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Predator-prey relationship in the vertical distribution of mites on grapevines

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ABSTRACT. Phytophagous mites can cause economic losses in many crops, including grapevines. The changes in their population levels may be associated with changes in the predator-prey relationship. Knowledge of the distribution of mites in plants is important for planning sampling strategies and facilitating control decisions. The aims of this study were to (i) evaluate the abundances of Tetranychus urticae (Tetranychidae) and Neoseiulus californicus (Phytoseiidae), and the correlations between them and environmental factors; (ii) determine their distribution on the top, middle, or base strata of the evaluated grapevines (Vitis vinifera, Chardonnay cultivar) and, additionally, report the first occurrence of damage caused by T. urticae in grapevine leaves in the state of Rio Grande do Sul, Southern Brazil. Sixteen samplings were conducted, divided between the 2018 and 2019 seasons. In each sampling, three leaves from the three strata of the plant (top, middle, and base) were collected from 20 plants, totaling 60 leaves per sampling. The predator-prey relationship and their association with environmental variables were evaluated with multivariate correlation, whereas the number of mites per plant leaf strata was compared using a generalized linear mixed model in R software. It was possible to observe the symptoms of damage caused by T. urticae attacks on grapevines in Southern Brazil, characterized by the presence of yellow spots and general yellowing of the vineyard. Our findings indicate that T. urticae and N. californicus individuals are strongly associated with each other regardless of the environmental variables, and such relationship occurs mainly on the lower strata (middle and base leaves) of grapevines. Thus, by taking into account the damage on the leaves of grapevines and the occurrence of T. urticae and N. californicus majorities on specific strata of these vineyards, we suggest that the lower strata of grapevines should be the priority targets for management strategies to control such mites.

Keywords: two-spotted spider mite; biological control; pest management.

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Introduction

Cultivated plants are subject to diverse biotic and abiotic stresses. Therefore, such stressors may affect the physiology of the plants, which may compromise their productivity. For example, it is known that grapevines (*Vitis vinifera* L. [Vitaceae]) may suffer serious damage caused by fungi, nematodes, bacteria, insects, and mites, which attain pest status in regions where they find suitable environmental conditions (Schruft, 1985; Duso & Lillo, 1996).

Among the myriad of mite species, the Tetranychidae family is of agricultural importance. *Tetranychus urticae* Koch, the two-spotted spider mite (TSSM), is the most agriculturally important phytophagous mite, feeding on more than 1,100 plant species and causing economic losses in many crops, including grapevines (Bolland, Gutierrez, & Flechtmann, 1998; Grbić et al., 2011). Many control strategies are used to combat infestations of *T. urticae*. Among them, biological control is an ecological alternative in organic and conventional farming systems and is very important for integrated pest management (IPM; Baker, Green, & Loker, 2020).

Predatory mites (Phytoseiidae) are important biological control agents (McMurtry, Moraes, & Sourassou, 2013). The use of phytoseiids in European vineyards proved to be effective in the 1980s (Schruft, 1985) and they have been continually used since then (Duso, Pozzebon, Kreiter, Tixier, & Candolfi, 2012). *Neoseiulus*

californicus (McGregor; Phytoseiidae) stands out among phytoseiids; this predatory mite is widely distributed worldwide (McMurtry & Croft, 1997), observed in high abundance, and can maintain the tetranychid mite population at below the economic damage thresholds (Sato, Silva, Raga, & Souza Filho, 2005; Tixier, Baldassar, Duso, & Kreiter, 2013).

Changes in the population levels of phytophagous mites may be associated with changes in the predatorprey relationships, cultivar (Duso & Vettorazzo, 1999), predominant microenvironment (Perring, Holtzer, Toole, & Norman, 1986), and/or concentration of leaf metabolites (Perring, Archer, Krieg, & Johnson, 1983; Karban & Myers, 1989). They may also show a preference for different strata owing to factors such as radiation, temperature, rain, and wind (Ferro, Chapman, & Penman, 1979). Phytophagous mites need to create refuges in the plant, move between strata, or leave it if they need to hide or escape from predators (Magalhães, Janssen, Hanna, & Sabelis, 2002). Although this displacement is related to the odor recognition of predators, they are able to chase their prey because they are more mobile and faster than phytophagous mites (Magalhães et al., 2002). Therefore, the evaluation of ecological relationships as predator-prey and the refugees through different strata on plants where such association occurs, may play an important role in the control of mites within orchards.

Knowledge of mite distribution on plants is important for planning sampling strategies to facilitate control decisions (Candolfi, Boller, & Wermelinger, 1992; Fitzgerald, Xu, Pepper, Easterbrook, & Solomon, 2008). Alatawi, Opit, Margolies, and Nechols (2005) developed a sampling strategy for *T. urticae* based on the distribution of mites on different parts of the plant, with control thresholds based on the average number of mites per leaf, from the proportion of infested leaves. A presence-absence sampling strategy was developed for *T. urticae* and *N. californicus* on strawberry in Argentina (Greco, Sánchez, & Liljesthröm, 2005) based on the spatial coincidence of the two species in greenhouse-cultivated strawberry crops (Greco, Liljeström, & Sánchez, 1999). Studies on the dispersal pattern among plants may provide data on incidence patterns, which would help to reduce the number of sampled plants and help to make decisions on the release site for predatory mites when necessary (Kumaran, 2011).

Tetranychus urticae is found in grapevine orchards worldwide, including some Brazilian regions such as the Southeast (Valadão, Vieira, Pigari, Tabet, & Silva, 2012) and Northeast (Domingos, Melo, Oliveira, & Gondim Jr., 2014; Ferreira, Andrade, Rodrigues, Siqueira, & Gondim Jr., 2015; Moraes & Flechtmann, 2008). However, until this study, it was not reported to cause damage to vines in the state of Rio Grande do Sul, currently the largest Brazilian producer of *V. vinifera* and *Vitis labrusca* L. grapes and wines (*Instituto Brasileiro do Vinho* [Ibravin], 2018). Thus, considering the importance of this mite in terms of the potential damage it can cause to cultures and the lack of information on this species on grapevines in Southern Brazil, it is important to analyze its occurrence and correlation with *N. californicus*. Similarly, it is necessary to evaluate the plant strata where this association occurs, mainly to define management strategies. Therefore, the aims of this study were to (i) evaluate the abundances of *T. urticae* and *N. californicus*, the correlation between them and environmental factors; (ii) determine their distributions on the top, middle, or base strata of the grapevines evaluated (*V. vinifera*, Chardonnay cultivar); and, additionally, report the first occurrence of damage caused by *T. urticae* in grapevine leaves in the state of Rio Grande do Sul, Southern Brazil. We expect that there is a strong correlation between the abundances of *N. californicus* and *T. urticae*, and predict that both mites are often found on a specific plant stratum rather than by chance.

Material and methods

The study was performed in an eight-year-old commercial vineyard (*V. vinifera*, Chardonnay cultivar) with a pergola training system and conventional management, which was naturally infested with *Tetranychus urticae*. The vineyard is located in the municipality of Garibaldi, in the Serra Gaúcha region, state of Rio Grande do Sul, Southern Brazil (29°13′10.2″ S 51°35′38.8″ W). This study was observational, characterizing a real case of mite infestation in the field, and was not a controlled field experiment. Sixteen collections were conducted, divided between the seasons of 2018 and 2019 (eight between January and March 2018 and eight between November 2018 and March 2019).

Samples were taken from the sixth plant in the sixth row, counted from the vineyard edge. Leaves were collected from the chosen plant and from six other plants in the same row, with an interval of two plants between each. The 9th and 12th rows were also sampled, leaving three rows between the sampled rows that were not assessed. From the 12th row onwards, only six plants were collected, totaling 20 plants/collection (Figure 1).

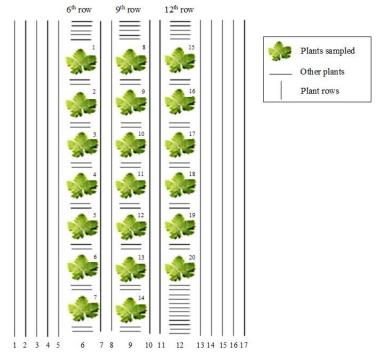


Figure 1. Schematic drawing of the position of grapevine plants sampled in the field.

In each collection, one leaf from each of three branches – one from the top, one from the middle, and one from the base stratum – of the plant were sampled, totaling 60 leaves/collection. Each leaf was individually placed in plastic bags, stored in polystyrene boxes containing reusable rigid ice under refrigeration, and taken to the Laboratory of Acarology, University of Vale do Taquari - Univates, Lajeado, Rio Grande do Sul State, Brazil for screening.

Regarding environmental variables, the maximum, mean, and minimum temperatures (°C); relative humidity (%); and rainfall (mm) were obtained from the nearest meteorological station, Estação Meteorológica São Gotardo, Garibaldi (http://clima.garibaldi.rs.gov.br/historico.aspx?EST_ID=2). The data obtained refer to an average of 24 hours on the day of each collection. The leaves collected were analyzed using a stereoscopic microscope (S6E-LED2500; Leica Microsystems) and the mites were removed from the leaves using a fine-tip brush. When more than 50 mites/leaf were found, the minimum number of mites mounted onto slides in Hoyer's medium (Jeppson, Keifer, & Baker, 1975) was 50 (maximum of 10 per slide), and the other specimens found were only counted. The mites were identified using an optical microscope with phase contrast and adequate dichotomous keys.

Data analysis

Abundance and predator-prey correlation

We calculated the mean number \pm SD (standard deviation) of *T. urticae* and *N. californicus* found per plant and per leaf. We performed a multivariate correlation analysis between the numbers of phytophagous mites (*T. urticae*) and predator mites (*N. californicus*) on the leaves of the vines and the following environmental variables: (i) mean temperature, (ii) minimum temperature, (iii) maximum temperature, (iv) relative humidity, and (v) rainfall. The correlation values (Spearman's) and concerning *p*-values were tested using the 'rcorr' function in the *Hmisc* package (Harrell Jr & Dupont, 2021).

Number of mites per plant stratum

We investigated where the majority of mites (dependent variable) were found in the plant (top, middle, and base strata: independent variables). Thus, we performed two GLMMs with a Poisson distribution and another with a negative binomial distribution to select the best model to explain our data. The random effects of GLMMs were the year, sampled plants, and repetitions. The GLMM with the best fit was chosen according to lowest Akaike Information Criterion (AIC) score, which adjusts each model's likelihood to take the number of parameters into account. The GLMMs were carried out using the 'glmer' (for Poisson distribution) and

'glmer.nb' functions (for negative binomial distribution) in the *lme4* package (Bates et al., 2015). We then computed the AIC scores from four GLMMs using the 'AICctab' function in the *bbmle* package (Bolker, R Core Team, & Giné-Vázquez, 2016). After that, we performed multiple comparisons among the plant strata using the 'glht' function, where Tukey's post-hoc test was used to determine the differences between the means in the *multcomp* package (Hothorn, Bretz & Westfall, 2008). All statistical analyses were performed using R software (Ihaka & Gentleman, 1996; R Core Team, 2018).

Results and discussion

Regarding the abundance, the average numbers of *T. urticae* found per plant and per leaf were 18.08 ± 85.38 and 6.03 ± 37.64 , respectively. For *N. californicus*, the average numbers found per plant and per leaf were 3.05 ± 9.24 and 1.02 ± 4.32 , respectively. The average temperature was 24.94 ± 6.37 °C (16-33.4°C), the relative humidity was $76.86 \pm 5.75\%$ (68.83-88.46%), and the rainfall was 53.79 ± 48.90 mm (0.04-151.40 mm).

The multivariate correlation analysis showed that the number of predator mites was positively and significantly correlated with the number of phytophagous mites, whereas the environmental variables were not correlated with mite quantity (Table 1).

The best model to determine the plant stratum most occupied by mites was the GLMM negative binomial (Table 2). After running the model, we found that mites are located at different strata on plants (GLMM negative binomial, p < 0.001, Table 2). Our multiple comparisons indicate that mites are selecting the strata nearest to the soil, as they were observed most on the middle and base strata (Table 2, Figure 2).

It was possible to visually observe the symptoms of damage caused by *T. urticae* feeding on grapevine leaves, characterized by the presence of yellow spots, in areas adjacent to the veins of the less-affected leaves (Figure 3A). On the most affected leaves, damage was generalized (Figure 3B), the vineyard showed general yellowing (Figure 3C), and it was possible to visualize mites without a microscope (Figure 3D). Abundant silk and empty casings left behind after molting were observed on both surfaces (Figure 3E-F).

N. californicus is capable of cutting silk wires with its chelicera, thus enabling it to feed on leaves highly infested with *T. urticae* (Shimoda, Kishimoto, Takabayashi, Amano, & Dicke, 2009). It can adapt to prey population fluctuations (Monteiro, 2002; Escudero & Ferragut, 2005; Greco et al., 2005; Liburd, White, Rhodes, & Browdy, 2007), with increased population reported in a region close to the study site during summer, which showed significant correlation with the presence of *Panonychus ulmi* (Koch; Acari: Tetranychidae – Toldi, Ferla, Dameda, & Majolo, 2013). Although it is a mite found in the region, *P. ulmi* was not found in this study.

		TU	NC	T. min	T. max	T. mean	RH	Rainfall	
	TU		0.005	0.53	0.33	0.87	0.33	0.43	-
	NC	0.96		0.48	0.38	0.71	0.38	0.38	
Correlation	T. min	0.19	0.20		0.70	0.53	0.03	0.01	n valuo
Correlation	T .max	-0.07	-0.05	0.10		0.05	0.07	0.81	p-value
	T. mean	0.20	0.28	0.48	0.74		0.53	0.33	
	RH	0.16	0.14	0.53	-0.23	-0.09		0.18	
	Rainfall	0.07	0.02	-0.07	0.10	-0.24	-0.09		

Table 1. Multivariate correlation between the numbers of mites and environmental variables on grapevine leaves.

TU: *Tetranychus urticae*; NC: *Neoseiulus californicus*; T. min: minimum temperature; T. max: maximum temperature; T. mean: mean temperature; RH: relative humidity. Yellow shading: values of correlation; green shading: *p*-values of correlation.

Table 2. Results of the GLMM (negative binomial distribution) and pairwise comparisons of the average number of mites on grapevine leaves.

Fixed effects	X^2	df	p-value	
Strata	104.09	2	< 0.001	
	Estimate	Std. Error	<i>z</i> -value	p-value
Base-Top = 0	1.612	0.185	8.690	< 0.001
Middle-Top = 0	1.803	0.184	9.794	< 0.001
Middle-Base $= 0$	0.191	0.150	1.277	0.202
Random effects	Variance	Std. Dev.	N	
Plants	0.072	0.269	20	
Visits	19.675	4.435	16	
Year	1.173	1.083	2	

Std. Error: Standard Error; z-value: Standard score, that is, standard deviations from their means. Negative values when the raw score is below the mean, positive when above; p-value: probability of finding z-scores by chance.

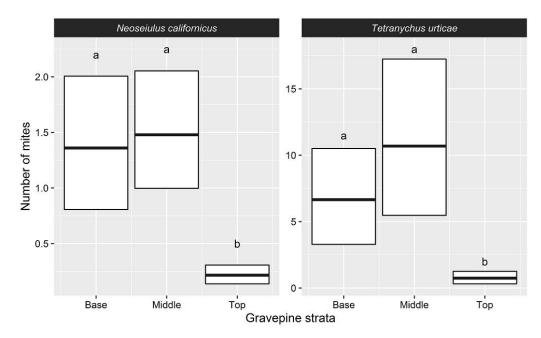


Figure 2. Pairwise comparisons of the average number of *Tetranychus urticae* and *Neoseiulus californicus* observed on the three different strata on grapevine leaves. Notes: Different letters indicate a significant difference (p < 0.001). Mean estimated via bootstrapping (999 repetitions) \pm standard deviation.

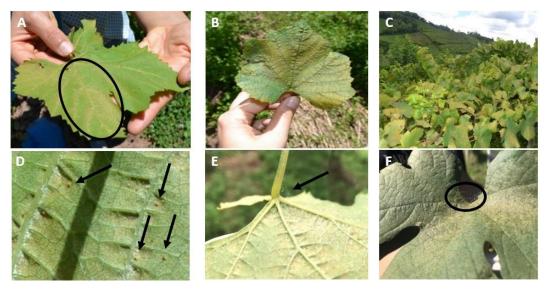


Figure 3. Symptoms of damage, visually observed, caused by *Tetranychus urticae* feeding on grapevine leaves* of Chardonnay cultivar. *Adaxial view: 3B, 3C, and 3F; Abaxial view: 3A, 3D, and 3E.

In strawberry plants, when the proportion of phytophagous/predator per leaf was 5:1, the number of active forms of *T. urticae* was significantly lower than the level of economic damage; however, in a 7.5:1 ratio, the final number of *T. urticae* reached the damage level, without exceeding it (Greco et al., 2005). In this study, a similar ratio was found: 6:1, being 6.03 *T. urticae* for 1.02 *N. californicus* per leaf, which is why it was possible to see symptoms of damage on the leaves, although the difference between strawberry leaves and vines must be considered. However, even though we found damage, there is no data on yield loss in *T. urticae*-infested grapevines in Brazil. In contrast, in India, the estimated loss due to damage by this mite was 47.2% (Veerendra, Udikeri, & Karabhantanal, 2015).

In this study, the number of predator mites was significantly correlated with the number of phytophagous mites, whereas the environmental variables tested were not correlated with mite quantity. The concentration of chemical components in the host plants may affect the population levels of herbivorous arthropods (Awmack & Leather, 2002), as well as the susceptibility of different clones of plants (Castro, Nuvoloni, & Feres, 2018). Castro et al. (2018) suggested that climatic factors played a secondary role in the population levels of phytophagous mites *Calacarus heveae* Feres (Acari: Eriophyidae) and *Tenuipalpus heveae* Baker (Acari:

Tenuipalpidae), considering that the population dynamics of these species are determined mainly by plant physiology owing to small variations in relative humidity and temperature and evenly distributed rainfall.

Reis et al. (2008) did not find significant correlations between the population levels of *Aceria guerreronis* Keifer (Acari: Eriophyidae) and phytoseiids evaluated with abiotic factors (temperature, humidity, and precipitation). Phytophagous mites play an important role in the population density of predators, and this can be an indicator of their potential as biological control agents in an IPM program (Castro et al., 2018).

Among the criteria (Walzer, Moder, & Schausberger, 2009) of an efficient predator is a high capacity for predation, a fast population increase and establishment on the plant, and adaptation to the climate location. Gotoh, Nozawa, and Yamaguchi (2004) argued that *N. californicus* was able to develop and reproduce successfully by feeding exclusively on *T. urticae* at temperatures of 18-20, 25, and 30°C, proving that this factor was of no influence, and Barber, Campbell, Crane, Lilley, and Tregidga (2003) did not find any differences in the predation rates of *N. californicus* in a relative humidity ranging from 55 to 93%.

In commercial strawberry farming in Argentina, *T. urticae* is the main pest and *N. californicus* is the main established predator, with high spatial coincidence (Greco et al., 1999). In this study, higher abundances of *T. urticae* and *N. californicus* were found on the middle and base strata. Walzer et al. (2009) verified that the distribution of *N. californicus* was similar to that of *T. urticae* – with most found in the basal and middle strata – and predation by *N. californicus* reduced the general population density of mites but did not affect its spatial distribution among the strata. It is possible that the leaves in the middle section offer an optimal niche between nutritional composition and shelter against the wind and rain. This movement seems to be controlled by a complex interaction between light intensity, temperature, and relative humidity (Gotoh, 1987), and it is known that precipitation has a negative impact on mite survival (Van de Vrie, McMurtry, & Huffaker, 1972).

Neoseiulus idaeus Denmark & Muma was only found on *T. urticae*-infested grapevine leaves (Domingos et al., 2014). Fitzgerald et al. (2008) found a higher incidence of *T. urticae* and *N. californicus* on older leaves of the plants, highlighted by a significantly positive association between both, and the incidence on different parts of the plant did not change over the sampling dates. On the grapevines evaluated, the highest predator abundance was found in the same strata as the highest phytophagous abundance, which can presumably be explained by considering that phytoseiids are driven by the search for food in the presence of odors emitted by their prey (Sabelis & Van de Baan, 1983), because *T. urticae* can look for more favorable parts of the plant, or because they were eventually attracted by volatiles emitted by the attacked plant (Sabelis et al., 1999). This suggests a significant dispersal capacity by the predator, as observed by Pratt, Monetti, and Croft (1998), as well as a high capacity to detect the presence of *T. urticae* in leaves, even when the mite has a low population density (Greco et al., 1999).

The spatial distribution of these species, as well as other characteristics, defines its spatial coincidence, which raises control success. Knowledge on the spatial coincidence, along with that of both species distribution patterns, is essential to assess the persistence of the system and the potential of the natural enemy to reduce the prey density (Greco et al., 1999).

The presence of *T. urticae* in the sampling area represents a risk to local viticulture, considering the economic importance of this species and the potential for damage and dispersal caused by tetranychids (Migeon, Nouguier, & Dorkeld, 2019). The symptoms of the damage caused by *T. urticae* on the grapevine leaves of Chardonnay cultivar were similar to those reported in other studies that evaluated infestations by this phytophagous mite on grapevine leaves. The attack by *T. urticae* on grapevines generally occurs in older leaves; the symptoms might be seen through chlorotic areas on the abaxial face and yellowish spots that develop a reddish appearance on the adaxial face (Schruft, 1985; Botton, Hickel, & Soria, 2003; Valadão et al., 2012).

Mites feeding on grapevine leaves cause damage to the cell membrane, as well as metabolic disorders and reduced amounts of chlorophyll (Sivritepe, Kumral, Erturk, Yerlikaya, & Kumral, 2009). After 24 hours, the damage is correlated with the increased expression of genes in the plant responsible for defense response and signaling, while the expression of genes responsible for photosynthesis and growth decreases (Díaz-Riquelme et al., 2016).

It is important for *T. urticae* IPM programs to evaluate mite distribution in the culture using an efficient sampling plan (Sanderson & Zhang, 1995), The main difficulties in surveying mites are inaccuracy, the size of mites, time spent counting, large populations, and profile of the sampled plant (Wilson et al., 1983; Bligaard, 2001; Opit, Margolies, & Nechols, 2003). The *Tetranychus urticae* populations in this study might have acquired the ability to survive and develop on the grapevines of Rio Grande do Sul. The ability to endure these environments might have been caused by inadequate vineyard management due to the intense use of acaricides, insecticides, and nonselective fungicides, considering that the control of tetranychids in vineyards

of Brazil is usually accomplished using pesticides, especially abamectin (Andrei, 2005). High *T. urticae* infestation on the studied Chardonnay cultivar occurred despite the presence of the predator mite, which suggests the need for monitoring. This event might have been caused by the pesticides used in conventional vineyard management; natural enemies might have been affected by the use of nonselective pesticides to *N. californicus* or other natural enemies present in these agroecosystems.

Conclusion

This work demonstrated that the population dynamics of mites in cultivated plants – in this case, grapevines – can vary greatly depending on the interactions between the different species of mites and their behavior (phytophagous vs. predator) and their preferred strata. Therefore, to ensure better vine quality and productivity, predator-prey dynamics must be observed. In addition, knowledge of the preferred stratum of both guilds can assist in future sampling studies and control strategies.

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