Cotton *Research and Development Corporation*

Project Title:

Management of resistance to synthetic insecticides in *Helicoverpa armigera*. Part 2 - New chemistry, other insecticides, biochemistry and genetics.

Project Number: DAN 81C

Research Organisation: NSW Agriculture

Principal Researcher: Dr Robin Gunning
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A final report prepared for the Cotton Research and Development Corporation

SUMMARY

INTRODUCTION

Chemical insecticides are currently essential for the control of *H. armigera* in cotton and are likely to remain an important component of control strategies for the foreseeable future. However, insecticide resistance in *H. armigera* is a major threat to the economic production of cotton in Australia. The development of resistance in *H. armigera* has been delayed by the Insecticide Resistance Management Strategy , but levels of pyrethroid and endosulfan resistance have gradually increased over recent years. As resistance to pyrethroids and endosulfan increases, so does the use of alternative chemicals, such as thiodicarb and organophosphates. It is essential that the use of these chemicals and any new chemical that become available is carefully managed to avoid, or delay resistance. This can only be achieved by establishing effective resistance detection and monitoring techniques and understanding the underlying resistance mechanisms.

OBJECTIVES

1. To develop resistance management strategies for organophosphates (OP's) carbamates, insect growth regulators (IGR's) and new insecticide groups, such as the pyrroles based on a sound understanding of resistance mechanisms

2. To evaluate the importance of a newly discovered pyrethroid resistance mechanism and develop low cost, rapid biochemical techniques for the detection of pyrethroid, carbamate and organophosphate resistance.

RESULTS

Mechanisms, and genetics of *H. armigera* resistant to organophosphate and carbamate insecticides are now well understood and appropriate resistance management strategies are now in place. Baseline toxicity data to some potential *H. armigera* control chemicals has been accumulated, as has information about possible resistance mechanism pathways.

Esterase mediated metabolism of pyrethroids was found to be the primary cause of resistance in *H. armigera* . Biochemical resistance detection methods have been developed to rapidly identify pyrethroid, carbamate and organophosphate resistance in *H. armigera.* These quick and low cost methods, have been successfully adapted for use as field kits for the detection of pyrethroid and carbamate resistance. Cotton industry personnel have found the kits to be a very useful aid in making effective spray decisions.

DISCUSSION AND RECOMMENDATIONS

All objectives for this project have been achieved. This and earlier studies by the author have demonstrated the capacity of *H. armigera* to develop a wide range of resistance mechanisms to conventional chemicals. Knowledge of these resistance mechanisms has been essential for developing effective resistance management strategies. This research has also resulted in rapid, low cost biochemical pyrethroid and carbamate resistance detection methods,. These methods have been successfully used in the field as resistance detection kits. The kits provide sufficient information to allow successful insecticide use against resistant *H. armigera*. There is a great demand for these kits in the cotton and other summer cropping industries. The need is so great that our laboratory, which has supplied the kits purely as an experimental tool, cannot satisfy all the requests for kits. Commercial production of the kits is therefore required.

Although the introduction of transgenic cotton will at least temporarily reduce insecticide use against *H. armigera,* the risks of resistance to transgenic cotton are so great that it is still necessary to use conventional insecticides to control *H. armigera* i. Clearly research in this area needs to be maintained, if cost effective control is to be preserved and to minimise the danger of *H. armigera* resistance to transgenic cotton. Conventional chemicals, as part of an IPM programme will be essential to the future control of *H. armigera* on cotton. The results of the present studies have provided, and will continue provide the scientific basis of an effective control strategy.

COMMUNICATION OF RESULTS

The results of this research have been communicated in the scientific literature, conference proceedings and in *The Australian Cotton Grower*

APPENDIX

Budget:

Total funds contributed by the Cotton Research and Development Corporation 1993/94 - 1995/96 were $168,920

SPECIAL CONSIDERATIONS

This project was greatly assisted by the collaboration of G. D. Moores and A. L. Devonshire (IACR-Rothamsted, UK). I am indebted to M.E. Balfe, N.A. Coleman and B. C. Crasswell (all of NSW Agriculture) for technical support. Thanks are also due to Mr A. McAlary (AGCRA), The Macquarie Valley Cotton Growers Association and The Cotton Consultants Association of Australia for assistance in testing the resistance detection kits.

ADDENDUM

ABSTRACT

Chemical insecticides are essential for the control of *H. armigera* in cotton, however, insecticide resistance in *H. armigera*, is a threat to the economic production of cotton. This can be combated by resistance management strategies, scientifically underpinned by effective resistance detection and by understanding underlying resistance mechanisms. This project had two objectives: Firstly, to develop resistance management strategies for organophosphates, carbamates, and new insecticide groups based on a sound understanding of resistance mechanisms. Secondly, to evaluate the importance of a newly discovered pyrethroid resistance mechanism and develop low cost, rapid biochemical techniques for the detection of pyrethroid, carbamate and organophosphate resistance.

This work has resulted in a sound understanding of the mechanisms of organophosphate and carbamate resistance in *H. armigera* and has led to appropriate resistance management strategies. Baseline toxicity data has been accumulated for new *H. armigera* control chemicals, as has information about possible resistance mechanisms. Esterase mediated metabolism of pyrethroids was found to be the primary cause of resistance in *H. armigera*. Biochemical resistance detection methods have been developed to rapidly identify pyrethroid, carbamate and organophosphate resistance in *H. armigera*. These rapid methods were successfully adapted as field kits for the detection of pyrethroid and carbamate resistance. Cotton industry personnel have found the kits to be a very useful aid to making effective spray decisions. There is a great demand for these kits and it recommended that the CRDC consider commercial production.

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OBJECTIVES

1. To develop resistance management strategies for organophosphates carbamates, insect growth regulators and new insecticide groups, such as the pyrroles based on a sound understanding of resistance mechanisms

2. To evaluate the importance of a newly discovered pyrethroid resistance mechanism and develop low cost, rapid biochemical techniques for the detection of pyrethroid, carbamate and organophosphate resistance.

RESULTS

(i) Pyrethroid esterase resistance mechanism in *H. armigera*

The importance of a pyrethroid esterase resistance mechanism in *H. armigera,* has been investigated with reference to synergism and population genetics. Resistant individuals have a greatly increased esterase titre because esterase enzymes, not occurring in susceptible individuals, hydrolyse and sequester pyrethroids (Fig. 1). The most resistant individuals (300 fold) have approximately 50 times more esterase enzyme than susceptibles. The increased enzyme production can comprise approximately 15 % of total body weight. This mechanism is almost wholly responsible for pyrethroid resistance in *H. armigera.* The esterase mechanism occurs in all pyrethroid resistant populations and can be detected in all life stages of the insect. Our studies indicate that the resistance enzyme is difficult to inhibit and thus, conventional esterase synergists, such as DEF and profenofos are ineffective. However piperonyl butoxide is a good inhibitor of this esterase. which can explain Pbo synergism of pyrethroids in *H. armigera*.. Hoping to by-pass this

resistance mechanism, we investigated the use of non-ester pyrethroids, with limited success, because detoxification mostly occurred via sequestration (which does not involve the ester binding site). We are presently collaborating with chemists at IACR-Rothamsted, UK to find a pyrethroid compound which is resistant to sequestration.

Genetic studies indicate that the resistance mechanism is as a result of amplification of a gene, which codes for esterase production. Resistant individuals can have one or more copies of the gene and as a consequence, the resistance factor is correlated to esterase activity. The more enzyme *H. armigera* have, then the more resistant to pyrethroids they are (Fig 2). The resistance mechanism is semi- dominant.

A consequence of gene amplification, is that resistance factor is correlated to the amount of enzyme produced. Thus H *armigera* forms discrete populations each of increasing resistance, depending on how many copies of the gene each individual possesses. Studies indicate that the majority of resistant *H armigera* belong to the groups with very low resistance factors (5-10 fold). These populations can be still be easily killed by the registered rates of pyrethroids. In the Macquarie Valley, pyrethroid use strategies were modified to take advantage of the low resistance and pyrethroids have been used successfully, despite high pyrethroid factors. The retention of a pyrethroid window in the Macquarie Valley has preserved relative susceptibility in *H. armigera* toward pyrethroids (Fig. 3).

(ii) Biochemical detection of pyrethroid resistance in the field

A rapid, low cost, biochemical technique for the detection of pyrethroid resistance in *H. armigera* larvae and eggs has been developed and was successfully trialed as a field kit in 1994/96 (Figure 4). The kit detects the overproduction of esterases in resistant individuals. It can successfully distinguish, by colour, between susceptible and resistant individuals, estimates resistance factor and according to the results, provides spray advice . Resistance data, generated by use of the kit, was verified by bioassay. Kits were located in the Macquarie Valley and at Bourke, Wee Waa, Moree and Goondiwindi. Feedback from users indicates that the kit is easy to use, and provides rapid , accurate results. It was considered very helpful in assisting growers and consultants to make successful spray decisions. The kit has been of particular use in the Macquarie Valley.

(iii) Insecticide resistance in carbamates, organophosphates

Extensive monitoring of thiodicarb, methomyl and profenofos resistance in *H. armigera* populations was carried out as part this project. Thiodicarb and profenofos resistance monitoring was financially assisted by Rhône Poulenc Rural and Ciba Geigy, Australia respectively.

Thiodicarb resistance

Thiodicarb resistance, which was first detected in 1993, is still increasing (Fig. 5). There were many carbamate control failures on cotton in 1995/1996. The

resistance mechanism was found to be an insensitive target site, acetylcholine esterase (Fig 6). This mechanism also confers resistance to methomyl (Fig. 7), and thus the resistance problem has been exacerbated by methomyl use against *H. armigera* on cotton and other summer crops. Genetic studies indicate that carbamate resistance mechanism is semi-dominant, so that individual *H. armigera* can be either heterozygous or homozygous for resistance (Fig. 8).

Resistant individuals can be rapidly identified by biochemical methods which detect carbamate insensitive acetylcholine esterases. Heterozygotes and homozygotes can be readily distinguished. Field and laboratory studies indicated that if there are 40% or more, of resistant homozygotes in a *H. armigera* population, then field control problems are liable to occur. A carbamate resistance management strategy, resulting from these data, was adopted by the TIMMS Committee.

Carbamate resistance detection kits

A kit for rapid, low cost biochemical detection of thiodicarb resistant *H. armigera* (Fig. 9), was introduced for field testing in 1995/96. The kit was of considerable assistance in making effective spray decisions. The kits were tested in the Macquarie Valley, Kingaroy and the Upper Namoi Valley There is a large demand in the cotton industry for these kits.

Organophosphate Resistance

Incipient profenofos resistance in field *H. armigera* has been known since 1987 and resistance frequency remains low (usually < 10%). We have identified the resistance mechanism as an insensitive target site acetylcholine esterase. This insensitive enzyme is distinct from that which confers resistance to carbamates and therefore there is no cross resistance between carbamates and organophosphates. Organophosphate resistance confers a considerable fitness deficit on *H. armigera*, larval growth rate of resistant individuals is a slower than in susceptible populations. Genetic studies indicate that resistance mechanism is recessive and this factor, combined with a real fitness deficit, means that organophosphate resistance should be relatively easy to manage, provided that this class of insecticides are not over used. The current resistance management strategy has recognised this, placing some limitations on the number of organophosphate sprays which can be used against *H. armigera* .

(iv) New chemistry

The potential of *H armigera* to develop resistance to new chemicals (pyrroles and the IGR's) has been investigated. Baseline toxicity data have been generated and studies of potential resistance mechanisms are partially completed. Studies with the pyrrole AC 606, 303 were greatly delayed by difficulties in obtaining radiolabelled insecticide and metabolites from Cyanamid. The results of these studies are subject to a three year secrecy agreement between NSW Agriculture and Cyanamid.

Chlorflurazuron and the pyrrole AC 606, 303 show no evidence of any esterase metabolism or mode of action via acetylcholine esterases. Experiments indicate that pyrroles require activation by monooxygenases and this suggests that *H. armigera* may use altered monooxygenases as a resistance mechanism.

Residue problems with chlorflurazuron, have meant that the compound is not likely to be re-used in Australia. As a consequence, the Japanese manufacturer of chlorflurazuron and ICI were not interested in assisting with this study and we were unable to obtain any radio labelled chlorflurazuron for further metabolic studies.

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(ii) See attached reprints.

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(i) *Scientific Publications*

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Gunning, R. V. , Moores, G. D. and Devonshire, A. L. (1996) - Esterases and fenvalerate resistance in Australian *Helicoverpa armigera* (Hübner) Lepidoptera: Noctuidae). *Pesticide Biochemistry and Physiology* 54: 12 - 23.

Gunning, R. V. , Moores, G. D. and Devonshire, A. L. (1996) - Insensitive acetylcholine esterase and resistance to thiodicarb in Australian *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). *Pesticide Biochemistry and Physiology* 55: 21 - 28..

Gunning, R.V. (1996) - Bioassay for detecting pyrethroid nerve insensitivity in Australian *Helicoverpa armigera*. *Journal of Economic Entomology* 89: 816 - 819.

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In *Piperonyl Butoxide Monograph* , (ed.) Glynne Jones, D, Academic Press (in press).

Gunning, R. V., Moores, G. D. and Devonshire, A. L. (in press) - Esterases and fenvalerate resistance in field populations of *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae). *Pesticide Biochemistry and Physiology*

(ii) *Other Publications*

Gunning, R. V. (1995) - Enlist in the war against Larvin resistance. *The Australian Cotton Grower* , 16 (2): 20.

Gunning, R. V. (1995) - New *Heliothis* resistance detection kit performs well. *The Australian Cotton Grower* , 16 (6): 20.

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(iii) *Conference Proceedings*

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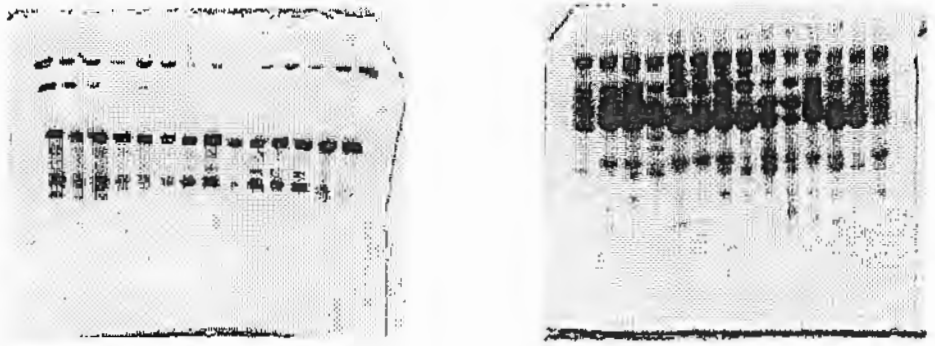
Gunning, R. V., Moores, G. D. and Devonshire, A.L. (1966) - The inhibition of insecticide resistance related esterases by PBO in *Helicoverpa armigera* and *Aphis Gossypii. Proceedings of the 20th International Congress of Entomology,* Florence, Italy

Pyrethroid resistance is correlated to increased esterase activity in *H. armigera*



Susceptible Resistant

Fig. 1

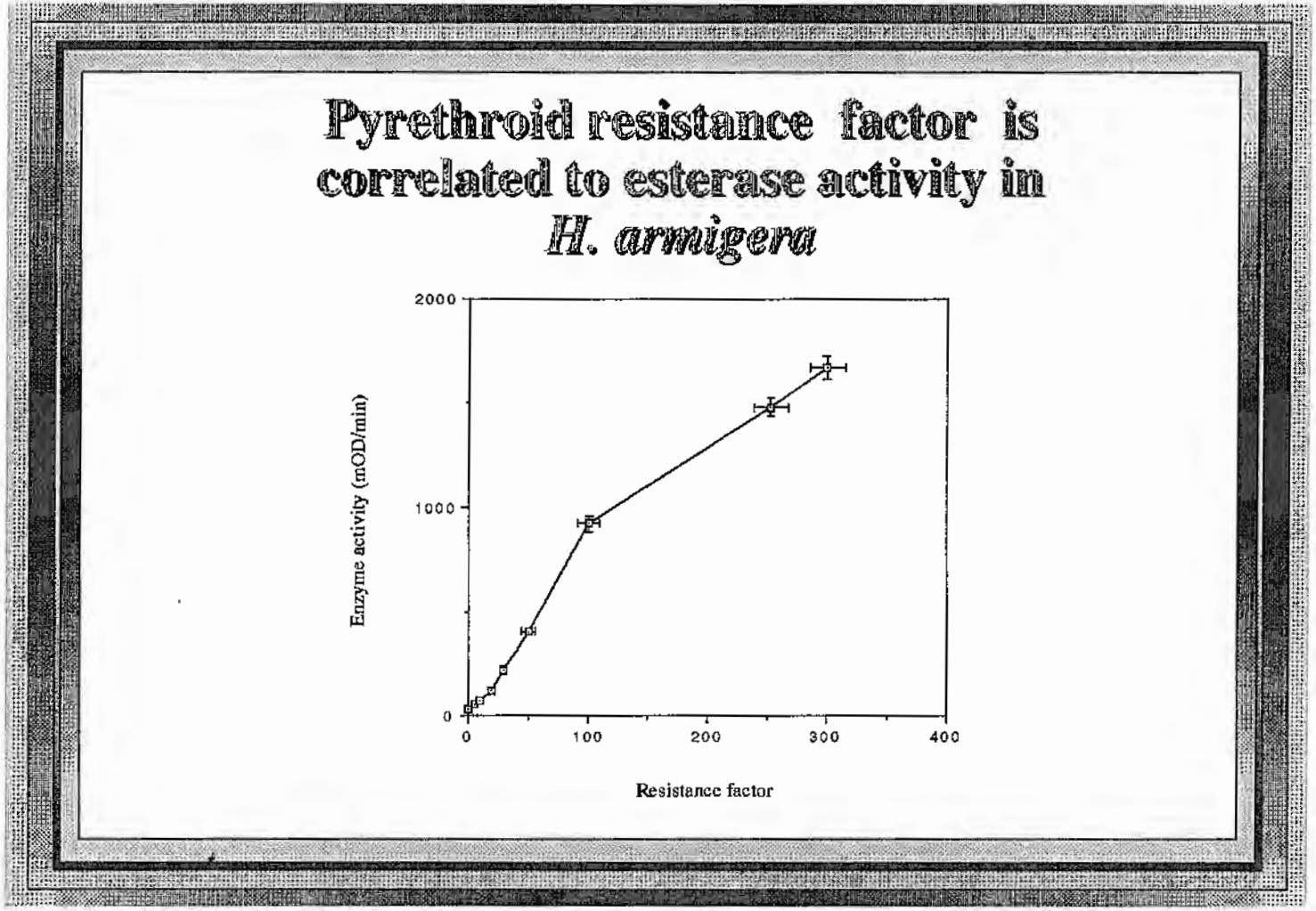

Pyrethroid resistance factor is correlated to esterase activity in H. armigera
Enzyme activity (mOD/min)
2000
1000
0
0
100
200
300
400
Resistance factor

Fig. 2

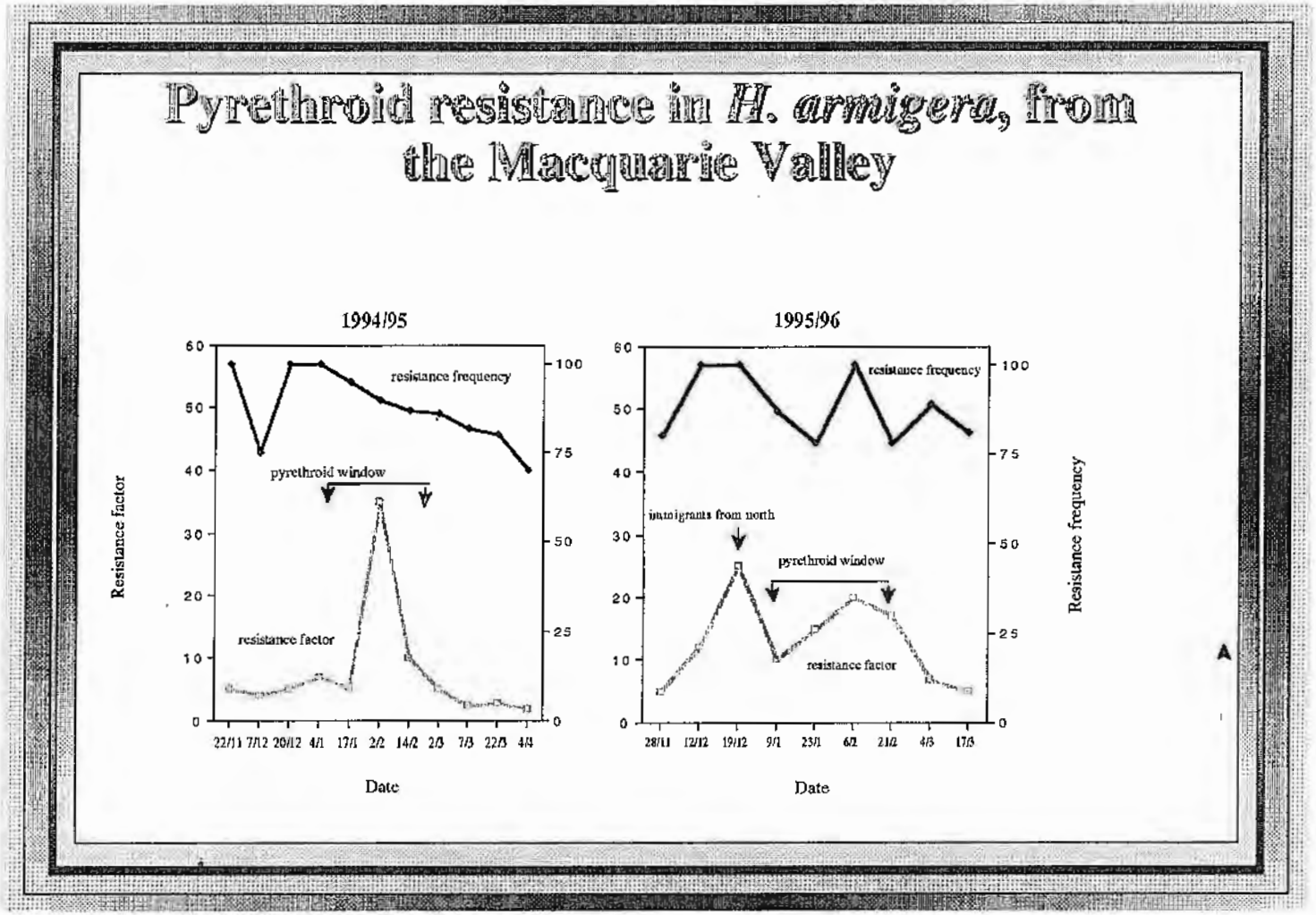
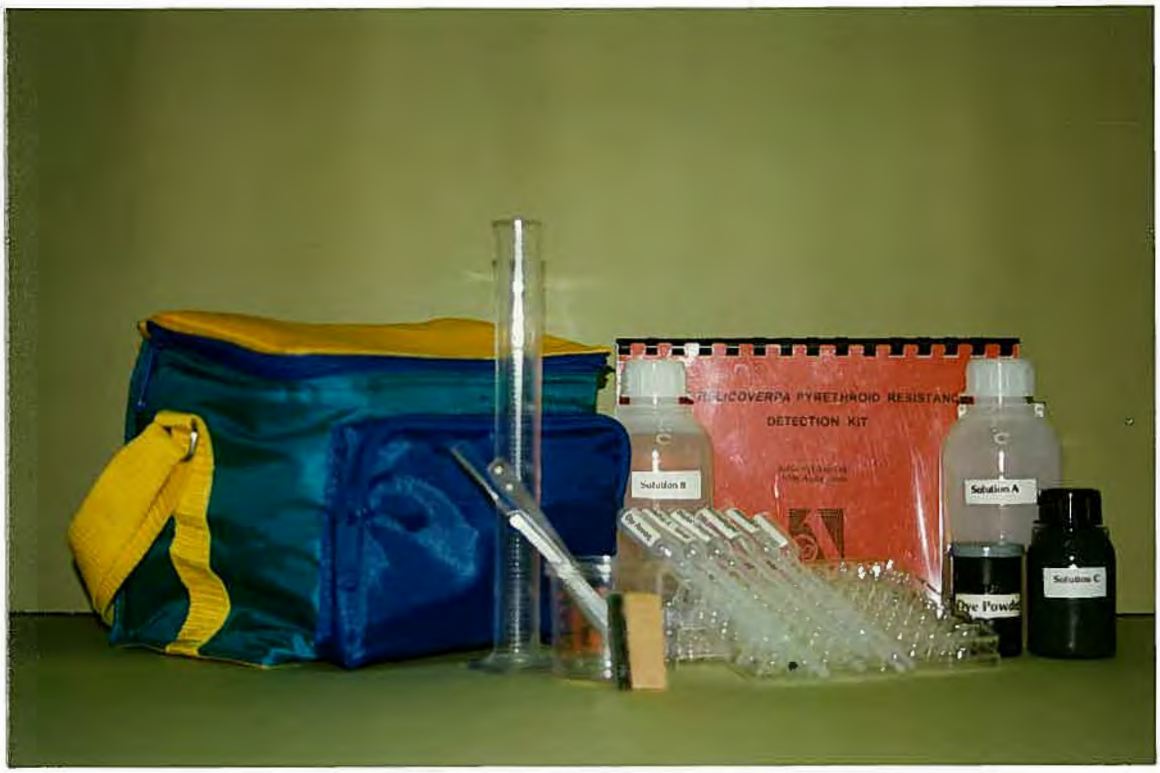

Pyrethroid resistance in H. armigera, from the Macquarie Valley
1994/95
1995/96
resistance frequency
pyrethroid window
resistance factor
immigrants from north
Resistance factor
Resistance frequency
Date
22/11 7/12 20/12 4/1 17/1 2/2 14/2 2/3 7/3 22/3 4/4
28/11 12/12 19/12 9/1 23/1 6/2 21/2 4/3 17/3

Fig. 3

Fig. 4

Pyrethroid resistance detection kit



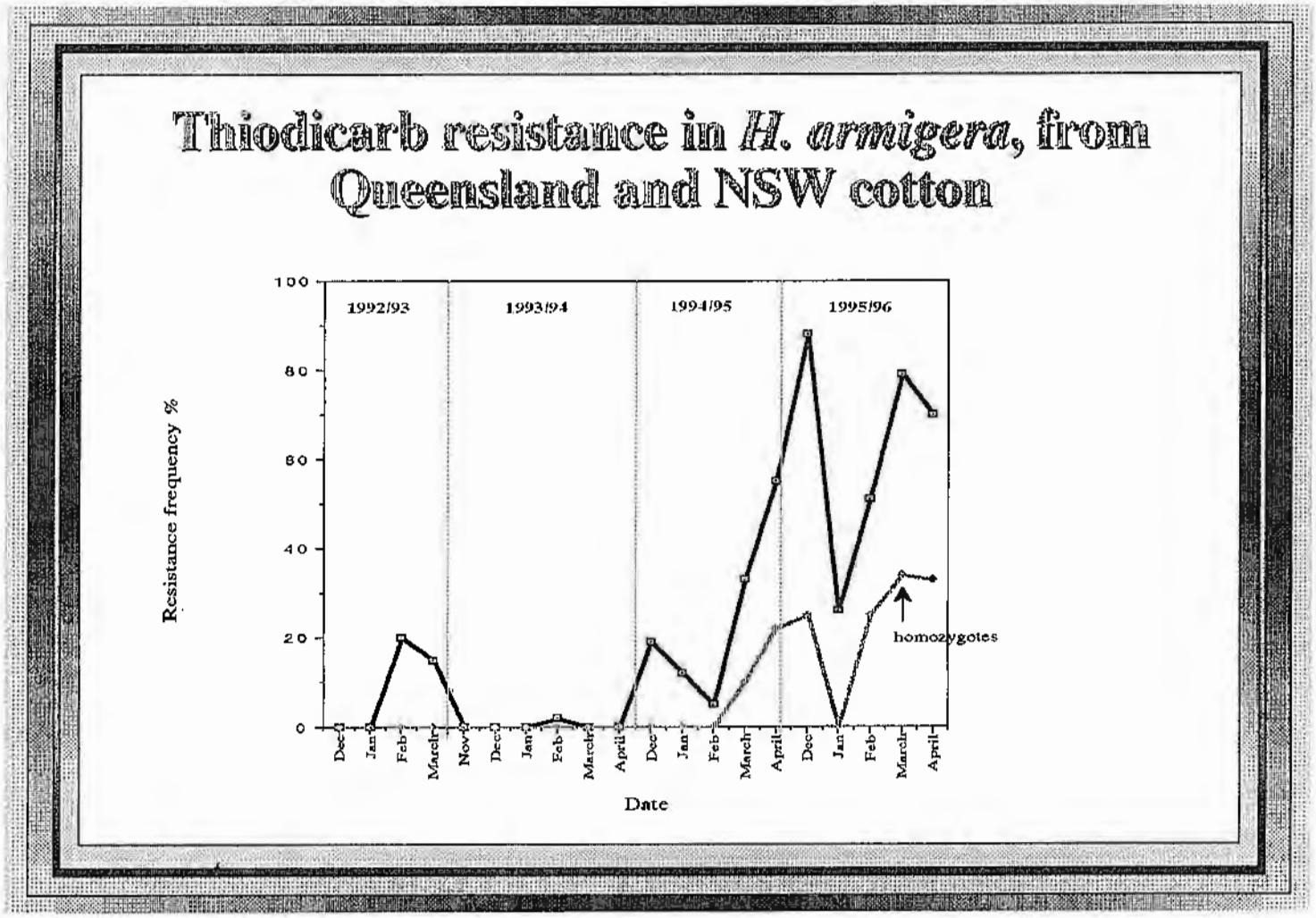

Thiodicarb resistance in H. armigera, from Queensland and NSW cotton
Resistance frequency %
100
80
60
40
20
0
1992/93
1993/94
1994/95
1995/96
homozygotes
Dec
Jan
Feb
March
Nov
April
Date

Fig. 5

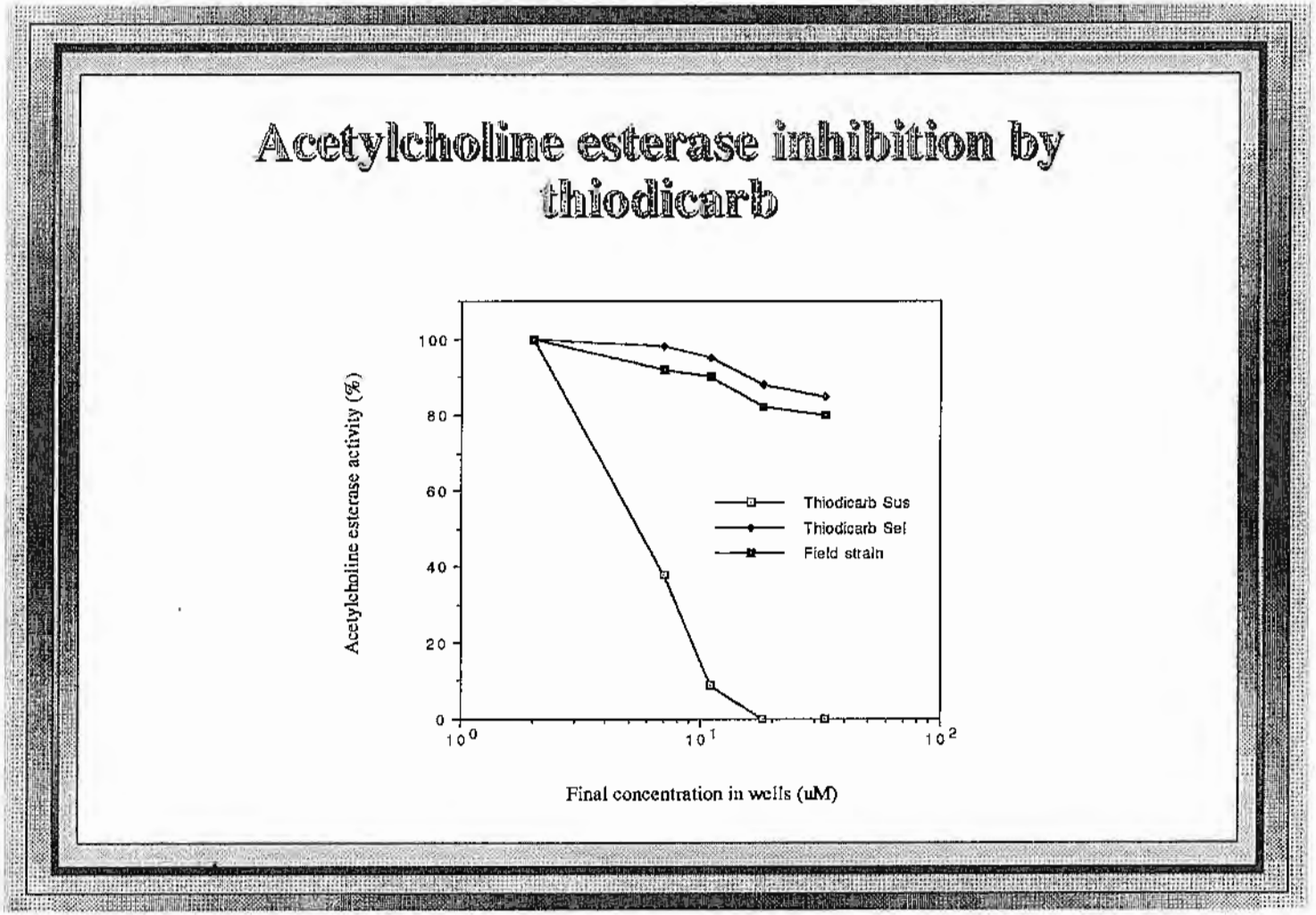

Acetylcholine esterase inhibition by thiodicarb
Acetylcholine esterase activity (%)
100
80
60
40
20
0
10^0
10^1
10^2
Thiodicarb Sus
Thiodicarb Sel
Field strain
Final concentration in wells (uM)

Fig. 6

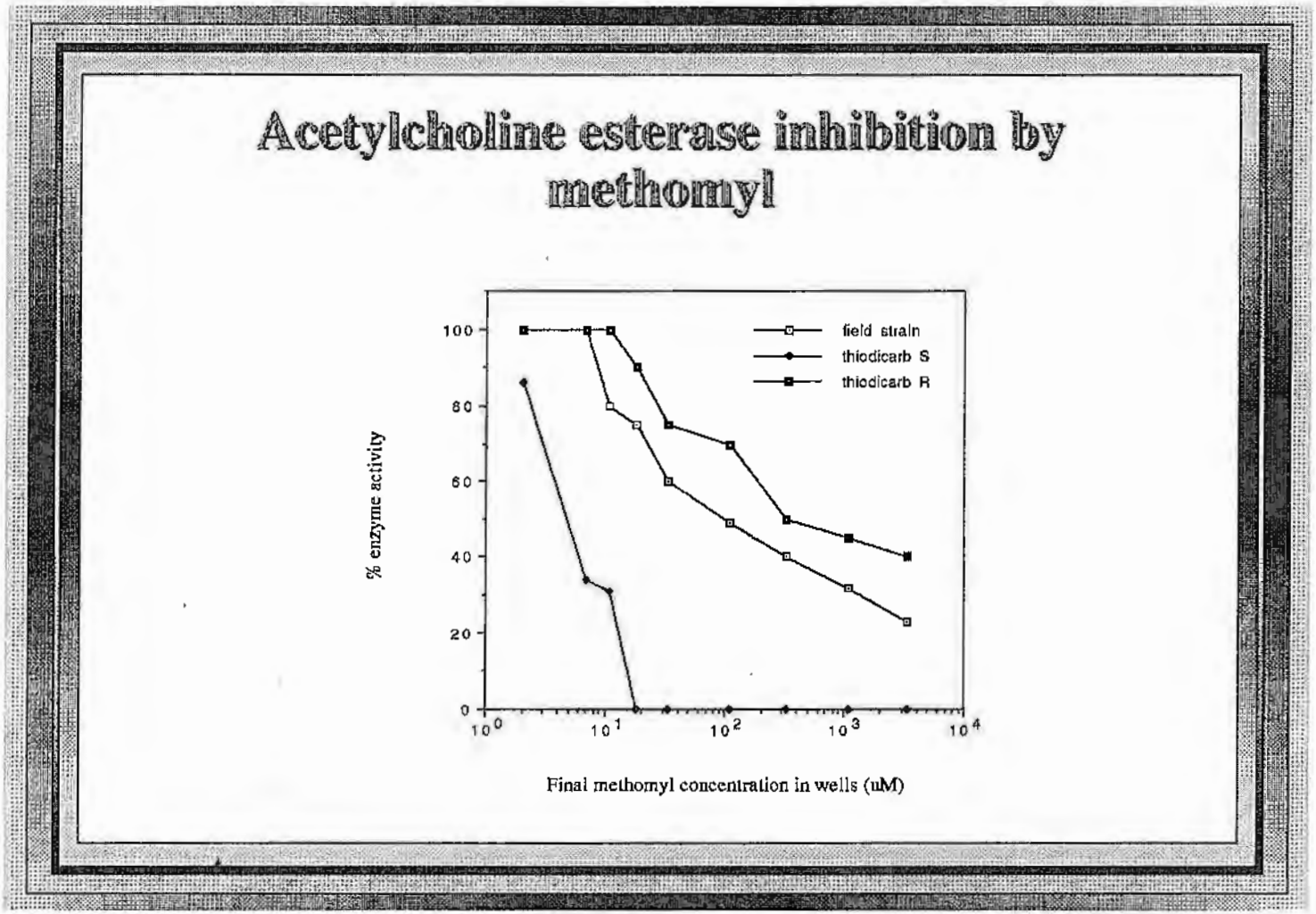

Acetylcholine esterase inhibition by methomyl
field strain
thiodicarb S
thiodicarb R
% enzyme activity
100
80
60
40
20
0
10^0
10^1
10^2
10^3
10^4
Final methomyl concentration in wells (uM)

Fig. 7

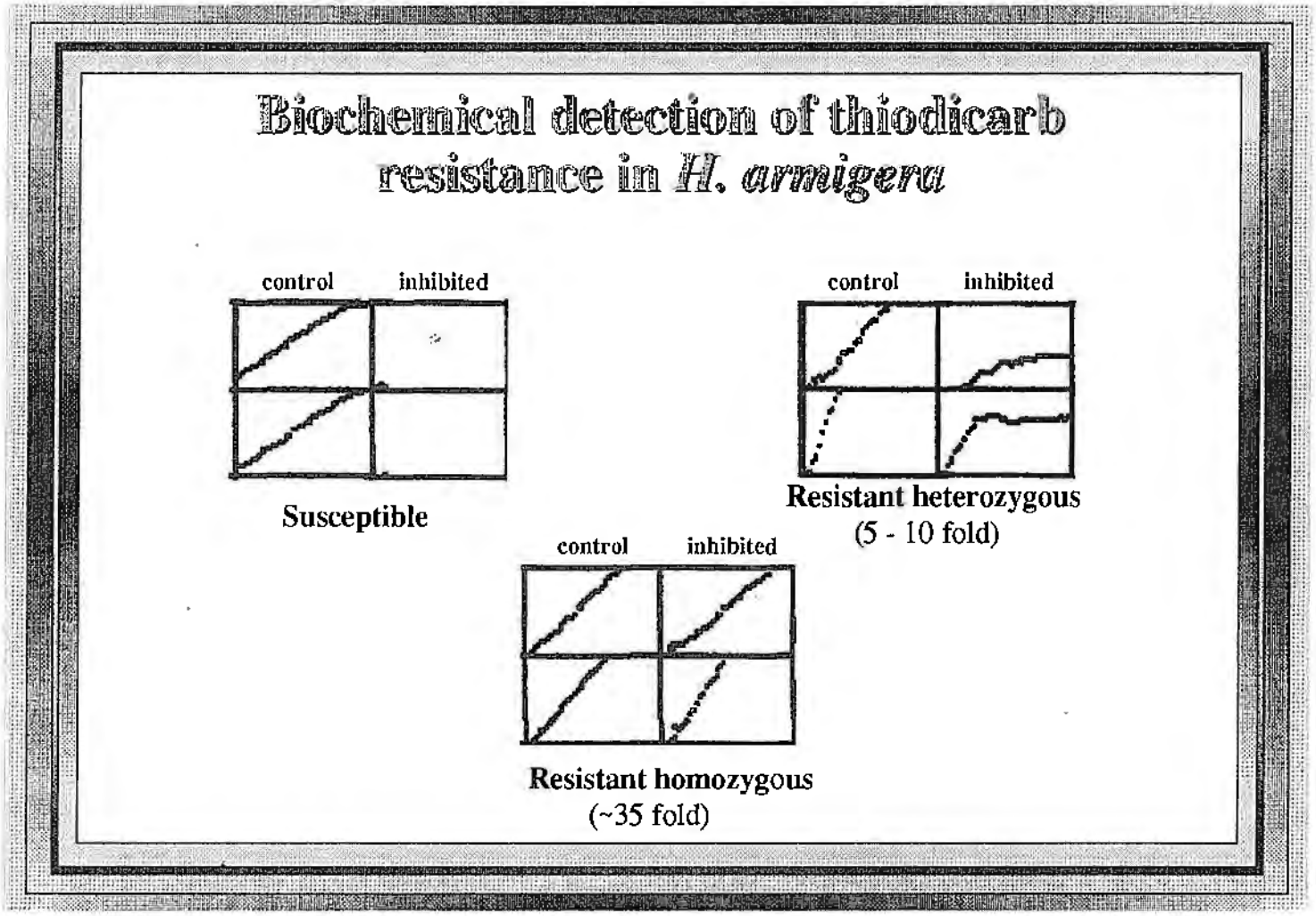

Biochemical detection of thiodicarb resistance in H. armigera
control
inhibited
Susceptible
control
inhibited
Resistant heterozygous
(5 - 10 fold)
control
inhibited
Resistant homozygous
(~35 fold)

Fig. 8

Fig. 9

Carbamate resistance detection kit