CROP PROTECTION PROGRAMME

CHEMICAL ECOLOGY AND MATING BEHAVIOUR OF THE MILLET PESTS *Coniesta ignefusalis* AND *Heliocheilus albipunctella*

R6693 (ZA0006)

FINAL TECHNICAL REPORT

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CONTENTS

EXECUTIVE SUMMARY

Millet is a major subsistence food crop in the Sahelian region of West Africa, particularly for household use by women. It is an important source of fodder and building materials and can provide a source of cash income for poorer households. Two insect pests, the millet stem borer, *Coniesta ignefusalis* Hampson (Lepidoptera: Pyralidae) and the millet head miner, *Heliocheilus albipunctella* (de Joannis) (Lepidoptera: Noctuidae), together cause annual yield losses estimated at more than US$207 million and affect the livelihoods of millions of the poorest people in Africa.

The Purpose of the project was to develop and promote improved methods for management of principal insect pests of cereal-based cropping systems in areas where they are a major constraint to production. The project contributed to this Purpose by investigating the chemical ecology of the two most important insect pests of millet in West Africa in order to determine the potential of pheromones and plant chemicals in management of these pests.

This project was a continuation of a previous project on monitoring and control of insect pests of millet (R5281, 1993-1996). Collaborative work by NRI and ICRISAT Sahelian Centre led to identification of the female sex pheromone of *C. ignefusalis* and initial studies on its use in the field.

Research activities were carried out in farmers' fields in Niger, in the laboratory and field at ICRISAT-SC, and in the laboratory at NRI.

Project outputs included the holding of a "Regional Training Workshop on the Use of Pheromone Technology in the Management of Millet Stem Borer" in Niger and production of a handbook "Use of Pheromone Traps for monitoring the Millet Stem Borer (*Coniesta ignefusalis)"* in English and French. Materials were provided for monitoring of *C. ignefusalis* with pheromone traps in the 12 countries of the West and Central Africa Millet Research Network (WCAMRN).

In further work on mating disruption of *C. ignefusalis,* new controlled release dispensers were developed which lasted for a whole millet season in the field. In replicated trials on 0.5 ha plots in farmers' fields during 1997 and 1998, these gave $\geq$99% disruption of communication but had no consistently significant effect on infestation or damage by *C. ignefusalis*.

Methods were established for rearing *H. albipunctella* in the laboratory and avoiding diapause for the first time. This made possible a detailed study of the mating behaviour of this species in laboratory and field. Earlier suggestions that female moths are attracted to the males were confirmed conclusively. The males make a buzzing sound and the characteristics and mechanism of this were described in detail. Behavioural studies indicated that the buzzing is the primary stimulus for attraction, and the quality of the sound is critical. No evidence for chemical stimuli was found in behavioural bioassays, and no biologically active components were found in male volatiles using GC-MS and GC-EAG. No trace of diethyl malonate could be found, although this was previously reported to be a potential pheromone component in extracts of genitalia of male *H. albipunctella*.

Laboratory studies showed a remarkable preference by female *H. albipunctella* moths to oviposit on millet panicles at 30% emergence over panicles at floral or grain-filling stages,

other parts of the millet plant or sorghum panicles. Initial results indicated that methanolic extracts of the panicles encouraged oviposition when applied to filter papers, suggesting that chemical stimuli played a major part in encouraging oviposition. Subsequent attempts to repeat this work were less successful, although significantly less oviposition occurred on panicles after they had been extracted with hexane or methanol.

Other results indicated that volatile chemicals from the millet panicles play a part in encouraging oviposition. Analysis of the composition of volatiles from millet panicles at different stages of development and sorghum panicles showed significant differences and highlighted nine components that might be responsible for encouraging oviposition on millet panicles at 30% emergence or for discouraging oviposition on other stages.

A new technique for assessing resistance/tolerance of millet to *H. albipunctella* has been developed by ICRISAT-SC and further refined.

Two studies carried out with farmers in Niger showed a low but significant level of awareness of the link between insect numbers, pest damage and yield loss. The ICRISAT damage rating scale could be adapted to give farmers a reliable method for estimating potential yield loss due to *H. albipunctella* and hence the need for control measures.

The project outputs have contributed to the project Purpose by developing and disseminating information and tools for monitoring *C. ignefusalis* throughout West Africa. Their use for early detection in farmers' fields is being investigated by ICRISAT. A suitable formulation for use of the pheromone of *C. ignefusalis* in mating disruption has been developed and evaluated and it is proposed to develop this further during a project in Niger, Nigeria and Burkina Faso funded by IFAD.

Project outputs have established there is little prospect of using manipulation of mate-finding behaviour in control of *H. albipunctella*.

In contrast, results on host finding and oviposition site selection and by *H. albipunctella* should be followed up with millet breeders. The marked preference for oviposition sites by female *H. albipunctella* would be a good target for use in development of resistant/tolerant varieties of millet. The findings on chemical stimuli for oviposition should be developed further and the relative importance of chemical and physical stimuli established. Farmer surveys confirmed *H. albipunctella* is the most damaging insect pest of millet in the Sahel, and development of resistant varieties appears to be the most appropriate approach to management of this pest by smallholder farmers in the Region.

The project produced seven peer-reviewed papers, one Handbook published in English and French and nine internal reports presented as draft publications.

BACKGROUND

Millet in the Sahelian region of West Africa

Millet, *Pennisetum glaucum* L., is a major subsistence food crop in the sahelian region of West Africa, particularly for household use by women, as well as being an important source of fodder and building materials. In many areas, production of millet beyond the immediate family needs provides an important source of cash income for poorer households. The millet stem borer *Coniesta ignefusalis* Hampson (Lepidoptera: Pyralidae) is the major chronic insect pest of millet in the region and the millet head miner, *Heliocheilus albipunctella* (de Joannis) (Lepidoptera: Noctuidae), was identified as a priority pest at the 1993 Consultative Group Meeting on sorghum and millet pests. Together they cause annual yield losses estimated at more than US$207 million and affect the livelihoods of millions of some of the poorest people in Africa. These pests are particularly difficult to control with conventional insecticides because the larvae spend most of their lives protected by host-plant tissue, and there is a need to develop appropriate, rational and environmentally-benign approaches to reduce crop losses caused by these pests.

Millet stem borer, *C. ignefusalis*

The millet stem borer, *C. ignefusalis*, is one of the two main insect pests of millet throughout the West African Sahelian and Soudanian zones (Harris, 1962; N'doye *et al*., 1984; N'doye and Gahukar, 1987; Youm *et al*., 1996). First generation larvae cause dead heart and stand loss, while the second and third generations cause lodging, disruption of the vascular system and inhibition of grain formation (Harris, 1962). In the sub-Saharan Africa region where pearl millet is the major staple crop grown by subsistence farmers, yield losses due to attack by *C. ignefusalis* range from 15% to total crop failure (Harris, 1962; Ajayi, 1990), and in Niger over 90% of stem borer infestation and damage on millet is caused by *C. ignefusalis* (Youm and Gilstrap, 1993, 1994).

Control by chemical means is not very effective and repeated applications are not possible in subsistence agriculture (Youm, 1990). Destruction of alternative hosts and crop residues is difficult to enforce because of the importance of these materials for construction, decoration and animal bedding in the Region (Harris, 1962). Manipulation of planting dates (Vercambre, 1978; Guevremont, 1983; Youm, 1990), field sanitation (Nwanze and Muller, 1989) and burning of stalks (Guevremont, 1983; Maiga 1984) have given inconsistent results. Although some tolerance has been reported in varieties producing a sticky secretion (N'doye, 1977) or increased tillering (Nwanze, 1985), there are no varieties showing useful levels of resistance. Natural enemies of *C. ignefusalis* have been described (see Youm *et al*., 1996), but significant parasitism develops too late in the growing season (Youm, 1990).

Female *C. ignefusalis* moths were shown to produce a sex pheromone that attracts the male moths (Bako, 1977; ICRISAT, 1989) and, in previous work by NRI and ICRISAT this was isolated, identified and synthesised (Beevor *et al*., 1999). Synthetic lures were optimised and an effective, locally-made trap developed (Youm *et al*., 1993; Youm & Beevor, 1995; Youm *et al*., 1997a), and the traps have been used extensively for monitoring the pest in the Region as part of the West and Central African Millet Research Network (Dakouo *et al.*, 1997; Youm *et al.*, 1997b). Some reduction in damage by *C. ignefusalis* was reported for mass trapping around village granaries with 25 traps/ha (ICRISAT 1994; 1995), and initial results on use of

the synthetic pheromone for control of *C. ignefusalis* by mating disruption were reported by Beevor *et al*. (1996). Using a PVC resin formulation of the pheromone components, it was shown that the attractive pheromone blend was more effective at disruption than two "inhibitor" compounds which reduce the attractiveness of the attractive blend, and essentially complete communication disruption was achieved with release rates of 0.64 gm/ha/day. The main limiting factor was lack of a suitable formulation as the PVC resin had a half life of only a few days under field conditions, making frequent replacement necessary.

Millet head miner, *H. albipunctella*

Larvae of the millet head miner moth, *H. albipunctella,* feed inside developing millet panicles and can cause yield reductions of over *50%* (Nwanze and Sivakumar, 1990, Krall *et al.*, 1995). Following the pioneering work of Vercambre (1978), a variety of potential management options have been researched (summarised in Nwanze and Youm, 1995). These include the complex of natural enemies, soil management, planting of short-season millet varieties and use of pesticides. However, at present, Sahelian farmers still lack any effective control measures against this pest.

Aspects of the biology of *H. albipunctella*, such as relatively low fecundity and mobility and its monophagous habits make it an ideal candidate for control techniques which interfere with its ability to mate successfully. However, information on the reproductive behaviour of *H. albipunctella* is both scarce and puzzling. Females have been observed with their ovipositors everted, supposedly releasing pheromone (Youm, unpublished), but previous attempts at isolating a pheromone using linked GC-EAG were unsuccessful (DG XII contract TSD.A.204.UK(H), 1989). Male *H. albipunctella* make a buzzing sound of approximately 130 Hz (Matthews, 1987; Keenlyside, 1988) and release a putative pheromone (diethyl malonate) during courtship (DG XII contract TSD.A.204.UK(H), 1989), but neither the buzzing nor the male 'pheromone' has yet been reported to affect female behaviour.

In the past behavioural and physiological research on *H. albipunctella* was difficult because an adequate culture technique was unavailable (Markham, 1985; Smith, 1989) and therefore a regular supply of adult insects was impossible to obtain. *H. albipunctella* was thought to be a univoltine species with an obligate diapause. In preliminary laboratory experiments at NRI, however, *c*. 95% of larvae that were reared under a LD 14:10 h photoperiod produced non-diapausing pupae. The regulation of diapause thus appeared to be facultative and under photoperiodic control which meant that a continuous culture in the laboratory should be possible without the need to select a non-diapausing strain. Subsequently, larvae were reared successfully on a chickpea based diet at NRI, although larval survival was still relatively low compared to survival of, e.g. *Helicoverpa* spp. and further research was required to improve the culturing technique.

PROJECT PURPOSE

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This project was a continuation of a previous project on monitoring and control of insect pests of millet (R5281, 1993-1996). Collaborative work by NRI and ICRISAT Sahelian Centre lead to identification of the female sex pheromone of *C. ignefusalis* and initial studies on its use in the field (Beevor *et al.*, 1996).

RESEARCH ACTIVITIES

Training of local counterparts

- A training course on use of pheromones in pest control was run at ICRISAT Sahelian Centre (ICRISAT-SC) for staff from ICRISAT and member countries of the West and Central African Millet Research Network (WCAMRN).
- A handbook was produced in English and French versions on use of pheromones in pest control.
- Staff at ICRISAT-SC were given on-the-job training in research techniques during visits by NRI staff.

Further development of the sex pheromone of *C. ignefusalis* for monitoring and control

Monitoring of C. ignefusalis

- The three components of the female sex pheromone of *C. ignefusalis* were synthesised at NRI and provided to ICRISAT for preparation of lures and distribution through the WCAMRN.

Mating disruption of C. ignefusalis

Trials of mating disruption against *C. ignefusalis* are described in Appendix 1. Research activities included the following.

- New pheromone dispensers were developed at NRI and release rates determined under laboratory conditions.
- The pheromone components were synthesised at NRI and formulated for field trials in Niger.
- Field trials of mating disruption were carried out on replicated plots of up to 0.5 ha in farmers' fields in Niger during 1996, 1997 and 1998. Trials were monitored by

measuring suppression of catches of male *C. ignefusalis* moths in pheromone traps in treated plots relative to catches in traps in untreated plots, by determination of percentage dead heart and infested hills on two occasions, and by recording numbers of larvae and exit holes in stems after harvest.

- Experiments were carried out in Niger to compare catches of male *C. ignefusalis* moths in traps baited with standard monitoring lures containing 0.5 mg of pheromone or the mating disruption dispensers containing 80 mg of pheromone.

Laboratory rearing of *H. albipunctella*

Work on rearing of *H. albipunctella* at NRI is described in Appendix 2 and included the following activities.

- Conditions to minimise pupal diapause were established.
- Conditions for successful oviposition were developed.
- Conditions for efficient maintenance of larvae in the laboratory were developed.

Mating behaviour of *H. albipunctella*

Description of behaviour during mating

Extensive field and laboratory studies were conducted on the mating behaviour of *H. albipunctella*, as described in Appendix 3. Research activities included the following.

- Natural mating behaviour of *H. albipunctella* was observed in millet fields in Niger during four millet growing seasons in 1996-1999.
- The rapid sequence of behavioural events immediately preceding copulation was clarified using observation and video filming in the laboratory at NRI.
- The buzzing call of male *H. albipunctella* moths was characterised in terms of temporal modulation and frequency spectrum in both laboratory and field in Niger and in the laboratory at NRI.
- During this work, another heliothine moth was observed ovipositing on millet in Niger and this was identified (Appendix 4).

Determination of roles of acoustic and chemical stimuli in mating

Chemical and behavioural studies were carried out at NRI to investigate the mechanism of mate location by *H. albipunctella*, as described in Appendix 5. Research activities included the following.

- Volatiles from male *H. albipunctella* moths were collected by solvent extraction and air entrainment methods.
- The collections were analysed by gas chromatography linked to mass spectrometry (GC-MS) and by GC linked to electroantennographic (EAG) recording from a female moth.
- A bioassay was developed for investigating the effects of both natural and artificial

chemical and auditory cues on behaviour of virgin female *H. albipunctella* moths in the laboratory at NRI.

Investigation of factors affecting oviposition site selection by *H. albipunctella*

Role of chemical components of millet panicles as oviposition stimulants

Experimental assessment of the importance of naturally occurring involatile and volatile compounds in millet panicles as oviposition stimulants was carried out in the laboratory at ICRISAT-SC as described in Appendices 6 and 7. Research activities included the following.

- Levels of oviposition by female *H. albipunctella* moths were compared in laboratory experiments with four different millet varieties at 30%, 50% and 100% panicle extension, flowering and grain-filling stages.
- The relative importance of polar and non-polar components of millet panicles as oviposition stimulants was assessed.
- The role of involatile components of millet panicles as cues in oviposition was investigated.
- The role of volatile components of millet panicles as cues in oviposition was investigated.

Investigation of the composition of volatiles from millet panicles

Work to determine the composition of volatiles from millet panicles at different stages of development and from different varieties is described in Appendix 8. Research activities included the following.

- Volatiles were collected from millet panicles at four different stages and from sorghum panicles.
- Volatiles were collected from millet panicles of four different varieties.
- Volatile collections were analysed by gas chromatography linked to mass spectrometry (GC-MS) and by GC linked to electroantennographic (EAG) recording from a female *H. albipunctella* moth.

Screening of millet varieties for resistance to *H. albipunctella*

Trials were conducted at ICRISAT-SC to assess millet varietal resistance/tolerance to *H. albipunctella* and the work is described in Appendix 8. Research activities included the following.

- A new technique developed at ICRISAT-SC using *H. albipunctella* eggs rather than larvae to infest plants was refined to give a more reliable method of infestation for assessment of resistance/tolerance of millet to this pest.
- The resistance/tolerance of 18 millet varieties was determined after artificial infestation with *H. albipunctella* eggs. Damage was scored on a scale of 1-10, and the percentage of eggs applied that developed to larvae or pupae (Larval Production Index

or LPI) was also recorded.

- The resistance/tolerance of 20 millet varieties to *H. albipunctella* was determined under conditions of natural infestation.

Assessment of farmers' perceptions of yield losses due to insect pests and likely adoption of control strategies.

- A survey was carried out to assess farmers' perceptions of losses due to insect pests (Youm and Owusu, 1998b).
- On-station and on-farm studies were carried out to develop a method by which farmers could assess damage due to *H. albipunctella* more consistently (Youm and Owusu, 1998a).

OUTPUTS

Training of local counterparts

Regional Training Workshop on the Use of Pheromone Technology in the Management of Millet Stem Borer

This workshop was planned and given by Dr Derek Russell (NRI) with Dr Youm and other staff from ICRISAT-SC from 30 September - 4 October 1996. Representatives took part from 12 West African countries - Benin, Burkina Faso, Chad, Côte d'Ivoire, the Gambia, Ghana, Guinea Bissau, Mali, Niger, Nigeria, Senegal and Togo.

The workshop included lectures on pheromone identification and use, handling of data from trials, use of GIS techniques and the socio-economic aspects of new pest management approaches. Practical sessions were held in laboratory and field on producing and using lures and traps. Each participant gave a presentation on the status and possibilities for use of pheromones in their country (e.g. Dakuou *et al.*, 1997; Youm *et al.*, 1997b).

Handbook "Use of Pheromone Traps for monitoring the Millet Stem Borer (Coniesta ignefusalis)"

Material used for the above Workshop was compiled and published as ICRISAT Information Bulletin No. 49 with 500 copies each in English and French (Youm *et al.*, 1998). These were distributed to most African countries by the African ICRISAT Centres.

On-the-job training of ICRISAT staff

During the project, 10 visits were made by NRI staff to ICRISAT-SC to assist with field work. These visits provided the opportunity to train further ICRISAT staff in specific aspects of using pheromones and carrying out investigations of insect behaviour.

Further development of the sex pheromone of *C. ignefusalis* for monitoring and control

Monitoring of C. ignefusalis

- The three components of the female sex pheromone of *C. ignefusalis* were synthesised at NRI and provided to ICRISAT for preparation of lures and distribution through the WCAMRN.

Mating disruption of C. ignefusalis

Results of work on mating disruption of *C. ignefusalis* are given in full in Appendix 1. These included the following.

- Polyethylene vials loaded with 80 mg of pheromone were shown to give constant, zero-order release of pheromone at 0.05 mg/day. This was considered to be suitable for field use, considering temperature data from Niger, and the vials were predicted to give a field life of at least three months, suitable for the millet-growing season. The vials are commercially-available, and filling and sealing could be automated. Field application was labour-intensive, but only one application per season was required. Polyethylene sachets released pheromone too fast in laboratory tests.
- Field trials were carried out in farmers' fields during 1996 used the 0.5 mg monitoring trap lures at 400 dispensers/ha renewed every three weeks, equivalent to 800 mg pheromone/ha/season. Significant but incomplete trap catch suppression was obtained (86.8 ± 2.6%).
- Field trials in 1997 and 1998 involved a single application of the new 80 mg dispensers on replicated 0.5 ha plots in farmers' fields. Trap catch suppression over the season was $\geq$ 99% in both years, e.g Fig. 1 for 1998. In 1997, the percentage dead hearts and infested hills were lower in the pheromone treated plots than in untreated plots, although not significantly so (Table 1). Numbers of exit holes and larvae of *C. ignefusalis* after harvest were significantly lower ($P<0.05$) in the plots treated with pheromone than in untreated plots, but infestations were low and the differences small (Table 2). In 1998, however, there were no significant differences between measures of damage and infestation between the plots treated with pheromone and untreated plots (Tables 3 and 4).
- In three replicated experiments carried out in 1997 on-station and in farmers' fields in Niger, catches of male *C. ignefusalis* moths in traps baited with the 80 mg mating disruption dispensers were 10-20% of the catches in traps baited with the standard 0.5 mg monitoring lures (Table 5).

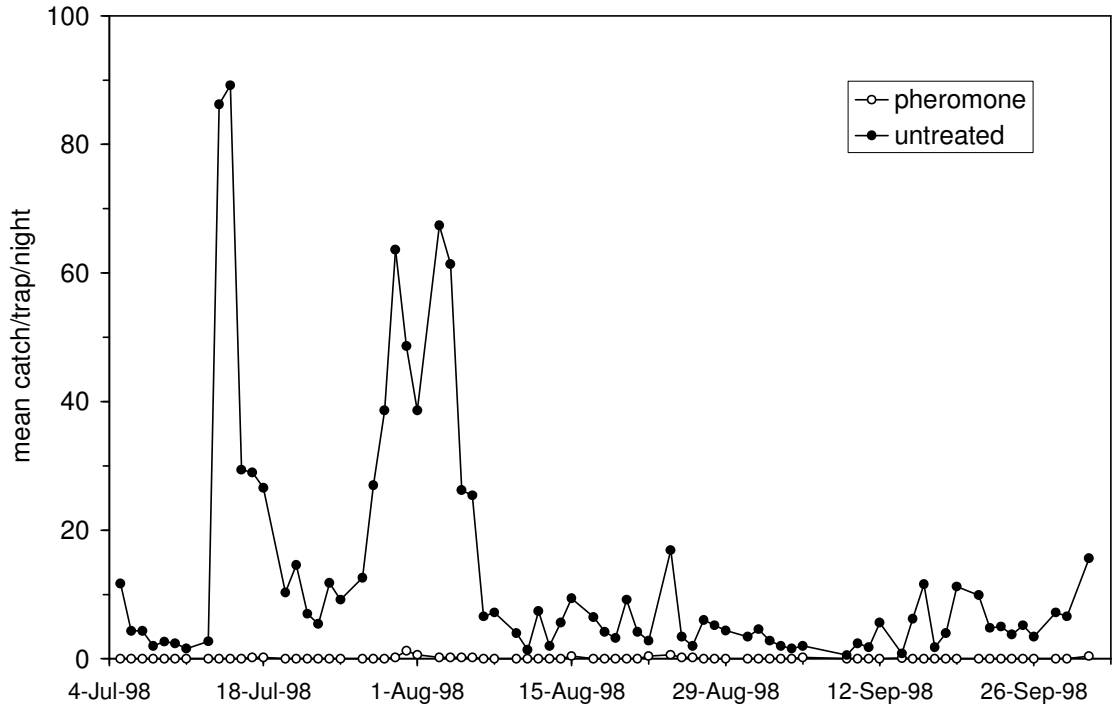

FIG. 1. Trap catches of male *C. ignefusalis* moths in treated and untreated areas during 1998 mating disruption trial (mean of five replicates).

TABLE 1. Mean percent dead hearts and infested hills (± standard error) at 40 and 70 DAS in 1997 mating disruption trial (four replicates; results from central 10 m x 10 m).

Treatment	% Dead heart		% Infested hill	
	40 DAS	70 DAS	40 DAS	70 DAS
Pheromone	0.45 ± 0.30	5.37 ± 4.51	1.35 ± 0.90	13.91 ± 10.91
Untreated	0.93 ± 0.32	7.08 ± 2.34	2.04 ± 0.84	18.81 ± 7.40

TABLE 2. Mean numbers of larvae and exit holes (± standard error) in 1997 mating disruption trial (four replicates; results from 50 stems; means followed by different letter in a column are significantly different at 5% level by DMRT)).

Treatment	Larvae	Exit holes
Pheromone treated	2.28 ± 0.22 a	1.79 ± 0.15 a
Untreated	2.88 ± 0.02 b	2.30 ± 0.13 b

TABLE 3. Mean percent dead hearts and infested hills at 70 and 90 DAS in 1998 mating disruption trial (five replicates; results from central 10 m x 10 m).

Treatment	% Dead heart		% Infested hill	
	70 DAS	90 DAS	70 DAS	90 DAS
Pheromone	1.99 ± 0.76	4.74 ± 2.12	9.18 ± 3.72	34.25 ± 13.61
Untreated	3.11 ± 1.27	4.14 ± 0.92	10.87 ± 2.84	28.00 ± 6.92

TABLE 4. Mean numbers of larvae and exit holes (± standard error) in 1998 mating disruption trial (four replicates; results from 50 stems; means followed by different letter in a column are significantly different at 5% level by DMRT)).

Treatment	Larvae	Exit holes
Pheromone	14.00 ± 9.24	31.20 ± 10.49 a
Untreated	3.60 ± 2.11	12.80 ± 3.72 b

TABLE 5. Mean catches of *C. ignefusalis* male moths in traps baited with 0.5 mg or 80 mg lures.

Location	Spacing (m)	No. nights	No. reps	Mean catch/trap/night ± SE	
				0.5 mg lure	80 mg lure
On-station	30-35	106	2	3.74 ± 0.04	0.56 ± 0.03
On-farm	30-35	106	3	10.25 ± 1.86	2.42 ± 0.19
On-station	100	56	2	12.99 ± 0.35	0.71 ± 0.17

Laboratory rearing of *H. albipunctella*

Work on rearing of *H. albipunctella* at NRI is described in Appendix 2.

- Although it was difficult to produce large numbers of moths regularly, cultures were maintained for up to 15 months at NRI.
- Few pupae entered diapause when cultures were maintained on a 14:10 light:dark cycle with temperatures 31°C:27°C and 60% rh. At lower temperatures significant diapause occurred.
- Female moths oviposited well on millet panicles but not on filter paper or cotton wool.
- Eggs were allowed to hatch on the millet panicles and the young larvae were

transferred within 6-8 hr of hatching to artificial diet in groups of 3-5.

- Under these conditions, larval development takes approximately 25 days and the pupal stage lasts 18-30 days.

Mating behaviour of H. *albipunctella*

Description of behaviour during mating

Results of research on the mating behaviour of *H. albipunctella* are given in full in Appendix 3.

- Mating occurs during the first 2-3 hours of the night. The pre-mating behaviour is unusual, since males perform a display that involves percussive vibration of the forewings and exposure of the genitalia, and receptive females are attracted to mate. Males display whilst perched on the lower leaves and stems of millet plants or on other low vegetation (<50cm), either singly, or in "lek" groups. Leks are commonest in relatively open areas, e.g. around the field margins. Females fly in directly from downwind and mating occurs almost immediately. Copulation lasts approximately 60 minutes.
- The male buzz consists of a continuous “trill” with a pulse repetition rate of 136/sec (at 27°C), with most power falling in the frequency range 1-8kHz. No ultrasonic component could be detected.
- Video analysis of mating behaviour in the laboratory showed that once the female has landed close to the male, she then approaches by walking whilst fluttering her wings. Typically the female approaches from behind, passing alongside and then crossing in front of the male, at which point he quickly extends his abdomen forwards and clasps the female by her abdomen tip. If the mating attempt is unsuccessful then the male typically does not pursue the female, but continues to buzz vigorously.

Identification of Masalia nubila

As described in Appendix 4, during 1999 another heliothine moth, very similar in appearance to *H. albipunctella* was observed during studies at ICRISAT-SC. This was identified as *Masalia nubila* Hampson (Lepidoptera: Noctuidae).

Although the larvae mine inside the millet panicle in a manner similar to *H. albipunctella*, *M. nubila* probably does not merit pest status under normal conditions. However, it is important for researchers and farmers alike that realise that eggs laid on millet panicles are not necessarily those of *H. albipunctella*.

Determination of roles of acoustic and chemical stimuli in mating

Appendix 5 describes investigations of the roles of acoustic and chemical stimuli in mating of *H. albipunctella*.

- Hexane extracts of excised genitalia of buzzing male *H. albipunctella* moths were made in the laboratory at NRI, in the laboratory at ICRISAT-SC and in the field at ICRISAT-SC. Volatiles from male *H. albipunctella* were collected by air entrainment at NRI.
- In analyses of these collections by GC-MS, diethyl malonate could not be detected (<<50pg/male). During previous work at NRI in 1985, this compound was found in similar extracts at levels of approximately 1 μg/male (Matthews, 1987).
- The analyses failed to show the consistent presence of significant amounts (>10 ng) of any other compounds that could be attributed to the male moths.
- In GC-EAG analyses of hexane extracts of *H. albipunctella* male genitalia, no components caused significant EAG responses from a female *H. albipunctella* moth.
- A behavioural bioassay was developed to study movement of virgin female *H. albipunctella* moths in the presence of chemical and/or auditory stimuli.
- Bioassay tests failed to demonstrate any attraction of virgin female *H. albipunctella* to hexane extracts from the genitalia of buzzing males (Table 6).
- Bioassay tests also failed to show any attraction of females to recordings of male buzzing (Fig. 2).
- However, virgin females were significantly attracted to live, buzzing males even when the latter were held in an extractive air stream to remove any volatile substances released by the males (Fig. 3).

TABLE 6. Response of virgin female *H. albipunctella* moths to hexane extract of male genitalia (6 replicates treatment and control; mean ± SE number of times female entered third of cage during 15 min; upwind is towards source)

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Source	Upwind	Downwind
Male extract	2.67 ± 0.95	3.33 ± 1.12
Hexane control	2.83 ± 0.83	1.83 ± 0.79

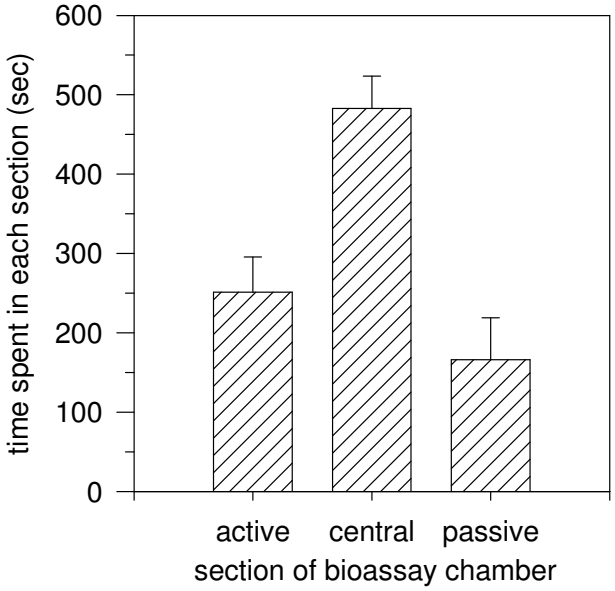


FIG. 2. Movement by virgin female *H. albipunctella* moths exposed to recorded male buzzing. (Playback of buzzing was from the "active third"; n=17; mean ± SE).

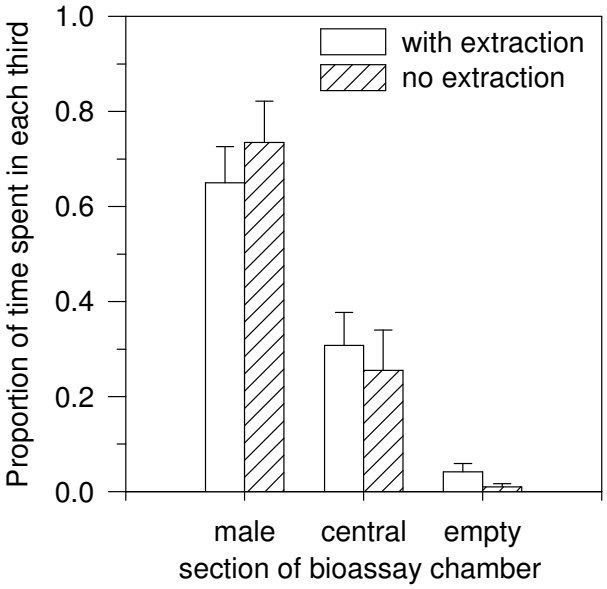


FIG. 3. Movement by virgin female *H. albipunctella* moths exposed to the buzzing of live males, held in an extractive airflow or without extractive. (Mean ± SE; n=14 with extraction, n=8 without; observation time 12 min).

Investigation of factors affecting oviposition site selection by *H. albipunctella*

Role of chemical components of millet panicles as oviposition stimulants

Results of research on oviposition stimulants for *H. albipunctella* carried out during 1997 are described in Appendix 6.

- In both choice and no-choice tests, millet panicles at 30% extension were favoured for oviposition by *H. albipunctella* females over panicles at 50% extension, 100% extension, flowering or grain-filling stages of four millet varieties (e.g. Table 7).
- In choice or no-choice tests, female *H. albipunctella* moths oviposited on millet panicles at 30% extension but rarely on millet leaves or stems or sorghum panicles (Table 7).

TABLE 7. Oviposition by female *H. albipunctella* on different substrates in choice test (3 replicates)

	Mean % total eggs ± SE				
Variety	30% ext	50% ext	100% ext	flowering	grain filling
3/4HK	57.2±2.48	30.3±1.26	12.5±3.73	0.0	0.0
MBH110	70.7±8.94	22.8±11.85	5.8±2.18	0.75	0.0
ICMV IS 89305	75.7±0.83	16.3±2.68	7.4±1.31	0.0	0.0
Chalakh	85.8±0.71	13.9±1.08	0.4	0.0	0.0

- In choice or no-choice tests, female *H. albipunctella* moths oviposited on filter papers impregnated with methanol extracts of millet panicles at 30% extension but rarely on filter papers impregnated with methanol extracts of millet leaves or stems or sorghum panicles (Table 8).

Table 8. Numbers of eggs (mean ± SE) deposited by *H. albipunctella* female moths on whole plant substrates or filter paper impregnated with methanol extract in choice (5 replicates) or no-choice (4 replicates) tests.

	Choice Test		No-Choice Test	
	plant	filter paper	plant	filter paper
Sorghum panicles	0.2 ± 0.18	0.0	0.0	0.0
Millet panicles	44.4 ± 7.38	5.4 ± 0.92	32.0 ± 2.9	16.8 ± 1.9
Millet leaves	0.2 ± 0.18	0.0	0.0	0.0
Millet stems	0.4 ± 0.36	0.0	0.5 ± 0.5	0.5 ± 0.5

Results of attempts to repeat and extend this work during 1999 are described in Appendix 7.

- A bioassay methodology was developed to assess the propensity of female *H. albipunctella* to oviposit. This involved solvent extraction of chemicals from newly emerged panicles (the favoured stage for oviposition) and then offering the "de-flavoured" panicle to females as a standard medium for oviposition in the presence or absence of volatile or involatile panicle components.
- Chemical extraction using hexane, a non-polar solvent, was found to render panicles less attractive for oviposition much more quickly (after 30 min, $\chi^2 = 3.99$, df = 1, p=0.046) than extraction using methanol, a non-polar solvent (after 24 hr, $\chi^2 = 6.26$, df = 1, p=0.012. This suggests that non-polar compounds in the panicle may be more important as oviposition stimulants.
- In contrast to the earlier work, "de-flavoured" panicles that were re-coated with panicle extract were not rendered more attractive for oviposition ($\chi^2 = 2.62$, df = 1, p=0.11) (Table 9).

TABLE 9. Oviposition by female *H. albipunctella* on millet panicles extracted with methanol and extracted panicles recoated with the methanol extract .

Treatment	No. with eggs	Mean no. eggs
Extracted panicle	12	26
Recoated panicle	6	32

- However, "de-flavoured" panicles presented to females along with a concealed fresh panicle were found to be significantly more attractive for oviposition than identical panicles presented alone ($\chi^2 = 4.48$, df = 1, p=0.034) (Table 10). This suggests that panicle volatiles have an important role in stimulating oviposition.

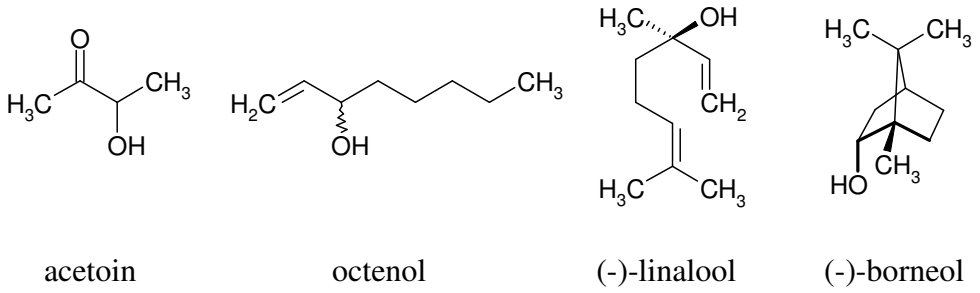
TABLE 10. Oviposition by female *H. albipunctella* on millet panicles extracted with methanol in presence or absence of concealed fresh panicles .

Treatment	n	No. with eggs present	Mean eggs/female
No panicles	32	13	27.2
Panicles present	33	21	31.1

Investigation of the composition of volatiles from millet panicles

Results of analyses of volatiles from millet panicles are described in Appendix 8.

- From GC-MS analyses of volatiles from millet panicles at various stages, 42 components were quantified as representing approximately ≥1% of the total volatiles. Of these components, 21 were identified. Comparison of the profiles by eye suggested that nine components represented a high proportion of the total volatiles in most cases, the remainder being made up of very small amounts of many of the other components. These major components were 2-hydroxy-3-butanone (acetoin), 1-octen-3-ol (octenol), (3*R*)-(-)-3,7-dimethyl-3-hydroxy-1,6-octadiene ((-)-linalool), (1*S*)-*endo*-1,7,7-trimethyl-2-hydroxy-bicyclo[2.2.1]heptane ((-)-borneol), 3-methylbutyric acid (isovaleric acid), hexanoic acid, an unidentified acid, nonanoic acid and decanoic acid (Fig. 4).



acetoin octenol (-)-linalool (-)-borneol

FIG. 4. Structures of the main, non-acidic components of millet panicle volatiles.

- The percentages of these nine components in volatiles from the four millet panicle stages and from sorghum panicles are shown in Fig. 5. For the millet volatiles, these accounted for ≥84% of the total volatiles. Volatiles from the 30% emergence stage favoured for oviposition by *H. albipunctella* were dominated by borneol with smaller amounts of linalool and octenol. In the floral and grainfilling stages the relative amount of borneol decreased and significant amounts of acetoin and the carboxylic acids appeared. The latter were even more prominent in the sorghum volatiles.

- Data from GC-MS analyses of collections of volatiles from 30% extension stages of panicles of four different millet varieties are summarised for the nine components referred to above in Fig. 6. Results for the 3/4HK agreed reasonably well with those obtained previously (Fig. 5). In all four varieties, borneol was the major component, although the amount varied with the percentage in volatiles from HH-VBC-PCV-2 > 3/4HK > EC87-PCV-2 > EC87-PCV-1. Proportions of octenol followed essentially the reverse pattern.

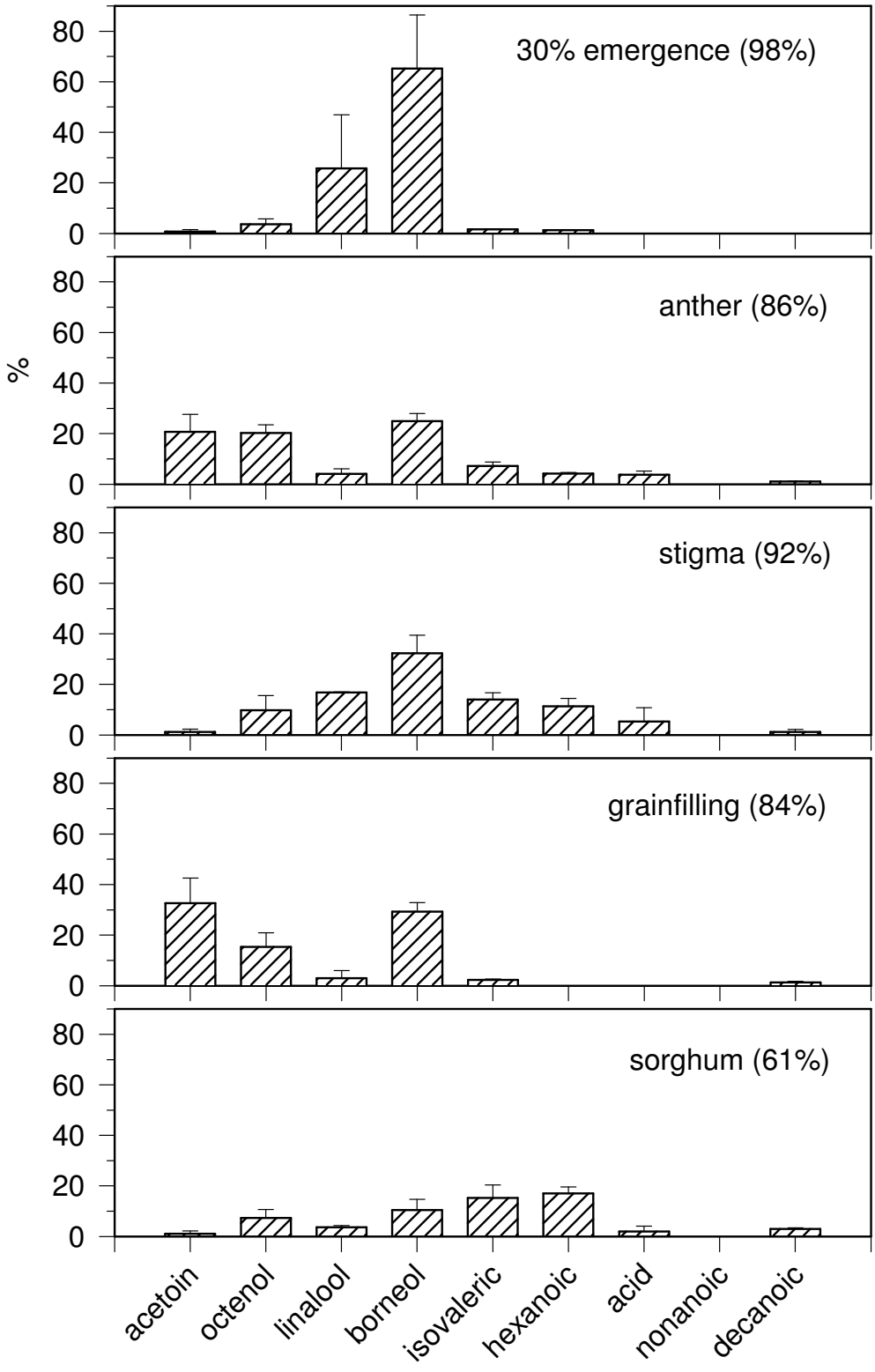


FIG. 5. Percentages of nine components in volatiles from four millet panicle stages and from sorghum panicles (amount of total volatiles accounted for by these components shown in brackets; graph shows mean and spread of results from duplicate samples, 3-24 hr collections).

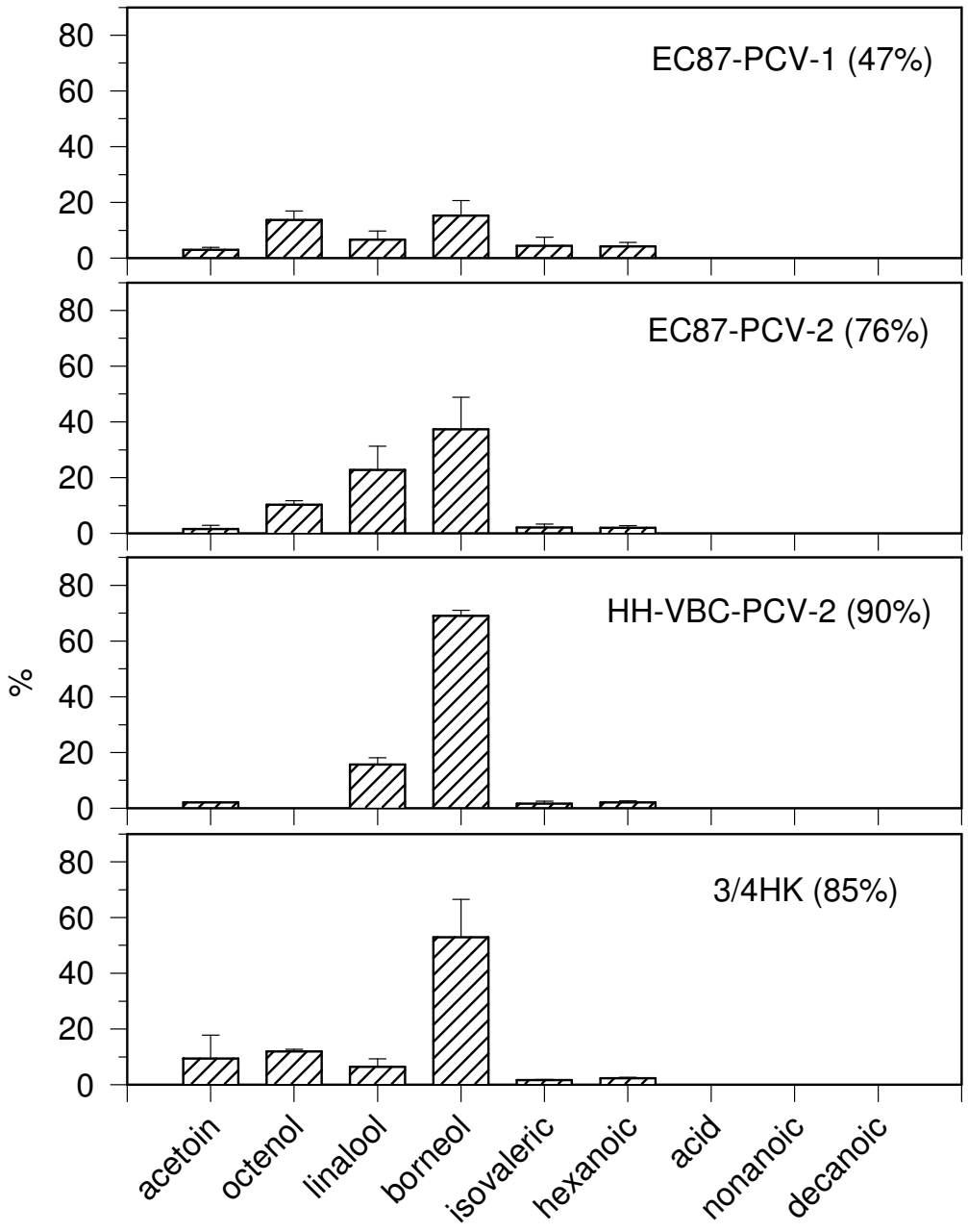


FIG. 6. Percentages of nine components in volatiles from panicles at 30% extension stage of four millet varieties (amount of total volatiles accounted for by these components shown in brackets; graph shows mean and spread of results from duplicate samples, 0-24 hr collections).

- In GC-EAG analyses of the collections of volatiles from millet panicles against female *H. albipunctella* moths, small EAG responses were observed to octenol (15.7 min), borneol (21.5 min) and *iso*valeric acid (20.7 min) (e.g. Fig. 7).

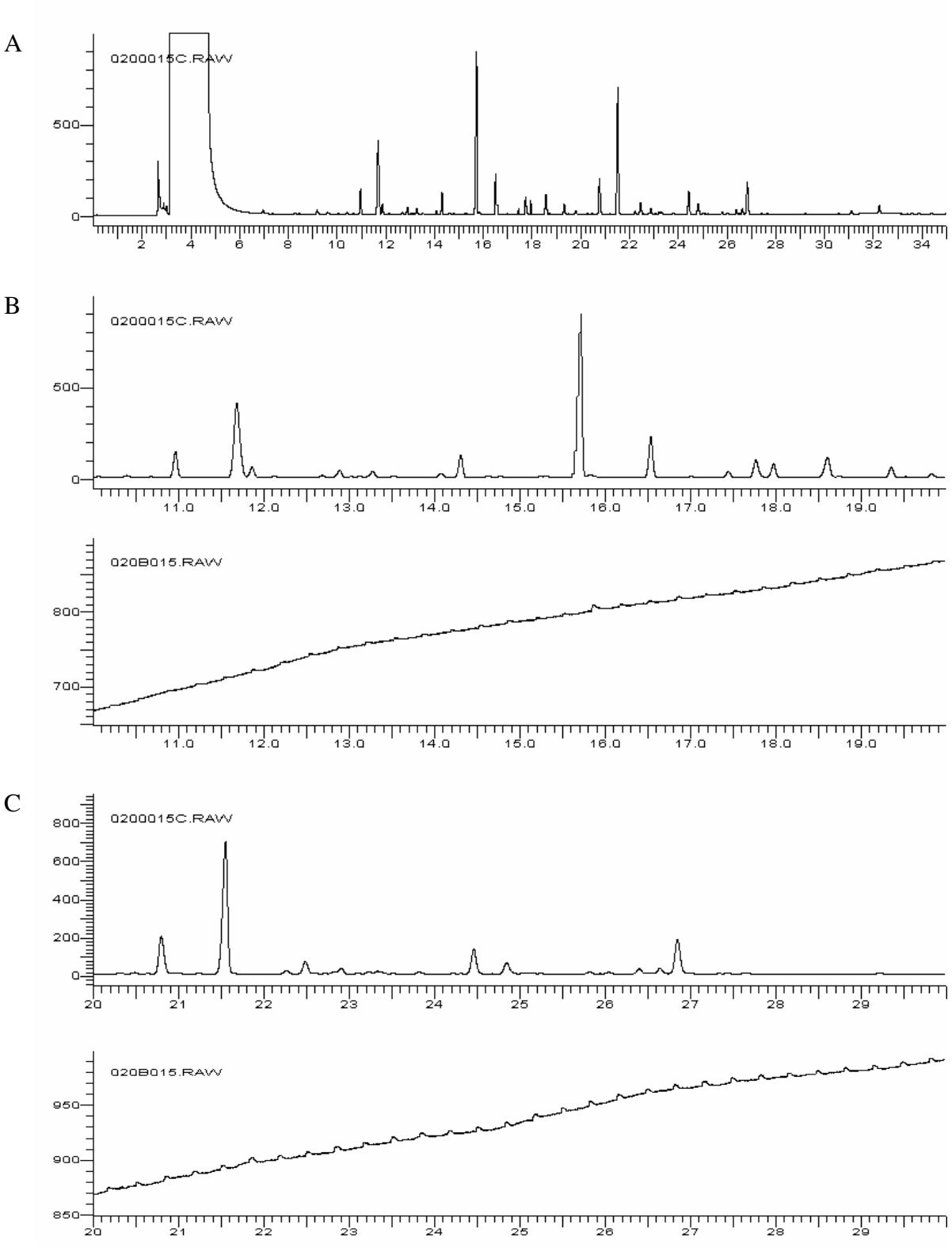


FIG. 7. GC-EAG analysis of volatiles from a 3/4HK millet panicle at the anther stage. (A) complete GC trace; (B) and (C) expanded GC (upper) and EAG (lower) traces.

Screening of millet varieties for resistance to *H. albipunctella*

Trials were conducted at ICRISAT-SC to assess millet varietal resistance/tolerance to *H. albipunctella* and the work is described in Appendix 9.

- An infestation technique involving the implantation of *H. albipunctella* eggs onto emergent millet panicles was refined by assessing the damage levels resulting from infestation with different numbers of eggs. It was found that damage was maximised when 40 eggs were implanted, and it is suggested that this should be the standard infestation level used in screening trials.
- Under artificial infestation, the damage ratings of 18 different millet varieties varied between 6.66 ± 0.78 and 2.66 ± 1.02, but these differences were not significant. Larval Production Indices varied between 36.7 ± 8.92% and 5.00 ± 3.96%, and these may provide a more sensitive measure of tolerance.
- Under natural infestation, damage ratings differed significantly between 4.27 ± 1.16 and 1.66 ± 0.19 amongst 20 varieties of pearl millet that were tested. Damage was lowest on a local variety but damage to a new ICRISAT variety, ICMV IS 990044 was not significantly greater ($P<0.05$).

Assessment of farmers' perceptions of yield losses due to insect pests and likely adoption of control strategies.

- In a survey of farmers in five villages near Sadore, Niger, a high proportion (83%) were aware of the importance of crop loss assessment and the link between insect numbers and damage. A surprisingly high number (15% overall, but 33% in one village) carried out some form of quantitative assessment of insect numbers and/or damage and compared this with yields in the previous season. Overall 33% of farmers surveyed used some form of chemical control, but the majority (55%) used traditional methods of control including use of insecticidal plant materials or prayers, mechanical picking of caterpillars (5%) or no control (7%) (Youm and Owusu, 1998b).
- In on-station and on-farm studies good correlation was found between millet yield and damage due to *H. albipunctella* assessed on a scale of 1 (no damage) to 9 (very high damage) for five millet varieties. A simplified scale of 1-5 was tested by farmers and found to give equally good results (Youm and Owusu, 1998a).

CONTRIBUTION OF OUTPUTS

The Project has contributed to the Purpose of developing and promoting improved methods for management of principal insect pests of cereal-based cropping systems in areas where they are a major constraint to production, by studying the chemical ecology of the two most important insect pests of millet in West Africa.

Training of local counterparts

Results of previous DFID-funded research on the pheromone of the millet stemborer, *C. ignefusalis*, have been published (Beevor *et al.*, 1999) and disseminated to representatives of ministries of Agriculture and extension services in 12 West African countries where this pest is a major constraint. A Workshop was held in Niger and a Handbook published in English and French (Youm *et al.*, 1998).

Pheromone traps are now used for monitoring *C. ignefusalis* in these countries as part of the West and Central Africa Millet Research Network (WCAMRN) (Youm *et al.*, 1997b; Dakouo *et al.*, 1997) Their use for early detection in farmers' fields is being investigated by ICRISAT (Ayaji and Prabhakar, 1997)

Further development of the sex pheromone of *C. ignefusalis* for monitoring and control

Research on mating disruption for control of *C. ignefusalis* was previously hampered by lack of a suitable slow-release formulation. A formulation has now been developed that lasts for the whole millet-growing season, could be produced commercially and is suitable for application by farmers without any specialised equipment. These sealed polyethylene dispensers were shown to maintain ≥99% communication disruption for up to three months in farmers' fields in Niger when applied at 400 dispensers/ha. In the replicated 0.5 ha plots used in these trials, effective communication disruption did not translate into consistently reduced infestation, damage or yield loss due to *C. ignefusalis*. This may have been because actual reduction in mating was less effective than the reduction in pheromone trap catch would indicate, or because immigration of mated female moths into the treated plots were negated any reduction of mating of females within the treated plots. The latter is considered to be more likely, and future trials should investigate whether mating disruption in larger plots can give effective control of *C. ignefusalis* (Appendix 1).

Dr Youm proposes to take up this approach in a project in Niger, Nigeria and Burkina faso funded by IFAD.

Mating behaviour of *H. albipunctella*

Techniques have been developed for continuous rearing of *H. albipunctella* in the laboratory without the onset of diapause (Appendix 2). This has made possible for the first time detailed study of the mating behaviour of this species in both the laboratory and field (Appendix 3).

Studies of the mating behaviour have conclusively confirmed earlier suggestions that virgin female moths are attracted to the males, rather than the other way round as in the majority of

Lepidoptera. This attraction is primarily mediated by sound. The mechanism of production of this sound has been established, and it seems that the quality of the sound is important for effective attraction. Furthermore, both behavioural and chemical studies showed no evidence for chemically-mediated attraction between the sexes. This is in contrast to previous studies at NRI (Matthews, 1987) in which diethyl malonate was isolated from genitalia of male *H. albipunctella* and proposed to be a sex pheromone. No trace of this or any other potential pheromone component could be found (Appendix 5).

On the basis of the present findings it would appear that the prospects for managing this pest by means of behavioural manipulation are not good. No long-range chemical pheromone exists that could be used in population monitoring, mating disruption or the trapping of mate-seeking females. In contrast, the acoustic stimuli from male buzzing seem to play the major role in mate attraction, and it appears that the quality of the acoustic signal is also critical, probably ruling out any possibility of using artificial buzzing in management of the pest.

Investigation of factors affecting oviposition site selection by *H. albipunctella*

Laboratory studies at ICRISAT-SC (Appendix 6) showed a remarkable preference by female *H. albipunctella* moths to oviposit on millet panicles at 30% emergence over panicles at floral or grain-filling stages, other parts of the millet plant or sorghum panicles. Initial results indicated that methanolic extracts of the panicles encouraged oviposition when applied to filter papers, indicating that chemical stimuli played a major part in encouraging oviposition. Subsequent attempts to repeat this work were less successful (Appendix 7), although significantly less oviposition occurred on panicles after they had been extracted with hexane or methanol.

Other results indicated that volatile chemicals from the millet panicles play a part in encouraging oviposition. There are no previous reports of the composition of volatiles from millet panicles. Analysis of the composition of volatiles from millet panicles at different stages of development and sorghum panicles showed significant differences and highlighted components that might be responsible for encouraging oviposition on millet panicles at 30% emergence or for discouraging oviposition on other stages.

Use of pest-resistant or tolerant varieties is one of the most effective, appropriate and environmentally-acceptable methods for reducing yield losses due to pests, particularly among poor farmers who cannot afford expensive inputs. Such resistance may arise from one or a combination of factors such as asynchrony of plant and pest development, lack of attractiveness of the plant to ovipositing females, physical or contact-chemical characteristics discouraging oviposition, tolerance of the plant to feeding by the larvae, or actual antibiotic properties of the plant against the pest.

Results of this project have demonstrated marked preference for oviposition by *H. albipunctella* female moths on a particular growth stage of the millet panicle even in a no-choice situation. Understanding of the reasons for this could permit breeding of varieties on which oviposition is not favoured/discouraged. Results to date indicate both volatile and involatile chemicals may play a role in this.

Screening of millet varieties for resistance to *H. albipunctella*

A new technique for assessing resistance/tolerance of millet to *H. albipunctella* has been developed by ICRISAT-SC and further refined. Application of known numbers of *H. albipunctella* eggs to millet panicles is easier to do and gives higher infestations and more reproducible results than previous methods using larvae or reliance on natural infestations.

This approach measures tolerance and/or antibiosis of the plant to *H. albipunctella*, separate from any effects on attraction of female moths or suitability for oviposition. It also permits measurement of both damage caused and the extent of larval survival. The latter may provide a more reliable index of tolerance to the pest.

Assessment of farmers' perceptions of yield losses due to insect pests and likely adoption of control strategies.

In this project it was planned to follow up previous work by Dr Baidu-Forson on farmers' perceptions of millet pests and control strategies (Youm and Baidu-Forson, 1995). However, as Dr Baidu-Forson left ICRISAT at the beginning of the project, only limited work was possible.

Two studies were carried out with farmers in Niger, focussing on approaches to crop loss assessment which had been highlighted as a need in previous DFID-funded work by Jago (1995). A low but significant level of awareness of the link between insect numbers, pest damage and yield loss was established (Youm and Owusu, 1998b). It was demonstrated that the ICRISAT damage rating scale could be adapted to give farmers a reliable method for estimating potential yield loss due to *H. albipunctella* and hence the need for control measures (Youm and Owusu, 1998a).

In this context, the finding of a related pest, *Masalia nubila,* in significant numbers in millet fields in Niger should be noted. Larvae and adults of this species could easily be confused with those of *H. albipunctella,* although *M. nubila* is not thought to nearly such a destructive pest. Further work to establish the occurrence and pest status of this species would be appropriate.

Follow-up indicated/planned

- Pheromone traps are being used by member of the WCAMRN to determine the distribution and *C. ignefusalis*. Their use for early detection in farmers' fields is being investigated by ICRISAT (Ayaji and Prabhakar, 1997).
- Now a suitable formulation for the pheromone is available, it is proposed to determine whether mating disruption of *C. ignefusalis* on larger areas can be used to reduce damage by this pest during a project in Niger, Nigeria and Burkina Faso funded by IFAD.
- There seems little prospect of using manipulation of mate-finding behaviour for control of *H. albipunctella*.
- In contrast, the results on host finding and selection by *H. albipunctella* should be followed up in collaboration with millet breeders at ICRISAT. The apparent marked

preference for oviposition sites by female *H. albipunctella* would seem to be a good target for use in development of resistant/tolerant varieties of millet. The findings of this project on chemical stimuli should be followed up and the relative importance of chemical and physical stimuli established. The farmer surveys carried out in this project provided support for the view that *H. albipunctella* is the most damaging insect pest of millet in the Sahel, and development of resistant varieties appears to be the most appropriate approach to management of this pest by smallholder farmers in the Region.

- The improved methods for assessing resistance/tolerance of millet to *H. albipunctella* will be used by ICRISAT in assessment of millet varieties.

Dissemination

Publications:

YOUM, O., TOURE, K., DARBOE, M., RATNADASS, A., MAHAMADOU, C.I., BALDE, M. and HALL, D.R. (1997) Monitoring pearl millet stem borer (*C.oniesta ignefusalis* (Hampson)) populations using pheromone-baited traps. Part I: Results from Mali, Gambia, Niger and Senegal. *International Sorghum and Millets Newsletter,* No. 38, 134-136. (Peer reviewed paper)

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Internal Reports

- Quarterly and Annual reports.
- Appendices 1-9 of this Report

Other Dissemination of Results

"Sound, scent and sex in the scourge of the Sahel". Presentation to Royal Entomological Society, London, by Dr S Green, November 1998.

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