirachtin A was absent in neem kernel extract, to physical effects of the oil properties of neem kernel extract. We conclude that neem kernel extract can be stored at high temperatures for at least 5 mo without significant reduction in overall effectiveness.

**KEY WORDS** azadirachtin A, heat degradation, high-performance liquid chromatography, post-harvest, *Vigna unguiculata*

THE NEEM TREE, *Azadirachta indica* A. Juss (Meliaceae), has long been studied as a source of medicines and insecticides (Mordue (Luntz) and Blackwell 1993, Ascher 1993). Neem's greatest potential usefulness is in the subsistence agricultural systems of the tropics. In many tropical regions, the neem tree grows readily and is an abundant multipurpose resource. Neem extracts can be effective crop protectants. However, the rapid photodegradation of the bioactive compounds in neem kernel extract (Schmutterer 1988) relegates its most effective use to postharvest storage, where exposure to light is usually minimized. Many scientists have demonstrated the effectiveness of neem for protecting stored products, especially cowpeas (*Vigna unguiculata* Walpers), an important source of protein in many West African countries (Sowunmi and Akinnusi 1983, Zehrer 1984, Pierrard 1986, Makanjuola 1989, Tanzubil 1991, Echindu 1991, Saxena and Saxena 2000), including Mali. Commercial neem has been shown to have no objectionable flavor or aroma in treated legumes traditionally prepared (Dunkel et al.

1995). "Neem flavor" is caused by terpenoids, which would be the same in both the commercial formulation and the crude neem kernel extract.

Most of the bioactive compounds in neem are concentrated in the seed kernels. Seeds are continually available in some areas, although seed production is restricted to once or twice a year in other areas. Storage of the seed, or fruit containing the seed, is a questionable practice because of the build-up of the potent carcinogen, aflatoxin B<sub>1</sub> (Chourasia and Roy 1991) and other problems (Sacande et al. 2000, Yakkundi et al. 1995). One possible alternative is long-term storage of neem kernel extract. However, whether neem kernel extract loses potency under normal subsistence farming storage conditions is not known. Subsistence farmers in Africa may be able to prepare neem kernel extract at their convenience and store it for extended periods if severe degradation does not occur.

Stark and Walter (1995) showed that heat degraded azadirachtins A and B in neem seed oil (Margosan-O and Neem-X, W.R. Grace and Company, Columbia, MD; Azatin, Agridyne Technologies Inc., Salt Lake City, UT., now Thermo Trilog) but have not demonstrated that this degradation reduces the effective-

<sup>1</sup> Current address: Department of Entomology, University of Georgia, Athens, GA 30602.

<sup>2</sup> Institut d'Economie Rurale, Bamako, Mali.

**Table 1.** Number eggs laid per female, adult mortality, and number of F<sub>1</sub> adults per female, in response to four concentrations of neem kernel extract

Treatment (g neem kernel extract per 100 g wet weight cowpeas)	Mean no. eggs laid/female ( $\pm$ SE)	Mean parent mortality during 3-day oviposition period ( $\pm$ SE) [percentage mortality] (adults inoculated 0–24 h post adult emergence)	Mean no. F <sub>1</sub> adults emerged/female ( $\pm$ SE)	Mean percent eggs developing to adults
Untreated	43.4 $\pm$ 1.3a	0c	25.1 $\pm$ 1.5b	57.8 $\pm$ 3.9
Water treated	48.6 $\pm$ 1.0a	0c	30.0 $\pm$ 2.0a	61.7 $\pm$ 4.3
10% neem kernel extract (0.34)	31.1 $\pm$ 3.0b	1.33 $\pm$ 0.8c [16.7]	12.5 $\pm$ 4.0c	40.2 $\pm$ 13.4
25% neem kernel extract (0.84)	33.2 $\pm$ 7.8b	2.33 $\pm$ 0.57bc [29.2]	4.2 $\pm$ 3.3d	12.7 $\pm$ 10.4
40% neem kernel extract (1.35)	16.2 $\pm$ 5.3c	5.0 $\pm$ 2.0b [62.5]	0d	0
55% neem kernel extract (2.36)	0c	8.0a [100]	0d	0

Means  $\pm$  SE followed by the same letter within a column are not significantly different ( $P \leq 0.05$ ; Student-Neuman-Keuls) ( $n = 3$ ); one-way ANOVA with model, response = dose.

ness of neem kernel extract for protecting crops in storage. Certain carriers and stabilizers have been used successfully to reduce degradation of azadirachtin A by heat in the laboratory (Kumar and Parmar 1999), but these are not readily available to subsistence farmers. A number of compounds in neem kernel extract have been shown to have bioactivity equal or greater to that of azadirachtin A (Lin-er et al. 1995). Furthermore, the physical properties of neem kernel extract may contribute to the effectiveness of the extract as an ovicide or a larvicide (Don-Pedro 1989, Pacheco et al. 1995, Rajapakse and Van Emden 1997). The capacity of the extract to protect stored cowpeas is not dependent on azadirachtin A content of the tree, so concerns about variation in azadirachtin A content of the trees among seasons or geographical locations can be largely ignored by farmers using neem kernel extract to protect their stored cowpeas. Two recent articles addressing the efficacy of extract after exposure to heat (54°C for 2 wk) against preharvest pest insects found no significant loss of efficacy (Ram-Niwias et al. 2000, Kumar and Parmar 2000). Other studies found slightly lower temperatures (40°C) to actually increase efficacy 10-fold (Kabar and Mwangi 2000). To our knowledge, no published studies have addressed the postharvest efficacy of neem kernel extract that is devoid of azadirachtin A or that has been exposed to high temperatures.

We hypothesized that prolonged high temperatures, as are commonly encountered in arid, low altitude, tropical and subtropical regions, e.g., the Sahel region of West Africa, portions of Mauritania, Senegal, Mali, Niger, and Chad, will reduce the azadirachtin A content and decrease the bioactivity of neem extract. The goal of our research was to examine the feasibility of prolonged storage of neem kernel extract for later treatment of stored products, particularly cowpeas. The most destructive pest in the cowpea system is the cowpea weevil, *Callosobruchus maculatus* (F.) (Ofuya and Bambigbola 1990). For this reason, we used the cowpea/cowpea weevil system to evaluate the reduction in neem kernel extract effectiveness under storage conditions, in particular, those conditions of temperature and humidity predominating in arid tropical and subtropical regions.

## Materials and Methods

**Extract Production.** Neem kernels used for extraction were gathered in September 1995 and 1996, from trees in the Sirakorola Arrondissement in southwestern Mali, West Africa. Another extract prepared in September 1996 was obtained in northern Benin. Extractions from neem kernels were performed in Mali using a manual cold press (GRAT 1993). The oil was filtered through a cloth mesh with an aperture diameter of 300–400 microns (one micron = 1/1000th mm). Extracts were stored at 28  $\pm$  1°C for 1 mo in a shaded room before being transported by air over a 3-d period, kept in dark and at 28  $\pm$  1°C during transport. Once the extracts arrived at Montana State University, they were stored at 5  $\pm$  0.5°C in the dark until used in bioassays.

**Insect Rearing.** A culture of *Callosobruchus maculatus* was obtained from the University of Wisconsin, Madison, WI, Department of Entomology, USDA-ARS Stored Product Insect Laboratory. The cowpea weevils were reared on cowpeas in 1-liter jars in a controlled environment at 28  $\pm$  0.5°C; 65  $\pm$  5% RH, photoperiod of 12:12 (L:D) h.

**Dose-Response Experiment.** Approximately 20  $\pm$  0.05 g of cowpeas (wet weight, 3% moisture) equilibrated at 28  $\pm$  0.5°C and 60% ( $\pm$ 5% RH) were apporportioned to each of 27, 40-ml glass vial, capped with copper mesh. Neem kernel extract (1995 acquisition from Mali) was mixed with deionized water in the proportions to obtain concentrations of 10, 25, 40, 55, 70, 85, and 100% in 15-ml glass vials with Teflon-coated silica septa. Untreated controls and controls treated with deionized water were prepared as well. Each treatment was replicated three times and neem kernel extract solutions for each replicate were prepared separately. For the total experiment there were 27 40-ml vials containing cowpeas. We reported (Table 1) only the lowest two concentrations that resulted in zero eggs oviposited. Seven-hundred-fifty microliters ( $\mu$ l) of the treatment were added to each 20 g of cowpeas in a Pyrex Petri dish (750  $\mu$ l was determined to exactly cover 20 g cowpeas). This resulted in neem kernel extract weights corresponding to 0.34, 0.84, 1.35, 1.85, 2.36, 2.87, and 3.37% of cowpea wet weight

**Table 2.** Mean number eggs oviposited per female parent of *Callosobruchus maculatus* (F.) in response to origin and storage temperature

Untreated (water-treated) control	Extract origin	Storage treatment					
		5°C for 5 mo		28°C for 5 mo		50°C for 2 wk and 28°C for the remainder of 5 mo	
		Percent (w/w) neem kernel extract					
		0.84	1.69	0.84	1.69	0.84	1.69
42.9 ± 1.7a (45.6 ± 3.5a)	Mali 1995	24.92 ± 1.62a	0	30.0 ± 1.18b	0	38.60 ± 1.07c	0
	Mali 1996	30.40 ± 0.59b	0	NA	NA	39.48 ± 0.65c	0
	Benin 1996	29.84 ± 1.10b	0	NA	NA	38.48 ± 0.62c	0

Means ± SE followed by the same letter, within rows and columns, are not significantly different ( $P \leq 0.05$ ; Student-Neuman-Keuls) ( $n = 5$ ). NA, not applicable.

(wt:wt). The Petri dish was covered and shaken vigorously for 10 s to thoroughly coat the cowpeas. The cowpeas were allowed to dry for 10 s on a Whatman No. 1 filter paper and then transferred to 40-ml glass vials capped with copper mesh. Five adult female and three adult male *C. maculatus* (0–24 h after adult emergence) were placed in each vial. After 3 d, the adult cowpea weevils were removed from each vial and the eggs were counted for each vial. The cowpeas were monitored daily for adult emergence. Eleven days after the first adult emergence, the cumulative number of emerged adults was divided by the number of parent females per vial (5) to determine the number of progeny per parent female.

**Heat-Degradation Experiments.** To examine the effect of heat on the efficacy of village-pressed neem kernel extract as a protectant for cowpeas, sealed 15-ml glass vials with Teflon-coated silica septa containing 10 ml neem kernel extract were stored under the following temperature regimes before the treatment of the cowpeas: Treatment I, continuous storage of all three neem kernel extracts for 5 mo at 5°C; Treatment II, storage for 2 wk at 50°C followed by ≈18 wks of storage at 28°C. In a third treatment, the Mali 1995 acquisition of neem kernel extract was stored for 5 mo at 28°C. Only the Mali 1995 acquisition was used for the third storage treatment because of insufficient amounts of the other neem kernel extracts. All vials were covered with aluminum foil to prevent photo-degradation. Treatments of 1.69% (wt:wt) neem kernel extract (the concentration typically used by Malian farmers) from storage temperature treatments I and II were applied to each of five 20-g replicates of

cowpeas. Because in the 1.69% neem kernel extract treatment we detected no oviposition, the test was repeated and treatments were applied at 0.84% (wt:wt) neem kernel extract. The bioassay procedure used in these heat-degradation experiments was the same as that used for the dose–response experiment described above.

**Stage-Specific Effects of Heat-Treated Extract.** Five 20-g replicates of cowpeas were each treated with either 1.69% or 0.84% (wt:wt) of each neem kernel extract at 1, 2, 3, 7, or 25 d after a 3-d exposure to five adult female and three adult male *C. maculatus*. Five additional 20-g replicates received five adult female and three adult male *C. maculatus* 28 d after treatment with either 1.69% or 0.84% (wt:wt) neem kernel extract. Thirty five days after infestation by *C. maculatus*, the total number of emerging adults in each vial were counted. Because *C. maculatus* eggs are affixed to the cowpea seed coat, even at the end of the experiment, the number of embryos that hatched and those that did not can be detected by observations through the *C. maculatus* egg chorion.

**High-Performance Liquid Chromatography.** A Shimadzu (Kyoto, Japan) LC-6A with a Spectroflow 757 absorbance detector and a Shimadzu Chromatopac C-R68 integrator was used for high-performance liquid chromatography (HPLC) analysis. The HPLC column used was a C-8 adsorbosil (5 μm) reverse phase (Alltech Associates, Inc., Deerfield, IL). Flow rate was 1 ml/min and UV absorbance measured at 214 nm. Azadirachtin A (90% AI) was obtained from W. Kraus (Department of Chemistry, University of Hohenheim, Stuttgart, Germany). Azadirachtin A (5.49 mg) was

**Table 3.** Mean number of adults per female parent of *Callosobruchus maculatus* (F.) in response to storage temperature and origin of neem kernel extract ( $r^2 = 0.92$ )

Untreated (water-treated) controls	Extract origin	Storage treatment					
		5°C for 5 mo		28°C for 5 mo		50°C for 2 wk and at 28°C for remainder of 5 mo	
		Percent(w/w) neem kernel extract					
		0.84	1.69	0.84	1.69	0.84	1.69
24.5 ± 1.0a (20.3 ± 1.9a)	Mali 1995	5 ± 3.02b	0	5 ± 3.82b	0	6 ± 3.30b	0
	Mali 1996	6 ± 0.55b	0	NA	NA	6 ± 0.96b	0
	Benin 1996	5 ± 0.42b	0	NA	NA	5 ± 0.62b	0

Means ± SE followed by the same letter within rows and columns are not significantly different ( $P \leq 0.05$ ; Student-Neuman-Keuls) ( $n = 5$ ). NA, not applicable.

Table 4. Mean adult emergence per female parent *Callosobruchus maculatus* (F.) 45 d after infestation in response to heated neem kernel extract applied to cowpeas at various beetle developmental stages ( $r^2 = 0.94$ )

Time of treatment (relative to infestation)	Neem kernel extract concentration (w/w)			
	Stage of development	Untreated controls	0.84%	1.69%
28 d prior	No insects present at treatment time, adult parents added 28 d after treatment	21.7a	6.1b	0c
1 d after	Embryo	21.6a	4.5b	0c
2 d after	Embryo	22.9a	4.5b	0c
3 d after	Embryo	21.6a	5.4b	0c
7 d after	First instar	23.4a	5.1b	0c
25 d after	Pupa	18.5a	21.1a	10.1b

Means within columns and within rows followed by the same letter are not significantly different ( $P \leq 0.05$ ; Student-Neuman-Keuls) ( $n = 5$ ).

dissolved into 5 ml of HPLC-grade methanol. Sample volume was 10  $\mu$ l, and an isocratic 50:35:15 H<sub>2</sub>O: MeOH:acetonitrile (AcN) mobile phase was used for analysis with pure AcN used for 20 min to flush the column between runs. The runs were repeated five times to ensure that the chromatograms were typical. Methanol was used only for the HPLC analysis and was not part of the *C. maculatus* bioassays. Because the goal of this research was to give Malian farmers a recommendation about the use of neem kernel extract which they can produce themselves without purchasing any materials, methanol would not be an option for extract formulation.

Raw neem kernel extracts from Benin and Mali (0.5 ml) were each mixed with 5 ml of HPLC-grade methanol and placed on an equilibrated solid phase extraction tube (Bakerbond C-18 solid phase extraction tubes, from J.T. Baker [Mallinckrodt] Co., Phillipsburg, NJ) to clean the samples for HPLC. No pressure was used to extract the neem kernel extract. The filtrate was collected and analyzed by HPLC as with the pure azadirachtin A. Ten microliters of 90% azadirachtin A were added to each raw neem kernel extract sample to confirm when azadirachtin A eluted. All HPLC runs were repeated five times to ensure the chromatograms were typical. Anisole (99%) was used as an internal standard to quantify azadirachtin A content in samples.

**Data Analyses.** One-way analysis of variance (ANOVA) and standard errors of all mean oviposition and adult emergence data were obtained using the SAS statistical program (SAS Institute 1988). For Table 1, the response variable was dose and the effects tested were number eggs laid per female and number of F<sub>1</sub> adults per female. For Tables 2 and 3, the response variable was heat treatment during storage of the neem kernel extract and neem kernel extract source. For Table 2, the effect tested was number eggs oviposited per female parent of *C. maculatus* and for Table 3 the effect tested was number adults produced per female parent of *C. maculatus*. For Table 4 the response variables were (1) stage of development at time of treatment, and (2) dose. The effect tested was mean adult emergence per female parent 45 d after infestation. The Student-Newman-Keuls test on the SAS program was used for multiple comparison tests of the different treatments with an alpha level = 0.05.

## Results

**Dosage-Response Experiment.** Neem kernel extract significantly affected adult emergence at all concentrations and a dose-dependent response was demonstrated (Table 1). None of the F<sub>1</sub> adults emerging exhibited any malformations, indicating that the growth regulatory effects of neem kernel extract reported by Mordue (Luntz) and Blackwell (1993) were probably not involved in the toxicity of neem kernel extract at the concentrations assayed. All treatments  $\geq 1.35$  g neem kernel extract per 100 g wet weight cowpeas (40% neem kernel extract) provided full protection, killing even parent beetles (Table 1). For all levels of neem kernel extract in which there was oviposition, there were two significantly different effects, reduction in oviposition and a further reduction in F<sub>1</sub> adult emergence (Table 1).

**Heat-Degradation Experiments.** All neem kernel extract samples from the three storage regimes resulted in no egg deposition and no F<sub>1</sub> emergence at 1.69% wt:wt concentration after 5 mo. Oviposition increased significantly after both of the heat treatments compared with unheated neem kernel extract ( $F$ -value = 144.96,  $df = 1$ ,  $P \leq 0.0156$ ; Table 2). The 0.84% wt:wt neem kernel extract, however, applied either after heat treatment followed by storage at 28°C, or after continuous storage at 28°C, still adversely impacted adult emergence ( $F$ -value = 0.06,  $df = 1$ ,  $P = 0.941$ ; Table 3). Of three West African village-prepared extracts, there was no significant ovipositional differences shown by female *C. maculatus* as a result of extract source except with the 1995 prepared Mali material that caused significantly less oviposition than from the same location 1 yr later ( $F$ -value = 4.85,  $df = 2$ ,  $P = 0.016$ ; Table 2). Of the three extract sources only the extract from Benin showed significant different (80% lower) adult emergence than the Malian extracts ( $F$ -value = 12.25,  $df = 2$ ,  $P = 0.0002$ ; Table 3).

**Stage-Specific Effects of Heat-Treated Extract.** The 1.69% wt:wt neem kernel extract treatments applied 1, 2, 3, or 7 d after oviposition completely prevented development of progeny to adults (Table 4). The 1.69% neem kernel extract applied while *C. maculatus* were in the pupal stage (25 d after oviposition) reduced adult emergence significantly compared with

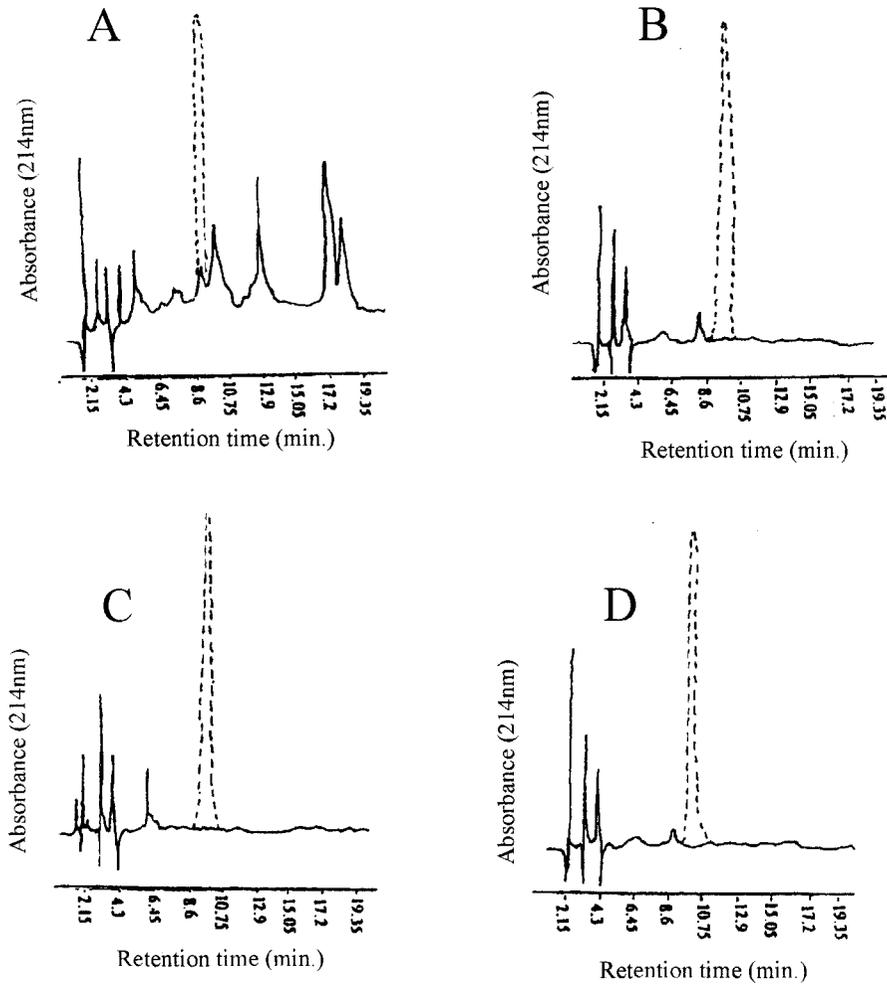


Fig. 1. High-performance liquid chromatograms of 90% pure azadirachtin A, Mali 1995, Mali 1996, and Benin 1996 acquisitions of neem kernel extract. (A) Solid phase extractions of raw neem kernel extract in methanol (Mali 1995 stored at 5°C). (B) Mali 1995 raw neem kernel extract 5 mo after heat treatment at 50°C for 2 wk and stored at 28°C for 19 wk. (C) Stored at 5°C in methanol (Mali 1996). (D) Stored at 5°C in methanol (Benin 1996). Dotted lines represent a 10- $\mu$ l injection of 90% pure azadirachtin A. Each chromatogram represents typical results of five runs.

the untreated control (Table 4). The 0.84% neem kernel extract treatments applied 1, 2, 3, or 7 d after *C. maculatus* oviposition also reduced progeny survival compared with the untreated control, except when they were applied 25 d after infestation (Table 4). However, the 0.84% wt:wt neem kernel extract applied while *C. maculatus* were in the pupal stage did not have a significant effect on  $F_1$  emergence compared with controls (Student-Newman-Keuls,  $P \leq 0.05$ ) (Table 4).

**High-Performance Liquid Chromatography.** Azadirachtin A and other unidentified compounds were degraded by the temperature regimes to which the neem kernel extracts were exposed (Fig. 1). High-performance liquid chromatography of the Mali 1996 and Benin 1996 acquisitions indicated no detectable azadirachtin A (Figs. 1B–D). Extracts from the Mali 1995 acquisition had a mean of  $5.0 \times 10^{-3}$  mg  $\mu$ l $^{-1}$

azadirachtin A (SE,  $3.2 \times 10^{-4}$ ) (Fig. 1A). The results demonstrate the absence of azadirachtin A content in stored neem kernel extract samples. Extracts with no azadirachtin A, such as the heat-treated Mali 1995 acquisition and the Benin and Mali 1996 acquisitions, were poor oviposition deterrents, but were as effective at reducing adult emergence as the extract containing azadirachtin A (Tables 2 and 3).

#### Discussion

The most important finding of these experiments was that the heat-degraded neem kernel extract prevented the development of *C. maculatus* eggs and larvae. The neem kernel extracts used in these trials significantly reduced adult emergence of *C. maculatus* at all concentrations tested. Storage at high temperatures (50°C for 2 wk) did not reduce neem kernel

extract effectiveness compared with neem kernel extract storage at 5 or 28°C. The 0.84% neem kernel extract demonstrated no reduction in toxicity to F<sub>1</sub> progeny, although oviposition did increase, indicating that the oviposition deterrence of the neem kernel extract had been reduced by heat.

In some respects, therefore, the heat degradation of neem kernel extract is an advantage for *C. maculatus* management over neem stored at lower temperatures. Heat-treated neem kernel extract apparently does not interfere with stimuli of the cowpea to illicit oviposition by female *C. maculatus*. Thus, more of the eggs are deposited on cowpeas coated with heat-treated neem kernel extract, which will eventually prevent the embryo from developing, than would be deposited on cowpeas to which nonheat-treated neem kernel extract had been applied.

Many vegetable oils are effective ovicides for *C. maculatus* (Don-Pedro 1989, Pacheco et al. 1995, Rajapakse and Van Emden 1997) and the physical properties of the oil of neem kernel extract may be responsible for its effects. Neem formulations sold in the United States are typically 3–4% azadirachtin and 30–40% neem kernel extract, but these formulations contain petroleum-based solvents at a rate of 60–65% (Align-XL and Azatin-XL, Agridyne Technologies, Salt Lake City, UT [now Thermo Trilog, Columbia, MD]). These materials, referred to on the label as inert, have alone been shown to be lethal to some aquatic nontarget insects and other macroinvertebrates (Dunkel and Richards 1998), although how insects respond on treated cowpea surfaces is not known. The neem formulation we tested was only water and neem kernel extract available on-farm/village in Mali.

Heat-treated neem kernel extract applied to cowpeas decreased survival of *C. maculatus* eggs and/or larvae. Pupae were not affected by low concentrations of neem kernel extract (0.84%) but some reduction in survival to adult was produced by the 1.64% concentration. Our results indicate that cowpeas treated up to 1 mo before infestation were able to resist *C. maculatus*. These results suggest farmer-applied treatments should result in reduced *C. maculatus* infestation of stored cowpeas regardless of the insect stage present at the time of application. Further, a meaningful level of *C. maculatus* control should be achievable even from stored neem extracts lacking measurable azadirachtin A content.

There are obvious differences in azadirachtin A content of the various sources of neem kernel extract, possibly related to phenotypic and genetic differences in the sources of the extracts. However, the lack of azadirachtin A in the 1996 acquisitions could be a result of exposure to high temperatures of storage before we received the samples.

In conclusion, all high temperature treatments altered the chemistry of neem kernel extract and significantly reduced oviposition deterrence, but did not significantly affect the toxicity of neem kernel extract to *C. maculatus* eggs and larvae. Because most embryos of *C. maculatus* exposed to heat-treated neem kernel

extract do not survive to become destructive larvae, the decrease in oviposition deterrence is not important in terms of the storage ecosystem. Therefore, neem kernel extract stored at high temperatures should be as effective at protecting cowpeas from *C. maculatus* as freshly prepared extract. These results are important to subsistence farmers who harvest and prepare their own neem kernel extract in the village. Our results indicate that where neem trees are readily available, neem kernel extract can be prepared and stored in the dark at least 5 mo until it is needed.

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