# LABORATORY SELECTION FOR RESISTANCE TO SPINOSAD IN THE COTTON LEAFWORM, *SPODOPTERA LITTORALIS* (BOISD)

BY **MOHAMED A. KANDIL, MANSOUR M. RABEI, ESSAM A. EWIS AND MOATAZ A. MAHMOUD**

*Economic Entomology and Pesticide Department, Fac. of Agric., Cairo University, Giza, Egypt*

*(Received 15-12-2004)*

## INTRODUCTION

The Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd), is one of the most notorious and destructive phytophagous insect pest in Egypt, not only to cotton, but also to other field crops and vegetables (Kandil *et al.*, 2003).

The availability and use of chlorinated hydrocarbons, cyclodienes, organophosphates, carbamates and pyrethroid insecticides for *S. littoralis* control have to a large degree been sequential. This has resulted in pests developing resistance to one intensively used insecticide after another. Resistance management is very important in a heavily treated crop such as cotton. History has shown that *S. littoralis* is one of the problematic pests in this regard. To break this cycle there is a need to use number of different insecticides having different mode of action, for evaluaing potentiality for insect resistance to and for resistance management (Young *et al.*, 2003).

The spinosyns, derived from the actinomycete, *Saccharopolyspora spinosa* were discovered in the 1980s; two of them, spinosyns A and D, have strong insecticidal activity (Thompson *et al.*, 1997) with low levels of mammalian toxicity and relatively little toxicity to non-target insects (Bret *et al.*, 1997 and Sparks *et al.*, 1998). Spinosyns probably act as an agonist at the post-synaptic cholinergic ion channels and GABA-gated ion channels (Salgado 1997). This novel mechanism that distinguishes this group of insecticides from all other insecticides is perceived to be of great interest from the standpoint of resistance management. This class of compounds has been recently introduced for pest management in Egypt. Therefore the research was carried out to explore the build up of resistance before manifestation in field populations. Moreover, the selected resistant strains to

spinosad were used for the investigation of the reversion of resistance after release from selection pressure and cross-resistance studies.

## MATERIAL AND METHODS

The used susceptible strain of *S. littoralis* was delivered by mass rearing conducted for many years at the Pesticides Department in the Faculty of Agriculture, Cairo University. Field strains were collected from two governorates Fayoum and Gharbia. All stages of *S. littoralis* were cultured and tested at 26 ± 1C. Larval stages were reared on castor leaves, while adults were provided with 10 % sucrose solution.

Formulated Tracer (spinosad: spinosyn A and D, 24% SC active ingredient) was a gift from Dow agroscience Egypt. Nudrine (Methomyl 90%), Pyrecal (Chloropyrifos 48%) and Sumi-alpha (Es-fenvalerate 5%) which are different insecticides with different mode of action have been chosen to evaluate cross-resistance.

In all tests the leaf dipping technique using castor leaves was adopted and 4$^{th}$ instar larvae, was used. This technique was achieved by dipping fresh castor leaves for 20 seconds in various concentrations of formulated spinosad. The treated leaves were then allowed to dry and a pair of leaves was placed into a jar with ten fourth instar larvae. Control larvae were fed on untreated leaves. Four replicates (10 larvae / rep.), for each concentration were allowed to feed on treated leaves for 48h. Larvae were considered dead when they were not able to move. Mortality was recorded at 48h post-treatment and corrected for natural mortality by Abbot's formula (Abbott, 1925) and data were analyzed using probit analysis (Finney, 1971).

Leaf-dipping technique was used to assess the susceptibility of different instars of cotton leafworm.

For selection pressure experiment the leaf-dipping technique was used as mentioned above. Survivors were reared up to the twentieth generation under the selection pressure of spinosad. The $LC_{50}$ of spinosad to each tested generation was used as a selection pressure for the same one.

Reversion of spinosad resistance after release from selection pressure was investigated after reaching a considerable level of resistance. A sub colony from each of the selected strains was allowed to relax from selection pressure, and susceptibility level to spinosad of the relaxed strains was tested every generation.

Cross-resistance to other insecticides was investigated in the spinosad resistant Gharbia and Fayoum strains of cotton leafworm after reaching a significantly high level of resistance to spinosad. Testing technique was the same as described before.

## RESULTS AND DISCUSSIONS

The results of feeding different instars of *S. littoralis* larvae on castor bean leaves treated with spinosad are summarized in table (1).

The susceptibility decreased with aging, the sixth instar larvae showed high tolerant level compared with the first and second instars. This means that this insecticide may be directed to control newly-hatched larvae.

### TABLE (I)

Susceptibility of different larval instars of *S. littoralis* to spinosad by using leaf-dipping technique.

| Larval instars | Slope ±S.E | $LC_{50}$ PPm (a.i) | $LC_{90}$ PPm (a.i) |
|---|---|---|---|
| 1st | 1.34 ±0.14 | 4.88 | 43.86 |
| 2nd | 2.01 ±0.17 | 10.09 | 43.61 |
| 3rd | 2.12 ±0.17 | 96.49 | 386.74 |
| 4th | 2.63 ±0.26 | 140.32 | 429.64 |
| 5th | 3.74 ±0.24 | 1976.20 | 4344.29 |
| 6th | 2.89 ±0.31 | 2871.35 | 7956.76 |

The present results are in complete accordance with Moulton *et al.* (2000) Who found that susceptibility to spinosad decreased significantly from second to third instar for all population evaluated. Furthermore the toxicity of abamectin (biopesticide) was decreased up to the fifth instars of *S. littoralis* but increased in the sixth instars (Christie and Wright, 1990).

The results indicating the development of resistance in the two cotton leafworm strains, Gharbia (Gh) and Fayoum (F) are shown in tables (2) and (3).

Selection for resistance of two strains with spinosad has resulted in reasonable levels of resistance after selection for 20 generations. At the $LC_{50}$ level the resistance ratio (RR) values were c.a. 30 and 38 folds for the F and Gh strains, respectively. Also at $LC_{90}$ level, RR values were c.a. 24 and 44 for the F and Gh strains, respectively.

## TABLE (II)

Development of spinosad resistance due to selection pressure towards Fayoum (F) field strain of cotton leafworm, *S. littoralis*.

| Strains and generation. tested. | Slope ±S.E | $LC_{50}$ PPm (a.i) | Resistance Ratio | $LC_{90}$ PPm (a.i) | Resistance Ratio |
|---|---|---|---|---|---|
| Susceptible | 2.63 ±0.26 | 140.32 | | 429.69 | |
| Parent (F) | 2.93 ±0.28 | 157.35 | 1.12 | 429.88 | 1.00 |
| G1 | 4.61 ±0.37 | 240.7 | 1.71 | 456.21 | 1.06 |
| G2 | 3.38 ±0.43 | 963.40 | 6.86 | 2306.26 | 5.36 |
| G3 | 2.69 ±0.29 | 1043.55 | 7.43 | 3120.6 | 7.26 |
| G4 | 3.01 ±0.33 | 989.70 | 7.05 | 2633.95 | 6.13 |
| G5 | --------- | --------- | --------- | --------- | --------- |
| G6 | 3.13 ±0.41 | 865.o9 | 6.16 | 2220.04 | 5.16 |
| G7 | 2.03 ±0.25 | 624.23 | 4.44 | 2664.33 | 6.20 |
| G8 | 2.34 ±0.20 | 689.28 | 4.91 | 2431.27 | 5.65 |
| G9 | 3.0 ±0.23 | 811.09 | 5.78 | 2166.47 | 5.04 |
| G10 | 2.76 ±0.25 | 1068.25 | 7.61 | 3112.83 | 7.24 |
| G11 | 2.70 ±0.22 | 1497.07 | 10.66 | 4452.98 | 10.36 |
| G12 | 2.94(±0.27) | 2127.85 | 15.16 | 5803.09 | 13.50 |
| G13 | 3.95 ±0.31 | 2615.17 | 18.63 | 5510.53 | 12.82 |
| G14 | 3.30 ±0.27 | 3168.44 | 22.58 | 7731.12 | 18.00 |
| G15 | 4.54 ±0.36 | 3257.04 | 23.21 | 6235.18 | 14.51 |
| G16 | 2.42 ±0.21 | 3750.81 | 26.73 | 12662.09 | 29.46 |
| G17 | ---------- | ---------- | ---------- | ---------- | ---------- |
| G18 | 3.16 ±0.31 | 3664.91 | 26.11 | 9300.54 | 21.16 |
| G19 | 3.89 ±0.27 | 3737.82 | 26.63 | 7967.62 | 18.54 |
| G20 | 3.27 ±0.26 | 4178.39 | 29.77 | 10287.18 | 23.94 |

The resistance ratio (fold) = LC50 of resistance strain / LC50 of susceptible strain

## TABLE (III)

Development of spinosad resistance due to selection pressure towards Gharbia (Gh) field strain of cotton leafworm, *S. littoralis*.

| Strains and generation tested | Slope ±S.E | $LC_{50}$ PPm (a.i) | Resistance Ratio | $LC_{90}$ PPm (a.i) | Resistance Ratio |
|---|---|---|---|---|---|
| Susceptible | 2.63 ±0.26 | 140.32 | | 429.69 | |
| Parent (Gh) | 3.07 ±0.23 | 212.39 | 1.51 | 554.89 | 1.29 |
| G1 | 2.89 ±0.25 | 330.63 | 2.35 | 916.82 | 2.13 |
| G2 | 1.32 ±0.20 | 488.15 | 3.47 | 4557.93 | 10.60 |
| G3 | 2.48 ±0.32 | 1543.42 | 11.0 | 5056.8 | 11.76 |
| G4 | 3.41 ±0.31 | 1593.56 | 11.36 | 3867.39 | 9.0 |
| G5 | --- | --- | --- | --- | --- |
| G6 | 4.18 ±0.48 | 906.77 | 6.46 | 1836.94 | 4.27 |
| G7 | 2.25 ±0.21 | 1352.44 | 8.71 | 4997.08 | 11.62 |
| G8 | 2.75 ±0.23 | 1475.17 | 10.51 | 4303.37 | 10.0 |
| G9 | 2.58 ±0.36 | 1804.64 | 12.86 | 5653.02 | 13.15 |
| G10 | 2.95 ±0.27 | 1846.24 | 13.15 | 5084.37 | 11.83 |
| G11 | 2.81 ±0.23 | 1939.18 | 13.81 | 5477.08 | 12.74 |
| G12 | 2.77 ±0.25 | 2620.27 | 18.67 | 7607.84 | 17.70 |
| G13 | 4.79 ±0.37 | 2995.80 | 21.34 | 5548.59 | 12.91 |
| G14 | 4.10 ±0.34 | 3721.81 | 26.52 | 7643.18 | 17.78 |
| G15 | 4.70 ±0.37 | 3936.07 | 28.05 | 7371.61 | 17.15 |
| G16 | 2.09 ±0.20 | 4482.28 | 31.94 | 18301.64 | 42.59 |
| G17 | --- | --- | --- | --- | --- |
| G18 | 3.98 ±0.32 | 4246.86 | 30.26 | 8900.78 | 20.71 |
| G19 | 2.71 ±0.24 | 4602.66 | 32.80 | 13636.71 | 31.73 |
| G20 | 2.35 ±0.24 | 5448.70 | 38.83 | 19089.28 | 44.42 |

The resistance ratio (fold) = LC50 of resistance strain / LC50 of susceptible strain

The progress of selection is clear in Tables (2) and (3). Selection concentrations have started from 240 and 330 ppm then gradually increased to 4178 and 5448 ppm for F and Gh strains, respectively. When selection with the $LC_{50}$ at the seventeen generation most of the successfully pupated insects could not emerge to normal adults. This has resulted in a drastic supression of the population of the next generation in the resistant strain. Thus we had to support the next generation with individuals from the previous selected generation. Generaly, it is clear from these results that both strains (F and Gh) can build up reasonable resistance to spinosad within 20-generation.

This result is inagreement with Young *et al.* (2000) who obtained a highly spinosad resistant strain (91.1 fold) of *Heliothis virescens* within 17 generations of selection when larvae were fed on treated cotton leaves. Recently, Young *et al.* (2003) built up resistance of *H. verescens* to spinosad within 13 generations to 316 fold. Also, Shono and Scott (2003) obtained a highly spinosad resistant strain (150 fold) of housefly within just 10 generations of selection.

The slope of regression lines showed a fluctuation, which increased in the following generation 1- 2- 13- 14 and 15 and decreased almost in the other generations. This may reflect the heterogenecity of the population as well as being accomplished by several sites or genes responsible for developing resistance in both strains. It has been stated by Georghiou (1972) that if there a genetic potentiality for development of resistance to a given insecticide, the rate at which development proceeds will depend on certain obvious important factors such as the frequency of R genes and their dominance, the selection pressure and the previous history of exposure to insecticides.

Results indicating the regime of relaxation of spinosad resistance after release from selection pressure in the two strains (F and Gh) are shown in Tables (4) and (5). The manner of relaxation of resistance was very similar in the two strains. The RR value decreased from more than 30 fold to almost 9.87 at F strain and from more than 38 fold to almost 11.52 at Gh strain.

## TABLE (IV)

Relaxation of spinosad-Fayoum resistant strain after release selection pressure in the $4^{th}$ instars larvae of cotton leaf worm.

| Relaxed Generation | Relaxed Strain | | | Resistance Ratio |
|---|---|---|---|---|
| | Slope ±S.E | $LC_{50}$ PPm (a.i) | $LC_{90}$ PPm (a.i) | |
| Original | 2.42 ±0.21 | 3750.81 | 12662.09 | 26.73 |
| 1 | 3.16 ±0.31 | 3664.91 | 9300.54 | 26.11 |
| 2 | 2.23 ±0.21 | 2193.76 | 8213.68 | 15.63 |
| 3 | 2.23 ±0.20 | 1774.57 | 6638.53 | 12.64 |
| 4 | 2.44 ±0.20 | 1386.32 | 4626.36 | 9.87 |

## TABLE (V)

Relaxation of spinosad-Gharbia resistant strain after release selection pressure in the 4th instars larvae of cotton leaf worm.

| Relaxed Generation | Relaxed Strain | | | Resistance Ratio |
|---|---|---|---|---|
| | Slope ±S.E | $LC_{50}$ PPm (a.i) | $LC_{90}$ PPm (a.i) | |
| Original | 2.09 ±0.20 | 4482.28 | 18301.64 | 31.94 |
| 1 | 3.98 ±0.32 | 4246.86 | 8900.78 | 30.26 |
| 2 | 2.41 ±0.22 | 2465.81 | 8385.46 | 17.57 |
| 3 | 2.02 ±0.20 | 2094.90 | 8978.90 | 14.92 |
| 4 | 2.16 ±0.19 | 1617.88 | 6343.88 | 11.52 |

This rate of reversion of resistance could probably be attributed to the incomplete homogenecity of the selected strains, so that the resistance individuals were not prevalent enough to keep the resistance level for long time and / or the susceptible individuals have biologically fitness than the resistant ones.

The results of cross-resistance spectra of spinosad – resistant F and Gh strains of cotton leafworm to three chemicals represent the main used conventional insecticides, Chloropyrifos, Methomyl and Es-fenvalerate, are presented in Tables (6) and (7). The results show no cross-resistance to conventional insecticides, evidenced by the RR values. This means that spinosad–resistant cotton leafworm were not resistant to all the conventional insecticides tested. These results are similar to those obtained by Liu and Yue (2000) who recorded, the absence of significant cross-resistance to spinosad on ALHF strain of housefly. Also, Wei *et al.* (2001) found that there was no cross resistance between spinosad and pyrethroids on Apyr-R strain of German cockroaches. At the same time Shono and Scott (2003) suggested that spinosad resistance in the respin strain of housefly was due to a unique resistance mechanism as a result of absence of cross-resistance.

## TABLE (VI)

Cross resistance spectra of spinosad-Fayoum resistant strain of cotton leafworm.

| Compound | S. strain | | FR. strain | | Resistance Ratio |
|---|---|---|---|---|---|
| | Slope ±S.E | $LC_{50}$ PPm (a.i) | Slope ±S.E | $LC_{50}$ PPm (a.i) | |
| Chloropyrifos | 2.14 ±0.18 | 3.49 | 2.39 ±0.23 | 4.43 | 1.26 |
| Methomyl | 2.62 ±0.18 | 88.66 | 2.97 ±0.21 | 104.07 | 1.17 |
| Es-fenvalerate | 3.12 ±0.22 | 4.11 | 1.62 ±0.15 | 5.89 | 1.43 |

FR: Fayoum resistance strain

## TABLE (VII)

Cross resistance spectra of spinosad-Gharbia resistant strain of cotton leafworm.

| Compound | S. strain | | GhR. strain | | Resistance Ratio |
|---|---|---|---|---|---|
| | Slope ±S.E | $LC_{50}$ PPm (a.i) | Slope ±S.E | $LC_{50}$ PPm (a.i) | |
| Chloropyrifos | 2.14 ±0.18 | 3.49 | 1.90 ±0.17 | 3.54 | 1.01 |
| Methomyl | 2.62 ±0.18 | 88.66 | 2.86 ±0.21 | 93.22 | 1.05 |
| Es-fenvalerate | 3.12 ±0.22 | 4.11 | 1.43 ±0.15 | 3.13 | 0.76 |

GhR: Gharbia resistance strain

## SUMMARY

Laboratory selection was carried out to develop resistance of cotton leafworm to spinosad insecticide. Two field strains from Gharbia (Gh) and Fayoum (F) were subjected to selection pressure. The results obtained are summarized as follow:

1- Testing the susceptibility of the different instars against a susceptible laboratory strain showed that spinosad exhibited high activity to the 1st and 2nd instars of cotton leafworm larvae.

2- Laboratory selection pressure applied for Gh and F strains with spinosad led to reasonable level of resistance to this compound after 20 selection generations. The RR of Gh and F selected strains reached 38 and 30 at the $LC_{50}$ level.

3- Release of selection pressure for four generations decreased the RR values of the selected Gh and F strains to ca. 11 and 9 after four relaxed generations.

4- No cross-resistance was detected to any of the tested conventional insecticides.

## REFERENCES

**Abbott, W.S. (1925):** A method of computing the effectiveness of an insecticide. (*J. Econ. Entomol., 18: 265 – 267*).

**Bret, B.L.; L.L. Larson; J.R. Schoonover; T .C. Sparks and G.D. Thompson (1997):** Biological properties of spinosad. (*Down to earth, 52: 1, 6-13*).

**Christie, P.T. and D.J. Wright (1990):** Activity of Abamectin against larval stages of *Spodoptera littoralis* Boisduval and *Heliothis armigera* Huber (Lepidoptera: Noctuidae) and Possible mechanism determining differential toxicity. (*Pestic. Sci., 20: 29 –38*).

**Georghiou, G. P. (1972):** The evolution of resistance to pesticides. (*Ann. Rev. Ecol. Syst., 3: 133 – 168*).

**Finney, D. J. (1971):** Probit analysis. 3rd ed. (*Cambridg University Press, Cambridg*).

**Kandil, M.A.; N.F. Abdel – Aziz and E.A. Sammour (2003):** Comparative toxicity of chlorfluazuron and leufenuron againt cotton leaf worm *Spodoptera littoralis* (Boisd). (*Egypt. J. Agric. Res. NRC, 2: 645-661*).

**Liu, N. and X. Yue (2000):** Insecticide resistance and cross-resistance in the housefly (Diptera: Muscidae). (*J. Econ. Entomol., 93: 4, 1269- 1275*).

**Moulton, J.K.; D.A. Pepper and T.J. Dennehy (2000):** Beet armyworm (*Spodoptera exigua*) resistance to spinosad. (*Pest Manag. Sci., 56: 842 – 848*).

**Salgado, V. L. (1997):** The modes of action of spinosad and other insect control products. (*Down to earth, 52: 1, 14 – 20*).

**Shono, T. and J. G. Scott (2003):** Spinosad resistance in the housefly, *Musca domestica*, is due to recessive factor on autosome 1. (*Pesticide Biochemistry and Physiology, 75: 1–7*).

**Sparks, T. C.; G. D. Thompson; H. A. Kirst; M. B. Hertlein; L. L. Larson; T. V. Worden and S. T. Thibault (1998):** Biological activity of the spinosyns, new fermentation derived insect control agents on tobacco budworm (Lepidoptera: Noctuidae) larvae. (*J. Econ. Entomol., 91: 1277 – 1283*).

**Thompson, G.D; K.H. Michel; R.C. Yao; J.S. Mynderse; C.T. Mosburg; T.V. Worden; E.H. Chio; T.C. Sparks and S.H. Hutchins (1997):** The discovery of *Saccharopolyspora spinosa* and a new class of insect control products. (*Down to earth, 52: 1, 1 – 5*).

**Wei, Y.; A.G. Appel; W.J. Moar and N. Liu (2001):** Pyrethroid resistance and cross-resistance in the German cockroach, *Blattella germanica* (L). (*Pest. Manag. Sci., 57: 1055- 1059*).

**Young, H.P.; W.D. Bailey; C.F.Wyss; J.J. Sheets; L.L. Larson; T.C. Sparks and R.M. Roe (2000):** Studies on the mechanism(s) of tobacco budworm resistance to spinosad (Tracer). (*Proceedings Beltwide cotton conferences*).

**Young, H.P.; W.D. Bailey and R.M. Roe. (2003):** Spinosad selection of a laboratory strain of the tobacco budworm, *Heliothis virescens* (Lepidoptera: Noctuide), and Characterization of resistance. (*Crop protection, 22: 265 – 273*).