Optimising the indigenous use of botanicals in Ghana

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Executive Summary

Botanicals with pesticidal properties are already used by farmers in many countries as a means of reducing the impact of insect pests on their stored grains and legumes. Farmers often prefer to use botanicals as opposed to commercial synthetics for a number of reasons. Botanicals do not need to be purchased and are, therefore, low-cost (requiring labour inputs but no direct financial outlay). Many farmers also believe that botanicals are safer to use and are more flexible in their usage than commercial synthetics. Botanicals are also often much more available than commercial products as they grow in the local environment. Our research has been able to show through farmer participatory trials that some of the plants traditionally used by farmers for stored product protection offer comparable protection to commercial synthetics. Farmers were able to make selections on which plants worked best due to their own comparative analysis and base further decision making on storage options with this new knowledge.

Plant secondary metabolites, which are responsible for bioactivity, are well-known to vary according to climatic, seasonal and geographic effects. Botanical materials were collected for eight different plant species within their normal habitat ranges. These were collected from three different times of year and from four different geographic locations in the Northern and Upper East Regions of Ghana. These samples were then analysed for changes in their phytochemical profile and their efficacy of control. Generally, collection time was more important than where it was collected from. Collection time or place had no real effect on level of efficacy for some plants, most notably *Securidaca longepedunculata* and *Cymbopogon schoenanthus*. Where the plants were collected from was important for some plants more than others. For example, *Ocimum americanum* was generally more potent when collected from the Upper East Region than the Northern Region. It is unknown whether this is due to climatic factors or genetic variations among varieties or sub-species in different areas.

Plants were found to act by two main actions, repellency and/or toxicity. Plants which are repellent are not always toxic. For example, if a farmer mixes a repellent plant in with grain which is already infested, the plant would have little effect in reducing the level of infestation. Repellent plants are good at preventing infestation on clean grain, and there is some evidence that a repellent plant may cause adults to leave infested grain, but generally should not be recommended to treat infested grain. Toxic plants were generally either effective against the egg and/or adult stages of primary pests (*Prostephanus truncatus*, *Rhyzopertha dominica*, *Sitophilus zeamais* and *Callosobruchus maculatus*). Plant species showed different levels and durations of efficacy depending on the species and commodity affected.

Reviving and modernising age-old farmer practice through the optimisation of ethnobotanicals has shown that farmers are more comfortable using plant materials than commercial synthetics and that botanicals can offer a similar level of control when certain guidelines are followed to their use. Our research has provided an understanding of plant chemistry and modes of action for plant species already used by many farmers in Ghana for stored product pest control. This has shown that these pesticidal plants can be used reliably and safely to treat grain and legumes when stored in small quantities at the farm level. Because of their indigenous use in Ghana, legal registration of these botanical products is not required for their promotion in Ghana. Knowledge on factors such as application concentration, method of application, preparation of botanicals and duration of control expected can be used to promote botanicals as a cost-effective and environmentally sustainable alternative to commercial synthetics.

Background

Small-scale farmers throughout sub-Saharan Africa continue to have problems protecting their harvested crops from insect infestation during storage. Traditional grain storage structures vary considerably and are unable to prevent insect infestation. Storage losses are typically patchy and can be a big threat to food security and household incomes particularly when losses are severe. Because of the risks associated with grain storage, farmers try to minimise their losses by selling their grain soon after harvest. Unfortunately, since most farmers sell their grain at this time, market prices are low as the market is flooded with recently harvested grain. Farmers could achieve a much higher

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price if they were to sell their grain later in the season, but they must control insect infestation of the grain over this time period.

Subsistence farmers often lack the financial resources to buy good quality commercial insecticides to protect their stored food, and their inappropriate use of conventional pesticides can result in risks to human and environmental health and promote insecticide resistance. Traditional storage methods using indigenous plants with insecticidal properties could, if improved, offer a safer, low-cost and more dependable method of storage protection while reducing the increasing reliance upon conventional pesticides. Farmers need information on botanicals to support their decision making with respect to the reliability of control they can expect when using a particular plant material to reduce insect infestation.

Research on farmer usage of plant materials in Ghana first started in 1996. This earlier collaborative work involving the Natural Resources Institute, the Ghanaian Ministry of Food and Agriculture and the Ghanaian Savannah Agricultural Research Institute surveyed the northern regions of Ghana to find out what, why and how plants were used by farmers. These surveys identified seventeen plant species commonly used by farmers for stored product protection (Brice et al., 1996). Confirming the identity of the pesticidal plants was an extremely important step because sometimes several local names could refer to the same plant species. The opposite was also experienced where a single local name could refer to a plant with very similar and closely related species within the same genus. The way farmers used the plants varied considerably. Some farmers would make a hot water extract of the plant which they pour over or dip their commodity into. Other farmers would use the plant material whole, whereas others would grind the plant into a powder (Table 1). Other differences occurred with the amount or concentration of material used to treat the commodity, and some farmers would admix botanicals while others would layer it with their commodity. Some farmers would mix two or more plant species together while others would reapply plant material as the storage season progressed. The plant parts used for any given species was the least variable factor, and, generally, all farmers would consistently use the leaves, roots or flowers for particular plant species. The big differences found in application methodology showed how innovative and experimental farmers could be. It also highlighted that communication among farmers may be a problem as no single method could claim superiority over another.

The survey asked farmers to assess different plant materials and synthetic pesticides against marker criteria (cost, effectiveness, availability, toxicity, ease of use, acceptability and versatility), and these results showed that some plant materials were preferred over others, either because of their availability or their ease of use. The survey showed that all farmers favourably rated the use of plant materials in comparison to commercial synthetics. This research also showed that 74% of villages visited during the survey showed some usage of plant materials as storage protectants. However, out of the three regions surveyed (Northern, Upper East and Upper West), the usage of plant materials was geographically biased towards the Upper East where the overall number of farmers using plant materials was highest. Based on these results, the usage of plant materials appears to be ethnically and culturally biased, constraining the availability of indigenous knowledge systems and preventing its uptake in other communities of northern Ghana (Cobbinah et al., 1999).

Initial screening trials with the plants against a range of stored product insect pest species showed that the efficacy of different plant species to control stored product insects was not correlated with the prevalence of farmer usage. In other words, the prevalence of the plants growing in the natural environment and other marker criteria were important factors in how widely a plant was used by farmers. For example, trials showed that the most effective plant material in controlling storage insects was *S. longepedunculata*, but this was not the most widely used plant by farmers. Laboratory and field trials showed that some of the plants were more effective in controlling storage insects, and the least effective plants were removed from further research trials.

A problem which became evident during this work was that the quality of pest control was often highly variable when using botanical treatments. As recommendations made to farmers need to be simple and trustworthy, a second phase of research was funded by the Crop Post-Harvest Programme, aimed at optimising the methods for using plant materials in the storage environment and identifying

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potential constraints to their promotion. This research project was initiated to address the researchable constraints that had been identified by previous CPHP research.*

Table 1 Previous CPHP-funded research was able to narrow down the list of 17 plant species that are commonly used by farmers in the north of Ghana for stored product protection to those 8 that were the most effective. These materials remain the focus of further research on pesticidal plants which have been selected through laboratory and field research that showed them to offer good insect pest control.

Local name varies among languages	**Latin name**	**Method of use cited by farmers**
Neem	*Azadirachta indica*	Most farmers use leaves, using either fresh or dried whole leaves, leaf powder, a paste or water extract, admixed or layered. Few farmers use seed oil because it's difficult and smelly to process. Many other uses as medicines, pesticides, soap.
Tikublaakum	*Cassia sophera*	Powdered leaves. Also used as green manure and as a supplement to animal fodder.
Lodel	*Chamaecrista nigricens*	Powdered leaves, admixed or placed at base. Veterinary and medicinal uses. Sometimes sold in markets.
Kulenka	*Cymbopogon schoenanthus*	Whole or powdered flower heads or entire plant. Sometimes sold in markets.
Lidikonja	*Lippia multiflora*	whole leaves and/or flowers, admixed or layered
Kpasiuk	*Ocimum americanum*	Whole or powdered mature plants, admixed or layered. Ceremonial and medicinal uses.
Palaga	*Securidaca longepedunculata*	Water from soaked roots, admixed powdered root bark. Many other uses, including water purification, ceremonial, medicinal, washing clothes. Sometimes sold in markets.
Kimkim	*Synedrella nodiflora*	Water from boiled leaves or whole plant, poured or immersed 20-30 sec., powdered leaves

Project Purpose

The purpose of this project was to develop strategies which improve food security of poor households through increased availability and improved quality of cereal and pulse foods and better access to markets. Specifically, the project purpose was to address issues that could prevent the increased usage of ethnobotanical materials for insect pest control.

Research Activities

In order to facilitate FTR review, project activities are summarised as 6 separate manuscripts following a peer-reviewed journal format

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Temporal and spatial changes in the phytochemistry of eight botanicals traditionally used for stored product protection and the effects on anti-insect activity

Abstract
Eight plant species traditionally used in Ghana for stored product protection were screened for potential changes to their phytochemistry when plant materials were collected from different areas or seasons. High performance liquid chromatography (HPLC) of crude methanol extracts showed that collection time and place affected the phytochemistry of *Cassia sophera*, *Azadirachta indica*, *Synedrella nodiflora* and *Ocimum americanum*, whereas *Lippia multiflora*, *Cymbopogon schoenanthus*, *Securidaca longepedunculata* and *Chamaecrista nigricens* showed no significant phytochemical differences among samples collected at different times or places. The repellent properties of the plants were measured using a behavioural bioassay with *Sitophilus zeamais* to determine whether this type of bioactivity was affected by the observed changes in phytochemistry. *S. zeamais* were not repelled by maize admixed with *C. nigricens* and *C. sophera* with all other botanicals showing varying degrees of repellency. The degree of repellency associated with plant samples collected from different times or places roughly followed changes in phytochemical profiles. Plants which showed no differences in phytochemistry (*C. schoenanthus* and S. *longepedunculata*) showed equal repellent properties. Plants which showed chemical changes with respect to collection time and/or place had respective changes in their repellency (*A. indica*, *S. nodiflora*, *O. americanum*). The implications of these results are discussed in the context of providing small scale farmers with reliable information on the use of plant materials for stored product protection.

Introduction
Botanicals are well-known to vary in the quality and quantity of active ingredients (Agerbirk et al., 2001; Azevedo et al., 2001; Isman, 1997). The impacts of phytochemical variability have been observed by many researchers testing botanicals against a range of target insect pest species (Prakash and Rao, 1997; Satasook et al., 1994; Marr and Tang, 1992). Anecdotal information from farmers in Ghana has suggested that plant materials used to protect grain from insect infestation do not always work in the same way, with some farmers claiming they work well while others indicate poor control. Many factors could account for these different farmer observations, such as different application methods or different levels of initial insect infestation before treatment. Farmer reports could also be explained if different species were growing in different regions or if the active ingredients were not present in plants collected from different areas. It was important to rule out that potential phytochemical variability did not significantly change the potency of plant materials collected in different places or at different times of the year. Because recommendations made to farmers need to be simple and trustworthy, a series of experiments were conducted which aimed to understand more specifically the chemical profiles of the plants and whether significant linked changes could occur in their bioactivity.

Materials and Methods
Phytochemical analysis
Plant materials (*Azadirachta indica*, *Cassia sophera*, *Lippia multiflora*, *Chamaecrista nigricens*, *Ocimum americanum*, *Cymbopogon schoenanthus*, *Synedrella nodiflora* and *Securidaca longepedunculata*) were collected from four different geographic areas in northern Ghana (near the towns of Tamale, Yendi, Bawku and Bolgatanga). Depending on the plant species and which parts were traditionally used by farmers, the leaves, small stems, flower heads/seeds and/or roots were collected. In each collection area, materials were collected from five different sites that were 10 - 20 km from each other. Plants were collected at three different times, roughly following different physiological stages of the plants. The first collection time was during the month of August, the new growth stage. The second collection occurred during the month of September, the flowering/fruiting stage. The third collection occurred during the months of October/November, the senescence stage. Plants were shade dried in Ghana and then shipped to the UK where they were stored in the dark at 12-13˚C.

Crude extracts of plants were prepared by grinding 100g of each plant species into a fine powder using an electric mill (HR2810A, Phillips). A 1g sub-sample of each 100g sample was mixed with 10ml of methanol in a glass vial. The vial was then gently shaken for a few seconds, and the plant material was allowed to extract for 24 hours at room temperature. After the 24-hour period, the extract was filtered (Whatman 93, 105mm diameter) using another 10ml of methanol to rinse the vial residues

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through the filter. Filtrates were placed into pre-weighed glass vials and evaporated under vacuum. After extract yields were calculated, 2ml of HPLC grade methanol was added to each vial to re-dissolve the extract which was then injected into a LiChrCART® 250-4 analytical column connected to a Waters™ 996 Photodiode Array Detector, Waters™ 717 Autosampler and Waters™ 600E system controller and analysed at 280nm. The number of major peaks in the HPLC profile were recorded. Differences in the peak profile and extract weights of samples from different areas and collection times were compared using ANOVA with a post-hoc LSD test.

Bioactivity assay
The behavioural responses of the maize storage pest, *Sitophilus zeamais*, were assessed by a one-way “choice” test bioassay to determine whether plants were repellent to the insect and whether the collection time and/or place of the plant material affected the insect’s behaviour in different ways. Ethovision video tracking, motion analysis and behaviour recognition system software (Noldus Information Technology b.v., Wageningen, NL) was used to monitor insect behaviour and quantify beetle movements in a bioassay in which insects could move between an open area and an area containing maize treated with powdered plant material. A camera placed above the experimental arena transmitted video information to a computer via a video-digitising-frame-grabber allowing an unmarked beetle to be tracked. The behavioural chamber arenas consisted of clear polystyrene boxes (124 x 82 x 22 mm, Azpack Ltd., Loughborough, UK) placed with an infrared light source beneath. Arenas were prepared by applying Fluon® to the inner sides of the arenas to prevent insects escaping. Two zones within each arena were defined as the “maize zone” containing treated or untreated maize and an “empty zone” containing nothing. Dried and pre-ground samples of plant material were admixed with 10 g of pre-conditioned maize (to experimental conditions of 27±2 ºC and 60±5 %rh) to give a 1.0% w/w concentration. The control trial consisted of untreated maize placed in “maize zone” of the bioassay dish. A single unsexed *S*. *zeamais* adult 7-14 days old was placed into the empty zone of each arena, and the position of the insect was tracked and recorded by the computer for 30 minutes with each treatment repeated six times. The average amount of time spent by the insect in the empty zone and the frequency of visits by the insect into the empty zone were taken as indicators of the degree of repellency of the botanical when admixed with the host's commodity. Data were compared using non-parametric analyses for unrelated samples.

Results
Phytochemistry
Phytochemical variability was more pronounced in some species than in others (Table 1). *C*. *sophera*, *A*. *indica*, *S*. *nodiflora* and *O*. *americanum* showed significant differences between the number of HPLC peaks and the extract yield, whereas *L*. *multiflora*, *C*. *schoenanthus*, *S*. *longepedunculata* and *C*. *nigricens* showed no significant differences among samples collected at different times or places. Variability among samples of *C*. *sophera* was accounted for by collection time 1 being different from the second and third collection time in both the number of peaks in the HPLC profile and in the mean extract weight. Differences in *A*. *indica* were shown to vary with time and place. Samples from Bolgatanga and Bawku were different from samples from Tamale and Yendi with respect to HPLC profiles but not with extract weights. Collection time varied for *A*. *indica* with both extract yield and HPLC profile showing that collection 2 was different from collections 1 and 3. *O*. *americanum* varied with collection time for both parameters with collection 1 different from collection 2 and 3. HPLC also showed that *O*. *americanum* varied by site with samples from Bawku different from the other three sites. *S*. *nodiflora* differed with collection time with all three collection times different from each other by HPLC and extract yield.

Repellency
Six of the eight species were shown to be repellent to *S*. *zeamais*, increasing the time that insects stayed away from maize treated with admixed powder (1% w/w) of the plant and the frequency of insects moving off of treated maize (Figure 1). In all treatments insects would enter the area where maize was present. In the case of the untreated maize (control) insects then usually remained within the maize and did not re-enter the empty zone, spending more than 80% of their time in the maize zone. Insects exposed to maize admixed with *C*. *nigricens* and *C*. *sophera* acted in a similar way to the untreated control. The other six plant species affected insect behaviour by increasing the time that insects spent in the empty zone and the number of visits to the empty zone when compared to the untreated control (Mann-Whitney U-test, $P < 0.05$) indicating that insects preferred to remain in the empty zone as opposed to the treated maize. *C*. *schoenanthus* and *S*. *longepedunculata* were the most repellent to *S*. *zeamais*.

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The effects of collection time and place where shown to occasionally affect the degree of repellency for some of the plant species. Ground leaves of *A*. *indica* were more repellent to *S*. *zeamais* when collected at time 2 and 3 than at collection time 1; however collection place did not change the repellent properties of the plant (Figure 2). *C*. *schoenanthus* and S. *longepedunculata* were found to be equally repellent no matter where or when the material was collected (Figure 3 and 4, respectively). *L*. *multiflora* was relatively more repellent when collected at time 3 (Figure 5). *O*. *americanum* was more repellent when collected at time 2 and 3 and from the area of Bawku (Figure 6). *S*. *nodiflora* was relatively more repellent when collected at time 3 (Figure 7).

Discussion

The results of these experiments have shown that general changes in phytochemical variability often follow changes in bioactivity. Plant species with little change measured in the phytochemical profiles of samples collected at different times or places (e.g. *S*. *longepedunculata* and *C*. *schoenanthus*) demonstrated equal effects in their repellent properties. Plants where phytochemical variability was noted (e.g. *A*. *indica* and *S*. *nodiflora*) also showed similar trends in their repellent properties.

However, the link between chemistry and activity was not always clear as shown with *L*. *multiflora* where no phytochemical variability was noted, while repellency was higher from collection time 3. It is important to observe that the phytochemical assessments made in this analysis were very crude, and visual assessments of HPLC profiles were used to determine the major peaks present only. All the plants assessed will contain many more compounds that were not resolved at this wavelength or at relatively smaller quantities, and this assessment method is, therefore, somewhat subjective. In the case of *L*. *multiflora*, the increased repellency noted in collection 3 could very easily be associated with relatively minor chemical components that were not recorded in the overall HPLC profile.

Repellency is only one potential mode of action by which these plants work to protect stored grain. Although two of the plant species (*C*. *nigricens* and *C*. *sophera*) did not demonstrate repellent properties against *S*. *zeamais*, previous research and research presented elsewhere in this report confirms that they are effective in controlling stored product pests. *C*. *nigricens* and *C*. *sophera* may indeed by acting through ovicidal or other toxic effects upon *S*. *zeamais* and be repellent against different target pests. As collection time did affect the chemistry of *C*. *sophera*, another bioassay would be required to determine whether these phytochemical changes affected its bioactivity. Our bioassay was principally designed to provide a quick assessment method to allow large numbers of samples to be tested. The repellency bioassay could be easily adapted for testing other stored product pests such as *Rhyzopertha dominica* or *Callosobruchus maculatus*.

The methodology does not establish which chemical components within the plant are responsible for bioactivity, but it does offer a relatively quick method to assess whether phytochemistry can vary and whether this variability can be used as a marker to assess quality of control. With some plants such links between phytochemistry and bioactivity could be useful in determining the insect pest control experienced by farmers using the plant to store their grain. For example, farmers from the area of Bawku could be likely to experience better control when using *O*. *americanum* than farmers in other areas. These methods have shown that chemical changes are potentially important and linked to the bioactivity of *A*. *indica*, *O*. *americanum* and *S*. *nodiflora*. Collection time and place do not appear to be important for the chemistry and bioactivity of *S*. *longepedunculata* and *C*. *schoenanthus*. These results will help inform farmers on the implications of their harvesting plants from certain areas or places.

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Table 1. Effect of plant collection time (n = 3) and place (n = 4) on the mean number of peaks detected by HPLC at 280nm in a crude plant extract and the extract yield obtained from 1g of ground plant material. Significant differences (ANOVA) between the number of peaks at different times/sites and the weight of plant extracts are indicated by * ($P < 0.05$).

Plant species	Parameter	Mean (±sem)	Grouping	P-value	F-value
A. indica	No. of peaks, 280nm	3.111± 0.228	Time	0.004*	6.264
			Site	0.002*	5.794
	Weight of plant extract, g	0.05±0.002	Time	0.001*	18.565
			Site	0.039	3.008
C. schoenanthus	No. of peaks, 280nm	8.833±0.398	Time	0.06	3.422
			Site	0.244	1.465
	Weight of plant extract, g	0.170±0.015	Time	0.3	4.542
			Site	0.067	3.915
C. nigricens	No. of peaks, 280nm	10.52±0.534	Time	0.07	3.45
			Site	0.055	5.07
	Weight of plant extract, g	0.23±0.005	Time	0.06	4.51
			Site	0.59	0.58
C. sophera	No. of peaks, 280nm	13.23± 1.186	Time	0.001*	22.007
			Site	0.231	1.504
	Weight of plant extract, g	0.147±0.024	Time	0.008*	5.674
			Site	0.169	1.827
L. multiflora	No. of peaks, 280nm	3.48± 0.201	Time	0.34	1.134
			Site	0.139	2.349
	Weight of plant extract, g	0.097± 0.028	Time	0.754	0.286
			Site	0.426	0.659
O. americanum	No. of peaks, 280nm	11.33±0.57	Time	0.001*	20.38
			Site	0.03*	3.041
	Weight of plant extract, g	0.18±0.007	Time	0.04*	4.67
			Site	0.57	0.621
S. longepedunculata	No. of peaks, 280nm	5.66±0.52	Time	0.48	0.956
			Site	0.721	0.41
	Weight of plant extract, g	0.34±0.004	Time	0.09	3.101
			Site	0.147	2.03
S. nodiflora	No. of peaks, 280nm	7.03±0.15	Time	0.01*	5.73
			Site	0.456	0.611
	Weight of plant extract, g	0.23±0.014	Time	0.001*	17.585
			Site	0.813	0.205

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Figure 1 Repellency bioassay showing effect of eight botanicals on the behaviour of *Sitophilus zeamais*. Insects could freely move between an empty zone and an area containing maize treated with powdered plant material (1% w/w). Insect movements were tracked over 30 minutes (n = 6).

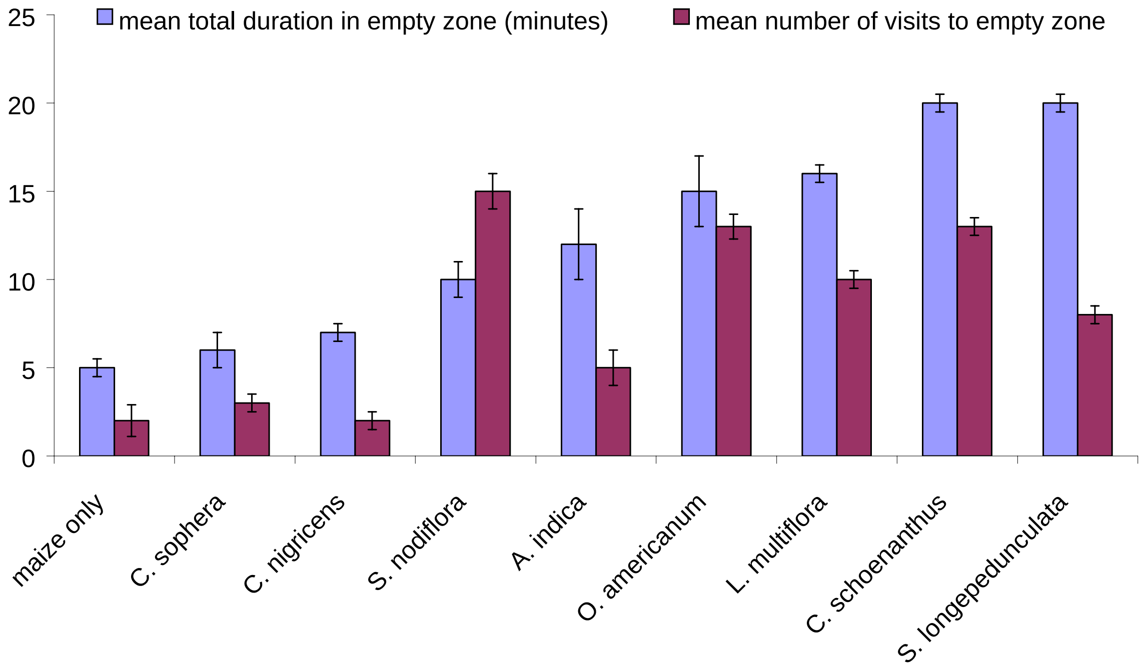

Figure 2 Effect of collection time and place on the repellency of *A. indica* against *S. zeamais*. Insects could freely move between an empty zone and an area containing maize treated with powdered plant material (1% w/w). Insect movements were tracked over 30 minutes (n = 6).

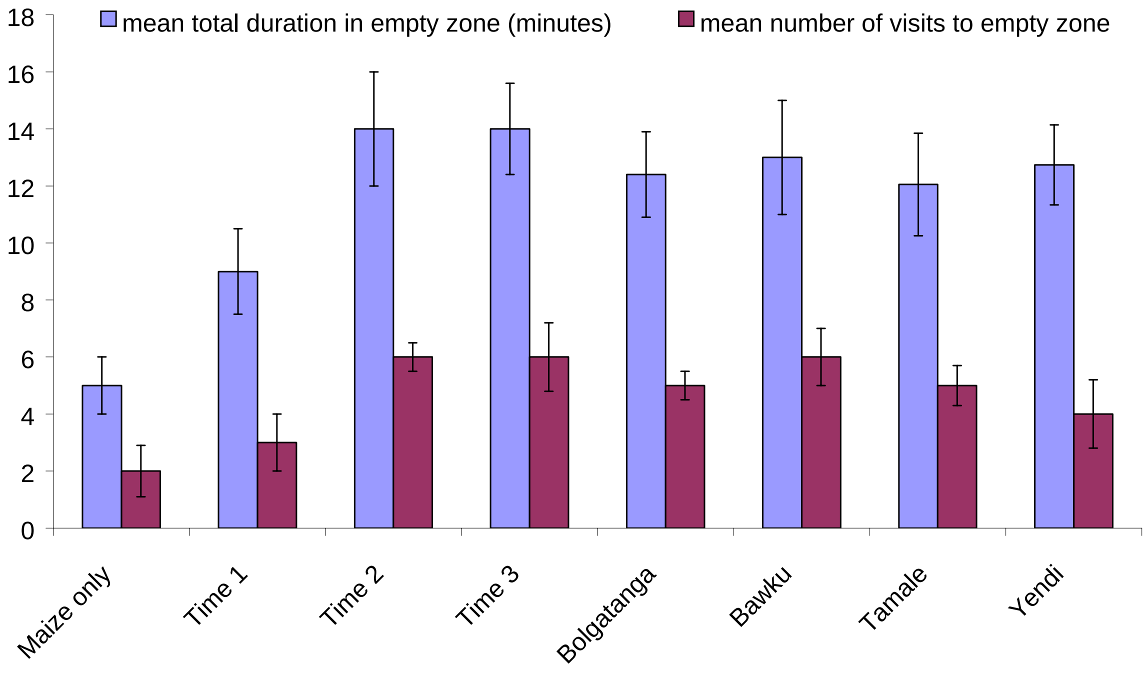

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Figure 3 Effect of collection time and place on the repellency of *C. schoenanthus* against *S. zeamais*. Insects could freely move between an empty zone and an area containing maize treated with powdered plant material (1% w/w). Insect movements were tracked over 30 minutes (n = 6).

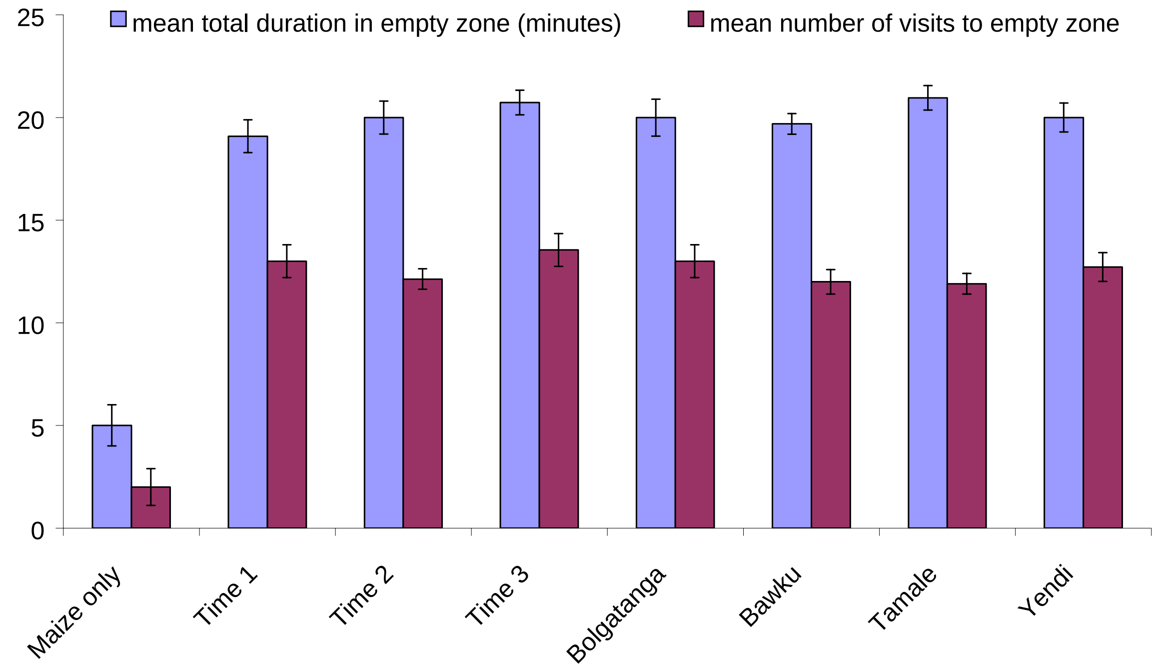

Figure 4 Effect of collection time and place on the repellency of *S. longepedunculata* against *S. zeamais*. Insects could freely move between an empty zone and an area containing maize treated with powdered plant material (1% w/w). Insect movements were tracked over 30 minutes (n = 6).

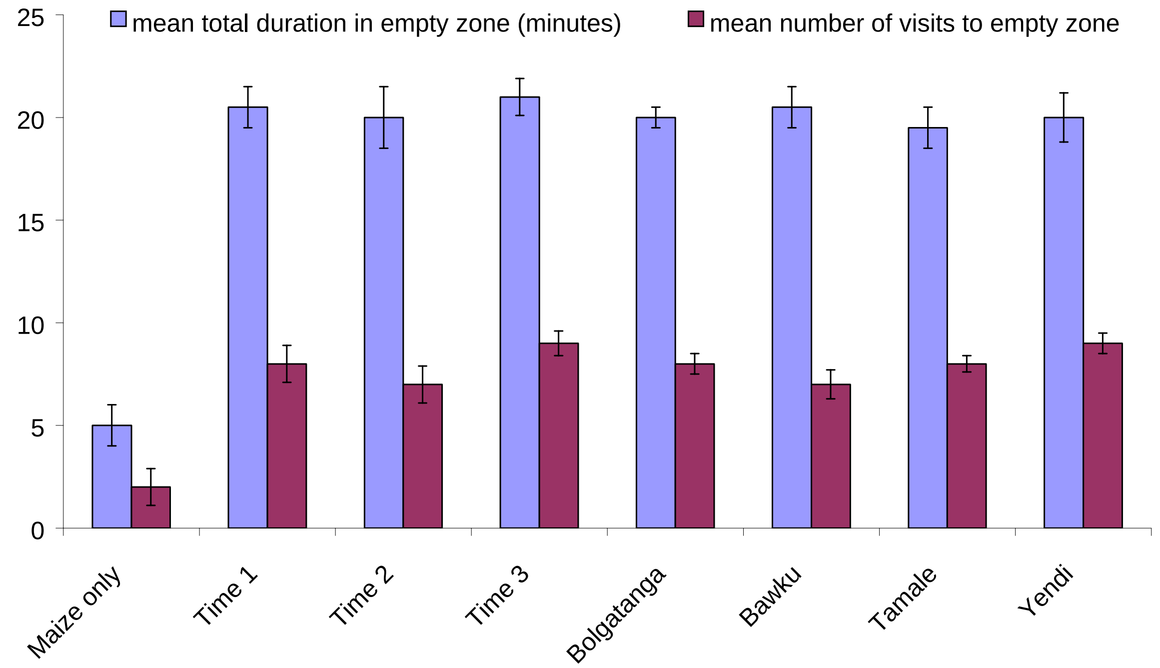

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Figure 5 Effect of collection time and place on the repellency of *L. multiflora* against *S. zeamais*. Insects could freely move between an empty zone and an area containing maize treated with powdered plant material (1% w/w). Insect movements were tracked over 30 minutes (n = 6).

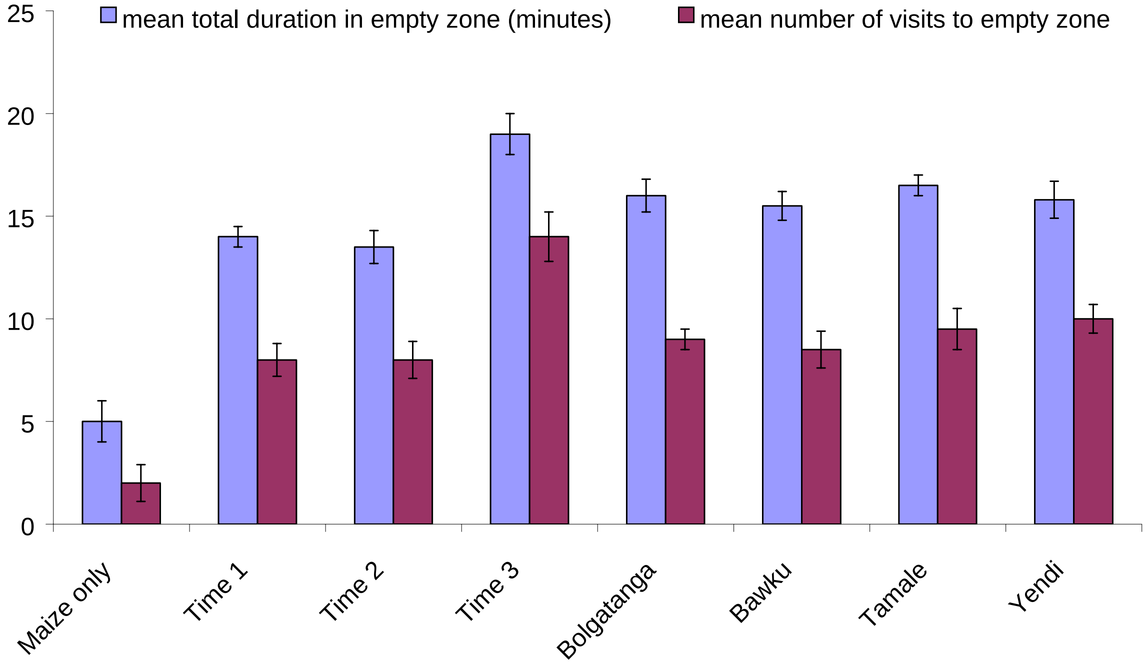

Figure 6 Effect of collection time and place on the repellency of *O. americanum* against *S. zeamais*. Insects could freely move between an empty zone and an area containing maize treated with powdered plant material (1% w/w). Insect movements were tracked over 30 minutes (n = 6).

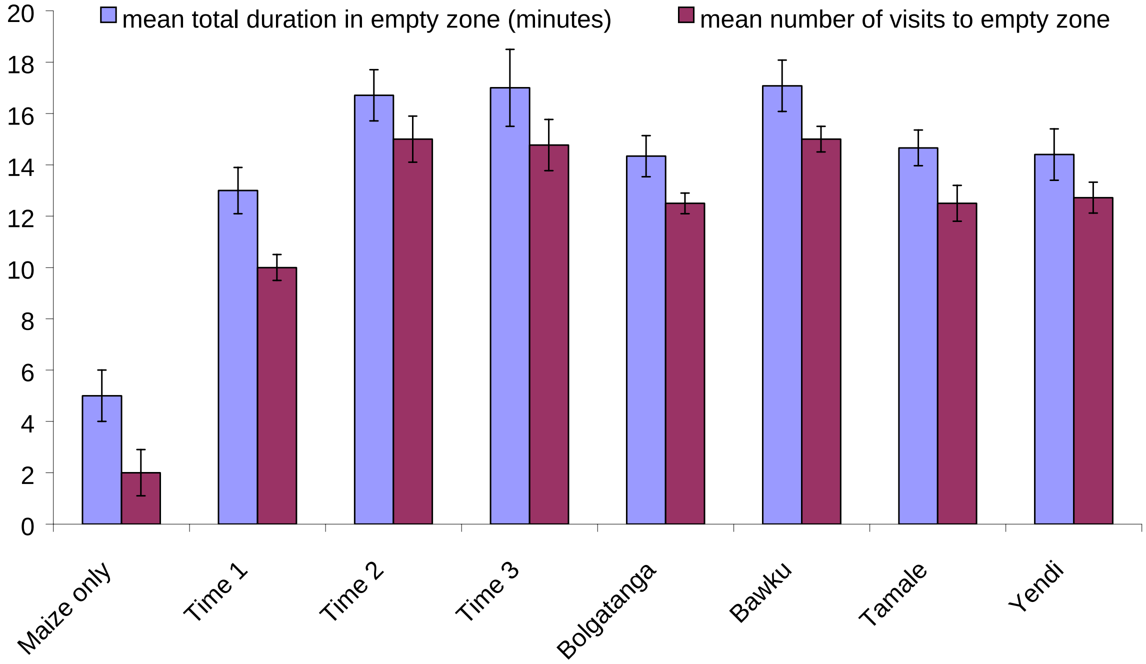

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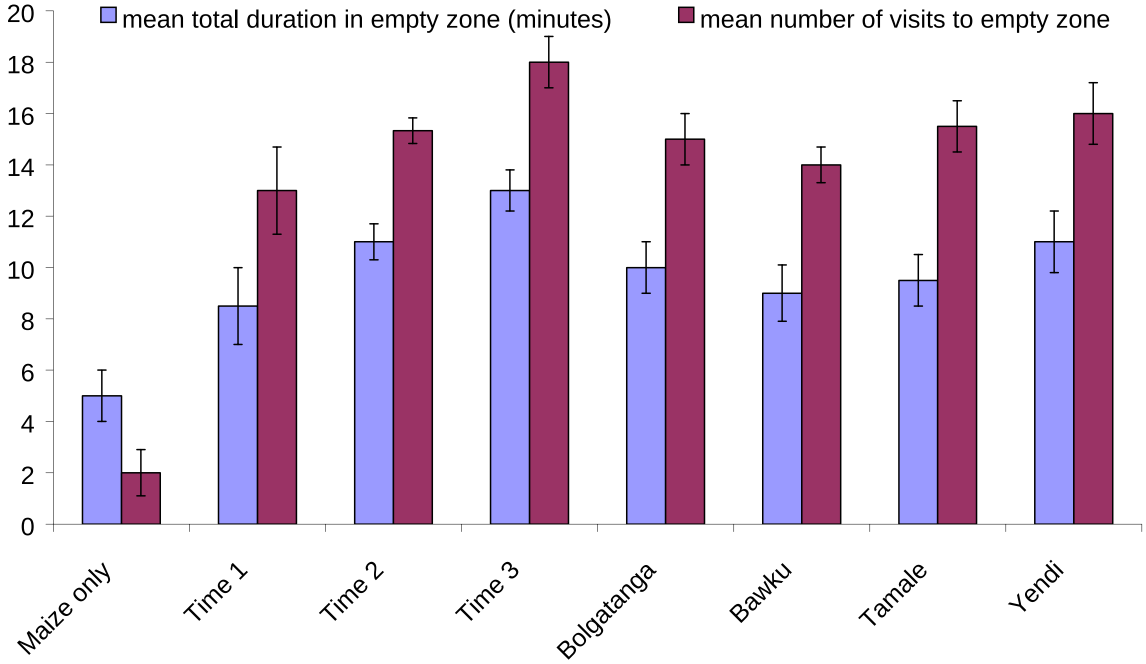
Figure 7 Effect of collection time and place on the repellency of *S. nodiflora* against *S. zeamais*. Insects could freely move between an empty zone and an area containing maize treated with powdered plant material (1% w/w). Insect movements were tracked over 30 minutes (n = 6).



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Assessment of botanicals used to protect stored grain and legumes under farm-store conditions in Ghana

Abstract
Farmer participatory trials evaluating eight plant species traditionally used for stored product protection in northern Ghana were implemented over three storage seasons (1999, 2000 and 2001). Trials involved 100 farmers from each of the Northern and Upper East Regions where the commodities evaluated were maize and cowpea, respectively. All the plant materials were shown to be effective in reducing insect damage levels with the best result achieved by *Securidaca longepedunculata* showing a 2 to 3-fold decrease in damage when compared to the untreated control. Farmer evaluations of plant materials roughly followed their efficacy; however, farmers tended to relatively overestimate the value of the least effective plants, and this may be influenced by their higher level of familiarity with these species. The implications of these results are discussed in the context of increasing the usage of botanicals for small-scale storage in the tropics.

Introduction
Research on the assessment of botanicals has been largely conducted in laboratory settings (Boeke et al., 2001). Since 1980, more than 1100 articles have been published that have assessed botanicals under laboratory conditions against a range of stored product insect pests (CAB Abstracts). Although this information is useful in establishing whether a botanical contains bioactivity against a target pest species, it does not necessarily equate with the efficacy observed when applied under local conditions. There are few studies published that have addressed the efficacy of botanicals under the complex ecological conditions experienced by farmers storing grain on-farm that are affected, among others, by variable temperature and humidity, different infestation pressures, different insect species, quality of grain and different storage structures (Stoll, 1986; Albert, 1992). In addition to making real assessments of botanical efficacy, farmer participatory trials have the added advantage of increasing communication between farmers, extensionists and researchers, providing feedback that can help improve they way grain is stored and optimising how botanicals are used. The objectives of this study were to evaluate and compare the efficacy of the botanicals under the small-scale storage conditions prevalent in northern Ghana, allowing farmers themselves to make assessments and choices in the botanicals that they used.

Materials and Methods
Farm trials were implemented over three separate storage seasons starting in the month of December when commodities were harvested. As trials used the farmer's own commodities, each trial ran for six months after which farmers require the grain for home use. Each seasonal trial involved 100 farmers each from the Northern Region (NR) and the Upper East Region (UER). Stored maize trials took place with farmers in the Tolon District of the NR, and cowpea trials took place in the Zebila District of the UER. Plant materials were harvested from wild areas in the locality, shade dried and ground to powder by MoFA extensionists and villagers (Figure 1). The botanicals used were *Azadirachta indica*, *Cassia sophera*, *Chamaecrista nigricens* (UER only), *Cymbopogon schoenanthus* (UER only), *Lippia multiflora* (NR only), *Ocimum americanum*, *Securidaca longepedunculata* and *Synedrella nodiflora*. Each farmer selected three different plant materials from the options available (Figure 2). Farmers placed 10kg of their harvested commodity into four different sacks (40 kg in total). Powdered material of the three chosen options was admixed separately (Figure 3) into three of the sacks (5% w/w), the fourth sack containing untreated commodity. All four sacks were stored within the granary or storehouse where all other commodity was stored for the household (Figure 4). Data on the number of insects found in a 300 g sub-sample and the number of damaged grains (2 x 100 grains) for each sack were recorded at the commencement of each trial and at one-month intervals over the duration of the trial. At the end of the trial farmers were asked to rank the three treatments that they used in the order that they observed worked best.

Results
Maize trials
A factorial analysis of variance using treatment, season, assessment time and their interactions showed that changes in damage levels were only related to the treatment and assessment time, allowing data to be combined across the three seasons (Table 1). The recorded damage was highest for the control with each botanical showing significant damage reduction levels (Figure 5). As expected, damage levels were strongly correlated with storage time for all treatments (Pearson = 0.831, $P < 0.001$), and were best represented by a linear regression ($R^2 = 0.69$, $P < 0.001$). At the

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end of the storage period, damage was lowest in maize treated with *S. longepedunculata*, followed by *A. indica*, *O. americanum*, *S. nodiflora*, *L. multiflora* and *C. sophera*. Insect numbers varied over the assessment period for all treatments with no clear trend observed in linear or quadratic regression analyses (P > 0.05). The cumulative total mean number of insects found in the untreated control over the six month period was higher than in any of the treatments (LSD, P< 0.01, Figure 6). The lowest total number of insects was found in maize treated with *S. longepedunculata*, followed by *A. indica*, *O. americanum*, *S. nodiflora*, *L. multiflora* and *C. sophera*. Farmer preferences for the botanicals varied, and although *S. longepedunculata* was most often ranked as their first choice, *O. americanum* obtained the highest ranking overall (Figure 9).

Cowpea trials
A factorial analysis of variance using treatment, season, assessment time and their interactions showed that changes in damage levels were only related to the treatment and assessment time, allowing data to be combined across the three seasons (Table 2). The recorded damage was highest for the control with each botanical showing significant damage reduction levels (Figure 7). As expected, damage levels were strongly correlated with storage time for all treatments (Pearson = 0.852, P < 0.001), and were best represented by linear regression (R^2 = 0.73, P < 0.001). At the end of the storage period, damage was lowest in maize treated with *S. longepedunculata*, followed by *C. schoenanthus*, *A. indica*, *S. nodiflora*, *O. americanum*, *C. nigricens* and *C. sophera*. Insect numbers varied over the assessment period for all treatments with no clear trend observed in linear or quadratic regression analyses (P > 0.05). The cumulative total mean number of insects found in the untreated control over the six month period was higher than in any of the treatments (Figure 8). The lowest total number of insects was found in maize treated with *S. longepedunculata*, followed by *C. schoenanthus*, *A. indica*, *S. nodiflora*, *O. americanum*, *C. nigricens* and *C. sophera*. Farmer preferences for the botanicals showed that *C. schoenanthus* was the most preferred overall *with C. nigricens* most often ranked as their first choice (Figure 10).

Discussion
The results of these trials indicated that the plant treatments gave similar levels of control over three storage seasons. This suggests that their overall reliability is high, and anecdotal information that has suggested variable results when using botanicals is more than likely due to changes in how the plant material has been used by farmers (e.g. variable concentration applied, different baseline infestation levels, plant material preparation methods). Depending on when and how farmers harvest their grain, insect infestation can occur before it is put into storage, and this could affect how well botanical treatments work. Although none of the plant materials were able to eliminate insect infestation, all the plant materials were able to significantly reduce damage levels. Variability in the results of botanical usage among farmers can not be ruled out as some farmers are more conscious of the effects of good hygiene and take more care to ensure better storage conditions than others.

The number of insects present in sub-samples of treatments at different assessment periods did not accurately represent the underlying damage levels found in each sample. This may be due to insects moving about in the sack or becoming concentrated in certain areas, and as only one sample was taken from each sack at each assessment period, the sub-sample may have failed to give a reliable method for determining overall insect numbers, explaining why insect numbers recorded appeared to randomly increase or decrease at progressive sampling times. Overall insect numbers recorded by combining the assessment period data was able to show that cumulative insect loads did vary between the treatments and control. As damage levels are essentially a more important assessment criteria, future trials may no longer need to assess insect numbers. Alternatively, methods for measuring insect numbers will need to be modified to reduce potential sampling bias.

The promotion of farmer usage of botanicals should not assume that because botanical pesticides are naturally derived that they are safe to use and consume by humans. Preliminary assessments of mammalian toxicity indicated that the plants should be relatively safe if only small quantities of plant material were ingested (Belmain et al., 2001). Many of the plants used for stored product protection have alternative uses as medicines or food spices, indicating that any serious vertebrate toxicity would have been established through the development of the ethnobotanical knowledge. The risk of consuming larger quantities of botanicals through storage protection would be reduced through winnowing, washing, processing and cooking. However, no research has established the levels of potential botanical residues remaining on treated grain before it is ingested. We, therefore, strongly recommend further research investigating potential botanical residue levels on treated food after

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processing and cooking before the institutional promotion of botanicals for stored product protection begins.

Farmer assessments and their choices of botanicals showed that prior experience and familiarity with certain species may be affecting their judgement over and above the results experienced during the trials. In the Northern Region, farmers were relatively unfamiliar with the use of botanicals when compared to farmers in the Upper East Region. NR farmers attributed relatively high preferences for *O. americanum* and *L. multiflora* despite their relatively poor results in reducing insect damage when compared to other botanicals. This may be due to the fact that both *O. americanum* and *L. multiflora* are used as medicines and are perhaps more readily recognised due to their availability and multiple uses. UER farmers have more experience using botanicals for stored product protection. However, similar discrepancies occur in the UER between the relatively poor level of control shown with C. nigricens and its high preference indicated by farmers. *C. nigricens* is a very well-known plant in UER with many uses, and it may be that farmers are rating the plant higher because of its history and familiarity. Farmer acceptability of botanicals is, therefore, likely to be influenced not only by demonstrated efficacy, but by other factors such as availability, familiarity and versatility.

Discussions with small-scale farmers indicate that they often prefer pesticidal plant materials over other forms of pest control during storage. However, individual farmer knowledge about different plants and how best to use them varies considerably. Research to date has been able to narrow down the list of plants, focussing upon those that work best and optimising their application methods for on-farm use. It is hoped that this knowledge can be widely disseminated to farming communities through partnerships with NGOs and farmer extension programmes operated by the Ghanaian Ministry of Food and Agriculture. One issue which remains unresolved is the accessibility and availability of the plant materials. This does affect farmer choices. Most of the plants are collected from the wild and some species are suffering from over-collection and environmental degradation. In order for these plants to be used sustainably, their propagation and cultivation potential as well as their conservation in the wild needs to be addressed. Some of the species such as *A. indica* and *S. nodiflora* could be considered weeds, and their invasive properties and prolific regeneration imply that neither plant is likely to become threatened. However, many of the species are potentially vulnerable with regard to their regeneration potential, showing sporadic and patchy growth due to widespread and uncontrolled annual fires. Promotion of botanicals will ultimately assist biodiversity conservation of the savannah by increasing its perceived value.

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Table 1 Factorial analysis of variation of insect damage to stored maize in farmer trials assessing six plant materials (plus control) over six months (plus baseline)

Source of variation	degrees of freedom	sum of squares	F probability
year (season)	2	6.270	0.401
botanical (treatment)	6	50464.135	<0.001
assessment time	6	324803.39	<0.001
year X botanical	12	32.754	0.655
year X time	12	33.025	0.601
botanical X time	36	39401	<0.001
year X botanical X time	72	201.394	0.868
Residual	7203	24687.88	
Total	7350	1148506	

Table 2 Factorial analysis of variance of insect damage to stored cowpea in farmer trials assessing seven plant materials (plus control) over six months (plus baseline)

Source of variation	degrees of freedom	sum of squares	F probability
year (season)	2	6.327	0.423
botanical (treatment)	7	88054	<0.001
assessment time	6	445600.48	<0.001
year X botanical	14	33.315	0.827
year X time	12	29.44	0.784
botanical X time	42	65244.87	<0.001
year X botanical X time	84	208.411	0.990
Residual	8232	30255.16	
Total	8400	629432.56	

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Figure 1 Plant materials used in all farmer trials were dried and then pounded to a powder so that they could be admixed with stored commodities. Previous research found this to one of the better methods of applying plant materials

Figure 2 Optimising how plant materials are used for stored product protection involves a) giving farmers a choice on which plants they will use during research trials they will manage in their own store using their own commodity. b) The plant materials being offered as choices to this farmer are (clockwise from top left) *Cassia sophera*, *Securidaca longepedunculata*, *Ocimum americanum*, *Lippia multiflora*, *Synedrella nodiflora* and *Azadirachta indica*. The plant materials are dried and ground to a powder before admixing with the stored commodity.

a)

b)

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Figure 3 Farmer trials involved adding powdered plant materials to sacks of the farmer's maize or cowpea. The farmers then admixed the plant with the grain by hand.

Figure 4 Sacks of commodity treated with plant materials were stored in traditional storage structures within the household compound.

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Figure 5 Effect of different plant treatments (5% w/w admixed powder) on insect damage levels to farm-stored maize (10kg/treatment) with 100 farmers participating (50 farmers storing each botanical) over three storage seasons

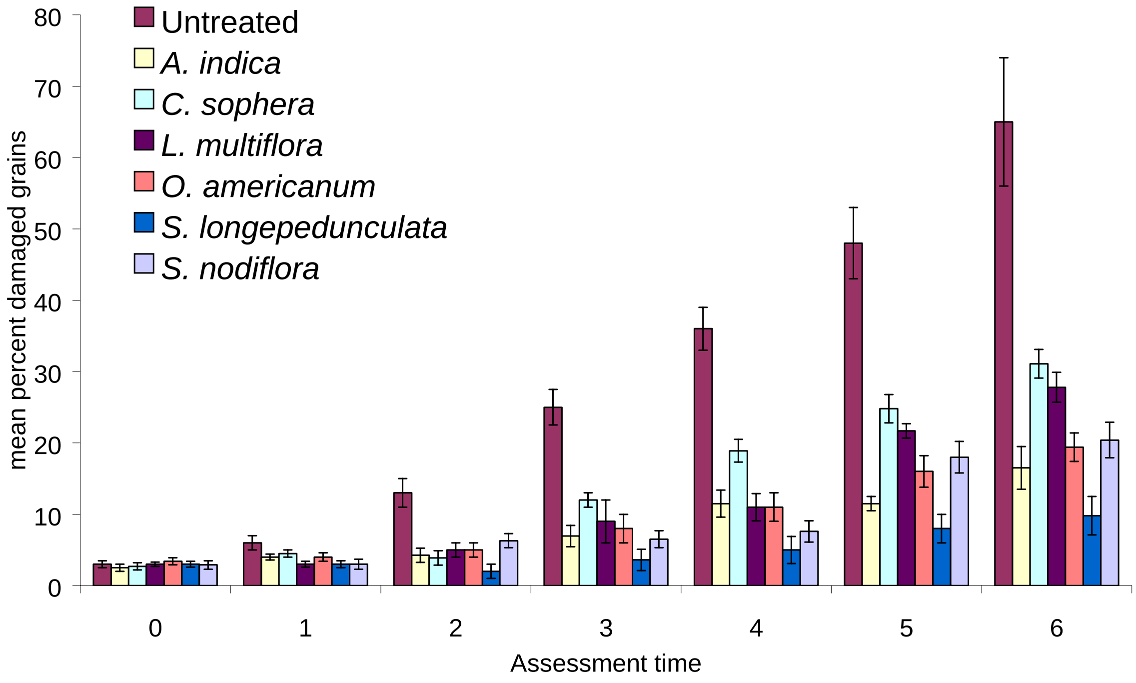

Figure 6 Cumulative mean total number of insects found in monthly assessments of 300 g sub-samples taken over a six-month period from fifty 10kg sacks of maize stored on farm with admixed powdered plant material (5% w/w) over three storage seasons

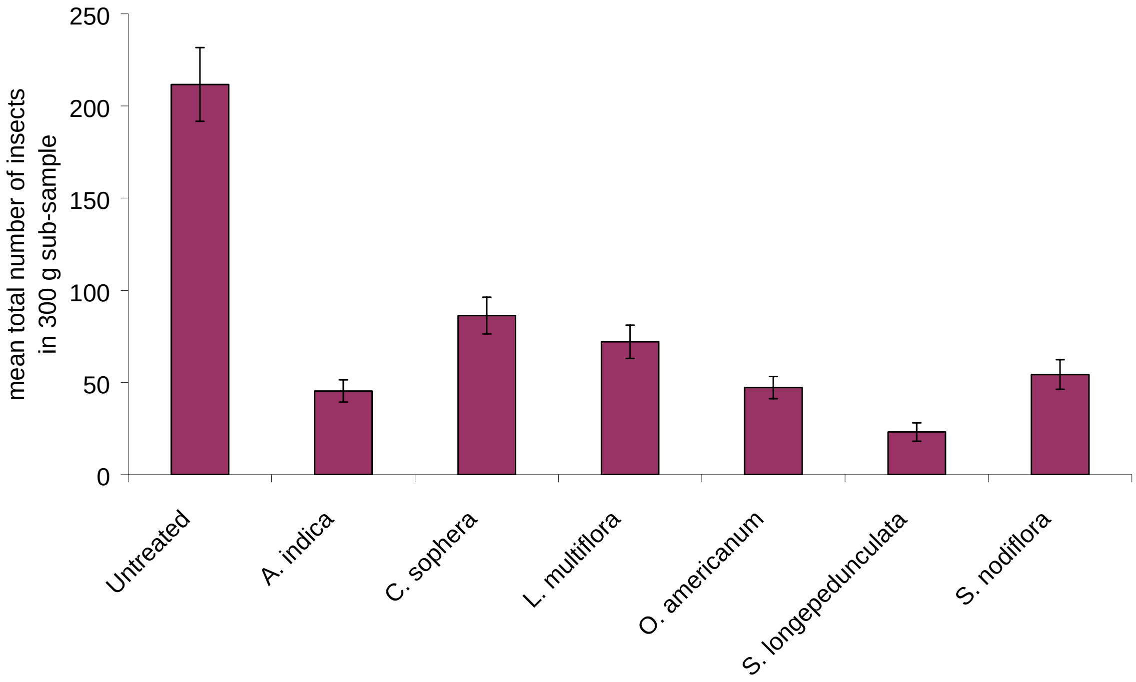

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Figure 7 Effect of different plant treatments (5% w/w admixed powder) on insect damage levels to farm-stored cowpea (10kg/treatment) with 100 farmers participating (42 farmers storing each botanical) over three storage seasons

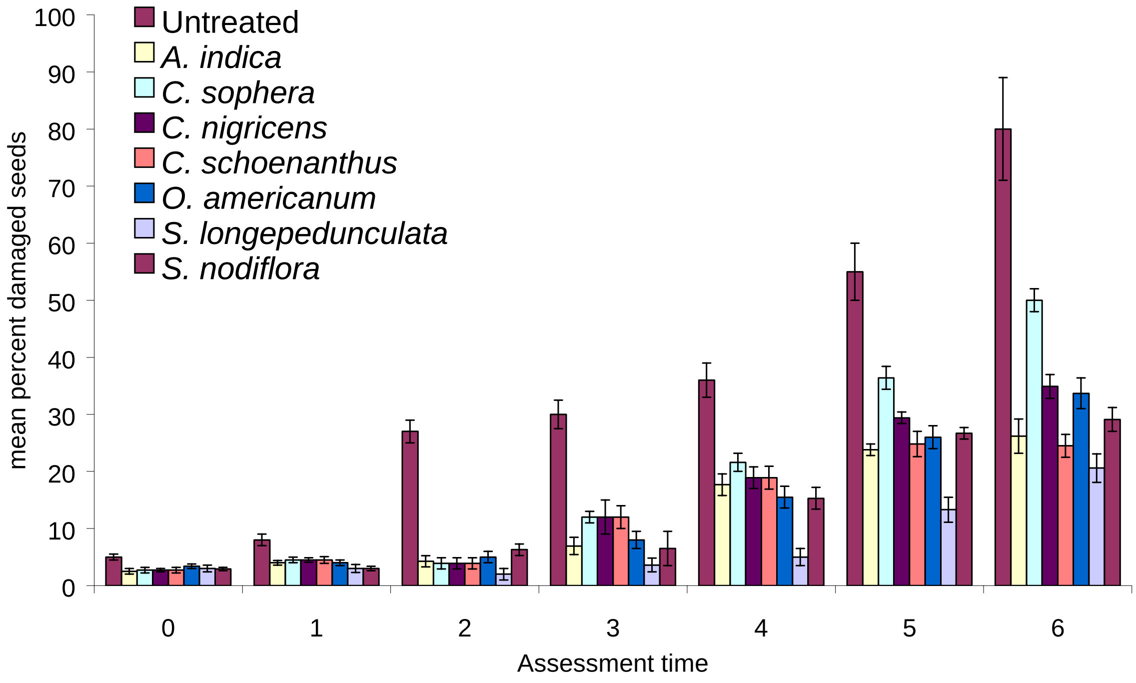


Figure 8 Cumulative mean total number of insects found in monthly assessments of 300 g sub-samples taken over a six-month period from forty-two 10kg sacks of cowpea stored on farm with admixed powdered plant material (5% w/w) over three storage seasons

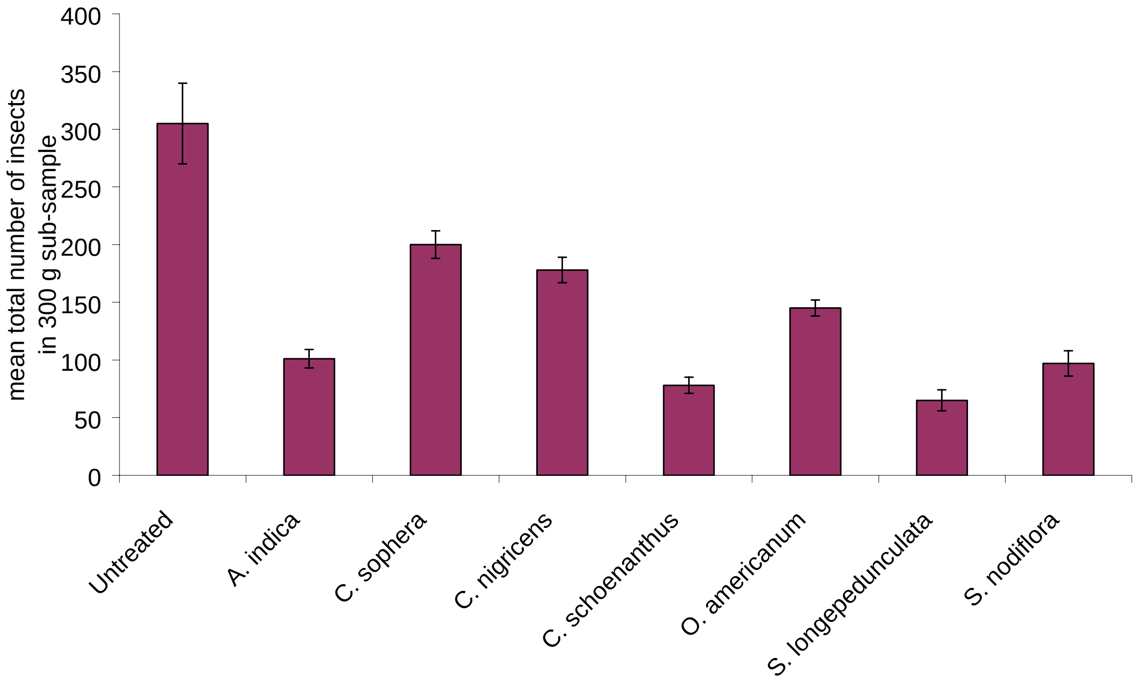


Figure 9 Botanical preferences of farmers growing maize who have been involved in on-farm evaluation trials in the Northern Region of Ghana

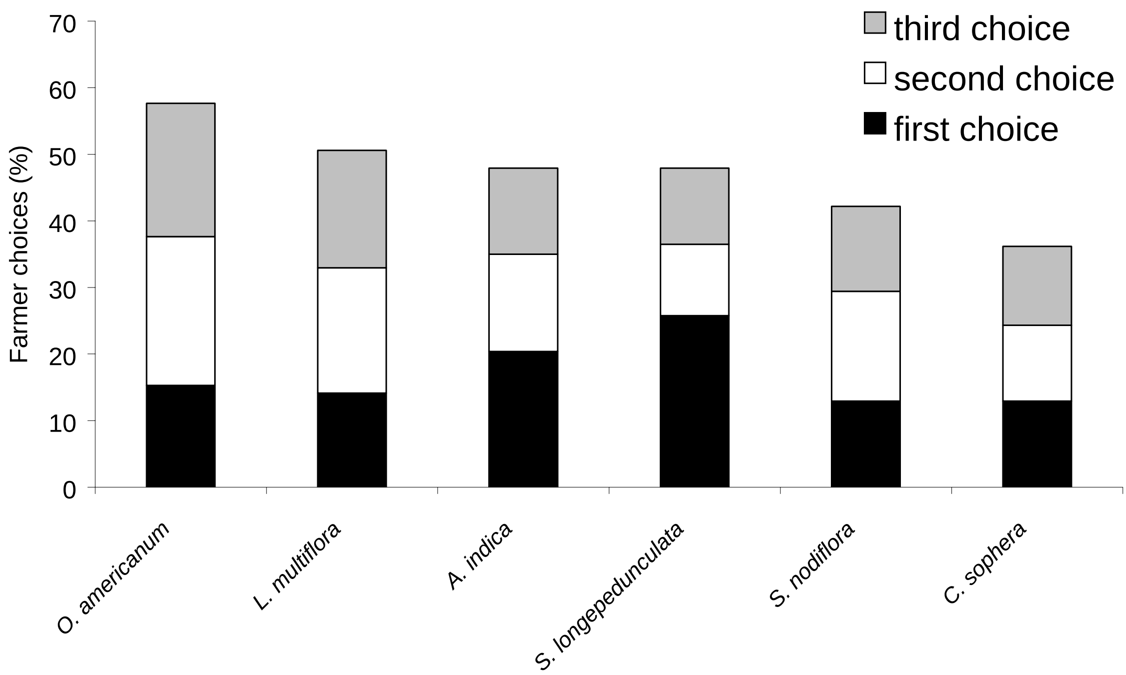
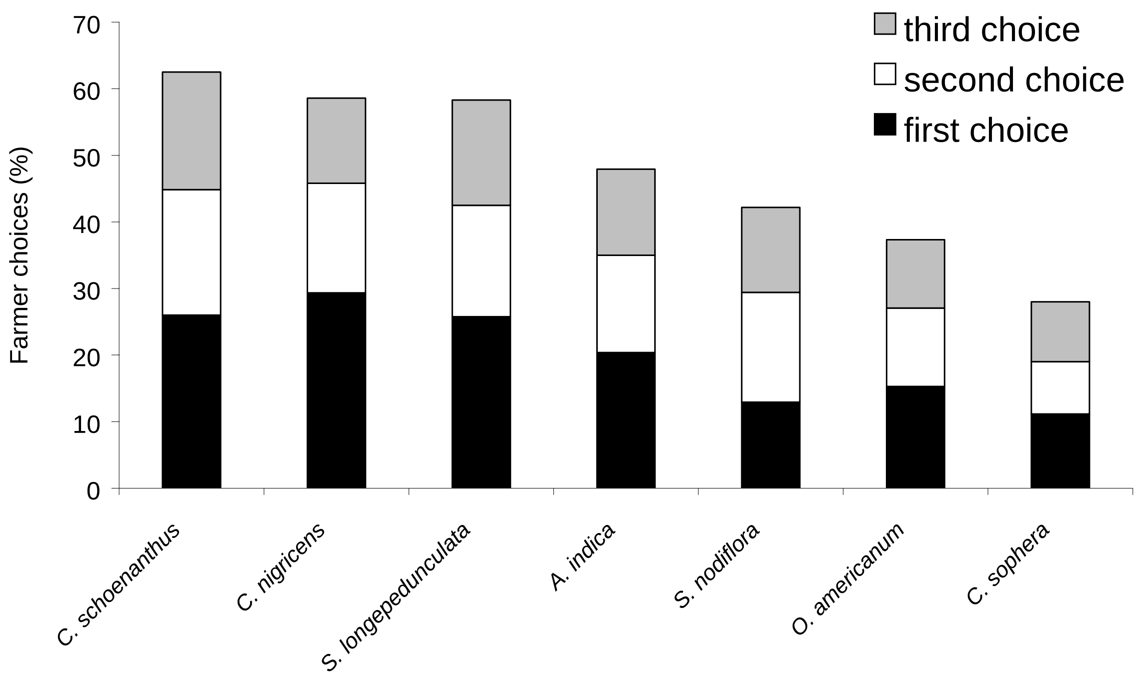


Figure 10 Botanical preferences of farmers growing cowpea who have been involved in on-farm evaluation trials in the Upper East Region of Ghana



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Behavioural responses of stored product insects to botanicals traditionally used for stored product protection

Abstract

A bioassay developed to assess host selection behavioural choices of *S. zeamais* between maize treated with plant extracts and untreated maize showed that eight botanical species (*Azadirachta indica*, *Cassia sophera*, *Lippia multiflora*, *Chamaecrista nigricens*, *Ocimum americanum*, *Cymbopogon schoenanthus*, *Synedrella nodiflora* and *Securidaca longepedunculata*) were deterrent to adult insects at relatively low concentrations (100 to 1000 ppm). Chromatographic separation of crude extracts of three of the plant species, *C. schoenanthus, L. multiflora and C. nigricens*, showed that bioactivity was associated with a number of compounds. Atleast nine compounds present in *C. schoenanthus* demonstrated deterrent effects on *S. zeamais* from a broad spectrum of compound characteristics. Bioactivity in *L. multiflora* was largely associated with more hydrophobic compounds present in a methanol extract. *C. nigricens* showed activity in atleast eight profile areas, and further investigation of one of those areas showed that deterrent activity was attributed to atleast four more compounds. Further isolation and characterisation of active constituents could lead to the identification of novel bioactive compounds or modes of action.

Introduction

Evidence from field and laboratory trials suggested that deterrence was one of the primary modes of action through which botanicals reduced insect problems in storage. Although deterrent compounds may or may not possess other modes of action, e.g. toxicity, the identification of deterrent compounds would help assess the safety of using the plant material for stored product protection and could potentially lead to the identification of novel compounds or modes of action and new commercial products. Knowledge about the bioactive components could also help assess whether related plants in the same genera that contain similar compounds could be used, reducing collection pressures on species that may be at risk of over-collection.

Materials and Methods

A bioassay was developed to present adult insects of *Sitophilus zeamais* with a choice between treated and untreated maize. Ground plant material (20 g) of eight different botanicals (*Azadirachta indica*, *Cassia sophera*, *Lippia multiflora*, *Chamaecrista nigricens*, *Ocimum americanum*, *Cymbopogon schoenanthus*, *Synedrella nodiflora* and *Securidaca longepedunculata*) was extracted in methanol (100 ml) at room temperature for 24 hours. The extracts were filtered (Whatman #1) and concentrated until dry under vacuum. The extract yield was calculated, and the extract was redissolved in methanol to make a solution of 100,000 ppm (parts per million). This solution was then diluted to make a dilution series of 10,000, 1,000 and 100 ppm. Maize (50 g) was mixed with each solution (2.5 ml) for one minute to thoroughly coat the grain, and treated grain was then left to dry in a fume cupboard for one hour. Untreated “control” maize was treated with methanol only (without any plant extract) to standardise experimental variables. Treated and untreated maize (5 g each) was placed separately into two of the partitions available in three-way petri dishes, 10 replicates per treatment (Figure 1). The control trial consisted of untreated maize in both compartments. Ten 7-14 day-old unsexed adult *S. zeamais* were placed into the last third of each petri-dish. Adult presence in the treated and untreated portions was recorded after 7 days.

This bioassay was used to assess the active components within crude extracts by chromatographic separation using HPLC. Three of the plant species that had the least known about their phytochemistry (*C. schoenanthus, L. multiflora and C. nigricens*) were prepared by extracting 100g of ground plant material in methanol (200 ml), filtered and dried as above. The extract was redissolved to 50,000ppm and injected into a LiChrCART® 250-4 analytical column connected to a Waters™ 996 Photodiode Array Detector, Waters™ 717 Autosampler and Waters™ 600E system controller. Preliminary assessments determined that compounds present in *C. nigricens* were best separated and resolved using 25% acetonitrile and 75% of 2% acetic acid, curve 9, detecting at wavelengths 313nm and 200nm. The crude extract of *C. nigricens* was separated into 15 different fractions. Further experiments led to the sub-fractionation of fraction 2 into 4 sub-fractions using 10% acetonitrile and 90% of 2% acetic acid at 250 and 300 nm. *L. multiflora* was analysed using 20% methanol and 80% water, curve 4 at 225nm and 285nm which separated the crude into 10 different fractions. *C. schoenanthus* was analysed using 20% methanol and 80% water, curve 6 at 212nm which separated the crude into 10 fractions. All HPLC fractions were dried to determine their yield and redissolved to a concentration referring back to a crude extract at 1000ppm.

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Results

None of the plant materials were able to induce 100% deterrence even at the highest concentration tested of 100,000ppm (Figure 2). However, deterrence was noted for all plant species and was statistically significant from the control at the lowest concentration of 100ppm for *A. indica*, *C. schoenanthus*, *L. multiflora* and *S. longepedunculata* with the remaining species significant at 1000ppm (Mann-Whitney U-test, n=10, P<0.01). Increasing concentration increased the deterrent effect for all the plant species which were best represented by linear regression models. Fractionation of the crude extracts of *C. schoenanthus, L. multiflora* and *C. nigricens* showed that compounds present in several of the fractions were deterrent to *S. zeamais* (Figures 3 to 5, respectively). Fractions where deterrency was noted all contained a number of chemical components. In order to pinpoint activity further fractionation of promising fractions was planned to isolate and characterise the active components. Further subfractionation of fraction 2 of *C. nigricens* into four subfractions showed that all four subfractions also contained bioactive compounds that caused behavioural deterrence of *S. zeamais* (Figure 6).

Discussion

The results of this experiment showed that all the plant materials were effective in deterring adult insects of *S. zeamais* at relatively low concentrations between 100 and 1000 ppm although 100% deterrence was not achieved at even very high dosages of 100,000 ppm with no obvious 'tail-off' observed. These results are comparable to the effects observed in other research using botanicals against target pests species (Weaver and Subramanyam, 2000). Our research suggests that farmers will achieve better control when higher concentrations of plant material are used and should aim to admix plant material with their grain at 5% or more, limited by the practicalities of botanical collection. Isolation and characterisation of the active constituents within botanicals can be a time consuming process as relatively large quantities of compounds are required to characterise compounds using Nuclear Magnetic Resonance (NMR) and to test their bioactivity in bioassays. Atleast nine compounds present in *C. schoenanthus* demonstrated deterrent effects on *S. zeamais* from a broad spectrum of compound characteristics. Bioactivity in *L. multiflora* was largely associated with more hydrophobic compounds present in a methanol extract suggesting more volatile components may be responsible for its action. *C. nigricens* showed activity in atleast eight profile areas, and further investigation of one of those areas showed that deterrent activity was attributed to atleast four more compounds. Further research to identify bioactive compounds is required as a means to assess the safety the botanicals for use in stored product protection. Through the UN Convention on Biological Diversity, potentially new commercial products that arise from these searches could lead to direct financial benefits to the communities that own the indigenous knowledge (Kate and Laird, 1999; Steinberg, 1998; Wrigley et al., 1997).

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Figure 1 Photograph of bioassay used to determine effect of botanical compounds on behavioural choices of *S. zeamais*. Treated and untreated maize were placed into separate compartments. Ten insects were introduced into the empty compartment with their location recorded after 7 days.

Figure 2 Effect of botanical concentration on the behavioural preferences of *S. zeamais* given a choice between maize treated with a plant extract and untreated maize. Control trials presented insects with a choice between two areas of untreated maize, resulting in the ten insects being evenly distributed between the two portions.

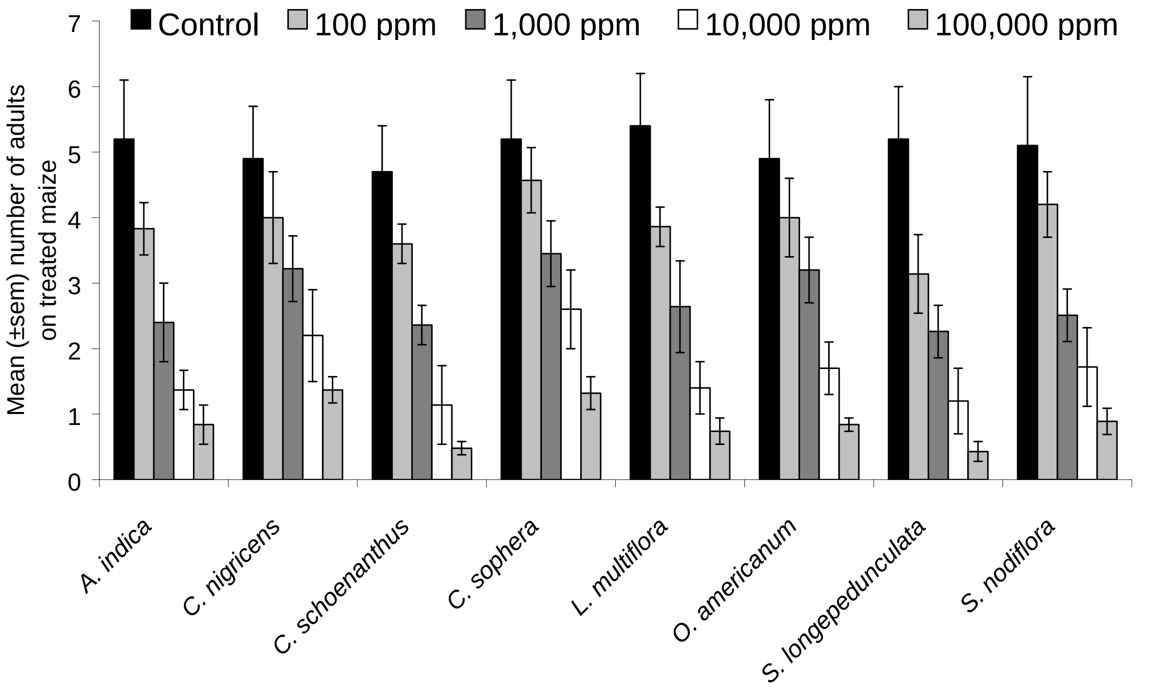

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Figure 3 Effect of chromatographically separated fractions of a methanol extract of *C. schoenanthus* on the choice preference behaviours of *S. zeamais* when given a choice between treated and untreated maize

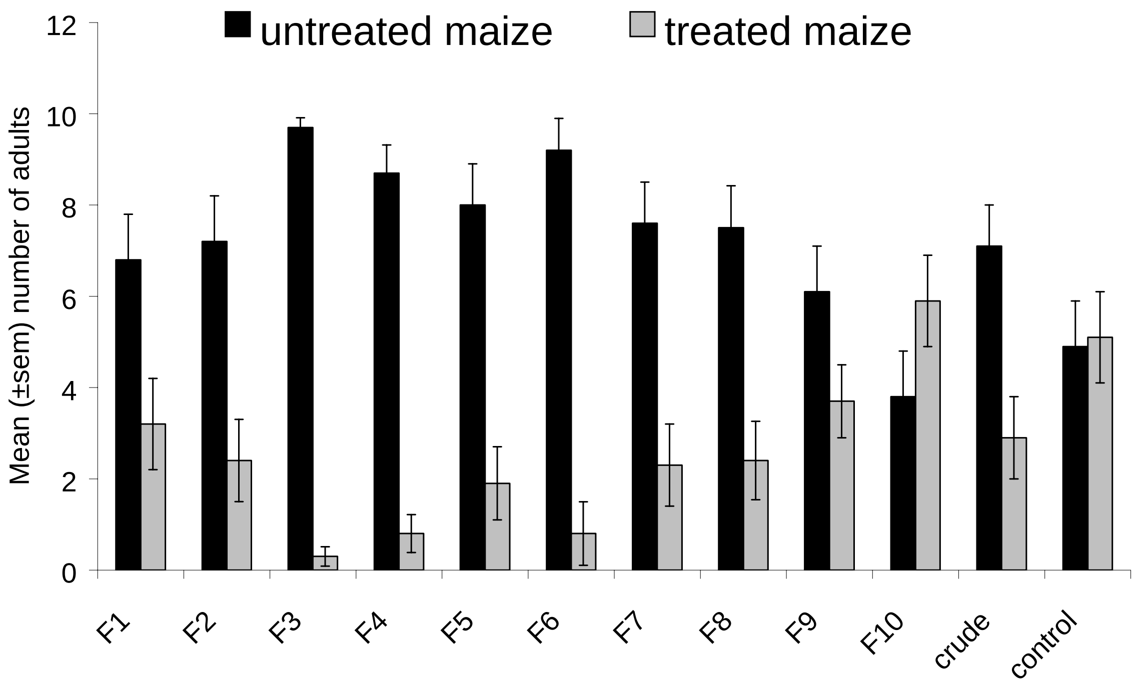

Figure 4 Effect of chromatographically separated fractions of a methanol extract of *L. multiflora* on the choice preference behaviours of *S. zeamais* when given a choice between treated and untreated maize

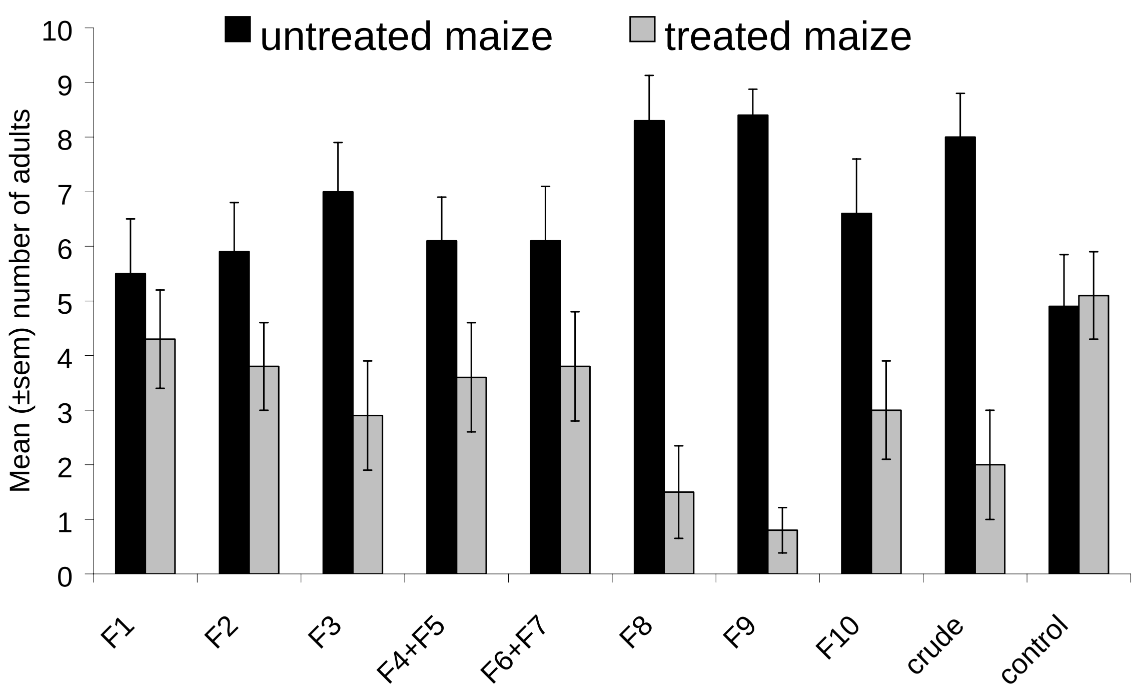

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Figure 5 Effect of chromatographically separated fractions of a methanol extract of *C. nigricens* on the choice preference behaviours of *S. zeamais* when given a choice between treated and untreated maize

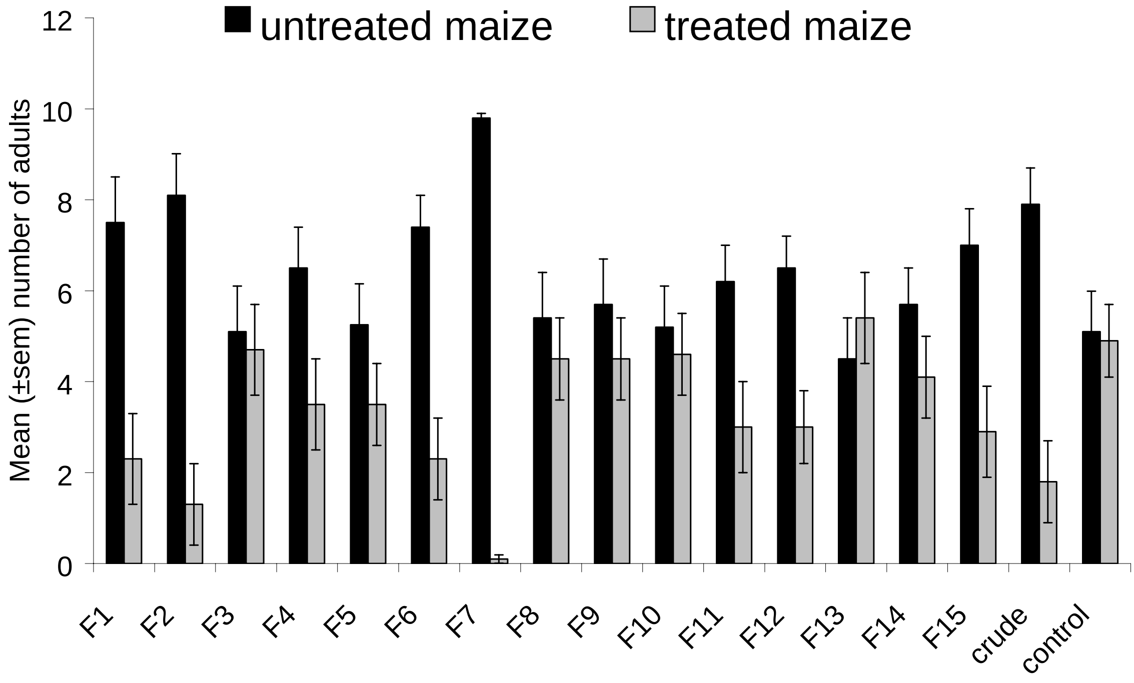
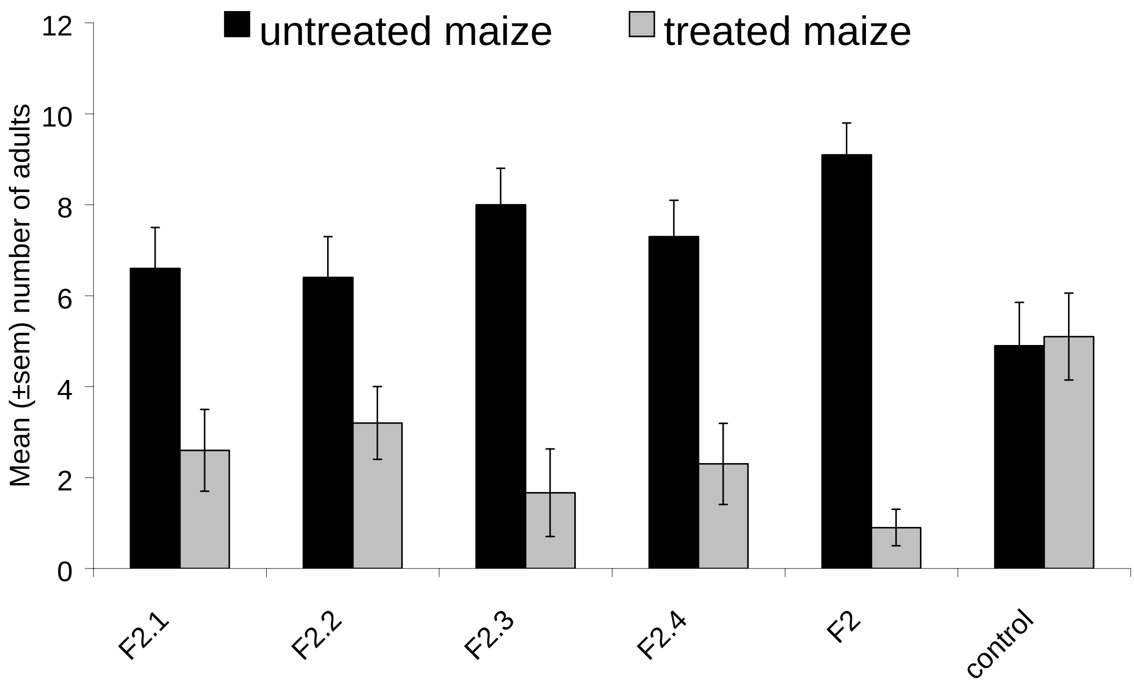


Figure 6 Effect of chromatographically separated subfractions of fraction 2 of *C. nigricens* on the choice preference behaviours of *S. zeamais* when given a choice between treated and untreated maize



Development of a quality control bioassay for botanicals used against the diamondback moth, *Plutella xylostella*

Abstract
The neem tree, *Azadirachta indica*, is the most widely used botanical for pest control in developing countries, and several commercial products are available based on extracting the active ingredients. The products vary in the level of the main active ingredient, Azadirachtin, and in their formulation. Other bioactive ingredients within commercial neem insecticides are usually not assessed and often considered as 'inert' materials. A method for assessing botanicals that is not based on phytochemical analysis was developed to distinguish subtle differences in bioactivity and overall efficacy. The diamond back moth, *Plutella xylostella*, was used as a model pest species to assess samples of neem collected at different times of year and from different places that were known to vary in their phytochemistry. The bioassay was able to distinguish activity variation of less than one order of magnitude among samples of neem collected in different seasons. The implications of these results are discussed in the context of improving the reliability of natural product usage for pest control in developing countries.

Introduction

Botanicals are well-known to vary in the quality and quantity of active ingredients, the results of which have been observed by many researchers testing botanicals against a range of target insect pest species (Satasook et al., 1994, Marr and Tang, 1992). The neem tree, *Azadirachta indica*, is the most researched insecticidal plant species with several commercial products available (Puri, 1999). Commercial products based on neem are often less costly than synthetic pesticides (Childs et al., 2001), making them more affordable for small-scale farmers in developing countries. Throughout South Asia and parts of Africa, there are increasing efforts to increase production of neem products (Wrigley et al., 1997). Many of these start-up ventures are relatively small producers serving a local market for neem-based insecticides. A problem suffered by both large and small-scale producers of neem-based insecticides is that the amount of the main active ingredient, Azadirachtin, can vary considerably depending on when, where and how the plant material is harvested. Some larger-scale production methods extracting Azadirachtin are able to chemically confirm the percent active ingredient and adjust this accordingly. However, even when the Azadirachtin content has been standardised within commercial products, there can be very large differences in efficacy between products (Weaver and Subramanyam, 2000). These differences are likely to be accounted for by formulation differences and, more importantly, by variable amounts of other chemical components present in neem that have also been shown to possess bioactivity.

A solution to the problem of variability in a market crowded with similar products is to use standard methods of assessing bioactivity. At the moment, no such standards exist for botanical products such as neem, and the widespread adoption of a quality control bioassay could provide the market in botanical products with the benchmarking it requires to increase and improve botanical usage. One of the most common targets of neem products in crop protection is the diamond back moth, *Plutella xylostella*, and our research objectives were to develop a bioassay that could be used to rapidly assess a botanical product for its efficacy against this pest and distinguish subtle differences in efficacy relative to phytochemical variability.

Materials and Methods

Neem leaves were harvested at three different times of year (July, September, November) and from four different geographic locations in northern Ghana (Tamale, Yendi, Bolgatanga and Bawku). The leaves were shade dried and shipped to the UK where they were stored as whole material in the dark at 10 ºC. Crude extracts of the leaves were prepared by grinding 100 g in an electric mill (HR2810A, Phillips). A sub-sample of 20 g of the powder was agitated for 30 seconds in 200 ml of distilled water and left to extract in darkness over 24 hours at 6 ºC (labelled as tube 1). A dilution series was prepared by placing 90 ml of distilled water into four containers (labelled as tubes 2 to 5). From the crude extract, 10ml of the suspension was transferred to tube 2. After mixing thoroughly, 10ml of tube 2 was transferred to tube 3, and so on for tubes 4 and 5 as per the below table. In order to test for the effects of phytochemical variability, 16 samples of neem leaves from different collection times and places were prepared in the same manner.

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Tube no.	Dilution factor	Amount of extract (mg/ml)
1 crude extract	1	100.00
2	10^{-1}	10.00
3	10^{-2}	1.00
4	10^{-3}	0.10
5	10^{-4}	0.01
6 control (distilled water only)	0	0.00

The bioassay for assessing bioactivity against *P. xylostella* was prepared using Chinese cabbage leaves. A cabbage leaf was dipped into each concentration of the sample (including crude and control), making sure that both sides were thoroughly wetted, and the leaves were hung up to dry. Once dry, each leaf was mounted in agar (1% tap water) placed in labelled 8oz pots with separate pots used for each treatment (3 replicates per concentration). After the agar had set, 30 second-instar *P. xylostella* larvae were placed on each leaf treatment. Larvae were left to feed for 24 hours on the treated leaves and then transferred onto fresh, untreated cabbage leaves mounted in agar (15 larvae per pot). The pots of larvae were examined after a further 24 hour period recording larval mortality.

Results

Linear and quadratic analyses of variance showed that the assumptions for analysis of variance are adequately satisfied when comparing the $\log_{10}$ levels of dilutions against the water control. Concentration affected the level of larval mortality with increasing concentration causing higher mortality. A linear contrast for the dilution levels was highly significant, indicating a clear linear relationship between mortality and concentration (Figure 1). All concentrations up to and including the 0.01 mg/ml concentration caused significantly higher mortality than the untreated control. A factorial analysis showed that variation among samples was principally accounted for by the collection time of plant material with variance estimates close enough to justify a combined analysis of samples within each time period (Table 1). The shape of response was similar across the collection times (Figure 2). The source of variation was shown to be accounted for by the higher mean value of mortality associated with samples from collection time 2 (15.7 dead larvae) which was significantly different from collection times 1 and 3 (11.5 and 12.77 dead larvae, respectively).

Discussion

Our research has confirmed that plant materials can vary in their bioactivity with regard to changes induced in their phytochemistry. Research on *A. indica* has previously confirmed that phytochemical variation is a phenomenon that can lead to changes in efficacy (Isman, 1997). Childs et al (2001) has reported that the lack of standard methodologies for estimating efficacy of neem products is a well-identified constraint to increasing the promotion of neem products. In the same report by Childs, research on the prioritisation of limitations of the use of neem by poor farmers showed that the lack of quality assured products and a perceived lack of effectiveness were hampering uptake. Our research has demonstrated that a relatively straight forward methodology can distinguish subtle differences between samples of neem. The bioassay, although specific to the diamond back moth, could be used as a baseline marker to assess the relative efficacy of neem products ensuring that standards are maintained by adjusting the concentration of the product accordingly. The bioassay can be implemented with no special equipment or solvents using the most simple of laboratory requirements to rear insects. Bioactivity can vary with target pest species; however, the same methodologies could be adapted to different insects by incorporating the neem extract on to a suitable host and evaluating morality over a suitable time-frame for the pest.

As has been highlighted in a number of reports, increasing the production and use of neem products will be dependent on a number of factors (Rejesus, 1995; Isman, 1999). Commercial entities wishing to capture a larger percentage of the market for neem pesticides will need to ensure that their product is trustworthy, giving similar levels of control over the production time-scale. Chemical analysis of the product requires expensive equipment and materials (HPLC and solvents) as well as highly specialised training making it more difficult for small scale producers to use such methods for quality control. Also because of the number of active ingredients acting synergistically within crude extracts of neem, standardising a neem formulation based on the quantity of Azadirachtin alone may fail to adequately ensure the quality of product efficacy. We, therefore, believe that quality control

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bioassays are not only more cost effective but also more capable of assessing the bioactivity of the product. Bioassays have the added advantage of being able to compare products that are derived from different botanical species and could help assess new competitor products of neem that are based on other plant species. The use of such bioassays should be incorporated into policy regulations and encouraged through commercial channels as a way to improve the indigenous use of pesticidal plant materials.

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Table1 Factorial analysis of all trials evaluating neem-treated cabbage leaves to induce mortality of 2nd instar larvae of *P. xylostella* (n=16)

Source of variation	degrees of freedom	sum of squares	F probability
collection time	2	937.99	<0.001
collection X trial	13	2349.21	<0.001
collection X trial X replicates	32	802.03	0.314
treatments (dilution series)	5	5962.53	<0.001
linear regression	1	320.28	<0.001
quadratic regression	1	5.05	0.636
collection X treatments	10	142.91	0.781
linear regression	2	11.75	0.770
quadratic regression	2	69.73	0.218
Residual	222 (3)	4980.73	
Total	284 (3)	15167.92	

Figure 1 Effect of concentration on the mortality of second-instar larvae of *P. xylostella* 24 hours after a 24 hour exposure period to cabbage leaves treated with a water extract of *A. indica* leaves. Larvae were transferred to untreated cabbage leaves after the first 24 hour period.

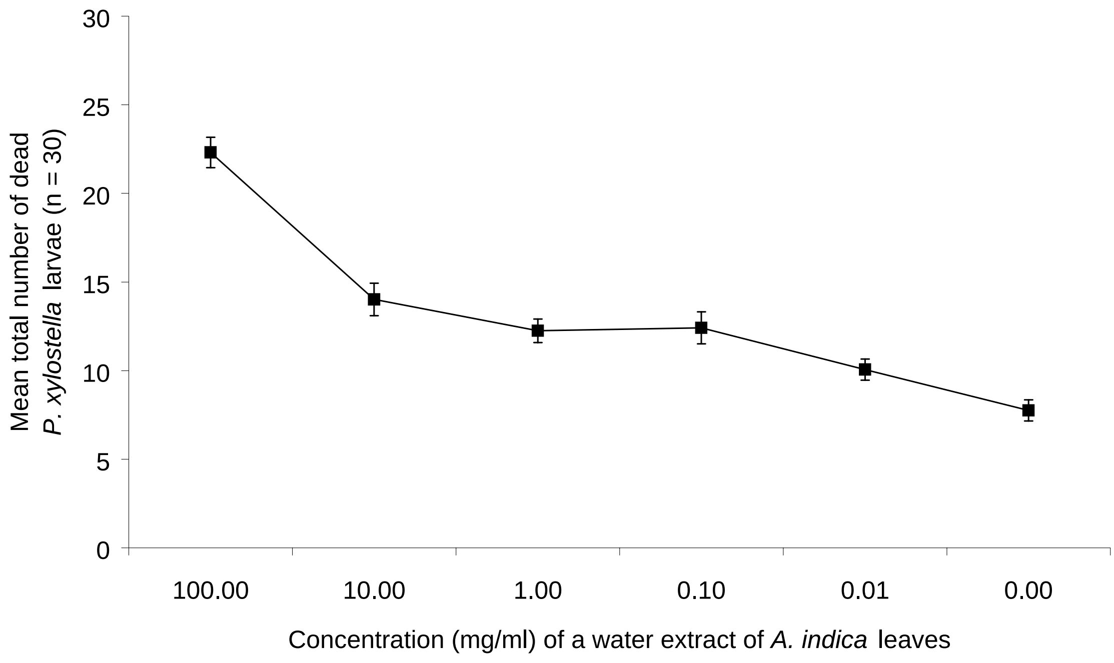

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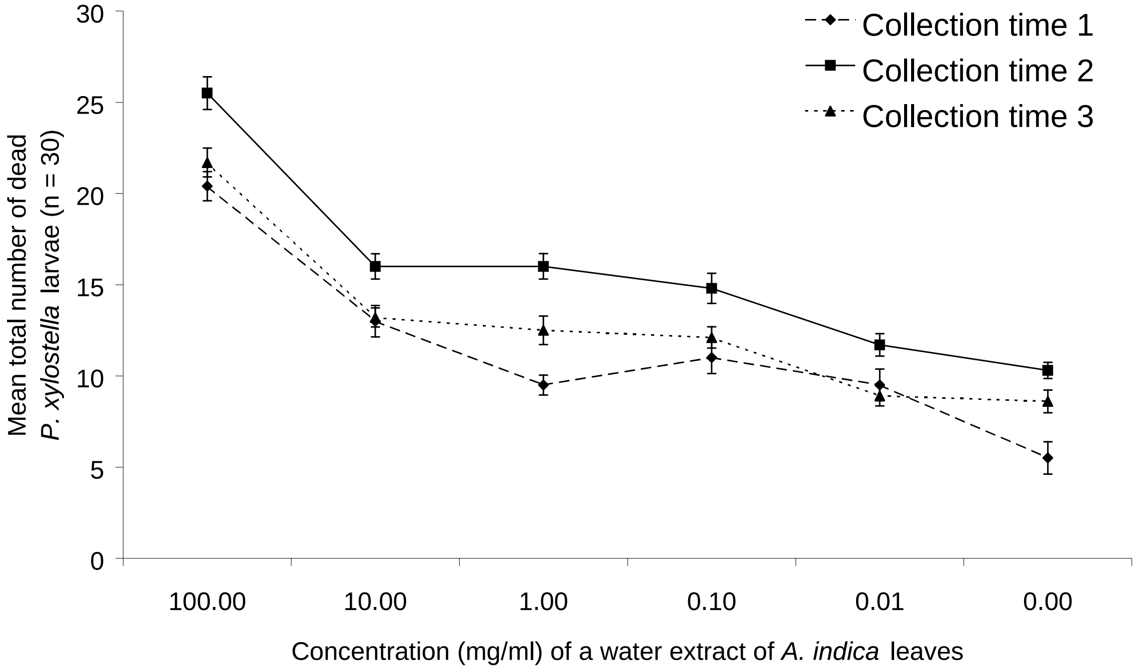
Figure 2 Effect of botanical collection time on the mortality of second-instar larvae of *P. xylostella* 24 hours after a 24 hour exposure period to cabbage leaves treated with a water extract of *A. indica* leaves. Larvae were transferred to untreated cabbage leaves after the first 24 hour period.



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Ecology of plant species used in storing grain in Ghana's northern savannah zone

Abstract
Ecological studies were carried out to determine the distribution, natural regeneration, germination and sustainability of eight plant species used in storing grains at farm level in the northern part of Ghana. *Azadirachta indica* and *Cassia sophera* were found to be ubiquitous and survive well in all localities. *Securidaca longepedunculata* is ubiquitous but more concentrated in the Guinea Savannah than the Sudan Savannah. *Synedrella nodiflora* and *Ocimum americanum* are also ubiquitous but have higher populations in the Sudan Savannah and in the transition to the Guinea Savannah (Upper East Region). *Cymbopogon schoenanthus* and *Chamaecrista nigricens* are mostly restricted to the Upper East Region. *Lippia multiflora* occurs only towards the southern portion of the Guinea Savannah (Yendi and Tamale). Natural regeneration of *S. longepedunculata* is generally low due to repeated wild fires. *O. americanum*, *C. nigricens*, *C. schoenanthus* and *L. multiflora* face threat of extinction in the likely event of being extensively used in grain storage. Intensive propagation is recommended for these species. With the exception of *A. indica*, *C. sophera* and *C. schoenanthus* all the other species recorded very low seed germination rates.

Introduction

One of the serious constraints to food security in most rural areas in the northern part of Ghana is the post-harvest losses of stored grain. This constraint is the result of infestation of farm store grain by insects. So far one exotic, *A. indica* and seven indigenous species, *C. nigricens*, *C. sophera*, *O. americanum*, *S. longepedunculata*, *S. nodiflora*, *C. schoenanthus*, and *L. multiflora* have been shown to offer good insect pest control. Apart from *A. indica* very little is known about the indigenous species being used by the locals as pesticides. For instance, are the species widely distributed within the northern savannah ecosystem? What are their preferred habitats? Is artificial regeneration possible? Is the exploitation of the species sustainable in the long term? If the exploitation of the species is to be sustainable in the event of their wide adoption then the knowledge about the regeneration ecology is as important as the extent of their use.

The objectives of the study were:
1) To find out the extent of distribution of the identified pesticidal plants in the northern savannah zone.
2) To determine the extent of natural regeneration of the identified pesticidal plant species.
3) To test whether the species can be artificially regenerated.
4) To determine the threat posed by the use of the plant species by the communities in the northern savannah zone.

Specific activities carried out to achieve the project objectives were:
1) Distribution and abundance of plant species
2) Characteristics of species distribution range.
3) Assessment of natural regeneration of species.
4) Soil seed bank.
5) Germination capacity and
6) Assessment of anthropogenic threat to species.

Materials and Methods

Natural distribution

Information was sought through existing literature and contacts with agricultural extension agents, forest guards, herbalists and rural farmers. Field visits were made to the sites of the species. In the case of *S. longepedunculata*, transepts were cut to the actual locations of the stands. At each site visited notes were taken of the extent of coverage of the species through direct measurements and ocular estimations, though the later has its associated limitations. For each site visited sample plots in the form of quadrants were laid for the purpose of assessing distribution of species in the localities visited. For shrubby species 4 x 4 metre plots were used while plot-less sampling was used for tree species (Kent and Coker 1992). At least ten random plots of each species were laid for each location where the species were spotted. Notes were taken of the habitats of the species in all sites visited.

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Natural regeneration
Ten quadrants each measuring 4 x 4 metres were randomly established in three different agro-ecological zones at locations where the species under investigation occur. These are Sudan Savannah (Bawku area), Sudan-Guinea Savannah Transition (Bolgatanga area) and Guinea Savannah (Yendi area). A 100 percent enumeration of all seedlings was done in all the quadrants. For shrubby annuals like *C. nigricens*, *S. nodiflora*, *O. americanum* and *L. multiflora* every plant was counted. For the tree species, however, only seedlings less than 2mm diameter were counted. Any seedlings above this diameter were considered as advanced regeneration and were, therefore, not considered as regeneration under this study. All the plots were laid in May just before the first rains. They were then monitored once every month until the end of November when all plants had began to wither and no new regeneration was expected.

Soil seed bank
Four 20 cm x 20cm plots were randomly demarcated in locations where the species under study were found to exist. Soil samples, collected from the surface down to 3cm soil depth were spread over sterilised river sand in plastic containers and watered twice daily (morning and evening). After two months the plants germinating in the soil were identified and counted. This experiment was limited to the species with tiny seeds.

Artificial regeneration
This study was limited to germination tests, and other means of propagation were not investigated. Treatments applied varied from species to species depending on the seed type. For *C. sophera*, *A. indica*, *C. schoenanthus*, *S. nodiflora*, *O. americanum* and *C. nigricens* seeds were tested for germination in three different media, namely, river sand (control), laterite soil and loamy soil. For each species and each treatment four plastic containers, each containing 25 seed-lots were set up and watered twice daily. This works up to 100 seeds per treatment for each species. In the case of *C. sophera* germination tests covered three different periods in the year due to its seed production almost throughout the year. These were March, June and October. The objective was to determine which period in the year produces the most viable seeds. For *S. longepedunculata* seeds 100 seed-lots were soaked in water for 24 hours while another 100 remained unsoaked (control). *C. nigricens* was tested using two different treatments, intact seeds (control) and 24 hours soaking in water. In all cases germination was occasionally monitored.

Threat assessment study
This study involved a rapid appraisal of different uses to which the identified species were made in the savannah zone, frequency of use, collection methods and whether the plants were cultivated or not. The Participatory Rural Appraisal method (PRA) involving interactions with the rural farmers was used to collect information on the sustainability of the species. Six communities located in six different districts (Table 4) were visited for the exercise. The selection of the communities were, however, biased towards the Upper East Region. This was based on the earlier report on post harvest constraints and opportunities by Brice et al. (1996) which showed the Upper East Region as the region in which the highest number of farmers use plant materials as protectants. A total of 144 farmers who were 40 years and above were involved in the exercise. However, the numbers differed from community to community. In each community the farmers were met as a group and questionnaires were administered in the form of discussions. Each farmer was given an equal opportunity to contribute in answering questions.

Results

Natural distribution and habitat
The natural distribution and relative abundance of the eight species under investigation are shown in Figure 1a-h. *A. indica* (Figure 1a) has a very wide distribution cutting across a wide range of micro sites ranging from water-logged areas to hard grounds in this vegetation zone. It occurs in the whole Sudan and Guinea Savannah areas of northern Ghana but with a higher concentration in urban areas than in villages due to its wide application for amenity planting in government ministries and bungalows. It is rarely seen on farmlands particularly in the Upper East Region where farmlands are under constant cultivation. It has, however, invaded most degraded areas even within forested sites. *C. nigricens* (Figure 1b) is restricted within the Sudan Savannah (Bawku and Garu District) and the Sudan-Guinea Savannah transition (Bolgatanga, Navrongo, Builsa and Bongo districts). It is mostly found on farm fields, compounds of houses and parklands where there is no tree cover. It rarely

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covers large areas wherever it occurs (0.1-0.4ha). It is seldom seen in water-logged areas. It is not found in the southern portion of the northern savannah zone. *C*. *sophera* (Figure 1c) is widely distributed in the northern savannah zone and even beyond, occurring around only human settlements and roadsides. It is rarely found in the wild. Stands of the species are well established on fertile sites as abandoned refuse dumps, undeveloped building plots and uncompleted buildings where human wastes are prevalent. It is relatively abundant in larger than in smaller towns probably due to differences in the levels of waste disposal in these areas. It is seldom found in smaller villages and single house settlements. *O*. *americanum* (Figure 1d) also occurs in human settlements throughout the savannah zone. It is generally seen on maize and groundnut fields and around houses. Stands of this species occur in small patches (about 0.5ha or less) with different stands widely apart. In the Sudan Savannah (Bawku District) the species covers even smaller patches (about 2-10m^2) compared with the Sudan-Guinea Savannah transition areas where larger patches of between 0.5 ha to 2 ha sometimes occur. *S*. *longepedunculata* (Figure 1e), is widely distributed in the Sudan and Guinea Savannah zones. However, while the species is only found in the remotest areas in the Sudan Savannah and its transition to Guinea Savannah, it occurs abundantly even on farmlands in the southern portion of the Guinea Savannah zone (Salaga, Damongo and Yendi areas) suggesting a higher collection pressure on the species in the former zones. Rocky and sandy soils are its preferred habitats. *C*. *schoenanthus* (Figure 1f) is generally located on hilly and gravely sites in the Sudan and Guinea Savannah portions of the Upper East Region, particularly areas stretching from Nakong on the Chiana-Tumu road towards the Upper West region and in the Navrongo district. *S*. *nodiflora* (Figure 1g) occurs throughout the whole northern savannah zone. It is found in abundance on almost every farmland, particularly in the Upper East Region but less abundant towards the southern portion of the Guinea Savannah. It is not seen in forested microsites. Natural regeneration is at its peak from the end of September towards October when farm clearing has been completed. *L*. *multiflora* (Figure 1h) occurs towards the southern portions of the savannah zone, particularly in Tamale, Yendi and Salaga areas. It is normally found along roadsides and fringes of settlements and farm fields. Table 1 shows the extent of coverage of individual stands of the species encountered in the different localities during the study.

Natural regeneration
The total number of seedlings emerging in the experimental plots are shown in Figure 2a-b. Seedling emergence was more highly prolific with *A*. *indica* than *S*. *longepedunculata* which had increasing numbers of emerging seedlings from the Sudan Savannah to the southern portion of the Guinea Savannah zone and vice versa. In contrast, natural regeneration of *S*. *nodiflora* showed a declining trend towards the southern part of the Guinea Savannah zone. The Sudan-Guinea Savannah transition (Bolgatanga, Navrongo and Bongo areas) had the highest number of seedlings of this species in the plots.

Soil seed bank
The results of the soil seed bank study is shown in Table 2. Grass was the single dominant genera regenerating in all the plots. *S*. *nodiflora* recorded the highest percentage seedlings in its own plot. Two of the species, *O*. *americanum* and *C*. *nigricens* did not have a single seedling germinating in their seed banks.

Artificial regeneration
Three species, *C*. *sophera*, *A*. *indica* and *C*. *schoenanthus* showed good germination. Almost all the species performed better in laterite soil than in the other soil media. Soaking for 24 hours did not improve germination of *S*. *longepedunculata* to any appreciable extent. Soaking for 72 hours, however, improved the percentage germination of *C*. *nigricens*. Figure 3 shows the germination of *C*. *sophera* seeds collected at different times of the year where collections from October had the highest percentage germination.

Threat Assessment
The results of the PRA conducted in selected communities (Table 5) indicated unanimity of respondents on the abundance of *A*. *indica* in all the communities covered. This was in spite of the fact that the species was widely used for grain storage, fuelwood, treatment of diseases, crop protection, veterinary applications, rafters and as chewing stick and the fact that collection is done all the year round. All the respondents indicated that collection was mostly limited to seeds and leaves except on a few occasions when whole plants (in the case of seedlings and saplings) were harvested.

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All of them said the species is cultivated in their localities, thus suggesting a high probability of sustainability of the species.

Only respondents from one community indicated the abundance of *C*. *nigricens* in their locality. The remaining respondents representing 80% said the species existed in small quantities in their localities. Besides grain storage, other uses like treatment of human diseases, veterinary applications and termite control in homes were mentioned though uses varied from community to community. In all the communities collection was done all the year round. 42% of the respondents said they removed the whole plant during harvesting while the rest harvest only the leaves.

C. *schoenanthus* was considered abundant by respondents in all the communities covered. Apart from grain storage it was found to be widely used for the treatment of various diseases, the elimination of lice, fermentation of Dawadawa, termite repellent in homes, as a disinfectant and for spiritual purposes. Collection was done all the year round in all the communities, the collection method being the removal of the whole plant. Respondents from only one community representing 20% of the respondents said the species was sometimes cultivated.

Approximately 60% of the respondents representing four communities admitted the relative scarcity of *O*. *americanum* in their localities while the remaining said it was abundant in their localities. Collection in all communities was done during the rainy season as it is available only within that season. 54% of the respondents said they removed the whole plant during harvesting while 26% removed leaves and seeds and 20% removed only leaves. It is not cultivated in any of the communities.

All the respondents in all the six communities said the plants of *S*. *longepedunculata* were few, and it was considered one of the 'difficult-to-find' plant species in all the communities. Uses like grain storage, treatment of varied human diseases, snake repellent, spiritual purposes and sale in the market for income were mentioned in all the communities. The species is harvested throughout the year. While 44% said they removed only the roots during harvesting, 35% said they removed both roots and leaves and 21% the whole plant, which they processed and sold in the market. Cultivation is not done in any of the communities.

All the respondents were unanimous on the abundance of *S*. *nodiflora* in their communities. Uses that the species are put to include grain storage, mosquito repellence, termite control in homes, processing of Dawadawa, treatment of human diseases and for spiritual purposes. Collection is done during the rainy season. Whole plant harvesting is the method that was mentioned as being generally used in all the communities. No cultivation was done in any of the communities.

Respondents in all the communities indicated that *C*. *sophera* were few in their communities. Only three applications of the species were mentioned in all the communities. These were medicinal, grain storage and veterinary applications. Collection was done all the year round with the removal of leaves and seeds being the general harvesting method. Only respondents one community mentioned the cultivation of the species.

Discussion

Species distribution patterns

The study indicates that there are three groupings of the eight species studied with respect to their distribution patterns. These are a) *A*. *indica* and *C*. *sophera* which are ubiquitous and do well in all localities, b) those which are ubiquitous but are more abundant in certain localities e.g. *S*. *longepedunculata*, *O*. *americanum* and *S*. *nodiflora* and c) those that are localised in distribution, eg, *C*. *schoenanthus*, *C*. *nigricens* and *L*. *multiflora*. This distribution pattern may be due to the fact that plant species differ in their tolerance of and requirements from the environments so that their distribution or abundance varies along environmental gradients (Swaine 1996). This concept is amply demonstrated in distribution maps of species (Hall and Swaine 1981; Hawthorne 1993) which show clear associations between species occurrence and known environmental conditions. Species distributions also show association with soil conditions, which are more often recognised on relatively smaller scales because extensive soil data are rarely available (Swaine 1996). The species distribution patterns as shown in this study are, therefore, possible because of slight variations in the intensity and duration of rainfall from along the corridors of Burkina Faso down to the southern portion of the northern savannah zone (Yendi, Salaga and Damongo areas). The results of the study,

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however, give room for testing the hypotheses as to which of the species are limited in their natural distributions by moisture and/or soil fertility.

Natural regeneration

The results indicate contrasting patterns of natural regeneration of the two tree species. While *A*. *indica* exhibits a prolific regeneration pattern, regeneration of *S*. *longepedunculata* appears erratic. *A*. *indica*, an invasive exotic species, has been found to fruit more than once in the northern part of Ghana (personal observation). Therefore, mature seeds sometimes fall from the trees after the bush fires and germinate with the onset of the rains. On the other hand, *S*. *longepedunculata*, an indigenous forest species, releases dry fruits in the peak of the annual fires and will probably not have prolific natural regeneration. However, in one of the areas in the Sudan Savannah where the experimental plots were laid there was a lot of saplings of the species. Personal communication with Azuwane Azure, a farmer in the Garu Timpane District, explained that those saplings emerged during years when no fire occurred so the 'lucky' seeds are able to germinate. The low regeneration of *S*. *longepedunculata* in the Sudan Savannah and its transition to Guinea Savannah is a clear indication of the imminent threat of extinction of the species in the drier and more populated part of the northern savannah zone. This will likely produce a cascading influence on the stocking in other parts of this ecozone.

Natural regeneration of *S*. *nodiflora* will continue to be high in so far as farmlands continue to undergo cultivation while *C*. *sophera* will also continue to exert its dominance in its preferred habitats in urban areas. For the other species, however, there appears to be some degree of unpredictability of regeneration every year .

Soil seed bank

The results show a poor representation of *C*. *schoenanthus* in its own seed bank with no seedlings of *O*. *americanum* and *C*. *nigricens* in their soil seed banks as opposed to species like *C*. *sophera* and *S*. *nodiflora*. The results give a true reflection of the relative densities of the species in their natural habitats. It is not clear why some species did not record any seedling growth in their own seed banks. It is possible that either the release of seeds into the soil is highly erratic or the seeds might have lost viability. This has important consequences on the regeneration capacity of the species. For such species artificial regeneration is critical to its sustainability.

Seed germination

With the exception of *A*. *indica* (tree), *C*. *sophera* (shrub) and *C*. *schoenanthus* (grass) all the other species recorded poor germination. It is not clear why a species like *S*. *nodiflora* which has prolific seedling regeneration under field conditions showed poor germination in the nursery. This may be due to some physiological adaptations of seeds of the species in its natural soil environment. The species may be positively photoblastic and, therefore, require high light intensity to germinate. The seeds of such species will not germinate if buried in the soil. For the other species, the results appear to have a direct co-relation with the field regeneration trend. All the seeds used in the germination tests were collected between January and March 2001. Germination tests done with seeds collected in October and November were not successful except with *C*. *sophera*, which had the highest germination for October collections. There remains a problem of collecting mature and viable seeds at the right time in this part of Ghana before they are destroyed by the bush fires. For most of the species studied, viable seeds are available between January and March when wild fires are rampant. It is hoped that future trials can achieve a higher percentage germination for *S*. *longepedunculata* as it has been suggested by Irvine (1961) that higher germination for *S. longepedunculata* can be achieved by using seeds which remain long on the tree. Further tests are planned to improve the germination of the species. Most of the species had the best germination in ordinary laterite soil; however, further tests need to be done to confirm this finding.

Species sustainability

The sustainability of every species depends on efficient harvesting methods, creation of suitable conditions for natural regeneration and propagation of the species to reduce pressure on its naturally existing stock. Therefore, the harvesting of a plant species without any replacement programme threatens its sustainability. It is clear from the threat assessment study that three species, *A*. *indica*, *C*. *sophera* and *S*. *nodiflora* could be very resilient to collection pressures in view of their colonising abilities. However, *S*. *longepedunculata* which has been described in the Upper East Region as a' difficult-to-find' species is not cultivated anywhere in the northern savannahs. In all the areas where

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this species was located in the Upper East Region the minimum distance from human settlement was about 8 kilometres. This shows how the species is rapidly getting out of reach of users in this region. Furthermore, with the current harvesting methods in the region, stems which undergo harvesting have little opportunity for survival. This has serious implications on the resource life of the species and the economies of those who survive on the sale of the plant. In the southern part of the savannahs, especially Yendi and Salaga areas, coppice shoots were found on cultivated farmlands and within easy reach of users. Unlike in the Upper East Region harvesting is done by the removal of only few roots so that it does not adversely affect the survival of the residual stems. The species, however, undergo another form of destruction through the cutting of the stems for yam stakes. This practise also has important consequences on the sustainability of the species in the northern savannah zone. In spite of the claim of abundance of *C*. *schoenanthus* in all the communities covered by the PRA, the collection method employed poses a severe threat to its sustainability, particularly in the Bawku area where the species is under so much pressure that it has no opportunity of seeding. The relatively low level availability of *C*. *nigricens* and *O*. *americanum* suggests their inability to withstand collection pressures in the likely event of their being heavily used for grain storage. With the endemic nature of *L*. *multiflora*, its widespread application would mean its introduction into other parts of the savannah zone provided its potency was not sacrificed.

On the basis of the results of the studies conducted it is concluded that:
1. While continuous cultivation is a threat to the continuous survival of species like *S*. *longepedunculata* it is a stimulant for the germination and growth of *S*. *nodiflora*.
2. Repeated annual fires is one of the greatest threats to the natural regeneration and survival of most of the plant species in the savannah ecozone.
3. Species like *A*. *indica* and *S*. *nodiflora* can be sustained for a considerably long time in the event of wide application for grain storage. *O*. *americanum* and *C*. *nigricens* will require interventions to prolong their resource lives. *C*. *sophera* holds promises of sustainability on account of its copious production of seeds and ease of germination.

It is recommended that:
1. The crusade against bush fires in the Ghana's northern savannah zone should be aggressively pursued to enhance natural regeneration and growth of the species.
2. Cultivation of the threatened species in home gardens should be promoted.
3. Further research aimed at improving germination of the species should be carried out.
4. Certain areas in the Sudan Savannah (eg. The Zawse Hills near Bawku) should be designated as 'Special Biologically Protected Areas' to ensure enhanced growth and supply of *C*. *schoenanthus*.
5. Intensive education on the conservation of the species must be vigorously pursued in the northern savannah zone.

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Table 1. Extent of coverage of stands of eight botanical species used to store grains in farm level storage systems in northern Ghana.

Species	Locality	Extent (hectares, ha)
A. indica	Tamale .	0.5 - 2.0
	Yendi	0.5 - 2.0
	Salaga	0.5 - 2.0
	Damongo	0.5 - 2.0
	Bolgatanga	0.4 - 4.0
	Bawku	0.5 - 3.0
	Sandema	0.5 - 2.0
S. longepedunculata	Tamale	0.5 - 4.0
	Yendi	1.0 - 3.0
	Salaga	1.0 - 3.0
	Damongo	1.0 - 3.0
	Bolgatanga (sakote)	0.5 - 1.0
	Sandema	0.2 - 0.5
	Bongo	0.2 - 0.5
C. nigricens	Bongo	0.1 - 0.2
	Bolga	0.2 - 0.4
	Chiana	0.1 - 0.2
	Bawku	0.1 - 0.3
	Garu	0.2 - 0.4
C. sophera	Tamale	0.5 - 1.0
	Yendi	0.5 - 1.0
	Salaga	0.5 - 1.0
	Damongo	0.2 - 2.0
	Bawku	0.2 - 0.5
L. multiflora	Tamale	1.0 - 2.0
	Yendi	1.0 - 2.0
	Salaga	1.0 - 2.0
O. americanum	Tamale	0.2 - 0.5
	Yendi	0.2 - 0.5
	Salaga	0.2 - 0.5
	Damongo	0.1 - 0.5
	Bolgatanga	0.25 - 2.0
S. nodiflora	Tamale	1.0 - 3.0
	Yendi	1.0 - 2.0
	Salaga	0.5 - 1.0
	Damong	0.5 - 1.0
	Bolgatanga	large and continuous coverage
	Bawku	large and continuous coverage
C. schoenanthus	Bawku	1.0 - 2.0
	Bolgatanga	1.0 - 2.0
	Tanga	1.0 - 4.0
	Chiana (Nakon)	1.0 - 5.0 or more
	Bongo	1.0 - 2.0

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Table 2. Soil seed bank status of six of the plants used to treat insect infestation in northern Ghana.

Plot type (species)	Life form/species	Number germinating	Percent of total
Cassia	*C. sophera*	19	11.5
	Grass spp.	36	21.6
	Unidentified	111	66.9
	TOTAL	166	
Synedrella	*S. nodiflora*	25	16.2
	Grass spp.	88	56.4
	Unidentified	43	27.4
	TOTAL	156	
Ocimum	*O. americanum*	Nil	Nil
	Okra	3	2.2
	Grass spp.	77	55.4
	Unidentified	57	42.4
	TOTAL	139	
Chamaecrista	*C. nigricens*	Nil	Nil
	Grass spp	31	30.1
	Unidentified	72	69.9
	TOTAL	103	
Cymbopogon	*C. schoenanthus*	3	4.5
	Other grass spp.	22	33.3
	Unidentified	43	62.2
	TOTAL	66	
Lippia	*L. multiflora*	11	8.4
	Grass spp.	69	52.7
	Unidentified	51	38.9
	TOTAL	31	

Table 3. Germination potential of eight plant species used in northern Ghana for the protection of grain in farm-level storage systems

Species	Treatment	Time to germination commencement	Time to germination completion	Percent germination
C. sophera	River sand	3	12	56
	Laterite soil	3	17	96
	Loamy soil	6	17	50
S. longepedunculata	Intact seeds	38	41	9
	Soaked 24 hrs	23	35	21
A. indica	River sand	12	27	32
	Laterite soil	12	27	36
	Loamy soil	12	24	64
C. schoenanthus	River sand	5	11	36
	Laterite soil	5	14	55
	Loamy soil	5	14	28
S. nodiflora	River sand	29	33	6
	Laterite soil	21	33	18
	Loamy soil	25	33	13
O. americanum	River sand	21	30	7
	Laterite soil	24	33	19
	Loamy soil	27	33	10
C. nigricens	Intact seeds	13	19	24
	Soaked 24 hrs	7	10	42
L. multiflora	River sand	15	27	10
	Laterite soil	12	27	20
	Loamy soil	18	27	9

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Table 4. Communities visited during the threat assessment study

District	Community	Number of farmers
Bawku West	Tanga	27
Bawku East	Garu	30
Kassena Nankana	Nangalikina	23
Bauilsa	Wiaga	38
Bongo	Bongosoe	11
Bolgatanga	Sgeaga	15
	Total	**144**

Table 5. Summary of PRA threat assessment in six communities. Each community response was compiled in aggregate form (n = 6)

Species	**Level of availability**			**# of**	**Collection frequency**		**Collection method**			**Cultivated**
	plenty	**few**	**none**	**uses**	**whole year**	**seasonal**	**leaf**	**root**	**whole**	**yes:no**
A. indica	6			9	6		6		3	6:0
C. nigricens	1	5		6	4	2	3		3	0:6
C. schoenanthus	5	1		8	6				6	1:5
O. americanum	2	4		7	3	3	3		3	1:5
S. longepedunculata		6		6	6		6	6	1	0:6
S. nodiflora	6			7	1	5	4		2	0:6
C. sophera		6		3	4	2	5		1	1:5

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Figure 1a. Distribution map of *A. indica* in northern Ghana

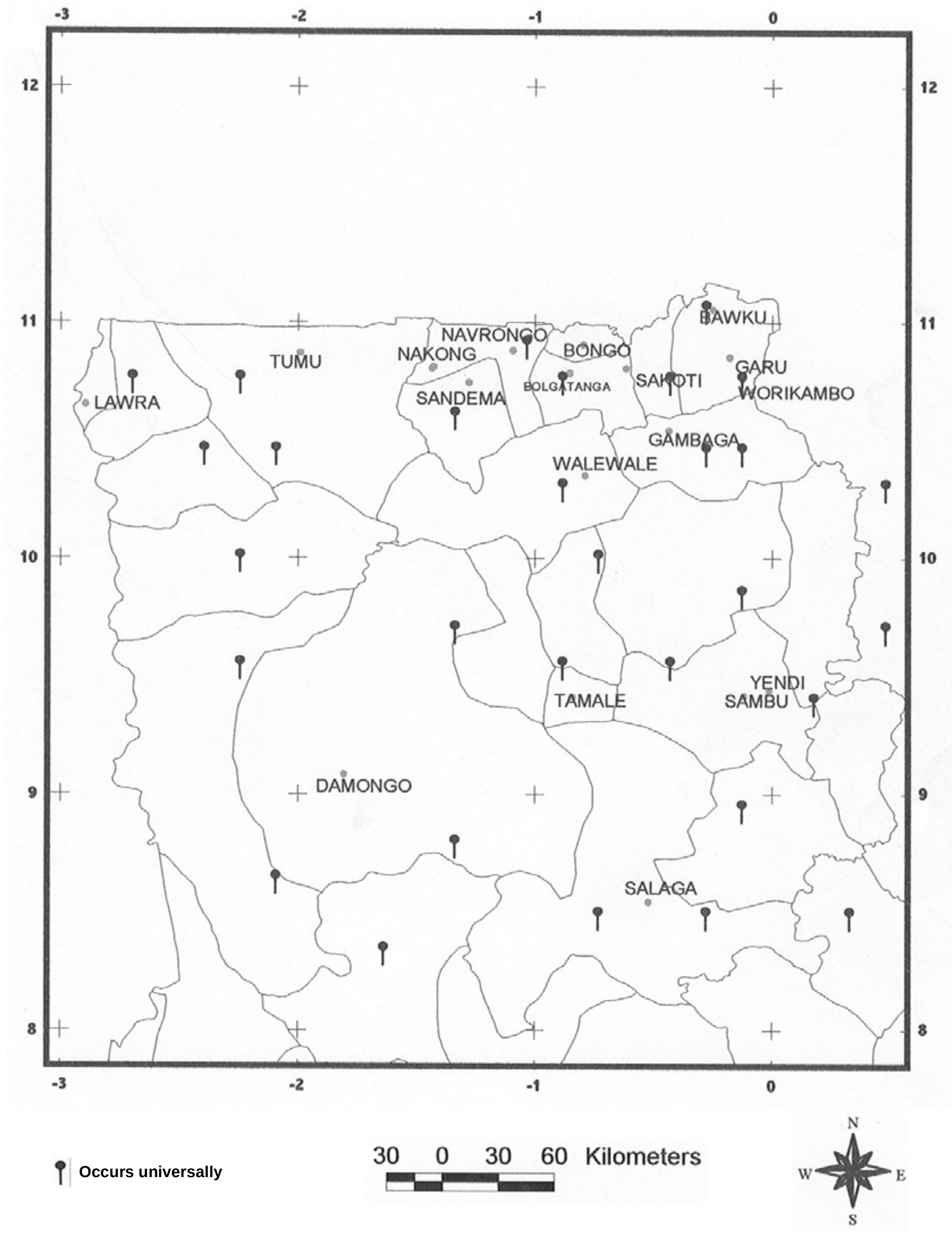

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Figure 1b. Distribution map of *C. nigricens* in northern Ghana

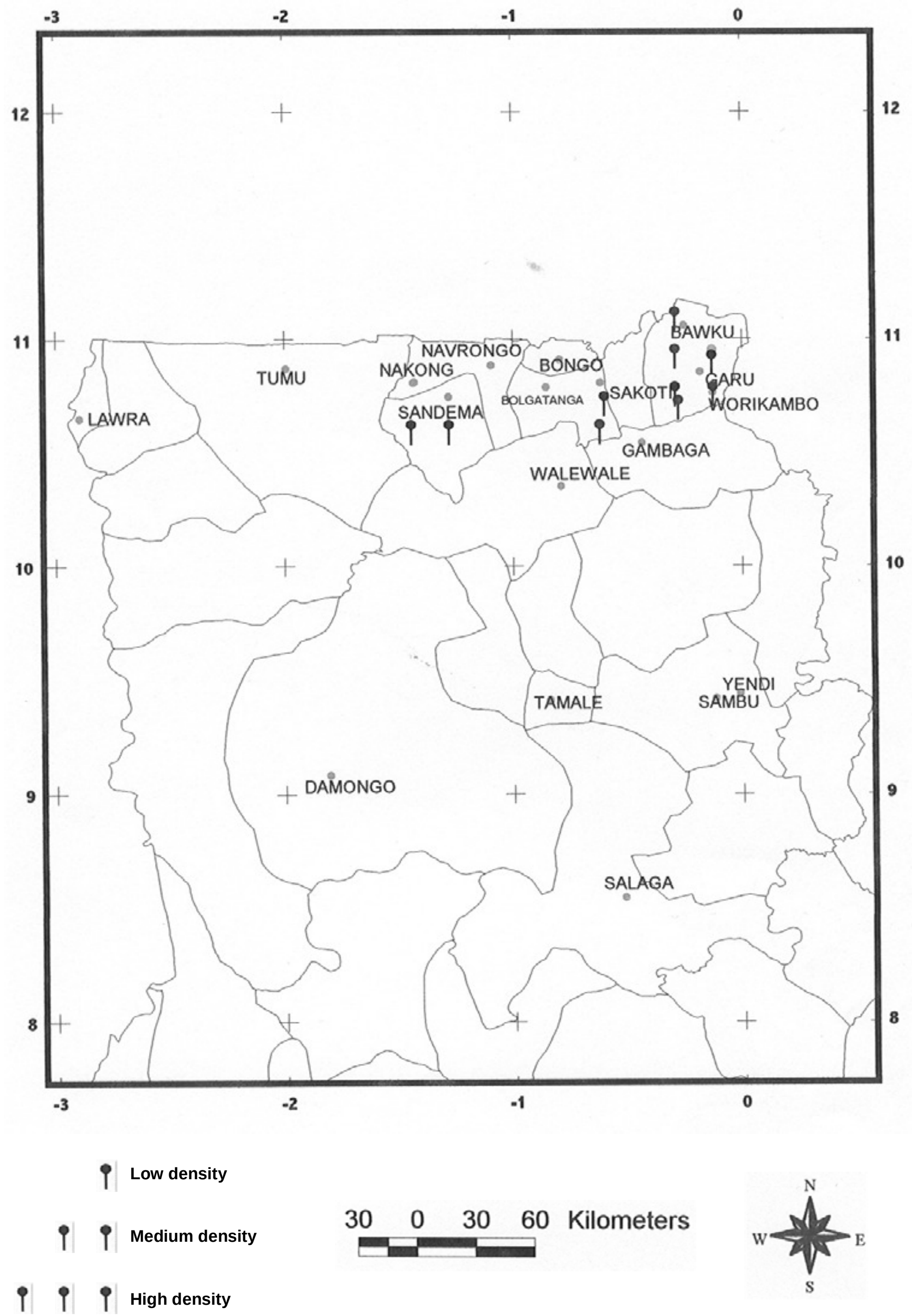

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Figure 1c. Distribution map of *C. sophera* in northern Ghana

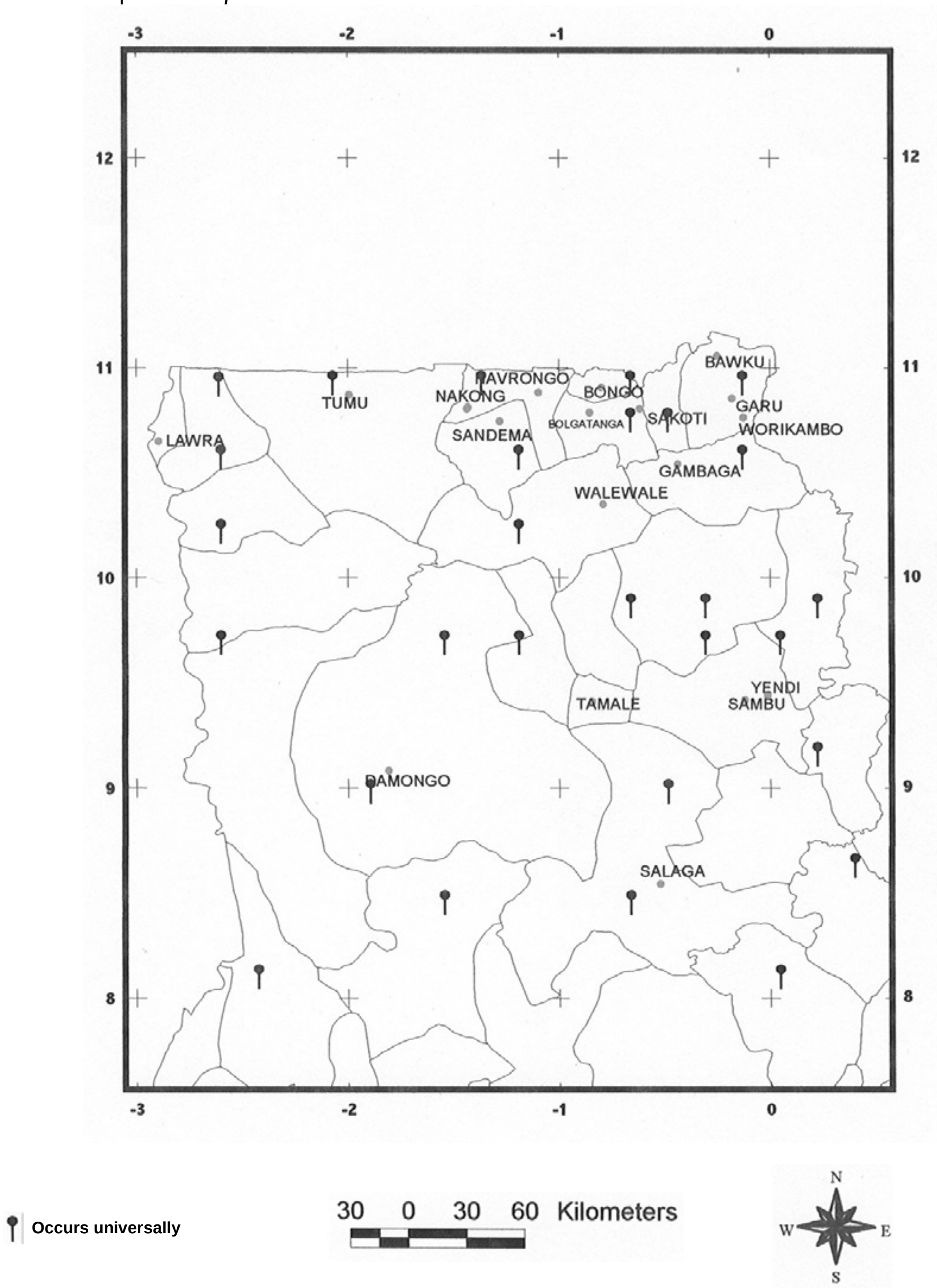

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Figure 1d. Distribution map of *L. multiflora* in northern Ghana

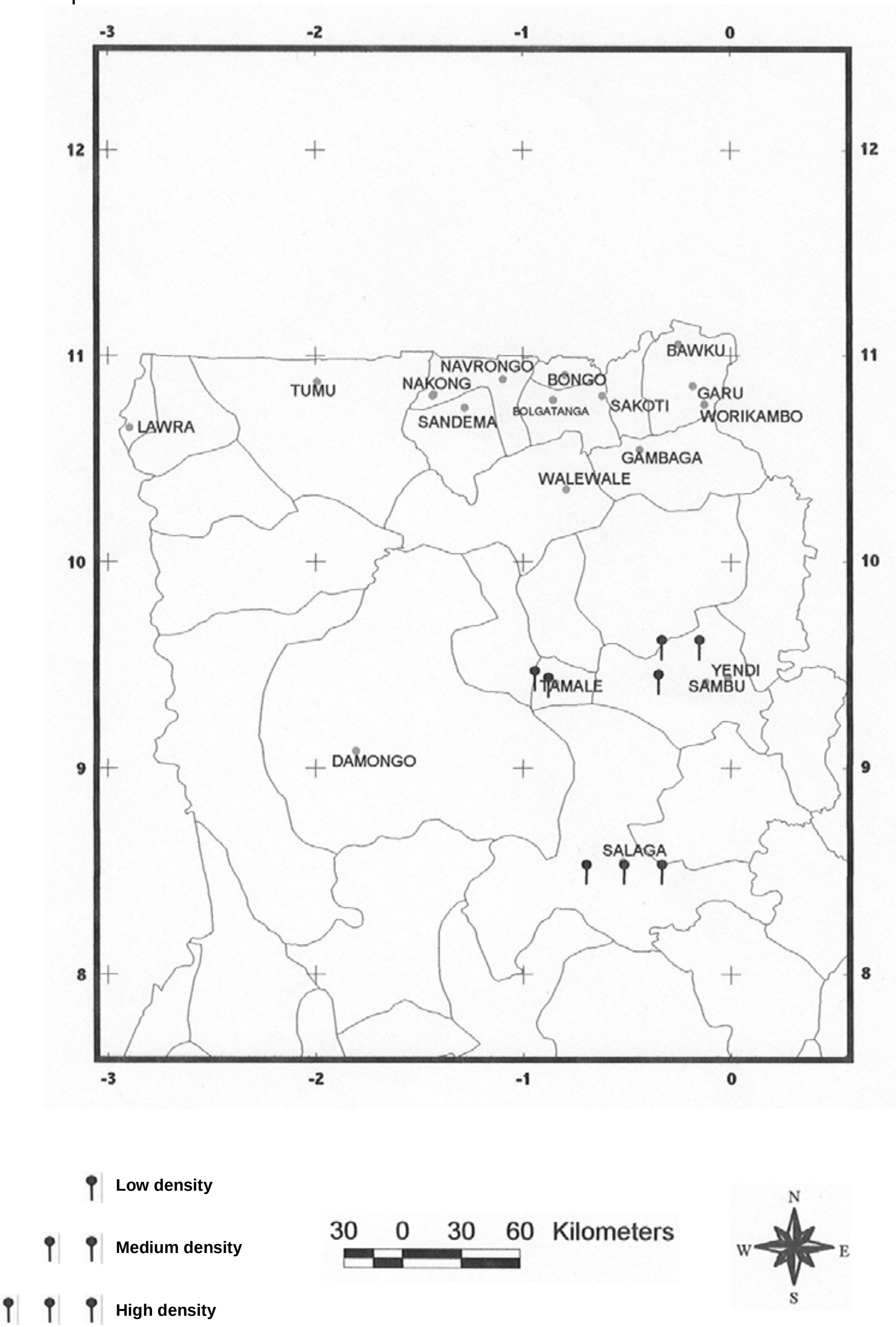

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Figure 1e. Distribution map of *O. americanum* in northern Ghana

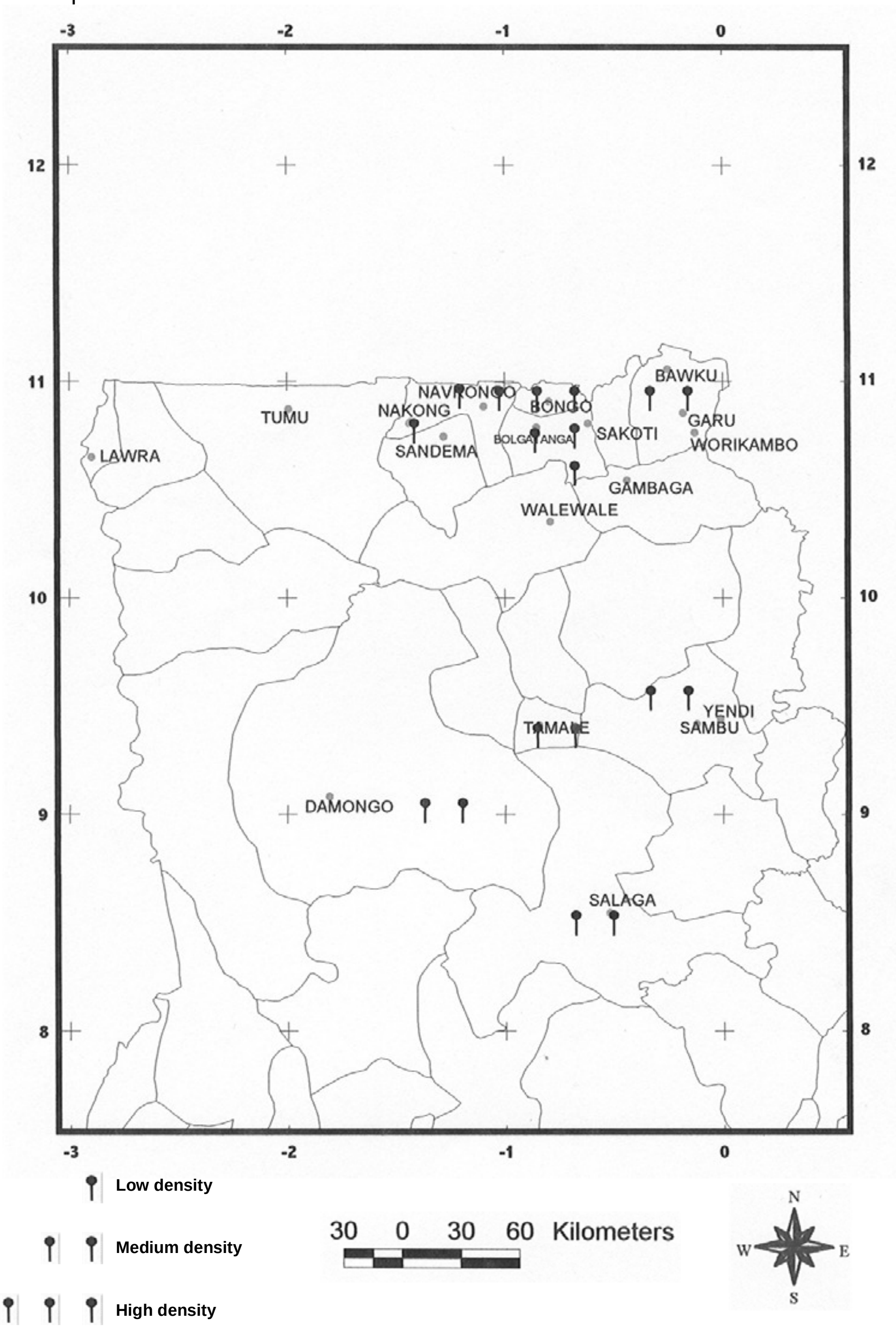

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Figure 1f. Distribution map of *S. longepedunculata* in northern Ghana

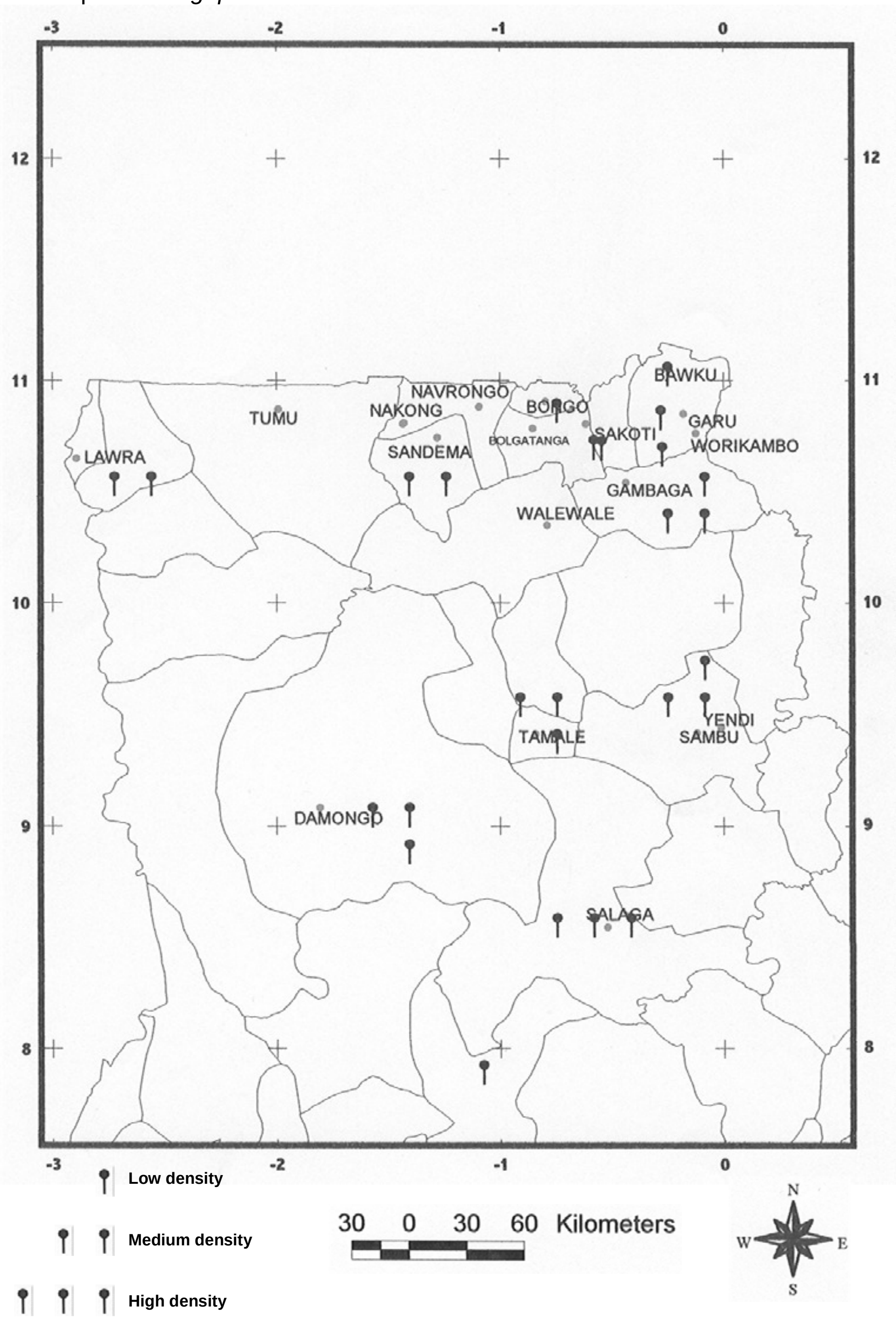

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Figure 1g. Distribution map of *S. nodiflora* in northern Ghana

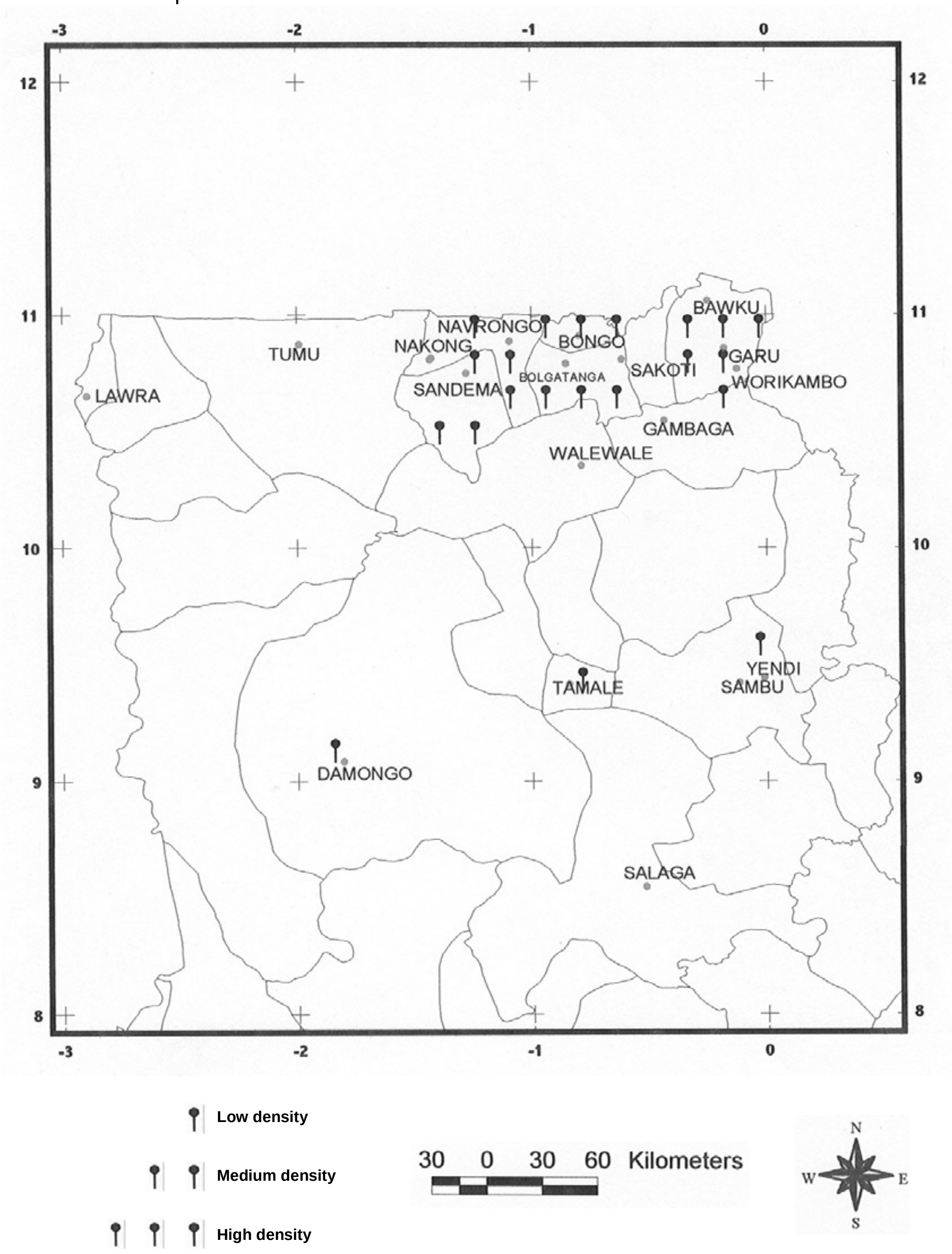

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Figure 1h. Distribution map of *C. schoenanthus* in northern Ghana

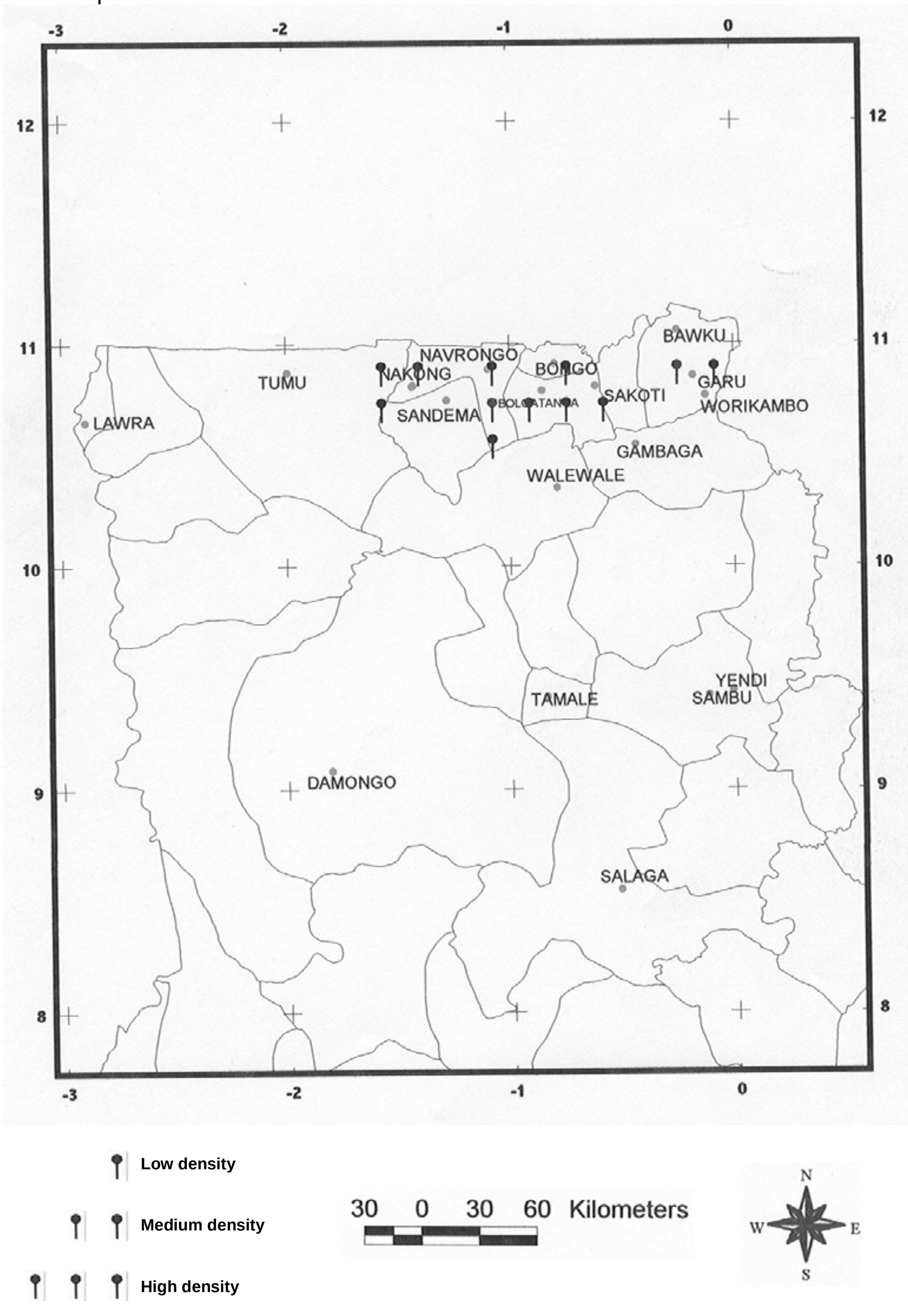

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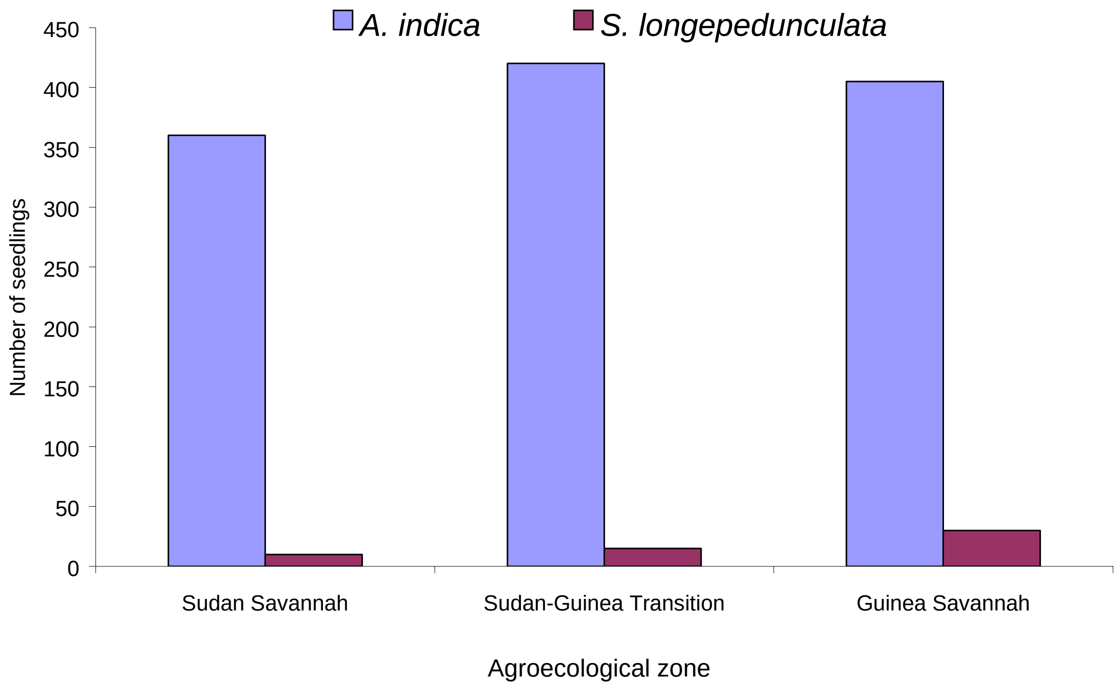
Figure 2. Natural regeneration in northern Ghana of a) tree species and b) perennial shrubs

a)



b)

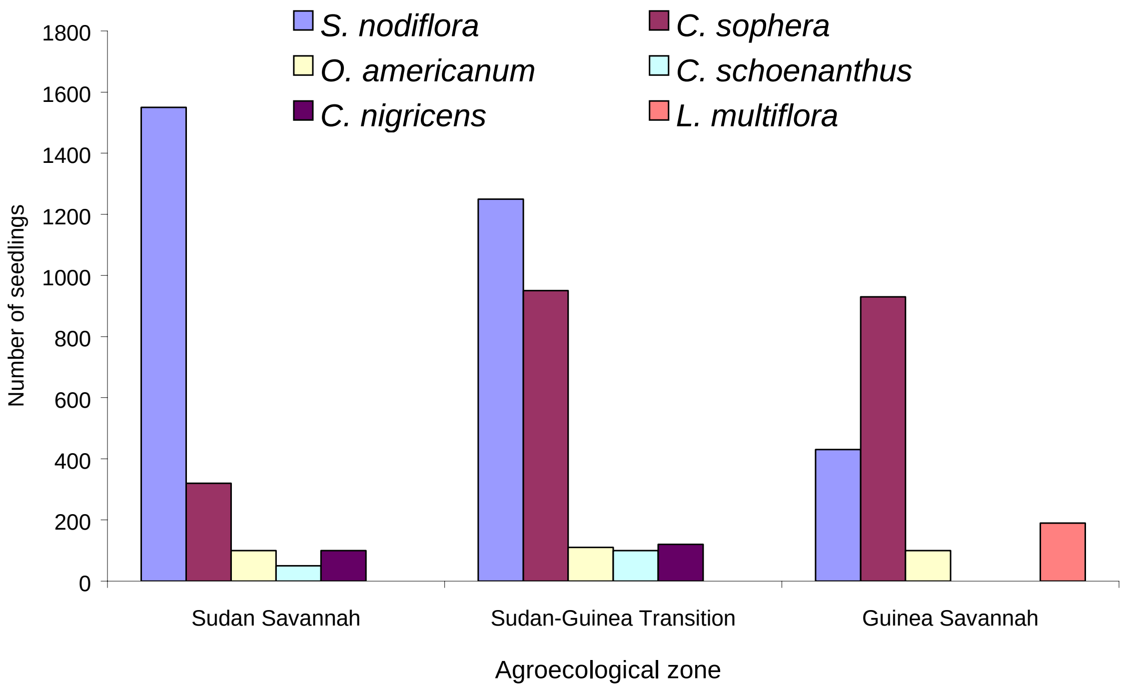


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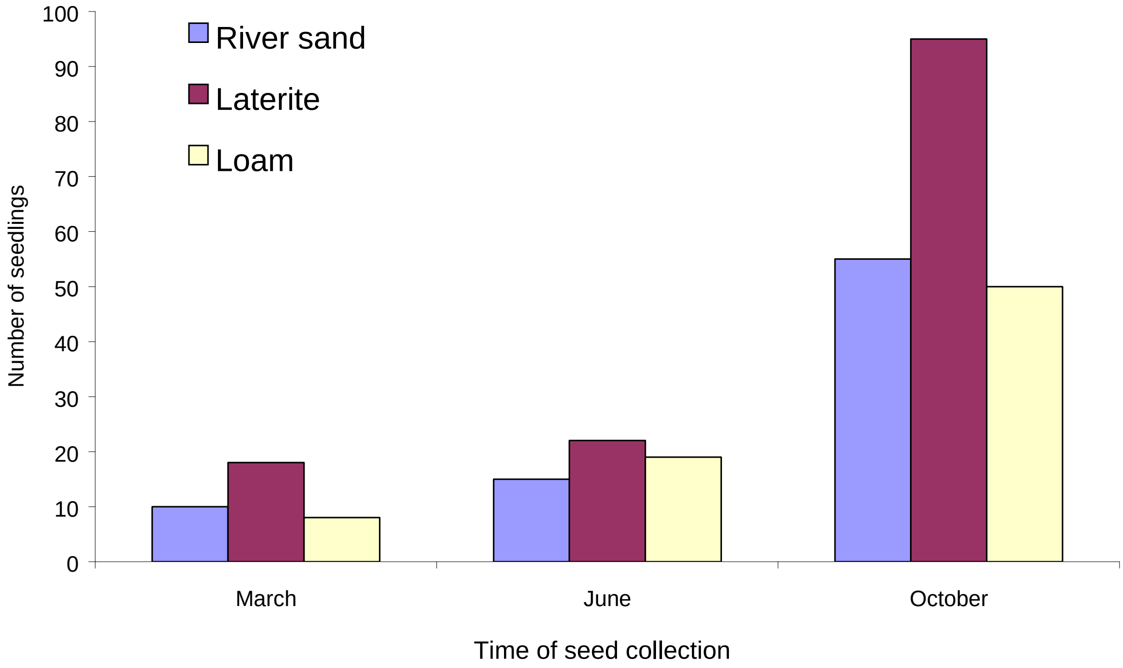
Figure 3. Germination potential of *C. sophera* seeds collected at different times of year in different soil media.



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Involving stakeholders in promoting indigenous knowledge in Ghana

In order to involve a wide cross-section of stakeholders who could be involved in the future development and promotion of research outputs from the project, a workshop was organised by the Ghanaian Ministry of Food and Agriculture (MoFA) and the Natural Resources Institute, UK (NRI). An entire day of discussions on the **Indigenous Use of Plants in Ghanaian Society** took place in Tamale in the Northern Region of Ghana, January 17th, 2002. Attendance was registered at over 80 participants including representatives from the Savannah Agricultural Research Institute (SARI), the University of Development Studies (UDS), the Environmental Protection Agency, 7 NGOs (ACDEP, CAPSARD, CARD, CARE, Technoserve, TRAX, World Vision), 30 MoFA extensionists and district officers, 21 farmer representatives from different villages in the Northern and Upper East Regions, and members of the local and national press.

The workshop objectives were to: 1) Make available the technical information on the activities undertaken by the project, 2) Increase farmer usage of plant materials for storage, 3) Determine what is still to be done to promote plant protection in the future - the pathways. Presentations were given by NRI and the Forestry Research Institute related to the results of CPHP-funded research on using botanicals for stored product protection. The organisers also invited speakers from SARI and UDS to discuss their work on the crop protection and medicinal/veterinary uses of plants, respectively. Discussions were very lively and often passionate, as everyone had personal experiences to share on the use of plants materials. Participants were strongly supportive of the CPHP research activities which confirmed many of their traditional practices as having sound and beneficial effects upon stored product protection. Participants were then divided into three groups with each group containing a cross-section of stakeholders, each to discuss and report on one of the following:

Should we encourage farmers to use botanicals? If so, then how do we do this?
The group response was, yes, we should encourage farmers to use botanicals. The reasons given were they are: readily available, effective (particularly for small-scale farmers), environmentally friendly, less costly compared to chemical pesticides, less toxic to humans, safer to handle, have many uses such as medicines or spices. Reassuringly, the methods described by the group for doing this were the same as those that had been used during the project involving PRAs, research, training, farmer participatory trials and dissemination of knowledge on optimal protocols for usage.

What methods can we use to bring knowledge about botanicals to farmers and their communities?
The group first listed the different methods which farmers used to receive messages which included: extension agents, personal communication, market centres, funerals and festivals, radio, meetings to transfer innovations, workshops and seminars, demonstrations, field days, exchange visits to other farmer fields, farmer field schools, farmer experimentation, NGOs, leaflets, PRAs. The group then asked the farmer members of the group to prioritise the methods which they prefer to receive messages, and these were: 1) radio, 2) extension agents, 3) community meetings. Further questioning revealed that farmers prefer to initially hear about innovations via the radio and then ask their extension agents about it. Farmers would prefer that extension agents stay in the community all the time to enhance direct contact.

Should we promote those plant species in places where they are not currently used?
The group response was, yes, with additional comments that this was in recognition of Ghanaian indigenous knowledge, production at location makes it favourable for distribution, botanicals are less persistent in the environment compared to synthetics, and lowers expenditure in storing food crops.

In conclusion, the workshop confirmed that often farmers are most-comfortable in accessing information from traditional sources. Innovative pathways of uptake promotion may, therefore, meet resistance by farmers who are well-adapted to risk reduction strategies and would perhaps view innovative pathways as too risky to get involved in by the majority of farmers. Because of the variety of different plants available that can be used for stored product protection and their different methods of application, increasing the usage of botanicals is best done using traditional instruction methods such as through extensionists, farmer field schools and on-farm trials. Using botanicals can not be simply explained in a leaflet or over the radio; however, farmers can use such sources of information to initially learn about botanicals, tapping into more detailed sources of information available through their extension agent. There is a certain pride among farmers to use local knowledge, and this can be

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exploited by NGOs and government agencies by using the knowledge about botanical pesticides generated in one community to transfer to other communities. The institutional promotion of pesticidal plants can help short circuit the communication barriers found among geographically isolated ethnic communities within Ghana.

Figure 1 Small groups of workshop participants discussed key issues affecting the use and promotion of botanicals in Ghanaian society. A mixture of farmers, extensionists, NGOs and national researchers all agreed that botanicals continue play an important role in stored product protection for small-scale farmers in Ghana, and research outputs should be promoted to increase their usage.

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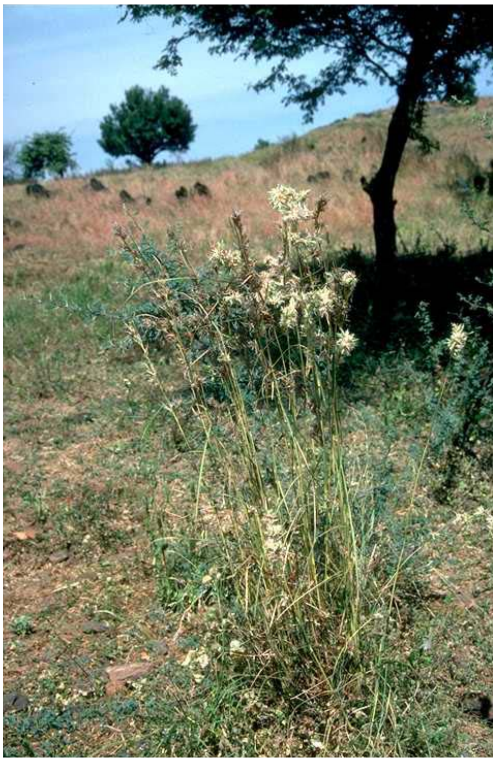
Photographs of the plant species recommended for further promotion for stored product protection

Cymbopogon schoenanthus



Chamaecrista nigricens

Synedrella nodiflora

Securidaca longepedunculata

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Ocimum americanum

Lippia multiflora

Azadirachta indica

Cassia sophera

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Outputs

This project had four outputs:
1) Low-cost alternatives to synthetic pesticides for controlling storage pests at farm-level investigated.
2) Data on the reliability and efficacy of botanical materials researched with respect to where and when the plant material was collected
3) Mechanisms by which botanicals prevent insect infestation of stored grain understood
4) Reliance upon synthetic pesticides reduced in order to improve human and environmental health

The project outputs have been achieved as described in the activities section. The goal of the project to "improve the efficiency of commodity storage and management, processing, marketing and credit systems" was addressed by increasing and improving the management options available to small-scale farmers who store their commodities on-farm. This will help farmers to minimise their post-harvest losses, storing their commodities for a longer period of time, and thereby selling their grain later in the season to obtain a higher price. Subsistence farmers can also be assured of preserving the quantity of grain for home consumption without resorting to the use of relatively expensive commercial synthetics. Because synthetic pesticides can be adulterated or misused, the project has provided improved indigenous methods which are more easily understood while reducing risks to human and environmental health.

Contribution of Outputs

The use of botanicals for post-harvest protection is patchy, with some regions/ethnic groups in Ghana having a higher utilisation than others. There is, therefore, great scope to promote the usage of botanicals in areas where the plants grow but are not widely used. Even in areas where usage is higher, farmers can benefit from the project outputs by improving the ways in which they use botanicals. Knowledge on which plants and application methods work best can be applied through existing extension channels, rural development programmes, farmer field schools and community action groups. Issues which could be important during the promotional phase are potentially unacceptably high plant residue levels on treated commodities, modified harvesting or propagation of plant species which are rare in the environment, development of small enterprises selling propagated material, biodiversity conservation, farmer and market acceptability.

List of publications currently produced from the project

OGENDO, J.O., BELMAIN, S.R., DENG, A.L. and WALKER, D.J. (in press) Comparison of toxic and repellent effects of *Lantana camara* L. with *Tephrosia vogelii* Hook and a synthetic pesticide against *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) in stored maize grain. *Insect Science and its Application*. 0: 000-000. [peer-reviewed]

OGENDO, J.O., DENG, A.L., BELMAIN, S.R., WALKER, D.J. and MUSANDU, A.O. (in press) Effect of insecticidal plant materials, *Lantana camara* L. and *Tephrosia vogelii* Hook, on the quality parameters of stored maize grains. *Journal of Food Technology in Africa*. 0: 000-000. [peer-reviewed]

JAYASEKARA, T.K., STEVENSON, P.C., BELMAIN, S.R., FARMAN, D.I. and HALL, D.R. (2002) Identification of methyl salicylate as the principal volatile component in the methanol extract of roots of *Securidaca longepedunculata* Fers. *Journal of Mass Spectrometry*. 37: 577-580. [peer-reviewed]

BELMAIN, S.R., ANDAN, H.F. and ATARIGIYA, J. (2002) Workshop on indigenous use of plants in Ghanaian society, 17 January 2002, Tamale, Ghana.
This workshop was attended by 81 participants including 21 farmer representatives from villages in the Northern and Upper East Regions of Ghana and staff from 15 public and private institutions in Ghana [English with translations provided in Dagomba, Dagbani and other local languages as required]

GHANA TELEVISION (2002) National news broadcast of workshop on indigenous use of plants in Ghanaian society held in Tamale on 17 January 2002. 10 minutes.

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BELMAIN, S.R. and STEVENSON, P.C. (2001) Ethnobotanicals in Ghana: reviving and modernising age-old farmer practice. *Pesticide Outlook*. 6: 233-238. [peer-reviewed]

BELMAIN, S.R. (2001) Using plant materials to control insects during storage. Leaflet distributed to 100 stakeholders during workshop in Ghana. 100 copies. [leaflet]

BELMAIN, S.R., NEAL, G E., RAY, D.E. and GOLOB, P. (2001) Insecticidal and vertebrate toxicity associated with ethnobotanicals used as post-harvest protectants in Ghana. *Food and Chemical Toxicology*. 39(3): 287-291. [peer-reviewed]

BELMAIN, S.R. (2000) Current developments with ethnobotanical insecticides from Ghana: reviving and modernising age-old practice. *21st International Congress of Entomology*. Brazil, August 2000. [30 minute key-note symposia address and abstract]

BELMAIN, S.R. (2000) Plants as protectants against storage pests. *New Agriculturist*. Website <http://www.new-agri.co.uk/00-3/focuson/focuson4.html> [web article]

BELMAIN, S.R., GOLOB, P., ANDAN, H.F. and COBBINAH, J.R. (1999) Ethnobotanicals - Future prospects as post-harvest insecticides. *Agro Food Industry Hi-tech*. 10(5): 34-36. [peer-reviewed]

COBBINAH, J.R., MOSS, C., GOLOB, P. and BELMAIN, S.R. (1999) Conducting ethnobotanical surveys: an example from Ghana on the plants used for the protection of stored cereals and pulses. *NRI Bulletin #77*. 15 pp. NRI: Chatham, UK. [bulletin]

GOLOB, P., MOSS, C. DALES, M., FIDGEN, A., EVANS, J. and GUDRUPS, I. (1999) The use of spices and medicinals as bioactive protectants for grains. *FAO Agricultural Services Bulletin #137*. 239 pp. FAO: Rome. [bulletin]

BELMAIN, S.R., GOLOB, P., ANDAN, H.F., ATARIGIYA, J. and CHARE, F.A. (1999) Toxicity and repellency of ethnobotanicals used in Ghana as post-harvest protectants. In*: 14th International Plant Protection Congress*, *Jerusalem*, *July 1999*. [15 minute oral presentation, abstract and poster]

BELMAIN, S.R., ANDAN, H.F. and ATARIGIYA, J. (1999, 2000, 2001) On-farm participatory research trials with 200 farmers/year providing education and promotion about traditional storage methods. [direct dissemination and training]

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