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**Resistance to insecticides in *Myzus persicae* (Sulzer) (Rhynchotha Aphididae)
in Italian peach orchards (*)**

Abstract - The results obtained by biochemical assays for esterases on samples of *Myzus persicae* (Sulzer) are reported. Aphid populations were collected in different peach growing areas in Italy, from 1991 to 1994. Fundatrices and fundatrigenies were considered in order to evaluate the changes which occur in the level of resistance to insecticides in aphid populations in the field on the primary host. In the samples of fundatrices the heterogeneity of resistance level appears higher than in the samples collected later, which resulted almost extremely resistant.

Riassunto - *Resistenza agli insetticidi in Myzus persicae (Sulzer) (Rhynchotha Aphididae) in pescheti italiani.*

Il lavoro presenta i risultati dei saggi biochimici per le esterasi su campioni di *Myzus persicae* (Sulzer). Le popolazioni di afidi sono state raccolte in diverse aree peschicole italiane dal 1991 al 1994. Sono state considerate le fondatrici e le fondatrigenie per valutare i cambiamenti dei livelli di resistenza agli insetticidi nelle popolazioni di afidi sull'ospite primario in pieno campo. L'eterogeneità dei livelli di resistenza è maggiore nei campioni di fondatrici che in quelli raccolti più tardi, risultati per lo più estremamente resistenti.

Key words: *Myzus persicae*, green peach-potato aphid, insecticide resistance, peach, Italy.

INTRODUCTION

The presence of the peach (*Prunus persica*) and the climatic conditions in Italy allow *Myzus persicae* to have a typical holocycle and the aphid lives on the primary host from its vegetative renewal. Late in the spring the winged forms leave the

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orchards and colonize the secondary hosts (potato, beet, tobacco, etc.), where they live for all the summer. In the last few years the problems of chemical control of *M. persicae* on peach trees have increased very greatly: in many cases numerous treatments have been applied without good results (Cravedi & Cervato, 1993).

Most of the research into resistance in other countries has been carried out on populations of the green peach aphid infesting herbaceous plants in glasshouses and in fields (Beranek, 1974; Devonshire, 1975).

The situation of resistance in *M. persicae* populations infesting the primary host is not well known. On the peach tree the amphigonic generation occurs in autumn and in order to better understand the results of the spring treatments against the fundatrices and fundatrigenies it is necessary to observe the situation at different times.

The fundatrices hatched from the overwintering eggs can have important modifications in their enzymes because of the amphigonic reproduction; the features of the amphigonic individuals depend on the selection made by the chemical treatments on the secondary hosts, from where they have arrived. Among the fundatrices in the same orchard different levels of susceptibility to the insecticides presumably exist. These differences can hypothetically continue to exist also in the following generations of fundatrigenies, if they are not selected by the chemicals applied.

The present research was begun in order to verify the validity of those hypotheses, to look for an explanation of the difficulties found in the field in controlling *M. persicae*, to characterize susceptibility to insecticides of its populations living on peach at different stages of the cycle and to show the changes that occurred.

MATERIALS AND METHODS

Samples

Aphids at different stages of their life cycle were collected in 1991-1994 at the end of the winter and late in the spring in peach orchards in some areas of North (Piedmont, Emilia-Romagna) and South (Abruzzo, Calabria) Italy (fig. 1).

In some cases in the same orchard two samples were collected during the season, before the aphids left their primary host to find the secondary hosts: firstly of fundatrices and then of fundatrigenies. The aphids were collected with a little brush; they were taken alive to the laboratory to be analysed immediately or frozen at -20°C for later assays.

Sometimes the fundatrices hatched from overwintering eggs were not found and only the colonies of fundatrigenies were analysed. These second samples were composed of about 50 wingless females, each one collected from a different colony far from the others.

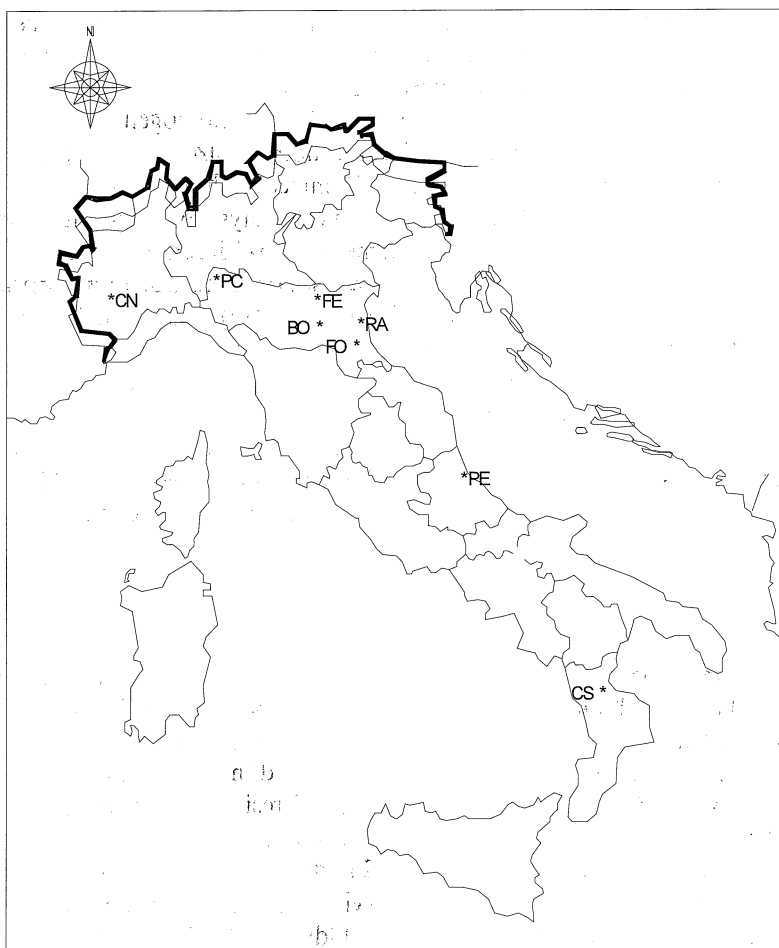


Fig. 1 - Peach growing areas considered in the research (CN = Cuneo, PC = Piacenza, BO = Bologna, RA = Ravenna, FO = Forlì, FE = Ferrara, PE = Pescara, CS = Cosenza).

When the colonies were numerous it was possible to carry out a biological assay besides the biochemical analysis.

Biochemical assays

The hydrolysing activity of single aphids was determined by a colorimetric assay (not immuno assay) which gives a broad measure of the amount of esterase present. There is a correlation between esterase activity and resistance level

(Needham & Sawicki, 1971); in fact, the esterases are responsible of insecticide inactivation, and this depends mainly on one esterase, E4 (Devonshire, 1977). These enzymes hydrolyse a model substrate, α -naphthylacetate (α -NA), and they are readily released into the soluble fraction of aphid homogenates.

The individual wingless adult aphids are homogenized in phosphate buffer (PB with pH7); a part of the homogenate is mixed with a revealing solution (α -NA in acetone diluted in PB) in a plate for microassays, so a great number of insects (88) may be analysed at the same time (Devonshire et al., 1992).

The reaction catalyzed by the enzymes takes 15 minutes at room temperature; then a colouring solution (a mixture of Fast Blue B Salt and SDS in water) is added and after 15 minutes (in the dark) the plate is read at 620 nm by a photometer (Titertek Multiskan MKII, Flow Instrument). Each optical density (O.D.) is corrected for the blank automatically.

Based on this reading each aphid is classified for its esterase activity in one of the four standard classes: S (susceptible), R1 (moderately resistant), R2 (very resistant) and R3 (extremely resistant) (ffrench-Constant & Devonshire, 1988).

RESULTS

In the nine peach orchards (2 in Piedmont and the others in Emilia-Romagna) considered in 1991 the absence of susceptible and moderately resistant aphids was observed at the end of the spring and five samples were extremely resistant. In the province of Ferrara the aphid populations collected in two orchards treated with different insecticides showed different levels of resistance; when acephate was used all the aphids were R3, while in the orchard, not too far away, treated with pirimicarb the sample was composed of R2 and R3 aphids (25% and 75% respectively). In a population sampled in the province of Bologna, never selected by insecticides during the spring, 75% of the aphids were R2 in mid May. A sample collected in a biologically controlled orchard near Forlì early in June was divided in the same proportion (50%) between the two highest classes of resistance. In the two populations sampled in mid-June in the main peach growing area in Piedmont, the province of Cuneo, the situation was different: where three treatments were applied (Borello) the aphids resulted all R3, while in Mellano orchard, treated only twice, 35% of R2 and 65% of R3 individuals were found.

In 1992 two series of samples were collected: fundatrices in mid-March and fundatrigenies in the late spring (table 1 and fig. 2).

The fundatrices collected in the province of Cuneo belonged to the different resistance classes; only two samples were composed of R3 individuals only. The colonies of fundatrigenies sampled in May in three of the same orchards, where pirimicarb and ethiophencarb were applied, were predominantly R3 aphids with

Table 1 - Results (% of the four classes of resistance) of the esterase assays on some samples of fundatrices (on the left) and fundatrigenies (on the right).

Year	Farm	Prov.	Date	S	R1	R2	R3	Date	S	R1	R2	R3
1992	Arnaudo	CN	17.03	0	0	0	100	20.05	0	0	0	100
	Bollati	CN	17.03	17	5	28	50					
	Borello	CN	17.03	0	0	6	94	06.05	0	0	6	94
	Mellano 1	CN	17.03	88	0	12	0	11.05	0	0	10	90
								20.05	0	0	10	90
	Mellano 2	CN	17.03	94	6	0	0					
	Bertorello	CN	18.03	0	0	0	100					
	Ricci	RA	13.03	20	40	20	20					
	Burzacchi	RA	13.03	0	13	31	56	04.05	0	0	0	100
	Mainardi	FE	23.03	25	25	0	50	11.05	0	0	0	100
	Golini	BO	16.03	0	0	6	94					
1993	Sola	CN	23.03	6	6	82	6					
	Fiandrino	CN	23.03	76	12	12	0					
1994	Sola	CN	16.03	0	0	0	100					
	Toselli T.	FE	15.03	0	0	0	100	24.05	0	0	0	100
	Toselli N.T.	FE	15.03	0	0	0	100					
	Testoni	FE	15.03	0	0	10	90	24.05	0	0	0	100
	Mainardi	FE	15.03	0	0	0	100	24.05	0	0	0	100
	Dallalpi	RA	07.03	0	0	50	50					
	Tassinari	RA	07.03	0	0	50	50	09.05	0	0	0	100
	Zauli	RA	07.03	0	0	10	90	30.05	0	0	0	100

only few R2 individuals. In some other cases a reinfestation after the treatments against the fundatrices did not occur late in the spring; as in the orchard of Bertorello farm, that was treated with metamidophos.

An analogous situation was observed analysing the samples from different peach growing areas in Emilia-Romagna (Ravenna, Bologna, Ferrara, Piacenza). Only the sample of fundatrices collected in the province of Bologna had mainly R3 aphids. The sampling repeated in May in an orchard in the province of Ravenna, never treated in the spring, and in one in the province of Ferrara, treated once, showed that the populations present in that period were composed of R3 aphids only. The fundatrigenies collected in the same period in an isolated small peach orchard, never treated, near Piacenza, which is not a typical growing area, were also all R3.

Three samples collected in April 1992 in Calabria, in the area around Cosenza, where climatic conditions determine a quicker development of the aphid than in Northern Italy, were composed of different classes of resistance. In one orchard the population was 27% R2 and 73% R3; in the second one 2% of the aphids were R1, 11% R2 and 87% R3 and in the last one 6% were susceptible, 57% R2 and 37% R3.

Two samples of fundatrices collected in Piedmont in March 1993 showed a good heterogeneity of composition (table 1). In this year the climatic conditions at the end of the winter in Emilia-Romagna were so bad that no sample of fundatrices was possible.

In the second series of samples, the aphids from the fundatrigenia colonies found on peach trees in Piedmont in May-June belonged to all the resistance classes except in one case where they were mainly R2 with a few R3 (9%). All the orchards were treated only once, in March.

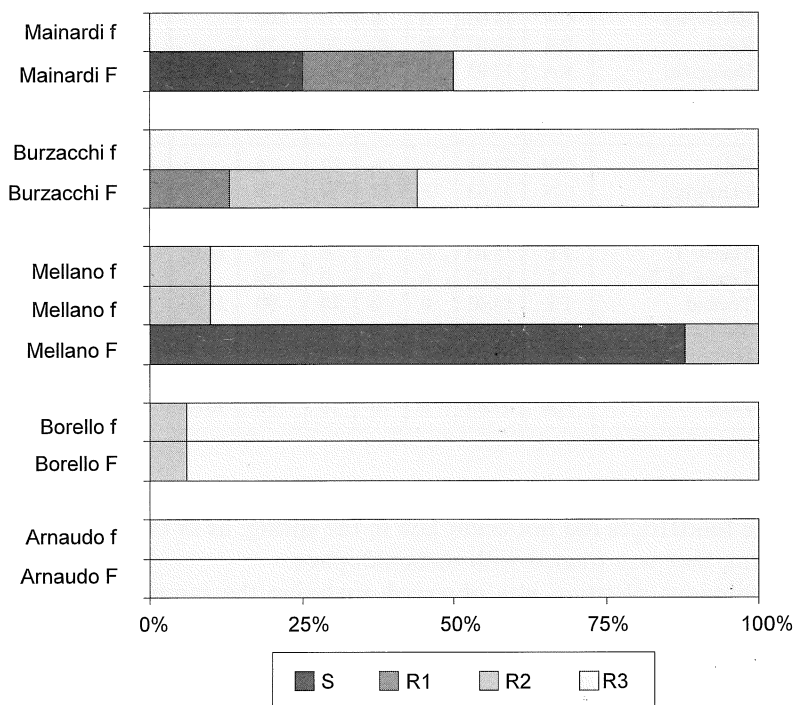


Fig. 2 - Composition of *Myzus persicae* populations in 1992 (F = fundatrices; f = fundatrigenies).

The samples collected in Emilia-Romagna in the same period were composed only of R2 (with values from 4% to 29%) and (predominantly) R3 individuals. In those orchards only one treatment was applied to control the fundatrices.

The only sample for 1993 from a peach orchard in Calabria was collected at the end of April and resulted on the whole extremely resistant (R3).

The fundatrices collected in 1994 in the province of Ferrara showed little variability only in one of the four orchards considered (10% R2 and 90% R3).

While in three farms in the province of Ravenna the fundatrices always belonged to the two higher resistance classes, but in different measures: 50% each in two orchards and 10% R2 and 90% R3 in the last one. The only sample from Piedmont in March was entirely R3 (table 1 and fig. 3).

The numerous samples (21) collected in Northern Italy in May-June were homogeneously extremely resistant. The only sample from peach orchards in South Italy in 1994 was collected early in June near Pescara, in Abruzzo, and it was composed of susceptible (4%), R1 (25%), R2 (25%) and R3 (46%) aphids.

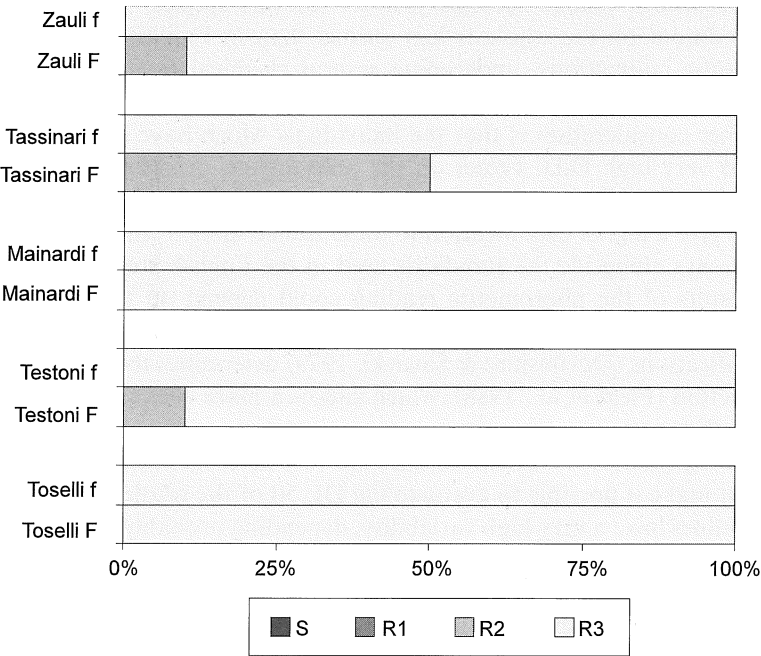


Fig. 3 - Composition of *Myzus persicae* populations in 1994 (F = fundatrices; f = fundatrigenies).

CONCLUSIONS

The observation of the decreased effectiveness of insecticide treatments was confirmed by the laboratory analyses of the esterase level in aphid populations sampled in different places. The results of the biochemical assays, which estimate the esterase content in individual aphids, generally showed a greater heterogeneity in the level of resistance in samples of fundatrices than in those collected in May-June period.

In fact the populations of *M. persicae* infesting peach trees, even if treatments were done early in spring, mainly showed high levels of resistance and many samples, for example all those collected in 1994, were composed only of R3 aphids.

The insecticides applied to control *M. persicae* are organophosphorus (acephate and metamidophos) and carbamate (mainly pirimicarb). Those chemicals are more or less quickly detoxified by the esterases and they show a similar effect on the change of resistance level. The susceptibility of the fundatrices is mainly caused by the selection pressure of the insecticides used for the protection of herbaceous plants, secondary hosts for this species. The situation, in fact, in Piedmont on the whole is less serious than in Emilia-Romagna, where the glasshouse cultivations, undergoing several chemical treatments, are more numerous.

A further consideration is that the individuals which have been classified as R3 showed very high O.D. values on the photometer, over the threshold fixed in the English classification (many times over 3,000). However it would not be justified to give a higher classification to these Italian aphids unless they are composed in assays alongside the standards used in the English studies.

The results of the photometric reading could depend on the presence of a change in the enzyme responsible for the resistance. In fact, a succession of E4 gene amplifications (Devonshire & Sawicki, 1979) determines the level of esterase overproduction (Field et al., 1988), which causes a more active detoxification of the toxic substances.

The biological assays on field samples are very heterogeneous and give results that do not make it possible to evaluate the DL 50 of the whole population. The obtained values have a very high variability, depending probably on the great heterogeneity of field populations.

In many situations, the treatments applied in the orchards, after the sampling of the aphids late in the spring, considerably reduced their presence even when the aphids were extremely resistant.

In some situations there was a variability in the populations that could be important if no selection pressure were made.

It would be necessary to observe the resistance to insecticides over large areas and using all available knowledge in order to obtain better results from the treatments.

In conclusion, this research has pointed out that photometric analysis is fast and good to evaluate the changes in resistance levels over time and to verify the efficiency of the defence strategies followed.

These results show that the insecticides usually used on peach act quite well in the moment of the application but they do not protect the plants from reinfestations for a long time. New kinds of active ingredients, with different action mechanism, could give longer protection.

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