International Journal of Tropical Insect Science Vol. 35, No. 4, pp. 172–184, 2015 doi:10.1017/S174275841500017X

Contact and fumigant toxicity of five pesticidal plants against *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) in stored cowpea (*Vigna unguiculata*)

Prisila A. Mkenda[1], Philip C. Stevenson[2,3], Patrick Ndakidemi[4]*, Dudley I. Farman[2] and Steven R. Belmain[2]

[1]Department of Biological Sciences, Sokoine University of Agriculture, Morogoro, Tanzania; [2]Natural Resources Institute, University of Greenwich, Chatham Maritime, Kent ME4 4TB, United Kingdom; [3]Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AB, United Kingdom; [4]School of Life Sciences and Engineering, The Nelson Mandela African Institution of Science and Technology, Arusha, Tanzania

(Accepted 26 May 2015; First published online 8 September 2015)

Abstract. Insecticidal activities of five pesticidal plant species, *Tephrosia vogelii*, *Dysphania* (Syn: *Chenopodium*) *ambrosioides*, *Lippia javanica*, *Tithonia diversifolia* and *Vernonia amygdalina*, which have been reported to control storage pests, were evaluated as leaf powders against *Callosobruchus maculatus* (Fabricius 1775) in stored cowpea. Their efficacy was compared with the commercial pesticide Actellic dust (pirimiphos-methyl) at the recommended concentration (50 g/90 kg), and with untreated cowpea seeds as a negative control. The plant powders were applied at concentrations of 0.01, 0.1, 1 and 3 g/10 g of cowpea seeds in 250 ml plastic containers (to measure contact toxicity), or 0.005, 0.05, 0.5 and 5 g tied in small muslin cloth bags and hung in 500 ml plastic bottles containing 10 g of cowpea seeds (to measure fumigant toxicity). Mortality of adults, oviposition deterrence, adult emergence, and percent seed damage were recorded. Complete protection of seeds and inhibition of adult emergence were achieved in Actellic dust-treated seeds; contact toxicity using leaf powders of *T. vogelii* at all concentrations, *D. ambrosioides* at concentrations of 0.1, 1 and 3 g and *L. javanica* at concentrations of 1 and 3 g; and fumigant toxicity using *D. ambrosioides* at concentrations of 0.5 and 5 g and *L. javanica* at a concentration of 5 g. Head space analysis of *D. ambrosioides* and *L. javanica* identified ascaridole and camphor, respectively, as components that could be responsible for the bioactivity of these plant species. These plants may, therefore, serve as effective but less harmful biopesticide alternatives to Actellic. Conversely, *V. amygdalina* and *T. diversifolia* were not effective, indicating that they should not be promoted for controlling bruchids in cowpea.

Key words: *Callosobruchus maculatus*, cowpea, contact and fumigant toxicity, pesticidal plants, post-harvest pest management, stored product pests

*E-mail: patrick.ndakidemi@nm-aist.ac.tz

Introduction

Cowpea, *Vigna unguiculata* (L.) Walp., is a widely cultivated legume that belongs to the family Leguminosae, subfamily Papilionaceae, Tribe Phaseoleae (Ileke *et al.*, 2013). In Tanzania, cowpea is grown mostly in semi-arid areas, including Shinyanga, Mtwara and Kigoma, due to its drought tolerance (Myaka *et al.*, 2002). It is also efficient in fixing nitrogen and enriching the soil (Brisibe *et al.*, 2011). Cowpea has nutritional benefits because it is rich in protein, making it suitable for many people who cannot afford to buy other protein sources such as meat and fish (Oparaeke *et al.*, 2006; Brisibe *et al.*, 2011). It is mostly cultivated as a grain legume, and is also widely used as a leafy vegetable. Cowpea production in Tanzania is relatively low and does not meet local demand, partly due to high insect infestation at both pre- and post-harvest stages (Swella and Mushobozy, 2007). The most important insect pest in stored cowpea is the bruchid, *Callosobruchus maculatus* (Fabricius 1775) (Coleoptera: Chrysomelidae) (Swella and Mushobozy, 2007). In Africa, 30–80% of cowpea production, valued at over US$ 300 million, is either lost or suffers damage annually as a result of bruchid infestation (Brisibe *et al.*, 2011). Losses attributed to *C. maculatus* in stored cowpea result in both quantitative and qualitative reduction, manifested by food and nutrition insecurity, and low incomes for commercially oriented farmers (Ileke and Olotuah, 2012).

Management of *C. maculatus* has been dominated by chemical control using fumigants and synthetic insecticides (Lale and Kabeh, 2004; Odeyemi *et al.*, 2006). However, the use of synthetic insecticides in crop protection programmes around the world has resulted in disruption of the environment, pest resurgences, development of resistance to pesticides, lethal effects to non-target organisms in agroecosystems, toxic residues in food and water bodies, as well as direct toxicity to users (Lale, 2001; Schafer and Kegley, 2002; Prakash *et al.*, 2008). This calls for an urgent need to develop alternative ecologically safer, economical, readily affordable and user-friendly pest control techniques, such as using locally available plants with insecticidal properties (Adedire *et al.*, 2011; Arannilewa *et al.*, 2006). In this study, the contact and fumigant toxicity of leaf powders derived from five known pesticidal plants, *Tephrosia vogelii* Hook. f., *Dysphania ambrosioides* (L.) Mosyakin & Clemants (Syn.: *Chenopodium ambrosioides* L.), *Lippia javanica* (Burm. f.), *Tithonia diversifolia* (Hemsl.) A. Gray and *Vernonia amygdalina* Delile, was evaluated.

Tephrosia vogelii is a deep-rooted, fast-growing leguminous, indigenous shrub found in the wild and cultivated in many parts of Africa as an inter-crop with maize or cover crop, to improve soil fertility through nitrogen fixation and to mine minerals from lower soil levels (Kamanula *et al.*, 2010; Grzywacz *et al.*, 2014). Two chemotypes of *T. vogelii* have been identified: chemotype 1, which contains rotenoids (deguelin, rotenone, sarcolobine, tephrosin and α-toxicarol) that are required for pest control efficacy, and chemotype 2 in which rotenoids are absent (Stevenson *et al.*, 2012). *Dysphania ambrosioides* originates from Mexico where it is used as a culinary spice and is now found across the Tropics. *Dysphania ambrosioides* has been used traditionally to preserve post-harvest grains from weevil attack, and for medicinal purposes (to treat intestinal worm infections, nervous infections, cough, pulmonary obstruction, typhoid, influenza, and skin and kidney infections) (Jamali *et al.*, 2006; Lans *et al.*, 2006; Nascimento *et al.*, 2006; Dembitsky *et al.*, 2008). The main bioactive constituents are ascaridole and various terpenes (Jardim *et al.*, 2008). *Lippia javanica*, sometimes called fever tea bush, is a woody shrub found across south-eastern Africa and is regularly used as a traditional medicine to treat minor ailments and microbial infections such as coughs, colds, fungal infections, respiratory diseases and skin infections (Samie *et al.*, 2010). The main active ingredients are quite variable, but contain linalool, carvone and myrcene (Viljoen *et al.*, 2004). *Tithonia diversifolia* is a woody herb or succulent shrub native to the New World, but now found across the Tropics. *Tithonia diversifolia* is often called the Mexican sunflower, and has been used traditionally as a medicine suitable for treating constipation, stomach pains, indigestion, sore throat, liver pains and malaria (García and Delgado, 2006; Obafemi *et al.*, 2006). It contains sesquiterpene lactones and diterpenoids that are known to have insecticidal properties (Adedire & Akkineye, 2004). *Vernonia amygdalina* has been traditionally used by nursing mothers to improve lactation, and has proven useful in diabetic patients (Anibijuwon *et al.*, 2012). It is a well-known African medicinal plant producing the anticancer agents vernodalin, vernolide and vernomygdine (Khalafalla *et al.*, 2009). In this study, these plants were tested against *C. maculatus* in cowpea and compared with pirimiphos-methyl (Actellic dust, 2%).

Materials and methods

Insect culture

The bruchids used to establish a laboratory colony of *C. maculatus* were obtained from infested cowpea seeds collected from a local market in

Morogoro, Tanzania. The bruchids were cultured at Sokoine University of Agriculture laboratory to obtain the parent stock of *C. maculatus*. These were then reared in the laboratory of Life Sciences and Engineering Department at the Nelson Mandela African Institution of Science and Technology (NM-AIST), Arusha, Tanzania, using disinfested cowpea seeds collected from farmers at Rombo District, Tanzania. The cowpea seeds were newly harvested, and free of insecticides. They were cleaned by washing with water, and then dried under sunlight and sorted to eliminate those already damaged by *C. maculatus*, before being disinfested by freezing at −20 °C for 48 h, with subsequent heating in an oven at 70 °C for 24 h. The disinfested cowpea seeds were air dried at room temperature to prevent mouldiness before the introduction of the insects, and were then stored in plastic containers with airtight lids.

Collection of plant materials

A botanist from the Forest Training Institute – Olmotonyi, Arusha Tanzania, carried out the identification of the pesticidal plants. Voucher specimens are deposited at NM-AIST. The plant species used in these trials were selected because of their: (a) traditional use by local farming communities, (b) high local abundance in northern Tanzania (where this research was conducted) and (c) information from published research (Isman, 2006).

Fresh leaves of the plants were collected from different locations around Arusha and Kilimanjaro, northern Tanzania. All *T. vogelii* collected for this study were confirmed to be chemotype 1. The leaves were rinsed in clean water to remove sand and other impurities, and then dried in the shade to avoid direct exposure to sunlight and possible decomposition of the bioactive compounds. They were ground into a fine powder using an electric mill, and the powders sieved to pass through 1 mm^2 mesh (Ileke and Olotuah, 2012). The powders were packed in plastic containers with airtight lids, and stored in the dark at ambient conditions of 25 ± 3 °C and 75 ± 5% RH.

Contact and fumigant toxicity of leaf powders to Callosobruchus maculatus

To test for contact toxicity, fine powders of *T. vogelii*, *D. ambrosioides*, *L. javanica*, *T. diversifolia* and *V. amygdalina* were admixed with cowpea seeds, disinfested as above, at the rates of 0.01, 0.1, 1 and 3 g/10 g of cowpea seeds in 250 ml plastic containers. A positive control of pirimiphos-methyl (Actellic 2% dust at a concentration of 50 g/90 kg), and an untreated negative control were included. The dosages were set at different orders of magnitude from low (0.1%) to high (30%), so as to obtain the minimum effective dose. For fumigant toxicity, 10 g of cowpea seeds were placed into 500 ml transparent plastic bottles with perforated lids to allow air circulation, but restrict entry or exit of bruchids. *Dysphania ambrosioides* and *L. javanica* leaf powders were the only plant species used in this experiment, as only their bioactive constituents are known to be volatile. Fine leaf powders of *D. ambrosioides* and *L. javanica* (at doses of 5, 0.5, 0.05 and 0.005 g) were put into small bags made using muslin cloth, then tied with a piece of thread and hung inside the 500 ml plastic bottle containing the cowpea seeds. As with the above toxicity test, this fourfold dose magnitude was used to determine the minimum effective dose required. For both toxicity and fumigant trials, five male and five female adults of *C. maculatus* (2 to 3 days old), were introduced into each container. Untreated cowpea seeds (negative control) were similarly infested. Six replicates of the treatments (including controls) were laid out in a complete randomized block design. Insect mortality was assessed every 24 h for 4 days (Ileke and Olotuah, 2012; Ileke *et al.*, 2013). For fumigant toxicity, the assessment was done without opening the containers.

On the 4th day, all insects, both dead and alive, were removed from each container and the eggs laid on seeds counted. Insects were determined to be dead when they failed to move any part of their body in response to gentle probing of the exposed abdomen with forceps. The experiment was kept for another 30 days to allow for the emergence of the first filial (F_1) generation. The number of days to first bruchid emergence, and the number of adults that emerged, were counted. The adults were removed daily for 14 days after the first emergence was observed (Denloye *et al.*, 2010). The percentage adult emergence (progeny development) was calculated using the formula:

$$\text{Percent progeny development} = \frac{\text{No. of adults emerged}}{\text{No. of eggs laid}} \times 100.$$

The number of undamaged and damaged seeds (perforated by bruchids), and the number of holes per seed were also recorded. Damage assessment was done through counting the total number of holes per cowpea seed. The number of holes per sub-sample of 10 randomly selected seeds, and the number of seeds with holes were recorded. Percentage damage (PD) was calculated according to Fatope *et al.* (1995) as:

$$PD = \frac{\text{Total number of sampled grains perforated}}{\text{Total number of sampled grains}} \times 100.$$

The Beetle Perforation Index (BPI) (Fatope *et al.*, 1995) was then calculated as:

$$BPI = \frac{\text{Percent treated cowpea grains perforated}}{\text{Percent untreated control cowpea grains perforated}} \times 100.$$

A BPI value exceeding 50% is regarded as enhancement of infestation by the beetle, or a negative ability of the plant material or insecticides tested (Ojiako and Adesiyun, 2013). Thus the Percent Protectant Ability (PPA) = 100 − BPI.

Collection of leaf volatiles and chemical analysis

Dry powdered leaf samples (5 g) were held in a 250-ml glass flask under laboratory conditions at approximately 25 °C, which was closed with aluminium foil. Volatiles were collected using a Solid-phase microextraction (SPME) fibre, coated with polydimethylsiloxane/divinylbenzene (Supelco, Sigma-Adrich, Poole, UK). The fibre was inserted through the foil and exposed to the headspace for 5 min. The fibres were desorbed using an Agilent 6890 gas chromatograph coupled to an Agilent 5973 mass spectrometer, and with a DB-5 fused silica capillary column (30 m length, 0.25 mm diameter and 0.25 µm film thickness) (Agilent Technologies, Wokingham, UK). Desorption was splitless, with helium at a constant flow rate of 1 ml/min as a carrier gas. The column temperature was held at 60 °C for 2 min then programmed to 240 °C at 6 °C/min. The ion source was held at 150 °C and the transfer line at 250 °C.

Data analysis

The bioassay data were subject to one-way analysis of variance (ANOVA) using the programme STATISTICA. Statistical differences between the means were separated using the least significant difference (LSD) test. Significance was set at the level of $P = 0.05$. Data on chemical analysis were analysed using ChemStation (Agilent).

Table 1. Mortality of adult *Callosobruchus maculatus* on cowpea seeds treated with different powders as contact insecticides

		[+]Mean % mortality + SE for 4 days			
Treatment	g/10 g cowpea seeds	Day 1	Day 2	Day 3	Day 4
Control	0	0.0 ± 0.00a	0.0 ± 0.00a	0.0 ± 0.00a	0.0 ± 0.00a
Actellic dust	0.01	100.0 ± 0.00g	100.0 ± 0.00g	100.0 ± 0.00j	100.0 ± 0.00m
Dysphania ambrosioides	0.01	5.0 ± 2.24ab	18.3 ± 3.07b	31.7 ± 3.07f	46.7 ± 2.11f
	0.1	55.0 ± 2.24f	86.7 ± 3.33f	100.0 ± 0.00j	100.0 ± 0.00m
	1	100.0 ± 0.00g	100.0 ± 0.00g	100.0 ± 0.00j	100.0 ± 0.00m
	3	100.0 ± 0.00g	100.0 ± 0.00g	100.0 ± 0.00j	100.0 ± 0.00m
Tephrosia vogelii	0.01	0.0 ± 0.00a	15.0 ± 3.42b	35.0 ± 2.24f	43.3 ± 2.11f
	0.1	15.0 ± 2.24c	40.0 ± 4.47d	58.3 ± 3.07h	71.7 ± 3.07j
	1	36.7 ± 2.11e	65.0 ± 2.24e	81.7 ± 1.67i	90.0 ± 0.00l
	3	36.7 ± 2.11e	63.3 ± 2.11e	83.3 ± 2.11i	93.3 ± 2.11l
Lippia javanica	0.01	0.0 ± 0.00a	1.7 ± 1.67a	10.0 ± 2.58c	16.7 ± 2.11c
	0.1	0.0 ± 0.00a	5.0 ± 2.24a	23.3 ± 2.11e	43.3 ± 2.11f
	1	25.0 ± 4.28d	40.0 ± 5.16d	56.7 ± 4.22h	65.0 ± 3.42hi
	3	38.3 ± 3.07e	61.7 ± 3.07e	80.0 ± 2.58i	81.7 ± 4.01k
Vernonia amygdalina	0.01	0.0 ± 0.00a	1.7 ± 1.67a	6.7 ± 2.11bc	8.3 ± 1.67b
	0.1	0.0 ± 0.00a	5.0 ± 2.24a	16.7 ± 3.33d	23.3 ± 2.11d
	1	23.3 ± 2.11d	38.3 ± 3.07cd	55.0 ± 2.24h	63.3 ± 2.11h
	3	28.3 ± 3.07d	45.0 ± 3.42d	58.3 ± 3.073h	70.0 ± 2.58ij
Tithonia diversifolia	0.01	0.0 ± 0.00a	0.0 ± 0.00a	3.3 ± 2.11ab	8.3 ± 1.67b
	0.1	0.0 ± 0.00a	5.0 ± 2.24a	10.0 ± 2.58c	20.0 ± 2.58cd
	1	10.0 ± 0.00bc	21.7 ± 1.67b	31.7 ± 3.073f	36.7 ± 3.33e
	3	15.0 ± 2.24c	31.7 ± 3.073c	46.7 ± 2.11g	55.0 ± 2.24g
One-way ANOVA (*F*-statistics)		356.312***	178.919***	221.310***	242.31***

*** Significant at the level of $P \leq 0.001$. Means in a column followed by different letters are significantly different from each other at the level of $P = 0.05$ using Fisher's least significant difference (LSD) test.
[+] Each value is expressed as mean ± standard error of six replicates.

Compounds were identified using the NIST Mass Spectral Database and by comparison to pure samples.

Results

Contact toxicity of leaf powders to Callosobruchus maculatus

Table 1 shows the mortality of *C. maculatus* 4 days after treatment with the leaf powders in comparison with Actellic dust, and the untreated control. The leaf powder of *D. ambrosioides* caused 100% mortality of adult *C. maculatus* at 10% concentration within 24 h, which was similar to the Actellic dust. Grains treated with 1 and 0.1% of *D. ambrosioides* caused 100% mortality by the third day and 46.7% mortality on the fourth day, respectively. The leaf powder of *T. vogelii* caused 43.3, 71.7, 90 and 93.3% mortality of *C. maculatus* at concentrations of 0.01 g/10 g, 0.1 g/10 g, 1 g/10 g and 3 g/10 g of cowpea seeds on the fourth day, respectively. The corresponding values for *L. javanica* and *V. amygdalina* by the fourth day at concentrations of 0.01, 0.1, 1.0 and 3.0 g/10 g of cowpea seeds were 16.7, 43.3, 65 and 81.7%, and 8.3, 23.3, 63.3 and 70%, respectively. The toxicity of the leaf powders of the various plants was, therefore, dosage dependent. However, the leaf powder of *T. diversifolia* caused a significant effect only at the highest dosage of 3 g/10 g of cowpea seeds causing 55% mortality on the fourth day, whereas at lower concentrations the mortality was below 50%.

Effect of leaf powders as contact toxicity on oviposition and progeny development of Callosobruchus maculatus

All the leaf powders of the target plants significantly reduced the number of eggs laid by *C. maculatus* compared with the untreated control (Table 2). Some of the plant leaf powders significantly suppressed progeny development. The leaf powder of *T. vogelii* completely inhibited the emergence of *C. maculatus* at all concentrations, similar to the Actellic dust, followed by *D. ambrosioides* that was able to inhibit adult emergence completely at concentrations of 0.1, 1 and 3 g/10 g of cowpea seeds. Complete inhibition of adult emergence was also observed in seeds treated with *L. javanica* at concentrations of 1 and 3 g/10 g of cowpea seeds. Oviposition and progeny development were high in untreated seeds, as well as in cowpea seeds treated with *T. diversifolia* and *V. amygdalina* at low concentrations (Table 2).

Table 2. Effects of leaf powders on oviposition and progeny development of *Callosobruchus maculatus* on cowpea seeds

Treatment	g/10 g cowpea seeds	+Mean no. of eggs laid	+Mean no. of adults emerged	+Mean % progeny development
Control	0	114.8 ± 2.01l	91.0 ± 1.46j	79.3 ± 0.56g
Actellic dust	0.01	3.7 ± 0.33a	0.0 ± 0.00a	0.0 ± 0.00a
Dysphania ambrosioides	0.01	35.7 ± 1.05e	24.2 ± 0.48b	67.9 ± 0.89d
	0.1	11.3 ± 0.71bc	0.0 ± 0.00a	0.0 ± 0.00a
	1	5.5 ± 0.56ab	0.0 ± 0.00a	0.0 ± 0.00a
	3	4.2 ± 0.31a	0.0 ± 0.00a	0.0 ± 0.00a
Tephrosia vogelii	0.01	38.0 ± 1.10e	0.0 ± 0.00a	0.0 ± 0.00a
	0.1	18.7 ± 1.38d	0.0 ± 0.00a	0.0 ± 0.00a
	1	5.8 ± 0.31ab	0.0 ± 0.00a	0.0 ± 0.00a
	3	4.5 ± 0.22a	0.0 ± 0.00a	0.0 ± 0.00a
Lippia javanica	0.01	101.7 ± 2.30ij	55.5 ± 0.99f	54.6 ± 0.54b
	0.1	67.3 ± 1.28g	36.7 ± 0.49d	54.9 ± 0.35b
	1	15.7 ± 1.23cd	0.0 ± 0.00a	0.0 ± 0.00a
	3	7.8 ± 0.60ab	0.0 ± 0.00a	0.0 ± 0.00a
Vernonia amygdalina	0.01	108.3 ± 3.30k	76.2 ± 1.89h	70.4 ± 0.43e
	0.1	92.8 ± 3.65h	63.2 ± 2.01g	67.7 ± 0.67d
	1	73.0 ± 2.89g	47.5 ± 1.86e	65.1 ± 0.59c
	3	51.0 ± 6.01f	32.7 ± 3.48c	64.5 ± 1.06c
Tithonia diversifolia	0.01	112.0 ± 1.88kl	84.5 ± 1.88i	75.4 ± 0.78f
	0.1	105.8 ± 2.06jk	73.5 ± 1.20h	69.5 ± 0.28e
	1	98.3 ± 3.20hi	65.8 ± 2.50g	66.9 ± 0.46d
	3	70.8 ± 2.34g	46.5 ± 1.54e	64.9 ± 0.19c
One-way ANOVA (*F*-statistics)		366.46***	606.76***	5736.7***

*** Significant at the level of $P \leq 0.001$. Means in a column followed by different letters are significantly different from each other at the level of $P = 0.05$ using Fisher's least significant difference (LSD) test.
+ Each value is expressed as mean ± standard error of six replicates.

Effects of contact toxicity of leaf powders on the number of days to first bruchid emergence

There was no bruchid emergence in *T. vogelii*-treated seeds at all concentrations, *D. ambrosioides* at concentrations of 0.1, 1 and 3 g/10 g of cowpea seeds, and *L. javanica* at concentrations of 1 and 3 g/10 g of cowpea seeds, over the whole period of the experiment. Some of the leaf powders increased the number of days to first bruchid emergence. In *D. ambrosioides*-treated seeds at a concentration of 0.01 g/10 g of cowpea seeds, the number of days to first bruchid emergence was 34 days compared with 28 days in the untreated control. In *L. javanica* at concentrations of 0.01 and 0.1 g/10 g of cowpea seeds, emergence was in 33 days, and it took only 29 days to first bruchid emergence in *V. amygdalina*-treated seeds at concentrations of 1 and 3 g/10 g of cowpea seeds (Table 3). There was no significant difference in the number of days to first bruchid emergence in seeds treated with *V. amygdalina* at concentrations of 0.01 and 0.1 g/10 g of cowpea seeds, and *T. diversifolia* at all concentrations compared with the untreated control seeds.

Damage assessment of cowpea seeds

Treatment with the synthetic pesticide, *T. vogelii* at all concentrations, *D. ambrosioides* at concentrations of 0.1, 1 and 3 g, and *L. javanica* at concentrations of 1 and 3 g/10 g of cowpea seeds, protected the seeds completely against bruchid damage (Table 4a), and the BPI in these treatments was zero (Table 4b). Lower number of holes per seed, damaged seeds and weight loss were recorded in treated seeds at higher concentrations compared with untreated seeds (Table 4a and b). A BPI above 50% indicates negative protectant ability, and from the results of this study, the untreated control is set at a WPI of 100%. Other treatments that recorded negative protectant ability included *L. javanica* at a concentration of 0.01 g, *V. amygdalina* at concentrations of 0.01 and 0.1 g and *T. diversifolia* at concentrations of 0.01, 0.1 and 1 g (Table 4b).

Fumigant toxicity of leaf powders to Callosobruchus maculatus

The fumigant toxicity of leaf powders to *C. maculatus* is presented in Table 5. Adult mortality significantly increased with the increasing concentration and days of exposure. The mortality ranged from 6.7 to 100% in the treated cowpea seeds compared with no mortality in untreated seeds on the fourth day after treatment. The highest value of 100% mortality was recorded in the treatment with *D. ambrosioides* at a concentration of 5 g/10 g of cowpea seeds after one day, and at a concentration of 0.5 g/10 g of cowpea seeds after two days; and in the treatment with *L. javanica* at a concentration of 5 g/10 g of cowpea seeds after three days of treatment (Table 5).

Fumigant action of leaf powders on oviposition and progeny development of Callosobruchus maculatus

The leaf powder of both plants significantly reduced the number of eggs laid by *C. maculatus* compared with the control (Table 6). The number of adults that emerged decreased with increasing

Table 3. Effects of leaf powders as contact insecticides on the number of days to first bruchid emergence

Treatment	g/10 g cowpea seeds	+Mean no. of days to first bruchid emergence
Control	0	28.0 ± 0.00a
Vernonia amygdalina	0.01	28.0 ± 0.00a
	0.1	28.0 ± 0.00a
	1	29.0 ± 0.00b
	3	29.0 ± 0.00b
Tithonia diversifolia	0.01	28.0 ± 0.00a
	0.1	28.0 ± 0.00a
	1	28.0 ± 0.00a
	3	28.0 ± 0.00a
Lippia javanica	0.01	33.3 ± 0.21c
	0.1	33.3 ± 0.21c
Dysphania ambrosioides	0.01	34.0 ± 0.00d
One-way ANOVA (*F*-statistics)		808***

*** Significant at the level of $P \leq 0.001$. Means in a column followed by different letters are significantly different from each other at the level of $P = 0.05$ using Fisher's least significant difference (LSD) test.

+ Each value is expressed as mean ± standard error of six replicates.

Table 4a. Effects of leaf powders as contact insecticides: damage assessment of cowpea seeds for only damaged seeds

Treatment	g/10 g cowpea seeds	+Mean number of damaged seed	+Mean % seed damage (PD)
Control	0	7.8 ± 0.31f	78.3 ± 3.07f
Actellic	0.01	0.0 ± 0.00a	0.0 ± 0.00a
Dysphania ambrosioides	0.01	3.5 ± 0.22bc	35.0 ± 2.24bc
	0.1	0.0 ± 0.00a	0.0 ± 0.00a
	1	0.0 ± 0.00a	0.0 ± 0.00a
	3	0.0 ± 0.00a	0.0 ± 0.00a
Tephrosia vogelii	0.01	0.0 ± 0.00a	0.0 ± 0.00a
	0.1	0.0 ± 0.00a	0.0 ± 0.00a
	1	0.0 ± 0.00a	0.0 ± 0.00a
	3	0.0 ± 0.00a	0.0 ± 0.00a
Lippia javanica	0.01	3.7 ± 0.42bcd	36.7 ± 4.22bcd
	0.1	3.7 ± 0.21bcd	36.7 ± 2.11bcd
	1	0.0 ± 0.00a	0.0 ± 0.00a
	3	0.0 ± 0.00a	0.0 ± 0.00a
Vernonia amygdalina	0.01	4.7 ± 0.21^{e}	46.7 ± 2.11e
	0.1	4.2 ± 0.17de	41.7 ± 1.67de
	1	3.8 ± 0.31cd	38.3 ± 3.07cd
	3	3.2 ± 0.40b	31.7 ± 4.01b
Tithonia diversifolia	0.01	7.7 ± 0.21g	76.7 ± 2.11g
	0.1	7.5 ± 0.22g	75.0 ± 2.24g
	1	7.2 ± 0.31fg	71.7 ± 3.07fg
	3	4.2 ± 0.54de	41.7 ± 5.43de
One-way ANOVA (*F*-statistics)		151.042***	151.042***

*** Significant at the level of $P \leq 0.001$. Means in a column followed by different letters are significantly different from each other at the level of $P = 0.05$ using Fisher's least significant difference (LSD) test.
+ Each value is expressed as mean ± standard error of six replicates.

concentrations. Progeny development was significantly suppressed by the fumigant action of the leaf powders, where *D. ambrosioides* at concentrations of 0.5 and 5 g and *L. javanica* at a concentration of 5 g completely inhibited the emergence of *C. maculatus*. In the other treatments, the progeny development ranged between 52 and 56% with the highest percentage of progeny development in the untreated seeds recorded at 70.2% (Table 6).

Fumigant action of leaf powders on the number of days to first bruchid emergence

There was complete inhibition of bruchid emergence in seeds treated with *D. ambrosioides* at concentrations of 5 g and 0.5 g/10 g of cowpea seeds, as well as in seeds treated with *L. javanica* at concentrations of 5 g/10 g of cowpea seeds (Table 7). In the other treatments, the number of days to first bruchid emergence increased from 34.7 days in the untreated seeds to 37 days in *L. javanica* at a concentration of 0.005 g, 39 days in *D. ambrosioides* at a concentration of 0.005 g, and 40 days in *D. ambrosioides* at a concentration of 0.05 g, and *L. javanica* at concentrations of 0.05 g and 0.5 g (Table 7).

Damage assessment of cowpea seeds

Complete protection of seeds was achieved in seeds treated with *D. ambrosioides* at concentrations of 0.5 g and 5 g and *L. javanica* at a concentration of 5 g in 500 ml bottles containing 10 g of cowpea seeds with a beetle perforation index of zero. Low number of holes per seed, damaged seeds and low weight loss were observed in all other treated seeds compared with untreated seeds (Table 8a and b).

Chemical analysis of Lippia javanica *and* Dysphania ambrosioides

Chemical analysis allowed the identification of several components in the volatile odour trapped onto SPME columns and analysed by GC–MS. The major volatile component in the odour of *L. javanica* was camphor. This compound occurred along with other minor components (including camphene, α-pinene, eucalyptol, *Z* and *E* α-terpineol, linalool, cymene, thymol, 2-carene, caryophyllene and α-cubebene). The volatile chemistry of *D. ambrosioides* comprised several components of similar relative quantities, including ascaridole, cymene, 2-carene and diethyl phthalate.

Table 4b. Effects of leaf powders as contact insecticides: damage assessment of cowpea seeds for holes, weight loss and perforation index

Treatment	g/10 g cowpea seeds	+Mean no. of holes per seed	+Mean % weight loss	+Mean BPI
Control	0	1.5 ± 0.07f	8.3 ± 0.45i	100.0 ± 0.00h
Actellic	0.01	0.0 ± 0.00a	0.0 ± 0.00a	0.0 ± 0.00a
Dysphania ambrosioides	0.01	1.2 ± 0.08bcd	5.2 ± 0.13e	44.7 ± 2.85bc
	0.1	0.0 ± 0.00a	0.0 ± 0.00a	0.0 ± 0.00a
	1	0.0 ± 0.00a	0.0 ± 0.00a	0.0 ± 0.00a
	3	0.0 ± 0.00a	0.0 ± 0.00a	0.0 ± 0.00a
Tephrosia vogelii	0.01	0.0 ± 0.00a	0.0 ± 0.00a	0.0 ± 0.00a
	0.1	0.0 ± 0.00a	0.0 ± 0.00a	0.0 ± 0.00a
	1	0.0 ± 0.00a	0.0 ± 0.00a	0.0 ± 0.00a
	3	0.0 ± 0.00a	0.0 ± 0.00a	0.0 ± 0.00a
Lippia javanica	0.01	1.3 ± 0.11bcde	6.0 ± 0.04f	55.3 ± 2.69ef
	0.1	1.2 ± 0.08bcde	5.0 ± 0.07e	46.8 ± 2.69c
	1	0.0 ± 0.00a	0.0 ± 0.00a	0.0 ± 0.00a
	3	0.0 ± 0.00a	0.0 ± 0.00a	0.0 ± 0.00a
Vernonia amygdalina	0.01	1.3 ± 0.07bcde	7.0 ± 0.07g	59.6 ± 2.69f
	0.1	1.2 ± 0.08bcd	4.0 ± 0.03d	53.2 ± 2.13de
	1	1.2 ± 0.10bc	2.8 ± 0.38c	48.9 ± 3.92cd
	3	1.1 ± 0.06b	1.2 ± 0.32b	40.4 ± 5.12b
Tithonia diversifolia	0.01	1.5 ± 0.05f	7.6 ± 0.22h	97.9 ± 2.69h
	0.1	1.4 ± 0.07ef	6.1 ± 0.04f	95.7 ± 2.85h
	1	1.3 ± 0.07de	5.1 ± 0.42e	85.1 ± 2.69g
	3	1.3 ± 0.08cde	2.6 ± 0.53c	46.8 ± 2.69c
One-way ANOVA (*F*-statistics)		131.497***	201.692***	282.564***

*** Significant at the level of $P \leq 0.001$. Means in a column followed by different letters are significantly different from each other at the level of $P = 0.05$ using Fisher's least significant difference (LSD) test.
+ Each value is expressed as mean ± standard error of six replicates.

Discussion

On the basis of properties required for controlling insects that feed on stored grains, e.g. toxicity to adults, reduction of oviposition and inhibition of adult emergence, the leaf powders of *T. vogelii*, *D. ambrosioides* and *L. javanica* showed the greatest potential as stored grain legume protectants. Products derived from *Tephrosia* had been shown to be effective in the control of a number of insect pests including cucumber beetle, leafhoppers, squash bugs, flea beetles, harlequin bug, spittle bugs, thrips, scales, mites, and some fruit worms

Table 5. *Dysphania ambrosioides* and *Lippia javanica* as fumigant insecticides: mortality of adult *Callosobruchus maculatus*

Treatment	g/500 ml bottle[2]	Mean % mortality + SE for 4 days[1]			
		Day 1	Day 2	Day 3	Day 4
Control	0	0.0 ± 0.00a	0.0 ± 0.00a	0.0 ± 0.00b	0.0 ± 0.00b
Dysphania ambrosioides	0.005	0.0 ± 0.00a	0.0 ± 0.00a	6.7 ± 2.11d	13.3 ± 2.11d
	0.05	0.0 ± 0.00a	18.3 ± 1.67d	33.3 ± 2.11f	33.3 ± 2.11f
	0.5	80.0 ± 3.65c	100.0 ± 0.00b	100.0 ± 0.00a	100.0 ± 0.00a
	5	100.0 ± 0.00d	100.0 ± 0.00b	100.0 ± 0.00a	100.0 ± 0.00a
Lippia javanica	0.005	0.0 ± 0.00a	0.0 ± 0.00a	1.7 ± 1.67bc	3.3 ± 2.11bc
	0.05	0.0 ± 0.00a	0.0 ± 0.00a	5.0 ± 2.24cd	6.7 ± 2.11c
	0.5	0.0 ± 0.00a	15.0 ± 2.24c	18.3 ± 1.67e	20.0 ± 0.00e
	5	16.7 ± 2.11b	73.3 ± 2.11e	100.0 ± 0.00a	100.0 ± 0.00a
One-way ANOVA (*F*-statistics)		783.437***	1425.739***	967.179***	1011.250***

*** Significant at the level of $P \leq 0.001$. Means in a column followed by different letters are significantly different from each other at the level of $P = 0.05$ using Fisher's least significant difference (LSD) test.
[1] Each value is expressed as mean ± standard error of six replicates.
[2] Each bottle contained 10 g cowpea seeds.

Table 6. Effects of fumigant toxicity of leaf powders of *Dysphania ambrosioides* and *Lippia javanica* on oviposition and progeny development of *Callosobruchus maculatus*

Treatment	g/500 ml bottle[1]	Mean no. of eggs laid[2]	Mean no. of adults emerged[2]	Mean % progeny development[2]
Control	0	116.2 ± 1.49i	81.5 ± 0.76g	70.2 ± 0.34e
Dysphania ambrosioides	0.005	97.0 ± 1.77g	55.8 ± 0.75e	56.7 ± 1.01c
	0.05	40.2 ± 0.91d	22.5 ± 0.43b	56.0 ± 0.29bc
	0.5	11.3 ± 0.71b	0.0 ± 0.00a	0.0 ± 0.00a
	5	5.0 ± 0.58a	0.0 ± 0.00a	0.0 ± 0.00a
Lippia javanica	0.005	107.0 ± 1.06h	59.2 ± 1.01f	55.8 ± 1.32bc
	0.05	91.0 ± 0.93f	49.2 ± 0.70d	54.1 ± 0.89b
	0.5	57.0 ± 1.18e	29.8 ± 0.48c	52.4 ± 1.14d
	5	19.8 ± 0.79c	0.0 ± 0.00a	0.0 ± 0.00a
One-way ANOVA (*F*-statistics)		1535.85***	2634.27***	1518.56***

*** Significant at the level of $P \leq 0.001$. Means in a column followed by different letters are significantly different from each other at the level of $P = 0.05$ using Fisher's least significant difference (LSD) test.
[1] Each bottle contained 10 g of cowpea seeds.
[2] Each value is expressed as mean ± standard error of six replicates.

(Muzemu *et al.*, 2011; Lina *et al.*, 2013). Stevenson *et al.* (2012) conducted a comprehensive analysis of the chemistry of *T. vogelii* and the closely related species *Tephrosia candida*, and indicated that rotenoids were required for their biological activity. Rotenoids have known low mammalian toxicities (Belmain *et al.*, 2012), making this species an organic alternative to commercial products.

The leaf powder of *D. ambrosioides* invoked 100% mortality of adult *C. maculatus* in stored cowpea seeds within 24 h. This insecticidal effect is in agreement with the findings of several workers (Tapondjou *et al.*, 2002; Chiasson *et al.*, 2004; Denloye *et al.*, 2010; Bossou *et al.*, 2013) who have reported the efficacy of this plant against various insect pests. The reduced oviposition with no adult emergence observed in seeds treated with *D. ambrosioides* at concentrations of 0.1, 1 and 3 g/10 g of cowpea seeds could be due to high adult mortality of *C. maculatus* that occurred within 24 h after treatment. These results indicate that *D. ambrosioides* may be used to protect stored cowpea against *C. maculatus* in small storage systems. A major chemical component of the *D. ambrosioides* identified in these experiments was ascaridole, which occurred along with cymene and 2-carene; and these results are in agreement with a previous report (Chu *et al.*, 2011). Chu *et al.* (2011) also showed ascaridole to be the active component against the storage weevil *Sitophilus oryzae*. This compound is also known to be toxic to a variety of other invertebrates, including mosquitoes (Bossou *et al.*, 2013) and cockroaches (Zhu *et al.*, 2012). Although *D. ambrosioides* may have some mild toxicity to mammals, it is a frequently consumed medicinal plant (Pereira *et al.*, 2010) and culinary herb. Therefore,

Table 7. Effects of fumigant toxicity of leaf powders on the number of days to first bruchid emergence

Treatment	g/500 ml bottle[1]	Mean no. of days to first bruchid emergence[2]
Control	0	34.7 ± 0.21b
Dysphania ambrosioides	0.005	39.3 ± 0.21d
	0.05	40.3 ± 0.21a
Lippia javanica	0.005	37.0 ± 0.26c
	0.05	40.2 ± 0.17a
	0.5	40.3 ± 0.21a
One-way ANOVA (*F*-statistics)		119.1***

*** Significant at the level of $P \leq 0.001$. Means in a column followed by different letters are significantly different from each other at the level of $P = 0.05$ using Fisher's least significant difference (LSD) test.
[1] Each bottle contained 10 g cowpea seeds.
[2] Each value is expressed as mean ± standard error of six replicates.

Table 8a. Damage assessment of cowpea seeds through fumigant action for only damaged seeds

Treatment	g/500 ml bottle[1]	Mean no. of damaged seeds[2]	Mean % seed damage[2]
Control	0	7.0 ± 0.37e	70.0 ± 3.65e
Dysphania ambrosioides	0.005	5.7 ± 0.21c	56.7 ± 2.11c
	0.05	4.7 ± 0.21b	46.7 ± 2.11b
	0.5	0.0 ± 0.00a	0.0 ± 0.00a
	5	0.0 ± 0.00a	0.0 ± 0.00a
Lippia javanica	0.005	6.3 ± 0.21d	63.3 ± 2.11d
	0.05	5.0 ± 0.00b	50.0 ± 0.00b
	0.5	4.5 ± 0.22b	45.0 ± 2.24b
	5	0.0 ± 0.00a	0.0 ± 0.00a
One-way ANOVA (*F*-statistics)		234.561***	234.561***

*** Significant at the level of $P \leq 0.001$. Means in a column followed by different letters are significantly different from each other at the level of $P = 0.05$ using Fisher's least significant difference (LSD) test.
[1] Each bottle contained 10 g cowpea seeds.
[2] Each value is expressed as mean ± standard error of six replicates.

D. ambrosioides may provide a suitable alternative to synthetics for controlling bruchids in cowpea, perhaps with the proviso that the seeds are washed thoroughly before consumption.

The study demonstrated that the leaf powder of *L. javanica* reduced oviposition and completely inhibited emergence of the F_1 generation of *C. maculatus*. *Lippia* also has potential as an environmentally benign alternative for the control of various insect pests (Shikanga *et al.*, 2009), and as with *D. ambrosioides*, is a less toxic alternative to *T. vogelii*. Chemical analysis identified several volatile chemicals, but the major component was camphor, along with 2-carene, α-cubebene, linalool, terpineol and eucalyptol. Of these compounds, camphor has known biological activity against other insect species including storage pests (Suthisut *et al.*, 2011), and therefore may account for the greatest effect of this plant. The leaves of *L. javanica* are used for a well-known beverage taken to alleviate the symptoms of flu, and so are likely to provide another effective alternative protectant against storage pests with little risk of human toxicity.

The results suggest that the volatile chemistry of *L. javanica* and *D. ambrosioides* has insecticidal properties that can be used for storage of cowpea against *C. maculatus*. It has also been reported that *D. ambrosioides* is an important plant species with repellent potential for controlling stored grain pests (Silva *et al.*, 2003; Denloye *et al.*, 2010). The repellent activity of *L. javanica* has been reported against mosquitoes (Lukwa *et al.*, 2009). Repellent botanicals are intuitively perhaps the most useful plants for crop protection as the chemistry is typically based on components less toxic to mammals (Isman, 2006). Furthermore, volatile compounds

Table 8b. Damage assessment of cowpea seeds through fumigant action for holes, weight loss and perforation index

Treatment	g/500 ml bottle[1]	Mean no. of holes per seed[2]	Mean % weight loss[2]	Mean BPI[2]
Control	0	1.5 ± 0.05d	8.3 ± 0.66e	100.0 ± 0.00f
Dysphania ambrosioides	0.005	1.2 ± 0.05b	5.6 ± 0.36c	80.9 ± 3.01d
	0.05	1.1 ± 0.04b	2.8 ± 0.09b	66.7 ± 3.01bc
	0.5	0.0 ± 0.00a	0.0 ± 0.00a	0.0 ± 0.00a
	5	0.0 ± 0.00a	0.0 ± 0.00a	0.0 ± 0.00a
Lippia javanica	0.005	1.3 ± 0.06c	6.5 ± 0.30d	90.5 ± 3.01e
	0.05	1.1 ± 0.04b	5.0 ± 0.18c	71.4 ± 0.00c
	0.5	1.1 ± 0.05b	3.3 ± 0.22b	64.3 ± 3.19b
	5	0.0 ± 0.00a	0.0 ± 0.00a	0.0 ± 0.00a
One-way ANOVA (*F*-statistics)		239.333***	115.694***	405.152***

*** Significant at the level of $P \leq 0.001$. Means in a column followed by different letters are significantly different from each other at the level of $P = 0.05$ using Fisher's least significant difference (LSD) test.
[1] Each bottle contained 10 g cowpea seeds.
[2] Each value is expressed as mean ± standard error of six replicates.

have low or no pesticide residue (Talukder *et al.*, 2004). The results show that subsistence farmers may use both *D. ambrosioides* and *L. javanica* as fumigants to protect stored cowpea against *C. maculatus* in small storage systems.

The reduced oviposition and lower F_1 emergence in cowpea seeds treated with *V. amygdalina* and *T. diversifolia* powder compared with untreated seeds demonstrates some pesticidal activity of these plants to *C. maculatus* (Adeniyi *et al.*, 2010; Oyedokun *et al.*, 2011; Anibijuwon *et al.*, 2012; Bernard *et al.*, 2012). Several compounds have been isolated from the leaves, stem and flowers of *T. diversifolia* (including sesquiterpenes, diterpenes, monoterpenes and alicyclic compounds), some of which have demonstrated biological activities against different species of insects (García and Delgado, 2006; Kuroda *et al.*, 2007; Obafemi *et al.*, 2009). However, as 3 g of plant material per 10 g of seed (30% w/w) were required for some efficacy, this relatively weak biological activity may have no practical value to farmers with the exception of treating smaller quantities of seed material saved for sowing. However, it is of value to know that not all plant species reported to have pesticidal activity actually provide much protection even at such high concentrations. The continuous use of such plants as grain protectants by local farmers should, therefore, be discouraged through education.

Bioactivity through contact toxicity may also be aided by the physical action of the powder formulations where particles may block spiracles of the test insects and cause death by asphyxiation (Denloye *et al.*, 2010; Ileke *et al.*, 2013). Furthermore, plant powders may cause abrasion of the insect cuticle and lead to water loss, stress and eventual death (De Sousa *et al.*, 2005). Previous work by Belmain *et al.* (2012) has shown that plant extracts coated onto seeds are less toxic than using plant powders at chemically equivalent concentrations.

The percentage of damage to seeds ranged between 0.0 and 78.3% in treated and untreated seeds. The BPI was effectively zero in seeds treated with the leaf powder of *T. vogelii* at all concentrations, *D. ambrosioides* at concentrations of 0.1, 1 and 3 g, and *L. javanica* at a concentrations of 1 and 3 g/10 g of cowpea seeds, which was similar to the effect of Actellic dust to prevent seed damage and protect cowpea from bruchid attack.

The results justify the use of leaf powder of *T. vogelii*, *D. ambrosioides* and *L. javanica* as plant-derived insecticides against *C. maculatus*. The leaf powder of these plants could be mixed with cowpea seeds before storage to prevent attack by this insect pest. *Dysphania ambrosioides* and *L. javanica* can also be used as fumigants in the storage rooms. According to the findings of this study, the minimum recommended rate is 0.1 kg of the leaf powder per 100 kg of cowpea seeds for *T. vogelii*, 1 kg leaf powder per 100 kg for *D. ambrosioides*, and 10 kg/100 kg of cowpea seeds for *L. javanica*. The availability of these plants, their non-toxicity to man or other mammals and the fact that they are eco-friendly, make them suitable candidates in post-harvest protection practices by resource poor farmers in sub-Saharan Africa.

Conclusion

Adult bruchids do not feed on stored cowpea seeds, but only deposit their eggs. As shown in this study, *D. ambrosioides*, *T. vogelii* and *L. javanica* have the potential for stored grain legume protection against bruchids by preventing oviposition. In addition, these plants were able to inhibit progeny development of *C. maculatus* at relatively low concentrations (1–10% w/w). This is important as both *D. ambrosioides* and *L. javanica* may provide effective, yet less toxic, alternatives to the more widely used *T. vogelii* by local farmers in Tanzania. The use of pesticidal plants that are naturally occurring has advantages over synthetic insecticides for the management of cowpea bruchids. For example, their natural levels of bioactive compounds are much safer to use than the highly concentrated amounts of active ingredients found in synthetic pesticides.

Acknowledgements

This study was funded by the McKnight Foundation as part of their Collaborative Crop Research Programme http://www.ccrp.org/ and we thank Canisius Kayombo, a botanist from Olmotonyi Forest Training Institute, who assisted in the identification of the plant species.

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