

10/89 - 9/91

✓ VQA

to director 30/10

## Final report on CSE6L as at June 1992

### SUMMARY

We have generated an antibody which is absolutely specific for *H. armigera* over *H. punctigera*. Specificity has been demonstrated in a number of ways. For example, the antigenic protein from *H. armigera* and its homologue from *H. punctigera* were purified and probed with the kit MAb. No detectable reaction was observed against *H. punctigera* under conditions where a very strong reaction was observed versus the *H. armigera* protein. The experiments indicate that a very high level of species-specificity is an inherent property of the kit antibody.

We have incorporated the antibody into a prototype ID kit which has now been tested under field conditions.

The kit is not yet of commercial quality and reliability. Nevertheless, for eggs collected from the field at a single time and place, there is a high correlation between the % *H. armigera* determined by the kit and as determined by rearing (Trowell et al. 1992). This indicates that the basic design of the kit is sound and further development is worthwhile.

We are working on reliability problems which have been thrown up in the field tests and have made significant progress in overcoming them. The problems are of the kind normally encountered in development of this type of test kit. Together with a partner experienced in immunodiagnostic production, we expect to be able to produce a kit of commercial quality.

### Problems encountered in field trials

Field trials of the kit by Neil Forrester and myself, during January 1992 identified four problems which had not been apparent in the laboratory.

These are:

1. Strong leaf peroxidases which completely obscure the test.
2. Strong larval peroxidases which can cause false positives.
3. Air oxidation of the test which progressively blurs the distinction between *armigera* and *punctigera*.
4. Chlorophyll staining which partly obscures the test.

It is worth emphasising that laboratory reared larvae and, with respect to 1) and 4), glasshouse grown cotton available to us out of season in Canberra do not exhibit these characteristics at a significant level.

We have also observed variation in the signal to noise ratio of the test which interacts with problems 3 and 4.

### Progress in solving problems

To date, we have solved problem 1 completely in a way which is transparent to users.

We have solved problem 2. Tests of our new method will now be performed in the field. The solution entails using separate procedures for eggs and for larvae. The procedure for eggs is given in the table below. To adapt the test to handle larvae involves the addition of 2 extra steps to the standard procedure and takes an extra 10 minutes.

Noted RS 10.11.92

Field test kit procedure used for trials from March 1992 onwards.

Step	Procedure	Incubation time	Comments
1.	Squash eggs/neonates.		
2.	Add conjugate to blocking solution and add to membrane.	5-10 min.	Add tap water to powdered protein followed by conjugate.
3.	Wash 3 times with tap water.	5 min.	Fill and empty, 3 times, over 5 min.
4.	Make up and add substrate.	≈2 min.	Mix two vials plus 2 drops from a dropper bottle.
5.	Rinse and score.	1 min	Ascorbic acid rinse.

We have succeeded in arresting the air oxidation (problem 3). When we can eliminate the variability in signal to noise ratio, this approach should be completely satisfactory.

We are continuing to work on the chlorophyll problem. Our strategy is first to minimise the transfer of chlorophyll to the membrane and second, to raise the signal strength of the reaction.

### Prospects

The four problems are all of the type we would have expected during this phase of the project, i.e. they are apparent under field conditions but not in the lab. Neil Forrester believes the test is not yet reliable enough to be released commercially and we agree with that conclusion. However we believe that the progress we are making, together with the additional expertise brought to the project by Dr. Skerritt's group, will allow us to produce a kit of commercial standard by Jan/Feb 1993.

### Problems identified in field trials

1. Leaf peroxidase, solved: agreed by NF
2. Larval peroxidase, believed solved (extra 2 procedures): sent for trial by NF
3. Oxidase, solved if signal strength increased: trialled by NF
4. Chlorophyll, believed solved: sent for trial by NF

• Solutions to 3 & 4 will be enhanced by greater signal strength and stability.

In addition to the technical progress made above we are conducting negotiations with Amrad, Panbio, CSL Diagnostics and Rhône-Poulenc regarding kit commercialisation. We will be approaching a small number of other companies. Abbott Agrochemicals have given a verbal commitment to market and distribute the kit. Other agrochemical companies may also be willing to fill this role.

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

### Notes added 28 September 1992

In line with a recommendation by CRDC, we are collaborating with Dr. Skerritt's group at CSIRO PI on aspects of commercial kit development. The timetable for commercialisation has been put back, although negotiations are proceeding.

We have succeeded in solving a number of the problems listed above. The test now takes nine minutes. Laboratory tests indicate that the problems of signal variability and leaf peroxidases have been overcome. We also have good indications that larval interference will also be overcome. Confirmation of these results does require testing under summer field conditions.

Patent proceedings have commenced.

*Steph Trowell*

Steph Trowell  
28 September 1992