ISRAEL JOURNAL OF ENTOMOLOGY Vol. VIII 1973

IMMUNOLOGICAL DIFFERENCES BETWEEN AN ORGANOPHOSPHORUS SUSCEPTIBLE AND RESISTANT STRAIN OF *TETRANYCHUS CINNABARINUS*[1]

S. BEN-EFRAIM,[2] G. HALPERIN[2] and A.S.TAHORI[3]

[2]Department of Human Microbiology, School of Medicine, University of Tel-Aviv, Tel-Aviv, Israel and [3]**Israel** Institute for Biological Research, Ness-Ziona, Israel.

ABS T R A C T

The serological pattern of a carmine spider mite *Tetranychus cinnabarinus* (Boisduval) strain resistant to organophosphorus (O-P) compounds and of a O-P sus- ceptible strain, was investigated by the microcomplement fixation and the agar gel double diffusion techniques. Antisera against the antigens of these strains were produced in rabbits. Quantitative differences were found in the antigenic pattern between the resistant and the susceptible strain. The fraction prepared from the sus- ceptible strain by mechanical disruption contained more antigenic material than the corresponding resistant fraction. On the other hand, the extract prepared by further ultrasonic treatment of the debris of the resistant strain was antigenically richer than the susceptible ultrasonic extract The differences observed were related to esterase- containing components.

[1]This research has been financed by a Ford Foundation Grant 6/B-VII.

INTRODUCTION

Resistance of spider mites to organophosphorus (O-P) compounds is a widespread phenomenon which has a great economic importance (Helle, 1965). In Israel, the carmine spider mite, *Tetranychus cinnabarinus* (Boisduval), has developed resistance to a number of acaricides including O-P compounds (Tahori and Raccah, 1970). Resistance of Acari to O-P compounds is usually due to the insensitivity of their acetylcholinesterase (AChE) to O-P compounds (Smissaert, 1964), and in the case of the carmine spider mite, it was found to be related to the size of the esteratic site (Zahavi et al, 1971). Development of resistance to pesticides in arthropods is connected with certain enzymatic changes (Brown and Pal, 1971). On the other hand, it is well known that enzymes, including AChE, are immunogenic. (Carter and Marx, 1958, Michaeli et al, 1969, Cinader, 1963).

Antibodies produced against certain enzymes may, in some cases, possess a specific neutralizing activity (Cinader 1967). Thus, the enzymatic changes occurring in resistant strains might be reflected in immunological differences.

Serological differences between insecticide-resistant and - susceptible strains have been reported for house flies in relation to DDT (Long and Silverman, 1965). The aim of the present investigation was to find whether serological differences can be detected between an O-P resistant and a susceptible spider mite strain, and whether this could be used to detect O-P resistance.

MATERIALS AND METHODS

Mite strains

The experiments were carried out with the carmine spider mite *Tetranychus cinnabarinus* (Boisduval), formerly known as *T. telarius* (L.) (Boudreaux, 1956). The O-P-susceptible *Amirim* and the O-P-resistant *Ayelet Hashahar* strains were used. Both strains were collected during the summer of 1966. Toxicological data on the strains and details of their rearing procedure are described elsewhere (Tahori and Raccah, 1970). Adult mites were collected from bean (*Phaseolus vulgaris*) leaves, by means of a hand aspirator and held in a

0.067 M sodium potassium phosphate buffer solution (pH 7.5) in a deep freezer.

Antigen preparation

Mites, in batches of several thousands, were ground thrice in a Griffin & George homogenizer in 2.5 ml of 0.067M ice-cold sodium potassium phosphate buffer (pH 7.5) for 30 sec. at maximal speed and cooled on ice between grindings. The homogenate was centrifuged at 7000 rpm at 4°C for 20 min. The soluble fractions derived from the resistant and susceptible strains were called *EX-R* and *EX-S* respectively. The remaining sediment was resuspended in 2.5 ml of the buffer solution and sonicated in an ice-cooled container for 2 min. by an NSE sonifier at 1A. The sonicate was then centrifuged at 7000 rpm at 4°C for 20 min. The supernatants derived from the resistant and susceptible strains were called *SON-R* and *SON-S* respectively. The *EX* and *SON* fractions were kept separately in a deep freezer. Protein (P) was determined by the method of Lowry et al., (1951) using crystalline bovine gamma globulin (BGG) as reference. The average yield of the *EX* fractions was 850 µg P/1000 mites and of the *SON* fractions 300 µg P/1000 mites.

Immunization schedule

Adult rabbits weighing 2 to 2.5 kg. were used. Four to nine injections at multiple sites of one of the fractions *(EX-S, EX-R, SON-R, SON-S)* in a total quantity of 1 ml, were administered subcutaneously during a period of 54 - 110 days. The first injection was given in Freund's Complete Adjuvant, followed by injections in Incomplete Freund's Adjuvant and intramuscular injections in phosphate buffer. The final antigen concentration injected ranged from 150 µg P/ml to 800 µg P/ml and the total quantity ranged from 1150 µg/P to 5235 µg/P per rabbit. During the immunization period, 5-11 cardinal bleedings were performed, starting from day 27 after the beginning of immunization. Sera obtained from the same rabbits before the start of immunization, were used as controls.

Serological tests

a) Micro-complement fixation tests were performed as described by Levine (1967) using commercial haemolysin (Bacto anti sheep haemolysin glycerinate - Difco) and a pool of guinea pig sera as complement. Haemolysin was titrated, using Kolmer's method (1951). The degree of haemolysis was determined by Klett photometer (green filter No. 54) in Klett units. The results were expressed in % complement fixation (C.F.) according to the formula :

$$\% \text{ CF} = \frac{\text{Positive control reading - tube test reading}}{\text{positive control reading}} \times 100$$

Each serum was tested in duplicate against homologous and heterologous antigen fractions using two fold serum dilutions ranging from 1:80. Within this range no anti-complementary effect was observed. The CF titer was expressed as the serum dilution which still gave significant inhibition of haemolysis (>10%). The optimal concentration of antigen was 5 µg P/ml for the *EX* fractions and 40 µg P/ml for the *SON* fractions.

b) Gel diffusion tests were carried out on microscope slides according to the Ouchterlony method (1958) and the modification of Jennings & Malone (1954). Special Noble Agar (Difco) made up to 0.8% in 0.15N NaCl and containing 0.01% merthiolate was used. The diameter of wells was 5mm and the distance between them was 4mm. The antigen concentrations used were 1030 µg P/ml for *EX* fractions and 700 µg P/ml for *SON* fractions. Readings were performed after incubation in a wet chamber for 48 hours at room temperature and for another 48h at 4°C. The precipitation lines were stained either with amido black or, for characterization of esterase activity, with β naphthyl acetate. (Uriel, 1964).

R E S U L T S

Complement fixation tests

The tests were performed with each of the individual bleedings of rabbits immunized with either *EX-S* or *EX-R* (2 animals per each extract) and *SON-S* or *SON-R* one rabbit per preparation). Immunization with *EX-S* induced formation of

higher titers against the homologous antigen *EX-S* than against the heterologous one *EX-R*. This difference in titers was detected with all the sera originating from bleedings performed at days 32, 41, 110 and 135 after the beginning of immunization (3 to 9 injections of antigen).

Injection of *EX-R* induced a lower response than *EX-S* and of the same titer against either *EX-R* or *EX-S* antigen. The similarity in titers was observed with all the sera tested: bleeding at days 34, 48, 64, 71 and 85 after the beginning of immunization (2 to 6 injections).

CF titers obtained after immunization with *SON-R* were higher both against *SON-R* and *SON-S* as compared with titers detected against the two *SON* preparations after injection with *SON-R*. In both cases, the sera were tested at day 93 after the beginning of immunization.

The maximal CF titers obtained are presented in Table 1.

Gel-diffusion tests

The results obtained by the CF method suggested the occurrence of some quantitative differences between the "resistant" and "sensitive" antigen preparations: We investigated this possibility further, by using the gel-diffusion technique. Anti *EX-S* and anti *EX-R* sera were tested against *EX-S* and *EX-R* antigens. Tests of these sera showed that the anti *EX-S* serum reacted better against *EX-S* and *EX-R* antigens than the anti *EX-R* serum. This was shown by the presence of an additional precipitation line between anti *EX-S* serum and either *EX-S* or *EX-R* antigens. (Fig. 1a and b).

The presence of more precipitation lines against anti *EX-S* serum can also be seen in Fig. 1c. A difference was also observed between *EX-R* and *EX-S* antigens with regard to their reaction against the anti *EX-S* serum: Line number 2 (marked with an arrow) between *EX-R* antigen and *EX-S* serum bifurcated between *EX-S* antigen and the corresponding serum (Fig. 1c). This line was one of the two lines which were shown to include esterase activity (Fig. 1d).

Tests were also performed with anti *SON-R* and anti *SON-S* sera against *SON-R* and *SON-S* antigens. The anti *SON-S* serum reacted better than *SON-S* serum against both antigens, as examplified in Fig. 1e.

The difference between these sera was due to the occurrence of antibodies in the anti *SON-R* serum against two fractions with esterase activity (Fig. 1f).

Comparison of reactions between *EX-S* antigen and various types of antisera showed also that the anti *SON-R* serum contained antibodies which could not be detected in the anti *SON-S* serum : compare in Fig 1g reactions between containers 1 and 2,3,4 and 5 (sera anti *EX-S* , anti *EX-R* anti *SON-R* towards the center and between container 6 (anti *SON-S* serum) and the center. A similar picture was also observed when *EX-R* was used as antigen.

D I S C U S S I O N

The results obtained show that some quantitative differences can be discerned between the antigenic pattern of an O-P susceptible and an O-P resistant strain of the carmine spider mite, both by CF and gel diffusion tests. It appears that these differences are related to the relative quantities of one or more esterase containing components. Differences between the two kinds of preparations could be detected by using antiserum obtained from the same rabbit.

A striking difference was found between the EX fractions obtained by mechanical disruption of mites and the SON fractions obtained by ultrasonic treatment of the debris left after separation of the EX . While EX-S contained more antigenic material than EX-R , the opposite was true with the SON fractions. These results indicate again that the difference between the susceptible and the resistant strain of mites lies in the relative quantity of a certain fraction. It might also be that the same esterase component is bound more strongly in a resistant strain and thus can be extracted better by the ultrasonic treatment than by simple mechanical disruption.

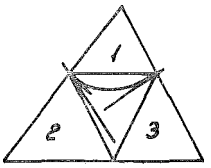
Zahavi et al. (1971) investigated the same mite strains for biochemical differences. They reported that the activity of AChE from resistant mite strains against propionylthiocholine was considerably lower than the corresponding activity of AChE from susceptible mite strains. However, they did not find additional protective mechanisms in the resistant mite strains. Motoyama et al. (1971) found that the mechanism of O-P-resistance in the predacious mite *Neoseiulus fallacis* was not associated with a modified cholinesterase but with a difference in the degree of esterase activity. The data of Zahavi et al. (1971) and of Motoyama et al. (1971) correlate with our finding that the immunological differences were only of a quantitative and not of a qualitative nature.

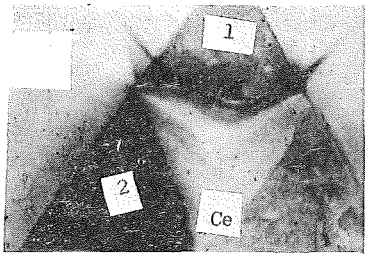
It is possible that the O-P-resistant and susceptible mite strains differ in a number of properties not connected with O-P-resistance. Thus, the differences observed between the strains in their antigenic pattern may not be related to the development of O-P-resistance. In order to draw conclusions on the relation between resistance to O-P compounds and antigenic make-up, more O-P-resistant and sensitive strains will have to be investigated.

TABLE 1 : Maximal CF (complement fixation) titer obtained in rabbits immunized with various preparations from mites.

IMMUNIZATION				CF RECIPROCAL TITERS	
Product	No. of inject.	Total Quant. Inj. µgP	Days after beginning of immunization	EXS	EXR
EXS	8	4600	110	5120	1280
	9	5235	135	10240	2560
EXR	6	3856	71	1280	640
				SON-S	SON-R
SON-S	6	3544	93	640	640
SON-R	7	2645	93	2560	2560

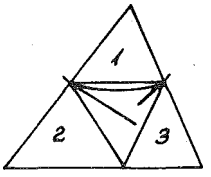
Fig. 1. a to g. Immunological characterization of an O-P resistant and susceptible spider mite strain.

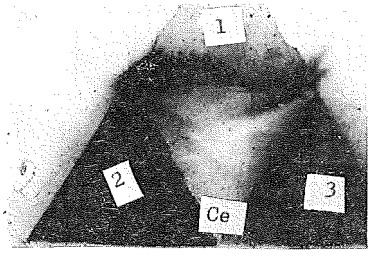




1a

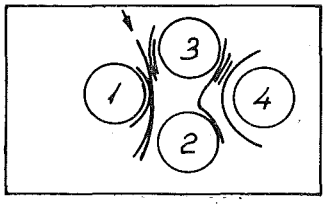
1 - EX-R 1030 µgP/ml
2 - anti EX-S serum
3 - anti EX-R serum

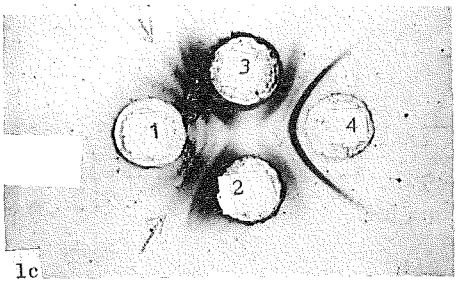




1b

1 - EX-S 1030 µgP/ml
2 - anti EX-S serum
3 - anti EX-R serum

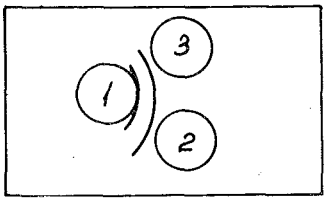


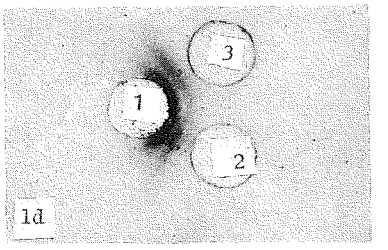


1c

1c

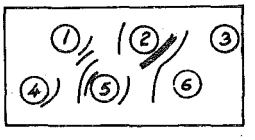
1 - anti EX-S serum
2 - EX-S 1030 µgP/ml
3 - EX-R 1030 µgP/ml
4 - anti EX-R serum

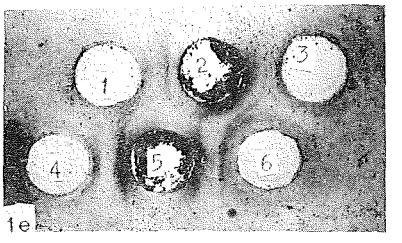




1d

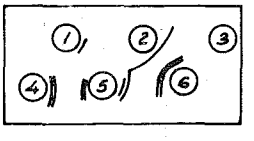
Esterase staining
1 - anti EX-S serum
2 - EX-S 1030 µgP/ml
3 - EX-S 1030 µgP/ml

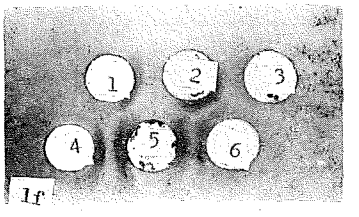




1e

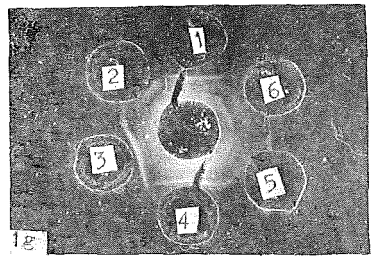
1 - anti SON-S serum
2 - SON-R µgP/ml
3 - anti SON-S serum
4 - anti SON-R serum
5 - SON-S 700 µgP/ml
6 - anti SON-R serum

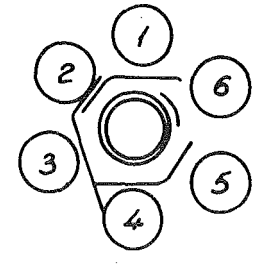




1f

Esterase staining same pattern as 1e.





1g

1.4 - anti EX-S serum
2.5 - anti SON-R serum
3. - anti EX-R serum
6 - anti SON-S serum
6e - EX-S 1030 µgP/ml.

R E F E R E N C E S

Boudreaux, H.B. 1956. Revision of the two-spotted spider mite complex *Tetranychus telarius* (Linnaeus). *Ann. Entomol. Soc. Amer.* 49:43-48.

Brown, A.W.A. and Pal, R. 1971. Insecticide Resistance in Arthropods. *World Health Organization. 491* pp.

Carter, M.K. and Marx, G.L. 1958. Immunological and enzymological specificity of antibodies produced by injection of purified acetylcholinesterase. *Proc. Soc. Exp. Biol. and Med.* (N.Y.) 99:712-715.

Cinader, B. 1963. Introduction: Immunochemistry of enzymes. *Ann. N.Y. Acad. Sci.* 103: 495-548.

Cinader, B. 1967. Antibodies to Enzymes-A discussion of the mechanism of inhibition and activation. In: "Antibodies to Biologically Active Molecules" (Edited by Cinader, B.) *Pergamon Press,* Oxford, pp. 85-137.

Helle, W. 1965. Resistance in the acarina: mites. *Adv. Acarology* 2:71-93.

Jennings, P.K. and Malone, F. 1954. Rapid double diffusion precipitin analysis *J. Immunol* 72:411-418.

Kolmer, J.A., Spaulding, E. and Robinson, H. 1951. Approved Laboratory Technic. *Appleton-Century Crofts,* New York. 827 pp.

Levine, L. 1967. Micro-complement fixation. In: "Handbook of Experimental Immunology". (Edited by Weir,D.M.) *Blackwell Scientific Publications,* Oxford and Edinburgh, 707 pp.

Long, C.F. and Silverman, P.H. 1965. Serological characterization of five strains of insecticide-resistant and susceptible house flies. *J. Econ. Entomol.* 58:1070-1074.

Lowry, O.H., Rosebrough, A.J., Farr, A.L. and Randall,R.J. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 193:265-275.

Michaeli, D., Pinto, J.D., Benjamini, E. and deBuren, F.P. 1969. Immunoenzymology of acetylcholinesterase-I. Substrate specificity and heat stability of acetylcholinesterase and of acetylcholinesterase-antibody complex. *Immunochemistry* 6:101-109.

Motoyama, N., Rock,G.C. and Dauterman,W.C. 1971. Studies on the mechanism of azinphosmethyl resistance in the predaceous mite, *Neoseiulus (T.) fallacis* (Family : Phytoseiidae). *Pesticide Biochem. Physiol.* 1:205-215. 215.

Ouchterlony, O. 1958. Diffusion-in-gel methods for immunological analysis. *Progr. Allergy* 5:1-78.

Tahori, A.S., & RACCAH, B. 1970. Resistance to acaricides in the carmine spider mite in Israel. *J. Econ. Entomol.* 63:545-548.

Uriel, J. 1964. The characterization reactions of the protein constituents following electrophoresis or immunoelectrophoresis in agar. In: "Immuno-Electrophoretic Analysis." (Edited by Grabar, P. & Burtin, P.) Elsevier Publishing Company, pp. 30-57.

Zahavi, M., Tahori, A.S. and Klimer, F. 1971. Insensitivity of acetylcholinesterases to organophosphorus compounds as related to size of esteratic site. *Mol. Pharm.* 7:611-619.