Using immunodot assay to test which predators feed on *Helicoverpa armigera* (Hubner) in Australian cotton.

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Introduction

With the advancement in production of monoclonal antibodies (MAbs) that are species specific the use of serological assay as a tool for assessing natural enemies has increased during the last decade (Greenstone 1996). Serological assay has been used widely overseas to estimate the potential of predators as biological control agents. It offers advantages over other predator assessment trials in that it provides a method of quickly screening a complex of predators and can be utilised to determine the stage of prey being consumed as well as the species of prey consumed. Furthermore, it offers a means of establishing when (at what time of year) predators are feeding on target prey. If high numbers of predators are found to consume target prey at certain times of a growing season the need for chemical control may be reduced at those times. This may aid in resistance management strategies.

The only study on predators of *H. armigera* using a MAb was done in India in pigeon pea (Sigsgaad 1996). To our knowledge, this technology has not yet been utilised on predators found in Australian cotton. Serological assay results provide a distinction between predators which have fed on a target prey (in this case *H. armigera*) and those that have not. This distinction has been obtained in laboratory trials at UNE (M-L. Johnson unpublished data) showing that the method is viable in laboratory conditions. However, the method still needed to be tested on field collected material to validate laboratory results.

The aim of this study was to determine how well a serological (immunodot) assay using a MAb could be utilised directly on field collected predators. This would help determine the practicality of the method as well as giving some initial information on which predator species feed on *H. armigera.*

Method

A MAb was developed as part of research that led to the production of the LepTon Test Kit (Trowell *et al.* 1992). The MAb was provided through CSIRO-Entomology, Canberra ACT, as part of an agreement with CSIRO and Abbott Laboratories. The immunodot assay method that was used followed Greenstone and Trowell (1994) with some modifications. As the primary antibody was hybridoma cell culture supernatant, it was used neat. The avidin\biotin steps were replaced with a conjugated horse radish peroxidase (HRP) whole linked Ig antibody (Amersham) and the substrate was replaced with a HRP chemiluminescence substrate (Boehringer-Mannheim). Adjustments to the ratio of reagents used in the protocol were made but are not discussed here.

In the field trials cotton was 'seeded' with *H. armigera* eggs in order to simulate a medium level of prey density (20 eggs per meter). Adult *H. armigera* were allowed to lay on paper towelling each morning on three occasions over one week in mid December 1998. The paper was collected each day and cut into rectangles so that each contained an average of 20 eggs. The trials were conducted at the Australian Cotton Research Institute, Narrabri, NSW on unsprayed irrigated Sicala V2 cotton with several sunflower rows that aided in reducing the effects of pesticide drift.

Each afternoon the paper rectangles were stapled to the second open leaf of cotton plants one meter apart in randomly selected rows. The following morning (9-11am) the papers were collected and stored on ice for future assessment of the number of eggs missing. Any predators on the paper that were observed feeding on eggs were collected, labelled and frozen in liquid nitrogen immediately. A bug check (EntomoLOGIC 1996: Anon 1996) was done to check if other *Helicoverpa* eggs were present in the field. These eggs were collected and assayed to see if any were *H. armigera*.

Fifteen x 20 meter rows were sampled for predators using a McCulloch D-Vac suction sampler. Each sample was frozen immediately and taken back to the laboratory for sorting and storage in liquid nitrogen. Predator species were stored individually to avoid any contamination of samples and all other insect species were stored in 70% ethanol for future sorting. Each field trial was then assayed on membranes that contained three positive controls of *H. armigera* eggs and three negative controls of predators starved of any *H. armigera* prey. Results from predators were scored positive (for *H. armigera* antigen) or negative by an independent assessor.

Results and Discussion

Table 1 shows the species and number of predators collected and the proportion of each that scored positive for *H. armigera* antigen. Numbers in the square bracket indicate the number of predators collected directly from the paper. These are included as part of the total proportion scoring positive for *H. armigera* antigen. The most abundant species present in the cotton were *Harmonia octomaculata* and *Diomus notescens*. Both were observed feeding on eggs on the papers and the assay results showed that the number of *D. notescens* scoring positive was very high. This number was much lower on the third sample day. The reason for this is unknown although it was noted that there were heavy rains on the previous night which may have affected their feeding behaviour. Another reason may be the effect of the abundance of alternative prey. This type of data will aid in interpretation of results from serological assay. Furthermore, some of the percentages that are high in other species are based on predators that were low in abundance. More replication would be of advantage in interpreting results for these species.

Table 1. The abundance of natural enemies and *H. armigera* sampled from unsprayed cotton each day. Numbers in parenthesis indicate the percent of each population scoring positive in the immunodot assays. Square brackets indicate the numbers collected directly form papers containing eggs.

Sample day Species	1	2	3
Harmonia octomaculata	28 (39%) [2]	43 (32%) [1]	35 (54%) [3]
Coccinella transversalis	7 (43%) [1]	8 (25%)	6 (0%)
Dicranolaius bellulus	9 (0%)	7 (28%) [2]	8 (0%)
Nabis kinbergii	1 (0%)	-	-
Diomus notescens	49 (86%) [2]	132 (86%) [2]	72 (8%)
Iridomymex vicinus	2 (50%) [1]	5 (100%) [3]	3 (100%) [3]
Pheidole sp.	5 (100%) [5]	2 (100%) [2]	-
Geocoris lubra	3 (33%)	2 (50%)	3 (0%)

Overall the proportion of predators that scored positive for *H. armigera* antigen indicated that the method works well on determining which species are feeding on *H. armigera* in the field. However, it is not known whether the paper attracted predators and therefore biased results. Further study would need to be done to eliminate this as source of bias. An index of the number of false negatives that occur as a result of predator protein content (Hagler *et al.* 1997) also needs to be included in future studies. The number of larger predators scoring positive here are likely to be an underestimation of predation.

All of the natural enemies collected directly from the paper containing eggs showed a strong positive reaction to *H. armigera* antigen. The exception to this was one soldier ant (*I. vivinus*). No negatives were provided in these trials for ants but a later test showed no false positives occurred on unfed ants (M-L Johnson unpublished data). Certainly the number of positives from ants warrants further investigation of the role of these species in control of *Helicoverpa*.

Whilst the samples taken here did provide useful information, the number of predators that could be handled each day was small in comparison to other studies (Sigsgaad 1996, Hagler and Naranjo 1994). This could be improved with the aid of better equipment and increased field assistance.

Future and conclusions

This trial shows that immunodot assay does have potential as an important tool for screening predators of *H. armigera* in cotton in Australian conditions. Further work needs to be done on eliminating false positives. This will aid in assessing the proportion of predator species that feed. The time that eggs can be detected after ingestion is also important in interpreting results. There are other antibodies available that may be more sensitive and therefore increase the time that eggs can be detected for.

The predators collected in this study definitely feed on *H. armigera* in the field and further work will enable us to determine whether other predators not found at this site and at this time of year also contribute to predation. Provided a method for sampling predators is established this technology can also be utilised on other crops where *H. armigera* is a pest.

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References

Anon (1996) EntomoLOGIC 96 manual. Publication compiled by CSIRO, Cotton Research Institute, Narrabri NSW and CRC for Sustainable Cotton Production.

Hagler, J.R. and Naranjo, S.E. (1994) Determining the Frequency of Heteropteran Predation on Sweetpotato Whitefly and Pink Bollworm Using Multiple ELISAs. *Entomologia experimentalis et applicata*. **72**: 59-66.

Hagler, J. R., Naranjo, S. E., Erickson, M.L., Machtley, S.A. and Wright, S.F. (1997). Immunological Examinations of Species Variability in Predator Gut Content Assays: Effects of Predator:Prey Protein Ratio on Immunoasssay Sensitivity. *Biological Control* **9**: 120-128.

Greenstone, M. H. (1996). Serological Analysis of Arthropod Predation: Past, Present and Future. *The Ecology of Agricultural Pests - Biochemical approaches.* W.O.C. Symondson and J. E. Liddell. London, Chapman and Hall: 265-300.

Greenstone, M.H. and Trowell, S.C (1994). Arthropod Predation: A Simplified Immunodot Format for Predator Gut Analysis. *Annals of the Entomological Society of America.* **87**(2): 214-217.

Sigsgaad L. (1996) Serological Analysis of Predators of *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae) Eggs in Sorghum-Pigeonpea Intercropping at ICRISAT, India: A Preliminary Field Study. *The Ecology of Agricultural Pests - Biochemical Approaches.* W. O. C. Symondson and J. E. Liddell. London, Chapman and Hall: 367-381.

Trowell S., Forrester N., Daly J., Garsia K., Bird L. and. Lang G. (1992) Developmental trials of the Heliothis ID kit. *Proceedings of the Sixth Australian Cotton Conference*, Broadbeach Queensland. ed. ACGRA, pp 209-216.