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Source: Florida Entomologist, 102(2) : 428-430

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.102.0222

Resistance to abamectin in field population of *Tetranychus urticae* Koch (Acari: Tetranychidae) associated with cut rose from state of Mexico, Mexico

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One of the major pests that affect stem roses is the twospotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), which attacks more than a thousand species of plants worldwide, and is considered difficult to manage (Grbić et al. 2011; Meena et al. 2013). Worldwide, the most common control method against this pest consists of acaricides (Van Leewen et al. 2010; Ilias et al. 2017). However, its short generation time (7.5 d at 27 ± 1 °C), and high reproductive rate (7.97 eggs per female per d) (Shih et al. 1976; Georghiou 1994), has promoted resistance to most of the compounds used for their control (Van Pottelberge et al. 2009; Ay & Yorulmaz 2010; Ay & Kara 2011; Khalighi et al. 2014; Kwon et al. 2015; Ilias et al. 2017; Pavlidi et al. 2017). This species has been considered one of the arthropod species, displaying the most reports of resistance to insecticides worldwide (Whalon et al. 2012).

For several decades, in the rose-producing region of the state of Mexico, Mexico, 1 of the active ingredients commonly used for combating *T. urticae* is abamectin, which was introduced into the market in the early 1990s. Initially, the biological effectiveness of this acaricide in the control of the two-spotted spider mite was high (> 95%). For this reason, it was used often. In 2009, *T. urticae* populations in this region were collected, and high levels of resistance to abamectin were documented (Aguilar-Medel et al. 2011). However, the growers increased the doses to reestablish the original level of control, leading gradually to higher levels of acaricide resistance, and then to larger numbers of cases of field-efficacy failures. Therefore, the objective of this study was to determine susceptibility to abamectin of 4 *T. urticae* populations from the flower-producing region of the state of Mexico.

Tetranychus urticae was collected in Apr and May of 2017 in commercial greenhouses where cut roses are produced and where records of abamectin use are kept. These greenhouses are located in the municipalities of Villa Guerrero, Tenancingo, Coatepec Harinas, and Ixtapan de la Sal in the state of Mexico. Four greenhouses were selected from each municipality, and at least 100 leaflets infested by two-spotted spider mite were collected from each. The leaflets were deposited in paper bags. They were taken to the Toxicology Laboratory of the Colegio de Postgraduados, Campus Montecillo, state of Mexico, Mexico. The specimens from the collection sites of each municipality were mixed, and the adults were transferred with a paintbrush to 35- to 40-d-old bean plants (*Phaseolus vulgaris* L., var. 'Flor de Mayo'; Fabaceae), which were then placed in cages (70 × 70 × 50 cm), covered with polypropylene fabric, Agribon® (19 g per m^2, Berry Plastics, San Luis Potosí, San Luis Potosí State, Mexico), to prevent escape of *T. urticae*, or inmigration of this or other arthropods. To obtain similar-aged mites, about 300 pairs of unsexed adults of the field-collected specimens were used to infest a bean plant ('Mayflower' var.) and allow them to lay eggs for 24 h. For each field-collection, 5 bean plants were infested. After this time, the adults were removed, so that the plants would be infested with only F1 eggs. As the susceptible population, a *T. urticae* colony was collected in the gardens of the Colegio de Postgraduados, Campus Montecillo; this population had been free of exposure to acaricides for 3 yr. Rearing was conducted under greenhouse conditions at a temperature of 27 ± 5 °C, 60 ± 10% RH, and a 16:8 h (L:D) photoperiod.

The bioassays were conducted with the commercial formulation Biomec® (abamectin, 18 g per L, emulsionable concentrate, Grupo Bioquímico Mexicano S.A de C.V., Saltillo, Coahuila State, Mexico). The required concentrations were prepared with distilled water that contained 0.25 mL per L coadjutant (Inex-A®, Cosmocel S.A., División Agrícola, San Nicolás de los Garza, Nuevo León, Mexico).

The leaf immersion bioassay for *Tetranychus* spp. adults proposed by the Insecticide Resistance Action Committee (IRAC) was used with slight modifications (IRAC 2009). Leaves were cut from the middle stratum of 35- to 40-d-old *P. vulgaris* var. 'Flor de Mayo' plants. Using a standard hole punch, 30 mm diameter discs were cut and placed underside (abaxial surface) down in a Petri dish that contained 3 mL 2% agar (Bacto™ Agar, Becton, Dickinson and Company, Mexico City, Mexico). With a paintbrush, 20 to 25 adult females 3- to 5-d-old were transferred to each leaf disc. To prevent mortality associated with handling, females that were walking were selected. Approximately 30 min after transfer, the mites were checked to assure they were not damaged, and if they were, the affected individuals were extracted. Later, the infested leaf disc was submerged in the respective concentration of acaricide for 2 s and gently shaken for 20 s to eliminate excess moisture. These submersion and shaking times were determined in preliminary tests, in which it was observed that they had no adverse effects on the treated individuals. Finally, the leaf discs were placed underside down in the Petri dishes.

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Initially, the range of concentrations that produced 0 to 100% mortality (biological response window) was determined, and later at least 6 intermediate concentrations were included to cover this range. The treated mites were kept under controlled conditions at 25 ± 2 °C, 55 ± 5% RH, and a 16:8 h (L:D) photoperiod. After 48 h of exposure, the mortality percentage was determined; a female mite was considered dead if it could not move more than twice the distance of its length (Sato et al. 2005). At least 4 replications were conducted on different d for each population; each replication included a control where the leaf disc was submerged in distilled water with 0.25 mL per L coadjutant with the same procedure described above. The maximum level of mortality accepted for the control without acaricide was 15%, and this was corrected using the Abbott formula (Abbott 1925).

The data on mortality were subjected to a Probit analysis with PROC PROBIT by means of the SAS statistical software (SAS 2016) to estimate slope, lethal concentration (LC) at 50 and 95% mortality and confidence limits at 95%. The values of relative response (RR) at 50 and 95% mortality were obtained with the quotient between LC_{50}/LC_{95} of each field population, and between LC_{50}/LC_{95} of the susceptible population, respectively. The population response was not considered statistically different when the confidence limits overlapped (Robertson & Preisler 1992).

The LC_{50} value for the susceptible population was 0.0012 mg per L, while LC_{95} was 0.008 mg per L (Table 1). Significant statistical differences were obtained in susceptibility of the field populations evaluated, relative to the susceptible population given that the confidence limits did not overlap either at the LC_{50} level or at the LC_{95} level (Table 1). The LC_{50} levels of the field populations fluctuated between 2.72 (Coatepec Harinas) and 25.37 mg per L (Ixtapan de la Sal), and the RR_{50} values were between 2,266 and 21,141 (Table 1). For LC_{95}, the values oscillated between 185.48 (Tenancingo) and 81,218 mg per L (Coatepec Harinas) with values of RR_{95} between 23,185 and 10,152,250 (Table 1). We consider that the high LC_{95} observed in the Coatepec Harinas population resulted from the small slope (0.36 ± 0.05) of its Log-Doses Probit line, in comparison with the observed value the susceptible one (2.00 ± 0.34). These results indicate high levels of resistance to abamectin in *T. urticae* field populations collected in the flower-producing region, which comprises the municipalities of Villa Guerrero, Tenancingo, Coatepec Harinas, and Ixtapan de la Sal in the state of Mexico, confirming that the control failures documented by growers in this region are due to resistance to abamectin that the two-spotted spider mite has developed. The indicated biotic potential of *T. urticae* expressed in its short life cycle and high reproductive rate, together with factors such as the high use rate of acaricides and low migration in greenhouses (Croft & Van de Baan 1988; Van Leeuwen et al. 2010), contribute to making the problem more severe in this type of production system (Ferreira et al. 2015).

Studies conducted in Mexico by Aguilar-Medel et al. (2011) have documented the response of twospotted spider mite populations to abamectin in this region, and suggest that the problem has become acute because of increasing dosages, and possibly to shortened intervals of application and scarce rotation of acaricides with different modes of action. Among the recommendations for minimizing the level of resistance, exclusion of abamectin from *T. urticae* management programs is proposed for sites where control failures have been observed. In addition, conventional acaricides with different modes of action (Georghiou 1994; IRAC 2018) and biorational products that have shown acceptable biological effectiveness (Silva-Flores et al. 2005; Attia et al. 2013) should be used, as well as other important factors in pest management, such as the use of natural enemies (Nicetic et al. 2001).

Resistance to abamectin is unstable in absence of selection pressure (Sato et al. 2005). Therefore, if its use is excluded for a given time, it is possible to decrease resistance to undetectable levels (Dennehy et al. 1990), and abamectin can be used again in two-spotted spider mite management. However, these actions should be grounded in studies that justify the renewed use of this acaricide, and the conditions of restriction of use to mitigate the evolution of resistance (Kwon et al. 2010).

In conclusion, the *T. urticae* populations collected in the municipalities of Villa Guerrero, Tenancingo, Coatepec Harinas, and Ixtapan de la Sal in the state of Mexico have high levels of resistance to abamectin, making it urgently necessary to implement integrated management programs to control this pest.

KVDA is grateful to the Consejo Nacional de Ciencia y Tecnología (CONACYT) for financial support for this research.

Summary

Cut rose growers in the municipalities of Villa Guerrero, Tanancingo, Coatepec Harinas, and Ixtapan de la Sal, in the state of Mexico, Mexico, have reported low effectiveness of abamectin in the control of *Tetranychus urticae*, possibly due to development of resistance. The objective of this study was to determine the response of 4 field populations and 1 population susceptible to this acaricide. The values of the lethal concentration (LC) at 50% of mortality fluctuated between 0.0012 to 25.37 mg per L, while the relative response (RR) at 50% of mortality of the field populations varied from 2,226 to 21,141. The values of LC at 95% mortality varied from 0.008 to 81,218 mg per L, while the RR at 95% varied from 23,185 to 10,152,250. These results reflect the levels of resistance to abamectin, and therefore, implementation of other alternatives in the management of *T. urticae* are recommended.

Key Words: resistance; ornamentals; macrocyclic lactone; acaricide

Table 1. Susceptibility to abamectin of populations of *Tetranychus urticae* Koch collected in cut rose in the state of Mexico, Mexico.

Population	n[a]	b ± SE[c]	LC_{50}[d] (CL 95%)[e]	RR_{50}[f]	LC_{95}[d] (CL 95%)[e]	RR_{95}[f]	χ^2 (df)[g]
Susceptible	747	2.00 ± 0.34	0.0012 (0.0008–0.0020)	—	0.008 (0.003–0.0404)	—	36.80 (6)
Villa Guerrero	970	1.01 ± 0.17	3.40 (1.75–18.89)	2,833	490.99 (56.37–4,087,153)	61,373	15.60 (4)
Tenancingo	1,371	0.78 ± 0.17	6.45 (4.02–11.46)	5,375	185.48 (67.36–1,227)	23,185	22.20 (5)
Coatepec Harinas	894	0.36 ± 0.05	2.72 (0.54–18.68)	2,266	81,218 (2,579–173,052,588)	10,152,250	20.40 (5)
Ixtapan de la Sal	967	0.58 ± 0.12	25.37 (7.37–375.84)	21,141	16,349 (766–648,970,731)	2,043,625	32.12 (5)

[a] = number of treated mites; b = slope value; [c] = Standard Error of slope; [d] = Lethal concentration = mg per L; [e] = Confidence limits at 95%; [f] = RR = Relative response (LC_{50} or LC_{95} of each field population; LC_{50} or LC_{95} of susceptible population); [g] = degrees of freedom.

Sumario

Productores de los municipios de Villa Guerrero, Tenancingo, Coatepec Harinas e Ixtapan de la Sal en el Estado de México, México han reportado una baja efectividad de abamectina en el control de *Tetranychus urticae* la cual posiblemente se deba al desarrollo de resistencia. El objetivo de esta investigación fue determinar la respuesta, a este acaricida, en cuatro poblaciones de campo y una susceptible. Los valores de la concentración letal (CL) al 50% de mortalidad fluctuaron de 0.0012 (Susceptible) a 25.37 mg por L (Ixtapan de la Sal), mientras que la respuesta relativa (RR) al 50% de mortalidad de las poblaciones de campo varió de 2,226 (Coatepec Harinas) a 21,141 (Ixtapan de la Sal). Los valores de la CL al 95% de mortalidad variaron de 0.008 (Susceptible) a 81,218 mg por L (Coatepec Harinas), mientras que la RR al 95% de mortalidad varió de 23,185 (Tenancingo) a 10,152,250 (Coatepec Harinas). Estos resultados demuestran un alto nivel de resistencia a abamectina, por lo que se recomienda la implementación de otras alternativas de manejo de *T. urticae*.

Palabras Clave: resistance; ornamentals; macrocyclic lactone; acaricide

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