Distribution of *Helicoverpa* (Lepidoptera:Noctuidae) eggs and larvae in INGARD and conventional cotton: Implications for sampling techniques

K.L. Abbott & G.P. Fitt
CRC for Sustainable Cotton Production and CSIRO Entomology, Locked Bag 59, Narrabri 2390 NSW.

Introduction

An important requirement for successful management of *Helicoverpa spp.* in Australian cotton is a reliable and effective monitoring system. Timely pest management decisions must be based upon knowledge of the pest population densities with a reasonable degree of accuracy. Effective sampling for all *Helicoverpa spp.* life stages can be time consuming and onerous, and any technique which reduces the amount of time spent scouting is welcomed, provided accuracy is not compromised.

Whatever sampling scheme is used must be based on an understanding of the distribution and feeding behaviour of *Helicoverpa* life stages and then be associated with a well validated threshold to allow management decisions.

Here we briefly review sampling techniques for cotton and present our first field results aimed at assessing the validity of these techniques with INGARD cotton.

Current Sampling Techniques

There are several methods of estimating pest population densities in a cotton field, and consultants utilise a broad range. These can involve sampling different numbers of plants, different parts of the plants, recording numbers on whole plants or using presence/absence techniques. A substantial body of research on sampling systems for Australian cotton (reviewed in Dillon & Fitt, 1995) has resulted in the currently recommended systems supported by entomoLOGIC. These techniques all require a minimum of 60 plants be checked per 100 hectares of cotton. Crops should be checked at least every three days, with the plants to be sampled selected using a stratified random technique in which six or more groups of five adjacent plants are checked at two randomly selected entry points. The spatial association of plant groups is specifically defined. The proportion of each plant checked can vary from terminal only or whole plants, and on each plant *Helicoverpa* life stages are recorded in six life stages: white eggs, brown eggs, very small larvae, small larvae, medium larvae and large larvae.

Over the 97/98 cotton growing season the most widely used technique for sampling conventional cotton was checking the whole plant and recording absolute numbers of

Helicoverpa spp. in the four life stage categories (Abbott, unpublished data), however, this method is time consuming and laborious if used throughout the season. Alternatively, pests can be recorded using a binomial (presence/absence) system rather than recording absolute numbers, saving considerable time for the consultant or pest scout. This method is based upon relationships between the proportion of plants infested and the density of the pest population in the metre sampled. The computer-based decision support package, entomoLOGIC allows the use of a number of sampling schemes, but the recommended approach is to sample the whole plant up to squaring, and then restrict sampling to the upper terminal portion of the plant, where the majority of *Helicoverpa spp.* eggs and larvae are to be found (Mabbett & Nachapong, 1984; Dillon & Fitt, 1995). EntomoLOGIC then generates estimates of the absolute pest density per metre from the terminal or presence/absence sampling.

There are advantages and disadvantages in all sampling techniques, and one would argue that time and labour would be one of the major disadvantages in intensive checking regimes. While checking whole plants and recording absolute numbers is beneficial in that the scout can assess damage to an entire cotton plant, inconspicuous very small larvae may be overlooked, and sampling must be carried out quite meticulously to ensure accuracy. Terminal sampling, on the other hand, saves time and is just as legitimate in estimating pest population densities if used in conjunction with entomoLOGIC. The presence/absence technique, although uncommon, is one which saves considerable time as it combines terminal sampling with only recording whether the respective life stage is present on each plant or not.

Presence/absence and terminal sampling techniques for *Helicoverpa spp.* were originally developed in the 1970's and released in SIRATAC system in 1979 (Ives & Hearn, 1987), and now appear in the decision support package entomoLOGIC. These procedures were re-validated in the mid 1980's for conventional varieties of cotton (Dillon & Fitt, 1995), but with the introduction of INGARD technology, concern has arisen about whether they are valid and effective methods for transgenic cotton, particularly for larvae.

On conventional cotton the application of pesticides is often timed to coincide with egg hatch, so egg densities are considered in decision making at certain times. Egg densities however, are less relevant in INGARD cotton, since larvae must feed on the plant to be controlled. Consequently there is a greater need to adequately scout for all stages of larvae, particularly the "very small" (1st -2nd instar) stage. Consultants are concerned that there appear to be different patterns of feeding and movement of larvae on INGARD cotton compared to conventional plants. There have even been suggestions of different egg distribution patterns on INGARD. Consequently we have commenced a study to address

these concerns and re-assess the validity of different sampling systems for INGARD varieties.

Why is it Important to be Accurate and Consistent?

The philosophy of integrated pest management with its emphasis on insecticide treatment only when necessary, requires that insect populations be reliably and consistently monitored. Accurate sampling must also be combined with well validated thresholds; we must be confident that the pest populations are at damaging thresholds (economic or physiological) before the application of an insecticide. It is important to note that sampling systems and thresholds go together; a threshold defined using one sampling scheme may not be valid if a very different sampling system is used. Sampling does not imply that we find every egg or larvae. A consistent method linked to a validated threshold for that method is ideal. Insufficient or inaccurate sampling could result in an over or under-estimation of the pest population in a field and mean that pesticides are applied unnecessarily or less commonly that crucial sprays are missed.

Where are the *Helicoverpa spp.*?

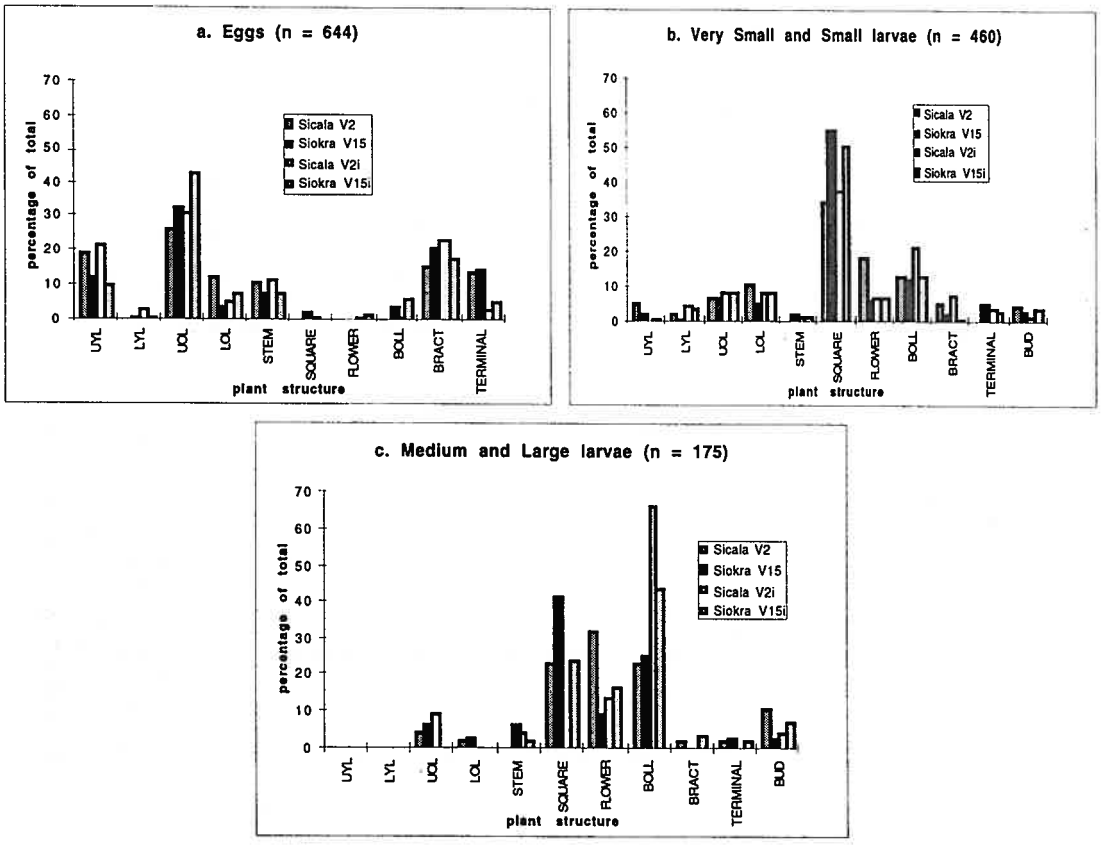
The development of effective sampling techniques requires knowledge of the distribution and behaviour of each life stage of *Helicoverpa spp.* within the cotton plant. This is quite well documented for conventional varieties of cotton (Mabbett *et al.*, 1979 & 1980; Uthamasamy, 1992) in Africa (Pearson, 1958; Ripper & George, 1965), Thailand (Mabbett *et al.*, 1979 & 1980), Indonesia (Karmawati, 1988) and Australia (Wilson & Room, 1982), but unavailable for varieties of INGARD cotton. The nature of INGARD cotton suggests that the distribution of larvae may be different compared to conventional cotton if the presence of Bt protein changes the mobility of larvae between feeding sites. Clearly if we know where on the plant the eggs and larvae of *Helicoverpa* are, we should know where to check. In these investigations, the plant was divided into various structures on which eggs and larvae were recorded. The structures were leaves (young and old, upper and lower surfaces), squares, flower buds, flowers, bolls, bracts, stems and the young terminal growing area.

These preliminary investigations into the distribution of *Helicoverpa spp.* life stages on both conventional and INGARD varieties indicate little difference in the distribution of eggs and larvae between the two types of cotton.

Figure 1 (a,b,c) shows the distribution of *Helicoverpa* life stages on 2 varieties of INGARD and conventional cotton. They show the changing distribution of life stages as development proceeds through a generation. Eggs are mostly found on the upper surfaces of leaves, both young and old; very small and small larvae are found on squares and

flowers, and medium and large larvae found on squares, flowers and bolls. Despite suggestions to contrary, we found very few eggs on flowers during the 1997-98 cotton season, and the proportion of larvae recorded feeding in flowers was no different on INGARD cotton when compared to conventional varieties.

Figure 1: Proportional distribution of *Helicoverpa* life stages on four varieties of cotton plants (97/98 season)

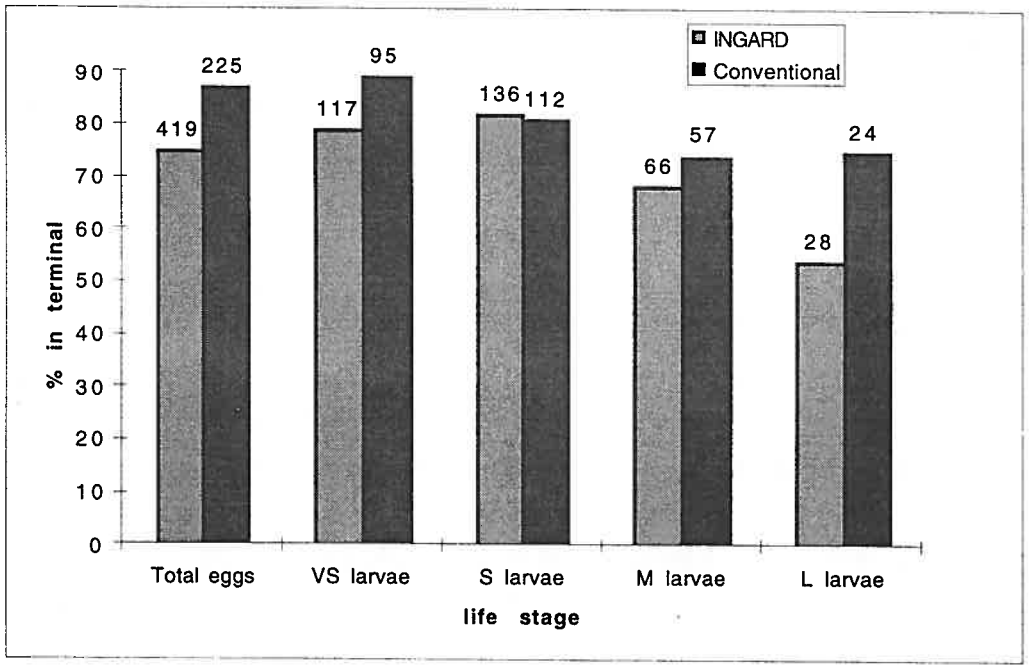


The terminal region was defined as any part of the cotton plant at or above node 3 (Phase I of the cotton resistance management strategy), node 5 (Phase II) and node 7 (Phase III). EntomoLOGIC defines the terminal region of the plant as the top 12 centimetres of the plant.

Over 70% of all *Helicoverpa* life stages were found in the terminal region of the plant, suggesting that the terminal portion of the plant (Figure 2) is a relatively good indicator of the numbers of pests that are present, and that conversion factors to relate to the whole plant can be easily calculated. However, the terminal region of the cotton plant varies from one crop scout to another. It is important that a rigorous definition is applied consistently.

Figure 2: Percentage of individuals within each Helicoverpa spp. life stage found in terminal region of the cotton plant (97/98 season)

(terminal: node #3, 5 & 7 and above in Phase I, II & III respectively)
Total numbers are displayed at the top of each respective column.



Data in Figure 2 suggests a slight difference in the proportion of eggs, very small larvae and large larvae in the terminal region of conventional and INGARD plants. However, more data is required before we conclude that this difference is significant and that a different conversion equation is needed for terminal sampling of INGARD.

Best Practice

Our research into the distribution of *Helicoverpa spp.* life stages on INGARD and conventional cotton and the validity of various sampling strategies is continuing. It is hoped that the information produced will allow consultants to efficiently and effectively check both INGARD and conventional cotton plants for eggs and larvae. Sampling pests in cotton, especially INGARD should not be a case of personal opinion, particularly as the industry moves closer to the adoption of "best practice". In pest management best practice starts with a clear and accurate assessment of the pest problem through sampling, and the system outlined in entomoLOGIC (whether it be whole plant or terminal sampling, binomial or whole count method), provide a benchmark for best practice.
Our initial results here suggest little difference between INGARD and conventional plants and we see no reason as yet why the established sampling systems will not be valid for

INGARD cotton. However, until we have statistically validated an effective and reliable sampling procedure it may be advisable to check whole plants and record absolute numbers so that accurate densities can be calculated. Remember also that no matter how accurate checking may be of an individual plant, there is no substitute for checking many plants distributed throughout a field.

References

Dillon, G.E. & Fitt, G.P. (1995) Reassessment of sampling relationships for *Helicoverpa spp*. (Lepidoptera:Noctuidae) in Australian cotton. *Bulletin of Entomological Research* 85: 321- 329.

Ives, P.M. & Hearn, A.B. (1987) The SIRATAC system for cotton pest management in Australia. pp. 251-270 *in* Teng, P.S. (Ed.) *Crop Loss assessment and pest management*. St Paul Minnesota, APS Press.

Karmawati, E. (1988) Within-plant distribution of *Heliothis armigera* (Hubner) eggs on cotton at Asembagus, East Java. *Industrial Crop Research Journal* 1(1): 26-31.

Mabbett, T. & Nachapong, M. (1984) Within-plant distribution of *Heliothis armigera* eggs cotton in Thailand. *Tropical Pest Management* 30: 367-371.

Mabbett, T., Dareepat, P., Nachapong, M. & Wangboonkong, S. (1979) Studies into the feeding distribution and movement of American Bollworm (*Helicoverpa armigera* Hubner). *Thai Journal of Agricultural Science* 12: 115-122.

Mabbett, T., Dareepat, P. & Nachapong, M (1980) Behaviour studies on *Heliothis armigera* and their applications to scouting techniques for cotton in Thailand. *Tropical Pest Management* 26(3): 268-273.

Pearson, E.O. (1958) The insect pests of cotton in Tropical Africa. *Commonwealth Agricultural Bureaux*, London. 335pp.

Ripper, W.E. & George, L. (1965) Cotton pests of Sudan. Blackwell Scientific Publications, Oxford, England. 345pp.

Uthamasamy, S. (1992) Spatial distribution of eggs and larvae of *Heliothis armigera* (Hubner) (Lepidoptera:Noctuidae) on four species on cotton, *Gossypium spp.* (Malvaceae). *Phytophaga* 4: 19-27.

Wilson, L.T. & Room, P.M. (1982) The relative efficiency and reliability of three methods for sampling arthropods in Australian cotton fields. *Journal of the Australian Entomological Society* 21: 175-181.