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Some aspects of the genetics of organophosphate resistance in *Tetranychus urticae* Koch

INTRODUCTION

Because of the frequent occurrence of resistance to insecticides, it has become of great importance that we gain knowledge of the genetic foundations of resistance.

Genetic analyses of resistance can give answers to the following questions: Will resistance develop rapidly? May we expect that resistance will decrease after some time? Is there a possibility of delaying resistance by adaptation of any spray programme?

There are various answers to the question whether after selection has ceased, reversion of organophosphate resistance in spider mites will occur.

Some authors (GARMAN, 1950; ABUL HAB et al., 1961); report such a reversion, others, however, have not found it (GASSER, 1957; SMITH, 1960; HELLE, 1962; WATSON et al., 1963). A very quick reversion of the systox resistance (DITTRICH, 1961) and of the TEPP resistance (SABA, 1961; HUWALD, 1965) in the Leverkusen strain after relaxation was reported. However, the Blaufelt strain has a stable systox resistance (DITTRICH, 1963b). The cause underlying the reversion in the Leverkusen strain was the decreased fitness of the resistant mites. The parathion resistance of this strain is largely attributed to a dominant major factor (HELLE, 1962); the systox resistance, on the contrary, to a single recessive factor with dominant semilethals (DITTRICH, 1963a). The above-mentioned differences between the parathion and systox resistances may be explained by the assumption that both are based on different genetic factors (HUWALD, 1965). Since systox resistance is accompanied by a high resistance to parathion, and since the reverse is true to a much lesser degree, there must be some relation between them.

Boll. Zool. agr. Bachic., s. II, v. 7, 1965.
(Atti del 5° Simposio europeo d'Acarologia, Milano, 23-25.IX.1965)

The degree of cross resistance which may be present, is usually determined through selection of a susceptible strain by means of an acaricide and subsequent determination of the resistance to other acaricides (HANSEN et al., 1963). Another possibility consists in taking a multiresistant strain and crossing it several times with a susceptible strain under low selection pressure. In this way, we may find out whether the resistance is determined by recessive or dominant factors and obtain data on the degree of linkage of the various resistance factors.

The *Tetranychus urticae* complex has three linkage groups. If the resistance to various insecticides is based on different factors, we have to assume that a linkage group may contain several resistance factors. New combinations of linked factors may be formed through crossing-over. However, owing to the absence of usable morphological characteristics, which might serve as markers, nothing is known about crossing-over in mites.

Let us suppose the following experiment to be made: susceptible females are crossed with males, which are resistant to 2 acaricides, say A_1 and A_2. The F_1 and the following generations are crossed back with susceptible males under low selection pressure of A_1. Now, if the resistance to A_1 and A_2 is largely determined by semidominant major factors, the following may take place:

In spite of the back-crossing, the resistance to A_1 is kept because the susceptible mites (50 %) are killed off by each selection. The resistance to A_2 may remain or disappear. If, after several back-crossings, the resistance to A_2 has disappeared, the factor for this resistance may be found either on other linkage groups or possibly on the same, but in the latter case at such a distance from the resistance factor for A_1, that no linkage results.

On the other hand, if the resistances to both A_1 and A_2 remain wholly or partially, then these two resistances must be caused by one and the same factor or by strong linkage between two factors. The latter can happen if these factors are at a short distance of each other, so that little crossing-over occurs. Crossing-over between the 2 factors can be prevented also by crossing-over reducers, e.g. inversions and translocations.

If the resistance to A_1 and A_2 is determined by multiple factors, the manner of back-crossing described above will decrease the resistance every time. According to the selection pressure exerted, the resistance will be reduced to normal or to a lower level.

Should resistance to A_1 and A_2 remain after back-crossings, then there must be an identical factor or a linkage between factors. We will disregard other possibilities such as cytoplasmatic inheritance and heterosis, which may cause residual resistance after several back-crossings.

In order to determine the genetic connection between meta-iso-systox, parathion and tedion resistance, we started with hybrids from susceptible females and resistant males. With these hybrids a back-cross programme (5x) was performed, under selection pressure of meta-iso-systox.

Materials and Methods

The multiresistant strain « Baardse » was collected during autumn 1963 in a rose house at Aalsmeer (Netherlands). Since then, it has been bred under laboratory conditions, on *Phaseolus vulgaris* at 25-28° C and about 70 % relative humidity, under constant light and without treatment by insecticides. The susceptible strain « Sambucus » has been bred under laboratory conditions since 1961. For toxicological

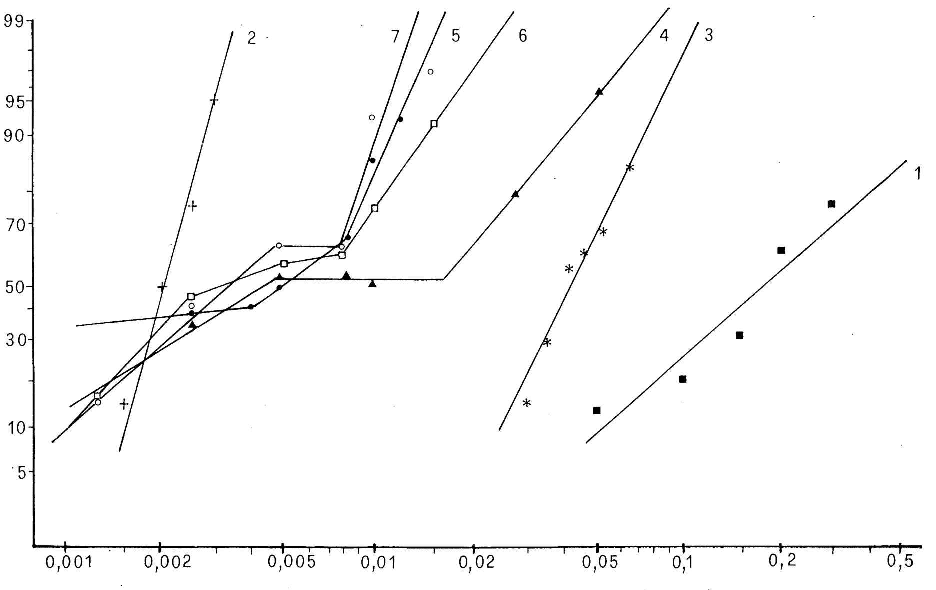

Fig. 1 - Meta-Iso-Systox susceptibility of the female mites of:
a) the Baardse and Sambucus strains (1 and 2);
b) the cross Sambucus × Baardse (3);
c) the backcrosses 1, 3 and 5 (Sambucus × Baardse) × Sambucus (4, 5, 6 and 7).
1. Baardse□ 2. Sambucus+ 3. Sambucus × Baardse✻ 4. backcross 1△ 5. backcross 3● 6. backcross 4□ 7. backcross 5○.

determinations of adult females the slide dip method was used. For the determination of d.m. lines usually 4 or more batches of 30-35 mites were dipped on subsequent days for 10 seconds in the test concentrations. Generally for each test concentration 130 to 200 mites were tested. After dipping, the batches were dried and placed in a thermostat of 30° C and 90 % relative humidity. After 24 hours the dead and living mites were counted. The percentages of mortality found were put on log-probit paper and a line estimated by the eye. Tedion concentrations have been tested according to the method of VAN ZON et al. (1963)

Crossing was done by placing females at the teleiochrysalis stage on detached leaf cultures and by adding a number of adult males (HELLE, 1962). When an egg wave had developed, we first collected new teleiochrysalids and then we put the leaf on a bean plant. After 2 to 6 days the mites, which had migrated to the plant, were used for toxicological tests. Selection took place as follows: after fecundation of the females the leaf was laid on a bean plant, which was dipped in 0,005 or 0,0025 % meta-iso-systox. After 2 days, the remaining mites were collected for supplying a new generation.

CROSSING PLAN

1) Sambucus virgins × Baardse males → F_1.

2) F_1 inter se → first back-cross generation B_1.

3) B_1 virgins × Sambucus males (selection with 0,005 % meta-iso-systox) → second back-cross generation B_2.

4) In the same way, the other back-crossings were done. The female parents of the B_5, however, have been selected with 0,0025 % meta-iso-systox.

RESULTS

After having been bred under laboratory conditions for about a year and a half, the Baardse strain was still very resistant to meta-iso-systox: LD 50 = 0.2 % (fig. 1). Moreover, this strain was resistant to parathion (LD 50>0.4 %), to tedion (LD 50>0.1 %) and to a lesser degree to kelthane. The Sambucus strain was susceptible for meta-iso-systox (LD 50 = 0.002 %), for parathion (LD 50 = 0.0014 %), for tedion (LD 50<0.0005 %) (VAN ZON et al. 1964) and kelthane.

The F_1 of the hybrid Sambucus × Baardse is much less resistant to meta-iso-systox than the original Baardse strain (LD 50 = 0.04 %). When the F_1 is crossed back with Sambucus males, the resistance is further reduced. In the third and following back-crossings a more or less stable condition is reached.

Table I gives the mortality of the female parents of the back-crossings after selection. The resistance to parathion remains in spite of the back-crossings (figs. 2 and 3).

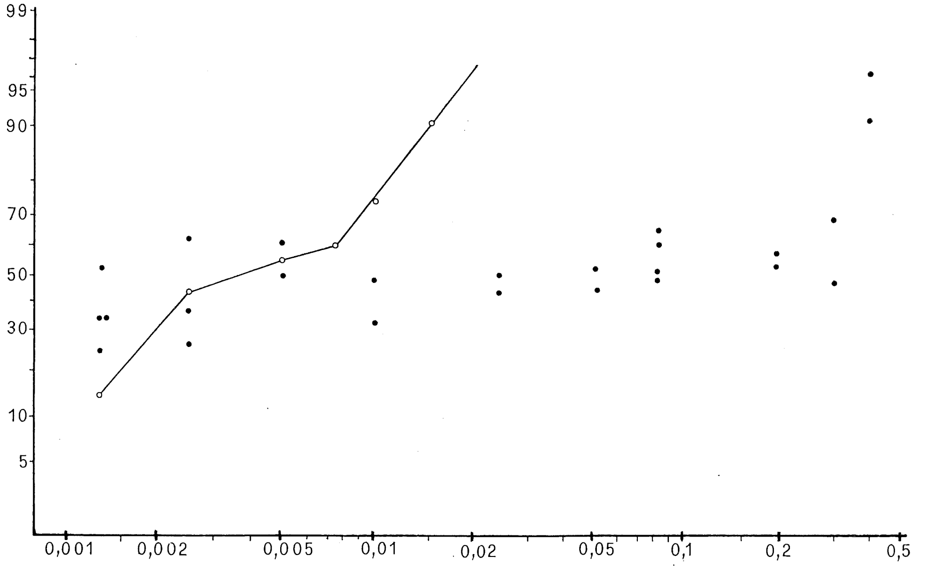

Fig. 2 - Susceptibility of the female mites from the 4th backcross to Parathion and Meta-Iso-Systox:
● = mortality percentages of ± 30 mites to Parathion;
○ = mortality percentages of ± 130 mites to Meta-Iso-Systox.

The resistance to tedion had disappeared after the fifth back-cross generation (LD 50$<$0.001 %).

Discussion

The gradual reduction of the resistance to meta-iso-systox caused by the back-crossings indicates that this resistance is determined by several dominant factors in the Baardse strain.

It is improbable that these factors would be semilethals, because in that case the strain would be difficult to breed, which was not true. Also the slope for F_1 would be flatter if the resistance had been determined by semilethals. Moreover, the stable resistance indicates the probability that homozygosity of some of the dominants occurs.

The susceptibility of the Sambucus strain is not obtained within 5 back-crossings. It is highly probable that the rather strong selection pressure is responsible in part for the factor (s) in favour of meta-iso-systox resistance. DITTRICH (1961) has established a polyfactorial resistance to systox and meta-systox. However, his resistant strain became susceptible with the use of a somewhat different selection procedure.

Within the parathion concentration ranges of 0.00125-0.3 % and 0.0025-0.1 % the mortality rates of B_4 and B_5 respectively are 50-70 %.

The presence of parathion resistant and susceptible mites indicates that in each back-crossing two genotypes are formed. This means that parathion resistance is largely caused by one factor or complex of closely linked factors. The selection with 0.005 % or 0.0025 % meta-iso-systox must have killed the parathion susceptible mites in each back-crossing.

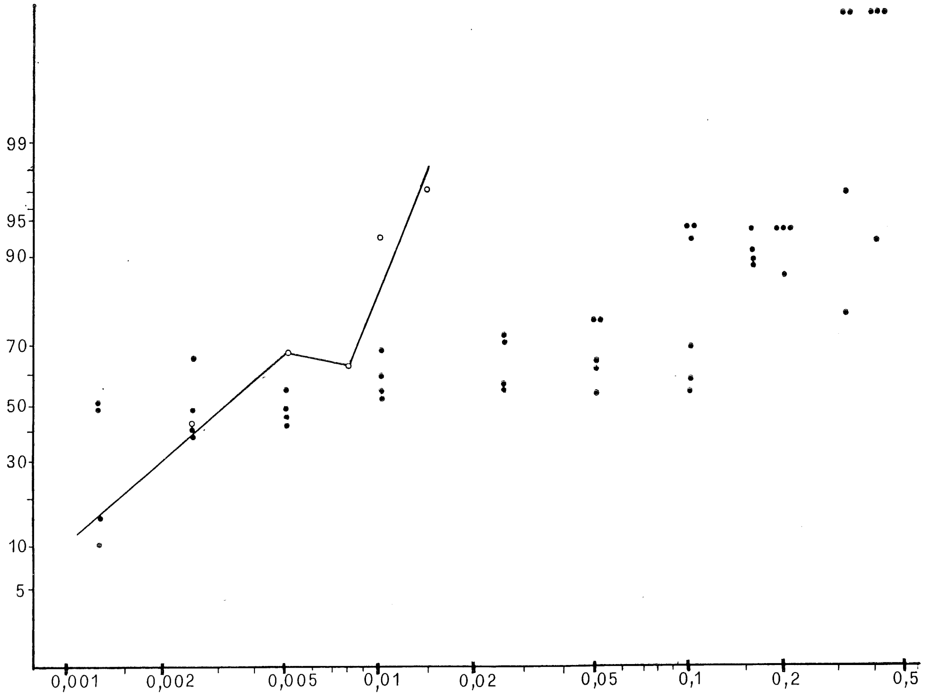

Fig. 3 - Susceptibility of the female mites from the 5th backcross to Parathion anl Meta-Iso-Systox:
● = mortality percentages of ± 30 mites to Parathion.
○ = mortality percentages of ± 130 mites to Meta-Iso-Systox.

These data suggest that the meta-iso-systox resistance is based on 2 genetic systems, which show an interaction. The first system is largely determined by a major factor; it lends a considerable parathion resistance to its carriers. The other system possibly interacts with the former one, and results in a high level of meta-iso-systox resistance. These data do not permit a conclusion as to the degree in which the parathion resistance is enhanced by this interaction.

As the tedion resistance has been removed by the back-crossing procedure, it can be concluded that the genetical make-up of this resistance is different.

TABLE I. - *Mortality rates of the female parents of the 2nd-5th backcrossings, selected by meta-iso-systox*

Back cross	Selection pressure	Mortality
1	—	—
2	0.005 %	62 %
3	0.005 %	67 %
4	0.005 %	66 %
5	0.0025 %	61 %

Acknowledgements.

The author is much indebted to Dr. P. A. VAN DER LAAN, Director of the Laboratory of Applied Entomology at Amsterdam, for providing facilities in his laboratory; to Dr. W. HELLE for his critical interest; to Mr. W. KUYKEN for technical assistance and to Mrs. DUVERT for improving the English text.

SUMMARY

A resistant strain of *Tetranychus urticae* Koch, which had been bred for one year and a half under laboratory conditions, without insecticide treatment, was still resistant to meta-iso-systox, parathion, tedion and kelthane. After crossing susceptible females with these multiresistant males and after back-crossing with susceptible males under selection pressure of meta-iso-systox, it appeared that the 5th back-crossing hybrids remained somewhat resistant to meta-iso-systox, but not to tedion. These hybrids showed a considerable resistance to parathion. It seems plausible that the meta-iso-systox resistance consists of two components, which show some interaction. One dominant factor underlies parathion resistance to a high level and is integrated to an unknown extent in the genetic basis of the resistance to meta-iso-systox.

RIASSUNTO

Un ceppo resistente di *Tetranychus urticae*, dopo essere stato allevato per un anno e mezzo in laboratorio senza subire trattamenti con insetticidi, manifestava ancora resistenza al metaisosystox, al parathion, al tedion e al kelthane.

Dopo avere incrociato femmine sensibili con maschi multiresistenti e dopo reincrocio sotto pressione selettiva con metaisosystox, è risultato che gli ibridi del quinto reincrocio rimanevano alquanto resistenti al metaisosystox e al parathion ma non al tedion.

Sembra che la resistenza al metaisosystox sia dovuta a due componenti, che mostrano interazione fra loro. Un fattore, dominante, induce un alto livello di resistenza verso il parathion e partecipa, in misura ignota, nella base genetica della resistenza al metaisosystox.

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