THE OVERWINTERING FOE:
WINTER POPULATIONS OF HELIOTHIS IN COTTON GROWING AREAS AND THE IMPORTANCE OF STUBBLE CULTIVATION.

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As major pests of cotton production, *Heliothis* spp. are of most concern to growers during the summer when they are active and damaging crops. Once autumn arrives and the crop is picked, *Heliothis* are soon forgotten. But they remain, secreted in their underground burrows, usually in low numbers, sometimes numerous, ready to emerge with the return of warm spring weather to haunt growers the following season. In this paper we will outline what is currently known about the overwintering phase of *Heliothis* populations, what a high survival of pupae means for regional population dynamics and pesticide resistance, and what growers might do about it.

PUPATION and DIAPAUSE

After feeding for 12-16 days, mature *Heliothis* larvae leave their food plants and burrow into the soil to pupate at depths of 2-22 cm, but usually in the range 5-10cm. Pupation depth is influenced by soil type, soil structure and moisture content. In compacted soil, pupae may often be found close to the surface, whilst in moist, friable earth they dig deeper. Each larva constructs a chamber in which to pupate and an emergence tunnel to the surface through which it will emerge as an adult. The structural integrity of this tunnel is crucial to its survival and emergence.

The position of pupae beneath crop residues has been described in detail elsewhere (Australian Cotton Grower May 1987). In row crops, many pupae are found immediately beside the row of plants, and the majority are concentrated in the central section of the hill, 20 cm either side of the row. Few pupae occur in furrows. In broadacre crops, pupae also tend to be concentrated immediately below their host plants.

In the temperate and subtropical regions, both species of *Heliothis* survive the winter mainly as diapausing pupae under their autumn hosts. Diapause is a state of arrested development which greatly extends the pupal period to about six months, preventing premature emergence in autumn and producing a synchronised emergence in spring. Some individuals survive the winter as non-diapausing pupae, while in some areas in some seasons there may be a small breeding population right through the winter. Pupae in diapause do not develop in response to prevailing temperature until the diapause is broken. Development normally recommences in late September. Adults begin to emerge in early October and may continue well into December (see Fig 1). Diapausing pupae are more tolerant of cold than non-diapausing ones, and are easily able to survive the soil temperatures experienced in winter. Diapause thus enables the persistence of populations during periods which are unsuitable for breeding or adult survival, and is an important attribute contributing to the pest status of *Heliothis* in agricultural systems.

In *Heliothis*, diapause is only induced if individuals experience the correct combination of temperature and photoperiod (hours of light per day) at the end of the larval stage or as prepupae. In most cotton-growing areas the declining temperatures and photoperiods of autumn provide the necessary signals to induce diapause, but the incidence of diapause decreases as we move further north, such that in tropical areas very few pupae diapause and breeding occurs year round. For both *H. armigera* and *H. punctigera* the optimal diapause-inducing conditions are temperatures less than 22°C combined with a photoperiod of 11.5-12.0 hours. In temperate regions some individuals enter diapause from early March onwards, the proportion increasing gradually until late April when 90-95% of the pupae formed enter diapause. The variable proportion of non-diapausing pupae formed in autumn, develop according to the prevailing temperature regime. Some emerge in late autumn (May) and probably die without reproducing, while the remainder emerge in early spring (September) before the bulk of the diapausing population. Because diapause is induced during the larval stage, the contribution of different hosts to the overwintering population will vary depending on whether they harbour larval populations during March and April when diapause may be induced. In most summer cropping areas these crops may be cotton, pigeon pea, late maize, sorghum or sunflowers.

SIZE OF THE OVERWINTERING POPULATION

During winter 1987 we conducted field studies in the Namoi/Gwydir area to determine the numbers of pupae overwintering under various crop residues, the levels of pyrethroid resistance in these populations, and the effect of cultivation on mortality.

Surveys were made in irrigated cotton crops, mainly in the Namoi Valley, and in dryland crops of sorghum, sunflower, maize and pigeon pea in the Namoi Valley, Bellata/Edgeroi area and the Biniguy/Croppa Creek area. Sites were chosen at random. We did not specifically select crops known to have harboured large numbers of larvae in the autumn. At each site, 10-25 metres of row were thoroughly excavated and all intact pupae or pupal remains were collected. Pupae were classified either as emerged, unemerged healthy, unemerged parasitised, or eaten (by predators). For those which had emerged it was possible to determine whether a moth or a parasite has emerged from the pupa.

Results of the survey are shown in Table 1. Overall some 1268 sq. metres of soil were searched at 89 sites, about half of them cotton. Perhaps surprisingly the survey showed that, in terms of pupal density and the areas grown, most of the overwintering population was under residues of cotton crops. Over 80% of all cotton blocks had some pupae, with an average density of 8600 pupae/hectare over all sites. Some sites had considerably more than this. The maximum density found under cotton being 45,000 pupae/hectare with up to 10 pupae in some individnal metres. It was noticeable that rank crops which had suffered late season *Heliothis* damage supported higher densities of pupae. Only 10% of pupae found under cotton were parasitised, predominantly by ichneumonid wasps (*Heteropelma scaposum* and *Pterocormus promissorius*). Pigeonpea was the only other crop to support densities of pupae similar to cotton, with an average of 8700 pupae/hectare for the 14 crops surveyed. However, a high proportion (73%) of these were parasitised (predominantly by *Heteropelma*), so the density of healthy pupae was much lower than under cotton. Maize also produced a large population of healthy pupae, but late maize was insignificant in area. Residues of other autumn broadacre crops, sunflower and sorghum, produced few or no overwintering pupae in that season, though both crops can support large populations of larvae and may produce significant numbers of pupae in some seasons. These results may not apply to other areas. For example, at Emerald cotton is often finished before *Heliothis* enter diapause and so the crop is unlikely to contribute substantially to the diapausing pupal population. Nevertheless in 1983, densities of up to 30 pupae/m^2 were collected under cotton at Emerald when the appearance of pyrethroid resistance led to large populations of larvae late in the season (Australian Cotton Grower 5(1): 22-25).

A second surprising result from the study was that ALL the overwintering pupae were *H. armigera*. We were unable to locate any overwintering populations of *H. punctigera* within our study area. Undoubtedly small numbers of *H. punctigera* pupae would be present, but our results strongly suggest that this species overwinters elsewhere and crops are infested each spring primarily by immigrants. It seems clear however that a large proportion of the overwintering population of *H. armigera* may occur under the cotton crop.

Our studies did not include areas of uncultivated autumn hosts; their patchy distribution and general scarcity during autumn making it extremely difficult to estimate pupal densities. In most cropping areas, *H. armigera* is abundant and concentrated in crop hosts in the autumn, while populations of *H. punctigera* decline dramatically in these areas at this time.

RESISTANCE LEVELS IN OVERWINTERING POPULATIONS

In order to determine the resistance level of the overwintering population, the adults which emerged from collected pupae were tested for pyrethroid resistance using a discriminating dose test. Results are expressed as the % of individuals which survived this discriminating dose. We have no indication of whether some subpopulations are more resistant than others.

The proportion of resistant individuals in overwintering populations was generally high, but varied according to crop type. On average 72% of pupae collected under cotton were resistant, whereas only 42% of those found under pigeon pea were resistant. Pupae under maize had an intermediate resistance frequency (52%), but this was based on only one site. The high frequency of resistance among pupae in cotton fields is not surprising, since the overwintering population is derived from larval populations present during mid-late Stage 3, when resistance levels are generally highest. Neil Forrester's studies show the frequency of resistance among eggs collected on cotton normally peaks 2-4 weeks into Stage 3 (1st-3rd weeks of March). These eggs become late instar larvae late in March or early April, when conditions are suitable to induce diapause. In seasons when alternative summer hosts of *Heliothis* are uncommon (or at least produce few adult *Heliothis*) the larval populations on cotton during stage 3 will be derived largely from the survivors of pyrethroid selection on cotton during Stage 2. In 1987 the peak resistance frequency of 60-65% was amongst eggs collected in the 2nd week of March (N. Forrester pers. comm.), leading to the high level of resistance amongst overwintering pupae found here.

IMPLICATIONS of OVERWINTERING PUPAL SURVIVAL for POPULATION DYNAMICS and PESTICIDE RESISTANCE

The size, location and survival of overwintering *Heliothis* populations in cropping areas has not previously received much attention. However these populations probably have a major impact on the density and resistance status of *Heliothis* populations in subsequent seasons. Intensive studies on the incidence of diapause, and the abundance and survival of pupae are now being conducted by Dave Murray (QDPI, Toowoomba). Many factors will reduce the survival of overwintering pupae; low temperatures, high soil moisture and extended flooding, parasites, predators, disease and cultivation. In many parts of the USA cotton belt, overwintering pupal survival is low (<5%), due to the combined effects of extremely low soil temperatures and high soil moisture. Winter soil conditions in Australia are far less severe. For example, at Narrabri, average soil temperatures at 5-10 cm never fall below 5°C, a temperature easily tolerated by diapausing pupae. Thus in many Australian cropping areas overwintering survival may be considerably higher than in other temperate cotton growing areas of the northern hemisphere.

We do not yet understand the importance of local survival of the overwintering population to either the abundance or resistance status of *Heliothis* spp. in the following season. As shown above substantial numbers of overwintering pupae of *H. armigera* can be found within the major cropping areas, and although some immigration of this species may occur, the survival of this local overwintering population may have a major influence on the abundance of *H. armigera* the following season. By contrast, overwintering populations of *H. punctigera* are extremely difficult to locate in cropping areas, yet that species reappears in large numbers each spring, suggesting immigration from elsewhere, perhaps from extensive winter breeding grounds in inland areas of Australia. This regular re-establishment of populations by immigrants from unsprayed native hosts outside the cropping areas may be one of the main reasons why *H. punctigera* has not developed pesticide resistance.

The high level of resistance in overwintering populations under cotton crops will maintain pyrethroid resistance in populations of *H. armigera*, if they are allowed to survive. High survival of these populations may lead to high levels of pyrethroid resistance in early generations the next season, which in turn may lead to spray failures when the pyrethroids are used later in the season. This scenario may have already occurred in the 1986/87 season. The noticeable reduction in the intensity of cultivation of cotton stubble during winter 1986, due to the low price of cotton and poor industry prospects at that time, may have allowed high survival of overwintering pupae, many of which could have been resistant for the reasons outline earlier. The increasing trend towards minimum till systems in cotton production may further exacerbate this problem.

As the time of writing (April 1988) our data on the abundance and resistance status of overwintering pupae is limited to one season (1987). Even so it seems clear that the removal of local overwintering populations acting as reservoirs of resistant *H. armigera* under cottou should help to reduce resistance levels in Stage 1 the following season. As has been repeatedly stressed, the success of the pyrethroid strategy is partly dependent on starting each season with a low level of resistance (<10%).

OVERWINTER SURVIVAL and the EFFECTS of CULTIVATION

What then can growers do to reduce the survival of overwintering pupae? The simplest answer is cultivation. Because of their position within the hills pupae are susceptible to any cultivation which disturbs the top 10-15 cms. of soil. To say that cultivation kills pupae is hardly new. However, to clearly define the effects of cultivation on overwintering survival in

Heliothis population models or models of pyrethroid resistance, we need a *quantitative* understanding of these effects on pupae.

Cultivation can kill pupae directly or may disrupt their emergence tunnel making it difficult or impossible for the emerged moth to reach the soil surface. Pupae exposed at the surface by cultivation are also eaten by birds or other predators or else eventually die from dessication. A number of different cultivation practices, including minimum tillage, are used in cotton. In order to measure the impact of cultivation an experiment was done to measure the effect of different levels of cultivation on mortality of a natural population of pupae.

The experiment centred on a naturally produced, high density population of pupae located on a commercial cotton farm. An area 200x120 metres was made available for experiments and was divided into 10 plots, each 12 rows wide. In this area a pretreatment count of pupae on August 7, made in 60 metres of row distributed uniformly over the entire area, showed an average density of 49,000 pupae/hectare. At this time approximately 19% of pupae had already emerged, probably being non-diapausing individuals which emerged in late autumn. Very few dead pupae were found. Five levels of cultivation (listed in Table 2) were applied during early September. Each treatment was replicated twice. The levels of cultivation were designed to cover a range of cultivation intensities, and do not represent all possible types of cultivation. Estimates of live pupal density were also made immediately after the treatments were applied (September 15). Emergence cages were installed in mid-September to record the numbers of moths emerging in the spring. Although the order of treatments was randomised over the area, there were considerable differences between treatments in the initial density of live pupae (Table 2). Estimates of overwinter survival are calculated from the based on the pre-treatment density of live pupae in each treatment and the combined number of moths and parasites which emerged, since the survival of a parasite in the pupal case indicates that the pupa had remained intact.

Levels of parasitism averaged 12-15% over all treatments. *Pterocormus promissorius* was the most common parasite in this population. This wasp parasitises the pupal stage of *Heliothis* and was observed actively searching for pupal burrows throughout the winter. The level of parsitism by *Pterocormus* increased from 4% in July to 15% in mid-October indicating it was able to locate pupae successfully.

Differences in overwinter pupal survival between treatments were highly significant ($F_{4,25}$=33.16, p<0.001). In the absence of cultivation (control treatment), survival was high (49.7%). All the cultivation treatments reduced survival, though the effect of direct drilled wheat was minimal. Aerially sown wheat would probably have even less effect. The post-treatment counts showed that direct drilled wheat reduced the density of live pupae by only 2.0%, while the treatments 3 and 4 directly killed 40-50% of pupae. No post-treatment counts could be done in the full cultivation. The remaining mortality occurred later as a result of disruption to the adult emergence tunnel, exposing the pupae to other mortality factors or trapping the adult in the soil after emergence. The marked difference in overall survival of pupae between treatments 3 and 4, which differ only in that stalks were pulled in 4, highlights the importance of disturbing the central section of the hill, where most of the pupae are located.

Here we tested only one time of cultivation, in early September. The effectiveness of different cultivations may vary depending on when they are applied during the winter. Significant rainfall after a cultivation which disturbed only the top 2-3 inches of soil (as the go-devil treatment here) may further reduce survival by filling disrupted pupal burrows with mud or by forming a crust thereby reducing adult emergence. Pupae in the experimental population began emerging in early October, with females emerging slightly earlier than males (Figure 1). 50% of females had emerged by the end of October, 50% of males by November 11, and 90% of both sexes by the end of November. However, some moths continued emerging until mid December, when the experiment was terminated, and small numbers of live pupae remained in the ground until at least the end of December. This extended emergence has survival value for *Heliothis* by ensuring that the entire population does not emerge at once into a spring environment unsuitable for breeding (eg. a dry spring). The timing of emergence also gives growers a considerable time (from picking till early October) in which to cultivate if necessary.

SURVEY OF THE FATE OF CROP RESIDUES (Winter 1987)

In addition to the experiment discussed above, we were interested to quantify the extent and level of cultivation of cotton residues in the Namoi and lower Gwydir valleys (Table 3). This was completed with the assistance of Neil Forrester (NSW Dept. of Agriculture, Narrabri). Almost 100 properties were visited in late September 1987 and the fate of 535 blocks of cotton was recorded. Each block was closely examined and rated for the intensity of cultivation according to a predetermined classification. This ranged from no cultivation (Class 1: stalks standing or slashed only) through various levels of disturbance to the hill (Classes 2-4) to Class 5 which had received a full cultivation (cross discing, chissel ploughing) completely destroying the old hills. Almost 30% of cotton blocks remained substantially uncultivated at September 30. We include here crops which had been direct drilled or aerially sown with a winter crop (wheat, barley, chickpeas, lupins) where the rows of stalks and central section of the hill were intact, which as shown above have little impact on survival, as well as those which were completely uncultivated. In fact over half of this category (13% of total blocks surveyed) were completely uncultivated, having standing stalks or been slashed only. In that year some 57% of cotton blocks were returned to cotton after having existing hills centre ripped and rebuilt (which would disturb many pupae) or being rehilled after a full cultivation, while 25% had been sown to rotation crops.

A much less extensive survey of other crop residues was done. From a total of 40 blocks surveyed, about 60% of the acreage of sorghum and sunflowers had been fully cultivated, while all but one crop of pigeonpeas had been cultivated.

Thus about a third of the cotton area surveyed had been largely undisturbed and the survival of any pupae under it would be high. However, it is important to stress that leaving cotton stubble uncultivated, and practices such as direct drilling or aerial sowing of rotation crops are undesirable only if significant numbers of pupae are present. These practices have some agronomic benefits and are acceptable if no pupae are present. What level constitutes a "significant" number of pupae is not clear. On average, *Heliothis* females may produce 1000 eggs during their reproductive lifetime of 4-6 days ie. about 200 eggs/night. The density of females needed to lay a total of 20 eggs/metre on cotton over 2-3 nights is thus only 500 females/hectare. A density of only 1 pupa/metre (10,000/ha) overwintering under cotton, which may produce 4000 moths in the spring, if uncultivated (allowing for 50% survival less 10% parasitism) could thus add significantly to the spring population of *H. armigera*. This calculation is not strictly correct since the adults emerging in spring utilise patchily distributed weed hosts and some spring crops, where larval survival may be quite low. Since we do not yet understand the link between densities of local overwintering pupae and the size of spring populations it is thus impossible to set a critical pupal density below which cultivation is unecessary, but densities of 1 pupa/metre or above certainly require some action. Even well below this level action may be warranted.

CONCLUSIONS

This research indicates that, in one season at least, cotton supported the bulk of the overwintering population of *H. armigera* in the Namoi Valley. Overwinter survival of these pupae is high if undisturbed. They may therefore contribute significantly to the size of spring populations and being highly resistant will act as a local reservoir for resistance. As part of the effort to manage resistance in particular, it would seem advisable for cotton growers to assess the density of overwintering pupae under cotton blocks before deciding on what to do with them. Pupae can be easily located by carefully scraping away the top 1cm. of soil to reveal the emergence tunnels. The tunnel can then be excavated to confirm that a healthy pupa is present. Inspection of 20 metres of row should be sufficient to confirm the presence of pupae. Many growers may be reluctant to search this amount of soil; 4-5 metres is more likely. Thus a useful rule might be that if 1-2 pupae are found in 4-5 metres, cultivation should be considered. However, where numbers of large larvae are present in late or rank crops late in the season, as seems the case in most areas, it is highly likely that significant numbers of pupae will be present. If this is true, then the block should receive an effective cultivation which at least disturbs the stalks and central section of the hill.

Table 1. The abundance and level of parasitism of *Heliothis* pupae under late summer crops in the Namoi Valley 1987.

Crop type	No sites sampled	Total metres searched	% Sites with some pupae	Pupae /ha	% parasitised	No healthy pupae/ha
Cotton	41	638	82.9	8600	10.4	8170
Pigeonpeas	14	205	53.0	8700	73.3	2958
Other legumes	3	30	0.0	0	-	-
Sunflowers	15	225	13.3	222	100.0	-
Sorghum	15	150	0.0	0	-	-
Maize	1	20	100.0	21000	18.9	19950

Table 2. The effects of different levels of soil cultivation on the survival of pupae overwinter.

Cultivation Treatment (after slashing)	Mean live pupae/metre (August 7)	Total moths and parasites emerged/metre	% Survival	Est.Moths/ ha.
1 No cultivation	4.37	2.17	49.7	18055
2 Direct wheat	4.10	1.75	42.7	15000
3 Go-devils	3.37	0.86	25.5	7500
4 Stalks pulled plus go-devils	2.67	0.34	12.7	555 *
5 Disced and chiselled twice	3.97	0.14	3.5	1388

* most individuals emerging in this treatment (10/12) were parasites

Table 3. Results from a survey of COTTON residues in the Namoi Valley 1987.

Total Properties surveyed	98
Total blocks of cotton surveyed	535
% Substantially uncultivated	29.0
% Completely uncultivated	12.7
% With existing hills cultivated and rebuilt	31.0
% Full cultivation	40.0

FIGURE 1 *Cumulative emergence of a natural population of H. armigera pupae. Myall Vale 1987.*