Kasetsart J. (Nat. Sci.) 28:136-141(2538)

Diagnostic Studies on the Resistance to Pyrethroid Insecticides in American Bollworm (Lepidoptera, Noctuidae) Strain from Western Thailand

Henri Caron[1], Jean-Paul Genay[2] and Pornpan Pooprompan[3]

ABSTRACT

Populations of *Helicoverpa armigera* collected from cotton fields in Kanchanaburi province were reared at LENI-CIRAD in Montpellier-France. To assess their level of resistance to synthetic pyrethroid insecticides, in comparison with LENI susceptible strain, the method of topical applications on larvae was used and data analysed by using CIRAD's designed "LD50" software.

Resistance ratios of Thai populations to esfenvalerate varied from 14.5 to 36.5. Twenty percent of the caterpillars belonging to two replications survived the application of doses higher than 60 g/g, which corresponds to 200 times the LD50 of the reference susceptible strain.

A comparison between topical applications on larvae and vial tests on adults was also made. The results indicated that the vial method could be used to determine the frequency of the resistance phenomenon and develop an on-farm diagnosis kit.

Key words: cotton, insecticides, resistance, LD 50, *Helicoverpa armigera*

INTRODUCTION

The resistance to insecticides of Thai populations of *Helicoverpa armigera* has been appraised by different authors (Mc Caffery *et al.*,1986; Ahmad, 1988): they mainly concern DDT and all the pyrethroids.

After very important losses on the seed-cotton yields in 1986, attributed to resistant populations of *H. armigera*, this insect pest had a highly heterogeneous spatio-temporal distribution, and the measurements of the resistance carried out since, gave very different results (Ouchaichon, 1986; DOA, 1988).

The first researches showed that resistance could be one of the factors responsible for the failure of insecticide treatments in the area covered by the project.

The first aim of our preliminary laboratory work, carried out in Montpellier, was to confirm the resistance, and to find reference data for further field diagnostic tests, that should allow a spatio-temporal study of the resistance for the coming cotton growing season.

The standard method used to determine the resistance is the one involving topical applications on larvae (Anon, 1970). The specific criterion is the resistance coefficient, which is the ratio between the LD50 of the resistant strain divided by the LD50 of the susceptible strain used as a reference. The tests based on the dose/mortality response curve cannot give the frequency of resistant insects and its variations.

Concerning the evaluation of the frequency of resistant moths in fields, diagnostic tests based on the use of 1 or 2 discriminating doses can be used on adults and larvae (Plapp, 1979; Mc Cutchen *et al., 1989).* Works by Roush and Luttrel (1989) showed, on *H. virescens*, a good correlation between the results of "vial" tests and resistance measurements obtained with other tests.

MATERIALS AND METHODS

The insects

The reference susceptible strain has been mass

1 Applied Entomology Research Unit, Annual Crops Department, CIRAD, Montpellier, France.
2 Development Oriented Research on Agrarian Systems Project, Kasetsart University, Bangkok 10900 Thailand.
3 Central Laboratory and Greenhouse Complex, Kasetsart University, Thailand.

reared for 15 years without any pesticide treatment at the LENI/CIRAD in Montpellier, France.

The Thai populations came from the Tha Sao area (Kanchanaburi province), and were collected in cotton fields during the 1991 growing season.

A first batch (pupae and larvae) were collected after a treatment with endosulfan in October 1991, some 68 days after sowing. From the 108 viable pupae received at Montpellier, 78 adults emerged, with a sex ratio of 1:1. The first generation reared at the lab (GL1) gave 289 viable pupae, with an emergence rate of 73%. A part of the GL2 (second generation) was kept for rearing, the others were used for toxicological tests at the 4th instar (30-50 mg).

The second batch (298 pupae collected in November on the same site), had low emergence rate, of 38 %. A part of the GL1 has been tested, the rest of the larvae were kept for rearing.

A strong virus infection decimated the GL3 (third generation) larvae, inducing a high mortality; furthermore, some GL2 pupae entered diapause (may be due temperature below 24°C during the last larval instar). These two problems significantly delayed the work program.

Toxicological tests

Topical applications on larvae: the insecticide used was esfenvalerate (technical grade active ingredient, pure at 96%), diluted in acetone.

The larvae were individually weighed (30-50 mg) then placed into individual lodges and fed with an artificial diet.

Four replications were made on the GL2 and only one test on the GL1. The treatments were made with 5 to 8 doses plus a control, with an average of 25 to 35 larvae per dose.

Each caterpillar received a 1µl droplet of the solution on the dorsal part of the thorax. The control batch just received pure acetone. Without any previous information, the range of tested doses had to be wide enough to take into account the heterogeneity of the field populations.

After the treatment, the larvae were kept in temperature controlled rooms (25°C); the mortality (dead or inactive caterpillars) is assessed after 24, 48 and 72 hours. The statistical analysis was done with the results at 48 h.

Diagnostic tests on adults: the methodology was set up in 1987 by Plapp *et al.* on *H. virescens*. Scintillation vials were used, in which 250 µl of the insecticide solution (the active ingredient at different doses being diluted in acetone) was applied and left to evaporate. The vials were prepared in large number in advance and kept in the dark.

Only one moth, male or female aged of 2 to 9 days, previously anesthesied with CO_2 if necessary, was introduced into each vial. After a few minutes, a piece of cotton imbibed with a sugar solution (10 %) was added into the vial; the vial was then closed with a gauze. The moths were kept at 25 °C and the mortality measured after 24 hours.

The insecticide used for this test was technical grade cypermethrin. To set up the reference dose/ mortality regression line, 7 doses with a minimum of 16 individuals per dose (sample size: 200) were used.

Statistical analysis

The dose/mortality regression line: the statistical analysis was made with the "LD50" software developed by CIRAD:

- the mortality of the control batch (Mt) permits to correct the observed mortality (Mo) of every dose according to Abbott's formula:

Corrected mortality:

$$Mc(\%) = 100 * [Mo(\%) - Mt(\%)] / [100 - Mt(\%)]$$

- the mortality is transformed in its probit. The software estimates a provisional regression line between mortality probit and the Log10 of the dose.

- by successive iterations at the maximum likelihood, it then determines the weighed regression line.

- in standard printing, the software gives the LD20, LD50 and LD90 values with their respective confidence intervals (p=5%) and draws the graph of the regression line.

- this software also allows:

  - to calculate a dose corresponding to a given mortality and vice-versa,

  - to test the parallelism of 2 regression lines (p=5%),

  - to draw on the same graph up to 6 regression lines.

- the LD50 value measures the activity of a poison, and the slope of the line is an indicator of the heterogeneity of the response to the poison of the tested sample; its value depends on the nature of the product (*i.e.*: $2 \leq \infty \leq 3$ for the pyrethroids; $4 \leq \infty \leq 6$ for the organophosphates with *H. armigera*); for a same product, and in the same experimental conditions, a lower slope reveals the heterogeneity of a sample.

Diagnostic tests: these tests only had a com-

parative value: at a given slope, between different populations, or a given place, successive dates.

In the case of the frequency of resistant individuals in a population is higher than 10%, a sample of 50 individuals per dose is enough.

Mc Cutchen *et al.* (1989) suggested as a resistance index:

$$\% \text{ resistance} = 100 - (Mc/Ms * 100)$$

where Mc = corrected mortality (Abbott) of the field population,
Ms = mortality of the strain susceptible to the discriminating dose.

RESULTS AND DISCUSSION

Determination of the resistance

Previous results in the laboratory permitted to directly select the appropriate range of doses of the poison (5% - 90% mortality) for the LENI strain (Figure 1). For the Thai strain, the doses of the successive replications were adjusted according to the results of the previous tests (TS 1: 0.14-35 g/g; TS 5: 0.3-62.5 g/g).

Results of the lethal doses and resistant ratios for the different generations are given in the Table 1.

The low values of the slopes and their high range of variation (0.48 - 1.45) of the regression lines for the Thai strain prove the expected heterogeneity of the population (Figure 2).

The first generation (GL1) and the second lab generation (GL2, with 4 replications) showed a high resistance to esfenvalerate, in comparison with the susceptible LENI strain: the resistance ratio varied from 14.5 to 36.5, and 20 % of the caterpillars of replications 3 and 4 survived to doses higher than 60 g/g, which corresponded to 200 times the LD50 of the reference susceptible strain. The difference between the control curve and the response curves of the Thai population is clear: the LD90 of the susceptible strain (S) was below the LD20 in 4 cases out of 5.

The fact that the resistance of the sample collected in November was similar to the one of the population collected one month before, seems to show that the problem was quite widespread over the area, and probably contributed to the inefficiency of some insecticide treatments observed during the season.

Esfenvalerate is not commercially available in the Tha Sao area where cypermethrin and deltamethrin are the most used; these results then confirm the previous works (Mc Caffery *et al.*, 1989). that were concluding to a widespread resistance to all the pyrethroids.

Even if this test cannot directly be compared with the previous ones, because of the experimental differences, our results are in accordance with their conclusions.

The next step of the work is to homogenize the resistant strain by maintaining a pressure of selection on each generation until the stabilisation of the response curve is obtained. A further field of investigation will be to study the mode of transmission of the resistance genes and their dynamics.

Diagnostic tests

Using adults, the first step was to set up the response curve to increasing doses of cypermethrin of the sensible LENI strain, in order to determine one or many discriminating doses to be applied to the resistant strain in the lab and to the fields populations.

Our results (Figure 3) confirmed the reliability of the method (probability of the Chi2: 97.95%); the

Table 1 Values of the lethal doses and resistance ratios for the different generations.

No rep.	Number of ind.*	Number of doses	Slope	LD50 g/g	LD90 g/g	Resistance ratio
LENI	189	6	2.77	0.27	0.79	-
GL1	125	5	0.73	6.6	373.0	24.4
GL2 1	358	6	0.48	3.49	1776.0	14.4
GL2 2	264	7	1.12	6.5	89.4	24.0
GL2 3	183	8	1.29	9.84	96.5	36.4
GL2 4	185	7	1.45	9.06	68.9	33.5

*: number of tested individuals (not including the control)
Resistance ratio: LD50 resistant strain/ LD50 susceptible strain
LD: lethal dose in g of drug per gram of larvae.

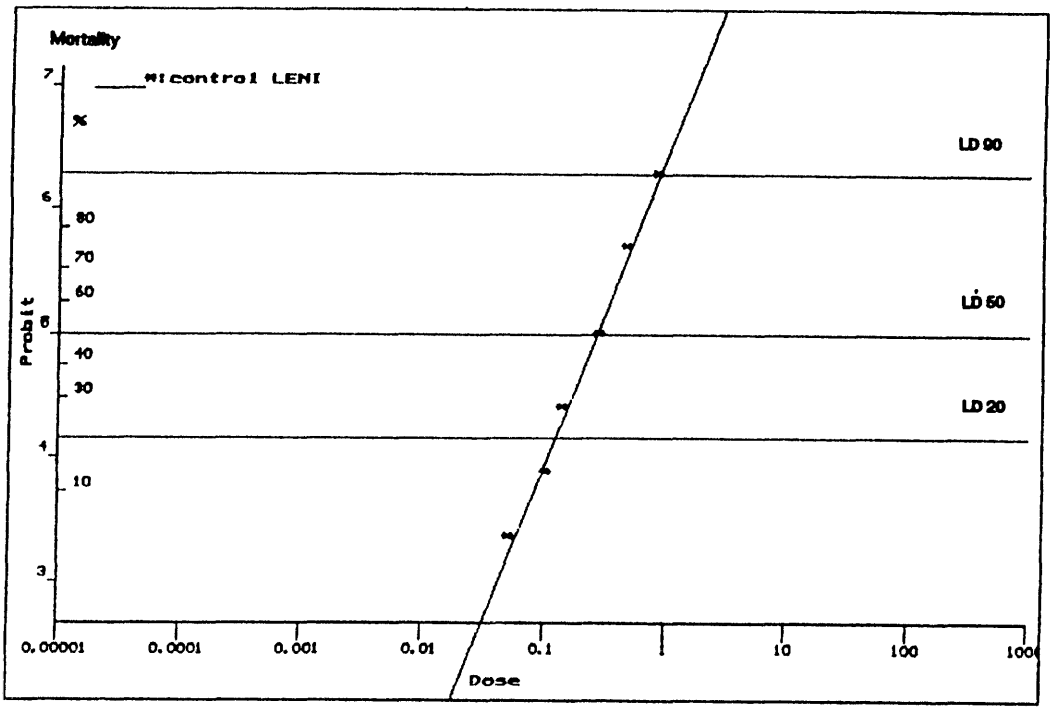

Figure 1 Dose/mortality regression line of the LENI reference strain.

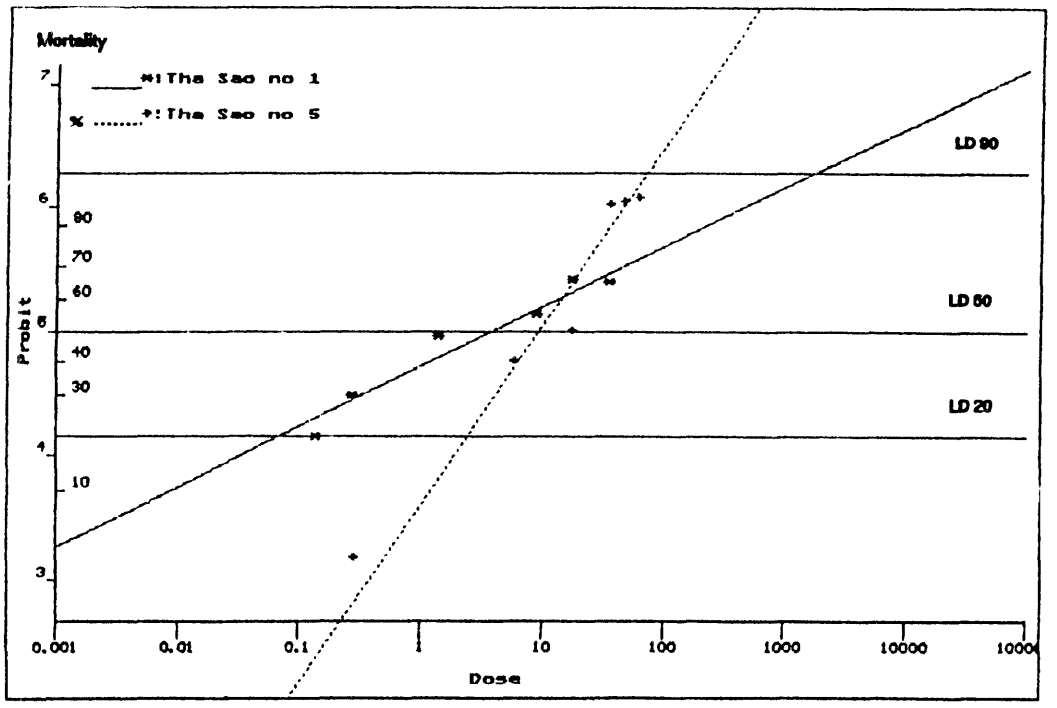

Figure 2 Dose/mortality regression lines of the Tha Sao population (replications 1 and 5).

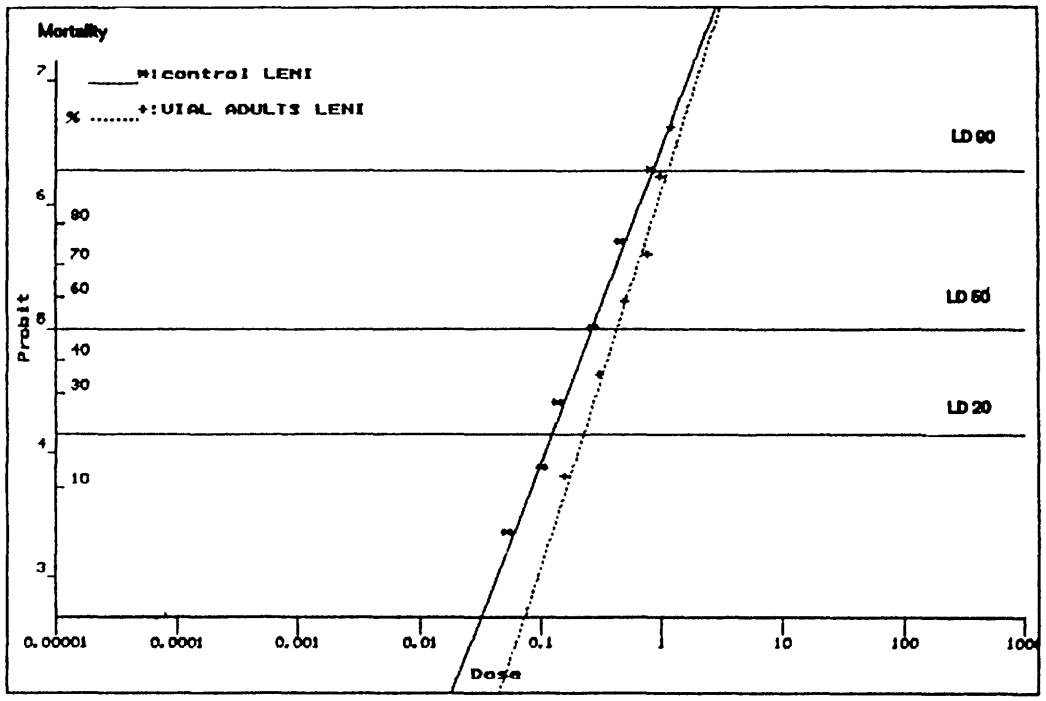

Figure 3 Dose/mortality regression line of the diagnostic test ("vial adults LENI") using adults compared with larvae ("control LENI").

slope of the regression line was similar to the one obtained with larvae.

These results did not show any significant difference of mortality according to the sex. As the theoretical works on the best choice of the discriminating dose are not all convergent (Roush and Miller, 1986; Halliday and Burnham, 1990), it was suggested to start to work with 2 diagnostic doses: 1µg/vial (DL86.5) and 2.5 µg/vial (DL99.8). By directly testing the resistant strain and the Resistant*Susceptible hybrid, two other doses to test in the field could be determined, depending on the results of the susceptible field strain (controlled by the treatments), then 1 or 2 doses could be kept.

CONCLUSION

These results indicated that the resistance phenomenon is very important, but not homogenous, even within a population coming from the same area.

That is a reason why diagnostic tests could be of great use in order to be able to easily estimate the resistance level at a given place and time.

This should allow to set up an efficient strategy to curb, or at least, to prevent further increase of the resistance level through better use of pesticides.

LITERATURE CITED

Ahmad, M. 1988. Mechanisms of insecticide resistance in *Heliothis armigera* (Hübner), (Lepidoptera; Noctuidae). PhD Thesis. University of Reading, England.

Anonymous. 1970. Standard method for detection of insecticide resistance in *Heliothis zea* (B) and *Heliothis virescens* (F). Bull. Ent. Soc. Am. 16:147-153.

DOA .1988. Annual report, Fiber Crop Division, Department of Agriculture, Bangkok, 201p.

Halliday W.R, K.P. Burnham. 1990. Choosing the optimal diagnostic dose for monitoring insecticide resistance. J. Econ. Entomol. 83(4):1151-1159.

Mc Caffery, A.R., M. Ahmad, C.H. Walker, R.T. Gladwell and K.S. Lee. 1986. Studies on resistance to insecticides in the cotton bollworm *Heliothis armigera* with special reference to the pyrethroids. Proc. 1986. Br. Crop Prot. Conf.-Pests and diseases-Vol.2 pp 591-598.

Mc Cutchen B.F., F.W. Plapp, S.J. Nemec and C.

Campanhola. 1989. Development of diagnostic monitoring techniques for larval pyrethroid resistance in *Heliothis* spp. (Lepidoptera, noctuidae) in cotton. J. Econ. Entomol. 82(6):1502.1507.

Ouchaichon, K. 1986. Toxicological test of insecticides on cotton bollworm. Research Document n 29 06 008 04 01 02 08 07. Department of Agriculture, Bangkok.

Plapp, F.W. 1979. Synergism of pyrethroid insecticides by formamidines against *Heliothis* pests of cotton. J. Econ. Entomol. 76:983-986.

Plapp, F.W. Jr, G.M. MC Whorter and W.H. Vance. 1987. Monitoring for pyrethroid resistance in the tobacco budworm in Texas 1986 ,pp 324-326 *In* Proceedings 1987, Beltwide Cotton Prod. Res. Conf.

Roush, R.T and G.L. Miller. 1986. Considerations for design of insecticide resistance monitoring programs. J. Econ. Entomol. 79: 293-298.

Roush, R.T and R.G. Luttrel. 1989. Expression of the resistance to pyrethroid insecticides in adults and larvae of tobacco budworm (Lepidoptera, Noctuidae): implication for resistance monitoring. J. Econ. Ent. 82(5):1305-1310.