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Development of sex pheromone traps for monitoring the legume podborer, *Maruca vitrata* (F.) (Lepidoptera: Pyralidae)

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Abstract

The Natural Resources Institute (NRI) and the International Institute of Tropical Agriculture (IITA) are collaborating to develop sex pheromone traps as monitoring tools for *Maruca vitrata*. The principal component of the pheromone is (*E,E*)-10,12-hexadecadienal. Our trapping experiments in cowpea fields in Benin have shown that the optimal synthetic blend also contains small amounts of (*E,E*)-10,12-hexadecadien-1-ol and (*E*)-10-hexadecenal. Polyethylene vial lures containing 0.1 mg of pheromone attracted more males than other combinations of dose or dispenser. Lures showed no loss of effectiveness for up to four weeks in the field. A water-trap made from a plastic jerry can was superior to commercial funnel- and sticky-trap designs and 120 cm was the optimum height for traps. Females comprised up to 50% of total catches with synthetic lures, though almost none were attracted to traps baited with live females. Preliminary observations indicated a temporal coincidence between catches in traps placed outside cowpea fields and the appearance of larvae in fields a few days later. Thus pheromone trap catches may predict larval infestations.

Introduction

The legume podborer, *Maruca vitrata* (F.) (syn. *M. testulalis*) (Lepidoptera: Pyralidae), is a pantropical pest of legume crops, particularly cowpea (Jackai 1995), pigeonpea (Shanower et al. 1999), and beans (Abate and Ampofo 1996). In West Africa without control measures, flower infestation rates by *M. vitrata* of up to 80% were reported by Afun et al. (1991) and seed damage rates of 50% by Dreyer et al. (1994).

Although the basic biology of *M. vitrata* has been studied extensively (Taylor 1967; Singh et al. 1990; Jackai et al. 1990; Onyango and Ochieng-Odero 1993), much remains to be understood concerning the behavior of this pest in the field, which has hindered development of IPM strategies in Africa (Jackai 1995) and Asia (Shanower et al. 1999). Pheromone-baited traps for *M. vitrata* could provide tools for monitoring the activity and movements of adults that would assist researchers in this respect. Bottenberg et al. (1997) provided some data relating to the population dynamics and migration of *M. vitrata* at three locations in West Africa, based on light trap catches. However, light

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traps are difficult and expensive to maintain, and catches of the target species have to be sorted from among other insects. Pheromone traps could be deployed more easily, cheaply, and in greater numbers than light traps. Moreover, pheromone traps are specific for the species of interest. Effective traps could provide simple alternatives to pest scouting by farmers or extension workers to time application of control measures.

Adati and Tatsuki (1999) recently reported (*E,E*)-10,12-hexadecadienal (EE10,12-16:Ald) to be an EAG-active component of the extract from female *M. vitrata* abdominal tips. Synthetic EE10,12-16:Ald was shown to be attractive to male moths in laboratory bioassays. The corresponding alcohol, EE10,12-16:OH, was also noted as being present at 3–4% of the aldehyde. Although no behavioral data were presented in relation to this compound, it was said to produce no increase in attraction. No field testing of the compounds was carried out. Previous analytical work carried out by the Natural Resources Institute (NRI) has confirmed the presence of EE10,12-16:Ald and EE10,12-16:OH as major and minor blend components, respectively. Results also suggested the presence of a third pheromone component, and subsequent laboratory and field bioassays indicated this was probably (*E*)-10-hexadecenal (E10-16:Ald) (Downham and Hall 2000, NRI unpublished data). Cross mating and cross attraction experiments with strains from Benin, India, Nigeria, Sri Lanka, and Taiwan, together with analysis of gland extracts from females of these strains, indicated that there is no geographic variation in the natural pheromone blend of *M. vitrata*.

In this paper we report the first successful field trapping experiments that used a blend of these compounds to catch *M. vitrata*. Experiments to develop further a practical trapping system are also reported.

Materials and methods

Trap and lure optimization experiments

Trapping experiments were carried out during 1998 and 1999 within cowpea fields at the IITA research station near Cotonou, Republic of Benin. Unless otherwise noted, traps were suspended from sticks using wire at a height of approximately 1.0–1.2 m. Synthetic lures were replaced every two weeks and, unless otherwise noted, they were shielded from sunlight to minimize isomerization by aluminum foil wrapped around them to leave only the lowermost surface exposed. The isomeric purity of pheromone components EE10,12-16:Ald, EE10,12-16:OH, and E10-16:Ald used in lures was > 99%. Trap catches were counted daily and trapped moths discarded at that time. Insecticides were not sprayed in the fields.

In the first experiment, four pheromone blends were evaluated. These were EE10,12-16:Ald alone or in combination with one or both of the two minor components, EE10,12-16:OH and E10-16:Ald, both of which were present at a level of 5% relative to the EE10,12-16:Ald. These synthetic blends were presented in polyethylene vial and rubber septa dispensers as lures in white, sticky, delta traps (Agrisense-BCS, Pontypridd, UK). For each of these, two doses, 0.01 mg or 0.1 mg, were compared, making 16 treatment combinations. These were compared with traps containing two virgin females confined to small wire-mesh cages and with unbaited controls. Females were two days old when placed in traps and were replaced every two days. Sticky card inserts in delta traps were replaced on a weekly basis. The experiment consisted of three cowpea fields, forming replicate blocks; in each, traps were positioned in a grid formation with 10-m spacing.

Four subsequent experiments included a comparison of lure age and shielding, two of different trap designs and one of trap height. The lures used in each of these were 0.1-mg polyethylene vials. Sticky, delta traps (Fig. 1a) were used in the lure age and shielding experiment; green plastic funnel traps (Fig. 1b) (Agrisense-BCS, Pontypridd, UK), with DDVP insecticide strips to kill trapped moths, were used in one trap design experiment and the trap height comparison. Four water-trap designs (Figs. 1c–1f), each constructed from cheap, locally obtained materials, were also evaluated in the trap design comparisons. A water-pan (Fig. 1c) trap was made from a green plastic bowl (20-cm diameter) and plate held 5 cm apart with steel wire. Others were made from a 1.5-liter clear plastic bottle (Fig. 1d) and 2-l and 5-l white plastic jerry cans (Figs. 1e, 1f) in which four windows had been cut from the sides. Lures were suspended within the center of each trap. A little soap powder was added to the water within each trap to reduce surface tension, and vegetable oil to reduce evaporation. In the lure age and shielding experiment, shielded and unshielded lures were pre-aged for two or four weeks before use by exposing them in sticky, delta traps. Each experiment was carried out to a randomized complete-block design with five replications. Traps within a replicate block were set out in lines or rectangular formations, the exact layout depending on the number of treatments being compared. Individual traps were positioned 20 m apart. Blocks were at least 50 m apart and were usually situated in separate fields.

During the trapping experiments, it is possible that there were some interactions between traps within replicate blocks. This may have occurred as individual pheromone plumes overlapped and moths, initially attracted by the plume of one trap, passed on to the plumes of other traps. This would have acted to blur treatment differences. However, the random positioning of treatments within blocks and night-to-night variation in wind direction would have meant that no systematic biases occurred.

For statistical analysis the total catches by each trap over the respective trapping periods were used. With the blend experiment and the lure age/shielding experiment, analysis involved the raw data, since these met the normality and constant variance assumptions. However, it proved necessary to transform data of the trap height (square root) and trap design ($\log_{10}[\times + 1]$) experiments. Analysis of variance was carried out using Genstat 5 for Windows© (release 4.1). Where this indicated statistically significant effects, treatment means were separated using the least significant difference (LSD) at the 5% level.

Observations relating pheromone trap catches to light trap catches and larval infestations

IITA operates a light trap (500 W mercury-vapor bulb) at its Cotonou station. During the relevant period this was situated several hundred meters from any experimental cowpea fields. Catches of *M. vitrata* were recorded on a daily basis and compared to those in pheromone traps forming part of the trap and lure optimization experiments. Weekly inspections for larvae were carried out in the fields containing traps from the optimization experiments; all individuals on four randomly selected plants per field were counted. On 22 September 1998, before the second cropping season began, a ring of 20 sticky, delta pheromone traps was established around the perimeter of the IITA station. These traps were baited with polyethylene vial lures containing 0.1 mg of the 3-component blend (100:5:5 ratio). They were placed 150 m apart and at least 80 m from the nearest cowpea field. Data from these traps were also compared to the light trap and other data.

Figure 1a. Sticky delta trap.

Figure 1b. Agrisense-BCS funnel trap.

Figure 1c. Water-pan trap.

Figure 1d. Plastic bottle water trap (1.5 liters).

Figure 1e. Two-liter bottle water trap.

Figure 1f. Five-liter jerry can water trap.

In a separate set of observations, conducted from mid-January to mid-March 2000, two pheromone traps (5-l jerry can design—see trap design experiments) were monitored three times/week in each of 10 on-farm plots. Plots were situated in the villages of Agonguey, Agbonou, and Wosounmé in the Ouémé valley in southeast Benin. The nearby river allows cowpea to be grown here in what is the off-season elsewhere in Benin. The size of plots varied from 400 to 600 m^2. Plots were not treated with insecticides but were situated within larger blocks that were treated. The cowpea variety in each case was Chawé Daho, which has a growing season of 90 days. Sowing dates were 30 November–15 December 1999, flowering commenced 9–25 January 2000, and harvest was 25 February–5 March 2000. At weekly intervals from 19 January to 21 February, 20 flowers per plot were inspected for the presence of larvae.

Results

Trap and lure optimization experiments

In the pheromone blend experiment, traps baited with lures containing all three of the proposed components, EE10,12-16:Ald, EE10,12-16:OH, and E10-16:Ald, caught significantly more male *M. vitrata* moths than those baited with one or two component blends or live females ($P < 0.05$), all of which attracted similar numbers of male moths. No males were captured in unbaited control traps (Table 1). Although the polyethylene vial dispensers loaded with 0.1 mg pheromone attracted slightly more males than other combinations of dose or dispenser (Table 2), there was no significant overall effect of dispenser type or dose ($P > 0.05$ LSD). About 20% of total catches in traps baited with synthetic lures were female moths although almost no females were attracted to live females or unbaited controls. The trends in respect of different blends, doses, and dispensers were similar to those for males (Tables 1 and 2).

When the attractiveness of lures of different ages was compared, separate analyses of variance indicated highly significant effects in respect of captures of both sexes ($P < 0.01$) (Table 3). Four to six-week-old lures were significantly less attractive to males than 0–2 and 2–4-week-old lures ($P < 0.05$); in respect of females, 0–2-week-old lures were significantly more attractive than both older sets of lures ($P < 0.05$). Analyses of variance showed that male captures were not influenced by shielding of the lures ($P = 0.75$), but female captures with shielded lures were significantly greater than with unshielded lures ($P < 0.01$). Captures of female moths made up 14% of the total in this experiment.

Table 1. Mean catches/trap of *M. vitrata* in the blend experiment at IITA, Cotonou, Benin, June–August 1998 (catches for synthetic blends averaged across dose and dispenser type).

Lure or ratio of components[†]	Males[‡]		Females[‡]	
	Mean	SE	Mean	SE
100:0:0	7.0 bc	1.4	1.3 cd	0.4
100:5:0	5.3 c	0.9	1.8 bc	0.4
100:0:5	8.9 b	1.1	2.9 b	0.6
100:5:5	33.1 a	2.4	5.3 a	0.9
2 × virgin females	5.8 c	0.8	0.2 d	0.1
Blank, control	0.0 d	0.0	0.0 d	0.0

[†](E,E)-10,12-16:Ald: (E,E)-10,12-16:OH: (E)-10-16:Ald.

[‡]Means within a column followed by a common letter were not significantly different (*P* > 0.05, LSD following ANOVA).

Table 2. Mean catches per trap of *M. vitrata* with synthetic dispensers in the blend experiment at IITA, Cotonou, Benin, June–August 1998 (catches averaged across different blends).

Lure dose/dispenser type	Males[†]		Females[†]	
	Mean	SE	Mean	SE
0.01 mg vials	12.3	3.3	2.5	0.5
0.1 mg vials	16.8	4.2	2.7	0.6
0.01 mg septa	13.3	3.4	3.2	0.9
0.1 mg septa	12.0	3.8	3.0	1.0

[†] There was no significant effect of dose or dispenser type on male or female catches (*P* > 0.05, F-ratio ANOVA).

Table 3. Mean catches/trap of *M. vitrata* with lures of different ages, shielded or not shielded from sunlight, at IITA, Cotonou, Benin, August–November 1999.

Lure age (weeks)	Shielding Yes/No	Males		Females	
		Mean	SE	Mean	SE
0–2	Yes	11.8 a	1.0	3.6 a	0.8
"	No	12.0 a	2.0	1.8 b	0.6
2–4	Yes	11.4 a	1.5	1.6 b	0.7
"	No	9.8 a	1.9	0.8 b	0.2
4–6	Yes	5.0 b	0.8	1.4 b	0.4
"	No	7.6 ab	1.4	0.4 b	0.2

In both trap design experiments, significant treatment effects were observed ($P < 0.05$). In the first comparison, the sticky, delta trap attracted the fewest moths of both sexes (Table 4). Three to four times more males were captured by the Agrisense-BCS funnel trap than the delta trap, but the locally constructed water-pan trap was most effective in capturing females (three times more than the delta trap). The sticky, delta trap was also less effective than two other locally constructed water traps in the second experiment. In this experiment, for both sexes, the 5-l and 2-l jerry can designs proved superior to both the delta trap and the 1.5-l bottle design (Table 5). Overall percentage captures of females in the two experiments were 46% and 35%.

The trap height experiment indicated that 120 cm was optimal in respect of catches of males (Table 6). Mean catches of males at this height were significantly greater than at 20 and 170 cm ($P < 0.05$), though not at 70 cm. Overall catches of females were around 11% of the total, and there were no significant differences in respect of trap height.

Table 4. Mean catches/trap of *M. vitrata* in the first trap design experiment at IITA, Cotonou, Benin, October–December 1998.

	Males		Females	
Trap design	Mean	SE	Mean	SE
Sticky, delta	3.0 b	1.6	3.2 b	1.5
Water-pan	7.6 ab	3.4	9.0 a	3.5
Funnel	11.0 a	4.0	6.4 ab	2.7

Table 5. Mean catches/trap of *M. vitrata* in the second trap design experiment at IITA, Cotonou, Benin, September–November 1999.

	Males		Females	
Trap design	Mean	SE	Mean	SE
5-l jerry	13.0 a	1.8	7.4 a	1.3
2-l jerry	10.8 a	2.0	6.0 ab	1.7
Sticky, delta	4.0 b	0.8	1.4 c	0.5
1.5-l bottle	5.0 b	1.1	2.8 bc	0.6

Table 6. Mean catches/trap of *M. vitrata* at different heights aboveground at IITA, Cotonou, Benin, July–October 1999.

	Males		Females	
Height (cm)	Mean	SE	Mean	SE
20	5.6 bc	1.2	0.2 a	0.2
70	6.8 ab	0.6	1.4 a	0.4
120	10.4 a	1.4	0.6 a	0.4
170	3.4 c	1.3	1.2 a	1.0

Observations relating pheromone trap catches to light trap catches and larval infestations

Catches in the light trap were always much greater than in individual pheromone traps, and while males predominated in pheromone trap catches, females tended to form the majority in the light traps. Within each type of trap, temporal patterns of catches of each sex were similar, so that catches of one sex accurately reflected the presence of the other.

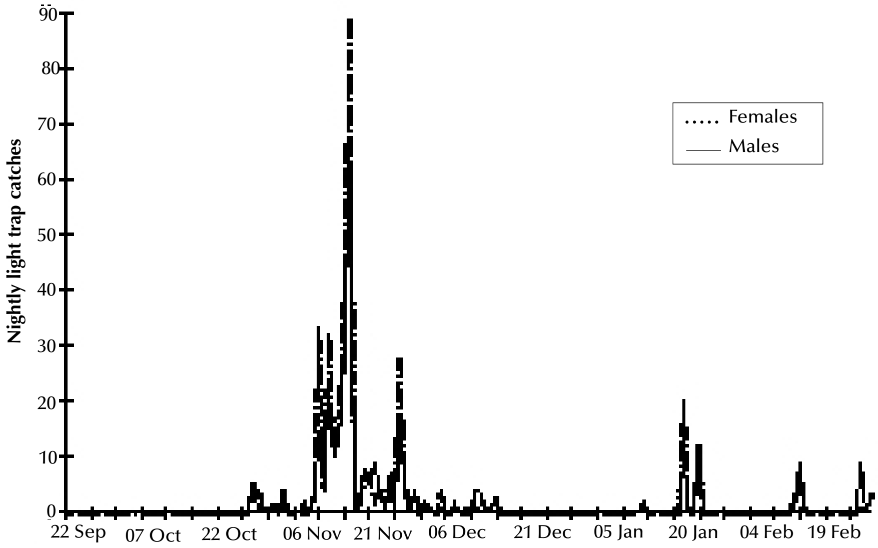
During two seasons of on-station trials at IITA-Cotonou, a general observation was made that the timing of catches in the light trap and those in pheromone traps within fields did not correspond closely. However, there was a better temporal correspondence between the light trap catches and those in perimeter traps (Fig. 2). This was notable at the start of the second season of 1998. Following several weeks of zero catches in the light trap and the perimeter pheromone traps, the latter detected the first small peak of moths at exactly the same time (29 October) as the light trap, although there appeared to be little subsequent quantitative correlation.

During this period, the first appearance of moths in traps in cowpea fields was at least 12 days after catches were first noted in the light trap and perimeter pheromone traps. These initial within-field catches were 33–50 days after the fields were sown. The first crop inspection, eight days after the initial catches in perimeter traps, showed that larvae were already present in each of three fields sampled at that time; but this was several days before within-field catches in two of the fields and simultaneous with the first catches in a third. Representative data for one field are shown in Figure 3 and can be compared to Figure 2. Trap catches within fields in the second season were confined to periods of 8–12 days.

Results from the on-farm observations in the Ouémé valley are summarized in Figure 4. Although overall catches were relatively low—rarely exceeding an average of 0.5 moths per trap per count—the timing of their onset across all plots was consistent. In eight of the 10 plots, the first catches were noted on 28 January, while first catches occurred in the remaining plots on the subsequent count three days later. Catches were evenly distributed across all 10 plots and three village sites until the end of February, when they began to decline. Males and females were trapped in approximately equal numbers. Larvae were only found on two dates: 9 and 14 February. On the first occasion they were noted in four plots, on the second they were observed in seven plots. Since some of the larvae were late instars it is probable that eggs were laid five to ten days after the first adults were trapped.

Discussion

From the results of the trap and lure optimization experiments an effective and practical trapping system for *M. vitrata* has now been developed for the first time. The best pheromone blend is a mixture of EE10,12-16:Ald, EE10,12-16:OH, and E10-16:Ald in the ratio 100:5:5. Although no significant differences were evident in respect of dose or dispenser, the 0.1-mg polyethylene vials would be expected to show the greatest longevity of the lures tested on the basis of dose and release rate characteristics. Our results indicate no loss of attractiveness for up to four weeks under field conditions. Therefore, these lures have now been adopted as standard for use in further work. The best trap height is 120 cm and the most effective traps are those produced from locally available plastic jerry cans. Not only are these relatively much cheaper than imported, commercial designs (US$0.30–0.80 as



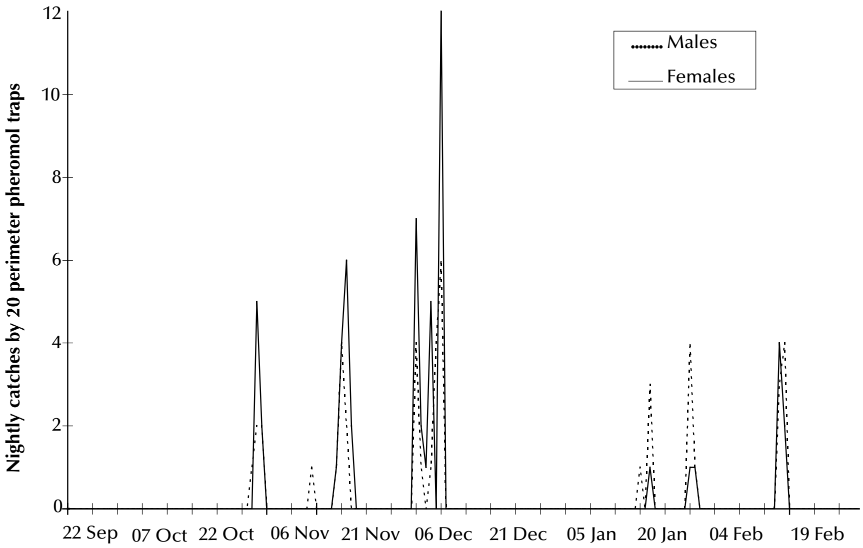


Figure 2. ***M. vitrata*** **catches in a light trap (top) and in 20 pheromone traps in noncrop areas around the perimeter of the IITA station (bottom) during and after the second cropping season in 1998.**

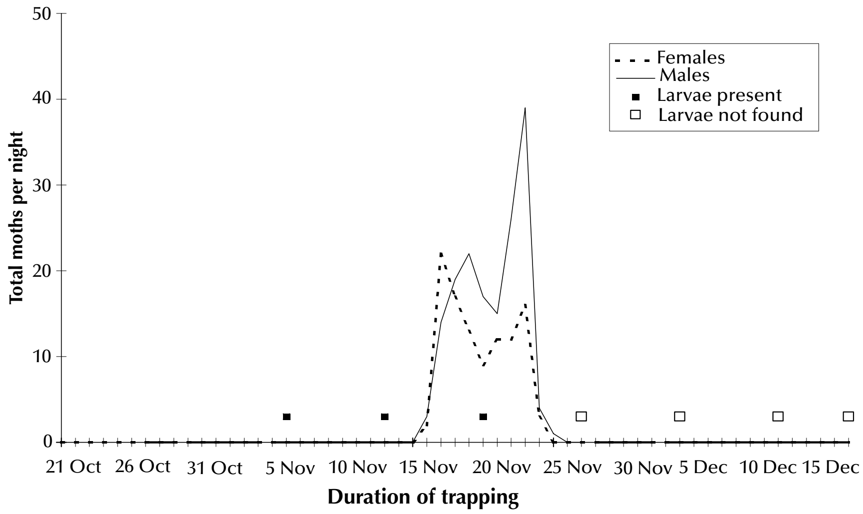


Figure 3. Total catches of *M. vitrata* by seven pheromone traps in field C3, forming one block of a pheromone blend experiment during the second cropping season in 1998.

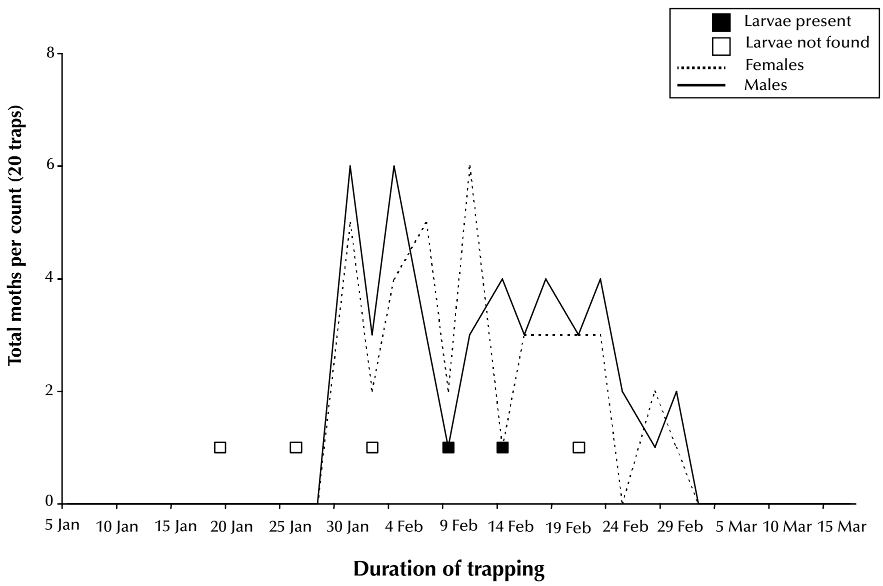


Figure 4. Total catches of *M. vitrata* by 20 pheromone traps in 10 on-farm plots in the Ouémé valley, Benin, 2000.

against approximately US$3.00 for sticky, delta traps and more for plastic funnel traps), they are easy to construct and robust in use.

Further work on trap and lure optimization is required. Experiments are planned to determine the effect of trap color and frequency of checking on trap catches. An experiment concerning isomeric purity of the pheromone blend components is currently underway and the results will be of particular relevance. If a lower level of purity can be used without a marked loss of attraction, it will be possible to reduce the cost of lure production to around US$0.50 per lure. This will be important in helping to ensure the economic viability of pheromone trap monitoring of *M. vitrata*.

The consistent capture of significant numbers of female moths with synthetic sex pheromone lures is, to the best of our knowledge, unprecedented. It could suggest an incomplete pheromone blend, but extensive analytical work with the natural pheromone, to be reported elsewhere, has failed to find any evidence for further blend components (M.C.A. Downham and D.R. Hall, unpublished data). Furthermore, incomplete pheromone blends generally produce lower catches of males, rather than co-attraction of both sexes. Thus a better explanation may lie in some unknown aspect of the species' mating behavior or ecology, and further work to explain the phenomenon would be very helpful. Regardless of explanation, catches of females may actually improve the predictive power of traps, since they should more accurately reflect local population events than males alone.

With regard to the practical use of traps for monitoring purposes, results to date indicate real potential. In the on-station trials at Cotonou, catches in pheromone traps outside cowpea fields preceded larval infestations in fields; in contrast, catches in traps within the fields occurred only after larvae appeared. Thus it seemed that traps near but outside fields might be better able to predict pest attacks. However, a subsequent trial at a different time of year in farmers' fields suggested that within-field traps could give early warning of larval infestations. Resolving the question of the best positioning of traps to detect immigrating moths will be the task of further work currently in progress at several on-farm sites around Benin, and soon to be extended to Ghana. This work is being carried out in association with the West African regional PRONAF (Projet de Niébé pour l'Afrique) project, a partnership between IITA and various NARS that aims to promote the transfer and implementation of research on cowpea to subsistence farmers. It will be necessary to determine the relationship between larval infestations and catches by pheromone traps with confidence. Whether any good quantitative correlation between catches and damage exists remains to be seen, but it seems likely that pheromone traps could be used as the basis for timing the application of control measures.

Afun et al. (1991) found that by using action thresholds based on larval/flower infestation rates to time insecticide applications on cowpea, the number of sprays could be reduced, relative to a calendar-based approach, with no loss of control and at reduced cost. Ultimately it is hoped that pheromone trap-based action thresholds could be used in conjunction with other promising sustainable control methods being developed through PRONAF, e.g., neem-based insecticides (W. Hammond, pers. comm.; see also Bottenberg and Singh 1996). Furthermore, Bottenberg (1995) found that many farmers are unable to link the adult stage of *M. vitrata* with the highly destructive larval stage. Pheromone traps could also serve a training role by assisting in making up this gap in knowledge.

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