Egg-laying behaviour in the millet head miner moth, Heliocheilus albipunctella (De Joannis): a preliminary assessment of the role of chemical cues in oviposition site selection.

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Introduction.

The millet head miner moth, Heliocheilus albipunctella (De Joannis), is rated as the most damaging pest of pearl millet panicles in the Sahelian region of West Africa (Nwanze and Youm 1995).

The flight period of the adult moth coincides with the peak of millet panicle emergence and flowering, which is towards the end of August in southern Niger. Gravid females oviposit into young panicles, laying their eggs either individually or in small clusters, stuck loosely to the rachis, or to the base of florets or their peduncles (Vercambre 1978). The panicle apex is the favoured region for oviposition, with 80% of eggs laid within 5cm of the tip (N'diaye 1985). After hatching, caterpillars feed and complete their larval development within the panicle. During this period the seed head also grows and develops, passing from emergence through flowering to grain-filling and maturity. The early larval instars eat into individual florets, whilst larger larvae consume peduncles, thereby killing the developing grains, and creating mines around the rachis which are evident as characteristic raised tracks on the panicle surface (Matthews and Jago 1993, Nwanze and Youm 1995). In the field, larval development takes 29-30 days (Vercambre 1978, N'diaye 1985). The fully grown caterpillar, which acquires a pink coloration at this time, then emerges nocturnally from the panicle and drops to the ground, where it burrows into the soil to pupate, usually within 25cm of the host plant (Youm 1995).

Several reports suggest that millet head miner damage is more severe on certain millet varieties than it is on others (reviewed in Youm and Kumar 1995). Youm and Kumar (1995) list the following potential mechanisms of resistance: "temporal escape" when flowering time is asynchronous with moth's main flight period, non-preference for oviposition, tolerance, antibiosis, and physical characteristics of the panicle such as the presence of bristles and the compactness of the seed head. Here we assess the importance of chemical cues in oviposition site selection by H. albipunctella, the millet head miner moth.

Chemical cues (i.e. plant odour/taste) are known to play an important role in the process of host plant selection by phytophagous insects (reviewed by Visser 1986). In the case of H. albipunctella, in the wild, females can be seen flying through millet fields from dusk onwards (Green et al. *submitted*), actively moving between millet panicles. An approach flight towards a panicle is followed either by rejection, with directed flight away, or by "initial acceptance" as manifest by the female landing on the panicle surface. Once landed, the female lowers her abdomen and applies the tip into the panicle surface, probing between the young florets, with an everted ovipositor (Fig 1). The female then walks slowly upwards, typically over a period of several minutes, toward the apex of the panicle, probing repeatedly. It is assumed that these movements correspond to the oviposition of individual eggs or egg clusters, but at present we have no quantitative information on the placement of eggs by individual females on individual panicles.

Figure 1. Female *H. albipunctella* ovipositing on 3/4HK millet panicle in the field.

The investigations described below form one part of a preliminary examination of the role of non-volatile and volatile panicle surface chemicals in oviposition site selection by female H. albipunctella, using a bioassay approach in the laboratory. The study was undertaken in order to follow up the findings of a previous study (Owusu et al. unpublished) which presented inconclusive evidence to suggest that female H. albipunctella would oviposit on filter papers that had been treated with panicle extract.

Methods.

This work was conducted at the ICRISAT Sahelian Center, Sadoré, Niger, during August-September 1999, a season in which H. albipunctella numbers remained very low. The short millet variety 3/4 HK was cultivated in a field plot and used as the standard in the following experiments.

Pre-emergent millet panicles in the field were enclosed within paper bags just prior to their emergence from the leaf sheath. By this means the panicles were shielded from potential damage or infestation before their use in experiments. Panicles were subsequently cut at approximately 30 cm below the base of the panicle. All leaves except the uppermost, which forms the leaf sheath, were removed.

Oviposition bioassays were performed overnight, using females that had been collected in a light trap during the previous night and subsequently maintained with access to water-soaked cotton wool. Two types of cage were used:. the larger type (henceforth referred to as "large cages") were wire mesh box cages (30x31x50cm), the "small cages" were cylindrical cardboard boxes (height 18cm x diameter 17cm) with nylon gauze lids (see Figure 2).

Panicles were placed into the cages, followed by female moths plus a water-soaked piece of cotton wool, towards the end of the afternoon. In large cages the panicles were secured upright in holders, but the smaller cylindrical cages were not quite tall enough to hold a vertically aligned panicle and so panicles were placed inside leaning at an angle approximately 30^{o} from vertical. The presence or absence of eggs, and number laid were scored for each panicle the following morning.

Polar and non-polar solvent extraction of panicles.

The relative importance of polar and non-polar compounds as oviposition stimulants was assessed as follows. Freshly cut panicles were soaked in either methanol or hexane (polar and non-polar solvents respectively) for periods of 2min, 30min and 24 hours, and then each was offered, along with an untreated control panicle, to females in "large cages" as a potential oviposition site. For hexane, soaking the panicle for 30 minutes rendered it significantly less attractive for oviposition (contingency $\chi^2_{(1\ d.f.)}$.=3.99, p=0.046). In the case of methanol however, 30 minutes soaking did not produce a significant effect, and 24 hours soaking was needed for a significant reduction (contingency $\chi^2_{(1\ d.f.)}$.=6.26, p=0.012). This suggests that non-polar compounds may be more important than polar compounds as oviposition stimulants.

Effect of re-coating methanol-extracted panicles.

Panicles subjected to 24 hours methanol extraction were re-coated by brushing with concentrated methanol extract, which was then allowed to dry by evaporation. These re-coated panicles were then tested pair-wise against control panicles that had been soaked in methanol for 24 hours, but not re-coated. "Small cages" were used. The presence or absence of eggs was scored the following morning. Data from the trials were combined to form a 2x2 contingency table.

	Eggs present	No eggs present
MeOH extract	12	26
MeOH extract + recoat	6	32

$\chi^2_{(1\ d.f.)}$=2.62, p=0.11

Figure 2. "Small cages", each containing two methanol-extracted panicles and a female H. albipunctella. The fresh panicle positioned on top of each lid provides a source of volatile chemicals - the smell of fresh panicle. The control treatment (no fresh panicle) was set up in a different room.

Hence recoating MeOH-soaked panicles with concentrated methanol, containing previously extracted polar compounds did not significantly affect their acceptability as an oviposition site. In contrast to Owusu's unpublished findings, however, here the non-significant trend was for re-coated panicles to be *less* attractive than panicles that were subjected to methanol extraction only.

Effect of panicle volatiles on female propensity to oviposit.

Two panicles that had been soaked in methanol for 24 hours and allowed to dry and then placed inside each "small cage". A female moth from the previous night's light trap catch was then added to each cage, along with a piece of water-saturated cotton wool. Females were exposed to panicle volatiles by the provision of a freshly cut young panicle, placed horizontally above the cage lid (see Figure 2). This lay beyond the reach of the female ovipositor. The control treatment, lacking any fresh panicles, was set up in a different room and the two treatments were performed separately on alternate evenings. The total number of eggs laid by each female was counted the following morning.

Results

	Panicles present	Control
No. of trials with eggs	21	13
No. of trials without eggs	11	20
Mean no of eggs/female	31.1	27.2

Comparing the tendency of females to oviposit in the two treatments, irrespective of the number of eggs produced, it was found that females were significantly more likely to lay in the presence of fresh panicle volatiles (contingency $\chi^2_{(1\ d.f.)}$=4.48, p=0.034). Since the distribution of egg/female is strongly skewed for both treatments, the medians for the two treatments were compared. These were not found to be significantly different (Mann Whitney U test: $U_{(32,33)}$=423, p=0.14).

Discussion.

A simple methodology for the bioassay of oviposition by H. albipunctella has been developed here which proved to be very effective. If more moths had been available then a wider programme of investigations with larger sample sizes would have been possible.

The main findings were:

1. A relatively shorter period of treatment in a non-polar solvent (hexane), compared to polar solvent (methanol), rendered the panicle unattractive as an oviposition site. This suggests that non-polar compounds probably play a more important role as oviposition stimulants.

2. Re-coating of methanol-soaked panicles with the extract did not re-render them attractive for oviposition.

3. In the presence of volatiles from fresh young panicles, females are significantly more likely to oviposit on panicles that have been subject to 24 hours methanol extraction.

Taken together, the above suggests that non-polar volatiles probably play a critical role as oviposition stimulants.

The investigations above are compromised to some extent by the fact that the female moths used in the bioassays were taken from a field light trap, and hence constituted a heterogeneous population with respect to age and mating status. The samples will have included virgins and aged females that had already oviposited, as well as recently-mated fully gravid females. This disadvantage is traded off, however, against a tremendous saving in effort to be made in culturing substantial numbers of moths.

The finding that panicle volatiles stimulate oviposition is very encouraging. This means that chemical factors play a role in oviposition site selection, and provided that there is heritable variation between millet varieties in their chemical profile, then there is a basis for the selective breeding of less attractive millet varieties as a management option against Millet Head Miner. The potential for development of highly attractive millet varieties also deserves

consideration, since these could be cultivated as trap plants in combination with less attractive varieties.

References Cited.

Bayoun, I.M., Gilstrap, F.E. and Youm, O. 1995. Assessment of the effectiveness of natural enemies of the millet head miner. International Sorghum & Millets Newsletter 36, 55-56.

Green, S.V., Owusu, E.O., Youm, O., and Hall, D.R. (submitted). Mating behaviour in the millet head miner moth, Heliocheilus albipunctella (de Joannis) (Lepidoptera: Nioctuidae: Heliothinae).

Matthews, M. and Jago, N.D. (eds) **1993.** Millet pests of the Sahel: an identification guide. Natural Resources Institute, Chatham, UK

N'diaye, A.1985. Étude de la biologie et du cycle vital de Raghuva albipunctella de Joannis (Lepidoptera: Noctuidae), ravageur de Pennisetum thyphoides S. & H. au Niger. Thèse Univ. Montreal, 120pp.

Nwanze, K.F. and Youm, O. 1995 (eds) Panicle insect pests of sorghum and pearl millet: proceedings of an international consultative workshop, 4-7 Oct 1993, ICRISAT Sahelian Centre, Niamey, Niger. ICRISAT Patancheru, A.P. 502 324, India.

Owusu, E.O. unpublished manuscript.

Vercambre, B. 1978. Raghuva spp. et Masalia sp., chenilles des chandelles du mil en zone sahelienne. Agronom. Trop. 33: 62-79.

Visser, J.H. 1986. Host odor perception in phytophagous insects. Ann. Rev. Entomol. 31: 121-144.

Youm, O. 1995. Bioecology of the scarab beetle Rhinyptia infuscata and millet head miner Heliocheilus albipunctella. p.115-124 In: K.F. Nwanze and O. Youm (Ed.s): Panicle insect pests of sorghum and pearl millet: proceedings of an international consultative workshop, 4-7 Oct 1993, ICRISAT Sahelian Center, Niamey, Niger. Patancheru 502 324, India: ICRISAT. 320pp.

Youm, O. and Kumar K.A. 1995. Screening and breeding for resistance to Millet Head Miner. p.201-209 In: K.F. Nwanze and O. Youm (Ed.s): Panicle insect pests of sorghum and pearl millet: proceedings of an international consultative workshop, 4-7 Oct 1993, ICRISAT Sahelian Center, Niamey, Niger. Patancheru 502 324, India: ICRISAT. 320pp.