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Entomology

Methods for Rearing *Heliocheilus albipunctella* in the Laboratory and Eliminating the Pupal Diapause

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Introduction

The millet head miner *Heliocheilus albipunctella* is one of the most damaging pests of pearl millet in the Sahel. During the past 15 years, considerable progress in the development of pest control measures has been achieved through increased knowledge of the ecology of this heliothine moth (Nwanze and Youm 1995, Kadi Kadi et al. 1998, Youm and Owusu 1998a, 1998b). Future research to improve control of the millet head miner could be enhanced through the development of reliable artificial rearing techniques. Moreover, the improved rearing techniques could be used for the assessment of biological control agents and for supporting millet breeding programs to advance head miner integrated pest management (IPM).

Breeding populations of *H. albipunctella* were established at the Natural Resources Institute (NRI), University of Greenwich, UK from eggs collected from Niger at the end of the 1996, 1997 and 1998 field seasons. Previous authors have reported difficulty in rearing *H. albipunctella* (Gahukar et al. 1986), and the process has remained problematic. However, from 1996 to 1998 we effectively increased the number of generations reared in each successive year, and the 1998 population was sustained until the end of the project, which was terminated after 15 months.

Methods and Results

Heliocheilus albipunctella cultures were maintained under environmentally controlled conditions. Relative humidity was kept at a constant 60%. A photoperiod of 14 h light and 10 h dark, with photophase light intensity changes, was used, and temperatures were maintained at 31°C and 27°C, respectively. Under this temperature regime few pupae entered diapause. The information

related to the life cycle and successful rearing methodology is summarized.

Adults to eggs. In the field, *H. albipunctella* females fly directly to buzzing males and mating takes place at the buzzing site, typically on the lower portions of the stem/ leaves of pearl millet (*Pennisetum glaucum*) or other suitable vegetation. In the laboratory, virgin adults of *H. albipunctella* mate readily, even in confined conditions. At NRI, adults were maintained in mating cages consisting of a perspex cylinder (15 cm diameter, 30 cm height) with a perforated metal lid. Up to 5 pairs of moths were placed in a single mating cage. Strips of fabric (disposable nappy liner) were suspended from the walls of mating cages to provide males with buzzing perches, and suitable mating sites. A layer of water-absorbent filter paper on the cage floor was moistened before the onset of scotophase to simulate the natural increase in relative humidity that occurs at dusk. It was estimated that 80% of moths mated under these conditions, provided a healthy partner was available. Successful matings were obtained with females for up to three nights after emergence, and with males for up to 5 nights post-emergence.

Access to free-water extends adult survival. In the field, *H. albipunctella* adults have been observed drinking from dew or rain drops on millet stems, and in our rearing culture water was provided in the form of water-soaked pieces of cotton wool that were placed in the cages, and also by the moistened filter paper used to increase the relative humidity. Moths also drank honey-water when provided.

Heliocheilus albipunctella is monophagous in the wild, with larvae mostly found on pearl millet panicles. This relates to the females' strict host specificity during oviposition, and this factor must be accommodated when rearing the moth. Cotton wool pieces provided for oviposition in the early stages of rearing were rarely observed with eggs. The only reliable method for obtaining eggs was to provide mated females with an erect millet panicle for oviposition on the nights following mating. Early stages of panicle development (up to flowering) are preferred for egg laying. Pearl millet was grown for this purpose in glasshouses at NRI during 1997–99. The supply of young panicles tended to be erratic; however, females readily oviposited on thawed panicles that had been stored frozen. Thus, a large stockpile of frozen panicles was kept for oviposition.

Typically, eggs are laid singly or in small clusters on the panicle and are attached to the base of the florets or to the peduncles (Vercambre 1978). They can readily be detached using a soft-bristled paintbrush or flexible forceps, after which they can easily be transferred onto larval diet for hatching. In this case the eggs should be placed on fresh diet 2 days after oviposition, and the surface of the diet block should be scratched to promote larval access into the media. Eggs that hatched on the cut panicles within the sealed plastic containers were similarly transferred onto the larval diet using a soft sable paintbrush. It is critical that the larvae are transferred soon after hatching, preferably within 6–8 h, otherwise high mortality can occur .

Larvae. Larvae were reared on a solid medium, based on chickpea (*Cicer arietinum*) flour, yeast and agar, which is the laboratory standard for rearing *Heliothis/ Helicoverpa*. At the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Niamey, Niger larvae were reared individually on medium contained in 35 ml plastic pots with cardboard lids that are permeable to water vapor and hence provide a safeguard against

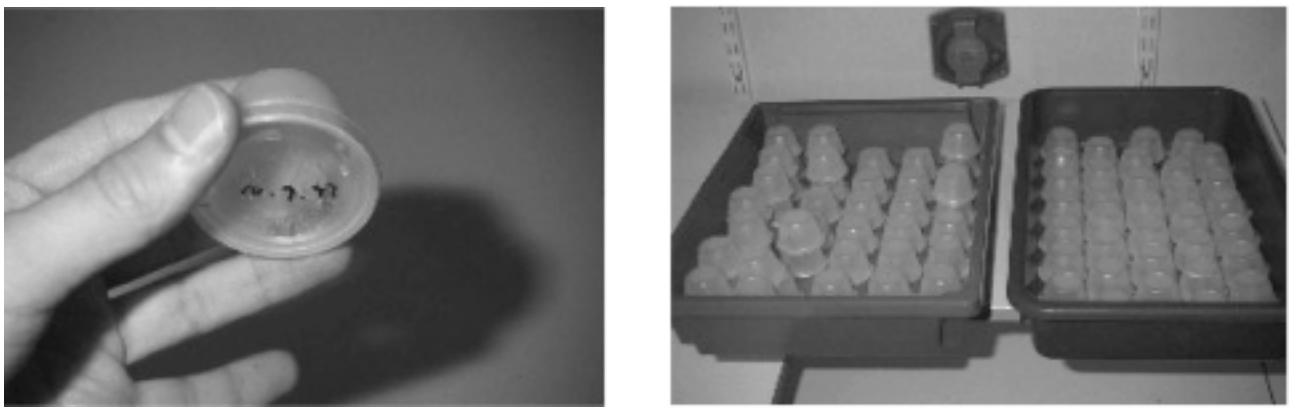

Figure 1. Rearing of *Heliocheilus albipunctella*: (left) rearing pot containing larval medium and first instar caterpillars; and (right) bulk rearing in pots and each pot contained 5 larvae initially.

excessive moisture and risk of fungal growth. At NRI a slightly different system was developed in which, initially up to 5 larvae were maintained per 35-ml pot, which saved a substantial amount of time and effort. These pots had plastic snap-on lids, pierced 4 times using an entomological pin to allow ventilation. By maintaining the pots in an inverted position (ie, lid down, see Fig. 1), escapes by the tiny first instar larvae were negligible. A new dice-sized piece of diet was added to the pot every 4 days. The old piece of diet, which typically contains the developing larvae, was left inside the pot. There is little larval cannibalism in *H. albipunctella*, except that full-grown larvae may eat freshly formed pupae, particularly if fresh medium is overdue. Hence, it is a good practice to reduce the larval number to 1 or 2 per pot when the final larval instar stage occurs. Bulk rearing of larvae was also tried, maintaining up to 25 larvae in 250 ml plastic tubs. But the productivity of this method was found to be inferior to the use of small pots, possibly because of larval-pupal cannibalism and higher pathogen transmission rates.

Hygiene is critical for the successful culture of *H. albipunctella*, especially during larval rearing. Bacterial and viral pathogens appeared to contribute substantially to the decline of the NRI culture in 1996 and to a lesser extent in 1997, and low-level incidence of infection was evident even when the culture was thriving. Consequently, all forceps and artists' brushes used to handle larvae were repeatedly dipped in *Virkon*® (a bleach-based disinfectant solution), and then rinsed and dried before re-use, during feeding sessions.

Larval development passed through 5 instars and took approximately 25 days under the conditions specified above. Final instar larvae turn green and then pink prior to pupation. In the field, the pre-pupal larva emerges from the panicle, drops to the ground and then burrows to a depth of about 25 cm before pupation occurs. In culture, at this stage the larval burrowing activities cause the medium to disintegrate and pupation occurs on the base of the pot, inside a silk-lined cavity within the particulate substrate.

Pupa and diapause. In the field, a pupal diapause suspends development for approximately 10 months, until the onset of the next season's rains. The pupa then returns to the soil surface where the pupal case splits and the adult emerges. In the laboratory cultures, pupae were collected every six days, and maintained resting on dry filter paper inside petri dishes. The pupae were observed daily for adult emergence.

Under the regime employed (31°C under 14 h light, 27°C under 10 h dark), few pupae entered diapause and these emerged 9–10 months after pupation. Earlier efforts to culture *H. albipunctella* at NRI, in which larvae were maintained at 26°C, had been unsuccessful because the majority of pupae entered diapause (J Colvin, NRI, UK, personal communication). It seems that there is a threshold rearing temperature during larval development at which diapause becomes inevitable, but this was not sought here, since the adopted regime inhibited diapause effectively. The non-diapausing pupal stage typically lasted 18–30 days under the present cultural conditions (see Fig. 2).

The sex ratio of emergent adults did not differ significantly from 1:1. There is potential for improving the likelihood of successful emergence. Even in the most successful rearing regime (1998–99) almost one-third of all emerging adults had either badly deformed wings or failed to emerge from the pupal case. Successful emergence might be enhanced if the pupae were buried in moist

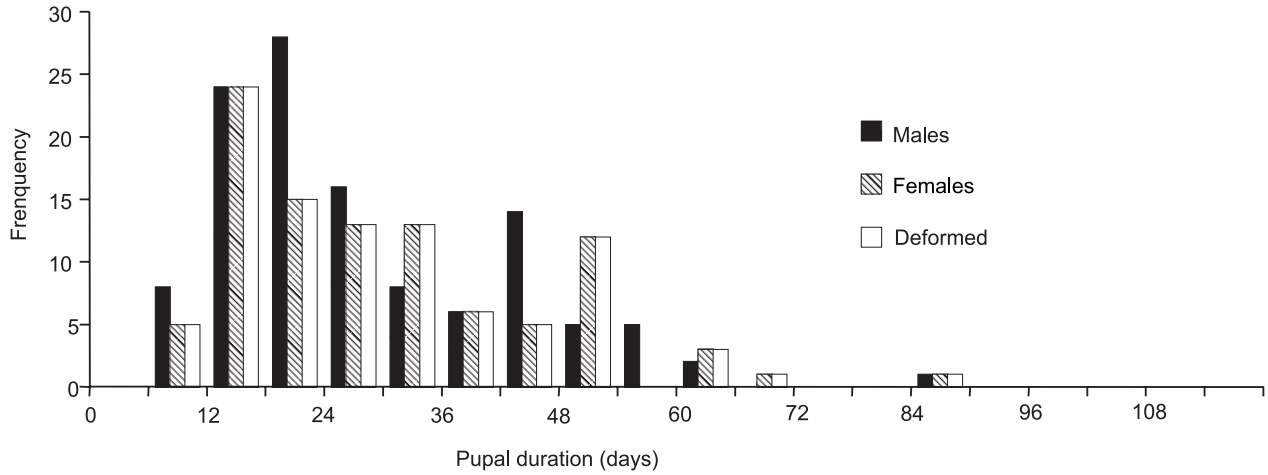

Figure 2. *Heliocheilus albipunctella* pupal duration under culture (31°C under 14 h light and 27°C under 10 h dark) during 1998.

sand, thereby simulating natural emergence conditions more closely.

Findings of these studies will be helpful in enhancing the rearing of the millet head miner in the laboratory and improve its management in the Sahel.

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Observations on Factors Affecting Attraction and Oviposition Preferences of the Millet Head Miner *Heliocheilus albipunctella* to Pearl Millet Panicles

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Introduction

The millet head miner *Heliocheilus albipunctella* is a serious insect pest of pearl millet (*Pennisetum glaucum*) in the Sahelian zone of West Africa. Females lay 20–50 batches of about 300–400 eggs on millet heads (Bernardi et al. 1989, Nwanze and Harris 1992). Eggs normally hatch in 3–5 days and the developing larvae feed on floral glumes and flower stems thus causing yield decrease.

Even though millet panicles serve as oviposition sites for the head miner, the mechanisms underlying this choice remain unknown. This article reports on laboratory experiments to investigate factors affecting host plant and head miner oviposition interactions.

Materials and Methods

Insects. Gravid female head miners were obtained from light traps (Robinson traps equipped with photosentivie cells with 125W mercury vapor bulbs) located at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) Sahelian Center, Niamey, Niger.

Panicle stages tests. The most suitable panicle stage for oviposition by the millet head miner was assessed using five plant growth stages, ie, 30% panicle extension, 50% panicle extension, 100% panicle extension, flowering stage and dough-filling stage. Panicle stages were arranged evenly in paper containers (27 cm height, 25 cm diameter) covered with nylon gauze. Ten adult females were used for a multi-choice test condition in the dark. The number of eggs laid on each panicle stage was counted the following morning. Five millet varieties were used in three replications. Positions of three pearl millet panicles in the cages were randomly assigned for each experiment.