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Bioassays of some insecticides on the green peach aphid (*Myzus persicae* (Sulzer)) in Northern Italy: a preliminary survey

Abstract - Samples of *Myzus persicae* strains were collected in different parts of Northern Italy during 1988 and 1989: biological tests to asses the susceptibility of these aphids to pirimicarb, ethiofencarb and heptenophos were carried out.
From the LD_{50} values calculated, it appears that different aphid strains exibhit different levels of sensitivity to the insecticides used in the tests.
Some strains, which have shown a reduced susceptibility towards an active ingredient, behave in a similar way towards others. *M. persicae* populations are evolving toward a resistant condition and it involves several active ingredients.

Riassunto - *Saggi biologici di alcuni insetticidi sull'afide verde del pesco (*Myzus persicae *(Sulzer)) nell'Italia settentrionale: un'indagine preliminare.*
Campioni di popolazioni di *Myzus persicae* sono stati raccolti in varie località dell'Italia settentrionale nel 1988 e nel 1989. Su tali campioni sono stati effettuati saggi biologici per valutare la sensibilità degli afidi al pirimicarb, all'ethiofencarb e all'heptenophos. Dalle DL_{50} calcolate emerge che queste popolazioni di afidi presentano diversi livelli di sensibilità agli insetticidi utilizzati nelle prove.
Alcune popolazioni, che hanno mostrato una ridotta sensibilità verso un principio attivo, si comportano allo stesso modo nei confronti degli altri. L'evoluzione verso una maggior resistenza delle popolazioni di *M. persicae* è in atto e sta coinvolgendo vari principi attivi.

Key words: *Myzus persicae*, bioassay, resistance, Northern Italy, peach.

INTRODUCTION

During the last few years it has become increasingly difficult to control *Myzus persicae* (Sulzer) infestations in the peach orchards of Northern Italy using insecticides.

Reports of reduced efficacy amongst the commonly used insecticides have prompted the close examination of this phenomenon in the laboratory.

There are several bioassay methods for insecticides (Busvine, 1971), however, the adopted technique was that of indirect application, which consisted of placing aphids on peach leaves treated with different rates of pesticide. A full description of this method, designed to test the susceptibility of *M. persicae* to organophosphorous and carbamate insecticides, was proposed by the I.R.A.C. (Insecticide Resistance Action Committee) and published in 1990.

MATERIALS AND METHODS

The aphids used for the tests were collected from peach orchards in Emilia-Romagna, Piemonte and Veneto (Northern Italy) in 1988-1989.

Most of these orchards are located within the principal peach coltivation areas in Northern Italy; however, in order to accentuate differences among the various peach growing areas, samples were also collected from orchards near Piacenza, an area where peach cultivation is not widespread.

Within peach growing areas different methods are used for controlling aphids, especially with respect to the type of insecticide used. This information is presented in table 1.

The insecticides used in the bioassays were: pirimicarb(1), ethiofencarb(2) and heptenophos(3).

In order to evaluate aphid susceptibility, peach leaf sprouts infested with *Myzus persicae* were collected at random and placed in plastic bags to be transported to the laboratory for testing during the same day.

Peach leaves were dipped for 10 seconds in a solution of the test chemical.

Concentrations used in the tests ranged from 62.5 mg/l to 1000 mg/l for heptenophos, from 31.3 mg/l to 1000 mg/l for ethiofencarb and from 5.8 mg/l to 750 mg/l for pirimicarb.

At each concentration 5 replicates (leaves) were used.

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(1) Commercial name: «Pirimor», produced by I.C.I. Solplant S.p.A.. Dispersable powder containing 23.7% pirimicarb ai.

(2) 1988: commercial name: «Croneton», produced by Bayer. Emulsifiable liquid containing 46% ethiofencarb ai; - 1989: commercial name: «Croneton 40 PB», produced by Bayer. Dispersable powder containing 40% ethiofencarb ai.

(3) Commercial name: «Hostaquich», produced by Roussel-Hoechst Agrovet S.p.A.. Emulsifiable liquid containing 50% heptenophos ai.

Table 1 - Collection sites for the M. persicae *populations tested, and active ingredients used by the farmers for aphid control.*

Province	Location	Active ingredients
BOLOGNA	Calcara	acephate pirimicarb
FERRARA	Ferrara	acephate
	Guarda Ferrarese	acephate demeton-methyl heptenophos pirimicarb quinalphos
	S. Biagio d'Argenta	acephate
FORLÍ	Cesena	acephate
	Ronta	acephate ethiofencarb pirimicarb
PIACENZA	Besenzone	acephate
	Castelvetro	pirimicarb
	Piacenza (C.S.S.)	heptenophos pirimicarb
	Piacenza (I.P.A.)	—
RAVENNA	Brisighella	methamidophos
	Fognano	pirimicarb
	Fossolo	acephate ethiofencarb heptenophos pirimicarb
	Godo	methamidophos pirimicarb quinalphos
	Massa Lombarda	ethiofencarb methamidophos pirimicarb
	Savarna	methamidophos
	Zattaglia	acephate pirimicarb
TREVISO	Treviso	acephate pirimicarb
VERCELLI	Borgo d'Ale	ethiofencarb

The control consisted of leaves dipped in distilled water for 10 seconds.

When dry, the petioles were wrapped in a pad of wet cotton wool and the leaves were placed in 9 cm plastic Petri dishes. 1 mm holes were made in the lids of the Petri dishes to facilitate air circulation.

Aphids were brushed from the peach sprouts and a random sample of 20 adults was placed on each treated leaf.

The sides of the Petri dishes were sealed with «Parafilm» to prevent escape of the aphids.

The dishes were kept in the laboratory at 22-26°C, 80% RH. After 24 hours aphid mortality was assessed and probit analysis performed on the data (Finney, 1971) enabling LD_{50} values to be determined.

In order to determine whether a relationship existed between the insecticide treatments employed by the growers and the susceptibility of the aphids, the samples were divided into four groups according to the insecticides used in the orchards they originated from: A: untreated; B: carbamates only used; C: both carbamates and organophosphorous compounds used; D: organophosphorous only used.

The LD_{50} values from the samples within the four groups were subjected to the Kruskal-Wallis non-parametric test. Heptenophos data was not included in this analysis owing to the small number of tests undertaken with this compound.

RESULTS

During 1988, 12 tests were carried out: 8 with pirimicarb and 4 with ethiofencarb. During 1989, 11 tests were carried out with pirimicarb, 3 tests were carried out with heptenophos, and 2 with ethiofencarb. The difference in the number of tests carried out with every insecticides is due to the difficulty in finding sufficient aphids for testing.

The results obtained in the tests are presented in table 2.

The population with the lowest measured LD_{50} value to pirimicarb was collected from an urban peach orchard in Piacenza in 1988, where no insecticide treatments had been applied.

This strain was tested on 3.V.1988 and an LD_{50} of 5.2 mg/l ai was measured.

A similar level of susceptibility was found in 1989 in a strain collected from S. Biagio d'Argenta (Ferrara): the LD_{50} was 5.6 m/l ai.

Higher LD_{50} values were encountered in two other populations; 40.5 mg/l and 39.0 mg/l from Cesena (Forlí, 20.IV.1989) and Massa Lombarda (Ravenna, 16.V.1989) respectively. Other *M. persicae* populations from different orchards gave LD_{50} values for pirimicarb between these two extremes.

All the ethiofencarb tests gave similar results. The lowest LD_{50} (25.5 mg/l) was obtained from aphids originating from Besenzone (Piacenza) on 6.V.1988.

The highest LD_{50} value was calculated for a strain from Brisighella (Ravenna) on 16.V.1989 (154.3 mg/l).

Other samples gave LD_{50} values between these two extremes.

Table 2 - Results of biological tests on M. persicae.

Insecticide	Date and number of the test	Province and location of origin of aphids populations		Slope of the log-probit line b (±se)	Ld_{50} a.i. (mg/l)	F.L. (95%)
heptenophos	1 21.IV.1989	Forlí	Cesena	3.31 (±0.32)	99.9	88.5-112.7
	2 17.V.1989	Ravenna	Brisighella	2.30 (±0.29)	146.2	115.2-187.7
	3 13.VI.1989	Bologna	Calcara	3.43 (±0.55)	93.0	71.6-111.6
ethiofencarb	4 22.IV.1988	Vercelli	Borgo d'Ale	3.26 (±0.51)	46.8	35.2- 55.8
	5 6.V.1988	Piacenza	Besenzone	2.14 (±0.36)	25.5	11.9- 39.4
	6 12.V.1988	Treviso	Treviso	1.85 (±0.16)	29.1	23.3- 35.0
	7 8.VI.1988	Ravenna	Fognano	1.57 (±0.19)	35.6	24.8- 46.8
	8 16.V.1989	Ravenna	Brisighella	1.30 (±0.22)	154.3	104.6-316.2
	9 12.VI.1989	Ravenna	Savarna	2.09 (±0.27)	51.1	40.4- 62.4
pirimicarb	10 28.IV.1988	Piacenza	Besenzone	2.76 (±0.25)	12.0	10.3- 13.7
	11 2.V.1988	Piacenza	Castelvetro	2.52 (±0.48)	12.7	7.3- 13.7
	12 3.V.1988	Piacenza	Piacenza (I.P.A.)	1.64 (±0.27)	5.2	2.6- 8.9
	13 11.V.1988	Treviso	Treviso	1.57 (±0.38)	11.8	3.1- 42.8
	14 16.V.1988	Ravenna	Godo	3.31 (±0.50)	12.2	9.6- 14.7
	15 16.V.1988	Ravenna	Massa Lombarda	1.38 (±0.37)	39.0	25.1-103.6
	16 8.VI.1988	Ravenna	Fognano	2.59 (±0.26)	14.3	11.9- 16.7
	17 28.VI.1988	Ravenna	Zattaglia	1.39 (±0.62)	11.4	#
	18 20.IV.1989	Forlí	Cesena	2.41 (±0.37)	40.5	33.4- 54.0
	19 16.V.1989	Ravenna	Brisighella (1st test)	2.84 (±0.21)	28.6	25.6- 32.0
	20 17.V.1989	Ravenna	Brisighella (2nd test)	1.29 (±0.33)	29.8	17.3-115.1
	21 22.V.1989	Ferrara	S. Biagio d'Argenta	1.85 (±0.23)	5.6	3.9- 7.3
	22 23.V.1989	Ferrara	Ferrara	3.03 (±0.32)	19.3	16.9- 22.0
	23 30.V.1989	Ravenna	Fossolo	3.65 (±0.35)	20.4	18.1- 22.8
	24 31.V.1989	Forlí	Ronta	4.57 (±0.71)	18.7	14.8- 23.1
	25 7.VI.1989	Piacenza	Piacenza (C.S.S)	4.43 (±0.56)	34.8	30.4- 39.2
	26 12.VI.1989	Ravenna	Savarna	1.99 (±0.23)	21.3	17.2- 25.6
	27 13.VI.1989	Bologna	Calcara	2.15 (±0.29)	8.1	5.7- 10.3
	28 28.VI.1989	Ferrara	Guarda Ferrarese	1.15 (±0.27)	6.7	2.6- 10.6

(#) Not calculable.

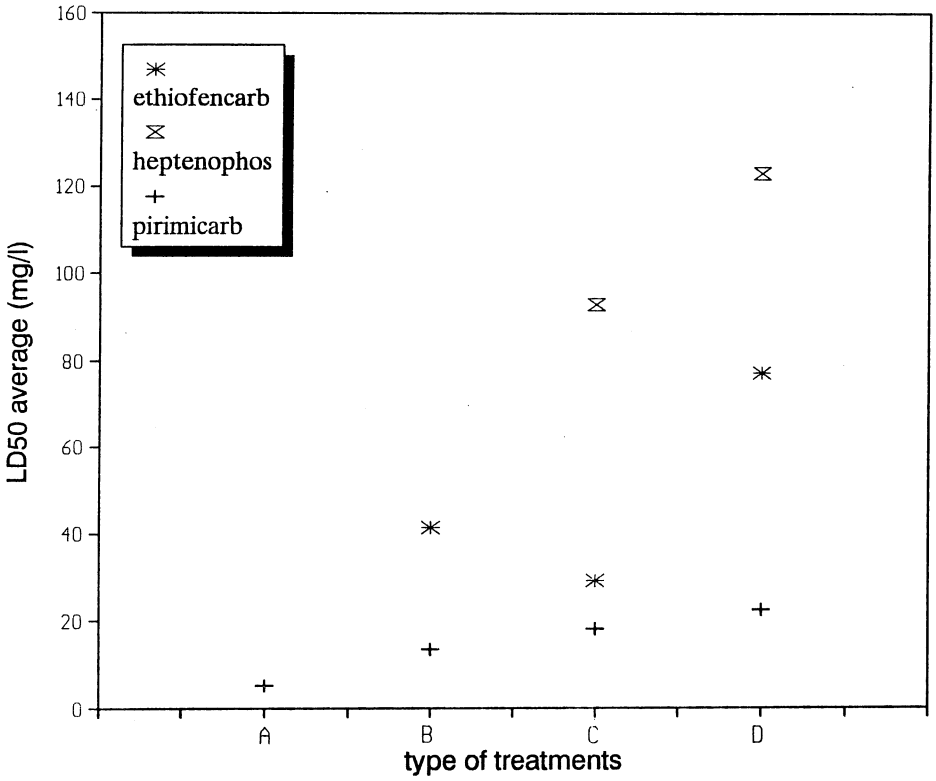

Fig. 1 - Distribution of the LD_{50} values in relation to the insecticides used in the orchards of origin of the aphid populations - A: not sprayed; B: sprayed only with carbamates; C: sprayed with carbamates and organophosphorous insecticides; D: sprayed only with organophosphorous insecticides.

Heptenophos was tested in 1989, however, only 3 tests were undertaken. Value for the LD_{50} ranged from 93.0 to 146.2 mg/l. The distribution of LD_{50} values in relation to the groups of insecticide used in those orchards is shown in figure 1. The data were subjected to the Kruskal-Wallis non-parametric test. The H parameter was not significant for the ethiofencarb test ($H = 0.86$, 2 df) or those performed using pirimicarb ($H = 3.7$, 3 df).

CONCLUSIONS

Two years experiments investigating insecticide resistance within *M. persicae* populations in Northern Italy has enabled some insight to be gained into the

susceptibility of aphid populations to the insecticides commonly used in those areas.

The population most sensitive to pirimicarb was found in an orchard where no insecticide treatment had been carried out. Where the use of pesticides is higher, *M. persicae* appeared less susceptible to pirimicarb.

Although fewer tests were undertaken with other insecticides, uniform results were obtained even when aphids were taken from sites far apart from each other.

Where populations had been subjected to several types of insecticide, a similar level of susceptibility was found, for example the strain collected from Brisighella (Ravenna) gave consistently higher LD_{50} values for pirimicarb, ethiofencarb and heptenophos.

Selection of resistant strains is taking place and several active ingredients appear affected by it.

The results obtained agree with the demonstration of the biochemical system given by Devonshire & Moores (1982), according to which the resistance of *M. persicae* to insecticides is conferred by an increase of esterase activity.

The activity of this enzyme affects not only organophosphorous, but also other classes of insecticides, such as carbamates and pyrethroids.

However, the esterase enzyme which is responsible for the detoxification of insecticides metabolizes organophosphorous insecticides at a faster rate than carbamates (Devonshire et al., 1983), such that the different groups of insecticide select for resistant strain of *M. persicae* at different rates (ffrench-Constant et al., 1987).

This may be demonstrated within certain limits with pirimicarb, with which the majority of tests were carried out: the lowest LD_{50} was obtained from a sample of aphids found in an untreated orchard located in an urban area. On the other hand, a sample from an orchard treated only with organophosphorous compounds gave the highest LD_{50} values. Furthermore, the LD_{50} values obtained for pirimicarb were found, on average, to increase from the population arising from untreated orchards, through those from orchards treated only with carbamates with the highest LD5O values arising in samples from orchards treated only with organophosphorous compounds (Figure n. 2).

Heptenophos although not in all the classes appeared to show the same tendency. In contrast, the LD_{50} values of ethiofencarb show a decrease where the sampled populations had been treated with insecticides from two chemical groups. However, there was an increase in the LD_{50} value for populations treated only with organophosphorous compounds.

The non-parametric analysis of these data do not indicate significant differences between the average LD_{50} values of populations treated in different ways. In spite of this, figures 1 and 2, and the results obtained by other authors

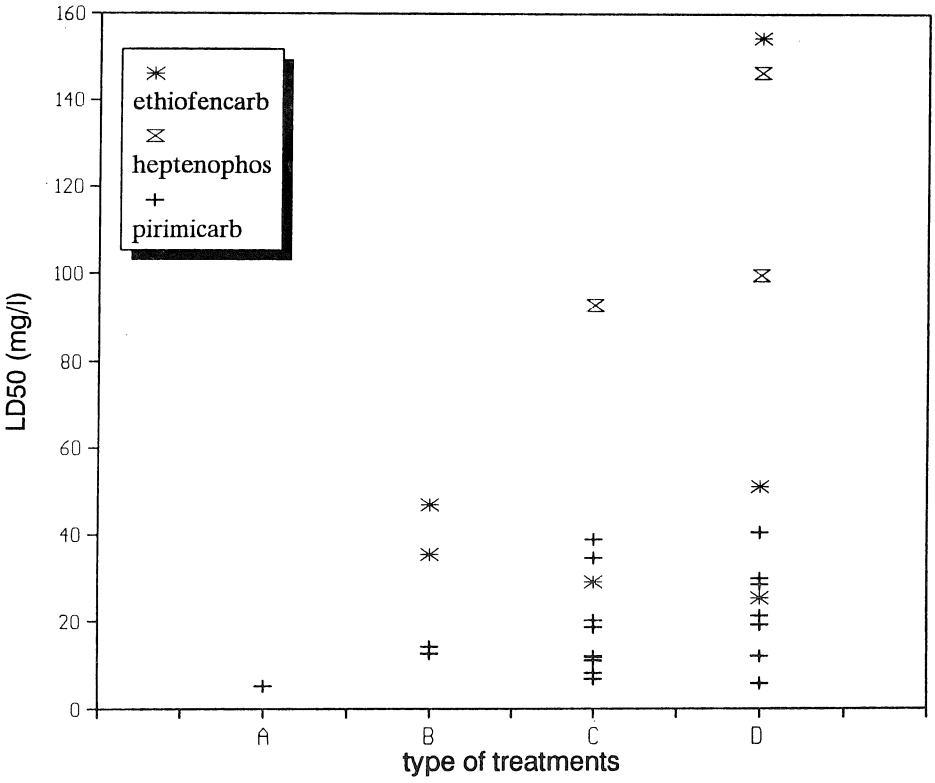

Fig. 2 - Distribution of the average of the LD_{50} values in relation to the insecticides used in the orchards of origin of the aphid populations - A: not sprayed; B: sprayed only with carbamates; C: sprayed with carbamates and organophosphorous insecticides; D: sprayed only with organophosphorous insecticides.

indicate that this tendency must be kept in account in the adopted control strategies and is an area which must be investigated throughly.

It is well known that a continuous selective pressure induces resistance in *M. persicae* even when products such as carbamates, which are normally the most effective aphicides, are used (ffrench-Constant et al., 1988). Furthermore, the life-cycle of *M. persicae* must be taken into account; and it is highly probable that each year there is a considerable mixing of populations and subsequent redistribution in the various areas.

The populations found on peach probably suffer the effects of treatments applied in the secondary hosts. For this reason it is possible to find less susceptible populations in areas where there are few peach orchards for

example those found in Piacenza (C.S.S.), which gave an LD_{50} value which was higher than those obtained from some populations collected in peach growing areas.

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