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Authors: Chacón-Hernández, Julio C., Cerna-Chávez, Ernesto, Aguirre-Uribe, Luis A., Ochoa-Fuentes, Yisa M., Ail-Catzim, Carlos E., et al.

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Resistance of four rose varieties to *Tetranychus urticae* (Acari: Tetranychidae) under greenhouse conditions

Julio C. Chacón-Hernández[1], *Ernesto Cerna-Chávez*[2], *Luis A. Aguirre-Uribe*[2], *Yisa M. Ochoa-Fuentes*[2], *Carlos E. Ail-Catzim*[3], *and Jerónimo Landeros-Flores*[2,*]

The rose, *Rosa hybrida* L. (Rosaceae), are decorative plants of urban landscapes throughout the world (Jaskiewicz 2006; Bidarnamani et al. 2015). In Mexico, the production of the rose represents an annual economic benefit of around 2,163,000 Mexican pesos. In 2018, exports reached a value of US $6,555,000. The USA is the largest consumer, importing 99.41% of the production in Mexico (SAGARPA-SIAP 2019). The quality of the flowers of these ornamental plants is susceptible to attack by pests and diseases, principally because the pests reduce the plant growth (Golizadeh 2017). The twospotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is one of the most economically important pests in roses in both greenhouse and open field conditions, feeding on more than 1,100 plant species (Grbic et al. 2011; Khajehali et al. 2011). Control of the twospotted spider mite mostly has depended on the use of insecticides and acaricide chemicals, but due to the pest's short life cycle, abundant progeny, and arrenotic reproduction, it can quickly develop resistance to these compounds (Van Leeuwen et al. 2010). This reason brought about the study of host plants resistant to attack by these phytophagous mites. The resistance of a plant to a pest can be through various mechanisms such as antixenosis, antibiosis, tolerance, or combinations of these. Antixenosis is the non-preference of a phytophagous pest to a resistant plant, and directly affects both feeding and oviposition of the pest. Antibiosis occurs when the phytophagous pest is negatively affected, especially in its biology, by secondary metabolites produced by resistant host plants. Tolerance is a polygenic trait that allows a plant to resist or recover from damage caused by a phytophagous pest (Smith 2005; Smith & Clement 2012).

Host plants with resistance to twospotted spider mite attack have been recorded in crops such as soybeans, *Glycine max* (L.) Merrill (Fabaceae) (Sedaratian et al. 2009); melon, *Cucumis melo* L. (Cucurbitaceae) (Shoorooei et al. 2013); beans, *Phaseolus vulgaris* L. (Fabaceae) (Shoorooei et al. 2018); rose, *Rosa* spp. (Rosaceae), among others (Golizadeh et al. 2017). The intrinsic growth rate (r_m) has been used as an indicator to assess the level of resistance of plants to attack of *T. urticae* in experiments performed under laboratory conditions (Sedaratian et al. 2009; Golizadeh et al. 2017; Shoorooei et al. 2018). This study proposed to evaluate the resistance mechanisms of 4 rose varieties to the attack of *T. urticae* under greenhouse conditions.

A twospotted spider mite colony was started with biological material obtained from the Laboratory of Acarology, Parasitology Department, Antonio Narro Agrarian Autonomous University, Saltillo, Coahuila, Mexico. Twospotted spider mite populations increased on bean plants that were maintained under laboratory conditions at 27 ± 2 °C, 60 ± 15% RH, and a 16:8 h (L: D) photoperiod.

Four rose varieties were used: 'Ojo de Toro,' 'Virginia,' 'Samuray,' and 'Keiro.' Ojo de Toro, Samuray, and Keiro are new varieties introduced and cultivated in Mexico, and Virginia is a variety known for its resistance to the attack of *T. urticae* (Flores-Canales et al. 2011). Ten 1-yr-old plants per variety were used, which were planted at a distance of 10 cm in a greenhouse. The plants were fertilized with NPK once a wk for 8 consecutive wk, and mono-ammonium phosphate (12-61-0) (36.10 g), ammonium nitrate (12-00-46) (35.16 g), and urea (46-00-00) (13.75 g) diluted in 20 L of water. From the ninth wk, fertilization was suspended to avoid the possible effect of macroelements on twospotted spider mite (Ribeiro et al. 2012; Alizade et al. 2016). Twenty-five d before the infestation of the plants with twospotted spider mite, the insecticide Dibrol® 2.5 EC was applied (1 mL of the product with L^{-1} of water, to eliminate the presence of other phytophagous pests. The study was conducted at 28 ± 4 °C and RH of 60 ± 15%. Seven d after the fertilization was suspended, each plant was infested randomly with 100 newly mated *T. urticae* adult females. Seven d after the infestation, a random sampling of the twospotted spider mite population was carried out on 9 leaflets per variety. The samples were processed in the laboratory, and after counting the twospotted spider mites, the leaflets were destroyed.

Antixenosis was evaluated by the non-preference of twospotted spider mite. The number of eggs, larvae, nymphs, and adults were counted under a stereo microscope (UNICO Stereo & Zoom Microscopes ZM180, Dayton, New Jersey, USA).

The growth rate was used as the parameter to determine the antibiosis.

$$r = \frac{1}{t}\ln\left(\frac{N_t}{N_o}\right) \quad (1)$$

Where N_t corresponds to the number of individuals at time *t* (d), and N_0 is the number of individuals at time 0 (initial cohort = 100 *T. urticae* adult females) (Birch 1948; Simoni et al. 2018).

Tolerance was determined in 2 ways: (1) leaf damage index, which was estimated visually on each leaflet by an arbitrary scale proposed by Nachman and Zemek (2002), where 0 = 0% damage (no feeding

[1]Instituto de Ecología Aplicada, Universidad Autónoma de Tamaulipas, División del Golfo 356, Colonia Libertad, CP 87019, Ciudad Victoria, Tamaulipas, Mexico; E-mail: jchacon@docentes.uat.edu.mx (J. C. C. H)
[2]Departamento de Parasitología, Universidad Autónoma Agraria Antonio Narro. Calzada Antonio Narro 1923, CP 25315, Buenavista, Saltillo, Coahuila, Mexico; E-mail: jabaly1@yahoo.com (E. C. C.); luisaguirreu@yahoo.com.mx (L. A. A. U.); yisa8a@yahoo.com (Y. M. O. F.); jlanflo@hotmail.com (J. L. F.)
[3]Instituto de Ciencias Agrícolas, Universidad Autónoma de Baja California, Carretera a Delta s/n CP 21705, Ejido Nuevo León, Mexicali, Baja California, Mexico; E-mail: carlos.ail@uabc.edu.mx (C. E. A. C.)
*Corresponding author; E-mail: jlanflo@hotmail.com

damage) and 5 = 81% to 100% feeding damage (a dense mark or wilt per whole bean disc consumption), and (2) through the estimation of chlorophyll loss, expressed as a percentage (Smith 2005). A Spad 502 chlorophyll meter (Konica Minolta, Osaka, Japan) was used to measure the amount of chlorophyll: $(SPAD_C - SPAD_T) / SPAD_C) \times 100$, where $SPAD_C$ represents the reading of the SPAD in the control plant (without twospotted spider mite) and $SPAD_T$ is the reading of the SPAD in the treated plant (infested with twospotted spider mite).

Normality and homogeneity of variance tests were used to apply the analysis of variance (ANOVA) of 1-way or its equivalent (the Kruskal-Wallis test). The significant differences were analyzed with the Tukey or Nemenyi multiple comparison tests ($P \leq 0.05$) (Wheater & Cook 2005; Pohlert 2014). Significance tests for the parameter among rose varieties was conducted using the jackknife procedure (Meyer et al. 1986). The R-project program (R Core Team 2018) was used for all analyses.

Antixenosis: The number of eggs laid, larvae, nymphs, and adults of twospotted spider mite were significantly different in the 4 rose varieties (Ojo de Toro – χ^2 = 12.341; df = 3; P = 0.0063; Samuray – χ^2 = 12.944; df = 3; P = 0.0047; Keiro – χ^2 = 24.733; df = 3; P = 1.756e-06; Virginia – χ^2 = 21.026; df = 3; P = 0.0001), respectively. Oviposition nonpreference was observed on leaflets of the Samuray variety (Table 1), and the number of larvae, nymphs, and adults were fewer in Ojo de Toro and Samuray varieties.

Antibiosis: Twospotted spider mite went through their development until becoming adults in the 4 rose varieties. There were significant differences among varieties for growth rate (r) (χ^2 = 20.274; df = 3; P = 0.0001). The highest (median) r of the twospotted spider mite was observed in the Keiro variety (0.3765), followed by the varieties Virginia (0.3289), Ojo de Toro (0.2440), and Samuray (0.2047) (Fig. 1). These values suggest that the Samuray variety showed the greatest resistance to the growing population of twospotted spider mite.

Tolerance: The damage caused by the twospotted spider mite was similar in the 4 varieties (χ^2 = 5.82; df = 3; P = 0.1207). In 7 d of interaction between the varieties and twospotted spider mite, the leaf damage index medians were 1 for the Ojo de Toro, Samuray, and Keiro varieties, and 2 for Virginia variety.

Chlorophyll loss percentage in the 4 varieties was significantly different from the control plants (F = 21.749; df = 3, 32; P = 7.25e-08). The greatest chlorophyll loss was recorded in the Virginia variety ($\bar{x} \pm SD$ = 45.50 ± 4.74%), followed by Keiro ($\bar{x} \pm SD$ = 30.44 ± 12.09%), Ojo de Toro ($\bar{x} \pm SD$ = 23.74 ± 7.25%), and Samuray ($\bar{x} \pm SD$ = 15.64 ± 6.59%) (Fig. 2). Therefore, Virginia was the most susceptible and Samuray the most resistant.

The effect of antixenosis was observed in the Samuray variety. The phytochemical (alkaloids, flavonoids, terpenes, and phenols) and morphological (trichomes, leaf thickness) factors of the plants adversely affect the behavior of the arthropods, leading them to late acceptance and possible rejection of a plant as a host (Smith 2005; Smith & Clement 2012). Flores-Canales et al. (2011) documented differences in the content of terpenes, essential oils, nitrogen, and in the leaf thickness of 13 rose varieties. These factors influence the suitability of plants to be used as hosts of different arthropods (Smith 2005; Flores-Canales et al. 2011, 2014; Golizadeh 2016; Golizadeh et al. 2017). The allelochemicals (alkaloids, flavonoids, terpenes, and phenols) produced by many plants and stored in cell walls of the leaves deter feeding and oviposition of arthropods (Smith 2005). The diversity and quantity of these metabolites in plants that affect the herbivory (e.g., oviposition, mate selection, and feeding) generally are due to their genetics and environmental influence (Städler 2002; Chapman 2003; Smith & Clement 2012).

In this study, the antibiosis of rose varieties on the twospotted spider mite was indicated by the growth rate (r). The highest values of r were found in the Keiro and Virginia varieties due to the higher survival of a significant number of eggs, larvae, nymphs, and adults; therefore, the probability of passing from one development stage to another is higher. Whereas in Ojo de Toro and Samuray varieties, r values were low due to lower fecundity (antixenosis); consequently, a smaller number of larvae, nymphs, and adults were recorded. The differences among r values of twospotted spider mite on rose varieties could be attributed to the levels of secondary metabolite produced by each of them, making them susceptible or resistant (Flores-Canales et al. 2011, 2014; Golizadeh 2016; Golizadeh et al. 2017). Flores-Canales et al. (2014) demonstrated that terpenes and tannins (secondary metabolites) extracted from rose varieties negatively impacted twospotted spider mite populations.

Chlorophyll loss in plant tissues (especially in leaves) is an indicator that allows one to determine the tolerance of a plant to attack by phytophagous pests (Smith 2005). Chlorophyll values in the 4 rose varieties were different; the Samuray variety showed the lowest values (resistant), whereas the Virginia variety showed the highest (susceptible) (Fig. 2). The Kruskal-Wallis test did not show significant differences by the leaf damage index in the 4 varieties studied, but the most considerable damage was observed in the Virginia variety (median = 2). These results are consistent with the values of chlorophyll loss caused by the feeding of twospotted spider mite (Fig. 2). In varieties Samuray, Ojo de Toro, and Keiro, the values of leaf damage index were equal (median = 1), indicating only initial damage in the leaflet due to the feeding of the larvae, nymphs, and adults of twospotted spider mite after 7 d of interaction. In the Virginia variety, feeding patches tended to coalesce, but only two-thirds of the leaflet was affected. The Samuray variety was the one that showed the least loss of chlorophyll and damage; therefore, it was the one that most tolerated the twospotted spider mite attack, showing excellent response to the injury caused by the mite. This response does not cause the evolution of resistance in target pest populations by involving only one plant response to phytophagous pests; therefore, an integrated pest management scheme may be included (Peterson et al. 2017).

In conclusion, the data obtained allow us to attribute the resistance of the rose varieties to attack of the twospotted spider mite to antibiosis, antixenosis, and tolerance. The Samuray variety was the most resistant to the mite, and Keiro and Virginia varieties were the most

Table 1. Descriptive statistics of 4 stages of *Tetranychus urticae* development on 4 rose varieties.

	Eggs				Larvae				Nymphs				Adults			
Varieties	*N*	Average	SD	Median*	*N*	Average	SD	Median*	*N*	Average	SD	Median*	*N*	Average	SD	Median*
Ojo de Toro	200	22.22	24.10	12 a	78	4.11	2.26	4 b	21	2.33	2.06	2 b	42	4.67	3.20	4 b
Virginia	147	16.33	11.80	15 a	33	8.67	6.20	8 ab	117	13.00	4.12	14 a	91	10.11	2.37	10 ab
Samuray	40	4.44	3.81	3 b	161	3.67	2.12	4 b	23	2.56	2.07	2 b	48	5.33	3.57	5 b
Keiro	125	13.89	6.03	12 a	41	17.89	14.86	15 a	127	14.11	11.20	9 a	191	21.22	11.31	22 a

SD = Standard deviation. *The medians of the varieties with different letters were significantly different (P ≤ 0.05; Kruskal-Wallis test and Nemenyi test).

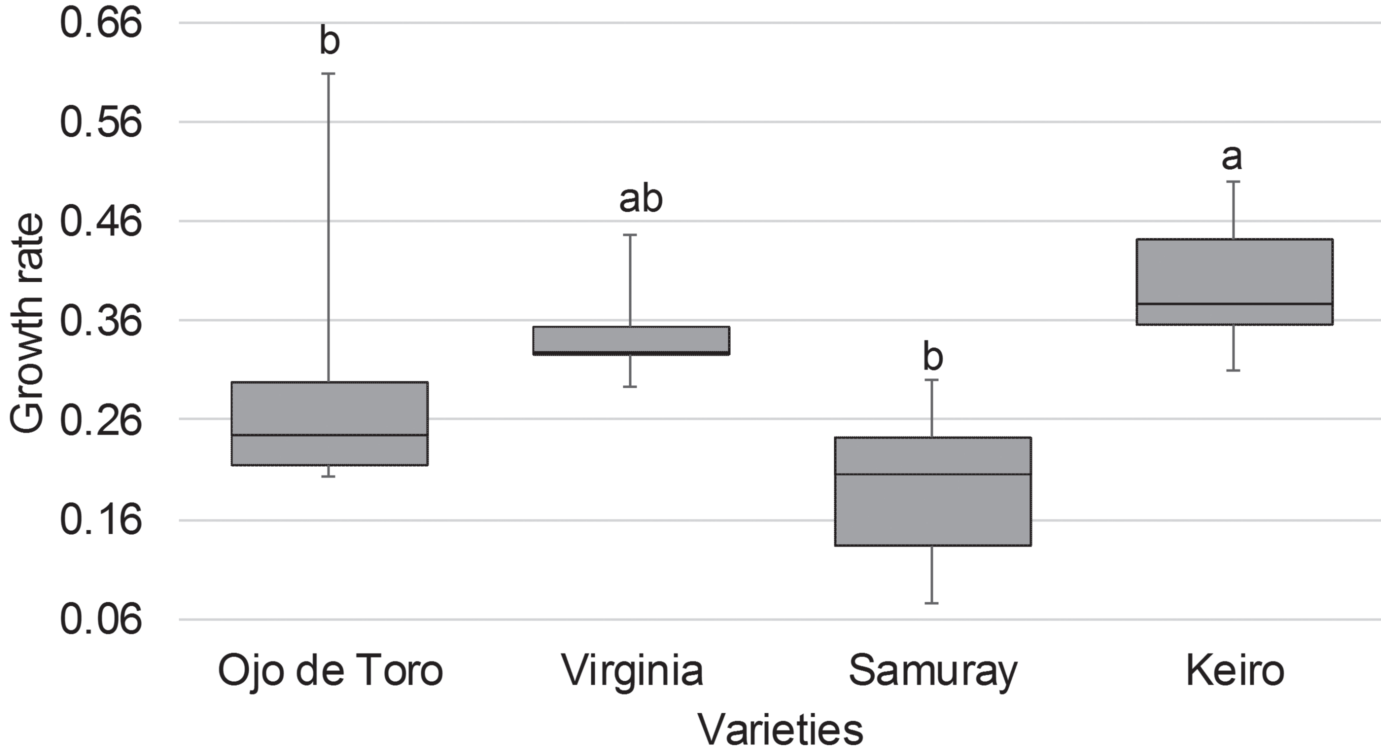

Fig. 1. Box-plot comparing growth rate (*r*) of *Tetranychus urticae* on 4 rose varieties. Varieties with different letters were significantly different (Nemenyi test, $P < 0.05$).

susceptible. These results are promising; however, further research on the morphology, detection, and quantification of secondary metabolites produced by the roses is required to be able to correlate them with their antibiotic, antixenotic, and tolerance effects to twospotted spider mite.

Summary

The rose varieties (*Rosa* spp.; Rosaceae), widely used to decorate urban landscapes, are susceptible to attacks by arthropod pests. The twospotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) is one of the most economically important pests in roses. The study proposed to evaluate the resistance mechanisms (antixenosis, antibiosis, and tolerance) of 4 rose varieties ('Ojo de Toro,' 'Samuray,' 'Virginia,' and 'Keiro') to twospotted spider mite under greenhouse conditions. The antixenosis was evaluated by non-preference of oviposition, and through the number of larvae, nymphs, and adults of twospotted spider mite; antibiosis by the growth rate (*r*); tolerance by chlorophyll loss and leaf damage index. Twospotted spider mite showed no preference to oviposit on plants of the Samuray variety (3 eggs per leaflet). Larvae, nymphs, and adults showed no preference to feed on Ojo de Toro and Samuray varieties. Twospotted spider mite recorded the lowest values in *r* (0.2047 d^{-1}) and the percentage of chlorophyll loss (15.64%) in the Samuray variety. The damage caused by twospotted spider mite in the 4 rose varieties was similar. The Samuray variety was the most resistant to twospotted spider mite, whereas the most susceptible varieties were Keiro and Virginia.

Key Words: antibiosis; antixenosis; tolerance; chlorophyll loss; leaf damage index; growth rate

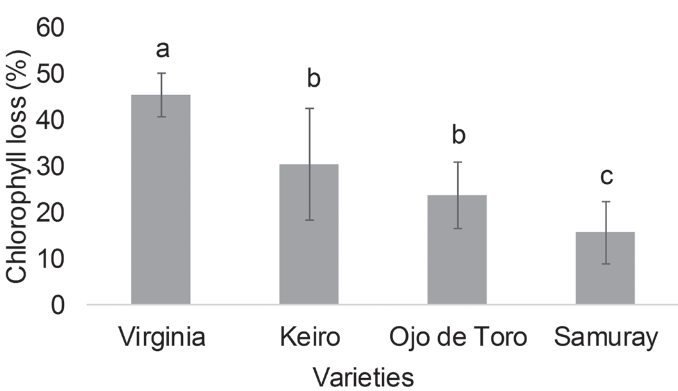

Fig. 2. Average (± SE) of the percentage of chlorophyll loss caused by the feeding of *Tetranychus urticae*. Varieties with different letters were significantly different (Tukey test, $P < 0.05$).

Sumario

Las variedades de rosas (*Rosa* spp.; Rosaceae), son ampliamente utilizadas para decorar paisajes urbanos, pero son susceptibles a los ataques de plagas de artrópodos. El ácaro de dos manchas, *Tetranychus urticae* Koch (Acari: Tetranychidae), es una de las plagas económicamente más importantes en rosas. La propuesta de este estudio fue evaluar los mecanismos de resistencia (antixenosis, antibiosis y tolerancia) de 4 variedades de rosas ('Ojo de Toro,' 'Samuray,' 'Virginia,' y 'Keiro') a ácaros de dos manchas bajo condiciones de invernadero. La antixenosis, se evaluó por la no preferencia de la oviposición y por el número de larvas, ninfas y adultos de ácaro de dos manchas; antibiosis por la tasa de crecimiento (*r*); tolerancia por pérdida de clorofila e índice de daño foliar. El ácaro de dos manchas no mostró preferencia por el oviposición en plantas de la variedad Samuray (3 huevos por foliolo). Las larvas, ninfas y adultos no mostraron preferencia por alimentarse

de las variedades Ojo de Toro y Samuray. En la variedad Samuray, el ácaro de dos manchas registró los valores más bajos en *r* (0.2047 d^{-1}) y en el porcentaje de pérdida de clorofila (15.64%). El daño causado por el ácaro de dos manchas en las 4 variedades de rosas fue similar. La variedad Samuray, fue la más resistente al ácaro araña de dos manchas, mientras que las variedades más susceptibles fueron Keiro y Virginia.

Palabras Clave: antibiosis; antixenosis; tolerancia; perdida de clorifila; índice de daño de la hoja; tasa de crecimiento

References Cited

Alizade M, Hosseini M, Modarres-Awal M, Goldani M, Hosseini A. 2016. Effects of nitrogen fertilization on population growth of two-spotted spider mite. Systematic Applied Acarology 21: 947–956.

Bidarnamani F, Sanatgar E, Shabanipoor M. 2015. Spatial distribution pattern of *Tetranychus urticae* Koch (Acari: Tetranychidae) on different *Rosa* cultivars in greenhouse Tehran. Journal of Ornamental Plants 5: 175–182.

Birch LC. 1948. The intrinsic rate of increase of an insect population. Journal of Animal Ecology 17: 15–26.

Chapman RF. 2003. Contact chemoreception in feeding by phytophagous insects. Annual Review of Entomology 48: 455–484.

Flores-Canales RJ, Mendoza-Villareal R, Landeros-Flores J, Cerna-Chávez E, Robles-Bermúdez A, Isiordia-Aquino N. 2011. Caracteres morfológicos y bioquímicos de *Rosa x hybrida* contra *Tetranychus urticae* Koch en invernadero. Revista Mexicana de Ciencias Agrícolas 3: 473–482.

Flores-Canales R, Róbles-Bermudez A, Cerna-Chávez E, Gómez-Aguilar R, Isiordia-Aquino N, Campos-Figueroa M. 2014. Relación de metabolitos secundarios de siete cultivares de rosal (*Rosa* sp.) con la dinámica poblacional de *Tetranychus urticae* Koch (Trombidiformes: Tetranychidae). Southwestern Entomologist 39: 797–804.

Golizadeh A, Ghavidel S, Razmjou J, Fathi SAA, Hassanpour M. 2017. Comparative life table analysis of *Tetranychus urticae* Koch (Acari: Tetranychidae) on ten rose cultivars. Acarologia 57: 607–616.

Golizadeh A, Jafari-Behi V, Razmjou J, Naseri B, Hassanpour M. 2016. Population growth parameters of rose aphid, *Macrosiphum rosae* (Hemiptera: Aphididae) on different rose cultivars. Neotropical Entomology 46: 100–106.

Grbić M, Van Leeuwen T, Clark RM, Rombauts S, Rouzé P, Grbić V, Osborne EJ, Dermauw W, Thi Ngoc PC, Ortego F, Hernández-Crespo P, Diaz I, Martinez M, Navajas M, Sucena É, Magalhães S, Nagy L, Pace RM, Djuranović S, Smagghe G, Iga M, Christiaens O, Veenstra JA, Ewer J, Villalobos RM, Hutter JL, Hudson SD, Velez M, Yi SV, Zeng J, Pires-daSilva A, Roch F, Cazaux M, Navarro M, Zhurov V, Acevedo G, Bjelica A, Fawcett JA, Bonnet E, Martens C, Baele G, Wissler L, Sanchez-Rodriguez A, Tirry L, Blais C, Demeestere K, Henz SR, Gregory TR, Mathieu J, Verdon L, Farinelli L, Schmutz J, Lindquist E, Feyereisen R, Van de Peer Y. 2011. The genome of *Tetranychus urticae* reveals herbivorous pest adaptations. Nature 479: 487–492.

Jaskiewicz B. 2006. The effect of the feeding of *Macrosiphum rosae* (L.) and *Chaetosiphon tetrarhodus* (Walk.) on the flowering of roses. Acta Agrobotanica 59: 515–520.

Khajehali J, Van Nieuwenhuyse P, Demaeght P, Tirry L, Van Leeuwen T. 2011. Acaricide resistance and resistance mechanisms in *Tetranychus urticae* populations from rose greenhouses in the Netherlands. Pest Management Science 67: 1424–1433.

Meyer JS, Ingersoll CG, McDonald LL, Boyce MS. 1986. Estimating uncertainty in population growth rates: Jackknife vs. bootstrap techniques. Ecology 67: 1156–1166.

Nachman G, Zemek R. 2002. Interactions in a tritrophic acarine predator-prey metapopulation system III: effects of *Tetranychus urticae* (Acari: Tetranychidae) on host plant condition. Experimental and Applied Acarology 25: 27–42.

Peterson RCD, Varella AC, Higley LG. 2017. Tolerance: the forgotten child of plant resistance. PeerJ 5: e3934. https://doi.org/10.7717/peerj.3934 (last accessed 1 Apr 2020).

Pohlert T. 2014. The Pairwise Multiple Comparison of Mean Ranks Package (PMCMR). R package. https://rdrr.io/cran/PMCMR/f/inst/doc/PMCMR.pdf (last accessed 1 Apr 2020).

R Core Team. 2018. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/ (last accessed 1 Apr 2020).

Ribeiro MGPM, Filho MM, Guedes IMR, Junqueira AMR, de-Liz RS. 2012. Effect of chemical fertilization on two-spotted-spider mite infestation and strawberry yield. Horticultura Brasileira 30: 673–680.

SAGARPA-SIAP – Secretaría de Agricultura Ganadería, Desarrollo Rural, Pesca y Alimentación-Servicio de Información Agroalimentaria y Pesquera. 2019. Panorama Agroalimentario 2019. Mexico City, Distrito Federal, Mexico. https://nube.siap.gob.mx/gobmx_publicaciones_siap/pag/2019/Atlas-Agroalimentario-2019 (last accessed 1 Apr 2020).

Sedaratian A, Fathipour Y, Moharramipour S. 2009. Evaluation of resistance in 14 soybean genotypes to *Tetranychus urticae* (Acari: Tetranychidae). Journal of Pest Science 82: 163–170.

Shoorooei M, Hoseinzadeh AH, Maali-Amiri R, Allahyari R, Torkzadeh-Mahani M. 2018. Antixenosis and antibiosis response of common bean (*Phaseolus vulgaris*) to two-spotted spider mite (*Tetranychus urticae*). Experimental and Applied Acarology 74: 365–381.

Shoorooei M, Lotfi M, Nabipour A, Mansouri AI, Kheradmand K, Zalom FG, Madadkhah E, Parsafar A. 2013. Antixenosis and antibiosis of some melon (*Cucumis melo*) genotypes to the two-spotted spider mite (*Tetranychus urticae*) and a possible mechanism for resistance. The Journal of Horticultural Science and Biotechnology 88: 73–78.

Simoni S, Angeli G, Baldessari M, Duso C. 2018. Effects of *Aculus schlechtendali* (Acari: Eriophyidae) population densities on Golden Delicious apple production. Acarologia 58: 134–144.

Smith CM [ed.]. 2005. Plant Resistance to Arthropods: Molecular and Conventional Approaches. Springer, Dordrecht, The Netherlands.

Smith CM, Clement SL. 2012. Molecular bases of plant resistance to arthropods. Annual Review of Entomology 57: 309–328.

Städler E. 2002. Plant chemical cues important for egg deposition by herbivorous insects, pp. 171–204 *In* Hilker M, Meiners T [eds], Chemoecology of Insect Eggs and Egg Deposition, Blackwell, Oxford, United Kingdom.

Van Leeuwen T, Vontas J, Tsagkarakou A, Dermauw W, Tirry L. 2010. Acaricide resistance mechanisms in the two-spotted spider mite *Tetranychus urticae* and other important Acari: a review. Insect Biochemistry and Molecular Biology 40: 563–572.

Wheater CP, Cook PA. 2005. Using statistics to understand the environment. Taylor and Francis, London, United Kingdom and New York, USA.