

Table 11. Quantities (ng) of EAG-active components in the volatiles¹ from host plants of the stemborers *Chilo partellus* and *Busseola fusca*

Compound	<i>Pennisetum purpureum</i> (N = 5)	<i>Hyparrhenia tamba</i> (N = 3)	<i>Sorghum bicolor</i> (N = 4)	<i>Zea mays</i> (N = 2)
Green leaf volatiles and other aliphatic compounds				
1 (E)-2-pentenal	0.2 ± 0.1	0.5 ± 0.1	0.4 ± 0.2	nd
4 hexanal	5.0 ± 5.0	1.3 ± 0.4	0.9 ± 0.1	0.3 ± 0.3
5 (E)-2-hexenal	4.7 ± 3.8	0.9 ± 0.5	0.3 ± 0.1	1.2 ± 0.2
6 (Z)-3-hexen-1-ol	12.3 ± 11.3	3.3 ± 3.0	1.7 ± 0.4	1.3 ± 0.4
7 3-methylbutyl acetate	0.4 ± 0.2	1.9 ± 1.0	0.4 ± 0.1	0.8 ± 0.0
8 pentyl acetate	0.4 ± 0.4	0.5 ± 0.3	0.3 ± 0.2	nd
13 6-methyl-5-hepten-2-one	2.4 ± 1.1	3.9 ± 2.3	1.2 ± 0.7	1.2 ± 0.5
14 octanal	3.4 ± 2.0	14.7 ± 10.2	2.0 ± 1.1	5.8 ± 2.3
16 (Z)-3-hexen-1-yl acetate	120.0 ± 117.0	23.1 ± 8.6	3.3 ± 1.5	1.3 ± 0.7
17 hexyl acetate	1.6 ± 0.8	3.4 ± 2.0	1.3 ± 0.6	2.2 ± 0.1
25 nonanal	4.5 ± 3.6	9.4 ± 6.7	5.4 ± 3.5	<0.1
34 decanal	8.7 ± 7.2	8.0 ± 6.8	7.7 ± 5.8	<0.1
Aromatic compounds				
3 toluene	0.4 ± 0.3	0.7 ± 0.1	2.0 ± 0.6	1.7 ± 0.4
10 benzaldehyde	0.3 ± 0.3	3.5 ± 1.9	0.1 ± 0.1	0.7 ± 0.1
12 propylbenzene	1.2 ± 0.8	3.6 ± 3.1	0.4 ± 0.2	2.7 ± 0.6
18 phenylacetaldehyde	1.7 ± 1.3	11.9 ± 6.1	0.2 ± 0.1	1.1 ± 0.4
21 acetophenone	3.0 ± 1.8	1.1 ± 1.1	0.3 ± 0.3	3.2 ± 3.2
23 methyl benzoate	0.9 ± 0.5	3.3 ± 2.0	1.0 ± 0.5	0.9 ± 0.5
28 2-ethylbenzaldehyde	nd	0.3 ± 0.3	nd	nd
29 3-ethylbenzaldehyde	2.0 ± 0.9	3.2 ± 1.8	4.4 ± 2.9	0.3 ± 0.3
30 4-ethylbenzaldehyde	0.1 ± 0.1	0.5 ± 0.5	0.6 ± 0.2	6.9 ± 6.9
31 naphthalene	0.8 ± 0.6	2.9 ± 2.2	0.8 ± 0.4	nd
32 methyl salicylate	3.2 ± 2.1	12.3 ± 8.3	0.8 ± 0.4	10.5 ± 10.5
33 4-allylanisole	0.1 ± 0.1	0.4 ± 0.2	0.2 ± 0.2	nd
35 indole	27.1 ± 20.7	1.6 ± 0.8	0.2 ± 0.2	2.7 ± 2.7
36 4-ethylacetophenone	3.5 ± 1.9	1.3 ± 0.7	nd	2.3 ± 2.3
37 eugenol	0.6 ± 0.2	0.3 ± 0.3	0.3 ± 0.3	nd
Terpenoids				
11 α-pinene ²	1.8 ± 1.1	1.8 ± 1.3	0.3 ± 0.2	5.1 ± 0.1
15 myrcene	0.7 ± 0.7	2.2 ± 1.9	0.3 ± 0.1	1.8 ± 1.8
19 1,8-cineole	nd	1.8 ± 1.3	nd	1.5 ± 0.2
20 limonene ²	1.4 ± 0.7	4.1 ± 2.3	2.7 ± 1.6	2.0 ± 0.7
22 (E)-ocimene	0.6 ± 0.3	1.7 ± 1.3	nd	6.2 ± 1.4
24 α-terpinolene	nd	0.1 ± 0.1	nd	nd
26 linalool ²	1.5 ± 0.9	14.2 ± 9.8	2.0 ± 0.9	0.6 ± 0.1
27 (E)-4,8-dimethyl-1,3,7-nonatriene	55.3 ± 19.1	21.0 ± 10.8	39.6 ± 11.6	2.9 ± 2.1
38 α-copaene ²	0.4 ± 0.4	1.1 ± 1.0	0.7 ± 0.4	1.4 ± 1.4
39 β-caryophyllene ²	21.5 ± 14.1	5.7 ± 2.7	19.5 ± 6.3	0.4 ± 0.4
40 bergamotene ²	0.9 ± 0.8	0.3 ± 0.1	2.3 ± 0.6	0.4 ± 0.2
41 (E)-β-farnesene	0.3 ± 0.3	1.3 ± 0.1	4.4 ± 1.5	0.2 ± 0.2
42 α-humulene	1.3 ± 0.9	0.5 ± 0.3	0.8 ± 0.3	nd
43 (E,E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene	3.0 ± 0.5	14.5 ± 5.7	0.8 ± 0.1	1.35 ± 1.35
Unknowns				
2 KI = 743	0.5 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	nd
9 KI = 911	nd	1.6 ± 1.0	0.3 ± 0.2	nd
Total volatiles	294.8	186.5	103.4	70.3

¹Compound numbers refer to the order in which they were eluted from an HP-1 GC column.

²The optical isomers of these compounds were not characterised.

Samples were collected for 10–12 h (starting from the 4th hour of the photophase).

The amounts of volatiles are estimates based on the total area under the reported peaks compared with the peak area of the internal standard (100 ng).

nd, below the detection limits of 0.05 ng.

2003 ICIPE Annual Scientific Report.) Bioassays of different chromatographic fractions of adsorbent-trapped collections of *Desmodium* root exudates had indicated that different sets of constituents are associated with the two activities and that the most potent radicle inhibitors are located in the polar region of the complex exudate blend.

Follow up efforts to isolate active allelochemicals from the root exudates of *Desmodium* led to the characterisation of three novel structurally related isoflavanoids of medium polarity, one with uncyclised and the other two with cyclised (furanoid) isopropenyl moiety in ring A. The two with isoproprenylfuran rings were active, one as a striga germination stimulant (I) and the other (II), with one of the phenoxy OH group methylated, as a moderate radicle inhibitor of the

germinated seeds. In addition, a polar post-germination inhibitor was isolated and shown to be a di-glycosyl flavone (III). These results suggested relatively strict structural requirements for striga germination and its post-germination inhibition, respectively. (See 2002–2003 ICIPE Annual Scientific Report.)

The focus during the last two years has been on comprehensive isolation and characterisation of candidate allelochemicals from *Desmodium* root extracts and evaluation of their bioactivities individually and in blends. Identification of active constituents of these fractions and if they act additively or synergistically would provide a useful basis of assessing the biotechnological potential of *Desmodium* genomics and the possible course such a venture could take.

The effect of the bulk organic extracts of *Desmodium* roots on *Striga* was compared with that of the aqueous root exudates. The polar MeOH extract, like the aqueous root exudates, was found to exhibit low striga germination stimulation activities but high post-germination inhibition. Acetone extract was found to be relatively active both as a germination stimulant and a post-germination radicle inhibitor, whereas the dichloromethane extract exhibited striga germination stimulation activities but did not inhibit the post-germination growth of striga (Figures 17 and 18). The results confirmed the previously observed pattern, with germination stimulation associated largely with the less polar extracts and post-germination inhibition with the more polar region.

Fractionation of the CH₂Cl₂ extract of *D. uncinatum* roots (vacuum liquid chromatography on silica gel and eluting with hexane-acetone mixtures with increasing proportion of acetone) yielded four fractions (A, B, C and D). Fractions A, B, C and D were eluted with 100% n-hexane; 5% acetone-hexane; 20% acetone-hexane and 50–100% acetone-hexane respectively. These fractions were tested for germination stimulation of *S. hermonthica* seeds (Table 12).

Table 12. Germination response of *Striga hermonthica* seeds to fractions of CH₂Cl₂ extract of *Desmodium uncinatum* roots at varying concentrations

Fraction	Percentage mean germination (± SE), n = 10	
	100 ppm	10 ppm
A	0.63 (0.63) d	0.4 (0.24) d
B	46.23 (1.88) bc	42.61 (1.25) c
C	57.64 (3.98) ab	50.22 (2.82) bc
D	67.80 (3.65) a	53.83 (4.30) bc
GR-24, 5 ppm	50.17 (4.66) bc	

Means with the same letter are not significantly different (P ≤ 0.05, Tukey's studentised range test).

Fractionation of the acetone root extract by *D. uncinatum* by vacuum liquid chromatography on C-18 reverse phase silica, yielded fractions ACF1 to ACF5 by eluting with 50% MeOH/H₂O, 75% MeOH/H₂O, 90% MeOH/H₂O, 100% MeOH and 100% acetone, respectively. The fractions were tested for their effects on germination and post-germination growth of *S. hermonthica* (Tables 13 and 14).

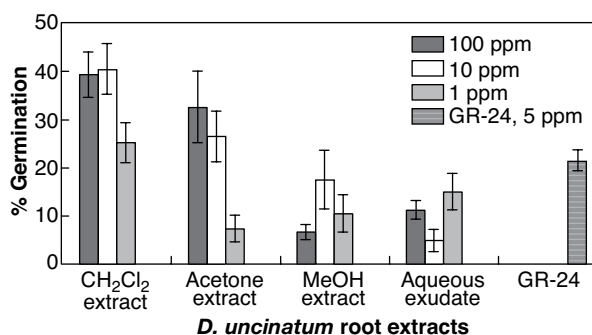


Figure 17. Germination response of *Striga hermonthica* seeds to *Desmodium uncinatum* root extracts at varying concentrations (values are means ± SE, n = 10)

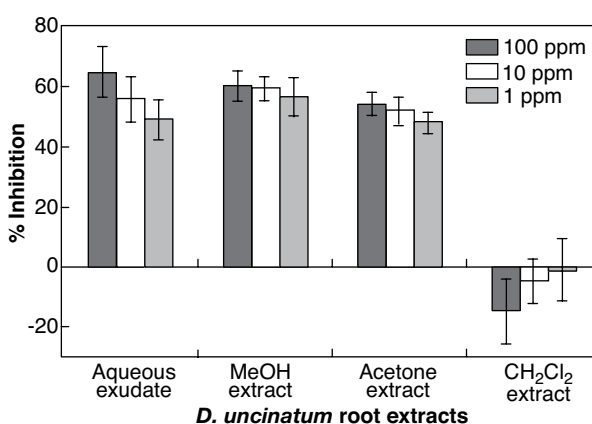
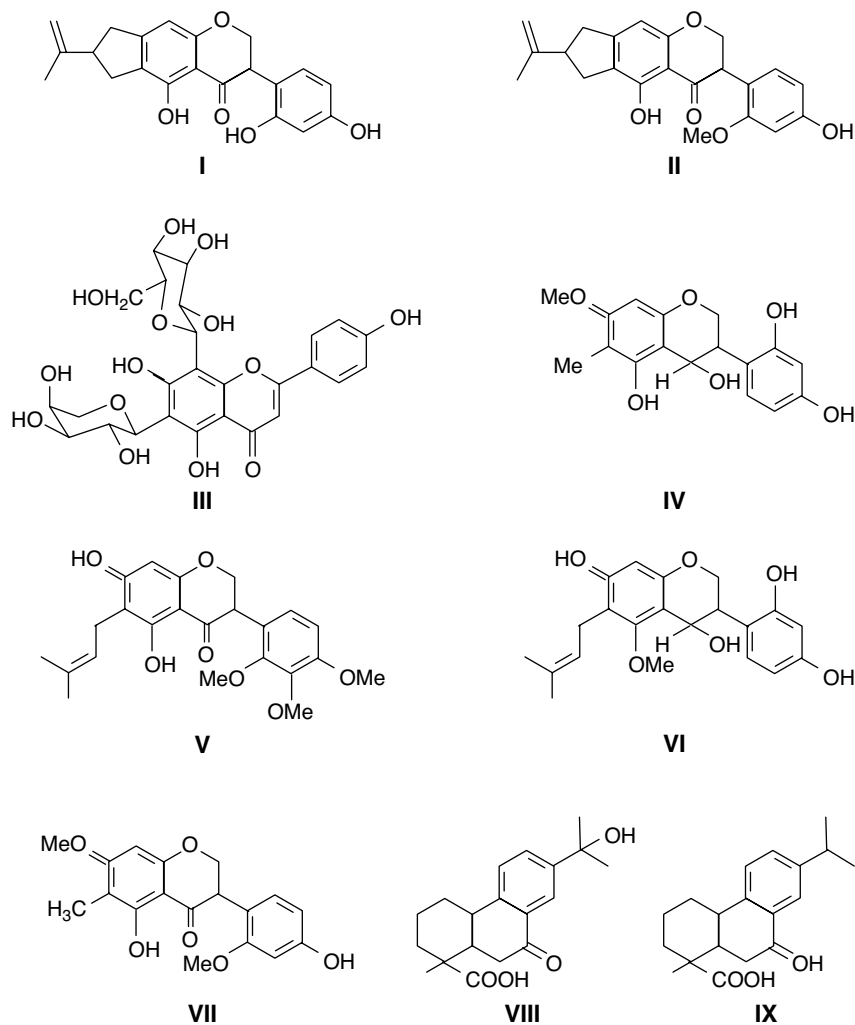


Figure 18. Radicle growth inhibition of germinated *Striga hermonthica* seeds exposed to the root extracts of *Desmodium uncinatum* at varying concentrations (values are means ± SE, n = 10)



Fractionation of fractions from CH_2Cl_2 extract on silica gel column chromatography followed by further purification on a semi-preparative HPLC on Ultrasphere C-18 column yielded four new isoflavonoids (**IV–VII**), which were characterised by 2D-NMR and mass spectroscopy, together with known abietane diterpenes, 7-oxo-15-hydroxydehydroabietic acid (**VIII**) and 7-hydroxycallitric acid (**IX**).

Table 13. Germination response of *Striga hermonthica* seeds to fractions of acetone extract of *Desmodium uncinatum* roots at varying concentrations

Treatment	Percentage mean germination (\pm SE), n = 16		
	100 ppm	10 ppm	1 ppm
ACF1	29.58 (1.85) cd	1.78 (1.20) f	0.00 (0.00) f
ACF2	46.66 (3.99) a	40.88 (3.03) ab	33.25 (3.34) bcd
ACF3	34.42 (3.56) bc	22.99 (2.64) de	23.47 (2.99) cde
ACF4	0.00 (0.00) f	2.46 (1.92) f	0.37 (0.37) f
ACF5	4.13 (1.53) f	2.79 (1.02) f	0.00 (0.00) f
GR-24, 5 ppm			16.97 (2.49) e

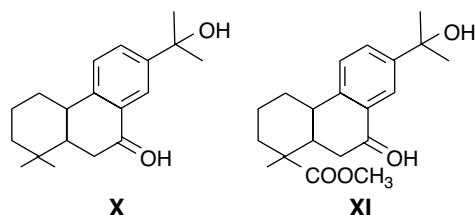
Means with the same letter are not significantly different ($P \leq 0.05$, Tukey's studentised range test).

Table 14. Radicle growth inhibition of germinated *Striga hermonthica* seeds exposed to fractions of acetone extract of *Desmodium uncinatum* roots at varying concentrations

Treatment	Concentration (ppm)	Radicle length (mm) (n = 16)	Percentage radicle length reduction
ACF1	100	0.20 (0.01) e	72.60
	10	0.27 (0.02) e	63.01
	1	0.36 (0.03) de	50.68
ACF2	100	0.31 (0.03) e	57.53
	10	0.58 (0.03) abc	21.23
	1	0.76 (0.03) ab	-3.42
ACF3	100	0.38 (0.03) cde	47.95
	10	0.56 (0.03) bcd	23.97
	1	0.71 (0.05) ab	2.74
ACF4	100	0.67 (0.05) ab	8.22
	10	0.75 (0.06) ab	-2.05
	1	0.74 (0.05) ab	-0.68
ACF5	100	0.73 (0.04) ab	0.68
	10	0.77 (0.04) a	-4.79
	1	0.76 (0.06) ab	-3.42
GR-24	5	0.73 (0.06) ab	0.00

Means with the same letter are not significantly different ($P \leq 0.05$, Tukey's studentised range test).

The most polar ACF1 fraction has been the major focus of sub-fractionation cycles involving semi-preparative HPLC (using C-4 reverse-phase column). A series of partially purified isolates have been obtained. Isolation of pure components from these has been a major challenge. Currently, we are using analytical C-4 reverse phase column to isolate pure compounds. So far, two compounds (**X** and **XI**) have been isolated and characterised, and are being assayed.



Two structural variants have so far been shown to be associated with post-germination activities: a moderately polar *O*-methylated isoflavanone (**II**) and a polar diglycosyl flavone (**III**). In all probability the most active ACF1 fraction of acetone extract also contains a series of glycosylated flavones or isoflavanones. The minor difference in their polarity has made their separation a very challenging task. We are currently deploying a combination of semi-preparative and analytical HPLC using C-4 bonded silica columns to build up enough stock of target compounds for spectral analysis and bioassays.

16. Commercial production of *Desmodium* seed

The Western Seed Company Limited in Kitale, Kenya in collaboration with ICIPE and its partners is undertaking a major drive for commercial seed production through smallholder farmers and local community groups. Farmers and communities were asked to select participating farmers and groups interested in income generation through seed production to become contract seed producers for the seed company. A planning workshop, attended by farmers' representatives, frontline extension staff and area chiefs was organised by Western Seed Company in collaboration with ICIPE, Rothamsted Research and KARI to lay down strategies for implementing the project. In-depth training to participating farmers from each location in cultivation of silverleaf, *D. uncinatum*, and greenleaf, *D. intortum*, and processing of seed materials was offered.

In the second year of the commercialisation of the seed project (2004), the seed company provided 250 g seed to each of 600 trained farmers in two districts of Kenya (Bungoma and Trans Nzoia) for planting. The Western Seed Company guaranteed it would purchase the *desmodium* seed from

the 600 contract farmers. The company cleaned the seed, checked germination and viability, and properly packed it. In 2005 Western Seed produced 3 tonnes of high quality desmodium seed. The company will continue purchasing desmodium seed from the contract farmers and cleaning and packing them for sale at an affordable price.

The seed company also checks germination viability of the seeds from time to time. Depending on demand, the seed company will expand the number of participating farmers. The Kenya Plant Health Inspectorate Service (KEPHIS) is responsible for quality control of the seed and planting material. Rothamsted analyses the airborne volatiles of these plants from selected samples from time to time using GC and GC-MS to establish that the quality of the product is maintained in terms of appropriate production of semiochemicals. Behavioural studies with stemborer females and parasitoids are also being conducted to ensure that the plants continue to produce volatile chemicals to repel stemborers, and that there is no genetic drift in seeds multiplied by farmers. Similarly, quality control of the striga-inhibiting effects of desmodium seed samples are being undertaken by ICIPE.

During the last five years two other pathways for propagation of desmodium have emerged: vegetative propagation among smallholder farmers and community-based seed production initiatives. These could evolve into important mechanisms for horizontal diffusion of the 'push-pull' technologies among resource-limited farming communities.

Future outlook

The habitat management 'push-pull' project is quite unique in the way that it has developed from the basic science to technology transfer, to farmer take-up, and spontaneous technology transfer between farmers. Although the experience to date has been restricted to maize-based farming systems and on-station sorghum-based farming systems, we believe that the general approach is applicable to a much wider range of pest problems, in a variety of crops (such as millet) and will be a model for other researchers in their efforts to minimise pest-induced yield losses in an economically and environmentally sustainable manner.

The 'push-pull' approach described here is now expanding into Kenya, Uganda and Tanzania. In Kenya, a farmer teacher programme has been initiated, where each trained farmer has to teach 5 new farmers each year. Sasakawa Global 2000, an international NGO, is helping to expand the 'push-pull' programme in Ethiopia. A pilot programme had been initiated in southern Africa, addressing stemborer control in the arid and semi-arid areas of the Northern Province of South Africa. Each region has, in addition to varying climate conditions and use of alternative cultivars, and some differences in crops that must be taken into account, gained considerable experience in this aspect by the pilot studies in various countries. However, wherever these approaches are developed for the specific needs of local farming practices and communities, it is essential that the scientific basis of the modified systems should be completely elucidated, otherwise there might be a drift from effectiveness to justifiable dissatisfaction on the part of the practising farmers. Every effort will be made to ensure that technology transfer follows the incorporation of these practices into other regions of Africa.

The 'push-pull' project is expanding in eastern Africa via smallholder farmers. However, the major constraint to widespread technology transfer has been availability of desmodium seed. Several pathways have emerged including involvement of a private seed company, community-based seed production, and vegetative propagation among farmers adopting 'push-pull' technologies. The relative merits of these pathways in stimulating autonomous diffusion of the technologies need to be analysed and compared. In addition, the role of different reinforcing interventions such as mass media, information bulletins, field days, farmer teachers, farmer field schools etc. need to be evaluated and the most cost-effective ones identified. The relationship between household socioeconomic status and land-labour ratio in different districts, and the performance of different diffusion mechanisms needs to be clarified.

Several new science-led maize production and protection technologies (IR maize, Bt maize and QP maize) have been developed by other research institutes, the effectiveness and sustainability

of which need to be compared with 'push-pull' strategies over a longer time scale. Questions relating to potential integration of these technologies or their complementarities have been raised and need to be evaluated in continued collaboration with other centres. Demonstrations of the relative productivity of integrated approaches and their socioeconomics, including possibility of forward linkages, will be an important objective of the future project as well as collaborative undertakings with other institutions.

For long-term sustainability of the 'push-pull' system and its placement on a strong scientific foundation, there is a need to: (i) develop tools for quality control of the performance of the 'push' and 'pull' components, (ii) enhance understanding of soil nutrient dynamics in long-term 'push-pull' fields, and (iii) study and solve emerging problems of a previously unrecognised pest (a pollen beetle attacking desmodium) and a disease of the companion crops (phytoplasma disease of Napier grass). Studies in these areas will be undertaken and tools that emerge will be optimised and incorporated into the 'push-pull' dissemination activities.

Output

Journal articles

Gohole L. S., Overholt W. A., Khan Z. R. and Vet L. E. M. (2005) Close-range host searching behavior of the stemborer parasitoids *Cotesia sesamiae* and *Dentichasmias busseolae*: Influence of a non-host plant *Melinis minutiflora*. *Journal of Insect Behavior* 18, 149–169. (Call No. 05-1801)

Studies were conducted on the host searching behavior of the larval parasitoid *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae) and the pupal parasitoid *Dentichasmias busseolae* Heinrich (Hymenoptera: Ichneumonidae), both of which attack lepidopteran (Crambidae, Noctuidae) cereal stemborers. The behavior of *D. busseolae* was observed in a diversified habitat that consisted of stemborer host plants (maize, *Zea mays* L. and sorghum, *Sorghum bicolor* (L.) Moench (Poaceae)) and a non-host plant (molasses grass, *Melinis minutiflora* Beauv. (Poaceae)), while *C. sesamiae* was observed separately on host plants and molasses grass. In previous olfactometer studies, *C. sesamiae* was attracted to molasses grass volatiles while *D. busseolae* was repelled. The aim of the present study was to investigate the influence of molasses grass on close-range foraging behavior of the parasitoids in an arena that included infested and uninfested host plants. *Dentichasmias busseolae* strongly discriminated between host and non-host plants, with female wasps spending most of the time on infested host plants and least time on molasses grass. Likewise, *C. sesamiae* spent more time on uninfested and infested host plants than it did on molasses grass in single choice bioassays. While on infested plants, the wasps spent more time foraging on the stem, the site of damage, than on other areas of the plant. Overall, the results indicate that presence of the non-host plant does not hinder close range foraging activities of either parasitoid.

Midega C. A. O., Khan Z. R., Van den Berg J. and Ogol C. K. P. O. (2005) Habitat management and its impact on maize stemborer colonization and crop damage levels in Kenya and South Africa. *African Entomology* 13, 333–340. (Call No. 05-1905)

We carried out an assessment of the impact of a diversionary maize stemborer management system on pest colonization, crop damage levels and crop yields. In this system, ovipositing moths are repelled by an intercrop and subsequently attracted to a discard perimeter crop. Studies were conducted at two sites in western Kenya and one site in South Africa. Treatments comprised two fields of maize intercropped with desmodium, and a Napier grass perimeter ('push-pull' system) and two fields of maize monocrop. Treatments were laid out in a completely randomized design at each site. Maize stemborer colonization, oviposition preference and incidence of stemborer larvae and pupae were significantly lower in 'push-pull' plots than maize monocrop plots at all sites. Similarly, the various crop damage levels were for most part significantly higher in maize monocrop than 'push-pull' plots. Maize yield per plant and per plot were mostly significantly higher in 'push-pull' than maize monocrop plots.

Mohamed M. H., Khan Z. R., Overholt W. A. and Elizabeth D. K. (2004) Behaviour and biology of *Chilo partellus* (Lepidoptera: Pyralidae) on maize and wild gramineous plants. *International Journal of Tropical Insect Science* 24, 287–297. (Call No. 04-1768)

The ovipositional response, larval orientation, larval settling, feeding, food assimilation, growth and development of maize stemborer *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) on a susceptible



maize (*Zea mays* L.) genotype (Inbred A) was compared to five wild plant species of the family Poaceae (Gramineae) in the laboratory and greenhouse. The intensity of oviposition differed among the test plants. *Pennisetum purpureum* Schumacher was the most preferred test plant for oviposition when offered in a two-choice situation with maize. Likewise, *P. purpureum* and *Sorghum versicolor* Andersson were the most preferred test plants for oviposition when offered in multiple-choice tests. In no-choice tests *C. partellus* responses to the test plants were not significantly different. The number of first instar larvae that settled on leaf cuts of test plants in Petri dishes was significantly higher on maize and *S. versicolor* than on the other test plants at 24 h after infestation in a multiple-choice test. No significant differences were observed in larval settling among wild grasses at 4 h and 24 h after infestation in a no-choice test. In a two-choice test there was no significant difference in the number of neonate larvae that settled on maize and *S. versicolor* at 1 h and 24 h after infestation. Feeding by fourth-instar larvae was significantly higher on maize than on *Echinochloa pyramidalis* (Lam.) and *Hyparrhenia rufa* (Nees) Stapf. Larvae fed on maize assimilated significantly more food than those fed on *E. pyramidalis*, *Panicum maximum* Jacq. or *H. rufa*. Larval growth and development was significantly faster on maize and *S. versicolor* in comparison to other test plants.

Book chapter

Khan Z. R. and Pickett J. A. (2004) The 'push-pull' strategy for stemborer management: A case study in exploiting biodiversity and chemical ecology, pp. 155–164. In *Ecological Engineering for Pest Management: Advances in Habitat Manipulation for Arthropods* (Edited by G. M. Gurr, S. D. Wratten and M. A. Altieri). CSIRO, Australia and CAB International, UK.

Research proposal

Promotion and dissemination of integrated pest and soil fertility management strategies to combat *Striga*, stemborers and declining soil fertility in the Lake Victoria basin (2005–2006). Funded.

Capacity building

PhD students

Charles Midega (Kenya) Impact of a habitat management system and Bt-maize on stemborer natural enemies and biodiversity of ground-dwelling arthropods and soil fauna in Africa. Kenyatta University, Kenya (completed).

Esther Njuguna (Kenya) Push-pull technology for the control of stemborers in the Lake Victoria region: Hidden impacts on the farming systems. University of Nairobi, Kenya (ongoing).

Lefulesele Lebesa (Lesotho) Towards development of traps for the control of blister beetles on desmodium: Studies on volatile profiles of the host plant and pests, and their influence on beetle behaviour. University of Pretoria, South Africa (ongoing).

Impact

- Over 3500 'push-pull' farmers have at least doubled their maize yields and increased milk production by 50%.
- Fodder production by 3000 'push-pull' farmers contributes in production of 1.5 million litres of milk annually.
- More than 600 smallscale farmers produce desmodium seed for income generation and are linked to a private seed company.
- Extra income from 'push-pull' fields has helped at least 500 farmers to send one child to a secondary school.
- By the end of 2009 at least 15,000 farmers will benefit from the 'push-pull' strategy.
- At least 3 tonnes of certified desmodium seed will be produced annually by 1000 smallscale farmers.