Egypt. J. Agric. Res., 76 (2), 1998.

COMPARATIVE CYTOLOGICAL STUDIES ON A SUSCEPTIBLE AND RESISTANT STRAINS OF PINK BOLLWORM, *PECTINOPHORA GOSSYPIELLA* (SAUND.)

MONA F. ROFAIL, A.E.M. EL-SORADY , AND AMIRA M. RASHAD

Plant Protection Research Institute, Agricultural Research Centre. Dokki, Egypt.

(Manuscript received 7 April, 1997)

Abstract

The parent strain of *Pectinophora gossypiella* (Saund.) was collected from cotton fields and reared in the laboratory for several generations free from insecticidal pressure. Selection of newly hatched larvae with cyanophos for 24 generations resulted in a 32.51-fold increase in resistance.

Cytological behaviour of chromosomes in meiotically dividing cells was studied at the 24th selected generation. Also, the cytological aberration in relaxed strain for one generation was studied.

Results show that the percentage of abnormal cells was 26.087 at the 24th selected generation. Relaxed strain showed a slight decrease of percentage of abonormalities (22%) as compared with the susceptible strain (2.8). The most frequent chromosomal aberrations were stickiness of chromosomes and lagging chromosomes. Irregular distribution of chromosomes was also observed in a few cells.

INTRODUCTION

Pink bollworm, *Pectinophora gossypiella* (Saunders) causes considerable damage to cotton in Egypt. The widespread and prolonged use of insecticides led to rapid development of resistance in field strains of *P.gossypiella*. Therefore, predictions or interpretations of rates of development or reversion of resistance are attainable without an understanding of the mode of inheritance of the resistance character. In order to understand how this pest develops resistance to pesticides, certain genetic studies has to be performed, especially on its cytogenics.

This study aims to verify the effect of continuous application of cyanophos against 1st instar larvae of *P.gossypiella* on the rate of development of resistance,

and to explore the relation between resistance and the cytological effects occurring in the gonads of the full grown larvae.

MATERIALS AND METHODS

1. Strains of pink bollworm

The susceptible strain (S) was obtained from the Bollworm Research Division, plant Protection Research Institute, Dokki, Giza (Rashad & Ammar, 1984).

The resistant strain (R) was obtained from the parent strain after being reared in the laboratory for several generations free from insecticidal pressure. It was selected by cyanophos for 24 generations using the newly hatched larvae at the LC30 level. 25th generation was left without insecticidal pressure to obtain relaxation strain (RS) for one generation (Rofail *et al.*, 1995).

2. Selection procedure

LC30 concentration was calculated for every generation and topically applied on artificial diet in glass tubes (2 x 7.5 cm). Every tube was infested with neonatal larvae and capped with a cotton plug. Concentration was adjusted and increased with the increase of resistance.

3. Cytological studies

Cyanophos selected strain larvae at generation 24 (G24), Relaxed line at generation 24 and untreated control larvae were collected for the cytological studies.

The techniques used were those adopted by North *et al.* (1981), Shalabi *et al.* (1983) and El-Sorady et la. (1992) with slight modifications. The gonads were obtained from larvae dissected in Belar's hypotonic saline solution, then placed into hypotonic colchicine solution at 37°C for 45 minutes. Each lobe of the gonads was divided into three or four pieces, placed on a slide and fixed by acetic acid (45%) for 3-10 minutes. The tissues were stained with aceto-orcein solution (2%) and the cover slips were positioned. The slides were slightly heated over a low flame and squashed by using thumb present with stripes of filter papers over the cover slips. The aceto-orcein stain was filtered each time through microfilter just before use to prevent any precipitation in the stain which may lead to the formation of artifacts. Prepared slides were stored in sealed slide staining dishes over bibulous paper saturated with acetic acid (45 %) at 2°C for 5 days.

RESULTS AND DISCUSSION

1. Toxicological studies

Data presented in Table 1 show the resistance level to cyanophos during selection in generations 17, 20, 24 and the relaxed G24. Resistance ratio was 37.35, 29.13, 32.51, and 27.38, respectively. The corresponding slopes of the regression line were 2.86, 2.37, 2.44 and 2.24 indicating a considerable degree of homogeneity toward the deveopment of cyanophos resistance. Results show a slight decrease in the resistance ratio in the relaxed G24 as compared with the selected one.

Table 1. Resistance ratio to Cyanophos in Cyanophos R-strain.

Generation	LC50	Slope	R.R.
Susceptible	3.577	1.79	
G12	133.62	2.86	37.35
G20	103.025	2.37	29.13
G24	116.30	2.44	32.51
Relaxation	97.97	2.24	27.38

2. Cytological studies

Observations indicated that the metaphase stage, Fig. 1, A & B was the highest frequent stage. The chromosomes were highly compact, and appeared as short rods with no details of structure or points of bending indicating the position of centromeres. Such observations might be due to the high construction of the chromosomes or to the presence of diffused centromeres (Davidson, 1974; Hassan, 1985).

Dividing meiotic cells of the male gonads of the pink bollworm displayed chromosomal aberrations, Table 2. Treatment increased such abnormalities. The most frequent aberrations induced by treatment were stickiness, Fig. 2 and lagging bivalents, Fig. 3. Irregular distribution of bivalent, Fig. 4 was also observed in few cells in all treatments and untreated control line. No abnormal cells with bridge formation were observed. Similar results were obtained by El-Wakil *et al.* (1988), El-Sorady *et al.* (1992) and Massoud *et al.* (1992).

The above-mentioned results showed that the percentage of abnormal cells was almost equal (26%) for cyanophos selected line in generation 24 (G24). On the other hand, the relaxation for one generation in Cyanophos selected strain in G24 showed a slight decrease in the percentage of abnormalities (22%). This observation

may be attributed to the stability of Cyanophos resistant strain with a considerable degree of homogeneity towards the development of Cyanophos resistance (Rofail *et al.*, 1995).

Table 2. The percentage of the different types of abnormalities in the abnormal meiotic cells of treated larvae of *Pectinophora gossypiella*.

Treatment	No.of divided cells	No.of abnormal cells	abnormal cells %	Type of abnormality		
				% Irregular distribution	% Stickiness	% Lagging
Cyanophos selected line G24	345	90	26.087	3.768	13.044	9.275
Relaxed line G24	255	55	21.569	4.314	9.804	7.451
Untreated line (Control)	325	9	2.768	0.615	1.230	0.923

Generally speaking, the chromosomal aberrations observed in treated cells were similar to treated cells from different organisms when subjected to different pesticides. These findings go in line with the results obtained by Devadas *et al* (1987), Jain and Sarbhoy (1987) in plants and Mishra and Benerjee (1987) and El-Wakil *et al.* (1988) in insects. Chromosomal stickiness is generally regarded as a physiological unspecific distrubance and has been attributed to the action of basic chromosomal proteins, e.g., histones (Devadas *et al.*, 1987).

Soliman and Al-Najjar (1984) suggested that stickiness of chromosomes in wheat cells treated with common fungicides may be due to the presence of attached methyl group in all the fungicides used in their experiment. Lagging chromosomes were due to delayed terminal, of perhaps, as a result of the stickiness of chromosomes. The same conclusion was reached by Kaur and Grover (1985). Another explanation was given by Jain and Sarbhoy (1987) on the phenomenon of lagging chromosomes. They stated that pesticide treatment hindered the chromosomes from reaching the poles and remained scattered in the cytoplasm.

It was obvious, however, that there were irregularities in chromosomal distribution in some treated cells. This may be attributed to the disturbance of spindle apparatus (El-Feel *et al.*, 1990).

In conclusion, chromosomal anomalies resulting from treatment were most frequent chromosomal aberrations causing stickiness of chromosomes and lagging chromosomes.

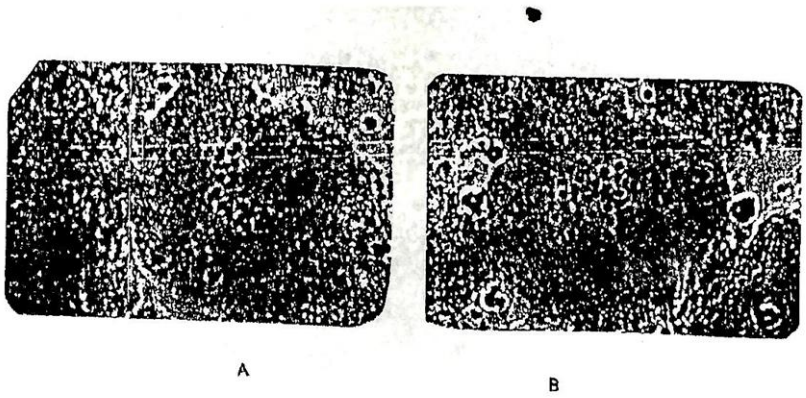

Fig. 1. Normal metaphase showing a complement of chromosomes in *P.gossypiella*:

(A) Side view (B) Polar view.

Fig. 2. Metaphase I showing sticky bivalents of *P.gossypiella* larvae .

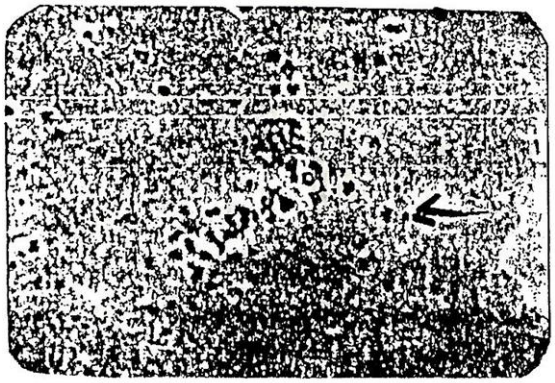

Fig. 3. Metaphase I showing lagging bivalents of selected *P.gossypiella* larvae.

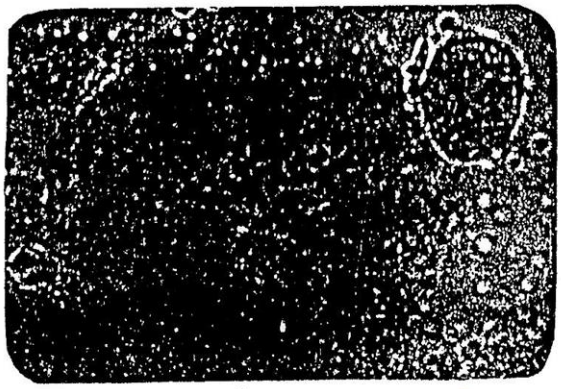

Fig. 4. Metaphase I showing irregular distribution of bivalents in selected larvae of *P.gossypiella* .

REFERENCES

1 . Davidson, G. 1974. Genetic control of insect pests. Academic Press, London, New York, pp. 124.

2 . Devadas, N., A. Sodanadam, R. Kishan Rao and K. Subhash. 1987. Induced somatic aberrations and chlorophyll mutation in Capsicum by insecticides. Cytologia, 52 : 235-241.

3 . El-Feel, E.A., H.I.H. Omar, A.E.M. El-Sorady and Y.H. Issa. 1990. Toxicological, enzymological and cytological effects of some insecticide mixtures against *Spodoptera littoralis* (Boisd.). Com. Sci. & Dev. Res., 30 : 31-49.

4 . El-Sorady, A.E.M, E.A. El-Feel and H.I.H. Omar. 1992. Cytological studies on the cotton leafworm *Spodoptera littoralis* (Boisd.) and the American bollworm *Heliothis armigera* (Huebner). Alex. J. Agric. Res., 37 (1): 107-121.

5 . El-Wakil, H.M.F., A.S. Soliman, O.A. Zaghloul and A.E.M. El-Sorady. 1988. Cytological studies on some natural populations of cotton leafworm *Spodoptera littoralis* (Boisd.). Minufia J. Agric. Res., 13 (2) : 1261-1280.

6. Hassan, A.H.M. 1985. Spermatogenesis and oogenesis in the Egyptian cotton leafworm, *S.littoralis* (Boisd.) (Lepidoptera : Noctuidae). Bull. NRC, Egypt, 10 : 455-463 .

7 . Jain, A.K. and R.K. Sarbhoy. 1987. Cytogenetical studies on the effect of some chlorinated pesticides. 1-Effect on somatic chromosomes of lens and pisum. Cytologia, 52: 47-53 .

8 . Kaur, P. and I.S. Grover. 1985. Cytological effects of some organophosphorus pesticides. II-Meiotic effects. Cytologia, 50: 199-211 .

9 . Massoud, M.A., A.E.M. El-Sorady, O.A. El-Gogary and H.A. Awad. 1992. *Bacillus thuringiensis* Berliner and Sabadilla as biological agents againts spiny and pink bollworms. J. Pest Control & Environ. Sci., 4 (2) : 25-46.

10. Mishra, P. and S. Banerjee. 1987. Chromosome aberrations induced by TEPA an alkylating chemosterilant in spermatocytes of *Callosobruchus chinensis* Linn., (Coleoptera : Bruchidae). Cytologia, 52 : 499-506 .

11. North, D.T., R.A. Leopold and D. Childress. 1981. Meiotic and mitotic chromosomes of the cotton boll weevil (Coleoptera: Curculionidae). Can. J. Genet. Cytol., 23 : 443-447.

12. Rashad, A. and E.D. Ammar. 1984. Mass rearing of the spiny bollworm *Earias insulana* (Boisd.) on semi-artificial diet. Bull. ent. Soc. Egypte, 65: 239-244.

13. Rofail. Mona F., Y.F. Ghoneim, A.M. Allam, Farida A. Ayad, Amira M. Rashad and Mary E. Keddis. 1995. Insensitive acetylcholinesterase. Alpha esterases and glutathione S-transferase activities as factors in resistance of pink bollworm to the organophosphorothioate Cyanophos. Egypt. J. Appl. Sci., 10 (9): 477-496.

14. Shalabi, H.G., N. Shaker, E. Badr, F. Taman and A. El-Sebae. 1983. Studies on meiotic and mitotic chromosomes of cotton leafworm *Spodoptera littoralis*. Proc. Int. Conf. Emv. Haz. Agrochem., II: 932-940.

15. Soliman, A.S. and N.R. Al-Najjar. 1984. Cytological effect of some fungicides and herbicides on bread wheat *Triticum aestivum* L. Proc. 2nd Mediterranean Conf. Genet. March, 831-844.

دراسات سيتولوجية مقارنة علي السلالات الحساسة والمقاومة لدودة اللوز القرنفلية *Pectinophora gossypiella*

مونا فكري روفائيل ، عبد السلام محمد الصردي ، أميره محمد رشاد

معهد بحوث وقاية النباتات ، مركز البحوث الزراعية ، الدقي ، الجيزة :

جمعت سلالة من دودة اللوز القرنفلية *Pectinophora gossypiella* من حقول القطن وربيت معمليا لعدة أجيال دون إستخدام أية مبيدات. وتم الانتخاب بمبيد السيانوفوس لمدة ٢٤ جيل علي اليرقات حديثة الفقس وأدي ذلك إلي زيادة المقاومة الي ٣٢,٥١ ضعفا. وبدراسة سيتولوجي كروموسومات الإنقسام الميوزي في الجيل الإنتخابي الرابع والعشرين وفي السلالة المسترخاه Re-laxed line لمدة جيل واحد وجد أن نسبة الخلايا الشاذة في الجيل الرابع والعشرون (٢٦٪)، أما السلالة المسترخاه لمدة جيل واحد فقد أظهرت انخفاضا طفيفا في نسب الخلايا الشاذة (٢١,٦ ٪) مقارنة بالسلالة الحساسة (٢,٨ ٪). وكانت أكثر الإختلافات الكروموسومية تكرارا هي ظهور الكروموسومات الملتصقة والكروموسومات المتلكأة أو المتأخرة، كما ظهر التوزيع غير المنتظم للكروموسومات في عدد قليل من الخلايا.