

DEVELOPMENT TRIALS OF THE *HELIOTHIS* ID KIT.

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At the last ACGRA conference in 1990, it was reported that the *Heliothis* ID project had been running for approximately six months and species-specific antibodies had not yet been obtained. Here we report the production of suitable species-specific antibodies, the design of a prototype kit, some successes and problems encountered in field trials and current work being undertaken to develop the kit to a commercial standard.

BACKGROUND

Heliothis management in the cotton industry is changing. The industry is awaiting the introduction of cotton varieties that have been genetically engineered with insecticidal genes. In the interim, growers continue to "hold the line" against resistance using a rotation of endosulfan, pyrethroids and a variety of more expensive insecticides. The synergist piperonyl butoxide (pbo) has been introduced to help overcome pyrethroid resistance. Bt may be used as an additive to pyrethroids and other insecticides. Despite these changes, the propensity of *H. armigera* to develop resistance to new control methods means that a resistance management strategy will remain vital after the commercial introduction of either genetically engineered cotton or new insecticides. Pyrethroids will continue to be a valuable component of any future strategy. Insecticide resistance is the principal threat to their continued use. If the industry can continue to manage this resistance, as it has over the last decade, it

will gain the benefits of cheap, non-toxic agents with minimal residue problems. Continued use of endosulfan is also desirable, at least in the short term, since it is the cheapest way of reducing selection pressure for pyrethroid resistance. As part of the answer to pyrethroid and endosulfan resistance, we are developing the *Heliothis* ID kit which will provide growers and consultants with the ability to discriminate between *H. armigera* and *punctigera* on a field by field basis and so avoid applying pyrethroids or endosulfan to a population that predominantly consists of *H. armigera*.

FUTURE USES FOR THE ID KIT

When a commercial version of the kit is available, it will open up a number of new pest management opportunities. These include the following:

1. To determine the optimum time to include synergists or additives (e.g. pbo or Bt) with pyrethroids or endosulfan.
2. To change the Insecticide Resistance Management Strategy to allow greater flexibility in the use periods for the pyrethroids and endosulfan. For example, the current inflexible strategy could be changed to a much simpler and more flexible one capable of being expressed in a single sentence: "Don't use pyrethroids or endosulfan on *H. armigera* dominant populations and don't use pyrethroids before Christmas." This would also free up the pyrethroid restrictions placed on sorghum growers for midge control.
3. To assess whether surviving larvae after a spray failure include a significant proportion of *H. punctigera*. This would indicate an application problem and influence the choice of chemical for a "clean up" spray.
4. To determine the species of *Heliothis* larvae in crops other than cotton, especially spring oilseeds, grain legumes and vegetable crops. This would

reduce restrictions on pyrethroid by growers of these crops in northern NSW. It will also assist growers of other crops susceptible to *Heliothis*, such as sunflowers, soybeans and tomatoes, to avoid potential control problems.

Besides these uses, the kit would also assist those studying the biology of *H. armigera* and *H. punctigera*; research vital to the cotton industry:

1. To determine whether the biologies of *H. armigera* and *H. punctigera* differ in particular respects, for example in the position of egg laying on cotton plants or the way that adjacent crops and spray regimes influence moth movement and egg-laying.
2. To allow relatively unskilled personnel to determine the species of overwintering *Heliothis* pupae and/or *Heliothis* moths easily (especially damaged moths in light traps.)
3. Possibly to assist in determining the role of predators in controlling *Heliothis*.

SPECIES-SPECIFIC ANTIBODIES

The basis of the test kit is a monoclonal antibody, developed by the Division of Entomology, which is specific for *H. armigera* over *H. punctigera*. The specificity has been demonstrated in a number of laboratory experiments where strong reactions are observed against all life-stages of *H. armigera*. Under normal laboratory conditions we can detect no reaction whatsoever against *H. punctigera*, making us confident that the antibody is truly specific. The specificity was also observed for samples collected over a geographically wide area, including *H. armigera* from India.

Furthermore the antibody is capable of detecting and discriminating individual eggs or neonate larvae. However the laboratory procedure

involved 9 steps and requires several hours to complete. We have therefore simplified and shortened the procedure for field use.

THE PROTOTYPE FIELD KIT

Two different versions of the kit were tested under field conditions during the '91/'92 growing season. The operating procedure for the second version is shown in Table I.

Table I. Test kit procedure used in field trials from March 1992 onwards.

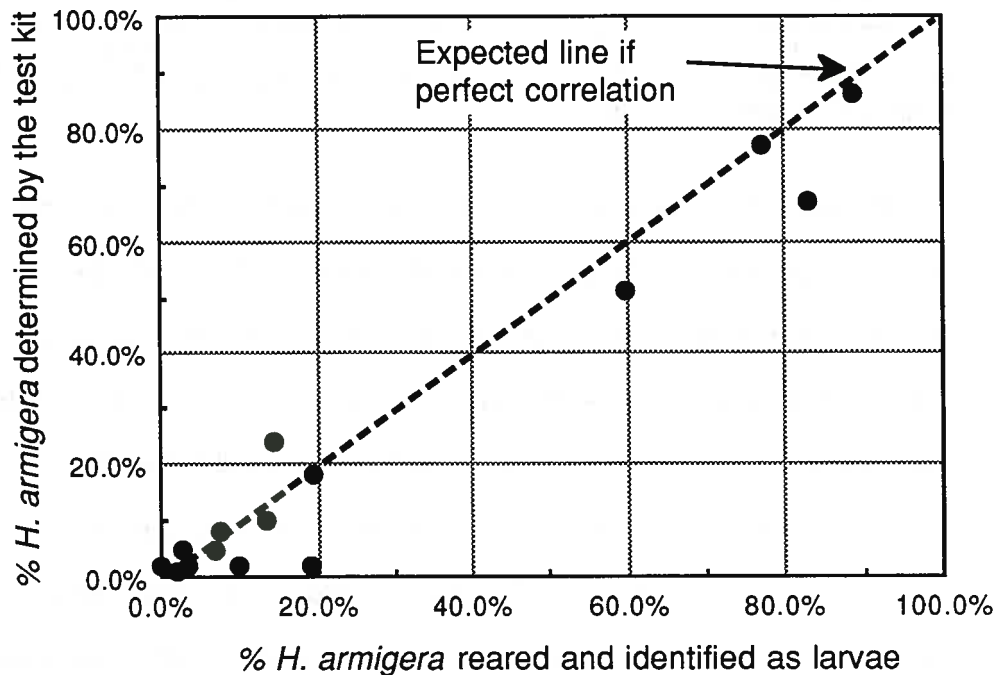
Step	Procedure	Incubation time	Comments
1.	Squash eggs/larvae onto membrane.		Performed during scouting
2.	Add conjugate to blocking solution, mix and pour onto membrane.	5-10 min.	Blocking solution is reconstituted by adding tap water to powder in tube.
3.	Wash 3 times with tap water.	5 min.	Fill and empty container 3 times over 5 min.
4.	Make up and add substrate.	≈2 min.	Mix contents of two vials, then add 2 drops from a dropper bottle.
5.	Rinse and score.	1 min	Stop solution provided for rinsing.

SUCCESSSES AND PROBLEMS

Field trials were conducted in order to answer two questions regarding the kit's performance. First, we wished to know how the results obtained with the kit under "ideal" conditions compare with those obtained by the traditional method, in which eggs or larvae are collected from the field and reared to the point (larger larvae or adults) where the species can be determined visually. These tests were conducted by NSW Agriculture and

Fisheries using *Heliothis* eggs collected over a wide geographical area. The trials concentrated on eggs because the test currently works best with this life stage and it is difficult to collect and transport reliably adequate samples of larvae. Absolute correspondence between the two methods was not expected because of sampling variation and potentially different survivorship of different egg-lays. Nevertheless when the kit was used under ideal conditions, and by experienced operators, both speciation methods gave very similar estimates of species composition (See Figure 1. and Table II).

Figure 1. Calibration of ID kit results obtained by squashing eggs against results obtained by rearing and identifying larvae from the same sample of eggs. Namoi/Gwydir, Jan-Feb 1992.



Note that the correlation between the two methods was approximately 95%.

Table II. Species composition of field samples determined in parallel by rearing and by using the ID kit. Namoi/Gwydir, Jan-Feb 1992.

Collection site	Collection date	% of <i>H. armigera</i> by rearing	(no. of larvae reared)	% of <i>H. armigera</i> by kit	(no. of eggs tested)
Hazeldean	16/1/92	0.0	(174)	2.0	(100)
Aloomba	5/2/92	1.0	(96)	2.0	(100)
Wraybourne	16/1/92	2.1	(189)	1.0	(100)
Courallie	4/2/92	2.6	(117)	5.0	(100)
Togo	20/1/92	3.5	(198)	2.0	(100)
Lammermoor	29/1/92	6.8	(220)	5.0	(100)
Carrington	23/1/92	7.5	(159)	8.0	(100)
Carrington	4/2/92	13.2	(68)	10.0	(100)
Calrossie	6/2/92	14.3	(15)	24.0	(100)
Glencoe	10/2/92	19.0	(232)	2.0	(100)
Yosemite	23/1/92	19.5	(128)	18.0	(100)
Glenara	19/2/92	59.8	(393)	51.0	(100)
Fairford	18/2/92	77.3	(44)	77.0	(100)
Terrawynia	18/2/92	82.9	(76)	67.0	(100)
Carrington	18/2/92	88.9	(63)	86.0	(100)

Samples from six additional collections were still being processed at the time of writing. The collection sites are Krui, Retreat, Telleraga, Abbey Green, Carsons and Togo.

The second aim of the tests was to define the performance of the kit under real field conditions, identify any shortcomings and provide the information necessary to remedy them. This set of tests was performed as a collaboration between NSW Agriculture and Fisheries, individuals within the cotton industry and the CSIRO Division of Entomology. Under field conditions we found that interference from a number of factors obscured the test reaction making it difficult or, in several cases, impossible to score accurately. The causes of these interference problems have been identified and fall into three classes:

1. Interference from enzymes present in the cotton leaf.
2. Interference from enzymes present in larvae.

3. Obscuring of the colour reaction by chlorophyll.

The level of interference observed in each case was much greater than had been observed using cotton plants or *Heliothis* grown in the laboratory. Field tests were conducted specifically to identify problems of this type. For example, freshly squashed *H. punctigera* larvae from the Narrabri area express an enzyme that catalyses a positive colour reaction, even if the specific antibody is omitted from the test procedure. Once stuck to the membrane, the interfering enzyme is rendered inactive over a period of days, allowing the test to be performed free from interference. We are developing methods to speed up the inactivation process so that it is complete in a few minutes.

CONTINUING DEVELOPMENT AND PROSPECTS

Throughout the 1991/'92 season we have made a number of changes to the test to improve its performance in the field. We have solved problem no. 1 and made considerable progress towards overcoming the remaining sources of interference. We are working towards eliminating the interference from larval enzymes and by enhancing the strength and stability of the test we believe we can solve the problem of larval interference. Future work will be performed in collaboration with Dr. Skerritt's group at the Division of Plant Industry, who can contribute techniques to enhance the kit's performance. We are also negotiating with a number of companies who have expressed an interest in manufacturing and distributing the kit. Our current plans are to conduct wide-scale trials of a commercial quality kit with collaborating growers and consultants in 1992/'93 and have the kit commercially available in 1993/'94.

NOTES

1. This work was carried out at the instigation of, and with financial support from, the Cotton Research and Development Corporation.
2. Accepted taxonomic practice is now to include *H. armigera* and *H. punctigera* in the genus *Helicoverpa* rather than *Heliothis*. The traditional names have been retained to maintain consistency for industry purposes.