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LABORATORY METHOD FOR TESTING SIDE EFFECTS OF PESTICIDES ON JUVENILE STAGES OF THE PREDATORY MITE, *PHYTOSEIULUS PERSIMILIS* [*ACARINA, PHYTOSEIIDAE*] BASED ON DETACHED BEAN LEAVES

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The method is based on detached primary bean leaves sprayed with the concentration recommended for the pesticide, or with demineralized water (controls). As soon as the spraying has dried, adult spider mites [*Tetranychus urticae* (Koch)] are placed on the leaves. The test mites are juvenile (0-48 h old) predatory mites, *Phytoseiulus persimilis* (Athias-Henriot), placed on the leaves immediately after the spider mites on which they feed. The duration of the test is 13 days, mortality of *P. persimilis* being recorded on day 9. The side effects of the pesticide are expressed as the reduction in egg production of *P. persimilis* during the whole test period. Results permit classification of the pesticides according to the 4 categories of harmfulness used by the IOBC working group. 27 pesticides have been tested, and results are compared with those of other workers. Finally, statistical analysis indicates that the reproductibility of the test is satisfactory.

Standardized methods for the testing of side effects of pesticides on beneficial arthropods are being developed in the IOBC working group "Pesticides and Beneficial Arthropods" (**Franz**, 1975).

The animals selected for test development are of economic importance, such as *Phytoseiulis persimilis* Athias-Henriot which is in use for biological control of spider mites (*Tetranychus urticae* (Koch)) in glasshouses in most European countries (**Van Lenteren** *et al.*, 1980). Several members of the group have been working on the development of a laboratory method for testing side effects of chemicals on the predatory mite *P. persimilis*. **Van Zon & Van Der Geest** (1978) submitted a test method at a working group meeting in Darmstadt that was almost identical to the one described by **Van Zon & Wysoki** (1978).

This method has now been further developed, and the result - described in this paper - has been accepted by the IOBC group as a standardized test method. It is a method of testing *P. persimilis* juveniles on fresh pesticide residue sprayed on primary bean leaves with untreated spider mites as prey. The evaluation of results is based on the oviposition rate of the predators.

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PRELIMINARY REQUIREMENTS FOR THE TEST

BIOLOGY OF THE PREDATOR, *PHYTOSEIULUS PERSIMILIS*

Phytoseiulus persimilis is a predatory mite (*Acarina, Phytoseiidae*), which lives on all stages of the spider mite *T. urticae*. Its origin is subtropical as it was originally imported from Chile, and it is unable to overwinter under temperate conditions (**McMurtry** *et al.*, 1970). Sex determination is parahaploid (all eggs are fertilized, but males are haploid while females stay diploid) and mating is necessary for oviposition (**Sabelis**, 1981).

The strain used shows the following characteristics at the experimental conditions : At 26 °C the oviposition rate is 4-5 eggs per ♀ per day and about 15 % of the offspring are males. At this temperature mature ♀♀ eat 40-45 spider mite eggs or 10-15 mature spider mites per day. Males and younger stages eat only about 1/4 of this. When food shortage occurs egg-laying decreases proportionally with food intake. Cannibalism arises when there is total lack of prey. The development from egg to egg-laying ♀ takes 4-5 days and passes through a larva, a protonymph, and a deutonymph stage. These features are in accordance with those of strains described in literature (**McMurtry** *et al.*, 1970, **Van Zon & Van Der Geest**, 1978 ; **Sabelis**, 1981), though the predation capacity and egg production rates are on a high level.

REARING OF THE TEST ANIMALS, *P. PERSIMILIS*

The predator strain has been in culture at the department for several years without any exposure to pesticides. It has the same origin as that of mites used by the Danish growers. The strain has - to our knowledge - not developed resistance to any pesticide, and certainly not to organo-phosphorous compounds.

The mass rearing takes place in a greenhouse where temperature is 20 ± 5 °C, humidity usually 70 ± 10 % R.H. (though it might reach 40 % R.H. or 90 % R.H. for short periods) and the light is natural in the summertime but artificially extended to 16 h from August until May. The predators (on leaves) circulate between greenhouse and refrigerator, so that there are always some spare mites in case something should break down. Once a week bean plants infested with spider mites are placed in a large cage (60 x 100 x 90 cm) with gauze walls and a glass ceiling, where older leaves with predatory mites are added. Leaves with predators are usually kept in the refrigerator for some time (max. 1 month) before being used again for the rearing. Mites for experiments are always taken directly from the greenhouse to eliminate any possible effect of cooling.

REARING OF THE PREY, *T. URTICAE*

The strain of spider mites used for the rearing of predators and for the experiments has also been in culture for several years, without exposure to pesticides.

Two rearing cages similar to that used for *P. persimilis* are placed in the same greenhouse as the predator cage. Once every week young bean plants with only 2-3 leaves are placed in the 1st cage, and older leaves with spider mites are laid on top of them. The old plants from this cage are moved to the 2nd cage, and the oldest plants to the predator cage. Thus there are always plants infested with spider mites at all stages, and leaves with many adult spider mites are always available. Spider mites are not kept in the refrigerator since it would be possible to pick some from the predator leaves if it should be necessary to restard the culture.

PREPARATIONS FOR THE TEST

EXPERIMENTAL CONDITIONS

Physical conditions

All experiments are carried out in controlled environment cabinets at 26 ± 1/2 °C, 90 ± 10 % R.H. and a 16L : 8D photoperiod.

Leaf quality

Primary leaves of *Phaseolus vulgaris* (L) variety "Fruco simplo" are used for all experiments. This variety has very smooth leaves which interfere neither with the mobility of the animals nor with the application of pesticides. The quality of the leaves is of paramount importance to the spider mite populations (**Van De Vrie**, 1972) as well as to the durability of the leaves. Therefore it is very important that all leaves are of the same quality in tests that are to be compared. Young, dark green, primary leaves are chosen that are roughly 5,5 cm wide at the widest part near the base.

Cleaning

To ensure that no residues of pesticides used earlier interfere with the test, leaves, cotton wool and tin foil dishes are discarded after each experiment, and all material to be reused is washed carefully in a detergent (Deconex 11®).

Residual analyses (made by **E. Kirknel** at the Danish Laboratory for Pesticide Analysis) have proved that this cleaning is sufficient to remove residues from glass surfaces (detection limit : 0.06 $\mu g/10\ cm^2$).

PREPARATION OF THE PREDATOR

Juveniles, 0 to 2 days old, have been chosen as a suitable stage with which to start the tests. They are considered most sensitive to the treatment - much more than the adult ♀♀ - and therefore results obtained with juveniles might more distinctly show the adverse effects of a pesticide on a predator population (**Van Zon & Wysoki**, 1978 ; **Hassan**, 1982a).

The juveniles are obtained by isolating *P. persimilis* eggs on leaves with spider mites 2 days before the test. On the 1st day of the test most of the eggs are hatched, the resulting mites being between 0 and 48 h old. 120 individuals are used in a test ; 10 on each bean leaf. The 12 x 10 individuals to be used are selected so that there will be about the same age distribution on all leaves. Since 120 is a big number to select, a systematic error might arise as a difference between e.g. the 1st 10 and the last 10 mites chosen. To minimize the consequences of this possible error, test leaves with different treatment are supplied with predators alternately.

PREPARATION OF THE PREY

Adult ♀♀ spider mites for the test are picked at random (under the binocular) from leaves taken from the mass rearing. The ♀♀ are moved from one leaf to another by means of a suction apparatus that allows a precision in counting of about 5 %.

PERFORMANCE OF THE TEST

EXPERIMENTAL PROCEDURE

A test unit comprises a tin foil dish (9 cm diameter) containing a detached primary bean leaf, underside up, resting on a pad of cotton wool. Six tin foil dishes are placed in a 20 x 30 cm plastic tray with water so that all test units are separated by a water barrier. The bottoms of the dishes are perforated to keep the cotton wool moist (fig. 1).

From the selected bean leaves the tips are cut off, if necessary, to give a standardized area of approximately 24 cm^2. Care must be taken that the leaf is placed in the centre of the cotton wool and does not touch the tin foil dish. Moreover, it must be in close contact with the cotton wool all around the edge in order to prevent mites from running out of sight. The centering of the leaf is also made to ensure a proper application of pesticide in the Potter Tower (**Potter**, 1952).

On the first day the test units are set up, sprayed and then left to dry for about 15 min.

Six leaves are treated with the concentration recommended for the compound concerned and 6 are treated with demineralized water. Units that received the same treatment are placed in 1 tray with water. Immediately after drying 60 adult ♀♀ of *T. urticae* (that have been picked at random from the stock) are placed on each leaf to establish a colony. When all leaves are supplied with spider mites 10 young *P. persimilis* are transferred to each leaf. Moving the animals directly from one leaf to another by means of a fine brush makes counting uncertain, therefore a method has been developed to move juvenile stage of *P. persimilis* from the rearing leaves to the test leaves : small circular pieces of filterpaper (5 mm diameter, made with an ordinary perforator) are soaked in water and placed on the rearing leaf. Under the binocular 10 mites are quickly moved to the paper, where their movements are impeded because of the water. They are counted again and immediately afterwards the paper is transferred to the test leaf. After some time (when all 12 test leaves have been supplied with predators) each filterpaper is examined under the binocular. Any mites that will not run off at once after being pushed with the brush are replaced. Any mites that leave the paper are considered to be in good condition. This method has improved the reproducibility in the controls considerably compared with lifting the mites one by one from one leaf to another. (See also the section "reproducibility of the test").

On the 2nd day no counting is done but the leaves are examined to make sure there is enough food for the predators. If the spider mites have run off the leaf and/or are dead because of the treatment, extra prey is added.

On the 3rd day the 1st adults of the predator will emerge, and it is then necessary to ascertain that at least 1 ♂ predator is present on each leaf to ensure mating and thereby egg production. On most leaves there will be 1 ♂ and a number of ♀♀, but occasionally there are 0 or 2 ♂♂ on a leaf. In the 1st case a ♂ will be added - either from another leaf that has received the same treatment and has 2 ♂♂ - or from the leaves where the stage rearing was performed. In the 2nd case nothing is done. If any *P. persimilis* eggs are present they are counted and removed. Extra spider mites are supplied if necessary.

Hereafter eggs are counted and removed about every 2nd day to record the total number of eggs laid. At the same time spider mites are added if necessary to make sure that no food shortage occurs.

On days 6, 7, 9, 10 and 13 the predators are counted : ♀♀, ♂♂ and eggs are recorded on each leaf and all eggs are removed. Extra spider mites are added if necessary. The number of adults on each leaf is usually constant during this part of the test period.

On day 13 the test is stopped.

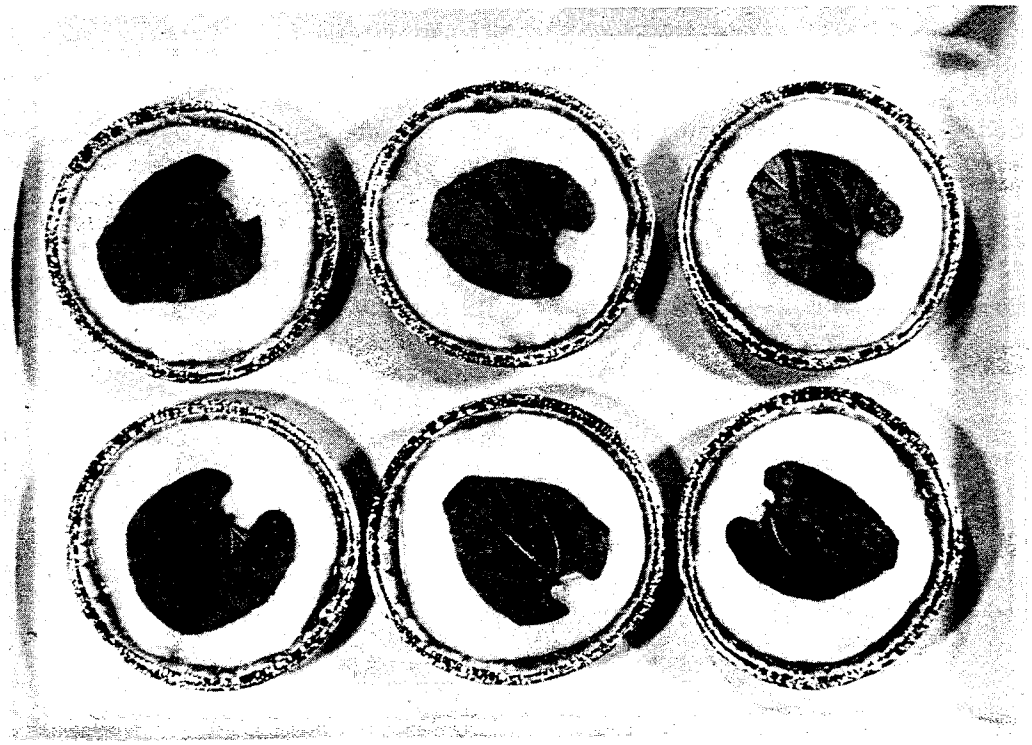

Fig. 1. Plastic tray with 6 bean leaves on cotton wool in tin foil dishes.

The following data are now available from each leaf :

1. Number of surviving *P. persimilis* on day 9.
2. Total number of eggs laid during the whole test period.
3. Mean number of eggs laid per ♀ during the period.

The last parameter is calculated by dividing the total number of eggs from a leaf by the number of ♀♀ alive on day 9 on that leaf. This can be done because the predators surviving till sexual maturity will almost always survive the entire experimental period.

APPLICATION OF THE CHEMICALS

A Potter Precision Spray Tower is used with a pressure of 0.68 atm. and 2 ml of solution for each unit, giving a deposit of 2.0 ± 0.2 mg/cm^2. The droplets are very fine and cover about 90 % of a glass plate without much overlap. The calibration of the tower is based on residual analyses of deposits from 12 sprayings with a 0.15 % Malathion solution.

To avoid risks to the operator even if highly toxic pesticides are used the test units need spraying without contamination of the tin foil dishes. An ordinary household cake slice is used to lift the cotton wool pads (with leaves) out of the dish. It is placed in the tower and after spraying the test unit is put back into the dish.

ASSESSMENT AND EVALUATION

Recording of survival and egg production of P. persimilis

Survival after 1 week and total egg production from maturity till the 13th day are relatively easy to measure and give reproducible measurements. Furthermore, these parameters give an overall picture not only of the vigour of the population during the test but also of its ability to regulate the spider mites in the long run. A big population in the test indicates an effective control in the greenhouse.

Calculation of the reduction in beneficial capacity

The reduction in total egg production during the test period has been chosen as a standard measure of the harmful side effects of pesticides. There are 2 reasons for this choice :

First, total egg production is the resultant of the mortality and the egg production for each of the survivors (the sex ratio - and survival in untreated - are very constant, giving 8-9 ♀♀ and 1 ♂ on most leaves. Exceptions from this will be discussed later).

Second, it gives a measure of the predation as the oviposition rate is proportional to predation in mature ♀♀ of *P. persimilis.*

It should be emphasized, though, that the total egg production does not measure the entire predation, as neither predation by young stages (the 1st 2 days of the test) nor predation by ♂♂ are included. Consumption by these are of minor importance as their predatory rate is about one fourth of that of the ♀♀, the larval stages last 3-4 days of the 13 day test period, and 80-90 % of the mites are females.

$$\text{Reduction } \% = \frac{\text{total egg prod. in untreated - total egg prod. in treated}}{\text{total egg prod. in untreated}} \times 100\ \%.$$

INTERPRETATION OF RESULTS

The IOBC working group has defined 4 categories of harmfulness of pesticides based on the reduction in predatory capacity they cause when used in the concentration recommended for practice :

1 = harmless, reduction $< 50\ \%$
2 = slightly harmful, reduction $= 50 - 79\ \%$
3 = moderately harmful, reduction $= 80 - 99\ \%$ and
4 = harmful, reduction $> 99\ \%$.

As described above, the decrease in beneficial capacity is measured by the reduction in total egg production per test unit during the test period. Results are analysed as follows. First, the difference between egg production on control and treated leaves (with the concentration recommended) is tested by an analysis of variance. If the difference is significant at the 5 % level, the percentage reduction in egg production caused by the pesticide is calculated from the mean of the 6 untreated and the 6 treated leaves.

RESULTS

Results of 27 tests are shown in table 1.

TABLE 1

The effect of pesticides on Phytoseiulus persimilis *juveniles investigated by the detached leaf method.*

	Pesticide	Concentration of pesticide %	Reduction in egg production %	Evaluation*
Insecticides /Acaricides	Aazomate (benzoximate)	0.03	NS	1
	Actellic 50 (pirimiphosmethyl)	0.05	100	4
	Ambush (permethrin)	0.005	100	4
	Asepta Lindaan (lindane)	0.014	41	1
	Dipterex (trichlorphon)	0.08	100	4
	Kelthane 35W (dicofol)	0.032	65	2
	Lannate (methomyl)	0.025	94	3
	Malathion NA (malathion)	0.0675	100	4
	Morestan (chinomethionate)	0.0125	84	3
	Peropal (azocyclotin)	0.025	47	1
	Sumicidin (fenvalerate)	0.0225	100	4
	Tedion V-18 (tetradifon)	0.018	NS	1
	Torque (fenbutatinoxid)	0.025	40	1
	Ultracid (methidation)	0.03	100	4
	Undeen (propoxur)	0.075	81	3
Fungicides	Afugan (pyrazophos)	0.012	72	2
	Bayleton (triadimefon)	0.025	NS	1
	Derosal (carbendazim)	0.03	99	3
	Morestan (chinomethionate)	0.0125	84	3
	Orthocide 83 % (captan)	0.1245	NS	1
	Plondrel 50W (ditalimfos)	0.0375	NS	1
	Ronilan (vinclozolin)	0.025	23	1
	Saprol (triforin)	0.0179	NS	1
	Thiovit (sulphur)	0.316	NS	1

TABLE 1 (continued)

	Pesticide	Concentration of pesticide %	Reduction in egg production %	Evaluation (a)
Herbicides	Aresin (monolinuron)	0.375	84	3
	Avenge (difenzoquat)	0.3	100	4
	Ramrod (propachlor)	0.65	87	3
	Semeron (desmetryne)	0.0625	52	2

(a) 1 = harmless, reduction < 50 % ;
2 = slightly harmful, reduction 50-79 % ;
3 = moderately harmful, reduction 80-99 % ;
4 = harmful, reduction > 99 %.

Most of the insecticides/acaricides tested are more or less harmful to *P. persimilis*. Only 5 out of 15 chemicals can safely be used in combination with the beneficial mites. These are Aazomate, Asepta lindane, Peropal, Tedion and Torque (category 1).

The fungicides are generally less harmful to the predators, as 6 out of 9 formulations are classified in group 1. Thèse are : Bayleton, Orthocide, Plondrel, Ronilan, Saprol and Thiovit.

Finally 4 herbicides are listed, none of which is harmless. It should be mentioned though that 3 of the herbicides, (avenge being the exception) made the leaves wither so much that the poor quality of the substrate might have affected the mites (food shortage being out of the question).

DISCUSSION

COMPARISON WITH OTHER RESULTS

To make the results useful in Denmark, the test mites are from the strain used by the Danish growers. Hence comparison with results from other tests is difficult.

Hassan (1982a) used a semifield method to compare the sensitivity of 2 strains of *P. persimilis* to pesticides. These strains had been reared without exposure to pesticides for years, as the Danish mites. **Hassan** used 11 pesticides and the 2 strains differed only with regard to 2 of these : in 1 case the pesticide was placed in category 2 and 3 - in the other in 4 and 3. Thus, different laboratory reared strains that have not been exposed to selection due to pesticides are likely to react in similar ways to such chemicals. Therefore the results presented in table 1 and results obtained by **Hassan** will be compared.

Hassan (not published) tested 1 of his strains with 15 of the formulations employed here using juvenile stages of *P. persimilis* and the method described in **Hassan** (1982a). He used bean plants in small pots infested with spider mites and juveniles of *P. persimilis* before spraying. Thus the predators were exposed to the pesticides as a direct spray while the method described here is a test of residuals. The measurement of reduction in beneficial capacity is based on egg production of predators in both tests.

The 2 methods gave the same results with 10 of the pesticides, 5 of which were placed in category 1 and 5 in category 4 by both. Four of the remaining pesticides were evaluated as more harmful by Hassan's method than by the present. The reason is probably the different way of applying the pesticide. In my experience a direct spray will affect *P. persimilis* more than a residual.

Only 1 pesticide gave a different result : Derosal (carbendazim) was classified as harmless by Hassan whereas the present method placed it in category 3, moderately harmful. The reason for this is not clear. In the test presented here the predators reacted in a remarkable way to Derosal : survival was not affected by the pesticide, but the mites did not develop into mature adults - and did not lay eggs. In fact, egg production was reduced with 99 %. (The experiment was done twice with an interval of 4 months. The results were identical). The compound might sterilize the ♀♀. Sterilization of ♂♂ would be concealed by the fact that untreated ♂♂ are added to leaves where no copulation is observed on day 3.

Hence, it is concluded that the 2 tests do give comparable results - and that strains of *P. persimilis* reared in the different laboratories do not differ much with respect to their tolerance to pesticides. The similarity of results obtained with different laboratory methods does not guarantee that the results reflect what would happen in a greenhouse. To investigate this experiments in glasshouses must be performed.

Reproducibility of the test

The test results were analysed for reproducibility in 2 batches, comprising the first 15 and the next 10 tests respectively.

The 15 tests gave results from 6 untreated leaves each, and if the method is reproducible the results from these 90 leaves should be similar since they were drawn from the same population. To investigate this, the parameter used for the evaluation of pesticides (the total egg production on each leaf) is partitioned into its components (survival and egg reproduction per female) and these are examined separately.

The chance of survival for each individual should be independent of the experiment from which it was taken, i.e survival in the pooled data should follow a binomial distribution.

The mean egg production per ♀ cannot a priori be expected to follow any special distribution. But its agreement with a normal distribution has been tested.

Finally an analysis of variance is made on the data of total egg production from all the usable tests.

Survival (fig. 2) :

A chi-square test of whether survival is binomially distributed gives $0.1 < P < 0.5$ ($df = 6$, chi-square = 8.76) for the 1st batch of experiments. On this background it seems reasonable to consider all leaves as drawn from 1 population.

In the 10 latest experiments the deviation from a binomial distribution is still smaller, as shown by the figure and $0.5 < P < 0.9$.

Mean egg production per female (fig. 3)

It is tested whether this parameter follows a normal distribution. The 1st 90 leaves did give a few extremely low and extremely high egg production rates, and hence the distribution deviates significantly from normality ($P < 0.01$; Kolomogorov-Smirnov D-test).

The 10 latest tests give better results as no extreme values are present, and the D-test yields a $P > 0.15$.

The change in results from the 1st 15 to the last 10 experiments is explained as follows :

1. The technique for transfer of young *P. persimilis* to the test leaves was changed after the 1st 15 tests using the method described on page 170.

2. The skills of the assistant have probably improved as a consequence of the practice given by the many identical experiments.

From the above results a practical conclusion has been drawn : the whole test should be discarded if survival is less than 4, or if egg production per ♀ is less than 20 or greater than 75 on any untreated leaf. Such irregularities may imply some fault during the performance of the test. The only exception to this rule will be cases where the mortality on pesticide-treated leaves is 100 %.

In accordance with this conclusion 9 of the 25 experiments were discarded and an analysis of variance was performed to detect whether the remaining 16 experiments were homogeneous with regard to total egg production per leaf. The resulting P-value was 0.0208 indicating a very low probability thal all the results were drawn from the same population. Therefore the basic data were examined once again to make out why the total egg production did not show the homogeneity of its components. The sex ratio was examined and it turned out that in 2 experiments there had been extraordinarily many ♂♂ (and consequently few ♀♀ to lay eggs). Instead of the usual 6-8 ♂♂ on 6 leaves there were 9 and 10 ♂♂ on 6 leaves in these 2 experiments. Another analysis of variance was therefore made on the remaining 14 experiments, and this one gave a $P = 0.2397$, indicating homogeneity of the results.

This means that another restriction must be imposed. In the few cases where the binomial distribution of the sex-ratio gives rise to more than 8 ♂♂ on 6 leaves the test should be discarded. Still the easy and otherwise reproducible registration of egg production is to be preferred to other ways of measuring the impact of pesticides on *P. persimilis*. In conclusion, the test is considered reproducible.

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RÉSUMÉ

Méthode de laboratoire pour évaluer les actions secondaires des pesticides sur les stades jeunes de l'acarien prédateur, *Phytoseiulus persimilis* [*Acarina* : *Phytoseiidae*] sur des feuilles détachées

Le principe de la méthode est de pulvériser des feuilles primaires détachées de haricot avec les doses recommandées de pesticides ou avec de l'eau déminéralisée pour le témoin. Dès que la pulvérisation a séché des araignées rouges adultes (*Tetranychus urticae*) (Koch) sont mises sur les feuilles. Les acariens soumis aux essais sont des jeunes stades, âgés de 0 à 48 h, de l'acarien prédateur, *Phytoseiulus persimilis* (Athias-Henriot) placés sur les feuilles aussitôt après les tétranyques dont ils se nourrissent. La durée de l'essai est 13 j, avec notation de la mortalité le

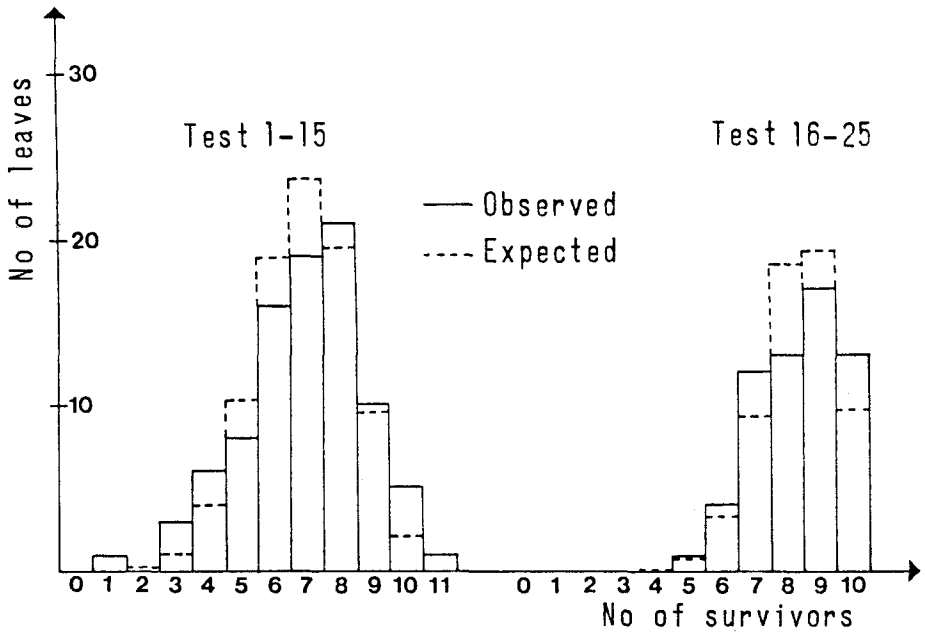

Fig. 2. Survival on control leaves from tests no. 1-15 and tests no. 16-25.
(Note : The leaf with 11 survivors is not included in the statistical test).

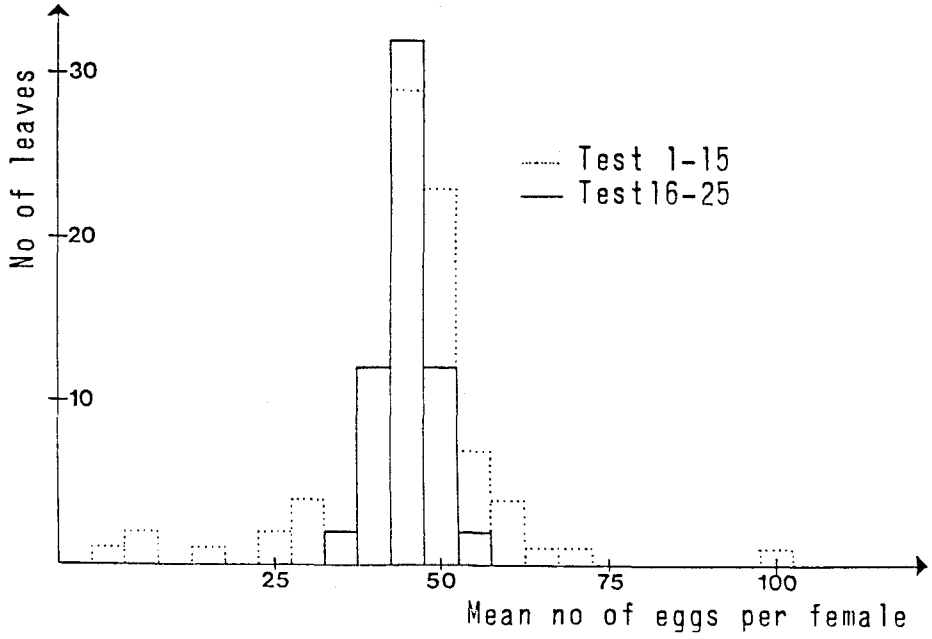

Fig.3. Egg production per ♀ on control leaves from tests no. 1-15 and tests no 16-25.

9e jour. Les actions secondaires des pesticides sont évaluées par la réduction de la fécondité de *P. persimilis* pendant toute la période de l'essai. Les résultats permettent la classification des pesticides dans les 4 catégories de nocivité utilisées par le groupe de travail de l'O.I.L.B. On a testé 27 pesticides et les résultats sont comparés avec ceux des autres chercheurs. L'analyse statistique montre que la reproductibilité du test est satisfaisante.

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