

EVALUATION OF ORGANIC CONTROL METHODS OF THE BEAN BEETLE, *OOTHECA BENNIGSENI*, IN EAST AFRICA

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ABSTRACT

Dry beans (*Phaseolus vulgaris*) are a major source of dietary protein and calories for the poor in East Africa. The increasingly abundant *Oothea bennigseni* (Coleoptera: Chrysomelidae) is a key pest that threatens bean production and jeopardizes farmers' harvest. Participatory research with farmers suggested the need for affordable and accessible organic pest control methods. The effect of diluted cow urine and aqueous extract from vernonia (*Vernonia lasiopus* var. *iodocalyx*) leaves was evaluated in three consecutive applications. Researcher-managed on-farm trials showed that cow urine reduced pest abundance for at least 24 hours. The aqueous vernonia extract reduced the insect abundance consistently for at least 7 days. Foliar damage at the peak time of infestation was significantly reduced by vernonia but not by cow urine. Future research needs to find ways to enhance and prolong the efficacy of natural substances and determine the relationship between adult abundance, larval population and bean yield.

Keywords: *Oothea bennigseni* Weise (Coleoptera: Chrysomelidae), *Phaseolus vulgaris* L. (Leguminosae), *Vernonia lasiopus* var. *iodocalyx* O. Hoffmann (Asteraceae), cow urine, lambda cyhalothrin.

INTRODUCTION

Dry beans (*Phaseolus vulgaris* L.) contribute up to 57% of recommended dietary protein and 23% of energy to the nutrition of some African people (Shellie-Dessert & Bliss 1991). Poor people rely on a diet of beans instead of meat (Wortmann et al. 1998), and therefore it is crucial to have a secure and adequate harvest of beans.

The Selian Agricultural Research Institute (SARI) in Arusha, Tanzania, hosts a bean entomology group of the Centro Internacional de Agricultura Tropical (CIAT). In 1996, they were approached by farmers, who noticed unexplained early senescence in their beans. Investigation discovered a high larval infestation by the bean beetle (*Oothea bennigseni* Weise (Coleoptera: Chrysomelidae)).

Oothea bennigseni is endemic to mainland Africa and is found almost exclusively on bean plants (*Phaseolus vulgaris* L.). Its biology has not been studied in detail, but Schneider (2002) and the entomology group at the SARI and CIAT (J.K.O. Ampofo, unpubl. data) found that the oval, about 7 mm long, shiny beetle oviposits up to eight egg batches of approximately 60 eggs/batch into the soil close to bean plants. Larvae emerge after 2-3 weeks and feed on the roots of beans. The larvae go through three instars,

which last 5 to 11 weeks each, before they pupate in an earthen cell within the soil. The teneral adults undergo a diapause until the onset of the following year's rainy season, when they emerge and start feeding on leaves of the newly planted beans. The adult beetle can cause extensive defoliation, and, with heavy infestation, may completely destroy a crop. Additionally, the feeding of the larvae on lateral roots causes wilting and premature senescence in bean plants (Abate & Ampofo 1996). Yield losses in the range of 18-31% are attributed to *O. bennigseni* in Tanzania (Karel & Rweyemamu 1984). *Ootheca* spp. are also reported to be a key pest in Zambia (Sithanatham 1989), Malawi (Ross 1998), Kenya, Burundi and Rwanda (Karel & Autrique 1989). Over the years, Tanzanian farmers have noticed increasing foliar damage by *O. bennigseni* to their young bean plants, but were unaware of the larval damage by the same insect until they were shown the larvae on the roots of the bean plants (Ampofo et al. 2002). Once they understood the insect's lifecycle they wanted to learn effective control methods. The scientists suggested management options based on cultural practices, but many farmers could not implement them, and requested research of traditional methods using local concoctions to be sprayed on the bean field. Treatments suggested by farmers were fermented cow urine and an extract from vernonia (*Vernonia lasiopus* var. *iodocalyx* O. Hoffmann (Asteraceae)). On-farm trials managed by farmers showed that those treatments indeed reduced insect abundance for varying periods of time. But the farmer-collected data showed inconsistencies that severely limited the statistical analysis. Therefore a researcher-managed experiment was conducted on a farmer's field with high *O. bennigseni* incidence in the past year. The three objectives were to: (1) determine if cow urine or vernonia extracts controlled infestation by *O. bennigseni*; (2) determine the duration of the efficacy of the two treatments; and (3) assess the treatment effect on larval abundance, foliar damage and bean yield.

METHODS

Adult *O. bennigseni* abundance

The experiment was conducted from April to July 2003 in a farmer's field of about 1500 m² at Tengeru/Camartec (Tanzania, Arusha region, Arumeru district, Patandi village, Duluti sub-village: 3°24'S, 36°47'E, 1205 m above sea level, mean of 1000 mm rain/year (bimodal), and a mean temperature of 21.5°C (unpublished data from a nearby flower-farm, 2002). Following the first heavy rain on 29 March, dry beans (cv. Lyamungo '91, Calima type), treated with Murtano® Dust (lindane 20%, thiram 26%) at 3 g/kg seed, were planted on 1 and 2 April. Inter-row spacing was 0.5 m and within-row spacing was 0.2 m with two seeds per planting hole. No fertilisers were applied. A randomised complete block design with six blocks was superimposed. Each block was surrounded by at least 2 m of bean plants and comprised four plots of 6×3 m, which were separated from each other by 2 m (or four rows) of beans. Each plot was subdivided perpendicular to the rows into two sub-plots of 3×3 m each. The natural infestation of the bean crop of this field was expected to be high, due to last year's high occurrence of *O. bennigseni*. Some adult *O. bennigseni* were observed on the soil surface during planting. A few volunteer plants, from last year's crop and distributed over the whole field, were left undisturbed to enhance *O. bennigseni* emergence. On 9 April, six emergence traps were placed randomly, each over a row of beans outside the trial area, and soil was put over the lower edges of the traps to close any potential escape holes between soil and frame. These traps were made of wooden frames in square pyramid form (ground surface 0.5×0.5 m and about 0.5 m high), covered with fine nylon mesh except for a hole at the apex where a plastic bag was fixed to a metal ring to collect the emerging insects. Traps were checked daily and trapped insects were freed. The traps were removed on 29 April, as the plants filled the entire trap.

The four treatments were:

- (1) cow urine from a dairy farm in Olasiti (Arusha), which was collected in the morning into a plastic container, and left fermenting for 6±1 days in the shade at ambient temperatures. Before application, the urine was diluted with water 1:3 (v/v) to reduce the risk of burning the bean leaves.

- (2) Aqueous extract of vernonia was prepared in the evening before the day of application. Young leafy branches of vernonia were collected in Olasiti and finely ground with a wooden pestle and mortar. The ground leaves were mixed with water 1:1 (w/w), using the same method as the farmer when using botanicals, and the slurry was kept overnight in an open plastic bucket. The next morning, the mixture was strained through a fine cloth and sprayed undiluted.
- (3) The standard insecticide lambda cyhalothrin (Karate® 50 g/litre EC) was bought from a local official supplier and used at the recommended application rate of 125 mg ai/litre.
- (4) Water was used as a control.

For the three applications of treatments, a randomly chosen sub-plot of each plot was sprayed with a thoroughly cleaned knapsack sprayer between 8 and 9 am with one of the four treatments (named test sub-plot). The other adjacent sub-plot was sprayed at the same time with clean water (named control sub-plot). About 0.5 ± 0.1 litres of liquid preparation was used for each sub-plot, which was the point where liquids started to run off. The bean plants between the trial plots were left unsprayed. The four treatments were first applied on 9 April, after counting all live *O. bennigseni* in all sub-plots. Counting was done with as little disturbance to the insects as possible, following each row of beans in each sub-plot. The primary leaves of most bean plants were fully opened and few *O. bennigseni* adults were leaf-feeding. Counting was repeated 60 ± 10 min after treatment and then daily between 9 and 10 am. It was attempted to count dead *O. bennigseni*, but this was only possible at the counting made 1 hour after application, since a day later, the bodies had disappeared. Because of dry weather after the first application, only very few *O. bennigseni* emerged in the following week and the second application was delayed. A drip irrigation system was installed and an equivalent of 20 mm rain total was used between 12 and 16 April to save the young bean plants from drought damage. However, this irrigation did not result in increased insect emergence. After the next rain on 16 April (30 mm) the numbers of *O. bennigseni* increased and the bean plants developed quickly. On 21 April the field was hand weeded before the second application of the four treatments on 22 April. The bean plants had reached the two-trifoliolate-leaves stage and this time 0.7 ± 0.2 litres of treatment was used for each sub-plot. The third and last application was on 30 April, at first flowering of the bean plants, and 0.9 ± 0.2 litres of treatment was used for each sub-plot. At this stage the *O. bennigseni* numbers started to drop naturally and no further applications were carried out. See Figure 1 for an overview of the time during the three applications of treatments (especially the rainfall and the irrigation) and the adult *O. bennigseni* abundance in the test sub-plots (for all four treatments).

Leaf damage

Oothea bennigseni adults make very distinct round feeding holes predominantly on the youngest leaves of the bean plant. Therefore only one leaf damage assessment was conducted. On 6 May, 6 days after the third application, leaf damage throughout the trial period was assessed by randomly collecting three primary leaves (corresponding to first application), three trifoliolate leaves from the centre of the bean plant (second to fourth trifoliolate leaf stage; corresponding to second application) and three trifoliolate leaves from the top of the bean canopy (corresponding to third application at flowering) from each test sub-plot for damage assessment. Leaf area loss by *O. bennigseni* in percentage of total leaf area was estimated from the leaves photocopied onto graph paper and rounded to 0, 1, 5, 10, 15, 25, 50, 75 or 100% loss.

Bean yield and *O. bennigseni* larvae abundance

At harvest on 8 July, the beans of each test sub-plot were threshed and the grain weighed. A day later, soil samples of approximate 0.02 m^3 (0.5 m long following a bean row and 0.2 m wide and 0.2 m deep) were collected from the centre of each test sub-plot. The soil was sieved (2 mm mesh) to separate and count *O. bennigseni* larvae. This method recovers about 80% of the larvae present (Ampofo & Massomo 1998).

Statistical analysis

For assessing insect abundance, univariate ANOVA (randomised complete block design with 4 treatments and 6 blocks, d.f.=15) was calculated for test and control sub-plots independently. The single count data were used for the analysis of abundance 1 hour after application and 1 day after application. Then the average numbers of insects on each sub-plot for the period day 1 until day 7 after application of treatments were used for an overall analysis. The LSD test was used for the separation of means. Univariate ANOVA of test sub-plot data was used for evaluation of leaf damage (average of the three leaf area loss measurements), larval counts and yield. Correlation between *O. bennigseni* abundance (average of the first seven days after each application) and the corresponding leaf area loss (average of three leaves) was calculated for each treatment separately and all data combined. Similarly, correlations between adult and larvae abundance, adult abundance and yield, larvae abundance and yield, as well as leaf area loss and yield were calculated. All correlations were calculated for the test sub-plot data only. Whenever a correlation coefficient was significant at 5% level, a regression analysis was conducted for detecting a possible difference of the regression coefficients. The software packages SPSS (version 9.2) and Microsoft Excel 2003 were used for all calculations.

RESULTS AND DISCUSSION

Adult *O. bennigseni* abundance

Climatic conditions and all disturbances in the bean field influenced *O. bennigseni* abundance. In particular, the abundance of *O. bennigseni* was reduced by treatment applications and heavy rainfalls as shown in Figure 1. There was also a general tendency of increasing insect abundance until end of April (before application 3), and a steady decrease afterwards. This is a typical population curve for *O. bennigseni* (Ampofa & Massomo 1998). The emergence traps caught an average of 2.3 *O. bennigseni* in 20 days. Although no measurements were made, it is assumed that a considerable number of beetles immigrated to the trial field from nearby bean fields, as higher insect numbers were observed at one end of the field shortly before application 2, but later the entire field seemed to be evenly infested. Few *O. bennigseni* were found after the first application of treatments, and no consistent result was obtained, therefore these data are not described. The data for each counting event of *O. bennigseni* adults are shown in Figure 1 (test

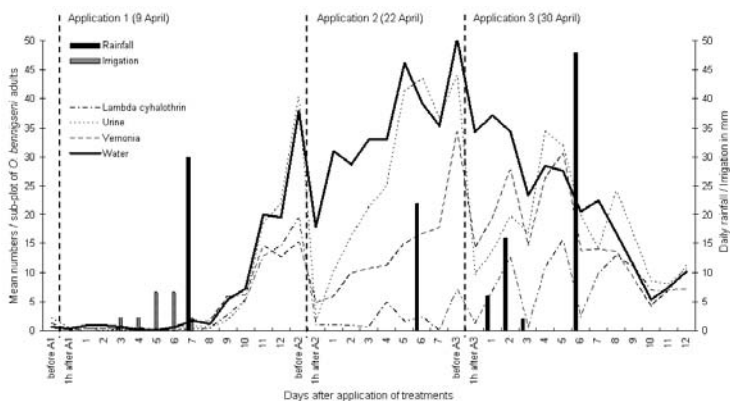


FIGURE 1: Mean adult *O. bennigseni* abundance per test sub-plot of 9 m² under four treatments (water (control), urine, vernonia and lambda cyhalothrin (standard)), and daily irrigation (mm) and daily rainfall (mm) during three applications of the treatments.

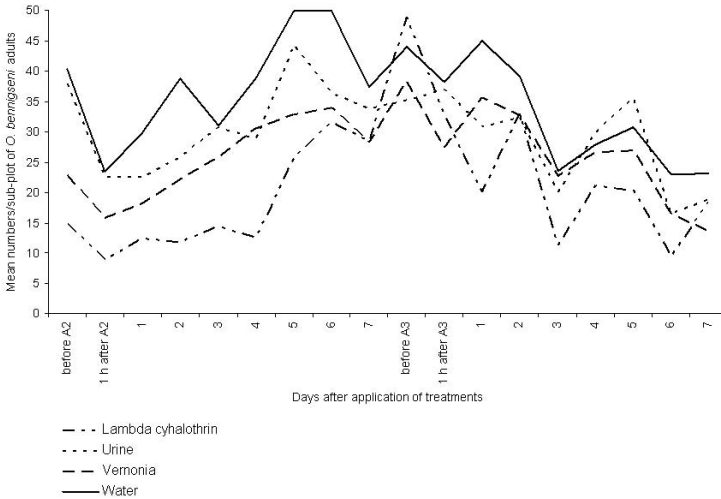


FIGURE 2: Mean adult *O. bennigseni* abundance per control sub-plot of 9 m² adjacent to the four treatments (water (control), urine, vernonia and lambda cyhalothrin (standard)) for applications two and three.

sub-plots, all three applications) and Figure 2 (control sub-plots for applications 2 and 3 only). It is apparent that all treatments had an effect on insect numbers in the test sub-plots (Fig. 1). Insects on urine treated plots dropped in the first hour after application from 40.3 to 1.5 in application 2, and from 44.0 to 9.7 in application 3. Thereafter the insect numbers increased over the next 3–4 days to similar levels as water. Vernonia treated test sub-plots revealed a less dramatic drop (15.3 to 4.8 and 34.3 to 14.2, for applications 2 and 3 respectively) and a slower recovery of the insect numbers over about 7 days. The standard lambda cyhalothrin reduced insect numbers to very low levels and kept them there for an extended period. Test sub-plots treated with the control (water) also experienced a reduction of insects 1 h after application compared to the pre-treatment count, but numbers recovered in about 1 day.

Results from statistical analysis are summarised in Table 1. Urine treated test sub-plots hosted fewer *O. bennigseni* than control test sub-plots 1 hour and 1 day after application, but only application 3 resulted in a significant difference for the period of 7 days after application. Vernonia treated test sub-plots contained significantly fewer *O. bennigseni* numbers than the control test sub-plot for 1 h, 1 day and 7 days after application 2 and 3. Lambda cyhalothrin treated test sub-plots resulted in highly significant differences for all durations and both applications. The control sub-plots reveal possible nearby effects of the treatments. While Figure 2 suggests reduced insect numbers in test sub-plots beside vernonia and lambda cyhalothrin treatments, only the lambda cyhalothrin test sub-plots contained significantly lower insect numbers than control sub-plots beside control treatments.

TABLE 1: Mean *O. bennigseni* abundance per sub-plot of 9 m² for treatment and control sub-plots at 1 h and 1 day after application of treatments, and for the average from day 1 until day 7. Treatments that are significantly different from the control (water) are shown by * (P<0.05) and ** (P<0.01).

Time	Urine	Vernonia	Lambda cyhalothrin	Water (control)	LSD P<0.05	LSD P<0.01
Application 2						
<i>Test sub-plots</i>						
1 h after application	1.5 **	4.8 **	1.0 **	17.8	9.2	12.7
1 day after application	10.2 **	5.8 **	1.0 **	31.0	14.3	19.8
7 days	27.7	12.4 *	1.6 **	35.2	19.6	27.1
<i>Control sub-plots</i>						
1 h after application	22.5	15.8	8.8 *	23.3	12.5	17.2
1 day after application	22.5	18.2	12.3 *	29.8	13.9	19.2
7 days	31.7	27.4	19.5 *	39.3	17.8	24.6
Application 3						
<i>Test sub-plots</i>						
1 h after application	9.7 **	14.2 **	1.2 **	34.3	10.3	14.2
1 day after application	13.7 **	19.5 **	6.8 **	37.2	11.7	16.2
7 days	21.5 *	21.0 **	8.3 **	27.7	4.5	6.3
<i>Control sub-plots</i>						
1 h after application	22.5	15.8	8.8	23.3	15.2	21.0
1 day after application	22.5	18.2	12.3**	29.8	16.8	23.2
7 days	31.7	27.4	19.5 **	39.3	6.7	9.2

It has been concluded that vernonia, urine and lambda cyhalothrin are effective at reducing *O. bennigseni* abundance between 1 and at least 7 days. Although urine is highly effective in reducing abundance directly after the application, it quickly loses this strong effect. The effect of vernonia lasts at least 7 days. Lambda cyhalothrin, a commercial insecticide, had a fast knock down effect, which was demonstrated by the discovery of dead beetles 1 h after application in these test sub-plots. It reduced insect abundance highly significantly for at least 7 days. Lambda cyhalothrin is insecticidal and known to have a repellent effect (Anon. 2000). No dead insects were discovered in urine or vernonia test sub-plots, which could either mean that there was no toxic effect or that it was slow acting (insects hide in soil before dying or had disintegrated before the next counting event). This may indicate a repellent effect of urine and vernonia. In contrast to the present work, fermented urine is reported to be an attractant for tsetse flies (Okech & Hassanali 1990). Liquid manure has also been found to change the composition of entomofauna in meadows, but the slight increase of Chrysomelidae under liquid manure compared to the control was not significant (Plewka 1986). There have been no published studies on the effect of vernonia on insect abundance. Further bioassays are needed to decide on the exact mode of action of urine and vernonia.

The results also show that lambda cyhalothrin significantly reduced insect numbers in areas adjacent to the treated sub-plots (i.e. the lambda cyhalothrin control sub-plots). A strong repellent effect could be responsible for the lower insect numbers in the control

sub-plots. However, another explanation for these results is that any *O. bennigseni* adults flying onto a lambda cyhalothrin treated plot are killed by the insecticide and these plots act as a “sink” for insects from adjacent areas. This opinion is supported by the fact that dead insects were found in lambda cyhalothrin test sub-plots. There was no significant reduction of *O. bennigseni* adults in control sub-plots for either urine or vernonia, which suggests that the effects are not as strong, or there are two divergent effects.

Bean leaf damage due to adult feeding of *O. bennigseni*

Insect abundance for the three applications together correlated closely with leaf area loss ($r=0.74$) for all treatments except lambda cyhalothrin. The regression analysis showed that regression coefficients did not differ significantly for each treatment (Table 2). This leads to the conclusion that there were no significant antifeeding effects of any of the treatments. Other studies report that some *Vernonia* spp. act as an insect feeding deterrent, with the active ingredients being specified as sesquiterpene lactones (Burnett et al. 1974; Rodriguez et al. 1976). The content of sesquiterpene lactones in *V. lasiopus* var. *iodocalyx* still needs to be established, but the present results indicate that it might be one of the species that lacks the feeding deterrent, as no antifeeding effect could be determined. There have been no published studies on the effect of urine on feeding behaviour of phytophagous insects.

TABLE 2: Parameters from correlation and regression analyses of adult *O. bennigseni* abundance per sub-plot of 9 m² on leaf area loss (%). Values are given for all treatments combined and for the four individual treatments, urine, vernonia, lambda cyhalothrin and control, over all three applications.

Treatment	N	Correlation coefficient	Intercept	Regression coefficient	Confidence interval for regression coefficient
All	72	0.74	0.80	0.125	0.098 – 0.152
Lambda cyhalothrin	18	0.23	0.71	0.151	-0.185 – 0.487
Urine	18	0.85	1.63	0.125	0.084 – 0.166
Vernonia	18	0.63	0.82	0.147	0.050 – 0.244
Control (Water)	18	0.89	1.06	0.148	0.108 – 0.188

Damage to primary leaves, corresponding to early damage after the first application of treatments, was generally low, which was a result of the low numbers of *O. bennigseni* present on the plants during this time. Urine treated plants had a higher loss of leaf area than the other treatments (Table 3). Although the leaves showed some signs of phytotoxicity by urine, the leaf area loss was certainly caused by *O. bennigseni* as the round holes are very distinct. The middle trifoliolate leaves corresponded to the second application of treatments. Leaf area loss in all treatments was still relatively low, in spite of higher insect numbers. Plants in vernonia treated sub-plots had a significantly ($P<0.05$) lower leaf area loss than the control. Plants in lambda cyhalothrin treated sub-plots had a significantly ($P<0.01$) lower leaf area loss than control plants. The upper trifoliolate leaves correspond to the third application of treatments. The differences between treatments in the leaf damage was less obvious at this time. Only lambda cyhalothrin treated plants showed significantly ($P<0.05$) less damage than the water treated plants. The correlation between *O. bennigseni* abundance and leaf area loss demonstrated that reducing abundance leads to less damage, and the different treatments resulted in some differences in leaf area loss, but the damage was generally relatively low.

In the study by Karel & Rweyemamu (1984), leaf area losses caused by an abundance of 0.54 *O. bennigseni* per plant were about 40%. In the present study the highest insect

abundance was 0.69 *O. bennigseni* per plant (average of insects in one sub-plot for seven counting events after the second application) and resulted in 13% leaf area loss only (average of three leaf area loss calculations). These calculations are dependent on the growth rate of the bean plant during the assessment period. In the present study the beans grew very fast after the dry spell, and the adult insects inflicted a comparatively lower damage than the insect abundance would suggest.

TABLE 3: Mean percentage leaf area loss for primary leaves (early attack/application 1), middle trifoliolate leaves (main attack/application 2) and upper trifoliolate leaves (late attack/application 3) after three treatments with water, urine, vernonia or lambda cyhalothrin. Separation of means was done by LSD (P<0.05 and P<0.01). Treatments that are significantly different from the control (water) are shown by * (P<0.05) and ** (P<0.01).

Time	Urine	Vernonia	Lambda cyhalothrin	Water (control)	LSD P<0.05	LSD P<0.01
Primary leaves	1.44 *	0.39	0.17	0.39	0.85	1.18
Middle trifoliolate leaves	5.83	3.50 *	2.00 **	7.33	3.77	5.21
Upper trifoliolate leaves	1.83	2.28	0.94 *	3.06	1.93	2.66

Larvae abundance of *O. bennigseni* at harvest and bean yield

Mean larvae numbers in the treatment sub-plots were not significantly different (P>0.05) between treatments (Table 4). The infestation was relatively low compared to a study in the neighbouring district (Hai, Kilimanjaro region) where Ampofo & Massomo (1998) estimated an average of 100 larvae/m². Adult *O. bennigseni* abundance was not correlated to larvae abundance (r=0.12), and larvae abundance did not correlate with the yield (r=0.28). No literature was found on the *O. bennigseni* larvae and bean yield relationship. However, Teixeira et al. (1996) reported that the larvae of *Cerotoma arcuata* Olivier (Coleoptera: Chrysomelidae), which is also root feeding, reduced yield more than the adult feeding on leaves, and their data implied a close correlation between larvae abundance and yield.

Grain yield in test sub-plots is shown in Table 4. In spite of the differences in abundance of *O. bennigseni*, no significant differences in yield between the different treatment sub-plots were found. The trials also showed that decreased adult *O. bennigseni* abundance did not correlate with increased yield (r=0.31). Karel & Rweyemamu (1984) measured a yield gain between 18 and 31% when using synthetic insecticides to control *O. bennigseni* compared to no treatment, but insect abundance was higher than in the present experiment.

TABLE 4: Mean *O. bennigseni* larvae abundance per 0.02 m³ soil and mean yield (kg/ha) after three treatments with water, urine, vernonia or lambda cyhalothrin.

	Urine	Vernonia	Lambda cyhalothrin	Water (control)	LSD P<0.05
Larvae abundance	58.3	33.3	50.0	36.7	36.5
Yield	1840	1710	1840	1830	300

CONCLUSIONS

The present study demonstrates that vernonia and cow urine are potential natural control substances against *O. bennigseni*. Reduced insect abundance was apparent in both treatments, but duration of the effect was short compared to lambda cyhalothrin. The reduction of *O. bennigseni* abundance led to significantly reduced foliar damage during the peak infestation in beans treated with vernonia.

Hongo & Karel (1986) and Karel (1989) reported similar results with different plant extracts, showing reduced foliar damage after treatment. Insect abundance was only measured once about 24 h after application of the treatments, so the results are inconclusive in regards to the duration of the treatment effect. In the present study, grain yields were generally high and no significant yield gain was achieved by any treatment. Beans are most sensitive to defoliation at the primary leaf stage and during flowering and early pod formation (Gálvez et al. 1977, Cardona et al. 1982). In the present study the main foliar damage occurred during the later vegetative stages, and no yield decline was measured.

Reduced adult beetle abundance did not directly result in reduced larval abundance in any of the four treatments. It is especially surprising that the standard lambda cyhalothrin did not reduce the larvae population. This means that adult insects must have been able to oviposit in the soil below the treated bean plants prior to dying or without getting in contact with the insecticide. More research is required to distinguish between larvae and adult insect damage, and understand the relationship between adult and larval populations.

While the effectiveness of urine and vernonia for the control of *O. bennigseni* have been demonstrated, further research into processes to enhance and prolong the effectiveness of these free and readily available natural substances should be explored, as farmers in Africa need affordable and sustainable methods to become more productive.

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