Sustainable use of neem in Malian villages
by David A Jenkins

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Entomology
Montana State University
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Abstract:
Semi-subsistence farmers in Mali, West Africa, are plagued with a variety of pests that attack crops in the field and while the crop is stored for later use. The utilization of the neem tree as a sustainable resource for Malian farmers has been examined with particular attention paid to the use of neem in the postharvest cowpea system. By exposing cowpeas to neem kernel extract and then to the major pest of cowpeas, the southern cowpea weevil, Callosobruchus maculatus, we were able to evaluate the efficacy of neem kernel extract as a postharvest protectant of cowpeas, as well as examine any fundamental problems with the use of neem as a crop protectant. Our investigations revealed that neem kernel extract successfully prevents infestation by C. maculatus for up to one month, even if the extract has been heated and the chemistry of neem kernel extract is altered. We also found that neem kernel extract is not miscible with water, so farmers must use potentially hazardous solvents, such as kerosine when applying neem kernel extract. Our investigations demonstrated that the addition of village-prepared soap did not improve the miscibility of neem kernel extract in water. We conclude that neem kernel extract is a valuable asset to Malian farmers, especially in the stored cowpea system. However, there are considerable problems to overcome in order to make the use of neem kernel extract a fundamental part of a Malian farmer's agricultural system.

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David Alan Jenkins

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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Date April 14 '98

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ABSTRACT

Semi-subsistence farmers in Mali, West Africa, are plagued with a variety of pests that attack crops in the field and while the crop is stored for later use. The utilization of the neem tree as a sustainable resource for Malian farmers has been examined with particular attention paid to the use of neem in the postharvest cowpea system. By exposing cowpeas to neem kernel extract and then to the major pest of cowpeas, the southern cowpea weevil, *Callosobruchus maculatus*, we were able to evaluate the efficacy of neem kernel extract as a postharvest protectant of cowpeas, as well as examine any fundamental problems with the use of neem as a crop protectant. Our investigations revealed that neem kernel extract successfully prevents infestation by *C. maculatus* for up to one month, even if the extract has been heated and the chemistry of neem kernel extract is altered. We also found that neem kernel extract is not miscible with water, so farmers must use potentially hazardous solvents, such as kerosine when applying neem kernel extract. Our investigations demonstrated that the addition of village-prepared soap did not improve the miscibility of neem kernel extract in water. We conclude that neem kernel extract is a valuable asset to Malian farmers, especially in the stored cowpea system. However, there are considerable problems to overcome in order to make the use of neem kernel extract a fundamental part of a Malian farmer's agricultural system.

INTRODUCTION

In a gesture of humanitarianism, developed nations have often donated or passed on advanced technologies intended to benefit subsistence farmers in developing nations. Through acculturation, the Western World has erased much valuable traditional knowledge that is more appropriate for these farmers' circumstances and replaced it with expensive, non-sustainable solutions that, in most cases, result in dependence upon further donations. Now, more advanced nations have the audacity to return to subsistence farmers their own traditional wisdom, disguised as high science from the latest western "think tanks."

A good example of this misconceived humanitarianism is pest management in tropical countries. Having seen our own yields increase, many agriculturalists from developed nations presumed that "advanced" pest control would also increase yields in developing nations, thereby remedying the problems of poverty and overpopulation. As we have seen, mis-managed use of pesticides not only aggravates pest problems and causes continual dependence upon them, but can also result in serious environmental and ecological imbalance. Furthermore, although regulation of insecticide use in developed nations tends to reduce the problems mentioned above, there is little or no regulation in developing countries. The great majority of environmental damage resulting from misuse of insecticides occurs in developing nations (WHO 1990). More importantly, cases of humans suffering toxic reactions and even dying as a result of mis-application of insecticides are rampant in the

developing world. WHO estimates that there are three million cases of acute pesticide intoxication annually worldwide (WHO 1990). It is reported that in Sri Lanka around 10,000 persons are hospitalized annually due to acute pesticide intoxication (Jeyaratnam 1993).

Only now are the "experts" of the developed nations looking back to the traditional wisdom that has sustained subsistence farming for thousands of years. Practices that were once dismissed as being behind the times are now being examined more closely for their merits in preserving crops, human welfare and the environment (Zehrer 1983; Weaver et al. 1994a; Weaver et al. 1994b).

At the forefront of traditional methods of pest management is the use of compounds derived from plants. Plants have long been embroiled in warfare against phytophagous insects and, as a result, have developed what might be called a highly advanced defense against herbivores and plant pathogens. Plants have shown themselves to be chemists extraordinaires, veritable factories of insecticidal compounds that have proven effective at reducing herbivory while impeding the development of resistance. An interesting group of these compounds are the terpenoids, a group of carbon-rich chemicals all having their synthesis based on the mevalonate pathway (Langenheim 1994). Terpenoids perform a variety of functions in the plant, including pigmentation, attraction of pollinators, attraction of parasitoids and predators of herbivorous insects, as well being toxic to the herbivores (Langenheim 1994). The plant kingdom's capacity to daunt persistent insect pests has been impressive and has inspired a movement to "green chemistry."

The neem tree, *Azadirachta indica* A. Juss (Meliaceae), is a native of Southeast Asia and the Indian Subcontinent that has shown much promise in the arenas of crop protection and medicine (Schmutter 1988; Ascher 1993). Marco Polo remarked on the magnificent teeth of the East Indians who chewed on neem twigs (Cordier 1929). Various parts of the neem tree have been used to treat ailments of diverse origins for millennia and seem to be particularly effective against diseases of bacterial or fungal origin (National Research Council 1992). Even more fascinating is the novel chemistry that has been elucidated from the neem tree and its varied biological activities against phytophagous insects (Ara et al. 1990; Gaikwad et al. 1990; Siddiqui et al. 1992; ; Ley et al. 1993). The compounds in neem have demonstrated powerful antifeedant effects against a very broad spectrum of phytophagous insects that compete with us for food and fibre (Kolb and Ley 1991; Mordue (Luntz) and Blackwell 1993; Ascher 1993; Verkerk and Wright 1993; Blaney et al. 1994; Lin-er et al. 1995). Neem has also shown a variety of other toxic manifestations, namely, inhibition of insect growth, deformations, inhibition of ecdysis, outright mortality (Mordue (Luntz) and Blackwell 1993), inhibition of protein synthesis (Timmins and Reynolds 1992) and inhibition of protein transfer (Moreira et al. 1994). This wide spectrum of toxic modes of action enhances neem's ability to slow down resistance development in phytophagous insects.

Although there have been very serious efforts to synthesize the active compounds for use in agriculture, the mixture of compounds occuring naturally in the neem tree has proved to be more effective than synthetics (Kolb and Ley 1991).

Interestingly enough, the antifeedant fragments of azadirachtin A demonstrate significantly less antifeedancy than the intact azadirachtin A molecule, demonstrating Nature's awesome powers of synthetic chemistry (Blaney et al. 1994). Furthermore, neem does not appear to be phytotoxic, nor does it appear to be toxic to vertebrates (although it has shown promise as a human spermicide) (National Research Council 1992) due to its specific modes of action (neem specifically acts on insect chemoreceptors and their endocrine system) (Jacobson 1988; Lin-er et al. 1995). All of this, plus neem's widespread distribution in the subsaharan region of West Africa (see Figure 1), presents the neem tree as an ideal candidate for subsaharan pest management.

Neem has already been in use in many countries to control field and storage pests, and farmers and scientists alike are demonstrating its effectiveness (Sowunmi and Akinnusi 1983; Makanjuola 1989; Echindu 1991; Tanzubil 1991). Applying the whole neem kernel extract, as opposed to selected compounds from neem, slows resistance development as there are many bioactive compounds in the neem kernel extract that display complimentary modes of action and may even potentiate or synergize each other, increasing neem's effectiveness (Kolb and Ley 1991; Blaney et al. 1994).

Not only may the numerous compounds in neem be effective, but the oil itself may be an important factor in toxicity to pest insects (Banken and Stark 1997). Many scientists have shown that various vegetable oils are effective at reducing infestations in stored products (Qi and Burkholder 1981), particularly against *C. maculatus* (Don-

Pedro 1989; Ivbijaro 1990; Pacheco et al. 1995; Lowery and Isman 1996; Rajapakse and Van Emden 1997).

Neem does have some shortcomings, the most prominent of which is the dilemma of formulation: neem kernel extract (NKE) does not dissolve in water, resulting in variable distribution when it is applied to crops or stored food stuffs. Some of its carriers used in commercial formulations have proven to be toxic to non-target organisms, and research on possible surfactants and alternate carriers is much needed (Dunkel and Richards, in press). Also, stored neem kernels are often infected with the potent human carcinogen and hepatotoxin, aflatoxin B_1 (Chourasia and Roy 1991).

Although we are aware that neem is currently effective in many storage situations, our aim is to optimize the use of neem so as to maintain its effectiveness. Are there optimal storage conditions for the NKE that will maintain its efficacy and reduce the probability of aflatoxin contamination? Should kernels or leaves be stored instead of the processed extract? Is there an inexpensive detergent or other surfactant available to the Malian farmer that can homogenize NKE in water, therefore making application more efficient?

HYPOTHESES TESTED

Our purpose in carrying out this project was to answer the questions posed above, to examine alternate methods that will optimize the use of neem in Malian villages, and to shed some light on the current formulation problems plaguing Malian farmers. We, therefore, tested the following hypotheses:

1) Storage of NKE under conditions of temperature typical of Malian villages will reduce the effectiveness of the NKE as a pest control agent.

2) Particular lifestages (embryo, larvae, pupal, and adult) of the pest insect will have varying susceptibilities to NKE. Special consideration of these varying susceptibilities will be necessary to apply the NKE effectively.

3) Village-prepared soap in Mali is a suitable surfactant that will evenly distribute the active agents of NKE in an aqueous solution.

To test these hypotheses we have chosen to use the stored cowpea system. Cowpeas, *Vigna unguiculata* Walpers, are an important source of protein in West Africa and are severely attacked by the cowpea weevil, *Callosobruchus maculatus* (F.), a pest wherever cowpeas are stored (Pierrard, 1986; Zehrer, 1983; Ofuya and Bambigbola, 1990; Tanzubil, 1991).

LITERATURE REVIEW

Assessment of Neem's Potential in Mali, West Africa

The present project is a result of a participatory assessment of Malian farming practices. Although scientists from all over West Africa had been investigating neem as a possible insect control agent, discussions with Malian villagers revealed that neem was still not being used in the typical Malian village prior to 1994 (Erbaugh et al. 1994). The neem tree was abundantly available to Malian farmers but it remained a forage for nomad livestock and firewood.

A series of participatory assessment surveys were employed to determine the most problematic crop pests in both preharvest and postharvest systems (Erbaugh et al. 1994; Dunkel and Warren 1994). In the postharvest system it was established that *C. maculatus* caused serious damage to stores of cowpeas (up to 60% loss in weight after six months), causing farmers to sell their cowpeas at reduced prices (Dunkel and Warren 1994). If the farmers had bad yields one year they would have to buy their cowpeas back at an inflated price (Erbaugh et al. 1995). The abundance of the neem tree in the region and the strong research supporting neem suggested that neem be used by these farmers to protect their crops in the field and during storage. Field applications of neem were tested at four sites in Mali (Erbaugh et al. 1995) and the results were impressive enough that farmers wanted to continue implementation of neem in storage and collaboration with additional experiments (Erbaugh et al. 1996).

Cowpea Weevil Management in West Africa

Because the cowpea weevil is such a problem in West Africa (Ofuya and Bambigbola 1990), many scientists in that part of the world have committed extensive study to the control of *C. maculatus*. In Nigeria, Ivbijaro found evidence that neem seed oil provided effective control of *C. maculatus* (Ivbijaro 1990). Makanjuola found that leaves and a kernel extract from neem were effective against several stored product pests, including *C. maculatus* (Makanjuola 1989). In Senegal, many farmers still depend on chemical controls that are not very effective (Pierrard 1986). Pierrard suggests that further research into local plants may provide an abundance of resources for crop protection with respect to *C. maculatus*. In Northern Ghana, Tanzubil found neem to be an integral part of the semi-subsistence farmer's arsenal against the cowpea weevil (Tanzubil 1991). Farmers in Togo often add sand, plants, and ashes to their stored cowpeas to protect the cowpeas from *C. maculatus* (Zehrer 1983). Zehrer (1983) recommended neem as a protective agent for stored cowpeas because of the abundance of neem trees in the area and the effectiveness of neem, outlasting the protection provided by the fumigant, phostoxin (Zehrer 1983). Although phostoxin and other fumigants are often used by West African farmers to protect their stored products, these chemicals do not have a lasting effectiveness, perhaps because farmers are not able to seal off the products effectively. In this case, the neem oil would provide protection that would not be subject to rapid disappearance; the oil would stay on the treated material. Furthermore, even though the adult bruchids do not have to

feed, there is evidence that antifeedants, such as those found in neem extracts, would be effective against ovipositing female bruchids because the females rely on chemoreceptors to give them a variety of information about a potential oviposition site (Messina et al. 1987; Wilson 1988).

Heat Degradation of Neem

Although current evidence suggests that, when applied, the neem oil would likely stay on the stored products, there is obvious concern about breakdown of the active compounds in neem oil over time. Stark and Walter (1995) demonstrated that azadirachtins A and B were lost when heated and were subject to even further breakdown by soil microbes. Knowing that these active components would be most certainly lost from neem oil applied to stored products or being stored for later use, it was important to ascertain whether these compounds hold potential for the protection of stored cowpeas. There is a body of evidence suggesting that compounds such as azadirachtin are not as important as previous literature might suggest. Many scientists have shown that neem oil and other vegetable oils are effective at controlling the cowpea weevil (Qi and Burkholder 1981; Don-Pedro 1989; Pacheco et al. 1995; Rajapakse and Van Emden 1997). If this is indeed the case, farmers using neem products to protect stored cowpeas need not be as concerned about any chemical variance inherent in the neem they use.

Life Stages of *Callosobruchus maculatus* Affected by Neem

The compounds in neem manifest a variety of actions against the "target" insects. There is antifeedancy, which may potentially be manifested against all stages of an insect (except the pupa, which does not feed). Also, there are growth regulatory effects, which may only be manifested in adulthood, and are usually more specific for a brief period of time just prior to the ultimate molt of the insect (National Research Council 1992; Mordue (Luntz) and Blackwell 1993). Other workers have also noted the importance of the life stage or age of the insect being treated with neem (Banken and Stark 1997). If the neem is only effective against a brief stage of the target pest's life cycle then it is imperative that farmers strategically apply neem when it will do the most damage to the pest population. In severe infestations where all life stages of the pest are represented in the infestation the neem could be applied at any time but only affect one cohort of pests on the stored cowpeas, making further applications necessary.

Formulation Problems and Solutions

Lastly, there is the problem of neem formulation. Reading through the vast literature on neem and its use, there is little expression of difficulty concerning the formulation of neem products. This is surprising because the major compounds in neem, such as azadirachtins A and B, are quite lipophilic, even though they are highly

oxygenated (Ley et al. 1993). Companies selling neem in the U.S. and Europe use petroleum-based solvents (Dunkel and Richards, in press) that have demonstrated toxicity to non-target aquatic macroinvertebrates. Scientists investigating neem have used commercial surfactants, such as Tween 20 and Triton or Teepol (Makanjuola 1989). Neem extractions used by scientists are often extracted with ethanol or other polar solvents and result in a polar fraction that is quite water soluble (Schroeder and Nakanishi 1987; Godrej et al. 1994; Locke et al. 1994). Hexane or other non-polar solvents are often used to remove much of the fatty acid portion that would cause miscibility problems (Schroeder and Nakanishi 1987; Godrej et al. 1994; Locke et al. 1994). The semi-subsistence farmer in Mali, however, does not have these tools available to improve the miscibility of NKE in water. The manual cold-pressed neem extraction that is currently available to villagers in Mali results in a highly lipophilic extract that is not miscible with water. It is imperative, then, that an inventory of locally available surfactants be made.

MATERIALS AND METHODS

Collection of Materials and Preparation of Extracts

Neem kernels used for extraction in these experiments were gathered in September 1995 and September 1996 from trees in the Sirakorola Arrondissment and the Mourdiah Arrondissment in northwest Mali. (See Figure 1.) A third extract was obtained from Benin. The extraction was performed using a manual cold press. All extracts were stored in Mali for one month at 28°C in a dark room before the extracts were transported by air to our lab in Bozeman, Montana. Once the extracts were obtained by our lab, they were stored at 5°C for 7 months until used in bioassays or for high-pressure-liquid-chromatography (HPLC) analysis.

Cultures of *C. maculatus* (F.), the cowpea weevils responsible for the extensive damage of stored cowpeas, were 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 1025cc jars topped with copper mesh. The cowpeas were purchased from the Bozeman Food Coop and were equilibrated for 1 month at 28°C and 65% RH in 1025cc jars topped with filter paper. After equilibration, a portion of the cowpeas were weighed, dried for 72 hours at 50°C and weighed again to determine the moisture content of the cowpeas. Moisture content of equilibrated cowpeas was determined to be 3% during all trials with no variation.

Ninety percent pure azadirachtin A was obtained from the laboratory of Dr. W. Kraus at the University of Hohenheim, Stuttgart, Germany. Forty ml glass vials for insect bioassays and a 25 microliter glass-wall syringe for use in HPLC were obtained from Supelco, Inc., Bellefonte, PA. A C-8 reverse phase HPLC column was obtained from Alltech Associates, Inc., Deerfield, IL. The Bakerbond C-18 solid phase extraction (SPE) tubes were obtained from J.T. Baker, Phillipsburg, NJ. The HPLC machine used in the chemical analyses was a Shimadzu LC-6A with a Spectroflow 757 absorbance detector = 214 nm and a Chromatopac C-R68 integrator from Shimadzu, Tokyo, Japan.

Determining Effective Concentrations

Cowpeas were equilibrated for 1 month at 38°C and 60% ($\pm$10%) relative humidity (RH) and 20 g ($\pm$0.05 g) of cowpeas were apportioned to each 40 ml copper mesh covered vial. NKE (Mali, 1995 acquisition, as this was the only acquisition we had at the time), stored at 5°C for two months, was mixed with deionized water at the following concentrations: 10%, 25%, 40%, 55%, 70%, 85% and 100%. Controls of water only and no treatment at all were also prepared. Equilibrated cowpeas were placed in a glass petri dish and 750 microliters of the given NKE concentration or control treatment were pipetted onto 3 replicates of cowpeas from the respective vials, with each replicate receiving its treatment from the same vials as all other replicates in that treatment. The petri dish was then covered

and shaken for 10 seconds so that the cowpeas would be thoroughly coated with the neem/water mixture. The cowpeas were then placed on a filter paper for 10 seconds to dry and replaced into the 40 ml glass vial. Five adult female and 3 adult male *C. maculatus* (0-24 hrs post emergence) were placed in the vial with the treated cowpeas. All adult *C. maculatus* were selected 5 days after the first adult emerged to control for any effects of cohort on oviposition patterns. After 3 days, the adult beetles were all removed and the total number of eggs in each vial was counted. Thirty-five days after the egg-counts commenced, the total number of adult beetles (progeny) were tallied. An analysis of variance (ANOVA) was performed to detect differences in concentration effects on oviposition (number of eggs) and progeny development (number of adult progeny).

The above procedure was repeated exactly except that in the second trial all replicates received their treatment concentration from a separate preparation of the concentration. This increased the chances that each replicate received the same amount of NKE whereas the first trial resulted in some replicates receiving more water and other replicates receiving more NKE.

Heat Degradation of Neem Kernel Extract

Having determined that the concentration of NKE used by Malian farmers in field applications and formulations (50% NKE in water, kerosine or other diluents) was effective at reducing infestations of *C. maculatus* in stored cowpeas when applied

at a rate of 1.69% NKE by weight, we selected this concentration and rate for later bioassays. However, on the chance that this concentration may be too high to be sensitive to chemical alterations in the NKE, we also used a lower treatment concentration of 0.84% NKE by weight.

Twenty grams (±0.05 g) of cowpeas were equilibrated for 1 month at 38°C and 60% (±10%) relative humidity (RH) and apportioned to each 40 ml, copper mesh-covered vial. NKE, stored at 50°C for 2 weeks and 28°C for 5 months, was mixed with deionized water with a volume ratio of 1:3 (NKE:water). Equilibrated cowpeas were placed in a glass petri dish and 750 microliters of the NKE and water mixture was pipetted onto the cowpeas. The petri dish was then covered and shaken for 10 seconds so that the cowpeas would be thoroughly coated with the mixture. The cowpeas were then placed on a filter paper for 10 seconds to dry and placed within the 40 ml glass vial. Five adult female and 3 adult male *C. maculatus* (0-24 hrs post emergence) were placed in the vial with the treated cowpeas. After 3 days, the adult beetles were all removed and the total number of eggs in each vial was counted. Thirty-five days after the egg-counts were begun, the total number of progeny was tallied. This procedure was repeated for cowpeas treated with NKE stored at 5°C for 5 months, NKE stored at 28°C for five months, and a control consisting of untreated cowpeas. Both extracts (Malian 1995 and 1996) as well as the separate extract from Benin were bioassayed after storage at 5°C for 5 months and 50°C for 2 weeks plus the remainder of 5 months at 28°C, but only the Malian 1995 acquisition was bioassayed after storage at 28°C for 5 months, due to restricted

supplies of NKE. Each treatment was replicated 5 times for each NKE acquisition.

In order to ascertain whether the effects the heated NKE had on *C. maculatus* were due to the chemistry of the NKE or the oil properties of NKE, we compared the effects of an inert mineral oil to the effects precipitated by the heat-treated NKE. Twenty grams ($\pm$0.05 g) of equilibrated cowpeas were apportioned to each of 30 glass vials (40 ml capacity). Five vials of cowpeas received no treatment but were infested for 3 days with 5 adult female and 3 adult male *C. maculatus*, 0-24 hrs post-emergence. Five vials of cowpeas received 750 microliters of a 25% NKE/deionized water mixture which was applied in the same manner as in the previous bioassays. These vials were infested with 5 adult female and 3 adult male *C. maculatus*, 0-24 hrs post-emergence. The last set of 5 vials of cowpeas received 750 microliters of a 25% mineral oil/deionized water mixture which was applied in the same manner as in the previous bioassays. These vials were also infested with 5 adult female and 3 adult male *C. maculatus*, 0-24 hrs post-emergence. All adults were removed after 3 days and the number of eggs enumerated. Thirty-five days after the initial infestation, adult progeny were counted in each vial. ANOVA was used to detect differences in mean number of eggs laid and mean number of adults produced.

Stage of *Callosobruchus maculatus* Affected by Heated Neem Kernel Extract

In order to find out if a particular life-stage of *C. maculatus* was especially susceptible to treatment with NKE, which had been exposed to high temperatures,

cowpeas were treated at different times before and after infestation by *C. maculatus*. Four treatments consisted of treating cowpeas with 1.69% NKE by weight 1 day, 2 days, 3 days and 7 days after infestation by *C. maculatus* (5 adult females and 3 adult males, 0-24 hrs post emergence). This would establish the effects of heat-degraded NKE on the early stages (embryonic and early larval) of *C. maculatus*. Another treatment consisted of infesting untreated cowpeas with 5 adult female and 3 adult male *C. maculatus*, 0-24 hrs post emergence. The embryos and larvae were allowed to develop for 25 days, which was typically the number of days required to reach pupation under our lab conditions. The cowpeas containing the pupal *C. maculatus*, were treated with 1.69% w/w NKE per cowpeas. Ten days after treatment, the number of adult progeny that eclosed was totalled to establish the effect of heat-degraded NKE on the pupal stage of *C. maculatus*. Because of the traumatic method of treatment (shaking the petri dish with the infested cowpeas) a control of untreated, infested cowpeas was shaken in the petri dish for 10 seconds and the total number of adult progeny was counted 10 days later.

To see if cowpeas treated with NKE would be protected from *C. maculatus* for more than 1 generation, cowpeas that had been treated 1 month earlier with 1.69% NKE by weight were infested with 5 adult female and 3 adult male *C. maculatus*, 0-24 hrs post-emergence. Thirty-five days after infestation, all adult progeny were counted. For all of these bioassays, parent *C. maculatus* were only allowed to oviposit for 3 days.

Village-Prepared Soap as a Surfactant

Malian soap was grated with a copper mesh and the powder was collected on a sterile filter paper. Twenty grams (±0.05 g) of cowpeas were equilibrated for 1 month at 28°C and 60% (±10%) RH and placed into each of 60 copper mesh-capped 40 ml glass vials. Two and a half milliliters of NKE (stored at 5°C, acquired from Mali presses, fall of 1996) was placed into a 15 ml flask. Seven and a half milliliters of deionized water was added to make a 25% mixture of NKE and deionized water. This 10 ml of NKE/deionized water mixture was to be used to treat all 5 replicates of the NKE treatment.

Two and a half milliliters of the NKE used above (stored at 5°C; acquired from Mali pressings in the fall of 1996) was placed in another 15 ml flask. Seven and a half ml of deionized water were added to make a 25% NKE and water mixture. To this mixture 10 mg of the Malian soap powder were added. This preparation was to be used to treat all 5 replicates in the 25% NKE + 10 mg soap treatment.

Two and a half milliliters of the NKE used above (stored at 5°C; acquired from Mali pressings in the fall of 1996) was placed in another 15 ml flask. Seven and a half milliliters of deionized water was added to make a 25% NKE and water mixture. To this mixture 55 mg of the Malian soap powder was added. This preparation was to be used to treat all 5 replicates in the 25% NKE + 55 mg soap treatment.

Two and a half milliliters of the NKE used above (stored at 5°C; acquired from Mali pressings in the fall of 1996) was placed in another 15 ml flask. Seven and a half milliliters of deionized water was added to make a 25% NKE and water mixture. To this mixture 360 mg of the Malian soap powder was added. This preparation was to be used to treat all 5 replicates in the 25% NKE + 360 mg soap treatment. Ten milliliters of deionized water was placed in another 15 ml flask. To this mixture 400 mg of the Malian soap powder was added. This preparation was to be used to treat all 5 replicates in the water + 400 mg soap treatment.

The last set of 5 vials received no treatment and was reserved as a control group. All vials were infested with 5 adult female and 3 adult male *C. maculatus*, 0-24 hrs post emergence. After 3 days, all parent beetles were removed and the number of eggs in each vial were counted. In all other trials except part of the first bioassay we made the NKE mixture for each replicate separately so that we could increase the probability that each replicate was receiving the same amount of NKE. This experiment was designed to show any changes in the variability between replicates by exaggerating this variability. The non-miscibility of the NKE in water caused some treatments to receive doses consisting almost entirely of water, whereas other doses to other replicates consisted almost entirely of NKE. Thirty-five days after infestation, all adults were removed from each vial and counted. An ANOVA program on SAS was used to determine differences in variance.

A calibrated sprayer was also used to measure differences in deposition of NKE mixed with water (25% NKE) when sprayed and the effects on deposition of

NKE by the addition of a surfactant. To do this, 9 watch-glasses (9 cm diam.) were placed in a 3 by 3 grid under the spraying apparatus. Each watch-glass in a row was separated from its neighbor by 3 cm. Each row of watch-glasses was separated by 18.5 cm. A velocity of 0.95 km/h was used in each spraying. This velocity, the slowest velocity on the mechanized sprayer, was selected due to the increased deposition of fluid, allowing the investigators to be more accurate in determining spray volumes. Treatments consisted of: 150 ml water only spray (control); 35 ml NKE and 115 ml water mixture; 35 ml NKE and 115 ml water mixture plus 3.5 g powdered Malian soap; 35 ml NKE and 115 ml water mixture with 0.25% by volume of X-77, a commercial surfactant. The amounts of Malian soap used were previously determined in the lab to be reasonable amounts for farmers to allot to crop protection. The amount of X-77 was the recommended rate used in most instances. These treatments were replicated 5 times and differences in deposition volumes along rows and along columns were analyzed using ANOVA.

Dried Neem Leaves as a Stored Cowpea Protectant

Cowpeas were equilibrated for 1 month at 28°C and 60% ($\pm$10%) RH. These cowpeas were then placed into copper mesh-capped 40 ml glass vials; each vial receiving 20 g of cowpeas ($\pm$0.05 g). A handful (approximately 10 g) of de-stemmed neem leaves that had been stored under nitrogen at -20°C for 1 year were placed in a blender for 1 minute. Ten vials received 0.6 grams of crushed, dried neem leaves.

Ten vials received 1.2 g of crushed, dried neem leaves. Another 10 vials received no treatment. All vials were then infested with 5 adult female and 3 adult male *C. maculatus*, 0-24 hrs post emergence. After 3 days all of the parent adults were removed and the number of eggs in each vial were counted. Thirty-five days after the initial infestation, all of the progeny adults were removed and counted. An ANOVA was performed to detect differences in the mean number of eggs laid and the mean offspring produced in each treatment.

HPLC Procedures

Azadirachtin Standard

Five point forty-nine milligrams of 90% pure azadirachtin A was dissolved into 5 ml of HPLC-grade methanol. Ten microliters of this solution was injected into the column using a 25 microliter syringe. An isocratic 50:35:15 H_2O:MeOH:Acetonitrile (AcN) mobile phase was used for analysis and pure AcN was used for 20 minutes to clean the column between runs, according to the methods of Hull et al. (1993). The runs were repeated 5 times to ensure that the chromatograms were typical. The flow rate was set at 1 ml/min; UV absorbance = 214 nm. Anisole was used as an internal standard to quantify azadirachtin A content (Thejavathi et al. 1995).

Neem Kernel Extract

NKE from Benin and Mali (0.5 ml) was mixed with 5 ml of HPLC grade methanol and placed on an Bakerbond octadecyl (c-18) solid phase extraction (SPE) column, equilibrated with 5 ml of methanol. No pressure was used to extract the NKE. The filtrate was collected for analysis and run on the HPLC according to the settings for the pure azadirachtin run. Ten microliters of 90% pure azadirachtin A were spiked in each NKE to see when azadirachtin A eluted. All HPLC runs were repeated 5 times to ensure that the chromatograms were typical.

RESULTS

Determining Effective Concentrations

C. maculatus demonstrated a dose-dependent response with respect to oviposition and adult progeny when treated with the various rates of NKE. The mean number of eggs per female and progeny per female decreased significantly with increasing concentrations of NKE ($p < 0.05$). The effective concentrations we selected to use in our bioassays were 1.69% NKE by weight and 0.84% NKE by weight. However, we do note the difference in variation between the two techniques we used, indicating that immiscibility of NKE in water could be an important problem in the utilization of NKE. (See Tables 1 and 2.)

The4 bruchids exposed to the 10% NKE treatment demonstrated significantly lower oviposition (means of 31.1 and 28.7 eggs in tables 1 and 2, respectively) and progeny (means of 12.5 and 8.9 progeny in tables 1 and 2, respectively) than either of the controls, but were significantly less effective than the higher concentrations of NKE. Bruchids exposed to the 25% NKE did not lay a significantly different number of eggs than the bruchids exposed to the 10% NKE but the progeny number had been reduced significantly (means of 4.2 and 1.9 in tables 1 and 2, respectively).

Table 1. Mean[1] number of eggs laid per female *C. maculatus*[2] and mean number of F_1 adults per female, resulting from a treatment concentration of 750 microliters of neem kernel extract. All replicates receiving their treatment from the same preparations (method of maximum variance). (n=3)

Treatment	Mean eggs laid/ female (x±SEM) (r^2=0.73)*	Mean F_1 adults emerged/female (x±SEM) (=0.72)**
Untreated	43.4±1.3 a	25.1±1.5 b
Water treated	48.6±1.0 a	30.0±2.0 a
10% NKE	31.1±3.0 b	12.5±4.0 c
25% NKE	33.2±7.8 b	4.2±3.3 d
40% NKE	16.2±5.3 c	0 d
55% NKE	0 c	0 d

[1] Means followed by the same letter within a column are not significantly different at the 0.05 level; Student-Neuman-Keuls)

[2] 5 adult female and 3 adult male *C. maculatus*, placed on 20 g of cowpeas 0-24 hrs post emergence for 3 days.

* For effects of NKE concentration on oviposition: F-value = 44.06; df = 5; $p < 0.000$.

** For effects of NKE concentration on F_1 progeny: F-value = 42.23; df = 5; $p < 0.000$.

Table 2. Mean[1] number of eggs laid per female *C. maculatus*[2] and mean number of F_1 adults per female, resulting from a treatment concentration of 750 microliters of neem kernel extract. All replicates received their treatment from separate preparations (method of minimal variance). n=3

Treatment	Mean eggs laid/ female (x±SEM) (r^2=0.97)*	Mean F_1 adults emerged/female (x±SEM) (r^2 = 0.91)**
Untreated	42.5±0.9 a	23.9±0.2 b
Water treated	42.5±1.1 a	29.1±1.3 a
10% NKE	28.7±0.4 b	8.9±3.9 c
25% NKE	26.2±1.9 b	1.9±1.9 d
40% NKE	1.7±0.6 c	0 d
55% NKE	3.2±3.2 c	0 d

[1] Means followed by the same letter within a column are not significantly different at the 0.05 level; (Student-Neuman-Keuls)

[2] 5 adult female and 3 adult male *C. maculatus*, placed on 20 g of cowpeas 0-24 hrs post emergence for 3 days.

* For effects of NKE concentration on oviposition: F-value =137.99; df = 5; $p < 0.000$.

** For effects of NKE concentration on F_1 progeny: F-value = 27.61; df = 5; $p < 0.000$.

Heat Degradation of Neem Kernel Extract

NKE from the various storage regimes was fully effective at the 1.69% NKE by weight treatment after 5 months (i.e., no eggs or F_1 emergence was observed). The bioassay using 1.69% NKE by weight may have been too high a concentration to be sensitive to the loss of azadirachtin A in NKE which was indicated by our HPLC analysis. To increase the sensitivity of our bioassays to changes in the chemistry, we also used 0.84% NKE by weight. Although oviposition increased significantly, the 25% NKE by weight applied 5 months after heat treatment and continuous storage at 28°C was still fully effective at keeping adults from emerging (See Tables 3 and 4, respectively; $p < 0.05$). Malian 1995 NKE stored for 5 months at 28°C still significantly reduced oviposition compared to controls (see Tables 3 and 4; $p < 0.05$), but demonstrated less oviposition deterrence than Malian 1995 NKE stored at 50°C for 2 weeks and at 28°C for the remainder of the 5 months. (See Tables 3 and 4.)

Location of the NKE also displayed significant effects at the 0.05 level for oviposition and progeny development. The 1996 acquisitions (both Mali and Benin) stored at 5°C for 5 months demonstrated significantly less ovipostion deterrency than the Mali 1995 acquisition stored at 5°C for 5 months. The same is true when the different extract acquisitions are stored at 50°C for 2 weeks and the remainder of the 5 months at 28°C; The 1996 acquisitions demonstrate significantly less oviposition deterrence (means of 30.4 and 29.8 eggs for Malian and Benin 1996 acquisitions,

respectively) than the Mali 1995 acquisition (mean of 24.9 eggs). Location of the acquisition had a significant effect on progeny development at the 0.05 level, but the temperature regimes the extracts were stored at had no significant effect on progeny development .

Table 3. Effect of storage temperature and locale of neem kernels on extract (0.84%w/w) efficacy in reducing oviposition of *C. maculatus*. Mean[1] (±SEM) number of eggs per parent female[2]. (n = 5)(r^2 = 0.90)

Location* and date of acquisition	Untreated controls**	5°C for 5 months**	28°C for 5 months**	50°C for 2 wks and 28°C for the remainder of 5 months**
Mali, 1995	44.3±1.3 a	24.9±1.6 c	30.0±1.2 b	38.6±1.1 a
Mali, 1996	44.3±1.3 a	30.4±0.6 b	-	39.5±0.7 a
Benin, 1996	44.3±1.3 a	29.8±1.1 b	-	38.5±0.6 a

[1] Means followed by the same letter are not significantly different ($p < 0.05$; Student-Neuman-Keuls).

[2] 5 adult female and 3 adult male *C. maculatus*, placed on 20 g of cowpeas 0-24 hrs post-emergence for 3 d.

* For effects of location: F-value = 4.85; df = 3; p = 0.0156.

** For effects of temperature: F-value = 144.96; df = 2: $p < 0.0001$.

Table 4. Effect of storage temperature and locale of neem kernel on extract (0.84%w/w) efficacy in reducing progeny of *C. maculatus*[1]. Mean[2] (±SEM) number of adults per parent female. (n = 5)(r^2 = 0.04)

Location* and date	Untreated Controls**	5°C for 5 months**	28°C for 5 months**	50°C for 2 wks and at 28°C for the remainder of five months[4]***
Mali, 1995	22.5±2.1 a	5.0±0.5 b	5±3.8 b	5.4±0.6 b
Mali 1996	22.5±2.1 a	5.5±0.5 b	-	5.8±0.9 b
Benin, 1996	22.5±2.1 a	1.0±0.6 c	-	1.2±0.7 c

[1] 5 adult female and 3 adult male *C. maculatus*, placed on 20 g of cowpeas 0-24 hrs post-emergence for 3 d.

[2] Means followed by the same letter are not significantly different ($p < 0.05$; Student-Neuman-Keuls).

* For effects of location: F-value = 12.25; df = 3; p = 0.0002.

** For effects of temperature: F-value = 0.06; df = 2; p = 0.9409.

The results of our mineral oil trial demonstrate that the mineral oil is just as effective as the heated NKE. (See Table 5.) These results suggest that it is the oil in NKE that exerts lethal effects on the *C. maculatus* embryo. Due to lack of available NKE we could not test the efficacy of non-heated NKE, which may have provided some interesting results in comparison with the mineral oil treatment.

Table 5. Effect of inert mineral oil as an oviposition deterrent and larval toxicant, compared to effect of neem kernel extract applied at the same rate. Mean[1] (±SEM) number of eggs or F_1 adults per parent female[2]. (n = 5)

Treatment	Untreated Control	25% Neem Kernel Extract	25% Mineral Oil
Eggs	41.8±0.8 a	38.1±1.9 a	35.1±1.6 a
Adult Progeny	21.2±1.6 a	5.2±1.5 b	7.3±2.3 b

[1] Means within rows followed by the same letter are not significantly different ($p < 0.05$; Student-Neuman-Keuls).

[2] 5 adult female and 3 adult male *C. maculatus*, placed on 20 g of cowpeas for 3 days 0-24hrs post-emergence.

Stage of *Callosobruchus maculatus* Affected by Heated Neem Kernel Extract

All 1.69% NKE by weight treatments applied 1 day, 2 days, 3 days and 7 days after oviposition by the bruchids were effective at keeping progeny from developing (0 adults emerged from all of these trials). The 1.69% NKE by weight applied while the bruchids were in the pupal stage (25 days after oviposition) reduced adult emergence significantly (mean = 10.1±1.1 adults per female when treated with 1.69% NKE by weight; mean = 18.5±1.3 adults per female when pupae are untreated: Student-Neuman-Keuls, $p < 0.05$). (See Table 6.) All 0.84% NKE by weight treatments applied 1 day, 2 days, 3 days and 7 days after bruchid oviposition were effective at reducing progeny survival. (See Table 6.) However, the 0.84%

NKE by weight applied while the bruchids were in the pupal stage did not have a significant effect on F_1 emergence (mean = 21.1±1.2 adults per female when pupae were treated with 0.84% NKE by weight; mean = 18.5±1.3 adults per female when pupae were untreated: Student-Neuman-Keuls, $P > 0.05$). (See Table 6.)

Table 6. Effect of heated neem kernel extract on various developmental stages of *C. maculatus*. Mean[1] (±SEM) number of adults per parent female emerging 45 days after infestation. ($n = 5$)($r^2 = 0.94$)

Time of treatment (relative to infestation[2])*	**Stage of development**	**Untreated controls****	0.84%w/w NKE[3]**	1.69%w/w NKE[3]**
28 days prior	**No insects**	**21.7±1.9 a**	6.1±1.5 b	0 c
1 day after	embryo	21.6±2.2 a	4.5±1.3 b	0 c
2 days after	embryo	22.9±1.7 a	4.5±0.9 b	0 c
3 days after	embryo	21.6±1.1 a	5.4±0.8 b	0 c
7 days after	1st instar	23.4±1.3 a	5.1±0.8 b	0 c
25 days after	pupa	18.5±1.3 a	21.1±1.2 a	10.1±1.1 b

[1] Means followed by the same letter are not significantly different ($p < 0.05$; Student-Neuman-Keuls).

[2] 5 adult female and 3 adult male *C. maculatus*, placed on 20 g of cowpeas 0-24 hrs post emergence for 3 d.

* For time of treatment effects: F-value = 20.99; df = 5; $P \leq 0.0001$.

** For concentration effects: F-value = 470.97; df = 2; $P \leq 0.0001$.

Village-Prepared Soap as a Surfactant

The addition of various concentrations of Malian soap did not reduce the variability of treatments (see Table 7), although when the solutions were prepared, all of them appeared to be homogenized (uniform color). The soap also had no apparent effect on the oviposition decisions of females. (See Table 7.) The spray table experiment also revealed that the addition of feasible amounts of Malian soap or X-77 does not homogenize NKE in water (95% CI of the slope of spray pattern when only water is sprayed is between -6.9 and -3.5: 95% CI for the slopes of all other mixtures is between 5.4 and 13.9). (See Figures 2-5.) The main difference is the higher levels of oil deposition toward the end of the spray run in the oil trials. The water only spray pattern is relatively even, demonstrating no trend similar to the oil and water spray patterns. Distance within rows showed no correlation with oil deposition (r^2 = 0.12) in any of the treatments, but distance between rows was a significant factor in all oil treatments ($r^2 = 0.80$, $p < 0.05$).

Table 7. Effects of using Malian village-prepared soap in reducing the variability of neem kernel extract efficacy and oviposition of female *C. maculatus*[1]. (n = 5)

Treatment	Mean[2] no. of eggs laid/adult female ± SEM
Untreated controls	30.2±1.9 a
750 microliters of 25% neem kernel extract[3]	11.3±3.6 b
750 microliters of 25% neem kernel extract[3] + 10 mg of Malian Soap	11.6±4.7 b
750 microliters of 25% neem kernel extract[3] + 55 mg of Malian soap	13.92±3.8 b
750 microliters of 25% neem kernel extract[3] + 360 mg of Malian soap	22.36±2.5 ab
400 mg of Malian soap	28.64±5.5 a

[1] 5 adult female and 3 adult male *C. maculatus* placed in vial with 20±0.05g of cowpeas and removed after three days.

[2] Means followed by the same letters are not significantly different at the 0.05 level (Student-Neuman-Keuls).

[3] Mali, 1996 acquisition.

Dried Neem Leaves as a Stored Cowpea Protectant

The neem leaves we evaluated and the concentrations we tried were not effective at reducing oviposition or adult survival when applied to infested cowpeas. ($p > 0.05$; Table 8.) The number of eggs laid per female were similar for the 2 dried neem leaf treatments and the control. Similarly, minimal differences in the number of progeny were obtained for the neem leaf treatments compared to the control treatments.

Table 8. Mean[1] number of eggs laid per adult female *C. maculatus*[2] and progeny survival on cowpeas treated with dried, crushed neem leaves.

Treatment	Mean no. eggs laid/female ± SEM	Mean no. adult progeny emerged/female ± SEM
Untreated control	32.6±2.0 a	20.7±2.7 a
6% by weight of dried, crushed neem leaves	30.2±1.3 a	19.0±1.9 a
3% by weight of dried, crushed neem leaves	34.5±1.7 a	22.1±0.9 a

[1] Means within a column followed by the same letter were not significantly different at the 0.05 level (Student-Neuman-Keuls).

[2] 5 adult female and 3 adult male *C. maculatus*, placed on 20 g of cowpeas 0-24 hrs post emergence for 3 d.

HPLC Quantification of Heat-Treated Neem Kernel Extract

HPLC revealed that there is a great deal of variability concerning the chemical composition of the various NKE acquisitions. (See Figure 6.) Only the September 1995 acquisition from Mali has azadirachtin A. The September 1995 acquisition from Mali was also the most repellent to ovipositing females, although its toxicity to developing larvae was not greater than that of other acquisitions or the heat-treated NKE that had undetectable levels of azadirachtin A. (See Tables 3 and 4.) We also noted that high temperature regimes (28°C and 50°C) effectively remove azadirachtin A. (See Figure 6.)

DISCUSSION

The importance of cowpeas to West African farmers and the destructiveness of the cowpea weevil to stored cowpeas have spurred many farmers in West Africa to assay a variety of potential protective agents. These include the use of pepper seed oil, *Piper guineense* (Ivbijaro 1990), ginger and cashew (Echindu 1991), sand, ash, plant materials and paprika (Zehrer 1983) and various neem products (Zehrer 1983; Makanjuola 1989; Ivbijaro 1990; Echindu 1991). Although neem is widely available to Malian farmers they have not currently implemented its use as a postharvest crop protectant to any degree. The interaction of scientists and farmers is fundamental to implementing the use of neem as a protectant of stored cowpeas in rural Mali.

Our laboratory work revealed many exciting dynamics that are important to semi-subsistence farmers protecting stored cowpeas in West Africa. Although the chemistry of neem kernel extract is very important in many situations, it is not necessary for the protection of cowpeas. We have shown that removing azadirachtin A by means of heat does not reduce the NKE's effectiveness at protecting cowpeas from bruchids. Thus, the farmer using NKE to protect stored cowpeas may prepare NKE at a convenient time without concern for loss of azadirachtin A or other important compounds that are dissipated by heat. We have also demonstrated that the effectiveness of NKE that has been heated is probably due almost entirely to the oil properties of the NKE. Although it is the oil that is effective in the postharvest situation, in the preharvest system neem's effectiveness is most likely highly

dependent on the chemical constituents. Therefore, farmers applying neem in the field must be aware that a great deal of variability in neem's effectiveness is likely, given that these compounds are dissipated by heat and the trees will produce varying amounts of these compounds.

We have demonstrated that the life stages of *C. maculatus* that are most susceptible to the deleterious effects of NKE are the early embryonic and larval stages. However, cowpeas treated 1 month prior to infestation can resist infestation, so farmers do not need to apply the NKE based on the life cycle of the bruchid.

Unfortunately, our results show that the preharvest benefits of neem in Mali are not as dependable as they are in postharvest applications. Malian soap does not appear to reduce variability of aqueous NKE effectively when administered at levels feasible for Malian farmers. This should strengthen the resolve of researchers to explore other methods of improving the miscibility of NKE in water. Although ultra-low-volume sprayers are not extensively available to Malian farmers, they may remedy the formulation problem by homogenizing applications. In the meantime, it can only be expected that Malian semi-subsistence farmers will continue to use kerosine and other petroleum based solvents in their applications. Currently there are trials underway to test the efficacy of the soap itself as a crop protection agent in the preharvest system.

Our tests indicate that the leaves of the neem tree do not provide significant control of *C. maculatus*. Many scientists, however, have demonstrated that neem leaves are effective against *C. maculatus* (Makanjuola 1989; Echindu 1991). The

contradiction of these results could very well be due to the fact that our neem leaves were not fresh. Larger applications may also prove to be effective. If fresh neem leaves do prove to be helpful postharvest protectants, Malian farmers will have a reliable source to count on when neem fruits are not available.

Finally, our analytical assays demonstrate that there is a great variation in the azadirachtin A content of NKE. NKE acquired in the fall of 1995 from Mali had measurable amounts of azadirachtin A. The other acquisitions (Mali, 1996 and Benin, 1996) did not have azadirachtin A at levels detectible by our HPLC assay, an important consideration for farmers using NKE to protect preharvest crops where chemical components are likely to play a greater role in crop protection.

These investigations have resulted in further field studies, conducted by Malian scientists and farmers. Farmers have adopted the use of neem in stored cowpeas on a preliminary basis.

CONCLUSIONS

Neem is an abundant resource in many subsistence and semi-subsistence settings in rural West Africa. Currently, the neem tree is most widely used for fuel in West Africa, but our studies show that the neem tree can provide extremely valuable crop protection to farmers in this area. Stored cowpeas can be protected by NKE even after storage at extreme temperatures. Furthermore, this protection extends for at least one generation of the cowpea weevil after treatment. In the postharvest situation, the farmer need not worry about variability in azadirachtin A content because it is the oil that protects the stored cowpeas.

Unfortunately, we were not able to overcome the miscibility problem we encountered. This could be a serious problem for Malian farmers applying NKE to crops in the field. We have learned, however, that the hulls of the neem kernel, which were removed in our experiments, may provide plenty of surfactant to keep the active agents, such as azadirachtin A, in solution in water (Saxena, personal communication 1997).

The leaves have not proved effective at all in our trials, despite the claims of many scientists that the leaves are effective. This may be due to our inability to test fresh leaves. Perhaps fresh leaves would be an effective resource for Malian farmers.

Although the neem tree does show promise, there are many other avenues to be researched and explored to improve sustainable agriculture in Mali and the rest of West Africa. Currently, there is a sustained interest in wild cultivars of our crop

plants because these plants appear better able to defend themselves against the insects and diseases that are plaguing our crops.

Plant chemistry appears to be a useful tool in the battle humans are embroiled in against insect pests for food and fibre, but the success of wild cultivars should not be attributed solely to the expression of bioactive compounds. A whole suite of ecological phenomena may account for the success of wild cultivars, including phenology, spatial occurrence, genetic variability, and compensatory strategies. Neem has shown itself to be a promising control agent, but it has been evaluated without consideration of equally important cultural measures. Perhaps, when neem is used with a variety of other tools, the Malian semi-subsistence farmer may advance into the millennium with the capacity to feed not only his or her family, but to reserve a portion of his or her harvest for commercial use. It is satisfying to know that, at least in some Malian villages, our research has been instrumental in pointing out that the potential exists for realizing commercial profit using the NKE system.