Development of a trap to contaminate variegated grasshoppers (*Zonocerus variegatus* L.) (Orthoptera: Pyrgomorphidae) with *Metarrhizium flavo-viride* Gams & Rozsypal in the field

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ABSTRACT

Locusts and grasshoppers are important pests of agriculture in West Africa. Application of chemical insecticides is the major control mechanism. However, concerns arising from human health problems and environmental hazards have put forward a strong case for biological control. A simple trap incorporating an attractant and a biological control agent, *Metarrhizium flavo-viride*, to contaminate the cassava pest, *Zonocerus variegatus*, is described. The attractant, an extract of the Siam weed, *Chromolaena odorata*, attracted significantly more adults and 5th /6th instars in the field than plant parts of *C. odorata* and cassava leaves presented whole or crushed in distilled water. At a mean *Z. variegatus* field population density of 1.1 m^{-2}, the mean trap catch was 27.2 $trap^{-1}$ day^{-1}. All insects trapped on the first day died from fungal infection within 10 days (LT_{50} = 6.1 days), with the need to replace the pathogen with a freshly prepared one after 7 days in the field. Trapped insects, infected or uninfected, showed a bimodal pattern of exit from the traps with peaks at 09.00 and 17.00 h. The pattern of spread of infected and uninfected insects after exiting from traps were similar. The farthest group mean displacements from traps were 34.2 and 36.3 m in 5 days for infected and uninfected insects, respectively, leading to a suggested trap density of 2 ha^{-1}.

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RÉSUMÉ

ADU-MENSAH, J.: *Développement d'un piège pour contaminer les sauterelles panachées* (Zonocerus variegatus *L.*) *(Orthoptera: Pyrgomorphidae) avec* Metarrhizium flavo-viride *Gams & Rozsypal dans le champ.* Locustes et sauterelles sont des insectes ravageurs importants de l'agriculture en Afrique de l'ouest. L'application des insecticides chimiques est le mécanisme de contrôle majeur. Toutefois, les inquiétudes provenant des problèmes de la santé sociale et les risques pour l'environnement avaient provoqué des arguments en faveurs de contrôle biologique. Un piège simple contenant un attractif et un agent de contrôle biologique, *Metarrhizium flavo-viride* pour contaminer l'insecte ravageur de manioc, *Zonocerus variegatus*, est décrit. L'attractif, un extrait de herbe Siam, *Chromolaena odorata*, attirait considérablement plus d'adultes et les stades 5e/6e dans le champ que les parties de la plante de *C. odorata* et les feuilles de manioc présentées intactes ou moulues dans l'eau déminéralisée. A un moyen de *Z. variegatus* la densité de population de champ de 1.1 m^{-2}, la prise de piège moyenne était 27.2 $piège^{-1}$ $jour^{-1}$. Tous les insectes pris au piège le premier jour mouraient d'infection fongique dans 10 jours (LT_{50} = 6.1 jours) avec le besoin de remplacer la pathogène avec une autre fraîche préparée après 7 jours dans le champ. Les insectes pris au piège infectés ou non infectées montraient un modèle bimodal de sortie des pièges avec les heures de pointe à 09.00 et 17.00 h les caractéristiques de dispersion des insectes infectés et non infectés après la sortie des pièges étaient semblables. Les déplacements moyens du groupe le plus éloigné de pièges étaient 34.2 et 36.3 m en 5 jours respectivement pour les insectes infectés et non infectés, menant une suggestion de la densité de piège de 2 ha^{-1}.

Introduction

Variegated grasshoppers, *Zonocerus variegatus* L., are among the most important pests of agriculture in West Africa (Geddes, 1990). Outbreaks are controlled by large scale applications of chemical insecticides. Integrated pest management (IPM) techniques still rely heavily on chemical spraying as the major control mechanism (Cunningham, 1992). The pesticides used have been predominantly non-persistent organophosphorus compounds. The strategy is to target nymphs and adults by aerial blanket spraying of large areas (Prior & Streett, 1997). This has led to concerns caused by human health problems, environmental damage, and high cost of control operations. Concerns about the detrimental effects would best be addressed by improving the safety aspect, which puts forward biological control as a viable alternative for consideration.

Several research organizations, e.g. The International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, The International Institute for Biological Control (IIBC), Ascot, Berks, UK, and Département de Formation en Protection des Végétaux (DFPV), Niamey, Niger, have developed oil formulations of fungal entomopathogens for the biological control of locusts and grasshoppers (Prior *et al.*, 1992). However, fungal spore production, handling and spray application technology may pose problems to small and medium scale farmers in developing countries. African cassava farmers have no history of application of agrochemicals. Thus, a system of trapping, using low-level and low-cost technology, might be an attractive proposition. In a novel approach, a sex pheromone trap has been combined with a fungal pathogen, *Zoophthora radicans* Brefeld., for the biological control of the diamond back moth *Plutella xylostella* L. (Pell, Macaulay & Wilding, 1993).

Nymphs and adults of *Z. variegatus* are attracted to flowers of *C. odorata* and extracts of plants containing pyrrolizidine alkaloids (PAs) (Modder, 1984a). Upwind movement of *Zonocerus* individuals in response suggests a chemical mediation (Boppré, Seibt & Wickler, 1984).

This study aimed at using the attractant effect of an extract of *C. odorata* to attract *Z. variegatus* to a trap loaded with a fungal pathogen, and allowing the eventual exit of the insect to disperse the spores in the immediate environment.

Materials and methods

Description of the trap

A wooden box (30 cm × 45 cm × 15 cm), made from plywood off cuts, was loosely fitted with a roof (30 cm high) of wire mesh covered with polyethylene sheet (Fig. 1). A hole (9 cm diameter) was made on each of the opposite sides of the triangle and fitted with 10 cm of the cap end of a

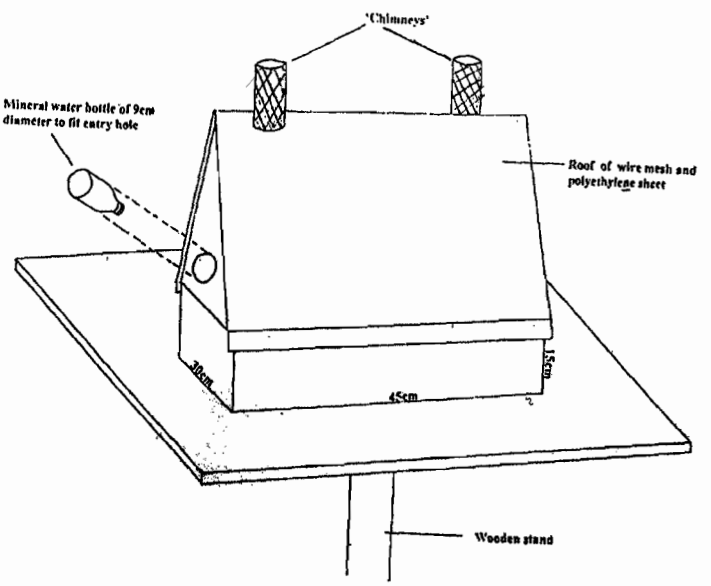

Fig. 1. The trap.

plastic bottle lined with a cloth laden with spores of a fungal entomopathogen, *Metarrhizium flavoviride* Gams & Rozsypal. This arrangement served as a valve to prevent trapped insects from exiting by the same route. Two 'chimneys' of glass tubes (10 cm × 3.5 cm) were pushed through the roof close to the median line. Wire netting was placed inside the tubes to facilitate the exit of trapped insects. Suspended from the center of the roof was a swab of cotton wool. Access to the cotton wool was by shifting the roof to one side. The trap was mounted on a platform supported by a wooden stand of the desired height.

Preparation of the spore-laden cloth

The *M. flavo-viride* isolate IIBC I91-609 used in the traps was taken from an infected *Z. variegatus*, which subsequently died of infection. It was the most virulent of three strains of the fungus tested against the insect with a median-lethal-time (LT_{50}) of 5.4 days at a dose of 10^5 conidia per insect delivered under the pronotum (Adu-Mensah, 1994).

A liquid medium containing 10 g glucose, 20 g brewer's yeast in 1 litre of tap water was autoclaved at 121°C for 20 min. The medium was inoculated with 75 ml of a spore suspension in sterile distilled water of 2-week-old cultures of *M. flavo-viride* at 6×10^6 spores ml^{-1}, and incubated at room temperature for 3 days in 250-ml Erlenmeyer flasks on an orbital shaker at 60 r.p.m. Absorbent cloths (16 cm × 30 cm) were dipped in the blastospore suspension and excess fluid drained. The cloths were hung on sterile glass rods in a tank, enveloped in sterile plastic cover (to maintain humidity), and incubated for 7 days in the dark at room temperature. The neck ends of plastic mineral water bottles (10 cm × 9 cm) were lined with the damp cloths and allowed to dry slowly in a laminar flow chamber for 2 days.

The attractant

The lure, an extract of *Chromolaena odorata,* was supplied by M. Boppré & O. Fischer of University of Frieburg, Germany. In preliminary field tests, 60 nymphs each of 1st/2nd, 3rd/4th, and 5th/6th instars and adults of *Z. variegatus* (field collected) were presented with either two drops of the lure or 5 g each of young *C. odorata* inflorescence, and the third fully expanded *C. odorata* and cassava leaves either whole or crushed in 10 ml distilled water in a no choice situation. Two drops of test samples on cotton wool were presented sequentially to the same group of insects, allowing 30 min in-between samples. Distilled water was presented as control. Materials were exposed for 25 min during which the number of insects attracted was recorded at 5-min intervals. Three replicates were used on different days with new set of insects and samples. The area under a frequency-time polygon was expressed as a ratio to the total area in a hypothetical case where all insects were attracted to a material throughout the duration of exposure. This figure was designated attraction index (AI) for comparison by ANOVA.

Field evaluation of trap

The trap was evaluated on a weil-maintained 1-ha cassava field (3 months old, 1m high) at Fanji in Mono Province, Benin. Population density of *Z. variegatus* (density of cassava plants × mean number of insects per plant) and indices of dispersion (variance of distribution / mean number of insects per plant) (Southwood, 1978) were estimated each day of trapping. Three groups of three traps each were prepared as follows:

Group 1: with two drops of lure on the cotton wool and a spore-laden cloth at the entry point.

Group 2: with two drops of lure on the cotton wool but no spore-laden cloth at the entry point.

Group 3: with no lure but with spore-laden cloth at the entry point.

Nine numbered positions were pre-selected to cover the whole field. The nine traps (with 'chimneys' blocked) were placed randomly on 1-m high platforms. Access to the entry points were provided by sticks placed in the soil at 45°

to the ground, and traps were set for inspection after 24 h. The trapped insects were removed for incubation in the laboratory. The dead insects were then placed in moist chambers to ascertain the causes of death (death caused by fungal infection produces mycosing cadavers after 3 days). Fresh lure was applied to the cotton wool (Groups 1 & 2) and trap positions randomized. The procedure was repeated consecutively for 10 days, with the same spore-laden cloths (Groups 1 & 3). The percentage mortalities of trapped insects were determined for each incubation day from pooled results (Group 1) after corrections for control mortalities (Group 2) by Abbott's formula (Abbott, 1925). Median-lethal-times (LT_{50s}) were computed by probit regression analysis (Finney, 1971).

Exit and dispersal of insects from traps

Two groups each of 150 field-collected adult *Z variegatus* (male to female ratio, short wing to long wing ratio all 1 : 1) were marked with quick-drying red and white enamel paint on the pronotum. One group was infected with the fungus by attracting the insects to a source of lure through a tunnel laden with spores. Both groups were kept for 24 h in traps with exit chimneys blocked and positioned side by side on 1-m high platforms of the same height as the cassava canopy in the center of a field planted at a spacing of 1m × 1m. The exit chimneys were opened at 0.700 h and insects that exited were recorded hourly. After 24 h, the field was examined for infected (white paint) and uninfected (red paint) grasshoppers. The distance from insects (dead or alive) to the trap from which they exited was measured by four pairs of assistants moving in four directions away from the traps. The insects were not disturbed but the exercise continued for 7 days. The experiment was repeated in a different location after 1 month and the results were pooled. Group displacements per day for each group was determined by the formula:

$$\sqrt{\sum (d^2)/n}$$ (Clark, 1962); where

d is displacement of each insect from the trap, and *n* is number of insects

Nutritional state of trapped insects

The gut contents of trapped insects were examined to ascertain the nutritional state of trapped insects and relate same to trap catches. The insects were fixed in 70 per cent alcohol before dissection and placed in one of eight categories according to the distribution of food in the gut: a - foregut only, b - midgut only, c - hindgut only, ab - fore- & midgut only, ac - fore- & hindgut only, bc - mid- & hindgut only, abc - all sections of gut, o - empty gut. The insects were placed in three groups and fat content was scored zero - for absent, 1 - moderate, 2 - abundant.

Results

Comparison of lure with plant parts

There were no significant differences between the attraction indices (AI) of the lure and plant materials tested for the 1st/2nd instars ($F = 2.408$, $P = 0.069$) and 3rd/4th ($F = 2.205$, $P = 0.09$) instars (Fig. 2). For 5th/6th instars, the AI of the lure was significantly higher than all materials offered ($F = 4.823$, $P = 0.004$), except cassava leaves presented whole or crushed. For adults, the AI of the lure was significantly higher than all materials tested ($F = 10.252$, $P = 0.0001$).

Performance of traps

The mean index of dispersion of *Z. variegatus* for the duration of trapping was 7.2 ± 1.1, with a mean population density of 1.1 ± 0.3 m^{-2} (Table 1). Mean trap catches of 27.2 ± 4.3, 23.7 ± 4.5, and 0.09 ± 0.19 trap^{-1} day^{-1} were recorded for traps in Groups 1, 2 and 3, respectively. Only 5th/6th instars and adults were trapped in a ratio male: female 1: 4 (1:6 adults, 1:2 late instars). Phagostimulatory responses were observed during which trapped insects attempted at biting the swab of cotton wool, the source of odour of the lure. All insects trapped on the first day (Group1) died within 10 days, with fungal sporulation on resulting cadavers after

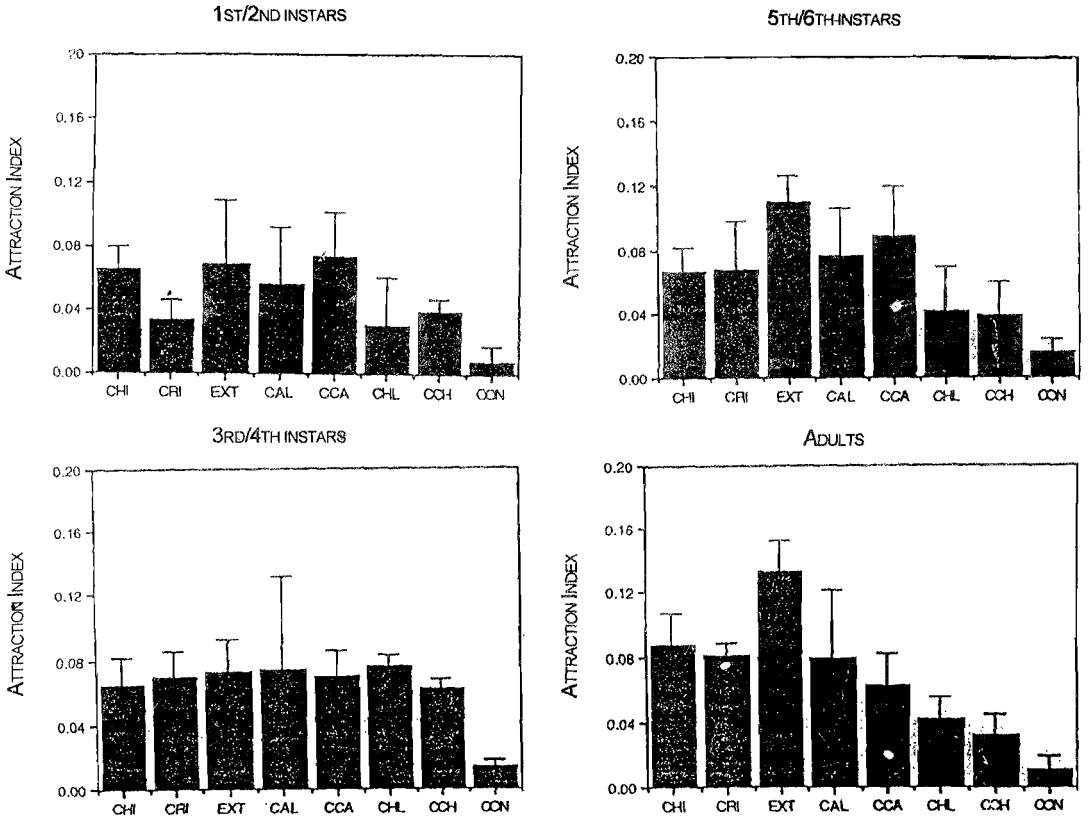

Fig. 2. Attraction indices of extract of *Chromolaena odorata* and plant materials presented whole or crushed in water to adults and nymphs of *Zonocerus variegatus*; CHI - *C. odorata* inflorescence; CRI - crushed inflorescence; CHL - *C. odorata* leaves; CCH - crushed *C. odorata* leaves; CAL - cassava leaves; CCA - crushed cassava leaves; EXT - extract of *C. odorata* (the lure); CON - control (water).

transference into moist chambers (Fig. 3). With the same fungal-laden cloths in traps, LT_{50s} for the first 7 days were between 6.1 and 8.3 days with no significant differences (Table 1). Results for the 8th to the 10th day were significantly higher ($P<0.05$).

Exit and dispersal of infected and control insects

The exit of infected and control-trapped insects showed a bimodal pattern with peaks at 09.00 and 17.00 h (Fig. 4). Group mean displacements per day, which measured the rates of spread of infected and control insects, were also similar, reaching a peak of 34.2 and 36.3 m for infected and control insects, respectively, in 5 days (Fig. 5).

Nutritional state of trapped insects

None of the trapped insects had any food in the foregut only or fore- and hindgut together only (Table 2). The proportion of insects with food in the mid- and hindgut only (66.67 %) was significantly higher ($P \leq 0.05$) than in each of the other categories. Of all the insects trapped, 83.3

TABLE 1

Distribution of Insects and Trap Catches on Each Day of Trapping

Day of trapping	*Mean no. of insects/plant*	*Index of dispersion*	*Insects m^{-2}*	*Catch per trap**			*LT_{50} (days)***
				G1	*G2*	*G3*	
1	16.6	8.1	1.2	26.3	25.0	0.0	6.1 ± 0.2a
2	15.1	5.9	0.8	17.7	20.7	0.0	7.3 ± 0.4a
3	16.0	6.0	0.9	25.0	26.0	0.6	7.1 ± 0.4a
4	14.9	8.4	0.8	30.7	26.0	0.0	7.4 ± 0.5a
5	17.3	6.3	1.1	28.0	19.7	0.0	7.6 ± 0.4a
6	21.2	7.5	1.4	34.7	22.3	0.0	8.3 ± 0.3a
7	17.8	8.8	0.9	26.3	14.7	0.3	7.6 ± 1.1a
8	19.1	6.1	1.6	28.3	25.7	0.0	11.8 ± 1.8b
9	23.7	6.9	1.1	26.7	24.0	0.0	11.5 ± 1.1b
10	14.3	7.5	1.6	28.0	32.7	0.0	11.7 ± 3.1c
Mean	17.6	7.2	1.1	27.2	23.7	0.09	8.6
±	3.0	1.1	0.3	4.3	4.5	0.19	2.0

Figures in a column followed by same letters not significantly different ($P \geq 0.05$).

* Traps in Groups 1, 2 and 3.

** LT_{50}, determined from catches by traps in Group1.

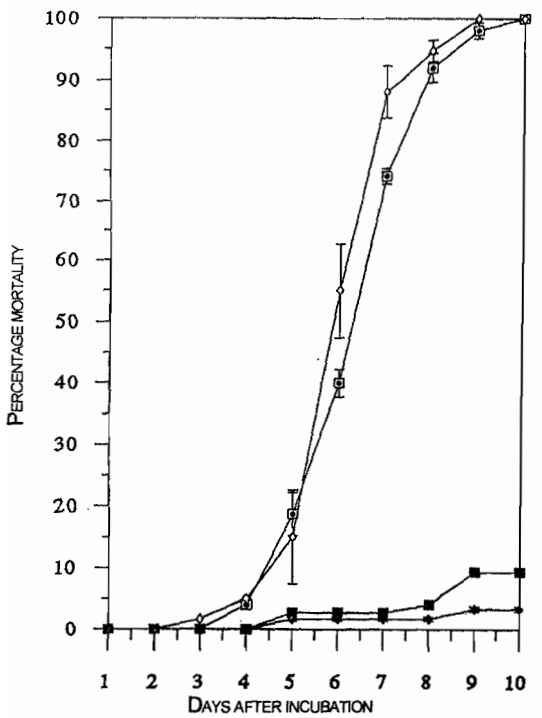

Fig. 3. Mortality - time curves for *Zonocerus variegatus* infected from traps placed in the field: adults (▣), control (■); nymphs (✧), control (◆).

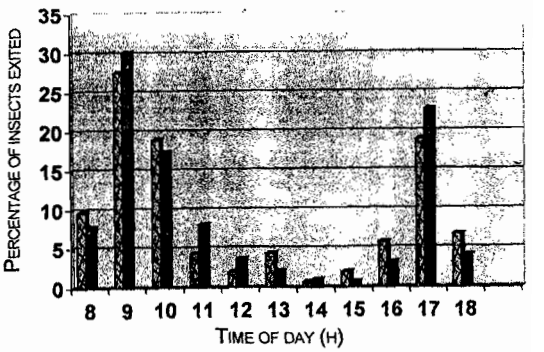

Fig. 4. Pattern of exit of infected (X) and uninfected (■) *Zonocerus variegatus* from traps in the field.

per cent had empty foreguts. This comprised insects in categories *b* (3.57 %), *c* (9.52 %), *bc* (66.67 %), and *o* (3.57 %). The long-term nutritional state of the trapped insects expressed as a ratio of fat score to maximum possible score fell within 65.4 - 83.3 per cent (Table 3).

Discussion

Attraction indices showed that the 1st to 4th nymphs of *Z. variegatus* were incapable of differentiating between different odours released by plant materials. This finding was supported by

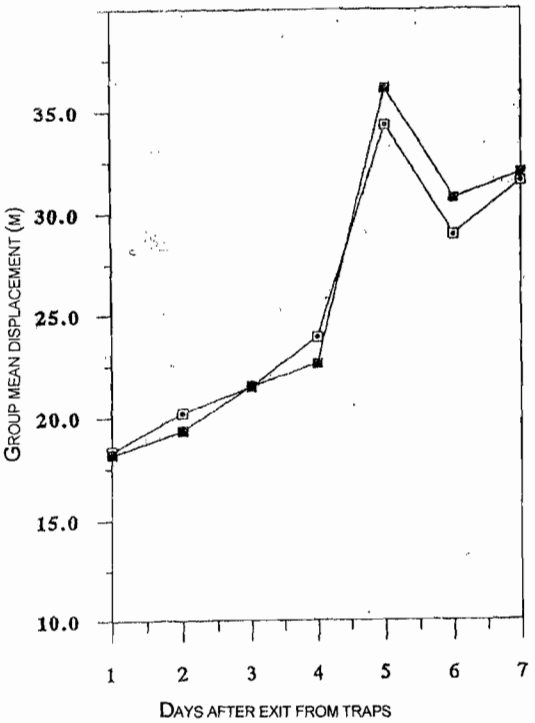

Fig. 5. Group mean displacements of infected (⊡) and uninfected (■) *Zonocerus variegatus* after exiting from traps in the field.

field trap catches. With an estimated population density of 1.1 insects m^{-2} and an index of dispersion greater than one, the grasshopper population in the study area was considered low and highly aggregated, which probably accounted for the low trap catches recorded. Densities in the range of 5 to 20 insects m^{-2} have been reported from several locations in southern Benin (Paraiso *et al.*, 1991). However, it must be stressed that the strength of the present strategy, apart from direct transmission from infected to uninfected insects, is secondary effect due to transmission of spores from mycoses of grasshopper cadavers killed by the pathogen. The carry-over effect may sustain a systematic dissemination of pathogens in the environment.

Differences in the number of male and female insects trapped warrant further investigation. Many workers have reported several examples of differences in the attraction of the different sexes to odours. For example, *Acrolepiopsis assectalla* males are attracted to leek and not females (LeComte & Thibout, 1981); traps baited with allylisothiocyanate catch only male cabbage root flies, *Delia brassicae* (Finch & Skinner, 1974); and only males of *Ostrinia nubilalis*, the European corn borer, are attracted to phenylacetaldehyde

TABLE 2

*Percentage of Trapped Insects with Food Located in Various Parts of the Gut**

Category	*Adults*		*5th/6th instars*		*Total*
	Males	*Females*	*Males*	*Females*	
abc	2.38(2)	9.52(8)	1.19(1)	1.19(1)	14.29(12)
ab	0.00	2.38(2)	0.00	0.00	2.38(2)
bc	4.76(4)	42.36(36)	7.14(6)	11.90(10)	66.67(56)
ac	0.00	0.00	0.00	0.00	0.00
a	0.00	0.00	0.00	0.00	0.00
b	2.38(2)	1.19(1)	0.00	0.00	3.57(3)
c	1.19(1)	3.57(3)	1.19(1)	3.57(3)	9.52(8)
o	0.00	2.38(2)	0.00	1.19(1)	3.57(3)
Total	10.71(9)	61.90(52)	9.52(8)	17.86(15)	100(84)

a - foregut only; **b** - midgut only; **c** - hindgut only; **ab** - fore- & midgut only; **ac** - fore- & hindgut only; **bc** - mid- & hindgut only; **abc** - all sections of gut; **o** - empty gut. **Percentage of insects with empty foregut (bc, b, c, and o) = 83.3 %.** Ratio of males to females: 1: 4 (adults, 1: 6 ; nymphs, 1: 2).

* Number of insects in parenthesis

TABLE 3

Nutritional State of Z. variegatus *Caught in Traps**

Insect type	*Fat score*			*Total score/max. score (percent)*
	Absent	*Moderate*	*Abundant*	
Adults				
Males	0.0	3.3 (3)	12.0 (6)	83.3
Females	0.0	36.0 (36)	32.0 (16)	65.4
Mean				74.4
5th/6th instars				
Males	0.0	4.0 (4)	8.0 (4)	75.0
Females	0.0	6.0 (6)	18.0 (9)	80.0
Mean				77.8

* Number of insects in parenthesis

(Cantelo & Jacobson, 1979).

The median-lethal-time (LT_{50}) of 6.1 days for insects infected in the traps the first day compare favourably with the 7 days from field applied 10^{12} spores ha^{-1} of *M. flavo-viride* formulated in oil (Lomer *et al.*, 1993). To enter the trap, an insect walks over 10 cm of spore-laden cloth and the extent of contamination of the tarsi, abdomen, and antennae may exceed that from conventional field application methods. However, it must be stressed that the relationship between LT_{50} and dose picked up is asymptotic (Bateman *et al.*, 1993). Therefore, beyond a certain point, extra spore picked up by the insects may not contribute much to infection. The combined effect of high temperature, humidity and light intensity might have interacted to accelerate spore inactivation in the 'tunnels' (Kaya, 1977). There is the need to replace the spore-laden cloth after 7 days in the field when subsequent differences in LT_{50} become significantly higher.

The diurnal activity rhythm of infected insects was similar to that of field populations reported by Modder (1984b) and Kaufmann (1965). The first of the two peaks of exit corresponds to the peak of feeding activity, the second to roosting. Similarities in the rates of dispersal in infected and non-infected insects imply that the former possess the same capacity to disperse up to the point of death. The grasshoppers wandered away from the point of exit for 5 days, before restricting to particular 'ambits' (Jackson, 1948). With the farthest group mean displacement of 34.2 m from traps for infected insects, a trap density of 2 ha^{-1} is suggested.

Insects with empty foreguts (83.3 %) might have entered the traps on the day of deployment in the field, since insects that were starved for 24 h had empty crops, although their mid- and hindguts were not empty (Tamu, 1990). They might have entered the trap during the first peak of activity at 9.00 h. Therefore, it is important that traps be set early morning before the peak activity elapses. Insects with completely empty guts (3.6 %) could have gone through apolysis while in the traps (Modder, 1977). The nutritional state of trapped insects fell on the lower side of 80 - 99 per cent for freshly collected insects (Modder, 1984b). For maximum growth and development, *Z. variegatus* was reported to use a wide range of food plants (Kaufmann, 1965; Modder, 1984b), which was not possible in the well-maintained plantation where the only source of food was cassava. The influence of the nutritional state of the field population on trap efficiency needs further investigation.

It may be concluded that the lure prepared from C. *odorata* is effective in attracting *Z. variegatus* into traps where they pick up inoculum of the fungal entomopathogen and then exit from traps to disperse the spores in the immediate environment. These preliminary results showed that the strategy could provide an effective way to deliver fungal entomopathogens to insect populations. However, this might not in itself alone be sufficient to sustain high levels of infective inoculum and thereby regulate the population of the pest. Three interdependent factors in such a situation must each be non-limiting: the causative agent (the pathogen), the susceptible host (the insect pest), and environmental conditions (temperature and humidity). Research on the use of extract of *Chromolaena* to attract *Zonocerus* is not conclusive. However, the strategy of combining a lure with a fungal pathogen is a novel approach with a potential which has also been demonstrated in the diamond back moth, *Plutella xylostella*, and its fungal pathogen, *Zoöphthora radicans* (Pell *et al.*, 1993).

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