Resistance to Bifenthrin and Resistance Mechanisms of Different Strains of the Two-Spotted Spider Mite (*Tetranychus urticae*) from Turkey

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Nine different strains of the two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) were collected on cotton from Adana, Antalya, Izmir, Manisa and Urfa in Turkey. Their responses to bifenthrin were investigated using conventional bioassay and biochemical assays. LC_{50} and LC_{90} values of bifenthrin were determined for all strains by using a residual bioassay with a petri dish–spray tower. Resistance ratios were determined by comparing the samples with a standard susceptible strain, GSS. The resistance ratios of the strains ranged from <1 to 669–fold (at LC_{50}). Of the investigated field strains, only three (two from Adana and one from Urfa) were resistant to bifenthrin. There was a correlation between esterase enzyme activity and bifenthrin resistance according to polyacrylamide gel electrophoresis and microtiter plate assays in the three resistant strains.
KEY WORDS: *Tetranychus urticae*; two-spotted spider mite; resistance; bifenthrin; esterase; cotton.

INTRODUCTION

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), a worldwide pest of many plant species, after having been exposed to many insecticides and acaricides for many years has developed resistance to a large variety of compounds (21). This resistance may develop quickly because of the mite's numerous annual generations and the high frequency of spray applications. Instead of using integrated pest management for controlling pests in cotton areas, Turkish producers prefer to use broad-spectrum insecticides that are effective against all pests. Such insecticides, when used against major pests, eliminate them as well as their natural enemies. As a result, secondary pests such as two-spotted spider mites became a major problem in cotton-growing areas. The evolution of resistance depends on gene flow among populations that colonize different habitats in a given area (21).

Excessive use of broad-spectrum pesticides with the same mechanism of action promotes the development of pest resistance in rapidly reproducing species, such as spider mites and aphids. It has often been reported that *T. urticae* developed resistance to many insecticides (9,12,14,20), making it imperative to choose suitable and effective methods for monitoring the development of pest resistance.

The petri dish bioassay method was initially used by Dennehy *et al.* (4) to monitor spider mite resistance to miticides. When using this method, pesticide residues retain their

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persistence, an advantage over other methods because the dishes can be prepared and stored for future use. For example, propargite residues applied by the Potter tower can remain as effective as freshly sprayed dishes for 30 d when stored at 4°C (12).

Bifenthrin is a broad-spectrum and contact-toxicity pyrethroid effective as an insecticide and acaricide. Bifenthrin has been registered and used in Turkey against such pests as the tobacco whitefly (*Bemisia tabaci* (Gennadius)), cotton spider mites (*Tetranychus cinnabarinus* Boisd., *T. urticae* Koch), cotton jassid (*Empoasca decipiens* (Paoli)) and cotton bollworm (*Heliothis armigera* Hubner) attacking cotton since 1988 (1). Besides bifenthrin and other pyrethroids, a number of organophosphate and carbamates insecticides have been registered and used in cotton fields for many years in Turkey.

Reduced susceptibility to bifenthrin in the two-spotted spider mite was determined by using the petri dish assay. We also used electrophoresis and microtiter plate total esterase methods to determine genetic differences among two-spotted spider mite populations and to detect differences in esterase patterns between strains that were susceptible or resistant to bifenthrin.

MATERIALS AND METHODS

Mite strains Adult spider mite females were collected on cotton plants growing at nine locations in Turkey during the summer of 1998–99 and a standard susceptible strain (GSS) obtained from the Rothamsted Experimental Station (England) (Table 1). All strains were kept on bean (*Phaseolus vulgaris* L.) leaves in a growth room at 25±2°C and a 16L:8D photoperiod.

TABLE 1. Collection site and date of *Tetranychus urticae* populations tested for their response to bifenthrin and their esterase activity

Strain name	Collection site	Collection date
GSS	Rothamsted Experimental Station (England)	2.VIII.98
DEN	Ç. U. A. F. Plant Protection Dept., experimental field	14.IX.98
TIM	Ç. Agricultural Research Institute, production field	14.IX.98
PAM	Adana, Cotton Research Institute, production field	15.IX.98
FET	Antalya, Fethiye cotton field	20.IX.98
SER	Antalya, Serik cotton field	19.VII.99
URF	Urfa cotton field	2.IX.99
TIR	Izmir, Tire cotton field	5.VIII.99
SEL	Izmir, Selçuk cotton field	5.VIII.99
MAN	Manisa, Harmandalı cotton field	6.VIII.99

Bioassay The effects of bifenthrin were tested using the methods of Kabir and Chapman (13) and Campos *et al.* (3). In each of the following experiments, technical grade bifenthrin (95% pure, Bayer Turk A.Ş) was dissolved in a mixture of acetone and water containing 0.02% Triton X-100 at a ratio of 1:1. This mixture was applied to the internal surfaces of the lids and bases of 50-mm-diam plastic petri dishes and allowed to dry for 30 min at 25±2°C. One ml of spray suspension was sprayed on each occasion onto every base and lid pair, using a Potter spray tower (Burkard Manufacturing Co. Ltd., Rickmansworth, Herts., UK) at 10 atm pressure and 3 sec settling time. Preliminary tests were conducted before each experiment to determine the range of concentrations that would produce 5–95% mortality. All experiments were conducted in three replicates of a seven- or eight-

concentration design (plus water including 0.02% Triton X-100 only, as control) with a sample size of 617–840 mites. Adult female mites (25–35) were transferred to each dish using a fine hair brush. The dishes were then closed, sealed with parafilm to prevent escape of mites, and placed in a growth room (16L:8D at 25±2°C). All experiments were assessed after 24 h; mortality, defined as the lack of movement when prodded, was assessed visually with the aid of a stereomicroscope.

Statistical analysis All data from each concentration–mortality experiment were pooled and subjected to probit analysis. LC_{50} and LC_{90} values with their 95% confidence level (CL) and slopes ± S.E. of the regression were estimated using the computer program POLO (16). Dose-response curves were plotted using percentage mortality rates and the log of the dosage by Microsoft Excel.

Biochemical assays

Electrophoresis: Vertical slab polyacrylamide gel electrophoresis was performed following the procedures by Walker (22) and Goka and Takafuji (6). The gels were 1 mm thick and 80 mm×80 mm in area. Acrylamide concentrations were 7.5% in separating gels and 3.5% in stacking gels. Adult female mites were homogenized individually in 10 μl of 32% (w/v) sucrose with 0.1% Triton X-100 in microtiter plates by a multiple-homogenizer (17). Electrophoresis was carried out at a constant current of 25 mA/gel at 5–8°C for ~1.5 h. Esterase was stained by placing the gels for 1 h in 0.4 (w/v) fast blue BB salt after incubating them for 30 min in a 0.02% (w/v) solution of α-naphthyl acetate in 0.2 M phosphate buffer (pH 6.5), which contained 1% acetone. All staining reactions were stopped by placing the gel in 7.5% acetic acid. The experiments were repeated at least ten times for each population.

Microtiter plate assay: Total esterase activity was determined by following the procedures of Devonshire *et al.* (5) and A.S. Velioğlu (1999, Ph.D. dissertation, Ankara Univ., Turkey). Each mite female was homogenized in 50 μl phosphate/Triton (20 mM phosphate buffer, pH 7, containing 1% Triton X-100) in separate wells of a microtiter plate. All mites and 12 controls (without mites) in each plate were homogenized simultaneously, using a multiple-homogenizer designed for flat-bottom microtiter plates, and left at room temperature for at least 15 min to ensure sufficient tissue solubilization. At least 48 individuals were used from each population.

The assay was started by adding fast blue RR salt in 0.2 M buffer (200 μl; stain-substrate solution), using an eight-channel micropipette. Stain-substrate solutions were prepared as follows: 0.030 g fast blue salt was solubilized in 50 ml phosphate buffer and then added to 500 μl α-naphthyl acetate (100 mM). Assays were performed using a kinetic microtiter plate reader (Anthos Labtec Instruments, Salzburg, Austria) that affords 37 absorbance readings (at 450 nm) automatically in the first 15 min of the reaction. Esterase activity of the mites in each well was determined in control reactions in which the average activity of the control wells was subtracted. Esterase activity of each population was plotted using the average esterase activity of individual mites from the same population, by Microsoft Excel.

RESULTS

Nine field-collected strains and one susceptible (GSS) strain were tested for bifenthrin resistance during the 1998–99 cotton seasons. The LC_{50} and LC_{90} values ranged from

13.96 to 10,802 mg l^{-1} and from 29.77 to 14,216 mg l^{-1}, respectively (Table 2). According to the LC_{50} values, two strains (FET and MAN) were susceptible, five strains (SEL, TIM, SER, TIR and DEN) had 1.28- to 3.15-fold resistance, and two strains (PAM and URF) were extremely resistant (564- and 669-fold resistance, respectively) to bifenthrin, when compared with the GSS strain. Also according to LC_{90} values, all strains were defined as resistant to bifenthrin; six strains (MAN, FET, SEL, TIM, SER and TIR) exhibited 1.36- to 3.35-fold resistance, one strain (DEN) exhibited 11.69-fold resistance, and two strains (URF and PAM) were extremely resistant (478- and 462-fold resistance, respectively). The differences in the mortality responses of the bifenthrin-susceptible and -resistant mite strains in petri dishes are shown in Figure 1. POLO tests for similarity between slopes of MAN and FET, SEL and TIM strains were accepted, indicating significant similarity. However, other strains were rejected – indicating significant differences. The distance between the average response curves for susceptible and extremely resistant strains is much greater than the distance between the susceptible strain and strains showing a low level of resistance.

TABLE 2. Concentration-response data for *Tetranychus urticae* to bifenthrin (a.i.) including a laboratory susceptible strain (GSS) and various populations collected from cotton fields

Strain	n[z]	Slope±S.E.	X^2 (df, n-2)	LC_{50} (mg l^{-1}) 0.95% CL[y]	LC_{90} (mg l^{-1}) 0.95% CL[y]	According to LC_{50} RF[x]	According to LC_{90} RF[x]
GSS	640	4.82±0.73	5.39 (5)	16.14 (12.17-18.81)	29.77 (25.75-38.38)	-	-
MAN	640	2.78±0.42	6.04 (5)	13.96 (8.25-18.16)	40.33 (31.79-62.39)	<1	1.36
FET	640	3.03±0.30	4.47 (5)	15.84 (13.58-17.99)	41.98 (36.04-51.36)	< 1	1.41
SEL	640	3.99±0.49	5.44 (5)	20.65 (16.84-23.83)	43.27 (36.38-57.95)	1.28	1.45
TIM	720	4.30±0.68	19.24 (6)	24.33 (9.71-31.53)	48.35 (37.40-117.09)	1.51	1.62
SER	640	2.15±0.34	0.92(5)	25.21 (20.05-30.44)	99.62 (71.93-177.46)	1.56	3.35
TIR	720	6.46±1.33	8.74 (6)	43.14 (31.33-48.96)	68.13 (58.60-111.17)	2.67	2.29
DEN	720	1.53±0.31	2.97(6)	50.86 (39.05-68.59)	348.07 (188.44-1,372.31)	3.15	11.69
URF	640	6.63±0.86	1.59 (5)	9,107 (8,499-9,645)	14,216 (13,001-16,333)	564	478
PAM	640	12.21±2.12	1.56 (5)	10,802 (10,383-11,212)	13,754 (12,864-15,628)	669	462

[z] n, number of mites in experiment.

[y] CL, confidence level.

[x] Resistance Factor (RF) = LC_{50} or LC_{90} of the field-collected strain divided by the LC_{50} or LC_{90} of the susceptible strain.

Biochemical assays

Electrophoresis: Up to five esterase bands (Est-1 to Est-5) were detected in the *T.*

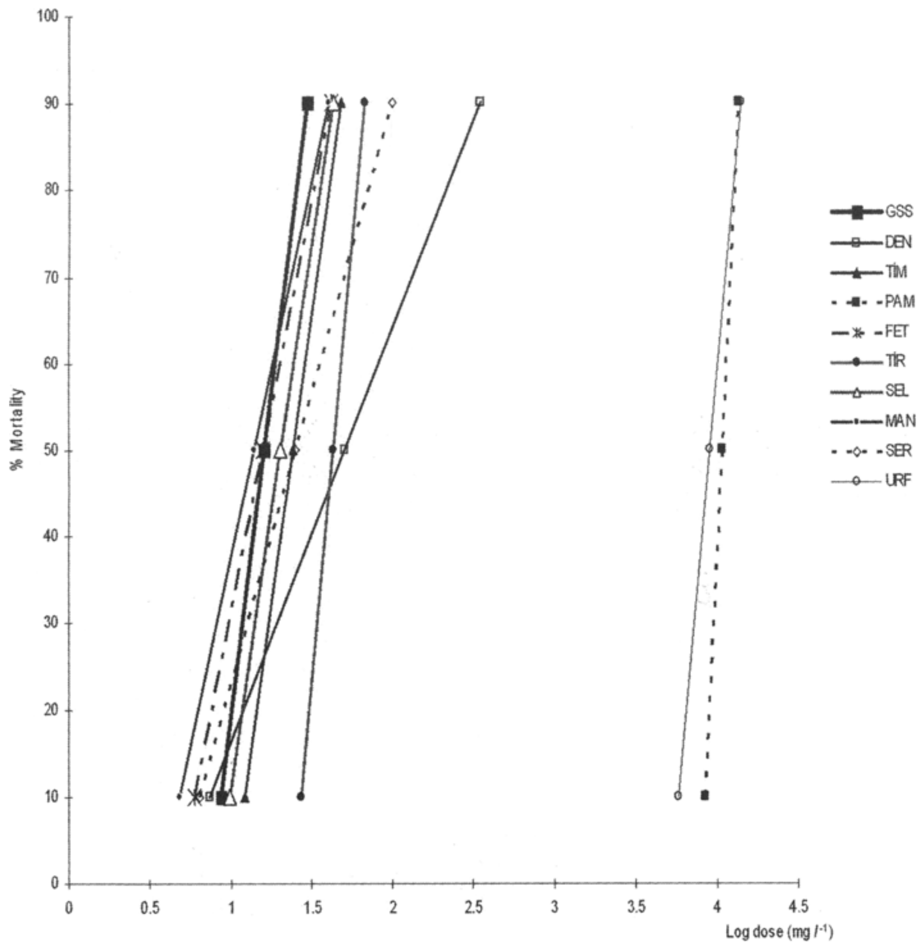

Fig. 1. Log concentration–mortality regression for a laboratory susceptible (GSS) and field-collected strains of *Tetranychus urticae* tested with bifenthrin.

urticae strains (Fig. 2), and their number and densities varied according to the strains. Five esterase bands ware detected in the DEN, TIR, SEL and URF strains (Est 1-5), four esterase bands in the TIM, FET and SER strains (Est 1-4), three esterase bands in the GSS strain (Est 1-3), two esterase bands in the PAM strain (Est-1 and Est-4), and only one esterase band in the MAN strain (Est-1). The Est-4 band of the PAM, DEN and URF strains showed extremely intensive banding patterns. The TIM, FET, TIR, SEL and SER had a light density Est-4 band and no Est-4 band was detected in the GSS and MAN strains.

Microtiter plate assay: Total esterase activity with α-naphthyl acetate was highest in the PAM, URF and DEN samples (Fig. 3), followed by the TIM, MAN, SER, SEL and TIR samples. The lowest activity was detected in the GSS samples.

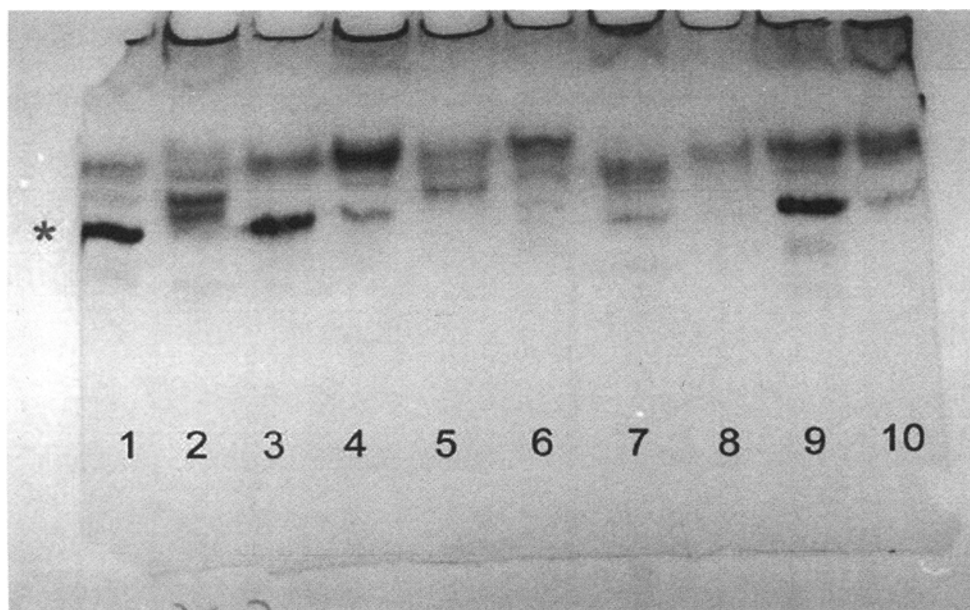

Fig. 2. Esterase zones in different *Tetranychus urticae* strains (1, DEN; 2, TIM; 3, PAM; 4, FET; 5, GSS; 6, TIR; 7, SEL; 8, MAN; 9, URF; 10, SER) *Est-4

DISCUSSION

According to the LC_{50} values, the MAN, FET, SEL and TIM strains showed almost the same level of susceptibility to the broad spectrum insecticide – bifenthrin, whereas the SER and TIR strains displayed low resistance to it. The DEN strain was heterogenic, because it had a very low log concentration–mortality slope value. A clear decrease in the slope is an indication that resistance has begun to develop and indicates that the population is heterogenic (11). The URF and PAM strains were homogenic and extremely resistant to bifenthrin. Selection pressure for resistance is generally high due to the frequent application of pesticides. Bifenthrin was used intensively in Turkey against whiteflies, bollworms and spider mites from the late 1980s to early 1990s under the commercial name Talstar EC 100. After bifenthrin became ineffective for controlling pests in the field, possibly due to resistance development in the mid 1990s, its importation and use in Turkey has been stopped since the year 2000. Similarly, resistance to bifenthrin increased progressively both in level (from 1.2- to 109-fold at LC_{50}) and abundance (from 20% to 90% of the strains) in three seasons and was linked to field control failures in Australia (10). Due to the fact that most organophosphates and pyrethroids used against the mite were also used against other cotton pests, *T. urticae* was strongly selected for resistance by the increased application of these pesticides (8).

In electrophoresis assay results, Est-4 bands were stained very dark in the extremely bifenthrin-resistant strains PAM and URF and the moderately resistant strain DEN. But Est-4 also showed light bands in the slightly resistant strains TIM, TIR, SEL, SER, and in the susceptible FET strain. Furthermore, the Est-4 band could not be detected in the GSS strain. However, in the other susceptible strain – MAN, the Est-4 bands were barely stained – according to its LC_{50} values. In the microtiter method, esterase activity was

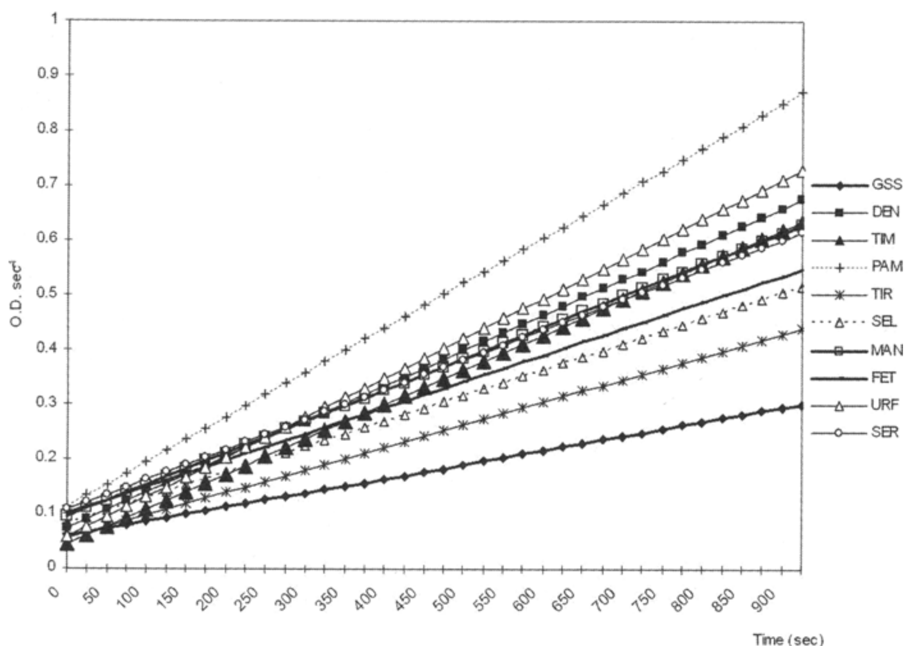

Fig. 3. Total esterase activity [optical density (O.D.)/sec] in a laboratory (GSS) and various field-collected strains of *Tetranychus urticae*.

highest for the PAM, URF and DEN strains. The highest esterase activity was detected in the bifenthrin-resistant strain PAM, followed by other resistant strains – URF and DEN. The lowest esterase activity was detected in the susceptible strain, GSS. However, in the other strains, the relationship between resistance to bifenthrin and esterase activity was not clear.

The increase in esterase activity could result from using various conventional insecticides, including organophosphates and pyrethroids. A positive relationship between esterase activity and resistance to organophosphate insecticides was observed in aphids (2,18,19), horn flies (7) and Kanzawa spider mite (15). However, it was recently reported that general esterase activity of *T. urticae* strain was increased in response to bifenthrin and γ-chalothrin but no significant change in general esterase activity was observed in response to dimethoate exposure (23). This shows that increased esterase activity of *T. urticae* is associated with exposure to pyrethroids rather than to use of organophosphates.

According to our results, there was a positive relationship between general esterase activity and high resistance to bifenthrin, but this relationship was not clear for low resistance to bifenthrin in *Tetranychus urticae*. In conclusion, based on these findings, we suggest that general esterases may play a role in conferring resistance to pyrethroids in two-spotted spider mites.

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