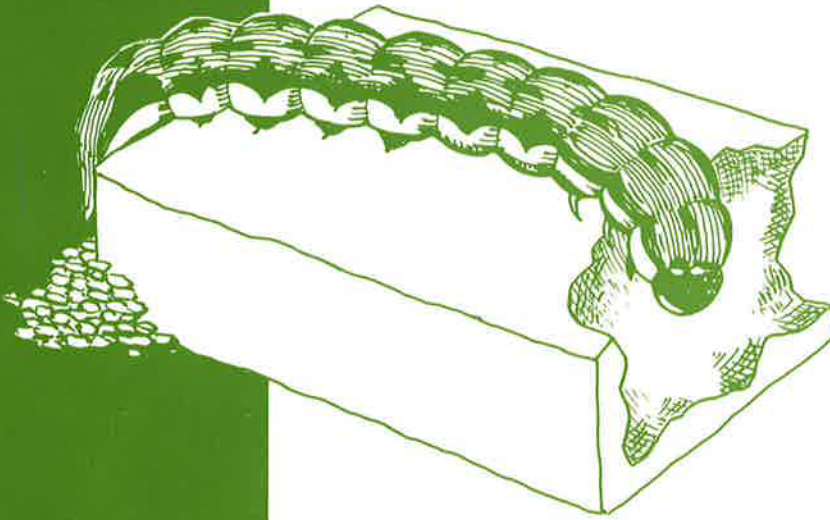


**THE LABORATORY CULTURE
AND DEVELOPMENT OF
*HELICOVERPA ARMIGERA***



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AND DEVELOPMENT OF
*HELICOVERPA ARMIGERA***

N. J. Armes, G. S. Bond and R. J. Cooter

Bulletin 57



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Summaries

SUMMARY

Techniques for the laboratory rearing of *Helicoverpa armigera*, an important pest of food crops in the Old World tropics, are described. Methods for rearing all stages of the insect are given, including recipes for artificial diets and recommendations for the recognition and control of disease. The effects of various environmental factors on development in the laboratory are described.

RESUME

Les techniques applicables à l'élevage en laboratoire de *Helicoverpa armigera*, qui est un fléau important des cultures vivrières dans les régions tropicales du vieux monde sont décrites. Il est fourni les méthodes d'élevage applicables à toutes les phases de développement de l'insecte, ainsi que des directives concernant les régimes alimentaires artificiels et recommandations en matière de reconnaissance et de lutte contre la maladie. Il est par ailleurs décrit les effets de divers facteurs d'environnement sur le développement en laboratoire.

RESUMEN

Se presenta una descripción de las técnicas de cría en laboratorio de la *Helicoverpa armigera*, importante plaga de los cultivos alimenticios en las zonas tropicales del viejo mundo. Entre los métodos para la cría de las distintas etapas del insecto se cuentan recetas para dietas artificiales y recomendaciones para el reconocimiento y control de la enfermedad. También se describe el impacto de distintos factores ambientales sobre su desarrollo en el laboratorio.

The laboratory culture and development of *Helicoverpa armigera*

INTRODUCTION

Noctuids of the genera *Helicoverpa** and *Heliothis* are among the most important Lepidopteran pests of both subsistence and cash crops in the New and Old Worlds. *Helicoverpa armigera* (Hübner), commonly referred to as the American bollworm or gram pod borer, is the most widely distributed of the group, occurring in Africa, Asia, Australia, Oceania and Europe. The polyphagous larvae attack cotton, maize, sorghum, sunflower, tomato, okra, and a range of legumes, notably pigeonpea and chickpea, but there are few reliable estimates of crop loss. In India, annual losses to pigeonpea and chickpea alone may exceed US\$ 300 million (Reed and Pawar, 1982), and a more recent estimate by Mehrotra (Indian Agricultural Research Institute, unpublished) suggests that total losses to pulses and cotton are likely to be more than US\$ 530 million per annum, with insecticides at an annual cost of US \$120 and US\$ 7.2 million being used on cotton and pulses respectively. In Queensland, Australia it is estimated that annual losses due to *H.armigera* and *Helicoverpa punctigera* (Wall.) amount to A\$ 25 million (Twine, 1989). Since 1972, there have been increasing crop failures resulting from the development of insecticide resistance in *H.armigera* (Wilson, 1974; Gunning *et al.*, 1984; Ahmad and McCaffery, 1988; McCaffery *et al.*, 1989). It is not therefore surprising that *H.armigera* has generated much research interest across the world and that the demand for laboratory reared insects for experimental use is increasing. The Natural Resources Institute (NRI), has over ten years' experience in keeping laboratory cultures of *H.armigera*, and this bulletin describes the methods used for long-term maintenance of a regular supply of healthy insects. Factors affecting the development of the life stages in the laboratory at NRI and reported in the literature are described.

REARING PROCEDURE

Environmental conditions

The insects are routinely reared in controlled environment rooms in a purpose-built insectary complex. The environmental conditions are, day: 25±2°C, night: 20±2°C, synchronized with a 14:10 hour light:dark photoperiod**. Two rooms are used, one humidified to 70 – 85% r.h. for holding moths and eggs, and the other for larvae and pupae where humidity in the room is not controlled, as

*The generic name *Helicoverpa* is now accepted for *H.armigera* (Hübner) which was formerly classified in the genus *Heliothis* (Matthews, 1991).

**In the United Kingdom, plant health and quarantine regulations set by the Ministry of Agriculture, Fisheries and Food specify that *H.armigera* must not be reared in photoperiods less than 13 hours light (i.e. LD 13:11) and temperatures below 23°C. This is to prevent pupal diapause which can occur if larvae are reared under short day-lengths (e.g. at LD 12:12 or below), particularly when coupled with low temperature (e.g. below 20°C).

larvae are fed on a moist diet in closed containers which ensures a locally humid environment. On occasion, the temperature of the larval rearing room is increased to day: 28°C, night: 25°C to hasten development of larvae and pupae.

Moths

Under the above temperature regime, adults emerge about 14-18 days after pupation; females usually start to emerge 1-2 days before males. Eclosion normally takes place during the scotophase. Immediately after emerging from the pupal case, moths climb up a nearby vertical surface and pass through three stages of wing drying: the crumpled wing stage, the butterfly stage and the quiescent moth stage. Sex of newly emerged adults can be determined by the colour of the forewings – in males the forewings are greenish whilst in females they are brown (varying from light to dark). In early work, males and females were selected on the morning after eclosion (moths tend to be least active during the morning and therefore easier to handle), and placed as individual pairs in 1-litre Kilner jars. A 9 cm Whatman No.1 filter paper is placed on the bottom of the jar and a strip (6 × 17 cm) of bonded viscose tissue ('nappy-liner') hung vertically inside to provide an oviposition substrate. Where nappy-liners are not available, medical quality close-woven gauze wadding can be used equally well. The glass lid is placed loosely on the jar to allow limited air exchange. Sucrose-based adult diet (see Appendix 1) is provided in a 5 ml glass vial with a cotton wool wick filling the mouth to prevent the moths from drowning. One diet pot is placed in the bottom of each jar and the diet changed three times a week.

The morning after eclosion, moths are transferred to cages (0.5 m wire frame cube covered with black cotton netting). A feeding dispenser consisting of a glass pot containing adult diet and a cotton wool wick is placed upside-down on the top of each cage with the wick hanging into the cage. On the third day after eclosion, males and females are mixed in a single cage. The moths are then observed during the late scotophase when calling and mating occurs (Kou and Chow, 1987) and single mating pairs removed to Kilner jars for oviposition.

Sucrose solution is preferable to diets based on honey, which tend to ferment, and if not changed frequently soon result in death of the moths. Methyl-4-hydroxybenzoate dissolved in a small quantity of ethanol is added to the solution to retard spoilage by moulds. It is advisable not to provide the moths with too much adult diet, nor within the first 12 hours following eclosion, as *H.armigera* will overfeed causing gross distension of the abdomen and premature death. Similar responses have been noted in other captive Lepidoptera (Friedrich, 1986). Furthermore, excessive feeding on sugars appears to stimulate an early over-production and subsequent oviposition of infertile eggs, causing females to become refractory to mating (Colvin, 1990). However, a small amount of a sugar diet is essential for complete maturation of the reproductive system and initiation of calling behaviour (Colvin and Gatehouse, in press).

In established laboratory cultures at peak oviposition 80 – >200 fertile eggs are laid per night. Eggs from individual pairs are kept separately so that the percentage of fertile pairs can be determined. This provides a useful indication over successive generations of changes in the viability of the culture. As a general guide, egg batches from 20-30 fertile pairs are required to maintain a viable, core, breeding culture, but would need to be increased if large numbers of insects are required for experimental purposes.

Under field conditions *H.armigera* mate in the early hours of the morning when the humidity is high, and in the laboratory adult longevity and mating success are usually enhanced when the moths are maintained in a humid environment (Callahan, 1962).

If humidification of the rearing facility is not possible, then humid conditions can be maintained in the moth containers by the inclusion of small pots containing distilled water and cotton wool wicks. (It is not so easy to achieve high humidity conditions by this method if large volume cages are used).

Inbreeding should be minimized as far as possible by promoting heterogeneous mating within the culture. This is best achieved by keeping sibling larvae in separate trays and pairing the resulting adults with individuals derived from another family. In this way it is possible to follow the lineages in succeeding generations, but this does become extremely complex after 3-4 generations! Bartlett (1985) provides more detailed guide-lines for retaining genetic diversity in insect cultures.

It has been found that rearing moths in individual pairs is preferable to keeping several pairs in larger containers, as mating success is increased (probably because of less interference from conspecifics), and it is easier to handle the moths when in smaller numbers. However, larger cage systems with multiple moth pairs have been used successfully in the NRI laboratories for reserve cultures and in other laboratories routinely (Griffith and Haskell, 1988; Teakle, 1991; McCaffery, Reading University, personal communication), so the choice of moth mating system can be adapted depending upon facilities and resources available.

Eggs

The eggs are pale yellow, 0.46-0.53 mm in diameter and mostly laid individually on the viscose strips or sometimes on the filter paper in the bottom of the jar.

Fertile eggs can be laid on the night following mating and as first matings generally take place between nights 2-4 the majority of eggs are laid within the first 12 days, peaking on nights 3 and 4. The viscose strip is changed every 1-2 days and any dead or dying moths removed so the paired adults can be discarded after 14 days. Occasionally however, it has been noted that field-collected cultures have exhibited delayed reproductive maturation, with peak egg production not occurring until nights 8-14. In such cases, if substantial numbers of eggs have not been produced in the first 10 days and the adults appear healthy, then it is worth waiting beyond 14 days before discarding them.

Each morning the tissue strips should be removed from the oviposition jars and the eggs surface-sterilized by immersing the whole strip in a 1.8% (v/v) solution of sodium hypochlorite for 5 minutes. The strips are then placed in a Büchner funnel and the hypochlorite solution pulled off under vacuum (a simple Venturi water-jet pump is sufficient). The funnel is filled with distilled water to rinse the eggs for 5 minutes, pulled dry and the rinsing repeated once more. The majority of the eggs remain attached to the strips; those which become detached can be collected by carefully rinsing the funnel with clean (preferably distilled) water. The funnel should be thoroughly cleaned between egg batches to prevent stray eggs from different strains contaminating subsequent egg washings. The viscose strips are left to dry in a laminar flow cabinet for about 30 minutes until they are only slightly damp, before being placed individually in 300 ml plastic pots. By avoiding putting excessively wet tissues into the pots condensation is minimized; this reduces the chances of the neonate larvae drowning and also reduces disease and fungal problems (see below). Similarly, the tissues should not be dried excessively as the eggs are more susceptible to desiccation after hypochlorite treatment, which affects the surface structure of the egg chorion. It is preferable to sterilize the eggs from individual pairs separately, at least for the core breeding culture, so that a rigorous check can be kept on the proportion of fertile pairs. Egg pots are labelled with pair details so that lineages can be traced as suggested above. Under these rearing conditions egg hatch normally occurs 3 days after oviposition; if the eggs remain unhatched after 5 days they can be discarded. Fertile eggs develop a brown ring on the second day, then the whole egg turns brown and they turn black on the third day. Infertile eggs remain yellow/brown and shrivel after a few days. If required to delay hatching eggs can be stored at 15 °C for 7-8 days with no noticeably adverse effects.

Larvae

Between 12-24 hours prior to hatching (that is, when the eggs have turned black) a 10 × 4 cm (approx.) strip of viscose tissue coated with larval diet (see Appendix 1) is placed in each pot. Neonate larvae usually eat some or all of the empty egg shell before moving to the larval diet. Coating the tissue strips with a thin layer of diet increases the surface area available for feeding compared with blocks of diet, and this greatly reduces cannibalism. The larvae are cannibalistic at all stages of development and are particularly vulnerable when moulting. When larvae reach the late 2nd instar (5-7 days after hatching), they are transferred individually, using a sable hair brush or blunt nosed spring-steel forceps, one per container, to either 5 cm plastic Petri dishes or 30 ml clear plastic pots (cheaper and available in bulk from catering suppliers) containing a 2 × 2 × 1 cm (approximately 7 g) piece of larval diet (see Appendix 1). Ventilation holes are made in the 30 ml pot lids as these are tight fitting and can otherwise result in suffocation of large larvae; Petri dish lids are loose fitting and no additional ventilation is required. Any larvae developing poorly are rejected at this stage. To reduce the risk of spreading pathogens, the brush/forceps must be sterilized in disinfectant between batches (see 'Disease control' below). Dishes/pots are arranged in 60 × 32 × 8 cm trays, usually 50 per tray, labelled with the strain details and the larval hatching date. Larvae are checked on alternate days and any unhealthy or dead larvae are eliminated by discarding the whole dish or pot without opening it. If it is likely that a larva will run out of food before reaching the pre-pupal stage the larval diet must be topped-up, but with experience this can be avoided by giving larger blocks of diet at the outset.

Pre-pupae

Under the above conditions, after approximately 23 days the larvae stop feeding, begin wandering and burrow into the remaining diet to form a 'pupation cell'. Here the larva becomes a pre-pupa which is characteristically shorter, fatter and grub like. The pre-pupal stage lasts for 2-3 days before pupation takes place.

Pupae

Once pupae have formed they should be left undisturbed for a few hours until the soft, newly formed cuticle (pale yellow-green), is fully hardened (red-brown), before removing them from the pupation cells using blunt nosed spring-steel forceps. Handling of newly formed soft pupae invariably results in injury. Deformed pupae are discarded at this stage. The pupae can be sexed by observing the abdominal characteristics (see Figure 1) described by Kirkpatrick (1961). The sex ratio should typically be 1:1. The sexes should be kept separately in 300 ml dishes, half filled with dry vermiculite (DSF grade) or sterilized, insecticide-free, saw-dust, with a maximum of 50 pupae per dish. Sand should not be used as this abrades the pupal cuticle, resulting in desiccation. The pupae should be transferred 3-4 days before the start of adult emergence to 36 cm high × 20 cm diameter perspex cylinders with ventilated lids (1mm mesh metal gauze). The bottom of each cylinder is filled with a 2 cm layer of vermiculite onto which are placed 50-60 pupae. If this number is exceeded the newly eclosed moths may disturb and injure each other.

In the most recent rearing programmes pupae are sexed and placed at different temperatures under conditions of reversed photoperiod (10 hours dark between 03.00 and 13.00 hrs). Males are held at 2°C higher temperature than females to promote synchronized emergence of both sexes.

Two 38 × 22 cm strips of nappy-liner are hung vertically down the inside of each cylinder for the newly emerged moths to climb up to complete wing expansion and cuticle hardening, which takes between 90-120 minutes.

H.armigera are generally imported from overseas as pupae and upon receipt these are surface-sterilized using the same concentration of hypochlorite solution wash followed by two rinses of distilled water as for eggs. If disease is a

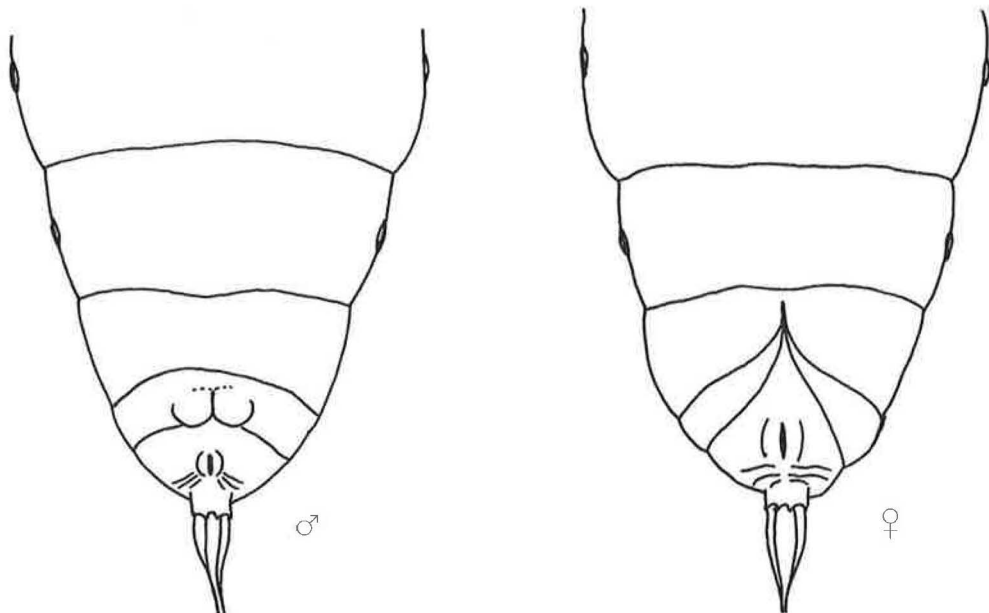


Figure 1 Ventral view of the abdomens of male and female *H.armigera* pupae to show sex determination

problem then it is recommended that pupae are surface-sterilized at each generation, but not until 2-3 days have elapsed since pupation by which time the pupal cuticle will have hardened fully.

If it is required to delay adult emergence, pupae can be kept at temperatures down to 12°C for about 2-3 weeks. If kept at too low a temperature or delayed for too long, a high proportion of moths will emerge crippled and mating success will be decreased.

A summary timetable of operations for a single generation of *H. armigera* in culture is shown in Figure 2.

Disease control

The three principal pathogens in established *H.armigera* cultures are:

- viruses – generally nuclear polyhedrosis virus,
- microsporidian protozoa – *Nosema* spp., (probably mostly *Nosema heliothidis* L and S); and
- fungi and bacteria – *Aspergillus* spp. fungi and probably a range of unidentified bacteria.

The first two are the most likely to occur when introducing field-collected insects into the rearing facility; fungi (commonly *Aspergillus* spp.), are generally associated with poor hygiene particularly in larval diet preparation or larval rearing under conditions of high humidity.

Nuclear polyhedrosis virus (NPV)

NPV infection is most evident in the larval stage. Infected larvae do not show symptoms until 1-2 days before death (Teakle, 1973). The initial signs are that the larva takes on a creamy appearance, death occurs within a few hours and the body contents rapidly liquefy. NPV can generally be eliminated from laboratory cultures providing routine hygiene procedures are maintained and larvae are reared in individual pots. In the rearing facility at NRI the following precautions are taken to ensure a virus-free culture:

- routine surface sterilization of eggs and pupae;
- rearing equipment and reusable containers are soaked in iodoform disinfectant (e.g. 'R62', Evans Vanodine International Ltd.), and then washed in

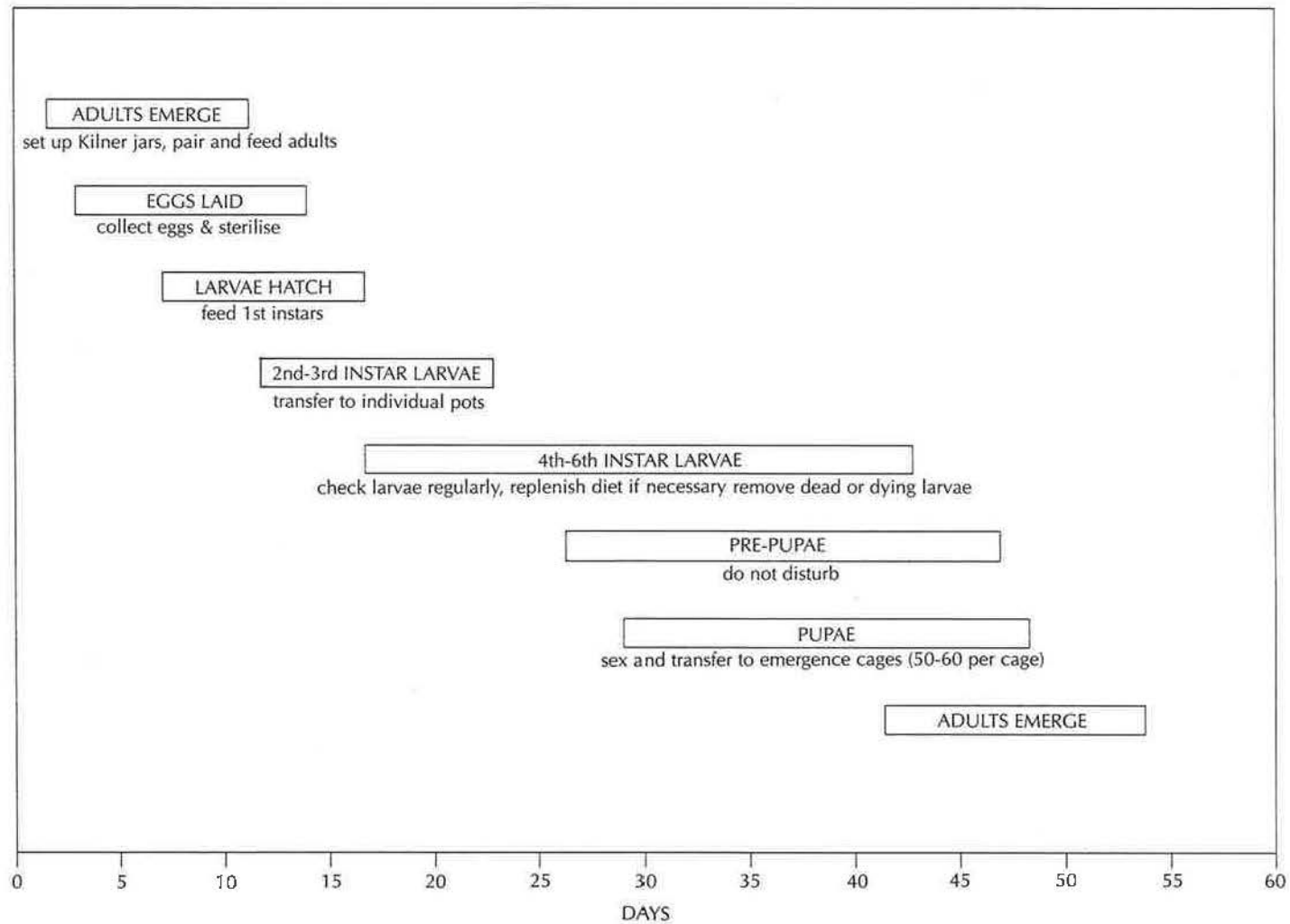


Figure 2 Summary timetable of operations for a single generation of *Helicoverpa armigera* in culture

a commercial cage washing machine with detergent and several hot (80°C) water rinses;

- disposable Petri dishes and 30 ml larval rearing pots are used only once;
- diseased and dead larvae are disposed of immediately by removing the whole dish from the rearing room without opening it;
- forceps and brushes used to transfer larvae are sterilized in concentrated iodoform disinfectant (or 5% sodium hypochlorite solution) at frequent intervals and before re-use on different batches of insects;
- semi-disposable 'Tyvek' suits and overboots are worn at all times in the insectary;
- bench surfaces are swabbed down with 70% isopropyl alcohol after each rearing procedure; and
- rearing room walls, floors and bench surfaces are thoroughly cleaned with 5% sodium hypochlorite solution once a month.

In countries where NPV is endemic strict adherence to hygiene procedures is essential. Germicidal UV lamps (output wavelength 254 nm) can be used to help reduce airborne virus and to sterilize bench surfaces. However such lamps should only be operated when the room is vacated because of potential harmful effects from UV light to the skin and eyes.

Despite these precautions it may still be difficult to prevent repeated re-introduction of the virus or eliminate the presence of a low level infection without more elaborate microbiological control procedures.

Microsporidia

Infection by *Nosema* spp. can be a more intractable problem than infection by NPV, as they can exist in a culture as a chronic condition often going unnoticed for a number of generations, but in the meantime may affect the results of experiments. Once established, the disease is difficult to eliminate, as it can be trans-ovarially transmitted from one generation to the next (Brooks, 1968) and is unaffected by the surface sterilization of eggs and pupae. Mass rearing techniques, where larvae are reared communally, as proposed by some authors (Giret and Couilloud, 1987; Griffith and Haskell, 1988), can augment the problem as the less vigorous, infected, larvae are eaten by healthy ones thereby increasing the spread of disease. The early symptoms of infection can easily be mistaken for inbreeding depression: increased development times and reduced fecundity and fertility over successive generations. At the larval stage acute infections result in cessation of feeding, with consequent loss of body weight. The larval cuticle appears shrivelled and darker than normal, and the larvae remain motionless except when touched. Eventually they die and the body contents rapidly liquefy much as they do in the terminal stages of NPV infections.

The basic principle for the control of microsporidia in culture is the selection of healthy lines. This can be achieved by careful screening of potential stock material (Waldbauer *et al.*, 1984) when establishing cultures from field-collected insects. When breeding pairs have produced sufficient fertile eggs they are killed and the abdominal contents of each insect are smeared onto a microscope slide and examined for *Nosema* spores (see Appendix 2 for preparation of slides). If spores are present then all the progeny from that pair are destroyed. Once the culture has been established in the laboratory for two generations the screening routine is relaxed, but dead and sick larvae must still be checked for *Nosema*. The insectary hygiene and sterilization procedures as described for NPV control also help to reduce the incidence of *Nosema*. The reader is referred to Weiser (1961) and Bulla and Cheng (1976) for detailed information on the biology and epidemiology of microsporidia.

Fungi and bacteria

Fungal and bacterial spoilage mainly occur on larval artificial diet. The following procedures will help to reduce the incidence of contaminant organisms:

- ensure that all equipment used in diet preparation is thoroughly cleaned;
- dry-heat sterilize the ground seed flour at 70°C for 3 hours;
- add to the diet ingredients an antibiotic (e.g. Aureomycin), methyl-4-hydroxybenzoate and sorbic acid reduce spoilage; and
- whenever possible keep uncovered trays of diet in a laminar flow cabinet to reduce the chances of contamination by airborne pathogens.

Excessive condensation in larval holding pots can sometimes occur, particularly if temperature fluctuations in the insectary are large; this will exacerbate microbial spoilage unless the pots can be ventilated.

Beauveria and *Nomuraea* spp. fungal infections are often seen in field-collected larvae, but only very occasionally in the F₁ and subsequent laboratory generations and these are not therefore considered to cause significant difficulties. The presence of these fungi is easily identified from the masses of white hyphae which cover the dead larvae.

Allergen containment

Laboratory staff who work with insect cultures over long periods may develop skin and respiratory allergic reactions. Allergy to locusts is particularly well documented (ODNRI, 1989), but the large quantities of hairs and scales shed in moth-holding cages may present some risk with prolonged exposure. Simple protective dust masks (conforming to British Standard BS6016 for fine dust, mist and fumes) should be worn when handling adult moths. Scales should be removed from cages and the insectary by vacuum and surfaces wiped down with damp clothes. Semi-disposable suits worn only in the rearing rooms prevent allergenic dust coming into contact with the skin, and providing changing facilities in the insectary reduces the risk of dust being transferred to other parts of the building.

DEVELOPMENT UNDER LABORATORY CONDITIONS

Moths

Adult longevity

Under laboratory conditions males generally live slightly longer than females (see Tables 1a and 1b). Longevity is largely determined by the availability of food. When provided with sucrose solution adults of both sexes live significantly longer than when unfed or fed only water (see Table 1a). Temperature over the range 20-35°C does not have any significant effect on longevity, although there is a slight tendency for moths to live longer at the lower temperatures (see Table 1b).

Fecundity

Adult feeding has a marked effect on both fecundity and mating success. Unfed and water fed moths produce less than 2% and 43%, respectively, of the total egg output of moths fed sucrose solution (see Table 2). Furthermore, none of the unfed and only 15% of water fed moths mated and oviposited fertile eggs, compared to 77% of sucrose fed moths. Multiple matings were only recorded in moths given sucrose solution, when up to 3 spermatophores were recorded in females from single pairings (see Table 2). Rearing temperature has an effect on both egg output and fertility in sucrose fed moths. When kept at constant

Table 1a Effect of adult diet on longevity of *H.armigera* at constant 24°C

Diet	Longevity (days ± 95% C.I.)			
	n	Male	n	Female
Unfed	20	7.2 ± 0.8	20	7.2 ± 1.1
Water	20	13.2 ± 1.1	20	12.6 ± 1.5
10% sucrose	22	18.9 ± 2.8	20	16.1 ± 2.2

Table 1b Effect of temperature on longevity of sucrose fed *H.armigera*

Temp °C	Longevity (days ± 95% C.I.)			
	n	Male	n	Female
20	20	15.1 ± 2.4	20	10.4 ± 1.8
25	19	13.6 ± 2.0	20	10.7 ± 1.6
35	21	11.6 ± 2.0	21	8.7 ± 1.4

temperatures in the range 20-35°C significantly fewer eggs were laid at 35°C than at 20° and 25°C (see Table 3). The daily pattern of oviposition at the three temperatures is shown in Figure 3. At all temperatures oviposition commenced on night 2 (night 0 being the night of emergence) with peak oviposition between nights 4-7. At all temperatures the percentage of females ovipositing exceeded 80% on nights 4-6. The oviposition period was inversely proportional to temperature, that is, 15 nights at 20°C, 11 at 25°C and 10 at 35°C. Egg hatch at 35°C was extremely low with only one batch of eggs from 21 pairs hatching (see Table 3).

There is agreement that adults generally need to feed on sugars as a prerequisite for egg maturation and mating (Hardwick, 1965; Topper, 1987). In the literature, estimates of fecundity are highly variable and probably reflect differences in the way the adults were kept in the laboratory. However, it is known that *H.armigera* females can lay several thousand eggs during their lifetime. For example Pearson (1958) found that the average number of eggs laid per female was 730, with a maximum of 1600 over an oviposition period of 10-23 days, and Hardwick (1965) recorded an average of 1702 with a maximum of 4394.

Table 2 Effect of adult diet on fecundity and mating success of *H.armigera* at constant 24°C

Diet	n	Mean no. of eggs laid/ female (± 95% C.I.)	Mean no. of eggs hatching (± 95% C.I.)	Percentage of females with no. of spermatophores:			
				0	1	2	3
Unfed	20	8.4 ± 6.4	0	100	0	0	0
Water	20	322 ± 137	59 ± 84	85.0	15.0	0	0
10% sucrose	22	749 ± 162	346 ± 121	22.7	50.0	22.7	4.5

Table 3 Effect of temperature on fecundity and mating success in sucrose fed female *H.armigera*

Temp °C	n	Mean no. of eggs laid/ female (± 95% C.I.)	Mean no. of eggs hatching (± 95% C.I.)	Percentage of females with no. of spermatophores:			
				0	1	2	3
20	20	994 ± 223	279 ± 196	25.0	70.0	5.0	0
25	20	839 ± 216	303 ± 233	20.0	45.0	30.0	5.9
35	21	236 ± 216	2 ± 5	95.2	4.8	0	0

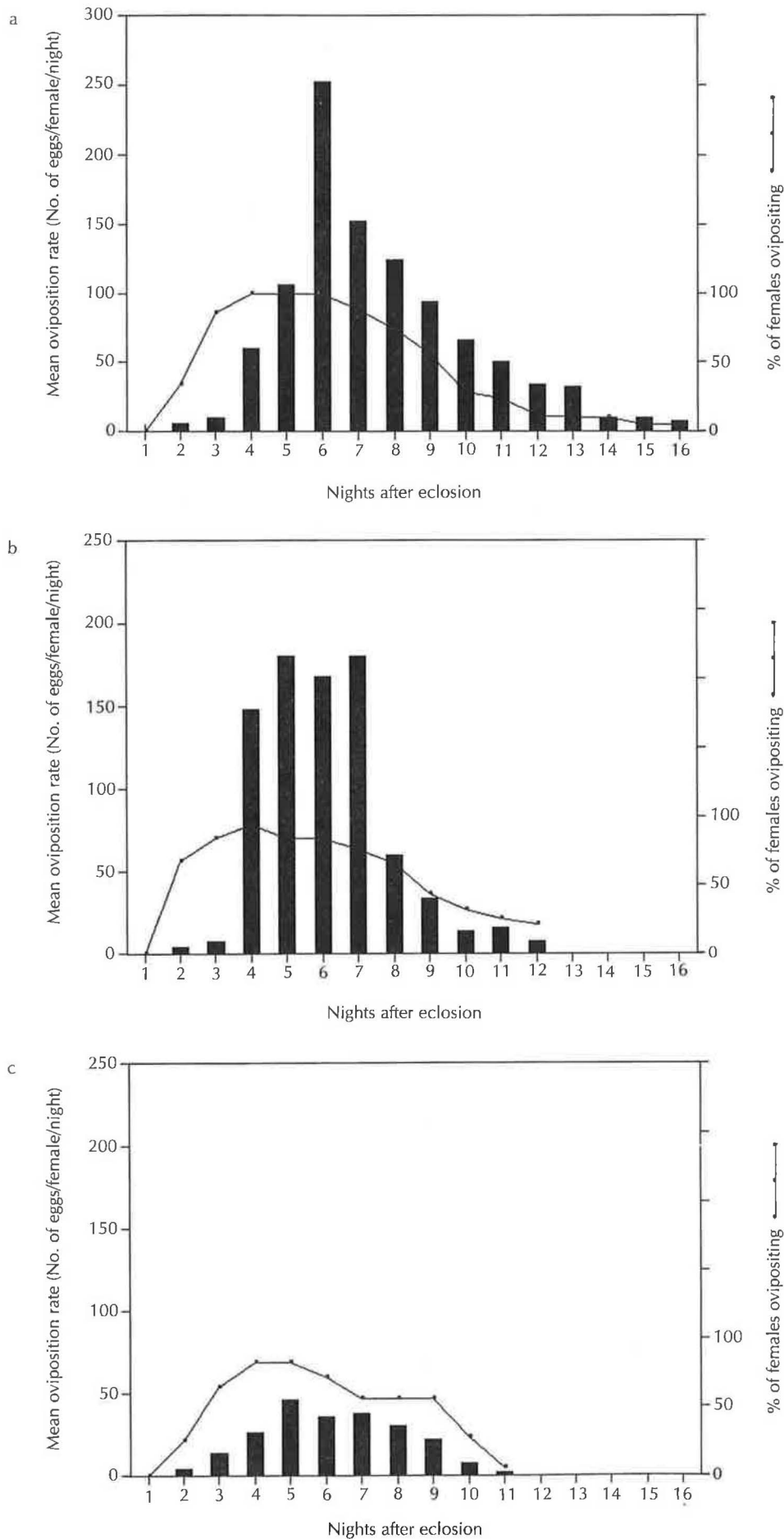


Figure 3 Pattern of oviposition of *H. armigera* at constant (a) 20°C, (b) 25°C and (c) 35°C

Eggs

Under normal rearing temperatures (20-30°C) the duration of the egg stage is 2-4 days which compares with the 2-5 (usually 3) days recorded in laboratory studies in India (Jayaraj, 1982) and Tanzania (Reed, 1965).

Relative humidity has no significant effect on egg viability. In an experiment where batches of eggs (not pre-treated with sodium hypochlorite) were subjected to humidities in the range 6-100 % r.h. for the duration of the egg period there were no significant differences in percentage hatch or duration of the egg stage. Humidity does however affect the survival of newly emerged larvae since they desiccate within a few hours of hatching at low humidity and in the absence of larval diet.

Larvae

Effects of larval diet

A wide variety of diets can be used to rear larvae successfully, but the majority currently in use in laboratories are refinements of the semi-synthetic diet developed by Vanderzant *et al.* (1962) and the wheatgerm diets of Berger (1963) and Bot (1966). Odindo (1981) describes a diet based on grass-meal which was used to rear *H.armigera* successfully for at least five generations. Our original diet was based on haricot bean flour but later, in a comparison of three flours, haricot, chickpea and sorghum, it was found that chickpea was superior in terms of larval development characteristics (see Table 4). Larval duration (including the pre-pupal period) was significantly shorter and larval survival greater on the chickpea diet than on the other two. There were however no significant differences in pupal weight between the diets but, on sorghum, many of the pupae were deformed and some exhibited 'metathely', a condition described by Wigglesworth (1959) as the retention of larval characters probably due to an abnormally high secretion of juvenile hormone at pupation. Chickpea, *Cicer arietinum*, has been used extensively as the base flour ingredient (Nachiappan and Subramanian, 1973; Nagarkatti and Prakash, 1974). Singh and Rembold (1988) found that the growth index was highest for chickpea, moderate for soybean and very low for maize diet. Much shorter development times have been recorded in other laboratory studies where the larvae were reared on natural host plants. For example, Dhandapani and Balasubramanian (1980) reported that the larval period ranged from 17 – 20 days on pigeonpea pods and tomato and Singh and Singh (1975) give 8-12 days on tomato and about 18 days on cotton and maize. Direct comparison between the data of various authors is often problematic as rearing temperatures and other conditions are not standardized and sometimes not even cited. Sometimes it is unclear whether or not the development times stated include the pre-pupal stage.

Effects of temperature

Over the temperature range 20-35 °C, the duration of the larval period decreases and mortality increases with increasing temperature (see Table 5). There are no significant differences in larval period between males and females.

Table 4 Effect of larval diet on period from egg hatch to pupation in *H.armigera* at constant 22°C

Larval diet	n	Mean duration of larval period (days \pm 95% C.I.)	Percentage of larvae surviving to pupation	Mean pupal weight (mg \pm 95% C.I.)
Chickpea	150	34.3 \pm 0.9	77.3	361 \pm 9
Haricot bean	150	37.5 \pm 0.6	75.3	370 \pm 8
Sorghum	150	41.8 \pm 1.0	61.3	355 \pm 8

Table 5 Effect of temperature on the duration and mortality of the larval stage in *H.armigera*

Temp °C	n	Mean duration of larval period (days ± 95% C.I.)		Percentage larval mortality
		Male	Female	
20	79	30.4 ± 1.1	31.6 ± 1.1	21.0
25	53	27.7 ± 2.5	25.6 ± 1.9	47.0
35	31	16.2 ± 1.0	17.8 ± 1.9	79.3

Effects of crowding

Larvae are highly cannibalistic, and in tests in which various numbers (up to 20) of 2nd instar larvae were confined per 300 ml container, only 1 or 2 larvae survived to reach the pupal stage (see Table 6). This result is similar to that recorded by Twine (1971) who noted that cannibalism begins as early as the first instar if larvae are kept at high density. Cannibalism can be reduced to some extent by increasing the surface area available for feeding by using strips of diet (Giret and Couilloud, 1987; Griffith and Haskell, 1988), or providing vertical sticks as refuges for the larvae (Giret and Couilloud, 1987). Tripathi and Singh (1989) did not find significantly higher mortality in batches of 25 larvae reared in 250 ml beakers on hydrated chickpea seed replenished twice daily compared with those reared individually. However, crowding at the larval stage did result in an increased proportion of moths which emerged deformed, probably through increased incidence of pathogens. Although maintaining larvae individually is time consuming and in the short term more expensive, 'mass rearing' is not recommended, as sick and diseased larvae are inevitably attacked and eaten by larger healthy individuals thereby rapidly increasing the problem of disease in the culture.

Instar determination

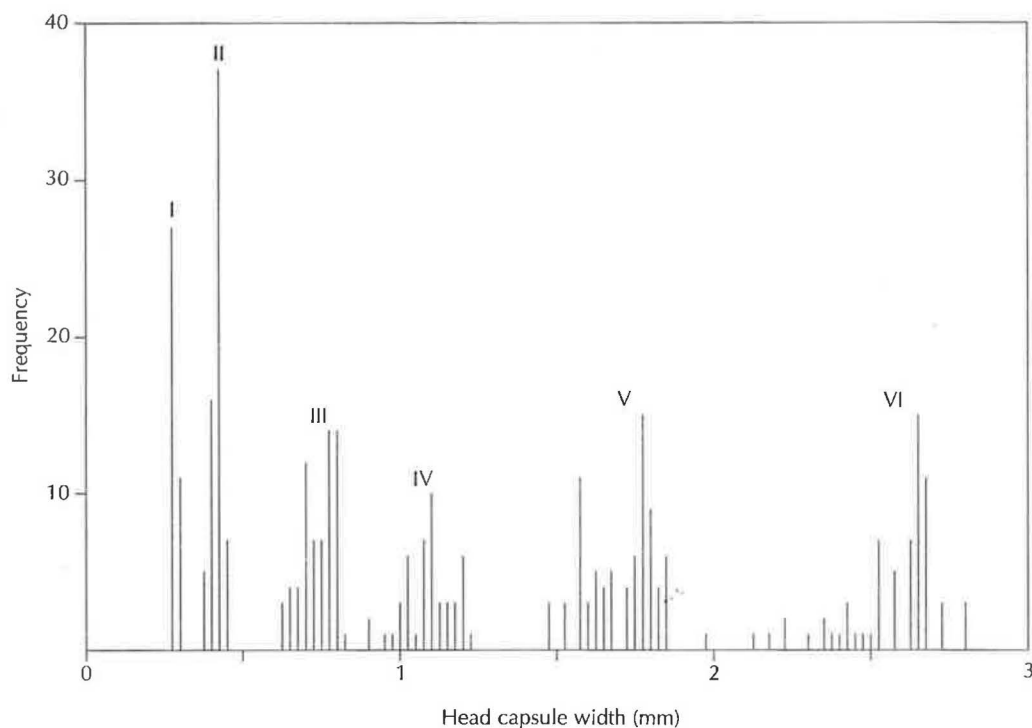
Head capsule diameters of a cohort of larvae reared on haricot bean artificial diet at 24°C were measured daily and the duration of each instar recorded. In addition ten larvae in each stadium were killed in order to measure the head capsules more accurately. In practice it was found that measurements on live larvae were quite adequate and it is these data that are presented in Table 7. The head capsule widths measured during the period from neonate to pre-pupal stage ranged from 0.27 to 2.63 mm, separable into six non-overlapping groups which corresponded with the six larval stadia (see Figure 4). Duration of larval instars and the pre-pupal stage are given in Table 8. The percentages of small (1st-2nd instar), medium (3rd-4th instar) and large (5th-6th instar) larvae on successive days after egg hatch are shown in Figure 5. On the basis of these data it is possible to estimate the approximate age range of a group of larvae from their head capsule size or estimate instar range from age. Under our rearing conditions there were always six larval instars, which is similar to the results of Reed (1965). Hardwick (1965), however, found that at a constant temperature of 25°C, 30%, 69% and 1% of *H.armigera* larvae passed through 5, 6 or 7 stadia respectively.

Table 6 Effect of larval population density on survival and pupal weight of *H.armigera* (each treatment replicated five times)

Population density at commencement (larvae/container)	Median number of larvae surviving to pupation	Percentage larval mortality	Mean pupal weight (mg ± 95% C.I.)
20	1	95	342 ± 41
10	1	90	313 ± 74
5	2	75	345 ± 51

Table 7Head capsule widths of *H.armigera* reared at a constant 24°C

Instar	n	Head capsule width (mm)		
		Mean \pm 95% C.I.	Range	Increment
1	50	0.27 \pm 0.002	0.25-0.29	–
2	65	0.40 \pm 0.005	0.36-0.44	0.13
3	64	0.75 \pm 0.013	0.64-0.84	0.35
4	47	1.10 \pm 0.023	0.90-1.24	0.35
5	68	1.74 \pm 0.025	1.50-1.88	0.64
6	66	2.63 \pm 0.044	2.00-2.88	0.89

**Figure 4** Frequency distribution of *H.armigera* larvae head capsule widths at constant 24°C

Undoubtedly variations in the number of stadia will arise in laboratory cultures depending upon such factors as rearing temperature and the nutritive value of the larval diet.

Pre-pupae and pupae

In the pupal stage, *H.armigera* can undergo a facultative diapause and therefore the duration of this stage depends upon whether or not diapause has been induced during the earlier life stages. Typically diapause can be induced by subjecting larvae to low temperatures and short photoperiods (Roome, 1979; Hackett and Gatehouse, 1982). In the laboratory rearing conditions of long days (14L:10D photoperiod) and moderate temperatures (20-30 °C) preclude diapause. The effect of temperature on the duration of the pre-pupal and pupal stages under laboratory conditions is shown in Table 9. As might be expected, the duration decreases with increase in temperature, but mortality and the percentage of deformed pupae also increase at higher temperatures. There was no difference between the sexes in the duration of the pre-pupal stage, but pupal periods were significantly shorter in females than males at 20° and 25°C, but not at 35°C. Jayaraj (1982) found that in non-diapausing pupae the pupal period ranged from 10.5-13.6 days (temperature not stated).

Table 8 Stadi al and pre-pupal periods for *H.armigera* reared at a constant 24°C

Instar	n	Stadial period (days)	
		Mean ± 95% C.I.	Range
1	67	3.9 ± 0.1	3-5
2	65	2.2 ± 0.1	2-5
3	64	2.6 ± 0.2	2-5
4	66	3.3 ± 0.3	2-6
5	67	4.0 ± 0.3	2-7
6	66	6.7 ± 0.4	4-11
Pre-pupa	41	3.1 ± 0.2	2-5

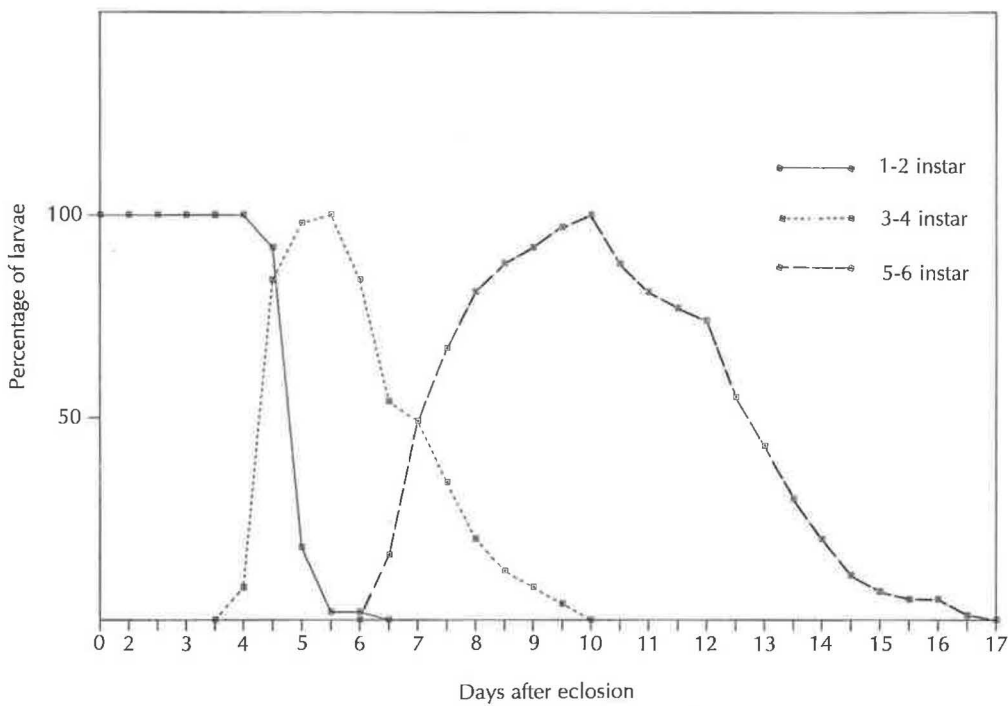


Figure 5 Percentages of small (1st-2nd instar), medium (3rd-4th instar) and large (5th-6th instar) *H.armigera* larvae on successive days after egg hatch at 24°C (only larvae surviving to pupation are included)

Table 9 Effect of temperature on durations of the pre-pupal and pupal stages of *H.armigera*

Temp °C	n	Mean duration of pre-pupal period (days ± 95% C.I.)	Mean duration of pupal period (days ± 95% C.I.)		Percentage of deformed pupae
			Male	Female	
20	65	5.0 ± 0.3	24.8 ± 0.3	22.6 ± 0.3	12.3
25	72	3.0 ± 0.4	17.8 ± 0.6	16.3 ± 0.7	21.2
35	62	1.8 ± 0.3	6.7 ± 1.0	6.7 ± 0.9	36.5

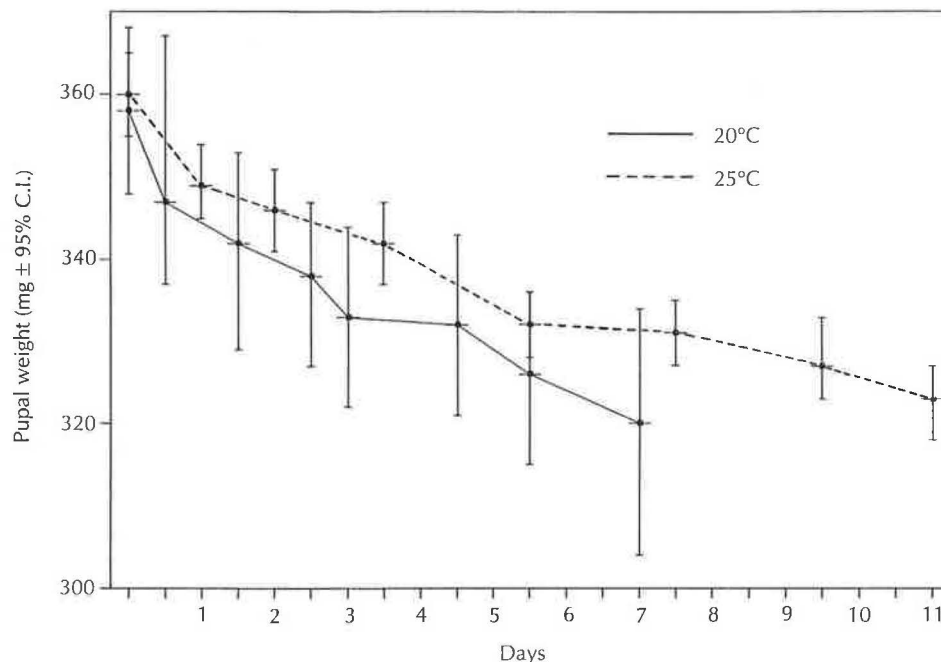


Figure 6 Change in weight of *H.armigera* pupae with time after pupation at constant 20° and 25°C. Error bars represent 95% confidence intervals

Pupal weight decreases from the time of pupation up to adult eclosion (see Figure 6). At 20° and 25°C, 30-50% r.h., average weight loss during the pupal stage was 10% and 11% respectively for pupae in the non-diapause condition. Pupal weights recorded for insects reared on the standard chickpea diet described (see Table 4) are similar to those obtained when larvae are reared on natural diets such as pigeonpea flowers and pods, chickpea foliage and sunflower heads (NRI, unpublished).

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Appendices

APPENDIX 1 PREPARATION OF DIETS FOR LABORATORY REARING OF *HELICOVERPA ARMIGERA*

(Recipes developed at NRI by Dr A. McCaffery (now at the School of Biological Sciences, University of Reading))

1 Vitamin mixture

The vitamin mixture is used in both the adult and larval diets.

Ingredients:

Nicotinic acid	Niacin	1.53 g
Calcium pantothenate (pantothenic acid calcium salt)	B ₃	1.53 g
Riboflavin	B ₂	0.76 g
Thiamine hydrochloride (aneurine hydrochloride)	B ₁	0.38 g
Pyridoxine hydrochloride	B ₆	0.38 g
Folic acid	Bc	0.38 g
D-biotin	H	0.31 g
Cyanocobalamin	B ₁₂	0.003 g
Distilled water		600 ml

Method:

Boil distilled water for 10 minutes in a 1000 ml flask, loosely plug with clean cotton wool and allow to cool to room temperature. Weigh out vitamins and add to a clean 500 ml volumetric flask. Make up volume to 500 ml with the cooled boiled distilled water. Keep refrigerated. Some of the vitamins are unstable if exposed to sunlight and therefore, if possible, an amber glass flask should be used, or clear flask wrapped in aluminium foil to exclude light. Providing the mixture is kept dark and refrigerated it will remain usable for several months. Some of the ingredients are insoluble in water and do not remain in suspension for long, so the mixture should be shaken well before use.

2 Adult diet

Ingredients:

Sucrose	50 g
Vitamin mixture (see 1 above)	10 ml
*Methyl-4-hydroxybenzoate	1 g
90-95% ethanol	1 ml
Distilled water	500 ml

Method:

Dissolve methyl-4-hydroxybenzoate in alcohol in a test tube and then combine all the ingredients in a 500 ml volumetric flask. Make up volume to 500 ml with the distilled water. Shake well until the sucrose has dissolved and pour into clean polythene wash bottles. Keep refrigerated.

*Fungicide

3 Larval diet

The quantities of water and agar may have to be adjusted at different laboratories in order to compensate for local variations in the quality of the chickpea flour and yeast used in the diet.

Ingredients:

a Chickpea seed, finely ground	300 g
b Ascorbic acid	4.7 g
c Linseed oil (refined)	12 ml
d Vitamin mixture (see 1 above)	10 ml
e *Sorbic acid	1.5 g
f *Aureomycin (as water soluble powder containing 5.5% chlortetracycline hydrochloride)	0.4 g active ingredient
g *Methyl-4-hydroxybenzoate (Nipagin M)	3.0 g
h 90-95% ethanol	3.0 ml
i Distilled water	400 ml
j Yeast (dried, bakers')	48 g
k Agar (powder)	17.25 g
l Distilled water (for yeast/agar)	700 ml

Method:

Put the 700 ml of water into a clean saucepan and bring to the boil. Slowly sprinkle in the agar, stirring all the time. Boil for 15 minutes. Meanwhile weigh and measure out all other ingredients. Dissolve methyl-4-hydroxybenzoate in the ethanol in a test tube and then combine ingredients (a)-(i) in a bowl, mixing thoroughly using a hand-held food mixer or commercial blender (e.g. 'Waring' blender). Once the agar has boiled for 15 minutes, add yeast while stirring. Remove from heat and pour into bowl containing ingredients (a)-(i). Mix continuously with food mixer/blender at high speed until an even consistency is obtained. This should be done reasonably quickly as the agar will start to set. Pour diet into shallow melamine or stainless steel trays (40 × 30 × 1.5 cm) to a depth of approximately 1 cm. Place trays in a laminar flow cabinet to cool and set. This generally takes between 30 to 60 minutes. Place trays with diet in clean plastic bags and keep in a fridge. If not required immediately the diet can be stored satisfactorily in a freezer providing bags are sealed.

4 Neonate larval diet

Neonate diet contains the same ingredients and is made in the same way as the larval diet above, except that after mixing all the ingredients together the diet is kept warm (but not boiling) on a pre-heated hot plate at 90° C to delay setting. Then strips of nappy-liner (viscose tissue, approximately 34 × 22 cm, are thoroughly immersed in the diet, promptly removed and hung vertically to drain off the excess diet. In this way a thin coating of diet is left on the liner which can then be cut into approximately 10 × 4 cm strips and kept in a fridge or freezer for later use.

APPENDIX 2 PREPARATION OF *HELICOVERPA* MATERIAL FOR SCREENING FOR MICROSPORIDIA

Method

Squash and smear insect tissue (e.g. eggs, larva, moth ovaries, etc.) onto a microscope slide. Leave to dry. Make up fresh 10% Giemsa's stain using distilled water buffered to pH 7.2. Fix slides in methanol for 1 minute. Rinse with distilled water. Place in stain for 30 minutes. Remove, drain and leave to dry at room

*These are antimicrobial agents. If Aureomycin is not available, ampicillin, streptomycin sulphate or penicillin V can be used equally well, up to 0.05% (w/w) of the larval diet (Sikorowski and Goodwin, 1985).

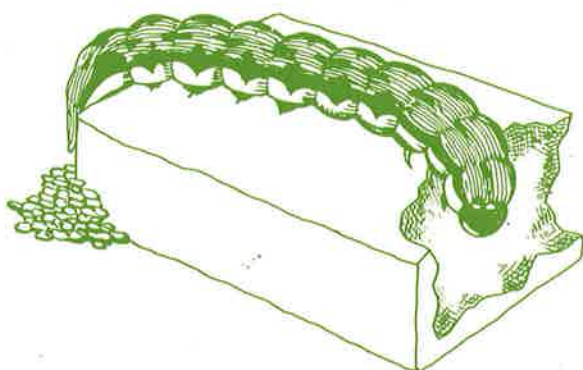
temperature. Examine preparation under compound microscope (10X eyepiece, 100X objective, bright-field oil immersion).

Microsporidia spores stain blue; they are oval in shape and of uniform size measuring 3-7 μm in length and 1.3-3.5 μm in diameter. Spores are obvious if present in moderate numbers. In particular look for the characteristic binucleate spores. Sporoblasts tend to stain pinker. Spores should not be confused with yeast cells which are of variable size and generally very abundant. Rod shaped bacteria stain dark and can be very numerous but are small (<2 μm in length).

Refer to Bulla and Cheng (1976) for details on the biology and identification of microsporidia.

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Helicoverpa armigera is an important pest of cotton, pulses and sorghum in the Old World

tropics. The development of resistance to insecticides is stimulating research into new management techniques for this species. To assist these efforts

The Laboratory Culture and Development of *Helicoverpa armigera* presents the experience gained at NRI in rearing and breeding this insect in the laboratory.