CROP PROTECTION

Abamectin Resistance in *Tetranychus urticae* Koch (Acari: Tetranychidae): Selection, Cross-Resistance and Stability of Resistance

Mário E. Sato, Marcos Z. da Silva, Adalton Raga and Miguel F. de Souza Filho

Instituto Biológico, APTA, C. postal 70, 13001-970, Campinas, SP, mesato@biologico.sp.gov.br

Neotropical Entomology 34(6):991-998 (2005)

Resistência de *Tetranychus urticae* Koch (Acari: Tetranychidae) a Abamectin: Seleção, Resistência Cruzada e Estabilidade de Resistência

RESUMO - Estudos envolvendo seleções artificiais com abamectim, relações de resistência cruzada e estabilidade da resistência foram realizados em *Tetranychus urticae* Koch para fornecer subsídios para um programa de manejo da resistência a abamectim. Seleções artificiais para resistência e suscetibilidade a abamectim foram realizadas em laboratório, utilizando-se uma população de *T. urticae*, coletada de um cultivo comercial de morangueiro em Atibaia, SP. Após cinco seleções para resistência e cinco seleções para suscetibilidade, foram obtidas as linhagens suscetível (S) e resistente (R) de *T. urticae* a abamectim. A razão de resistência (CL_{50} R/ CL_{50} S) obtida alcançou valores de 342 vezes. A toxicidade de oito acaricidas foi avaliada nas linhagens R e S, observando-se diferenças significativas entre as duas linhagens, para as CL_{50}s dos produtos milbemectin, fempropatrim e clorfenapir. Foram obtidas correlações significativas entre as CL_{50}s de abamectim e milbemectim, indicando resistência cruzada entre esses acaricidas. Não foi detectada resistência cruzada com os acaricidas fempiroximate, ciexatim, propargite e dimetoato. A resistência de *T. urticae* a abamectim mostrou-se instável na ausência de pressão de seleção. Para todas as populações estudadas (com freqüência inicial de 75, 50 e 25% de ácaros resistentes), a porcentagem de ácaros resistentes caiu para níveis iguais ou inferiores a 15% em seis meses. Os resultados indicam que milbemectim deve ser evitado em programas de manejo da resistência de *T. urticae* a abamectim.

PALAVRAS-CHAVE: Ácaro rajado, manejo da resistência, controle químico

ABSTRACT - Studies on artificial laboratory selections with abamectin, cross-resistance relationships, and stability of resistance were carried out with *Tetranychus urticae* Koch to provide basic information for an abamectin resistance management program. Selections for resistance and susceptibility to abamectin were performed in a population of *T. urticae*, collected from a commercial strawberry field in the State of São Paulo, Brazil. After five selections for resistance and five selections for susceptibility, susceptible (S) and resistant (R) strains of *T. urticae* to abamectin were obtained. The resistance ratio (R/S) at the LC_{50} reached 342-fold values. The toxicity of eight acaricides was evaluated in the R and S strains, observing significant differences (at LC_{50}) between R and S strains for milbemectin, fenpropathrin and chlorfenapyr. Significant correlation was detected between the LC_{50}s of abamectin and milbemectin, indicating cross-resistance between these acaricides. No cross-resistance was detected for the acaricides fenpyroximate, cyhexatin, propargite and dimethoate. The stability of abamectin resistance was also studied under laboratory conditions. Abamectin resistance was unstable in the absence of selection pressure. For all studied populations (with 75, 50 and 25% of initial frequency of resistant mites), the percentage of resistant mites decreased to levels equal or lower than 15% in six months. The results indicate that milbemectin should be avoided for managing abamectin resistance in *T. urticae*.

KEY WORDS: Two-spotted spider mite, resistance management, chemical control

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is an important agricultural pest with a global distribution. Its phytophagous nature, high reproductive potential and short life cycle facilitate rapid

resistance development to many acaricides often after a few applications (Cranham & Helle 1985, Keena & Granett 1990, Devine *et al*. 2001, Stumpf & Nauen 2001). Failure in the chemical control of *T. urticae* caused by resistance have been reported in several countries for compounds, such as organophosphates (Sato *et al.* 1994), dicofol (Fergusson-Kolmes *et al*. 1991), organotins (Edge & James 1986, Flexner *et al.* 1988); hexythiazox (Herron & Rophail 1993), clofentezine (Herron *et al.* 1993); fenpyroximate (Stumpf & Nauen 2001, Sato *et al.* 2004) and abamectin (Beers *et al.* 1998).

Abamectin is currently used in Brazil to control insects, like *Alabama argillacea* (Hübner), *Liriomyza huidobrensis* (Blanchard), *Phyllocnistis citrella* Stainton, *Tuta absoluta* (Meyrick) and mites, such as *T. urticae*, *Tetranychus ludeni* Zacher, *Polyphagotarsonemus latus* (Banks), *Panonychus ulmi* (Koch), *Aculops lycopersici* (Massee) in several crops (e.g. cotton, citrus, apple, water melon, strawberry, cucumber, potatoes, tomatoes, and ornamental plants) (Andrei 2005).

Intensive applications of abamectin have been used to control the two-spotted spider mite in some crops such as strawberry and ornamental plants, in the state of São Paulo. Recently, some growers have observed low efficacy and shortened residual control with abamectin, indicating a possible problem of resistance development. Although various aspects of abamectin resistance in *T. urticae* have been studied during the last ten years (Campos *et al.* 1995, 1996, Beers *et al.* 1998, Stumpf & Nauen 2002), there is no information about abamectin resistance in this pest in Brazil.

Exploitation of new chemicals and the judicious use of acaricides from different modes of action are currently the best approaches to overcome problem of resistance. One of the new compounds to be used to control *T. urticae* is milbemectin, which is a mixture of two macrolide compounds, milbemicyn A_3 and milbemycin A_4. Milbemectin belongs to the same class as the acaricide abamectin and presents activity against all life stages of a broad spectrum of phytophagous mites (Dekeyser 2005).

This study reports on selections for resistance and susceptibility to abamectin in *T. urticae* under laboratory conditions. In addition, the paper presents results of toxicity tests comparing the response of the resistant and susceptible strains of the spider mite to several acaricides recommended to control *T. urticae* in Brazil. The possible cross-resistance between abamectin and milbemectin was analyzed. Furthermore, the stability of abamectin resistance in *T. urticae* was examined to provide basic information for the definition of an effective resistance management strategy for this pest.

Material and Methods

Mite Strains. The original population of *T. urticae* was collected from a commercial strawberry (*Fragaria* sp.) field in Atibaia county, SP, in September 30, 1999. After collection, the mites were reared continuously on bean plants, *Canavalia ensiformis* L., under laboratory conditions at 25 ± 1ºC, 70 ± 5% RH and a 14h photoperiod.

Toxicity Tests. These tests were based on the method described by Knight *et al*. (1990). Twenty adult females of *T. urticae* were placed on a bean leaf disc (4 cm diameter) on water soaked cotton in a petri dish (9 cm diameter). The prepared suspension of acaricide (2 ml) was sprayed onto the leaf disc mites using a Potter spray tower (Burkard Manufacturing, Rickmansworth, Herts, UK), at 68.9 kPa. Preliminary tests indicated that 1.6 mg/cm^2 of distilled water was sprayed on the leaf disc with this volume and pressure. Thereafter, the mites on the leaf disc were kept at 25 ± 1ºC and a 14h photoperiod for 48h after treatment. Individual mite survival was determined by touching each mite with a fine brush. Mites which were unable to walk at least a distance equivalent to their body length were considered dead. Each experiment was replicated at least three times. Pooled data were subjected to Probit analysis (POLO PC) (LeOra Software 1987) and LC_{50} with respective 95% CL were estimated (Finney 1971).

Selection for Resistance. Females of the original population were selected for resistance to abamectin under laboratory conditions from December 1999 to June 2000. Fifty adult females on bean-leaf disc were sprayed with abamectin (Vertimec® 1.8% EC: Syngenta Crop Protection) using the Potter spray tower, as described above. Increasing concentration of abamectin were used for each selection [9.0, 13.5, 18.0, 21.6 and 27.0 mg of active ingredient (A.I.) / L of distilled water] so that 20 to 40% of female mites survived for the succeeding generations. Survivors after 48 h were used to initiate the next generation. At least 1,950 mites were used in each selection. The intervals between selections varied from 24 to 28 days.

Selection for Susceptibility. The purpose of selection for susceptibility was to remove the gene responsible for abamectin resistance, and then to produce a strain more susceptible to the insecticide. The selections for abamectin susceptibility were conducted with *T. urticae* gravid females from the original population, from December 1999 to July 2000. The mites were placed individually on a bean leaf disc (2.5 cm diameter) on water-soaked cotton in a petri dish for 48h. Each female oviposited on average 12.8 eggs. After this period, the female was transferred to another leaf disc arena and treated with abamectin using the Potter spray tower. Decreasing concentrations of abamectin were used for each selection (2.16, 1.26, 0.72, 0.36 and 0.18 mg of A.I. / L of distilled water), causing mortality of 30% to 45%. Only progeny of dead females were used to produce the next generation. At least 210 adult females were used in each selection. The intervals between selections varied between 27 to 35 days.

Cross-Resistance. Cross-resistance relationships between abamectin and seven other acaricides were evaluated on selected resistant (R) and susceptible (S) strains of *T. urticae*. The pesticides used were milbemectin (Milbeknock® 50 EC, emulsion concentrate, 50 g of milbemectin/L; Iharabras S.A. Chemical Industries), fenpropathrin (Danimen® 300 CE, emulsion concentrate, 300 g of fenpropathrin/L; Iharabras

S.A. Chemical Industries), chlorfenapyr (Citrex®, suspension concentrate, 240 g of chlorfenapyr/L; Basf S.A.), fenpyroximate (Ortus® 50 SC, suspension concentrate, 50g of fenpyroximate/L; Hokko do Brasil), cyhexatin (Hokko Cyhexatin® 500, wettable powder, 500 g of cyhexatin/L; Hokko do Brasil), propargite (Omite® 720 CE BR, emulsion concentrate, 720 g of propargite/L; Uniroyal Chemical), and dimethoate (Perfekthion®, emulsion concentrate, 400 g of dimethoate/L; Basf S.A.). All chemicals (except milbemectin) were commercially available in the State of São Paulo. Milbemectin (Milbeknock®) was provided by Iharabras S.A. Chemical Industries, Sorocaba City, State of São Paulo. With the exception of milbemectin, all the tested acaricides are recommended to control *T. urticae* in Brazil.

The bioassay method used for all chemicals was the same as described earlier for abamectin (toxicity tests), except for the pyrethroid fenpropathrin. Because pyrethroids are known to cause mites to abandon treated leaves (Mochizuki 1994), the conventional method was slightly modified for fenpropathrin. In this case, adult females were introduced onto a small bean leaf disc (2.5 cm diameter) on a wet filter paper in a petri dish. Immediately after pesticide treatment, the disc was placed on another untreated disc (5 cm diameter) on water soaked cotton in a petri dish. By this method, mites which escaped from the treated disc were caught on the untreated disc. Mortality was assessed 48h after treatment in the same manner as described earlier. For propargite, the evaluations were carried out after 72h. All experiments were repeated at least three times.

The mortality data of each acaricide for selected S and R strains of *T. urticae* were subjected to probit analysis (POLO PC) (LeOra Software 1987). The cross-resistance relationships between each chemical and abamectin were analyzed based on the overlapping or not of 95% confidential intervals of LC_{50} values, estimated for each acaricide for S and R strains. The resistance ratio (R/S) was calculated by the division of LC_{50} of R strain and LC_{50} of S strain.

In the case of milbemectin, we also evaluated the LC_{50} of this acaricide in seven populations of *T. urticae* with different susceptibilities to abamectin (LC_{50}s = 0.31, 1.17, 1.40, 4.36, 31.82, 43.31 and 58.10 mg of A.I. / L of distilled water). These populations were obtained during the selection process for resistance and susceptibility to abamectin, from mites of the original population collected from strawberry field in Atibaia county. The number of mites used to estimate each LC_{50} value for abamectin and milbemectin were equal or higher than 300. The relationship between LC_{50} of abamectin and LC_{50} of milbemectin for different populations of *T. urticae* was examined with bivariate correlation analysis (Ayres & Ayres 2003).

Stability of Resistance. For this study, the frequencies of abamectin resistance were evaluated monthly in three populations with different initial percentage (25%, 50% and 75%) of resistant mites, from August 2000 to March 2001. These populations were obtained with different proportions of mites from the selected R and S strains of *T. urticae*. Each population was kept on bean plants (*C. ensiformis*) (cultivated in plastic pots) free of any pesticide treatment, in transparent plastic recipients with 32 x 42 x 48 cm. The recipients were maintained under 25 ± 1ºC, 70 ± 5% RH and a 14h photoperiod. At this temperature, the duration of the developmental period (egg to adult) and the mean generation time (*T*) of *T. urticae* are around 9.8 and 16.2 days, respectively (Saito 1979).

The initial population (R + S) in each recipient was of 1,000 mites (adult females). The *T. urticae* population in each box (with at least 25 bean plants) was much higher than 1,000 mites during all the period of the study.

The evaluations were carried out by observing the percentage of survival of mites after 48h from the pesticide application, with the use of discriminating concentration of 4.79 mg of abamectin (A.I.) / L of distilled water. This concentration was slightly higher than the LC_{99} of abamectin estimated for the selected susceptible strain of *T. urticae*. The discriminating concentration was able to kill about 100% of susceptible mites without affecting the resistant mites. The bioassay method was the same as described earlier (Toxicity Tests). The experiment was constituted by four replicates and the total of 240 adult females of each population were used during each monthly evaluation.

Data of percentage of survival (X) of each population, collected for six months, were transformed in arc sin $\sqrt{X/100}$ and analyzed using ANOVA of two factors (population and time) with interactions ($\alpha = 0.05$).

Results

Selections for Resistance and Susceptibility. After five selections for resistance, the LC_{50} of abamectin increased from 4.36 mg to 58.10 mg of A.I./L (Table 1). Regarding the selection for susceptibility, after five selections, the LC_{50} of the acaricide decreased from 4.36 mg to 0.17 mg of A.I./ L (Table 1). After the selection process, the final resistance ratio (RR) reached 342 at LC_{50}.

Cross-Resistance. The activities of eight different pesticides against both strains (R and S) of *T. urticae* are shown in Table 2. The highest resistance ratios (at LC_{50}) were observed for abamectin (342) and milbemectin (16.3). The results indicate associated resistance between these two chemicals. In the case of fenpropathrin and chlorfenapyr, the abamectin resistant population presented only a slightly higher LC_{50} than the susceptible population, indicating that this possible associated resistance is of minor importance. The resistance ratios for fenpropathrin and chlorfenapyr were respectively of 3.20 and 2.23. No cross-resistance was detected for the acaricides fenpyroximate, cyhexatin, propargite, and dimethoate.

The experiment of the toxicity of milbemectin to populations of *T. urticae* with different susceptibilities to abamectin indicated high correlation ($F = 222$; Degree of Freedom = 1, $P = 0.0002$; $R^2 = 0.978$) between the LC_{50}s of milbemectin and abamectin. Populations with higher resistance to abamectin also presented higher resistance to milbemectin (Fig. 1).

Stability of Resistance. The results indicate that abamectin

Table 1. Selection for resistance and susceptibility to abamectin, in a population of *T. urticae* from a commercial strawberry field, Atibaia, SP: estimation of LC_{50} (mg of A.I./L of water) and slope.

Selection number	Concentration (mg of A.I./L)	N[1]	LC_{50}[2] (mg of A.I./L) (95% CL)	Slope ± SEM	χ^2	D.F.[3]
For resistance						
0[4]	-	360	4.36 (3.47 – 5.62)	1.55 ± 0.087	0.66	3
1	9.0	420	9.12 (7.38 – 11.22)	1.56 ± 0.068	1.79	5
2	13.5	360	18.80 (15.41 – 23.20)	1.71 ± 0.086	1.47	4
3	18.0	360	31.82 (25.83 – 38.94)	1.68 ± 0.110	2.26	4
4	21.6	360	43.31 (35.54 – 54.09)	1.82 ± 0.059	0.66	4
5	27.0	360	58.10 (48.03 – 71.04)	1.80 ± 0.050	0.37	4
For susceptibility						
0	-	360	4.36 (3.47 – 5.62)	1.55 ± 0.087	0.66	3
1	2.16	360	1.40 (1.19 – 1.69)	2.22 ± 0.120	2.72	4
2	1.26	360	1.17 (0.99 – 1.41)	2.17 ± 0.062	0.67	4
3	0.72	360	0.31 (0.25 – 0.37)	1.95 ± 0.230	5.25	3
4	0.36	360	0.19 (0.17 – 0.23)	2.87 ± 0.092	1.31	4
5	0.18	360	0.17 (0.15 – 0.21)	2.10 ± 0.210	6.77	4

[1]Total number of mites used
[2]Lethal concentration (95% confidential limit)
[3]Degrees of freedom
[4]Before selection

resistance is unstable in the absence of selection pressure, in laboratory conditions (Fig. 2). For all populations (with 75, 50, and 25% of initial frequency of resistant mites), the percentage of resistant mites decreased to levels equal or lower than 15% in six months. For the population with initial frequency of 75% of resistant mites, a significant decrease in resistance frequency was observed from the third month after the beginning of the experiment. For the other two populations (initial frequencies: 50% and 25%), a significant decrease was observed from the second month.

The interaction between the factors population and time was significant ($F = 6.37$, Degree of Freedom = 10, 54; $P < 0.001$), indicating that the pattern of decline of abamectin resistance was different for these three populations of *T. urticae*, during the six months of evaluation. Higher decrease in resistance frequencies were observed for the population with higher initial frequencies of resistant mites (Fig. 2). If we consider the decrease in percentage of resistant mites, the average rate of decrease for the interval from 75% to 15% was around 10% per month. However, the rate of decrease from 25% to 0% was approximately 4.2% per month.

Discussion

The original population of *T. urticae*, collected from strawberry field in Atibaia county, state of São Paulo, was already moderately resistant to abamectin, even before the selection process in the laboratory. The initial LC_{50} (4.36 mg of A.I./L) was 25 times higher than the LC_{50} (0.174 mg of A.I./L) observed after the selection for susceptibility. Abamectin has been used in this strawberry field for almost ten years and recently, was the most frequently applied acaricide in this field. Abamectin had been sprayed at least six times during 1999, before collecting this population of mites.

The maximum LC_{50} of abamectin observed after the selection pressure was 58.1 mg of A.I./L, which corresponds to a concentration 4.3 times higher than the recommended

concentration (13.5 mg of A.I./L) of this chemical to control *T. urticae* on strawberry in Brazil.

The positive cross-resistance observed between abamectin and milbemectin in *T. urticae* seemed likely because of the similarity in mode of action of these pesticides. Both compounds potentiate glutamate and GABA (gamma-

Table 2. Toxicity tests with different acaricides, using the resistant and susceptible populations of *T. urticae*: estimation of LC_{50} (mg of A.I./L of water), slope and resistance ratios.

Acaricide	Strain	N[1]	LC_{50}[2] (mg of A.I./L) (95% CL)	Slope ± SEM	χ^2	D.F.[3]	RR[4] at LC_{50}
Abamectin	R[5]	360	58.10 (48.02 – 71.03)	1.80 ± 0.050	0.38	4	341.76
	S[6]	360	0.17 (0.15 – 0.21)	2.10 ± 0.212	6.77	4	-
Milbemectin	R	300	7.34 (5.99 – 9.22)	1.81 ± 0.093	3.71	3	16.31
	S	360	0.45 (0.36 – 0.59)	1.51 ± 0.066	5.09	4	-
Fenpropathrin	R	360	7.25 (6.01 – 8.89)	1.88 ± 0.073	3.77	4	3.20
	S	360	2.27 (1.84 – 2.74)	1.91 ± 0.075	3.27	4	-
Chlorfenapyr	R	360	5.98 (5.03 – 7.08)	2.15 ± 0.077	5.39	4	2.23
	S	360	2.68 (2.34 – 3.08)	3.17 ± 0.114	2.66	4	
Fenpyroximate	R	360	21.71 (18.20 – 25.72)	2.24 ± 0.082	2.94	4	1.34
	S	360	16.21 (13.33 – 19.20)	2.20 ± 0.086	1.83	4	-
Cyhexatin	R	360	73.30 (63.39 – 84.74)	2.82 ± 0.099	2.45	4	1.31
	S	360	56.11 (48.50 – 64.72)	2.90 ± 0.105	2.03	4	-
Propargite	R	360	87.02 (74.73 – 102.04)	2.55 ± 0.089	7.50	4	0.84
	S	360	103.08 (88.03 – 121.15)	2.45 ± 0.087	2.36	4	-
Dimethoate	R	360	3,469.07 (2,888.20 – 4,196.26)	1.92 ± 0.072	6.88	4	1.11
	S	300	3,126.31 (2,601.22 – 3,726.17)	2.16 ± 0.099	1.66	3	-

[1]Total number of mites used.
[2]Lethal concentration (95% confidential limit)
[3]Degrees of freedom
[4]Resistance ratio (LC_{50} of resistant strain divided by LC_{50} of susceptible strain)
[5]R strain of *T. urticae* after selecting five times for resistance to abamectin.
[6]S strain of *T. urticae* after selecting five times for susceptibility to abamectin.

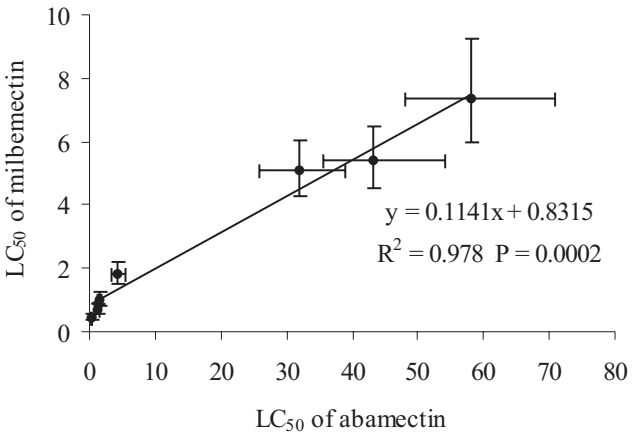

Figure 1. Relationship between LC_{50} of abamectin (mg of A.I./L of distilled water) (n ≥ 360 mites per population) and LC_{50} of milbemectin (mg of A.I./L of distilled water) (n ≥ 300 mites per population) for various populations of *T. urticae* with different susceptibilities to abamectin. Bars represent the 95% confidential limit of LC_{50} values for abamectin (horizontal bars) and milbemectin (vertical bars).

amino butyric acid) gated chloride-channel opening, leading to paralysis and death of pests (Shoop *et al.* 1995, Bloomquist 2001). Milbemectin is a fermentation product of *Streptomyces hygroscopicus* sub sp. *aureolacrimosus*. Milbemycins and avermectins are characterized by the presence of a 16-membered lactone ring. The difference between milbemycins and avermectins is a disaccharide substituent at carbon 13, present in the avermectins and absent in the milbemycins (Shoop *et al*. 1995).

The abamectin resistance mechanisms were not studied in the present research and therefore it is not possible to confirm the existence (or lack) of cross-resistance with other acaricides. By definition, cross-resistance refers to the existence of a sole mechanism underlying resistance to two or more compounds. In the case of abamectin and milbemectin, due to the increased resistance to the second

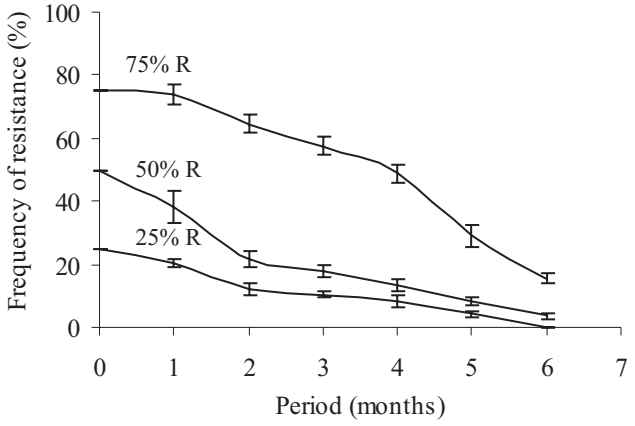

Figure 2. Variation in the frequency of resistance (percentage of resistant mites with SEM) to abamectin in *T. urticae*, under laboratory conditions (25 ± 1ºC, 70 ± 5% RH and a 14h photoperiod).

compound by selection for resistance in the first and the fact that this *T. urticae* population had never received any treatment with milbemectin, there are strong indications of cross-resistance between these acaricides. However, not much can be inferred for other compounds (such as fenpropathrin and chlorfenapyr) concerning cross-resistance with abamectin. The small number of chromosomes (n = 3) in *T. urticae* (Helle & Bolland 1967) increases the possibilities of multiple-resistance development. The selection with an acaricide (e.g. abamectin) may select populations resistant to another group of acaricides (e.g. pyrethroids), if the genes responsible for resistance to these two groups are located in the same chromosome (Omoto 1995).

Abamectin resistance in *T. urticae* was also reported by several authors (Campos *et al.* 1996, Beers *et al.* 1998). Stumpf & Nauen (2002), investigating enzymes involved in abamectin resistance in the two-spotted spider mite, observed that resistant strains (NL-00 and COL-00) presented severalfold higher MFO (cytochrome P450-dependent monooxygenase) activity than the susceptible strain GSS. Abamectin resistance in strain NL-00 was strongly synergized by PBO (piperonyl butoxide) and DEM (diethyl maleate), suggesting that MFO and GST (glutathione *S*-transferases) may be involved in abamectin resistance (Stumpf & Nauen 2002).

Stability of acaricide resistance has been studied for several compounds in *T. urticae* and other species of mites (Inoue 1980, Omoto *et al.* 1995, Sato *et al.* 2004, Stumpf & Nauen 2002). Dicofol resistance was shown to be unstable in *Panonychus citri* (McGregor), in the absence of selection pressure (Inoue 1980). Lower variations in resistance frequency were observed for populations with low percentage of resistant mites (Inoue 1980), corroborating the results obtained in this study with abamectin resistance in *T. urticae*.

Although the abamectin resistance frequency decreased from 75% to less than 15% in six months in our population of *T. urticae*, abamectin resistance was shown to be stable in the laboratory at least over six months in a Dutch strain (NL-00) of two-spotted spider mite, collected from roses (Stumpf & Nauen 2002). These reports indicate that the instability of abamectin resistance can not be generalized for all populations of *T. urticae* in Brazil.

The instability of resistance, as observed in this strain of spider mite, is considered favorable for the management of resistance (Dennehy *et al.* 1990). The instability of abamectin resistance may explain the relatively high efficacy of this chemical in crops such as strawberry, in the state of São Paulo, where abamectin has intensively been used during the last ten years. In this aspect, the period from one season of strawberry to another (more than six months) is probably enough for the reestablishment of susceptibility in populations of *T. urticae* in the field. The immigration of susceptible (or resistant) mites from other host plants may also affect the reestablishment of susceptibility in field conditions (Miller *et al.* 1985, Dunley & Croft 1992).

One of the strategies to prolong the efficacy of this acaricide in the field is the rotation of abamectin with other acaricides, such as fenpyroximate, cyhexatin and propargite.

For these chemicals no positive cross-resistance was detected. This strategy is probably very interesting in the case of abamectin, considering the instability of resistance.

Estimates of selection intensity and factors leading to declines in resistance frequencies such as immigration and fitness differences can be used to suggest minimum intervals between acaricide applications to preserve susceptibility of field populations and to determine appropriate rotations of acaricides (Martinson *et al.* 1991).

Further studies on the instability of abamectin resistance in *T. urticae* under field conditions are necessary. An improved understanding of abamectin resistance in the two-spotted spider mite is important to maintain the lifetime of this chemical for the control of this pest in several crops in Brazil.

Acknowledgments

The authors are thankful to FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) for the financial support to this research (Process # 1999/11926-2), and to CNPq-Brazil (Conselho Nacional de Desenvolvimento Científico e Tecnológico do Brasil) for providing the scholarship to Marcos Zatti da Silva (PIBIC/CNPq/IB).

Literature Cited

Andrei, E. 2005. Compêndio de defensivos agrícolas. 7ª ed., Editora Andrei, São Paulo, 1141p.

Ayres, M. & M. Ayres Jr. 2003. BioEstat 3.0 Aplicações estatísticas nas áreas das ciências biológicas e médicas. Sociedade Civil Mamirauá/CNPq, Belém, 291p.

Beers, E.H., H. Riedl & J.E. Dunley. 1998. Resistance to abamectin and reversion to susceptibility to fenbutatin oxide in spider mite (Acari: Tetranychidae) populations in the Pacific Northwest. J. Econ. Entomol. 91: 352-360.

Bloomquist, J.R. 2001. GABA and glutamate receptors as biochemical sites for insecticide action, p. 17-41. In I. Ishaaya (ed.), Biochemical sites of insecticide action and resistance. New York, Springer, 361p.

Campos, F., D.A. Krupa & R.A. Dybas. 1996. Susceptibility of populations of twospotted spider mites (Acari: Tetranychidae) from Florida, Holland, and the Canary Islands to abamectin and characterization of abamectin resistance. J. Econ. Entomol. 89: 594-601.

Campos, F., R.A. Dybas & D.A. Krupa. 1995. Susceptibility of twospotted spider mite (Acari: Tetranychidae) populations in California to abamectin. J. Econ. Entomol. 88: 225-231.

Clark J.M., J.G. Scott, F. Campos & J.R. Bloomquist. 1994. Resistance to avermectins: Extent, mechanism, and management implications. Annu. Rev. Entomol. 40: 1-30.

Cranham, J.E. & W. Helle. 1985. Pesticide resistance in Tetranychidae, p 405-421. In W. Helle & M.W. Sabelis (eds.), Spider mites: Their biology, natural enemies, and control. vol. 1B, Amsterdam, Elsevier, 458p.

Dekeyser, M.A. 2005. Review acaricide mode of action. Pest Manag. Sci. 61: 103-110.

Dennehy, T.J., J.P. Nyrop & T.E. Martinson. 1990. Characterization and exploitation of instability of spider mite, p.77-91. In M.B. Green, H. Lebaron & W.K. Moberg (eds.), Managing resistance to agrochemicals: from fundamental research to practical strategies. Washington, Am. Chem. Soc., 497p.

Devine, G.J., M. Barber & I. Denholm. 2001. Incidence and inheritance of resistance to METI-acaricides in European strains of the two-spotted spider mite (*Tetranychus urticae*) (Acari: Tetranychidae). Pest Manag. Sci. 57: 443-448.

Dunley, J.E. & B.A. Croft. 1992. Dispersal and gene flow of pesticide resistance traits in phytoseiid and tetranychid mites. Exp. Appl. Acarol. 14: 313-325.

Edge, V.E. & D.G. James. 1986. Organo-tin resistance in *Tetranychus urticae* (Acari: Tetranychidae) in Australia. J. Econ. Entomol. 79: 1477-1483.

Fergusson-Kolmes, L.A., J.G. Scott & T.J. Dennehy. 1991. Dicofol resistance in *Tetranychus urticae* (Acari: Tetranychidae): Cross-resistance and pharmacokinetics. J. Econ. Entomol. 84: 41-48.

Finney, D.J. 1971. Probit analysis. 3rd ed., London, Cambridge University Press, 383p.

Flexner, J.L., P.H. Westigard & B.A. Croft. 1988. Field reversion of organotin resistance in the twospotted spidermite (Acari: Tetranychidae) following relaxation of selection pressure. J. Econ. Entomol. 81: 1516-1520.

Helle, W. & H.R. Bolland. 1967. Karyotypes and sex determination in spider mites (Tetranychidae) Genetica 38: 43-53.

Herron, G.A. & J. Rophail. 1993. Genetics of hexythiazox resistance in two spotted spider mite, *Tetranychus urticae* Koch. Exp. Appl. Acarol. 17: 423-431.

Herron, G.A., V. Edge & J. Rophail. 1993. Clofentezine and hexythiazox resistance in *Tetranychus urticae* Koch in Australia. Exp. Appl. Acarol. 17: 433-440.

Inoue, K. 1980. Relationship between dicofol resistance and fitness in the citrus red mite, *Panonychus citri* (McGregor). J. Pestic. Sci. 5: 165-175.

Keena, M.A. & J. Granett. 1990. Genetic analysis of

propargite resistance in Pacific spider mites and twospotted spider mite (Acari: Tetranychidae). J. Econ. Entomol. 83: 655-661.

Knight, A.L., E.H. Beers, S.C. Hoyt & H. Riedl. 1990. Acaricide bioassay with spider mites (Acari: Tetranychidae) on pome fruits: Evaluation of methods and selection of discrimination concentrations for resistance monitoring. J. Econ. Entomol. 83: 1752-1760.

LeOra Software. 1987. POLO-PC: A user's guide to Probit or Logit analysis. Berkeley, 20p.

Martinson, T.E., T.J. Dennehy, J.P. Nyrop & W.H. Reissig. 1991. Field measurements of selection for twospotted spider mite (Acari: Tetranychidae) resistance to dicofol in apple orchards. J. Econ. Entomol. 84: 7-16.

Miller, R.W., B.A. Croft & R.D. Nelson. 1985. Effects of early season immigration on cyhexatin and formetanate resistance of *T. urticae* (Acari: Tetranychidae) on strawberries in Central California. J. Econ. Entomol. 78: 1379-1388.

Mochizuki, M. 1994. Variations in insecticide susceptibility of the predatory mite, *Amblyseius womersleyi* Schicha (Acarina: Phytoseiidae), in the tea fields of Japan. Appl. Entomol. Zool. 29: 203-209.

Omoto, C. 1995. Resistência de *Brevipalpus phoenicis* (Acari: Tenuipalpidae) aos produtos químicos na citricultura, p.179-188. In C.A.L. de Oliveira & L.C. Donadio (eds.), Leprose dos citros. Jaboticabal, FUNEP, 219p.

Omoto, C., T.J. Dennehy, C.W. McCoy, S.E. Crane & J.W. Long. 1995. Management of citrus rust mite (Acari: Eriophyidae) resistance to dicofol in Florida citrus. J. Econ. Entomol. 88: 1120-1128.

Saito, Y. 1979. Comparative studies on life histories of three species of spider mites (Acari: Tetranychidae). Appl. Entomol. Zool. 14: 83-94.

Sato, M.E., N. Suplicy Filho, M.F. de Souza Filho & A.P. Takematsu. 1994. Resistência do ácaro rajado *Tetranychus urticae* (Koch, 1836) (Acari: Tetranychidae) a diversos acaricidas em morangueiro (*Fragaria* sp.) nos municípios de Atibaia-SP e Piedade-SP. Ecossistema 19: 40-46.

Sato, M.E., T. Miyata, M. da Silva, A. Raga & M.F. de Souza Filho. 2004. Selections for fenpyroximate resistance and susceptibility, and inheritance, cross-resistance and stability of fenpyroximate resistance in *Tetranychus urticae* Koch (Acari: Tetranychidae). Appl. Entomol. Zool. 39: 293-302.

Shoop, W.L., H. Mrozik & M. Fisher. 1995. Structure and activity of avermectins and milbemycins in animal health. Vet. Parasitol. 59: 139-156.

Stumpf, N. & R. Nauen. 2001. Cross-resistance, inheritance, and biochemistry of mitochondrial electron transport inhibitor-acaricide resistance in *Tetranychus urticae* (Acari: Tetranychidae). J. Econ. Entomol. 94: 1577-1583.

Stumpf, N. & R. Nauen. 2002. Biochemical markers linked to abamectin resistance in *Tetranychus urticae* (Acari-Tetranychidae). Pestic. Biochem. Physiol. 72: 111-121.

Received 23/III/05. Accepted 22/VI/05.