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INHIBITION OF SPERMATOGENESIS AND DNA-SYNTHESIS IN MALE GONADS OF *Pectinophora gossypiella* (SAUNDERS) IN RESPONSE TO THE DEVELOPMENT OF CYANOPHOS-RESISTANCE

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ABSTRACT

Selection of newly-hatched larvae of the pink bollworm *Pectinophora gossypiella* (Saunders) (Lep., Gelechiidae), field strain reared in laboratory for several generations free from insecticidal pressure, with Cyanophos for 32 generations, besides three relaxed generations (G33-G35) was carried out. The effect of development of Cyanophos-resistant, relaxed and susceptible strains on both male gonad relative nuclear DNA-content and the proliferation of differential stages of spermatogenesis was studied.

The obtained results showed that Cyanophos, as a selective agent, possesses a high resistance potential to the pink bollworm. The selection indicated a considerable degree of homogeneity towards the development of Cyanophos resistance and achieved 42.76 fold at the 32nd generation. While the third relaxed generation (G35) showed the highest slope of 3.1 accompanied by high decrease in resistance (27.80 fold) leading to rapid return to resistance reversion, nearly equals to G20 to be susceptible again.

Moreover, the achieved results showed that the development of Cyanophos resistance increased the percentages of male gonad cells having 2C-DNA-content and the abnormal ones having more than 4 C-DNA-content. Classifying the differential stages of spermatogenesis indicated that the proportions of stem cells, spermatogonia A & B and spermatocytes were the highest in the resistant strain. On the other hand, the development of Cyanophos-resistance induced the least percentages of cells having 1C-, 3C- and 4C-DNA-content and associated with the decrease of young & mature spermatids, immature & mature spermatozoa, proleptotene, pachytene and certain cell division stages (diakinesis, G2 and metaphase I).

INTRODUCTION

The pink bollworm, *Pectinophora gossypiella* (Saunders) (Lep. Gelechiidae) is one of the most serious pests attacking cotton at the mid and end of its growing season. The economic loss occurs as a result of its infestation which reduces the yield, and gives low fiber quality (Willcoks, 1961). Recently, resistance to organophosphorus compounds has been detected in field populations of the pink bollworm in Egypt (Omar and Keddis, 1987; Ayad *et al.*, 1994). However, the efficiency of many potent insecticides has been reduced and some are no longer used due to the development of resistance (Auda, 1986).

Despite the widespread interest in resistance management, few studies to explain the development or reversion of resistance were attained. Therefore, genetic toxicological studies have to be performed (Rofail *et al.*, 1998). Previous investigations on the efficacy of certain biopesticides on the relative testicular nuclear DNA-content, its synthesis and the differential stages of spermatogenesis against some cotton insect-pests, were conducted by Massoud and Moustafa (1993) and El-Sorady *et al.* (1995).

The knowledge concerning the explanation of the effect of continuous selection for the insecticide resistance on testicular nuclear DNA-content, stages of spermatogenesis and infertility, is still very limited. Therefore, the present study deals with the effect of continuous application of Cyanophos against the 1st instar larvae of *P. gossypiella* on the rate of resistance development. The aim of the present

investigation is also to throw more light on the delayed effect of selection on the relative testicular nuclear DNA-content and the proliferation of the differential stages of spermatogenesis of the pink bollworm.

MATERIALS AND METHODS

I. Tested insecticide:

Cyanophos (Cyanox®) 50% EC.: [O-4-Cyanophenyl O, O-dimethyl phosphorothioate; 4-(dimethoxy- phosphinothioloxy benzonitrile] supplied by Sumitomo Co.

II. Tested Insect strains:

Newly-hatched larvae of a susceptible strain (S) of *Pectinophora gossypiella* (Saund.) were obtained from the Bollworm Research Division, Plant Protection Research Institute, Dokki, Giza, and were reared in the laboratory for several generations free from any insecticidal pressure according to Rashad and Ammar (1984).

The resistant strain (Rs) was selected by Cyanophos for 32 generations using the LC_{30} level. The used LC_{30} 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, while the relaxed strain (Rx) was obtained by rearing the 33rd generation for three more generations free from the insecticidal pressure.

III. Bioassay Technique:

Several concentrations of Cyanophos were sprayed into petri-dishes (9 cm diam) using a hand atomizer. Treated surfaces were left to dry. Thirty neonates were transferred with a fine brush to each treated dish. The dishes were covered with a tissue paper, then again covered with their covers and kept at 27 ± 2°C. After one hour from exposure, treated and check larvae were transferred individually on a semi-artificial diet, poured into glass tubes (2 x 7.5 cm), covered with cotton and kept under the previous constant condition. Mortality was recorded after seven

days. The LC_{50} and slope values were determined using the method by Busvine (1957).

IV. Preparation of Cytological Gonad-Cells:

Cytological investigation was conducted for each of the three strains (S, Rs and Rx). From each strain, four replicates, each of 20 larvae of the pink bollworm, at the 4th instar stage were collected for investigating the gonads of the male larvae. The removed gonads were squashed between two slides, for the nuclear image analysis. The slides were stained for one hour at 20°C with Schiff reagent (BDH) after hydrolysis in 6 N HCl for one hour at 20°C. Feulgen stained cell preparations were stored in the dark at 4°C until analysis. A micro-photometric scanning analysis system, which is composed of micro-computer and a transmission microscope with motorized stages, coupled to an electro-optical system, was performed for the nuclear image analysis.

Twelve parameters were generated for each cell including five densitometric and seven texture characteristic parameters (Appendix 1). The biological definition and significance of these parameters were interpreted according to Brugal (1984). The discrimination between the different cell-types, classified by the learning set, was analyzed automatically by using F-test, discrimination function and to obtain classification rate.

V. Classification of Larval Germinal Cells

The germinal cells were classified practically and manually with micro-photometric scanning microscope to build up the learning set. They were divided into 10 types in untreated larvae according to the histological and cytological information of feulgen stained nuclei (young spermatid, mature spermatid, immature spermatozoa, mature spermatozoa, stem-cells, spermatogonia A, spermatogonia B, spermatocytes, proleptotene and pachytene), besides cells in three stages of division (diakinesis, G2 and metaphase I).

Then, the multi parameter analysis involving 12 parameters was conducted to discriminate automatically between these 10 cell-types, 3 stages of division and the cell groups depending on their nuclear DNA-contents as Arbitrary units (A.U.). The discrimination between the different cell-types, groups and stages which were classified by the

learning set, was analyzed automatically by using F-test, discrimination function to obtain good classification rate.

RESULTS AND DISCUSSION

I. Toxicological Studies:

Table (1) presents the developmental resistance ratio (RR) of *P. gossypiella* to Cyanophos during selection for 32 generations (G0-G32), besides 3 relaxed generations (G33-G35). The estimated LC_{50} values were3.58, 5.18, 103.02, 153.08 and 99.54 ppm in the susceptible strain (G0), G6, G20, G32 and the relaxed generation (G35), respectively. These increases in the LC_{50} values resulted in developmental increases in the calculated resistance ratios, whereas, they were 1.45, 28.78, 42.76 and 27.80-folds at the 6th, 20th, 32nd and 35th generations, successively.

Table (1). Resistance ratio to Cyanophos in the pink bollworm during selection for 32 successive generations.

Generation	LC_{50} ppm	Slope	R.R*
G0	3.58	1.79	-
G6	5.18	1.48	1.45
G20	103.02	2.37	28.78
G32	153.08	2.88	42.76
G35**	99.54	3.10	27.80

* R.R. (Resistance ratio) = LC_{50} for Rs strain/ LC_{50} for S-strain.
** G35 The relaxed generation obtained by rearing the 33rd generation

for three generations free from the insecticidal pressure. It is obvious that increase in resistance level from G0-G32 indicates a considerable degree homogeneity towards the development of Cyanophos resistance. Nevertheless the corresponding slopes of the regression line were 1.79, 1.48, 2.37, 2.88 and 3.1 in generations G0, G6, G20, G32 and G35 (the

relaxed generation), respectively, indicating also a considerable degree of homogeneity for developing Cyanophos resistance. However, the data showed a slight decrease in the slope at the 6th generation indicating a considerable tolerance, while it increased to 2.88 in G32 indicating a high response to selection. Also, the G35, which was reared free from Cyanophos selection pressure, showed the highest slope of 3.1 accompanied by high decrease in resistance ratio leading to rapid return to resistance reversion, nearly equals to G20, to be susceptible again.

In conclusion, Cyanophos, as a selective agent, possesses a high resistance potential to the pink bollworm for 32 generations. Abdel Hafez *et al.* (1994) and Rofail *et al.* (1998) found that selection of the pink bollworm by Cyanophos resulted in 3.19-fold tolerance in G8 and 32.51-fold resistance in G24, respectively.

II. Effect on the Relative Nuclear DNA-Content:

The feulgen relative nuclear DNA-content of the cell-population of the larval gonads of the pink bollworm was determined in the susceptible (S), resistant (Rs) and relaxed strains (Rx) (Table 2 and Fig. 1). The obtained results showed major tetramerous DNA distributions (1C-, 2C-, 3C-, and 4C- DNA-content distributions) besides two new abnormal group distributions [cells which contain less than 1C- and more than 4C- DNA content] at the three lines of selection.

The first distribution corresponds to 1C-DNA-content of haploid cells at about 200-350 arbitrary units (A.U.) and also represents the cells in spermatids and spermatozoa. The second profile refers to 2C-DNA-content of diploid cells at about 400-550 A.U. and represents G0/G1, spermatogonia A & B and spermoatocytes. The third peak shows the 3C-DNA-content at about 600-850 A.U. which corresponds to the cells of S-phase for both spermatogonia A & B as well as to the cells in the last DNA replication phase of spermatocytes. The fourth distribution indicates the 4C-DNA-content at about 900-1100 A.U. which represents G2 and M-phase of meiotic proliferation of spermatogonia A & B and spermatocytes, in addition to the cells belonging to Leptotene and pachytene of meiotic cell-division.

With respect to these distributions of nuclear DNA-content in gonad cells population, the data declared that the susceptible strain contained the highest percent of cells having 1C- (25.23), 3C-(23.86) and 4C-DNA-content (30.89) and the least of cells having 2C-(16.93), less

Table (2). Effect of the development of Cyanophos resistance and its relaxation on th distribution of male gonad cells groups depending on their nuclear DNA-content.

Groups of gonad cells depending on their nuclear DNA-content	% groups of gonad cells			
	A.U	Control	Resistant	Relaxed
Cells having less than 1C		1.53	1.95	2.28
(Necrotic cells)	50	0.00	0.78	0.38
(0-150 A.U)	100	0.38	0.39	0.76
	150	1.15	0.78	1.14
Cells having 1C		25.22	14.73	17.80
(200-350 A.U)	200	2.77	4.26	3.03
	250	10.36	5.81	6.82
	300	8.68	3.88	6.06
	350	0.38	0.78	1.89
Cells having 2C		16.93	47.67	29.17
(400-550 A.U)	400	0.77	1.16	1.52
	450	4.23	14.73	5.30
	500	8.08	17.44	12.88
	550	3.85	14.34	9.47
Cells having 3C		23.86	15.51	18.56
(600-850 A.U)	600	3.46	1.55	3.79
	650	4.62	2.71	3.03
	700	5.00	3.49	2.65
	750	3.85	3.88	3.41
	800	3.85	2.33	3.41
	850	3.08	1.55	2.27
Cells having 4C		30.89	15.13	27.56
(900-1100 A.U)	900	5.00	1.55	4.45
	950	5.77	3.11	6.82
	1000	10.00	5.04	7.58
	1050	7.04	3.49	5.68
	1100	3.08	1.94	3.03
Cells having more than 4C		1.53	5.05	4.65
(1150-1400 A.U)	1150	0.38	1.55	1.98
	1200	0.77	1.16	1.52
	1250	0.38	0.78	0.76
	1300	0.00	0.78	0.38
	1350	0.00	0.39	0.00
Total		99.96	100.04	100.00

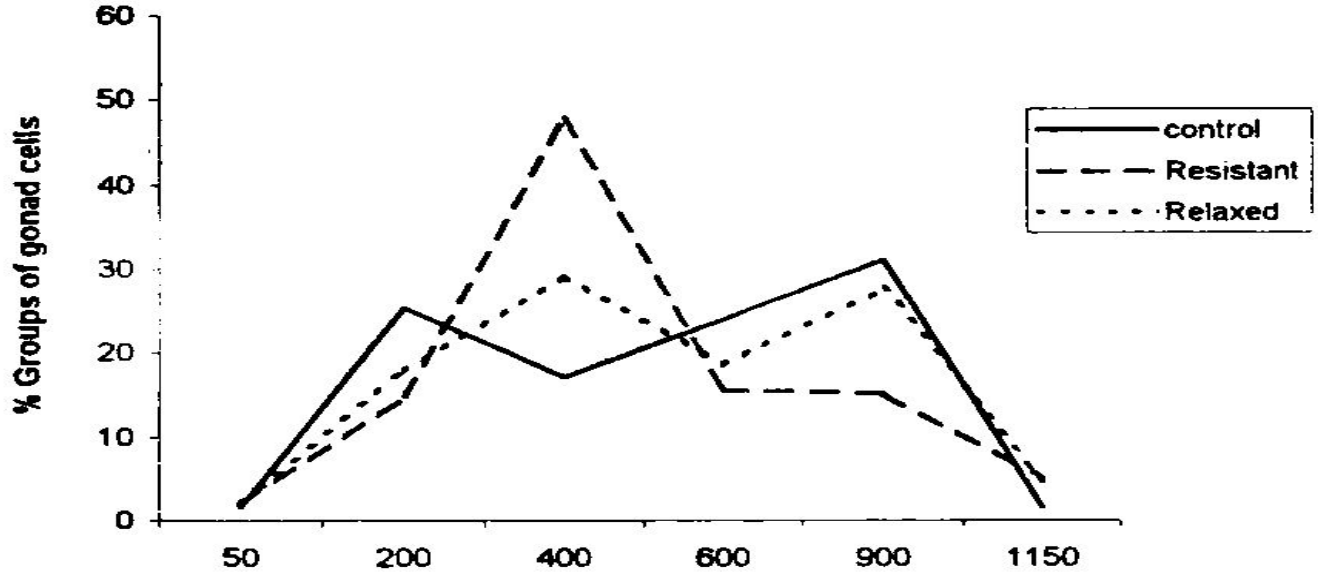

Figure (1). Effect of the development of Cyanophos resistance and its relaxation on the distribution of male gonad cells groups depending on their DNA-content

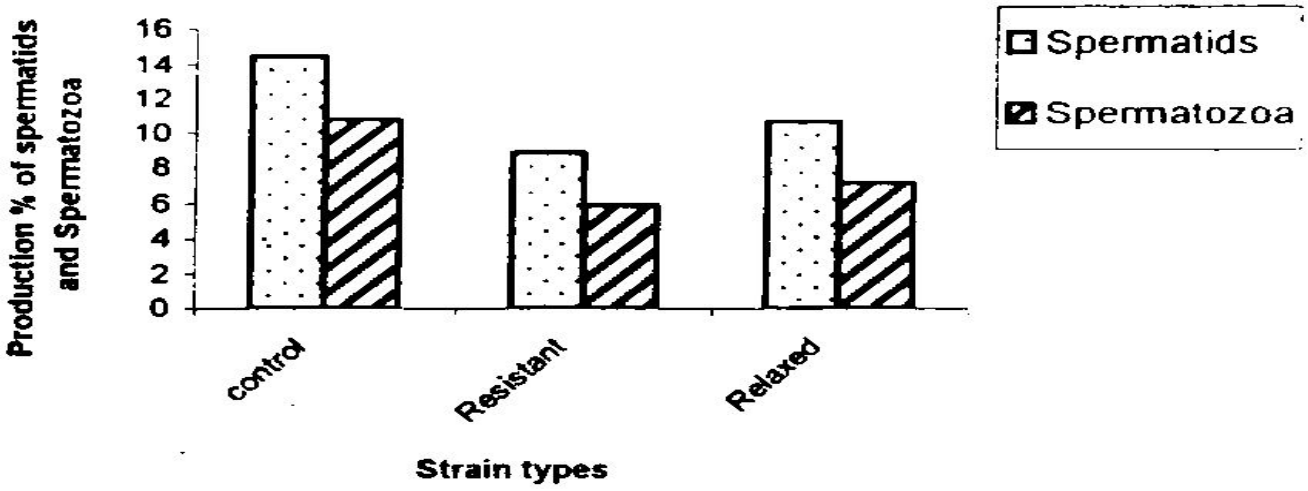

Figure (2). Effect of the development of Cyanophos resistance and its relaxation on the production of immature & mature spermatids and spermatozoa.

than 1C- (1.53) and more than 4C-DNA-content (1.53). While the Cyanophos resistant strain exhibited the highest percent of cells having 2C- (47.67) and more than 4C-DNA-content (5.05), and the least percents of cells having 1C-(14.73), 3C- (15.51), 4C-DNA-content (15.13). Meanwhile the relaxed strain gave the highest percent of cells having less than 1C-DNA-content (2.28) and intermediate percents of the other groups.

So, the foregoing results shown in Figure (1) could indicate obviously that the development of Cyanophos-resistance reduced the haploid cells having 1C-DNA-content which refers to spermatids and spermatozoa indicating the deficiency of the fecundity in males resulted from successive Cyanophos-selection. Moreover, the proportion of the triploid cells-group (3C-DNA-content) was decreased showing an inhibition of DNA synthesis in spermatogonia A & B and in spermatocytes divisions as somatic and germ cells, respectively. In addition the tetraploid cell-group (4C-DNA-content), belonging to leptotene and pachytene, was highly affected which apparently indicates also the effect on G2 and M-phase of mitotic proliferation of spermatogonia A & B and spermatocytes.

It is necessary to take into consideration that, in case of the resistant strain the percent of cells having more than 4C-DNA-content was the highest (5.05) when compared with the susceptible or relaxed strains. So, it could be recognized that the development of that resistance may be associated with chromosomal aberrations, especially the chromosomal stickiness or lagging which may lead to the increase of the cells having more than 4C-DNA-content or the cells having less than 1C-DNA-content. These expectations coincide with the results achieved by Massoud *et al.* (1992) who indicated that *B. thuringiensis* produced chromosomal aberrations ranging from 17.33 to 23% of the male gonad-cells of *Earias insulana* (Lep., Noctuidae) and *P. gossypiella*.

Also, Rofail *et al.* (1998) showed that the percentage of abnormal cells was almost equal (26%) for Cyanophos selected line in the 24th generation (G24). They also stated that the relaxation for one generation in Cyanophos selected strain showed a slight decrease in percentage of abnormalities.

Also, if some sectors of the DNA had replicated twice and others not at all, then some cells will contain high concentration of DNA and others low concentration of DNA The development in Cyanophos-

resistance induces the least percent of cells having 3C- and 4C- DNA-content, which indicates the inhibition effect on DNA-synthesis in cells (S-phase) and in cells which are going to begin their DNA replication (M-phase and proleptotene stage), respectively. Meanwhile, the results showed the effect on DNA-synthesis and its replication, while the DNA-replication and the repair appear to be associated with DNA-ligase (David *et al.*, 1982). Moreover, DNA polymerase is by no means the only enzyme involved in DNA replication (Stent and Calendar, 1976).

Finally, it could be recognized that the Cyanophos-resistance should be associated with the inhibition of DNA-ligase or/and DNA polymerase.

As a matter of fact, it is well-known that, DNA and histone together make up about two-thirds of the weight of most chromosomes. Also, most of the prominent basic protein in which DNA is combined, is histones, polypeptide chains about 50 to 200 amino acids in length, three basic amino acids (argenine, lysine and histidine) which make up nearly 25% of the total amino acids residues (Stent and Calendar, 1976). Selection pressure with Cyanophos may affect histone and reduce the content of these three basic amino acids which finally could lead to the decrease of DNA-synthesis or/and DNA histones or DNA-abnormalities. These findings are in agreement with the results obtained by Tayeb *et al.* (1996) who reported that amino acids (i.e. arginine, histidine, lysine and others) in the larval haemolymph of treated *Agrotis ipsilon* (Lep., Noctuidae) with triazophos (an organophosphorus compound) were decreased significantly.

III. Classified Stages of Spermatogenesis Affected by Cyanophos Selection:

The male larval gonad cells were automatically classified, using the discriminating methods of analysis, into ten major different cell-types, besides three division stages. Also, two abnormal cell-types appeared in the three tested strains, the first was the cells having less than 1C-DNA-content (necrotic cells), which their DNA-content-was low (less than 200 A.U.), while the second was cells having more than 4C-DNA-content, which their DNA-content-was high (more than 1300 A.U.).

Table (3) represents the percentages of 15 cell-types or stages. The data illustrate that the proportions of most cell-types were the highest in the male larval gonad cells of the untreated line. The important cell-

types, whose fecundity is correlated with it, are young & mature spermatids, and immature & mature spermatozoa. It was observed that their proportions were 6.50 & 7.95 and 5.48 & 5.29, respectively. On the

Table (3). Effect of the development of Cyanophos resistance and its relaxation on cell-type or stages of spermatogenesis.

Cell-types	% stages of Spermatogenesis		
	Control	Resistant	Relaxed
Necrotic cells	1.53	1.95	2.28
Young spermatids	6.50	3.83	4.50
Mature spermatids	7.95	5.01	6.14
Immature spermatozoa	5.48	3.09	3.67
Mature spermatozoa	5.29	2.80	3.49
Stem-cells	1.86	4.77	2.91
Spermatogonia A	3.56	9.53	5.83
Spermatogonia B	4.23	11.92	7.29
Spermato cytes	7.28	21.45	13.14
Proleptotene	23.86	15.51	18.56
Pachytene	4.64	2.27	4.14
Diakinesis	5.25	2.57	4.63
G2	19.15	9.38	17.14
Metaphase I	1.85	0.91	1.65
Cells having more than 4C-DNA content	1.53	5.05	4.64
Total	99.96	100.04	100.01

other hand, these percentages in the resistant strain were the least (3.83 & 5.01 and 3.09 & 2.80, respectively). While the relaxed strain exhibited the intermediate proportions in that respect (4.50 & 6.14 and 3.67 & 3.49, successively) as shown in Fig. 2.

So, it is important to declare that, the development of resistance is concurrent with the homogeneity and reduced the fecundity, which for that reason the rearing of the resistant strain in this study was blocked and stopped at the 32nd generation.

Moreover, at the beginning of male gonad cell-differentiation, it was also observed that discriminated cell-types or stages (metaphase I, G2, diakinesis, pachytene and proleptotene) were increased in the susceptible strain (S) than in those of resistant (Rs) or relaxed (Rx) strains (Table 3). So, if the first stages in resistance strain were decreased at the beginning of proliferation, then it is expected that the final stages will also be decreased as what was found in the final formed spermatozoa.

On contrast, the percentages of stem-cells, spermatogonia A & B, and spermatocytes were the highest in Rs strain followed by Rx and S strains. In the resistant strain, the percentages of these cell-types were 4.77, 9.53, 11.92 and 21.45 followed by 2.91, 5.83, 7.29 and 13.14 in relaxed strain and 1.86, 3.56, 4.23 and 7.28 in the control, respectively.

Noticeably, while the fore-mentioned cell-types which were increased in the Rs, were reduced in the final cell-types, especially spermatids and spermatozoa. The increase of these cell-types (stem-cells, spermatogonia A & B and spermatocytes) could be due to the blocking or delayed effect in these differentiation steps. The treated cells appeared to be retracted in the restriction point (Baserga, 1985), where cells started to begin its DNA replication. This blocking resulted in the retract of these cell-types which were prevented to continue and form spermatids and spermatozoa. This could also be due to the delay of synthetic rate of DNA or the prolongation of the duration of G0 and G1. As a matter of fact, Emara *et al.* (1991), Farrag (1992), Moustafa *et al.* (1993), and El-Sorady *et al.* (1995) reported observations which more or less agree with and support these results.

On the other hand, a high reduction in the prolesptotene cells (15.51%) was observed in Rs strain compared with that determined in S strain (23.86%) or with the Rx strain (18.58%). It is well-known that proleptotene has a role in S-phase especially in DNA-replication. Also, DNA-replication and repair are associated with DNA lygase (David *et al.*, 1982) and with DNA-polymerase (Stent and Calendar, 1976). Thus, the differentiation effect on stages of spermatogenesis could be due to the effect on DNA-lygase or-polymerase enzymes. These findings are merely

in agreement with the findings of Massoud and Moustafa (1993) and El-Sorady *et al.* (1995).

In addition, two new cell-types were found in the male larval gonad-cells of the three lines of selection. These new cell-types were cells having less than 1C-DNA-content (necrotic cells) and cells having more than 4C-DNA-content. The frequencies of necrotic cells were the least in the control (1.53%), the highest in the relaxed strain (2.28%), while they were intermediate in the resistant strain (1.95%). However, the frequencies of cells having than 4C-DNA-content were the least in the control (1.53%), the highest in the resistant strain (5.05%) and intermediate in the relaxed strain (4.64%). Generally, the total of these percentages of both abnormal cell-types was higher in the resistant and in relaxed strains (7.00 and 6.92, respectively) than in the control strain (3.06).

The aberrations which occur in the chromosomes may be due to the disturbance happening in the DNA-replication or in the meiosis division, which should lead to a high or low DNA content (Stent and Calendar, 1976). However, sticky chromosomes are generally regarded as a physiological unspecific disturbance and have been attributed to the action of basic chromosomal proteins, e.g., histones (Devadas *et al.*, 1987) or to the basic amino acids (Stent and Calendar, 1976; and Tayeb *et al.*, 1996).

Jain and Sarbhoy (1987) reported that the chromosomes could not reach the poles and remained scattered in the cytoplasm as a result of chemical treatments. It was also obvious, that there were irregularities in chromosomal distribution in some treated cells. This may be attributed to the disturbance of the spindle apparatus (El-Feel *et al.*, 1990).

Determining the nuclear DNA-content of larval gonad-cells indicated that the development of Cyanophos-resistance increased the percentage of cells having 2C-DNA-content and the abnormal gonad cells having more than 4C-DNA-content. As well as the classified stages of spermatogenesis showed the highest proportions of stem-cells, spermatogonia A & B and spermatocytes in the resistant strain.

On the other hand, the Cyanophos resistant strain induced the least percentage of cells having 1C-, 3C- and 4C-DNA-content. Also, Cyanophos-resistance was associated with the decrease of young & mature spermatids, immature & mature spermatozoa, proleptotene, pachytene and certain cell-division stages (diakinesis, G2 and metaphase

I). So, from the fore-mentioned results, it could be concluded that the development of Cyanophos-resistance may be associated with the deficiency of fecundity, blocking or delayed effect on certain cell-types of spermatogenesis, delaying the rate of DNA synthesis, prolongation of the duration of G0 and G1, the effect of DNA-replication and repair, the effect on DNA- lygase and polymerase and the effect on the disturbance of DNA replication leading to chromosomal aberrations.

In conclusion resistance may have been developed as a result of differential gene activity of the genome, which leads to an increase of production of an enzyme detoxifying the pesticide, or as a result of some alteration in the sensitive receptor sites affected by the pesticide. This resistance is manifested as a result of gene regulation and expression. Gene expression in eukaryotes can be regulated at different levels i.e. transcriptional control, RNA processing control, transport control, m RNA translation control, RNA degradation control, and protein degradation control. (Russell, 1996). Such changes leading to resistance have some influence on development and differentiation, leading to the observed changes in cell frequencies.

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Appendix 1

Each Feulgen stained nucleus was described by 12 parameters:

- Nuclear Area (NA).

Parameters from the optical density histogram:

- Integrated Optical Density of the Nucleus (IOD).
- Mean Optical Density of the Nucleus (MOD).
- Standard Deviation of Nuclear Optical Density Histogram (SDODH).
- Skewness of the Nuclear Optical Density Histogram (SODH).

Texture parameters:

- Local Mean.
- Second Moment of the Matrix Coefficients (SMMC).
- Standard Deviation of the Matrix Coefficients (SDMC).
- Contrast (CON).

Parameters from the run-length section matrix:

- Short-Run Emphasis (SRE).
- Gray-Level Distribution (GLD).
- Run Percentage (RPC).

الملخص العربي

تثبيط تكوين الحيوانات المنوية وتخليق DNA في خلايا البراعم التناسلية الذكرية نتيجة لتطور صفة المقاومة في دودة اللوز القرنفلية لمبيد السيانوفوس

تم دراسة تأثير انتخاب العمر الاول من يرقات دودة اللوز القرنفلية لصفة المقاومة لمبيد السيانوفوس لمدة ٣٢ جيل ، ثم عدم المعاملة لمدة ٣ أجيال (السلالة المسترخاه) علي معدل المقاومة . كذلك تم دراسة تأثير تطور صفة المقاومة علي محتوي خلايا البراعم التناسلية الذكرية من DNA وعلي تكون " تشكل " مراحل تخليق الحيوانات المنوية .

أظهرت النتائج أن مبيد السيانوفوس كعامل انتخابي أدي إلي ظهور عالي لصفة المقاومة في دودة اللوز القرنفلية . وأن الانتخاب أعطي درجة عالية ملموسة من الاستجابة لتطور صفة المقاومة ، حيث زاد معدل المقاومة إلي ٤٧.٧٦ ضعف (في الجيل رقم ٣٢) ، بينما أظهر الجيل الثالث (السلالة المسترخاه – الناتجة بعد توقف الانتخاب) أعلي ميل في درجة الاستجابة صاحبه نقص كبير في درجة المقاومة (٢٧,٨٠ ضعف) مؤديا إلي تحول سريع في انعكاس المقاومة تجاه الحساسية مساويا تقريبا لما حققه الجيل رقم ٢٠ .

علاوة علي ذلك أوضحت النتائج أن تطور صفة مقاومة السيانوفوس أدي إلي زيادة النسب المئوية لمجاميع خلايا البراعم التناسلية الذكرية التي تحتوي علي 2C من محتوي DNA وكذلك الخلايا الشاذة التي تحتوي علي 4C من محتوي DNA ، كما أظهر تقسيم مراحل تشكل تخليق الحيوانات المنوية أن نسب Stem-cells ، A & B Spermatogonia ، Spermatocytes كانت الأعلى في السلالة المقاومة . وعلي الجانب الأخر كانت نسب الخلايا المحتوية علي 1C ، 2C ، 3C ، 4C من محتوي DNA هي الأقل في السلالة المقاومة بالإضافة إلي نقص نسب مراحل تكوين الحيوانات المنوية مثل Proleptotene, & mature Spermatozoa, young & mature Spermatids . metaphase I ، G2 ، diakinesis ، Pachytene ، immature