Resistance of *Aphis gossypii* (Homoptera:Aphididae) to Insecticides

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ABSTRACT

The cotton aphid Aphis gossypii is a serious cotton pest in some regions of Greece. Recently, reports from different regions suggest that it can no longer be controlled with applications of insecticides that were formerly effective, especially organophosphates and pyrethroids. Laboratory biossays were conducted from 1993 to 1997 to determine the susceptibility of cotton populations to the most commonly used insecticides. LC_{50}s were determined using the dip test method (FAO, 1979), with assessment of mortality after 24 hours. The results show that resistance to insecticides has spread to many different regions. Of fifty tested populations 25%, were resistant to pirimicarb, showing up to 16-fold resistance and 13% were resistant to carbosulfan with up to 42-fold resistance. Some populations were very susceptible to fenvalerate and some very resistant. Cross-resistance between the pirimicarb and triazamate a carbamoyl-triazole has been observed. The newly introduced insecticide, imidacloprid, was effective enough in cotton except in a few cases where some tolerance was evident. Populations that were resistant to pirimicarb and carbosulfan were susceptible to imidacloprid and no cross-resistance was observed. LC_{50}s for organophosphates, pyrethroids and carbamates were positively correlated with the previous use of these insecticides in the region where the aphids were collected. This study reports the incidence of insecticide resistance in Aphis gossypii to several insecticides over a three year period in Greece.

Introduction

Surveys in Greece have shown that the following species of aphids attack cotton: *Aphis gossypii* (Clover), *Aphis craccivora* (Koch), *Aphis fabae,* (Scopoli), *Myzus persicae*, (Sulzer), and *Macrosiphum euphorbiae* (Thomas). The cotton aphid *A. gossypii* is a very serious pest and has been found in almost all the cotton growing regions in Greece. It is the most predominant aphid, causing severe damage in some years. An extensive survey by the Hellenic Cotton Board in Greece in 1994 found that the population of A. *gossypii* has two peaks, one in spring and the other in summer. The areas with most severe problems were central Macedonia and west Greece, where 15 - 20% of cotton was attacked in spring, and 16 - 18% in summer. In contrast, cotton in Thessalia and central Greece had fewer attacks. Only 1.5 - 2% in spring and 4 - 10% in summer. In the first two areas, aphids were more abundant because the temperatures are usually lower and the relative humidity higher compared with Thessalia, where the temperatures are very high during the summer and the climatic conditions are not favourable for the development of the aphids. Recently, the aphids have developed resistance to many insecticides. Reports from several different parts of the world suggest that cotton aphids cannot be controlled any longer with applications of insecticides that were formerly effective (Kerns, 1992; Suzuki *et al.*, 1993). Apparently insecticides often fail to give satisfactory control of the aphids because of cross - resistance and farmers increase the doses and the number of spray applications. Cotton crops in Greece also receive frequent applications of insecticides directed against other pests e.g. *Bemisia tabaci*, *Helicoverpa armigera* and *Pectinophora gossypiella,* and these insecticides can also select for resistance in *Aphis gossypii.* The objectives of this project were to establish base line data for comparison of resistant populations from different areas, to monitor changes in the level of resistance from year to year and to determine the distribution and severity of resistance in the cotton aphid.

Material and Methods

During 1993 -1997 more than fifty populations of *Aphis gossypii* were collected from cotton fields and tested for insecticide resistance. In the bioassays, commercial formulations of carbosulfan, carbofuran, imidacloprid, pirimicarb, triazamate and fenvalerate were used (Table 1). These insecticides were the most frequently used by farmers. Laboratory bioassays were conducted using a rapid dip test method, modified from that described by FAO (1979). At least four replications were used and in each replication, batches of 20 apterous adult aphids were put between two strainers and immersed for 10 seconds in an insecticide solution of known concentration. Control aphids were dipped in distilled water only. After being partly dried, the aphids were tipped on to a leaf disc on wet filter paper in a petri dish and kept at 25°C in a 16:8 hrs L:D photoperiod. Mortality estimates were made after 3 and 24 hours. Individuals that were unable to stand up or did not make forward progress when prodded were scored as dead. LC_{50}s were estimated by using Probit Analysis (Finney, 1971) with the MSTAT-C statistical program. If control mortality was > 20% data for that bioassay were not used. Two LC_{50}s were considered significantly different if their 95% confidence limits did not overlap.

Results and Discussion

In Greece, *A. gossypii* has developed resistance to organophosphates (OP) and pyrethroids (PY), and only carbamates (CA) still give satisfactory protection. Therefore most of the farmers use carbosulfan, benfuracarb and other carbamate insecticides, often in low doses of 0.7 per 1 litre of commercial products. The results of the bioassays are in agreement with the performance of the insecticides used in field sprays. Triazamate can be used in practice to control aphids resistant to OPs and PYs, but a few populations have high resistance to triazamate despite the fact that it has not been used commercially. The occurrence of the insensitive acetylcholinesterace (Ache) mechanism that gives resistance to pirimicarb, also confers resistance to triazamate. This cross-resistance has been well documented in the aphid *Myzus persicae* (Moores *et al* 1994). Pirimicarb has been used extensively in the past for controlling the cotton aphid, so this resistance mechanism may be *quite* widespread in field populations. Rongai (1996) reported high level of resistance to pirimicarb in *A. gossypii* collected from potato crops in Italy. They also found that carbosulfan was very toxic to the cotton aphid. However, results from the current study have demonstrated that some populations 12.5 % of the tested populations had developed up to 42 -fold resistance to carbosulfan, and 25% up to 16-fold resistance to pirimicarb (Table 4). Reports from other countries have also recorded resistance of *Aphis gossypii* to carbamates (Hollingsworth *et al.,* 1994; Yanchao *et al.,* 1994). The cotton aphid also have been known to use all the known mechanisms of resistance (Zhaojun, 1993). If carbamates continue to be used in Greece exclusively for the control of the aphids, they are going to become progressively less effective. The new insecticide, imidacloprid (Abbink, 1991; Elbert *et al.,* 1996), gives excellent control against aphids (Dewar, 1992; Nauen, 1996) even controlling aphids resistant to other insecticides. In our studies populations from the regions of Achialos EA1 and Lianovergi EA3 that were resistant to carbosulfan and pirimicarb, were susceptible to imidacloprid (Table 2). The results indicate that imidacloprid can be used in field applications to control cotton aphids that are resistant to OP, CA and PY insecticides. To ensure the continued effectiveness of this insecticide, it must be used rationally and only applied once per season as a folliar spray. There are some indications from the results of the bioassays in 1997 and 1998 that populations tested with imidacloprid were heterogeneous with shallow - slopes and high Chi - square values (Table 3) and an average 15 fold resistance. However its effectiveness in practice is still satisfactory. The properties of imidacloprid its the mode of action are well documented (Leicht, 1996). Imidacloprid has also sublethal effects on aphid fecundity e.g. by inhibiting feeding (Devine *et al.*, 1996). In this way, low levels of resistance can be overcome by these properties of imidacloprid. Nauen (1996) reported low levels of resistance to imidacloprid up to 12 - fold *in M. persicae* and *Myzus nicotianae.*

The cotton aphid has developed resistance to pyrethroids in many parts of the world (Zhaojan, 1993; Hollingsworth, 1994). In our studies the response of two populations to fenvalerate collected 5 km apart showed that both had very high LC_{50} and 8.6 fold resistance difference (Table 2). Natural enemies that are sometimes abundant, reduce many aphid populations and control most infestations. If the natural balance is disrupted by the insecticides, aphid populations increase quickly. Near harvest, they may become so numerous that the honeydew deposited on the fiber reduces yield and quality. The use of insecticides that could disrupt beneficial insect activity should be avoided (Kabissa, 1996).

Recently, the use of insecticides in cotton has been substantially reduced and decisions to apply sprays have been made very carefully after considering the presence of the natural enemies in the fields. In order to prolong the life span of the new insecticides and to maintain the effectiveness of the currently used insecticides against cotton aphid, insecticide resistance management is a necessary part of an IPM strategy used successfully in cotton in Israel for other pests (Horowitz *et al.,* 1994). Because the problem of resistance is universal, to be manage successfully, it needs collective efforts, with scientist of different disciplines working together, as in the programme for resistance ENMARIA (Denholm and Jespersen, 1998). Also the main effort must be concentrated on preserving natural enemies (Weathersbee and Hardee, 1994), by using alternative available methods to control aphids.

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Table 1. Insecticide used on *Aphis Gossypii* in the dip test bioassays.

Active ingredient	Commercial product	Insecticide group
Pirimicarb	Pirimor 50 WG	Carbamate
Carbofuran	Curater 200 SC	Carbamate
Carbosulfan	Marshal 25 EC	Carbamate
Triazamate	Aztec 14 EW	CarbamateTriazole
Methamidophos	Tamaron 600 SL	Organophospate
Fenvalerate	Sumicidine 30 EC	Pyrethroid
Imidacloprid	Confidor 200 SL	Chloronicotinyls

Table 2. Insecticides toxicity (LC_{50}s) on apterous adults of Aphis Gossypii by the dip test method with assessment mortality in 24 hours in 1993.

Insecticides	slope ± S.E	LC_{50} (ppm[1])	95%FL		$LC_{90's}$ (ppm[1])
Imidacloprid	1.21±0.39	1.99	8	0.5	22
Methamidophos	0.51±0.10	192.06	718	51	>1000
Carbosulfan	1.21±0.45	0.24	1	0.1	3
Fenvalerate	1.49±0.71	233.91	507	107	>1000
Triazamate	0.94±0.25	46.01	131	16	828
Carbofuran	1.32±0.37	9.54	17	5	93

Table 3. Insecticides toxicity (LC_{50}s) on apterous adults of Aphis gossypii by the dip test method in 24 hours, tested populations from different regions in 1997.

Locations	slope ±SE	LC_{50} (ppm[1])	95% FL (ppm[1])	x^2 (df=2)	RR[2]
		Imidacloprid			
Volos	0.50±0.14	14.28	3.09 - 65.93	2.07	12
Volos	0.54±0.22	33.72	5.74 -198.22	3.47	28
Achillio	0.62±0.19	8.69	2.39 - 35.56	1.63	7
		Carbosulfan			
Plati	0.62±0.41	0.01	0.01 - 0.37	1.19	0
Magnisia	0.75±0.20	0.55	0.17 - 1.79	4.29	0

[1]The LC_{50}s values are expressed in ppm active ingredient of commercial formulation.
[2]Resistance Ratios (RR) were calculated by dividing the LC_{50} of each resistant population by the pooling values of LC_{50}s of the susceptible populations which had overlapping confidence limits.

Table 4. Insecticide toxicity (LC_{50}s) on apterous adults of Aphis Gossypii by the dip test method in 24 hours, tested populations from different regions in 1996.

Locations	Slope± SE	LD_{50} (ppm[1])	95% FL (ppm[1])	χ^2 (df=2)	RR[2]
		Carbosulfan			
Arahos EA 1	0.35±0.12	0.03	0.01 - 0.75	1.350	
Volos EA 1	1.32±0.27	0.04	0.02 - 0.07	0.523	
Lianovergi EA 3	1.94±0.34	0.05	0.03 - 0.07	0.358	1
Lianovergi EA 2	2.07±0.34	0.05	0.04 - 0.07	1.888	
Lianovergi EA 1	1.93±0.33	0.07	0.05 - 0.10	0.008	
Arahos EA 2	1.29±0.24	0.51	0.29 - 0.89	6.284	
Iraklia EA 1	1.38±0.26	1.46	0.81 - 2.61	1.240	8.1
Achialos EA 1	2.48±0.49	7.65	5.34 -10.95	0.287	42.5
		Imidacloprid			
Volos EA 1	1.98±0.51	0.03	0.02 - 0.05	0.249	
Lianovergi EA 2	0.96±0.13	0.42	0.22 - 0.81	4.670	
Lianovergi EA 1	1.17±0.18	0.99	0.56 - 1.74	0.928	
Achialos EA 1	0.81±0.17	2.20	0.77 - 6.24	0.924	
Lianovergi EA 3	1.47±0.23	2.31	1.39 - 3.83	3.301	
		Fenvalerate			
Lianovergi EA 1	0.23±0.10	<0.01	0.00 - 0.55	1199	
Triazamate					
Lianovergi EA 1	0.66±0.51	663.7	1.46-274630	0.033	
		Pirimicarb			
Velestino EA 1	0.75±0.12	0.74	0.19 - 2.85	1.407	
Lianovergi EA 1	0.97±0.18	4.53	2.41 - 8.52	0.548	1
Lianovergi EA 2	0.37±0.11	9.22	1.57 -54.01	0.339	
Lianovergi EA 3	0.37±0.09	11.10	3.13 -39.72	1.936	15.9

1. The LC_{50}s values are expressed in ppm active ingredient of commercial formulation.
2. Resistance ratios (RR) were calculated by Dividing the LC_{50} of each resistant population by the pooling values of LC_{50}s of the susceptible that had overlapping confidence limits.