CROP PROTECTION


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


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 (CL50 R/ CL50 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 CL50s dos produtos milbemectin, fenpropathrin e clorfenapir. Foram obtidas correlações significativas entre as CL50s de abamectim e milbemectin, indicando resistência cruzada entre esses acaricidas. Não foi detectada resistência cruzada entre os acaricidas fenpyroximate, cyhexatin, propargite e dimethoate. A resistência de *T. urticae* a abamectim mostrou-se instável na ausência de pressão de seleção. Para todas as populações estudadas (com frequência 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 milbemectin deve ser evitado em programas de manejo da resistência de *T. urticae* a abamectim.

PALAVRAS-CHA VE: 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 LC50 reached 342-fold values. The toxicity of eight acaricides was evaluated in the R and S strains, observing significant differences (at LC50s) between R and S strains for milbemectin, fenpropathrin and clorfenapir. Significant correlation was detected between the LC50s 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), dicrof (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), *Phylocnistis citrella* Stanton, *Tuta absoluta* (Meyrick) and mites, such as *T. urticae*, *Tetranychus ludeni* Zacher, *Polyphagotarsonemus latus* (Banks), *Panonychus ulmi* (Koch), *Aculus lycopersici* (Massa) 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, milbemycin A₃ and milbemycin A₁. 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 determination 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 ± 1°C, 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/cm² 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 ± 1°C 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 LC₅₀ 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 (Vertimek® 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%.

**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 (Milbemectin® 50 EC, emulsion concentrate, 50 g of milbemectin/L; Iharabras S.A. Chemical Industries), fenpropatrin (Danimen® 300 CE, emulsion concentrate, 300 g of fenpropatrin/L; Iharabras...
S. A. Chemical Industries), chlorfenapyr (Citrex®, suspension concentrate, 240 g of chlorfenapyr/L; Basf S.A.), fenpyroximate (Ortus®, 50 SC, suspension concentrate, 50 g of fenpyroximate/L; Hokko do Brasil), cyhexatin (Hokko Cyhecatin® 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 LC50 values, estimated for each acaricide for S and R strains. The resistance ratio (R/S) was calculated by the division of LC50 of R strain and LC50 of S strain.

In the case of milbemectin, we also evaluated the LC50 of this acaricide in seven populations of T. urticae with different susceptibilities to abamectin (LC50 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 LC50 value for abamectin and milbemectin were equal or higher than 300. The relationship between LC50 of abamectin and LC50 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 ± 1°C, 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 LC50 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 √X/100 and analyzed using ANOVA of two factors (population and time) with interactions (α = 0.05).

**Results**

**Selections for Resistance and Susceptibility.** After five selections for resistance, the LC50 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 LC50 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 LC50.

**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 LC50) 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 LC50 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; R2 = 0.978) between the LC50 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

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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. Abamectin has been used in this strawberry field for almost ten years and recently, was the most frequently applied acaricide in this field. Abamectin was sprayed at least six times during 1999, before collecting this population of mites.

The maximum LC50 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.

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

$^{(1)}$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.
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 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).

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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.

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