Methods for rearing *H. albipunctella* in the laboratory and breaking diapause.

Breeding populations of *H. albipunctella* were established at NRI, from eggs brought back to the UK from Niger at the end of the 1996, 1997 and 1998 field seasons. Previous authors reported difficulty in culturing this moth (Gahukar *et al* 1986), and the process remained problematic throughout the present project. However, at NRI the number of generations reared successfully before the culture died out increased in each successive year of the project, and the population founded by the 1998 cohort of eggs was sustained for 15 months until the end of the project. Unfortunately, the premature demise of the culture in 1996 and 1997 curtailed several laboratory experiments.

The culture was maintained in a controlled environment room running on a 14:10 hour light:dark cycle, with phased light intensity changes at dawn and dusk. Humidity was fixed at 60% RH. Temperature was maintained at 31^{o}C during day time, and 27^{o}C at night time. Under this temperature regime few pupae enter diapause (see below). The life cycle and successful rearing methodology are summarised together by life stages below.

1. Adults to eggs.

In the field, female *H. albipunctella* fly directly to buzzing males and mating takes place at the buzzing site, typically low down on the stem/leaves of a millet plant or amongst other low vegetation. In the laboratory, virgin adult *H. albipunctella* mate readily, even in quite confined conditions. At NRI, adults were maintained in cages consisting of a perspex cylinder (diameter 15cm, height 30cm), 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 to provide males with buzzing perches, and suitable mating places subsequently. A filter paper layer on the cage floor absorbed water, which was squirted into the cage before the onset of scotophase, simulating the natural increase in relative humidity at dusk. We estimate that 80% of moths mated under these conditions, provided a healthy partner was available. Successful matings were obtained with females up to three nights after adult emergence, and with males up to 5 nights post-emergence.

Access to fluids extends adult survival. In the field, adult *H. albipunctella* are often observed drinking from dew or rain drops on millet stems, and in the culture water was provided in the form of soaked pieces of cotton wool placed in the cage, as well as squirting water into the cage at around dusk. Moths also drank honey water if this was provided.

H. albipunctella is monophagous in the wild, with larvae found only on millet panicles. This relates to females' strict host specificity during oviposition, and this factor must be accommodated when culturing the moth. Only occasionally did females lay on pieces of cotton wool provided for that purpose in the early stages of culturing. The only reliable means found of 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. Millet was grown for this purpose in glasshouses at NRI during years 2-4 of the project. The supply of young panicles tended to be erratic, but since females laid readily on panicles that were thawed from frozen this presented no problem. A large stockpile of frozen panicles was kept for oviposition.

Eggs are laid singly or in small clusters on the panicle, attached to the base of the florets or to the peduncles (Vercambre 1978). They can readily be detached using a soft bristled paint brush or flexible forceps, after which they may be transferred onto larval diet for hatching. In this case the eggs should be placed on fresh diet 2 days after laying, and the surface of the diet piece should be scratched to promote larval access into the media block. The method we have most often used at NRI has been to leave the eggs on the cut panicle, which is placed inside a sealed plastic container, and then allow hatching to take place on the millet head. The young larvae can then be transferred onto the larval diet using a soft sable paint brush. It is critical that they are transferred soon after hatching, preferably within 6-8 hours, or else high mortality results.

2. Larvae.

Larvae were reared on a solid media, based on chickpea flour, yeast and agar, which is the laboratory standard for rearing *Heliothis / Helicoverpa*. At ICRISAT, Niger, larvae were reared individually in 35ml plastic pots of media, with cardboard lids that are permeable to water vapour and hence provide a safeguard against excessive moisture and risk of fungal growth. At NRI a slightly different system was developed in which 3 or 4 larvae initially were maintained per 35ml pot, which saved a substantial amount of time. These pots had plastic snap-on lids, pierced 4 times with an entomological pin to allow ventilation. By maintaining the pots in an inverted position (i.e. lid down, see Figs 1, 2) escapes by 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, is left inside the pot. There is little larval cannibalism in *H. albipunctella*, except that freshly formed pupae may be eaten by full-grown larvae, particularly if fresh media is overdue. Hence it is good practice to reduce the larval number to 1 or 2 per pot once they reach the final larval instar. Bulk rearing of larvae was also tried, maintaining up to 25 larvae in 250ml 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, through 5 instars, takes 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 c.25cm before pupation occurs. In culture, at this stage the larval burrowing activities cause the media to disintegrate, and pupation occurs on the base of the pot, inside a silk-lined cavity within the particulate substrate.

Figure 1. Rearing pot, containing larval media and first instar caterpillars.

Figure 2. H. albipunctella bulk rearing Each pot contains 5 larvae.

3. Pupa and diapause.

In the field, pupal diapause has the effect of suspending further development some 10 months, until the onset of the next season's rains. The pupa then burrows back up to the soil surface, the pupal case splits and the adult emerges. In culture pupae were collected every six days, and maintained on dry filter paper inside petri dishes. Checks for adult emergence were made daily.

Under the regime employed here (31^{o}C x 14hours light : 27 ^{o}C x 10hours dark) few pupae entered diapause (those few that did emerged 9-10 months after pupation). Earlier efforts to culture *H. albipunctella* at NRI, maintaining larvae at 26 ^{o}C, had been frustrated because the majority of pupae entered diapause, (J.Colvin, pers.comm). It seems likely there is a threshold rearing temperature during larval development at which diapause becomes inevitable, but this was not sought here since the regime that was adopted inhibited diapause so effectively. The non-diapausing pupal stage typically lasted 18-30 days under the present culture conditions (see Figure 3 below, representing 1998-9 data).

The sex ratio of emergent adults did not differ significantly from 1:1. There is clearly scope for improving the likelihood of successful emergence. Even in the most successful rearing regime (1998-9) almost one third of all emerging adults had either badly deformed wings or else failed to properly emerge from the pupal case. Successful emergence might be enhanced if the pupae were buried in moist sand, thereby simulating natural emergence conditions more closely.

Figure 3. *H. albipunctella* pupal duration under culture (L:D 14:10h, L:31degC D:27degC)