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Monitoring *Tetranychus urticae* Koch (Acari: Tetranychidae) resistance to abamectin in vineyards in the Lower Middle São Francisco Valley

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ABSTRACT

In the Lower Middle São Francisco Valley, *Tetranychus urticae* Koch is controlled by the application of acaricides; however, the intensive use of these products in this region has caused control failures in the field. In the present study, concentration–response curves were constructed periodically to monitor the toxicity of abamectin to *T. urticae* in two vineyards over two years. Diagnostic concentrations of 1 mg and 9 mg of abamectin/L water were established based on the monitoring period to detect *T. urticae* resistance in different vineyards in the region. Concentration-response curves were obtained for abamectin, bifenthrin and carbosulfan for the populations considered resistant to abamectin. *T. urticae* were confined in arenas on cotyledonary leaf discs from jack bean (*Canavalia ensiformis* L.) that had been immersed in acaricide solution. Mite mortality was assessed after 48 h of exposure to the acaricides. The lethal concentration (LC) values varied over time in both of the vineyards studied, which was most likely a result of crop management. An additional 35 vineyards were sampled, and 20 additional populations were established. The results indicated that 45% of the populations exposed to the 9 mg/L abamectin diagnostic concentration experienced less than 80% mortality and were considered resistant to abamectin. The frequency of resistant mites ranged from 4.1% to 80.4%. The resistance ratio ranged from 2406-fold to 8272-fold compared to susceptible populations in the laboratory. Resistance to bifenthrin was also confirmed in the present study, though resistance to carbosulfan was not. No cross-resistance between abamectin and bifenthrin was observed though this requires further investigation.

1. Introduction

The two-spotted mite *Tetranychus urticae* Koch is an important grapevine pest in several parts of the world (Schruft, 1985; Duso et al., 2010; James and Prischmann, 2010), including the Lower Middle São Francisco Valley (Haji and Alencar, 2000; Oliveira and Moreira, 2009; Domingos et al., 2014). *T. urticae* causes chlorotic spots on leaves that can become reddened, necrotic and dry. High infestations can cause defoliation and browning (Carmona, 1996; Haji et al., 2001b; Botton, 2005; Oliveira and Moreira, 2009). Other mite species are also important pests in the region and include the broad mite *Polyphagotarsonemus latus* (Banks) and the mango spider mite *Oligonychus mangiferus* (Rahman and Sapra) (Haji and Alencar, 2000; Domingos et al., 2014).

Grapes produced in the Lower Middle São Francisco Valley are mainly destined for export. To meet the export market demands, the product must be certified by different entities whose standards meet the requirements of importing countries (Fachinello, 2001; Haji et al., 2001a; Silva et al., 2001). Certification provides assurance to consumers regarding product quality (Fachinello, 2001; Pinheiro and Adissi, 2007). However, only three acaricides that are registered in Brazil for the control of grapevine mites by the Ministry of Agriculture, Livestock and Food Supply (Ministério da Agricultura, Pecuária e Abastecimento – MAPA) are also accepted by importing countries: abamectin, bifenthrin and carbosulfan (AGROFIT, 2013). Farmers avoid using more than one acaricide because they must also use insecticides to control thrips and fungicides to control the downy mildew fungus *Plasmopara viticola*

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(Berkeley and M. A. Curtis) Berlese & De Toni and grape powdery mildew fungus *Erysiphe necator* Schwein, which are important diseases affecting grapevines. Thus, farmers tend to use a broad-spectrum acaricide such as abamectin that controls tetranychids (*T. urticae* and *O. mangiferus*) and tarsonemids (*P. latus*). Apparently, this practice has promoted the intensive use of abamectin in the region, which has elevated the selective pressure on these populations and increased the number of reports of control failures in the field as a result of the emergence of populations with a high frequency of resistant individuals.

T. urticae stands out among arthropods for being resistant to a large number of acaricides/insecticides (Whalon et al., 2008), and resistance mechanisms have been recently reviewed (van Leeuwen et al., 2010). Cross and multiple resistant species have also been reported for this mite in several parts of the world (Sato et al., 2005; Kim et al., 2006; Kwon et al., 2010; Nicastro et al., 2010). The short life cycle, reproductive mode and high biotic potential of *T. urticae* favor the rapid development of resistance to several acaricides (Stumpf and Nauen, 2001). In field populations, resistance can be easily and quickly assessed through toxicity tests using discriminating concentrations (Roush and Miller, 1986). These concentrations cause mortality in most of the susceptible individuals of a population, which differentiates these from resistant individuals (Kabir et al., 1991; Shah et al., 2002; Yu, 2008; Sato et al., 2009). The discriminating concentration is expressed by values that are usually between the lethal concentrations (LCs) LC_{95} and LC_{99} of susceptible populations (Halliday and Burnham, 1990). Monitoring of *T. urticae* resistance using discriminating concentrations was performed for abamectin and hexythiazox in the United States (Knight et al., 1990), for clofentezine and fenbutatin oxide in Australia (Herron et al., 1997), for propargite in New Zealand (Shah et al., 2002), and for bifenazate, acequinocyl, abamectin, milbemectin, bifenthrin, cyflumetofen, etoxazole and spiromesifen in the Netherlands (Khajehali et al., 2011). In Brazil, tests using discriminating concentrations were conducted in the state of São Paulo using abamectin, fenpyroximate and milbemectin in *T. urticae* populations from several crops (Sato et al., 2009; Nicastro et al., 2010).

The aim of the present study was to provide information on the frequency of *T. urticae* resistance to abamectin in grapevines in the Lower Middle São Francisco Valley; concentration–response curves were periodically performed to establish diagnostic concentrations and monitor the toxicity of abamectin to *T. urticae* in the region studied. Toxicity tests were also conducted for bifenthrin and carbosulfan, which are the other acaricides registered for *T. urticae* control in grapevines in Brazil, to assess if cross-resistance occurs in the region studied.

2. Materials and methods

2.1. Tested acaricides

The experiments were conducted using the acaricide abamectin (Kraft 36 EC, Cheminova Brasil Ltda., São Paulo, Brazil), bifenthrin (Talstar CE, FMC Química do Brasil Ltda., Batatais, Brazil) and carbosulfan (Marshal 400 SC, FMC Química do Brasil Ltda., Campinas, Brazil).

2.2. Monitoring abamectin toxicity to T. urticae over time in two vineyards

2.2.1. Obtaining and maintaining T. urticae populations

Grapevine leaves were collected from two properties in the municipality of Petrolina, state of Pernambuco that reported control failures using abamectin: population 1 (9°20′10.86″S; 40°38′51.43″W) and population 2 (9°17′45.82″S; 40°32′51.47″W). The samples were collected from July 2011 to September 2013 with two to three month intervals between collections. Leaf samples were transported to the Laboratory of Acarology of the Federal Rural University of Pernambuco (Universidade Federal Rural de Pernambuco - UFRPE) in paper bags that were placed in Styrofoam boxes. In the laboratory, the mites were transferred to jack bean plants (*Canavalia ensiformis* L.) that were maintained at 25 °C ± 1 °C temperature and 85 ± 10% relative humidity for a 12 h photoperiod.

2.2.2. Abamectin bioassay

Toxicity tests were conducted according to Method N°4 of the series of methods to test susceptibility from the Insecticide Resistance Action Committee (IRAC, 2009). A series of 10-fold dilutions of abamectin (0.01; 0.1; 1; 10; 100; and 1000 mg L^{-1}) was prepared to establish an "all or none" response. The acaricide application was conducted by immersing *C. ensiformis* cotyledonary leaf discs (5 cm diameter) for 5 s in a beaker containing the different abamectin concentrations. The control treatment corresponded to the immersion of leaf discs in distilled water. After immersion, the discs were dried at room temperature for 30 min. The experimental unit consisted of a 9 cm diameter Petri dish, into which 1 cm high polyethylene foam and filter paper (both 9 cm diameter) were placed. The leaf discs were placed on the filter paper, and the edges were covered with paper towel pieces to prevent the mites from escaping. The Petri dishes were moistened with distilled water to maintain leaf disc turgidity. Ten *T. urticae* adult females (F1 generation) were transferred to each experimental unit. Each concentration and the control treatment had three replicates, for a total of 30 mites per treatment. Mortality was assesses 48 h after treatment by counting the total number of live and dead mites per replicate. Mites that did not walk at least a distance corresponding to their body length after being touched with an N°000 brush were considered dead. The mortality percentage of the concentrations was corrected for the mortality of the control (Abbott, 1925).

Based on the "all or none" response assays, seven to eight concentrations diluted by a factor of two were established for the definitive bioassays. These bioassays were performed as aforementioned, but the entire procedure was repeated twice on different days for a total of 60 mites per concentration. The mortality data were subjected to Probit analysis (Finney, 1971) after correction for control mortality (Abbott, 1925). The software POLO-Plus 2.0 (LeOra-Software, 2005; Petaluma, USA) was used to obtain the concentration–response curves. The resistance ratios (RR_{50}) of the resistance populations were calculated for a 95% confidence interval (CI) using the method described by Robertson and Preisler (1992).

2.3. Monitoring abamectin resistance in T. urticae populations over the Valley area

2.3.1. Obtaining and maintaining T. urticae populations

Grapevine leaves were collected from 35 commercial plantations in the Lower Middle São Francisco Valley from January 2013 to April 2013 for obtaining and establishing *T. urticae* populations under laboratory conditions. The sampling sites were georeferenced with the aid of a global positioning system (GPS) (Fig. 1). The leaf samples were transported to the Embrapa Semi-Arid (Embrapa Semiárido) facility in paper bags that were placed in Styrofoam boxes. In the laboratory, the mites were transferred to *C. ensiformis* plants that were kept at 25 °C ± 1 °C temperature and 85 ± 10% relative humidity for a 12 h photoperiod. As a susceptible standard, a *T. urticae* population collected from *Gossypium hirsutum* L. plants in the municipality of Piracicaba, São Paulo State, was used (22°42′48.22″S; 47°37′34.03″W). This population was collected in

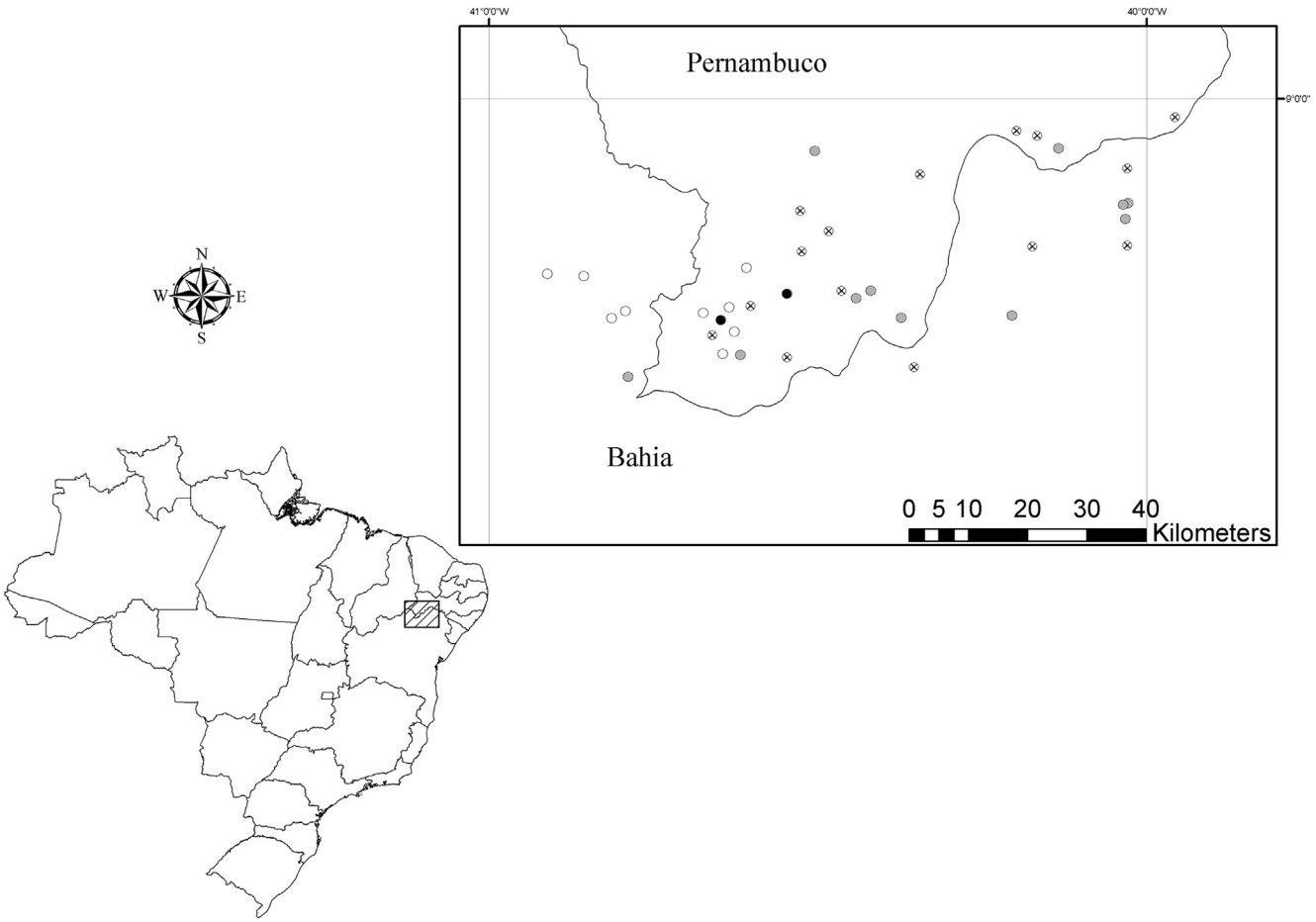

Fig. 1. Locations of collections of *T. urticae* populations in grapevine plantations in the Lower Middle São Francisco Valley. Vineyards monitored over time (represented by black circle); vineyards with populations considered resistant (represented by white circle); vineyards with populations considered susceptible to (represented by gray circle) and vineyards with no *T. urticae* populations (represented by circle with x).

2000 and maintained in the Laboratory of Acarology of the UFRPE with no insecticide pressure since then.

2.3.2. Bioassay

For this monitoring, diagnostic concentrations of abamectin of 1 mg/L and 9 mg/L were respectively used based on the LC_{50} and LC_{95} midpoint estimates from overtime monitoring experiment (item 2.2.). The LC_{95} estimate average virtually matched the field recommended dose of abamectin (AGROFIT, 2013). The procedures were similar to the experiment previously described regarding acaricide application, arena manufacturing (experimental units) and mite confinement and assessment. Each bioassay corresponded to one population with three treatments; two corresponded to the diagnostic concentrations (1 mg/L and 9 mg/L) and another corresponded with distilled water only (control). Each treatment consisted of five experimental units totaling 50 mites per treatment. Each bioassay was repeated three times on different days for a total of 150 mites per treatment. After mite confinement, the experimental units were placed at 25 ± 1 °C temperature and 85 ± 10% relative humidity for a 12 h photoperiod. The percentage mortality of the concentrations was corrected for the control mortality (Abbott, 1925). The population that had less than 80% mortality when exposed to the 9 mg/L abamectin diagnostic concentration was considered resistant to this acaricide.

2.4. Bioassays of bifenthrin and carbosulfan toxicity to T. urticae populations resistant to abamectin

For the populations considered resistant to abamectin (mortality < 80% in the 9 mg/L diagnostic concentration), toxicity tests were performed according to Method N°4 of the series of methods for susceptibility tests from the Insecticide Resistance Action Committee (IRAC, 2009). Bifenthrin and carbosulfan concentrations were diluted by a factor of 10 (0.1; 1; 10; 100; 1000 and 10,000 mg) per liter of solution. Acaricide application, arena manufacturing, mite confinement and assessment and data analysis were performed in a similar manner as the preliminary test for abamectin toxicity monitoring. From the preliminary tests, seven to eight concentrations diluted by a factor of two were established among concentrations that caused approximately 0 and 100% mite mortality. All of the acaricide application, arena manufacturing, mite confinement and assessment and data analysis procedures were similar to the bioassay for abamectin toxicity monitoring. A correlation was performed between the LC_{50}s and LC_{95}s of bifenthrin and abamectin for each population resistant to both acaricides using the PROC CORR procedure in the program SAS (SAS Institute, 2002; Cary, USA).

3. Results

3.1. Monitoring abamectin toxicity to T. urticae over time in two vineyards

The LC_{50}, LC_{80} and the LC_{95} of population 1 ranged from 0.46 to 2.98, 0.93 to 6.85 and 1.79–15.55 mg/L, respectively. The LC_{50}, LC_{80} and the LC_{95} of population 2 ranged from 0.62 to 4.53, 1.38 to 10.15 and 2.90–21.91 mg/L (Table 1), respectively.

3.2. Monitoring abamectin resistance in T. urticae populations over the Valley area

Thirty-five farms in the Lower Middle São Francisco Valley were visited, but *T. urticae* was not found in 15 farms (Fig. 1). The 1 mg/L diagnostic concentration caused less than 50% mortality to populations 3, 4, 5, 6, 7, 8, 9, 10, 11, 15 and 17 and higher than 50% mortality to the remaining populations (populations 12, 13, 14, 16, 18, 19, 20, 21 and 22). The 9 mg/L diagnostic concentration caused less than 80% mortality to populations 3, 4, 5, 6, 7, 8, 9, 10 and 11, which were considered resistant to abamectin; and more than 80% mortality to the remaining populations (12, 13, 14, 15, 16, 17, 18, 19, 20, 21 and 22), which were considered susceptible to abamectin (Fig. 2). The frequency of resistant mites when subjected to the 9 mg/L diagnostic concentration ranged from 4.14% to 80.40%.

The populations considered resistant to abamectin when assessed by concentration–response curves showed resistance ratios ranging from 2406-fold (Population 10) to 8272-fold (Population 8) compared to a susceptible population in the laboratory (Table 2).

3.3. Bioassays of bifenthrin and carbosulfan toxicity to T. urticae populations resistant to abamectin

The carbosulfan toxicity tests estimated an LC_{50} of 715 and 2132 mg/L and LC_{95} of 2462 and 11,095 mg/L for populations 5 and 6, respectively. The resistance ratios ranged from 1- to 3-fold compared to the susceptible population. The bifenthrin toxicity tests estimated an LC_{50} of 1411 to 4271 mg/L for populations 8 and 3, respectively, whereas the LC_{95} varied from 8378 to 24,792 mg/L for populations 6 and 3, respectively. The resistance ratios ranged from 162- to 489-fold compared to the susceptible population (Table 3). The correlation between the LC_{50}s ($R^2 = 0.24$; $P = 0.50$) and LC_{95}s ($R^2 = 0.16$; $P = 0.65$) of abamectin and bifenthrin were not significant (SAS Institute, 2002).

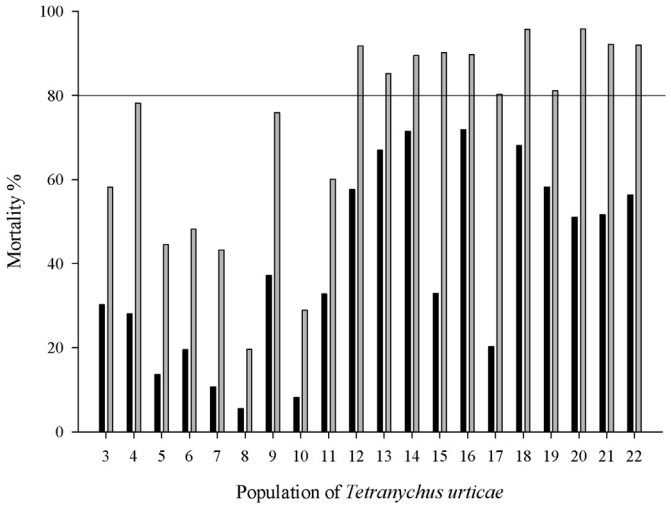

Fig. 2. Percentage mortality of *T. urticae* populations collected in vineyards of the Lower Middle São Francisco Valley subjected to diagnostic concentrations of abamectin –1.0 mg/L (black bar) and 9.0 mg/L (gray bar).

Table 1
Abamectin toxicity over time for two *T. urticae* populations collected in grapevines of the Lower Middle São Francisco Valley from July 2011 to September 2013.

Population	Collection date	N[a]	χ^2 (DF)	Slope ± SE[b]	LC_{50} (95% CI)[c]	LC_{80} (95% CI)[c]	LC_{95} (95% CI)[c]	RR_{50} (95% CI)[d]
1	Jul/11	355	0.67 (4)	1.83 ± 0.30	0.63 (0.33–0.90)	1.83 (1.44–2.36)	5.00 (3.63–8.51)	1.4 (0.6–3.1)
	Aug/11	345	1.79 (4)	1.98 ± 0.28	0.97 (0.63–1.28)	2.59 (2.14–3.31)	6.58 (4.85–10.65)	2.1 (1.0–4.5)
	Oct/11	299	0.62 (4)	2.81 ± 0.24	0.46 (0.15–0.72)	0.93 (0.58–1.22)	1.79 (1.36–3.64)	–
	Feb/12	354	2.67 (4)	2.50 ± 0.24	1.09 (0.92–1.30)	2.73 (2.19–4.04)	4.95 (3.85–7.12)	2.4 (1.2–4.9)
	Apr/12	347	2.49 (4)	2.94 ± 0.29	1.09 (0.93–1.97)	2.12 (1.84–2.61)	3.98 (3.14–5.53)	2.3 (1.2–4.8)
	Jun/12	330	2.05 (4)	2.01 ± 0.21	1.29 (1.02–1.59)	3.39 (2.83–4.42)	8.49 (6.19–13.33)	2.8 (1.3–5.7)
	Sep/12	367	5.35 (4)	2.13 ± 0.19	1.05 (0.77–1.39)	2.62 (2.20–3.60)	6.22 (4.00–13.18)	2.3 (1.1–4.6)
	Nov/12	349	4.48 (4)	2.83 ± 0.17	1.81 (1.32–2.42)	5.16 (3.98–8.38)	14.01 (8.58–31.72)	3.9 (1.9–7.9)
	Feb/13	340	3.61 (4)	1.68 ± 0.19	0.85 (0.63–1.00)	2.69 (2.20–3.60)	8.04 (5.59–13.83)	1.8 (0.9–3.8)
	May/13	343	5.16 (4)	2.44 ± 0.21	2.66 (2.04–3.48)	5.87 (4.66–9.03)	12.50 (8.29–24.62)	5.7 (2.8–11.5)
	Jul/13	345	2.22 (4)	2.25 ± 0.20	2.90 (2.44–3.44)	6.85 (5.80–8.78)	15.55 (11.67–23.06)	6.2 (3.0–12.6)
	Sep/13	357	1.80 (4)	2.37 ± 0.21	2.98 (2.53–3.50)	6.74 (5.75–8.50)	14.67 (11.21–21.11)	6.4 (3.1–12.9)
2	July/11	346	2.26 (4)	2.18 ± 0.22	3.51 (2.85–4.22)	8.52 (7.23–10.84)	19.86 (14.91–29.71)	7.5 (3.7–15.4)
	Aug/11	344	1.61 (4)	2.40 ± 0.22	4.53 (3.79–5.36)	10.15 (8.68–12.76)	21.91 (16.80–31.52)	9.7 (4.8–19.7)
	Oct/11	335	1.58 (4)	2.43 ± 0.32	0.62 (0.47–0.77)	1.38 (1.18–1.74)	2.96 (2.25–4.51)	–
	Feb/12	356	5.44 (4)	3.32 ± 0.35	0.92 (0.71–1.17)	1.49 (1.31–1.82)	2.90 (2.08–5.38)	1.8 (0.9–3.6)
	Apr/12	348	3.95 (4)	2.15 ± 0.20	0.89 (0.73–1.06)	2.18 (1.84–2.81)	5.16 (3.84–7.80)	1.9 (0.9–3.9)
	Jun/12	351	2.44 (4)	2.08 ± 0.19	1.55 (1.28–1.86)	4.30 (3.60–5.60)	9.61 (7.12–14.51)	3.5 (1.8–7.1)
	Sep/12	315	2.25 (4)	2.04 ± 0.21	1.76 (1.43–2.12)	4.44 (3.75–5.70)	11.25 (8.17–17.81)	3.8 (1.8–7.7)
	Nov/12	352	1.38 (4)	2.31 ± 0.22	1.83 (1.53–2.17)	4.24 (3.62–5.35)	9.44 (7.14–13.76)	3.9 (1.9–8.0)
	Feb/13	356	4.35 (4)	2.48 ± 0.21	2.70 (2.13–3.42)	5.90 (4.80–8.48)	12.42 (8.61–21.96)	5.8 (2.8–11.7)
	May/13	352	2.37 (4)	1.77 ± 0.17	2.52 (2.04–3.07)	7.53 (6.14–10.26)	21.41 (14.86–35.72)	5.4 (2.6–11.0)
	Jul/13	356	2.37 (4)	2.11 ± 0.19	2.30 (1.92–2.73)	5.75 (4.84–7.40)	13.76 (10.22–20.66)	4.9 (2.4–10.0)
	Sep/13	345	2.81 (4)	2.09 ± 0.20	1.76 (1.43–2.11)	4.65 (3.55–6.79)	10.77 (7.99–16.33)	3.8 (1.8–7.7)

[a] Total number of mites used to calculate the concentration–response curves.
[b] Slope and standard error.
[c] Lethal concentration (mg/L) and 95% confidence interval.
[d] Resistance ratio: ratio (95% CI) of the LC_{50} between resistance and susceptible populations, calculated using the Robertson and Preisler (1992) method.

Table 2
Abamectin toxicity for *T. urticae* populations considered resistant in the Lower Middle São Francisco Valley.

Population	N[a]	χ^2 (DF)	Slope ± SE[b]	LC_{50} (95% CI)[c]	LC_{95} (95% CI)	RR_{50} (95% CI)[d]
Susceptible	477	4.33 (6)	0.70 ± 0.06	0.00068 (0.00044–0.00106)	0.149 (0.076–0.451)	–
10	330	0.36 (4)	1.62 ± 0.19	1.66 (1.20–2.13)	16.41 (11.31–28.62)	2406 (1367–4235)
11	345	6.68 (4)	1.84 ± 0.17	2.19 (1.43–3.15)	17.71 (9.86–54.73)	3230 (1904–5481)
5	349	4.35 (4)	2.38 ± 0.20	2.29 (1.79–2.92)	11.28 (7.71–20.41)	3387 (2026–5663)
6	341	3.23 (4)	2.22 ± 0.21	3.00 (2.47–3.59)	16.55 (12.46–24.53)	4433 (2630–7471)
9	331	7.65 (4)	1.84 ± 0.17	3.76 (2.52–5.80)	29.22 (14.94–112.78)	5545 (3273–9396)
7	314	5.98 (4)	1.91 ± 0.18	4.32 (2.99–6.16)	31.34 (17.76–89.53)	6370 (3761–10790)
3	338	7.08 (4)	2.30 ± 0.20	4.68 (3.38–6.59)	24.17 (14.61–59.91)	6903 (4118–11571)
4	340	8.25 (4)	1.87 ± 0.18	5.15 (3.30–7.73)	38.70 (20.66–140.12)	7590 (4487–12839)
8	339	3.57 (4)	1.87 ± 0.18	5.61 (4.55–6.81)	42.24 (29.97–68.43)	8272 (4883–14015)

[a] Total number of mites used to calculate the concentration–response curves.
[b] Slope and standard error.
[c] Mean lethal concentration (mg/L) and 95% confidence interval.
[d] Resistance ratio: ratio (95% CI) of the LC_{50} between resistance and susceptible populations, calculated using the Robertson and Preisler (1992) method.

4. Discussion

The results of the present survey clearly show that abamectin has been intensively used in the Lower Middle São Francisco Valley to control *T. urticae* in grapevines, and the management of resistance to this acaricides has been neglected. Out of the 20 *T. urticae* populations tested, 9 (45%) were shown to be resistant to abamectin when exposed to the 9 mg/L diagnostic concentration. Resistance to abamectin has also been observed in other surveyed populations using diagnostic concentrations lower than those used in the present study (Stumpf and Nauen, 2002; Khajehali et al., 2011). In Brazil, monitoring of *T. urticae* resistance through diagnostic concentrations of abamectin (4.79 mg/L) and fenpyroximate (46.3 mg/L) has been performed with 29 populations collected in different crops in 15 municipalities of the state of São Paulo (Sato et al., 2009). The authors observed a frequency of abamectin and fenpyroximate resistant individuals of up to 82% and 95%, respectively. The initial frequency of resistance genes and intensity of acaricide selective pressure may result in different resistance levels among populations of a certain region (Osakabe et al., 2009). However, resistance to abamectin has been shown to be unstable (Stumpf and Nauen, 2002; Sato et al., 2005, 2009), which leads to a decreasing frequency of resistant individuals after relaxing sprays, favoring management.

The climatic conditions of the Lower Middle São Francisco Valley favorably influence *T. urticae* biology. It is a region with high mean annual temperatures and low relative humidity (da Silva et al., 2009). The *T. urticae* life cycle at 30 °C is approximately 7 days (Adb El-Wahed and El-Halawany, 2012). The mean annual temperature in the Lower Middle São Francisco Valley over the last four years was 27 °C, and the relative humidity was 54% (EMBRAPA, 2013). These conditions in association with the high reproductive potential of *T. urticae* increase the population growth rate and number of annual generations and, consequently, favor the increased frequency of resistant individuals because of the intense use of abamectin. *T. urticae* can develop up to 37 generations per year under these climatic conditions (Riahi et al., 2013). In other wine producing regions of Brazil, such as the Serra Gaúcha (municipality of Bento Gonçalves) in the state of Rio Grande do Sul, the climate is temperate and humid and the mean annual temperature is 16 °C (Tonietto et al., 2012), which might explain why *T. urticae* is not considered a mite pest in this region and there are no reports of resistance of this pest to acaricides in grapevines of that state.

Most *T. urticae* resistant populations in the present study were located on the western side of the map, and most of the susceptible populations were located farther east (Fig. 1). In the Lower Middle São Francisco Valley, the predominant wind direction is from east and southeast to west (WINDFINDER, 2013), which may have influenced the distribution of resistant populations in the west part of the region. The distance and destination of mites that disperse by wind are important factors that influence population distribution (Kennedy and Smitley, 1985; Osakabe et al., 2005, 2008). Dispersion studies with mites of the family Tetranychidae suggest that wind is the main dispersion strategy over long distances (Bell et al., 2005; Bergh, 2001). The dispersion and colonization behavior of mites also affects the distribution of acaricide resistance genes (Grafton-Cardwell et al., 1991).

The LC values fluctuated in both vineyards monitored over time (Table 1). The wine production of the studied region is directed to external and domestic markets; however, in the second semester of the year, it is primarily destined for export because in this period, the Northern Hemisphere is in the off season, which contributes to a higher trade value (Araújo, 2004; Lazzaroto and Fioravanço, 2013). A reduction in the LC values was observed in the beginning and middle of the second semester in both monitored vineyards (Table 1). This is the time of year before the flowering and fruiting of grapevines. During this period, acaricide application is reduced because it is necessary to comply with the maximum residue limits allowed by law in the exported fruits. However, in vineyard 1, whose production in the first semester is destined for the domestic market, there was a consistent and progressive increase in LC values over the studied period. Coincidentally, in this vineyard, the producer kept legumes such as *C. ensiformis* and weeds between the grapevines inter rows to protect the soil, and some of these plants were *T. urticae* hosts. Although the producer controlled the mite in the vineyard, the mites must have been exposed to abamectin subdoses in non-target plants (*C. ensiformis* and weeds), favoring the increase in resistance. These mites might have subsequently migrated to the vineyard with a higher frequency of resistance. Apparently, keeping a clear grapevine crop favors resistance management.

The *T. urticae* populations resistant to abamectin tested in the present study also showed resistance to bifenthrin. Likely, resistance to abamectin and bifenthrin may have developed concurrently in the populations here assessed, as observed by Ferreira et al. (2015) in other *T. urticae* populations to abamectin and METI group. The bifenthrin dose recommended for *T. urticae* control is 50 mg/L. This concentration is much lower than the LC_{50} estimated in the present study, which shows that the bifenthrin dose recommended by the manufacturer for *T. urticae* control is insufficient to control the mite. In Belgium, a *T. urticae* population was exposed to successive bifenthrin pulverizations in rotation with other acaricides, and this population subsequently showed resistance ratios to bifenthrin of approximately 2000-fold (van Leeuwen

Table 3
Toxicity of carbosulfan and bifenthrin to *T. urticae* populations of the Lower Middle São Francisco Valley considered resistant to abamectin.

Product	Population	N[a]	$\chi 2$ (DF)	Slope ± SE[b]	LC_{50} (95% CI)[c]	LC_{95} (95% CI)[c]	RR_{50} (95% CI)[d]
Carbosulfan	Susceptible	360	2.35 (4)	2.23 ± 0.19	715 (606–846)	3902 (2913–5786)	–
	5	332	3.49 (4)	3.08 ± 0.28	720 (622–833)	2462 (1960–3350)	1.0 (0.8–1.3)
	4	327	6.54 (4)	1.80 ± 0.17	751 (515–1120)	6123 (3213–21283)	1.1 (0.8–1.4)
	10	349	4.68 (4)	2.57 ± 0.22	1079 (847–1378)	4700 (3234–8477)	1.5 (1.2–1.9)
	11	360	6.52 (4)	2.13 ± 0.18	1094 (796–1518)	6455 (3875–15944)	1.5 (1.2–1.9)
	3	360	6.54 (4)	1.80 ± 0.16	1303 (903–1876)	10563 (5788–32341)	1.8 (1.4–2.4)
	7	344	4.62 (4)	2.26 ± 0.20	1324 (1021–1729)	7076 (4617–14110)	1.9 (1.5–2.4)
	9	335	5.96 (4)	2.41 ± 0.20	1448 (1091–1931)	6931 (4491–14347)	2.0 (1.6–2.6)
	8	349	4.56 (4)	2.65 ± 0.23	1588 (1259–2015)	6612 (4591–11752)	2.2 (1.8–2.8)
	6	341	3.64 (4)	2.29 ± 0.21	2132 (1783–2527)	11095 (8377–16277)	3.0 (2.3–3.8)
Bifenthrin	Susceptible	360	1.02 (4)	2.76 ± 0.30	8.7 (7.3–10.2)	34 (27–49)	–
	8	315	3.40 (4)	2.12 ± 0.20	1411 (1158–1699)	8378 (6162–12804)	162 (125–208)
	7	343	4.02 (4)	2.07 ± 0.20	1413 (1062–1822)	8744 (5791–16984)	162 (126–208)
	6	358	3.88 (4)	2.32 ± 0.21	1585 (1337–1865)	8070 (6134–11733)	182 (143–230)
	4	311	6.09 (4)	1.97 ± 0.20	1724 (1166–2437)	11780 (6851–32827)	197 (152–256)
	9	360	1.36 (4)	2.23 ± 0.21	1753 (1457–2076)	9523 (7211–13959)	201 (157–256)
	10	345	0.94 (4)	2.45 ± 0.22	2060 (1734–2423)	9622 (7420–13679)	236 (186–299)
	11	360	6.11 (4)	1.87 ± 0.17	2844 (2000–3979)	21432 (12337–58061)	326 (253–419)
	5	336	5.81 (4)	2.71 ± 0.23	3669 (2796–4829)	14795 (9974–28460)	420 (334–528)
	3	360	6.54 (4)	2.15 ± 0.18	4271 (3122–5962)	24792 (14792–61876)	489 (385–622)

[a] Total number of mites used to calculate the concentration–response curves.
[b] Slope and standard error.
[c] Mean lethal concentration (mg/L) and confidence interval at 95%.
[d] Resistance ratio: ratio (95% CI) of the LC_{50} between resistance and susceptible populations, calculated using the Robertson and Preisler (1992) method.

et al., 2005). Conversely, in the Netherlands, 60% of the *T. urticae* populations resistant to abamectin were also resistant to bifenthrin (Khajehali et al., 2011).

The underlying mechanisms of resistance in the populations evaluated in this work have not been studied yet. Resistance of *T. urticae* to abamectin has been recently associated to point alteration at the position G326E in the glutamate-gated chloride channel (GluCl) (Dermauw et al., 2012). Additionally, Riga et al. (2014) showed that a cytochrome P450-dependent monooxygenase (CYP392A16) was also associated with high levels of resistance to abamectin. However, these authors did not find any activity of this enzyme towards bifenthrin in *T. urticae*, which may suggest no cross resistance between both acaricides, and thus, our finding strengths the hypothesis of concomitant resistance development. Resistance to bifenthrin has been showed to be linked to both para sodium channel mutations (Tsagkarakou et al., 2009) and to increased esterase metabolism (van Leeuwen and Tirry, 2007). The hypothesis of target site alteration is very likely in the Valley populations because no association with abamectin resistance was observed. Therefore, it will be important to evaluate such mechanisms in the Valleys' populations using existing diagnostic tools or to identify potential novel mutations present in those mite populations.

Among the three products registered for *T. urticae* control on grapevines in Brazil, carbosulfan was the only product to which the tested populations were not resistant. Although resistance to carbosulfan was not observed, the LC_{50} values estimated in the present study were higher than the concentration recommended for *T. urticae* control, which is 400 mg/L. The recommended concentration might not be efficient in the field even with a laboratory-susceptible population. However, carbosulfan is not used in the studied region because its metabolites (carbofuran, 3-hydroxy-carbofuran and 3-ceto-carbofuran) (Nigg et al., 1984; Soler et al., 2006) can be detected in residue analyses, making grape sales and export difficult. Carbofuran is banned from use in most of the world, including the European common market, which is an important export market (RAS, 2011).

In conclusion, the failures of *T. urticae* control with abamectin in grapevines of the Lower Middle São Francisco Valley are associated with resistance. Resistance to bifenthrin was also confirmed in the present study. Carbosulfan is not used by the producers in the region because of the possibility of detecting carbofuran in residue analyses of grapes designated for the external market. Therefore, an increase in the number of acaricides registered for *T. urticae* control in grapevine crops in Brazil could facilitate resistance management.

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