

# Abundance and distribution of *Aculus schlechtendali* on apple orchards in Southern of Brazil

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## Original research

### ABSTRACT

The dispersion of insects and mites can be favored by many factors, including the increasing circulation of plant materials. Special attention is needed to the eriophyoid mites, which have a greater potential as introduced species, due to their physical and biological characteristics. *Aculus schlechtendali* (Nalepa) (Eriophyidae, Apple Rust Mite - ARM) is considered an important apple pest in several countries, being recently reported for the first time in Brazil. This study aimed to carry out a survey of the abundance and distribution of ARM in the Southern region of Brazil, in the cultivars Fuji, Gala and Eva, grown in the states of Rio Grande do Sul (RS), Santa Catarina (SC) and Paraná (PR). In addition, Moran's I autocorrelation was used as an analytical tool to assess the spatial dependence between the sample points. A total of 94 orchards were sampled in 19 municipalities, distributed in the three evaluated states. Regarding cultivars, there were 40 orchards of Fuji cultivar, 43 of Gala and 11 of Eva. At each one, 20 plants were selected, from which four leaves were collected, totaling 80 leaves/orchard. The screening and identification of the mites occurred at the Laboratório de Acarologia at the Universidade do Vale do Taquari – Univates, and the statistical analysis were made using generalized linear mixed models with subsequent paired analysis, using R-software. A total of 1,647 specimens of ARM were found in 66 orchards located in 17 municipalities, with an average number (mean  $\pm$  standard deviation) of  $24 \pm 55$  mites/orchard,  $44 \pm 83$  in Fuji,  $10 \pm 19$  in Gala and  $17 \pm 21$  in Eva. The average number of mites differed between cultivars and states, with the lowest number in the cultivar Gala and in Rio Grande do Sul. No spatial autocorrelation was observed between the points, indicating that the dispersion of ARM in Southern Brazil has occurred at random, without a predefined pattern that would indicate a possible hotspot. The presence of this species serves as an alert for the apple production industry, regarding the distribution of a species previously not reported in the region. The recognition of the presence, abundance and distribution of this species will help in the monitoring and future management decisions, as well as the understanding of the distribution pattern.

**Keywords** apple rust mite; adventive mite species; apple chain

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## Introduction

The presence of pathogens and organisms considered as invasive pests has been a major threat to food security worldwide, with losses in quality and reduction in production yield estimated at 30-40% (Fisher *et al.* 2012; Flood 2010; Strange and Scott 2005). The analysis of the propagation of these organisms in agriculture must consider where they are found, where they occur and how they are spreading (Savary *et al.* 2019).

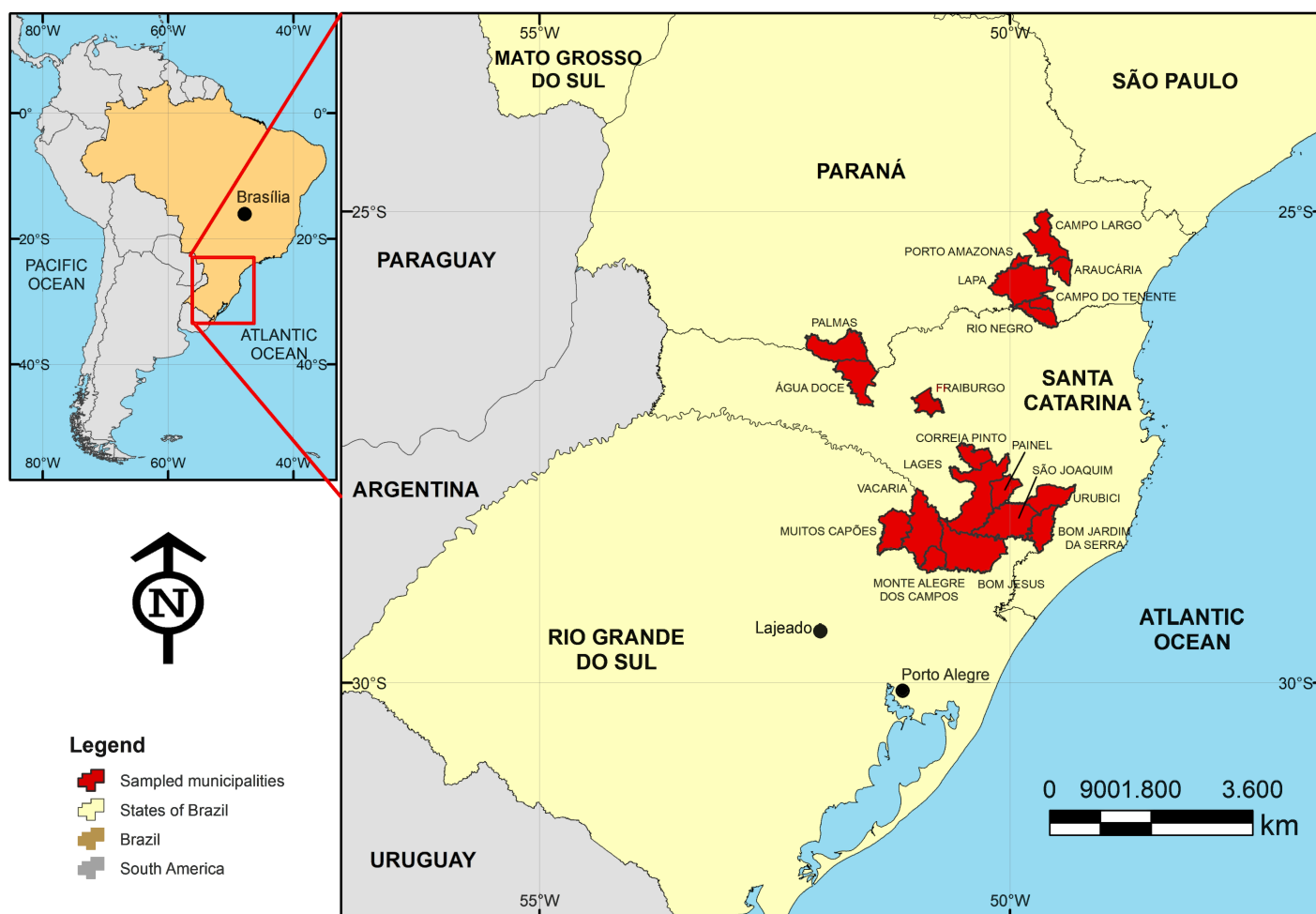
Dispersal of insects and mites through international and regional borders can be favored by the increase in circulation of plant materials. There's a scarcity of information about the loss in revenue suffered by the affected countries (Bebber *et al.* 2014; Sileshi *et al.* 2019). Phytophagous mites, specially eriophyoids, have a greater potential as adventive mite species (AMS) (Navia *et al.* 2010), which include many exotic species that can cause economic and ecological problems around the world (Wheeler and Hoebeke 2009).

These mites can become invasive, as they are hard to be detected, easily adapting to new host plants, have favorable reproductive characteristics and disperse by wind (Navia *et al.* 2007). They can also survive in adverse conditions during the dispersion, like solar radiation, high water transpiration, low temperatures/concentrations of oxygen and the absence of food (Valenzano *et al.* 2019). Two species of phytophagous mites, previously considered quarantine pests, were recently introduced in Brazil: *Raoiella indica* Hirst (Tenuipalpidae, Red Palm Mite - RPM) (Navia *et al.* 2010) and *Aculus schlechtendali* (Nalepa) (Eriophyidae, Apple Rust Mite - ARM) (Ferla *et al.* 2018).

*Aculus schlechtendali*, considered an important apple pest in many countries, feeds in flowers, fruits and leaves, using a stylet that penetrates the plant's epidermal cells (Duso *et al.* 2010; Krantz and Lindquist 1979). Light-colored spots appear in the infested leaves and end up merging, causing a rusty aspect in the attacked spots (Danelski *et al.* 2015; Hoy 2011). When the population reaches more than 50 mites/cm<sup>2</sup>, it can reduce photosynthesis, therefore setting the control risk threshold for this species at 10-40 individuals/cm<sup>2</sup> of leaf surface (Hoy 2011). The infested leaves can wither and fall with severe mite damage, which can also inhibit plant growth and causing brown spots in the fruits' peel, known as 'apple russetting' (Alford 2014; Danelski *et al.* 2015), affecting the physiological activities of plants and the aesthetical quality of the fruits (Easterbrook 1996; Hoyt 1969; Spieser *et al.* 1998; Walde *et al.* 1997).

Among the reasons for the success of apple culture in Brazil, centered in the highest regions of the three Southern states, are good environmental conditions for fruit production with high acceptance by consumers and the constant search for better production alternatives (Kist 2019a). The 2016/2017 crop year registered an apple production record in Southern Brazil, with the highest volume being produced in Santa Catarina (SC), followed by Rio Grande do Sul (RS) and Paraná (PR) (Kist 2019b). According to the data provided by Epagri (Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina), the Gala cultivar represents 56% of production in the last five years in Brazil, followed by Fuji, with 39%, and other cultivars with 6% (Assmann 2019). The cultivar Eva, the product of the cross between the cultivars Anna and Gala (Hauagge and Tsuneta 1999), is within 6% of the production of other cultivars (IAPAR 1999).

Considering that ARM is a recently reported species in Brazil, with an unknown population abundance and distribution, we hypothesize that ARM is present in the three Southern Brazil states, and its abundance varies amongst the different cultivars. The aim of this exploratory analysis was to carry out a survey of abundance and distribution of ARM in the three apple producing states in Southern Brazil, within cultivars produced in these regions. Additionally, we checked for spatial autocorrelation (dependence) between the sampling points, to determine if there is an ARM infestation hotspot.



**Figure 1** Location of the municipalities sampled in the states of Rio Grande do Sul (RS), Santa Catarina (SC) and Paraná (PR).

## Materials and methods

The study was carried out in the three apple producing states in Southern Brazil, Rio Grande do Sul (RS), Santa Catarina (SC) and Paraná (PR). A total of 94 orchards were sampled in 19 municipalities. Specifically, 27 orchards were located in four municipalities in RS, 39 in eight municipalities in SC, and 28 in seven municipalities in PR (Figure 1). Regarding cultivars, there were 40 orchards of Fuji cultivar, 43 of Gala and 11 of Eva (Table S1 – Supplementary material).

At each orchard, 20 plants were sampled once, in March 2019 at harvest point, with four leaves collected from each one, totaling 80 leaves/orchard. The first sampled plant was the sixth plant of the 6th row counted from the orchard edge, with an interval of two plants between them, totaling four plants/row in five selected rows.

The leaves were packaged in polyethylene bags, properly labeled and stored under low temperatures with reusable rigid ice (Gelox®), until they were sent to the Laboratório de Acarologia of the Universidade do Vale do Taquari – Univates, in Lajeado (RS), and screening was performed under a stereomicroscope (40x). The ARM specimens found were mounted on microscope slides using modified Berlese's medium (Amrine and Manson 1996) and kept at 50-60°C, to clarify the specimens and dry the medium. Mites were identified using optical microscope with phase contrast and the identification were made according to Amrine and Manson (1996), Baker *et al.* (1996) and de Lillo *et al.* (2010).

## Data analysis

### Average number of ARM per states and apple cultivars

It was compared if the average number of ARM would be different between the three states (Rio Grande do Sul, Santa Catarina and Paraná), as well as between the three apple cultivars (Eva, Fuji and Gala). Therefore, two generalized linear mixed models (GLMMs) with Poisson or negative binomial distribution were fitted. Both GLMMs had the average number of ARM as a response variable, while the states and the apple cultivars were included as fixed factors (predictor variable). Additionally, the municipalities and sampling places were considered as random effects. The GLMMs were carried out using the ‘glmer’ and ‘glmer.nb’ functions from the lme4 package (Bates *et al.* 2015). After that, the best-fitted LMM was chosen according to the smallest score of the Akaike Information Criterion (AIC) that was computed and compared using the ‘AICctab’ function from the bbmle package (Bolker and R Core Team 2016).

After choosing GLMMs with Poisson distribution, multiple comparisons were made among the three states and the three apple cultivars using the ‘glht’ function, with differences tested with Tukey (Holm = p-value adjustment method) in *multcomp* package (Hothorn *et al.* 2008). All analyses were performed in R (Ihaka and Gentleman 1996; R Core Team 2018).

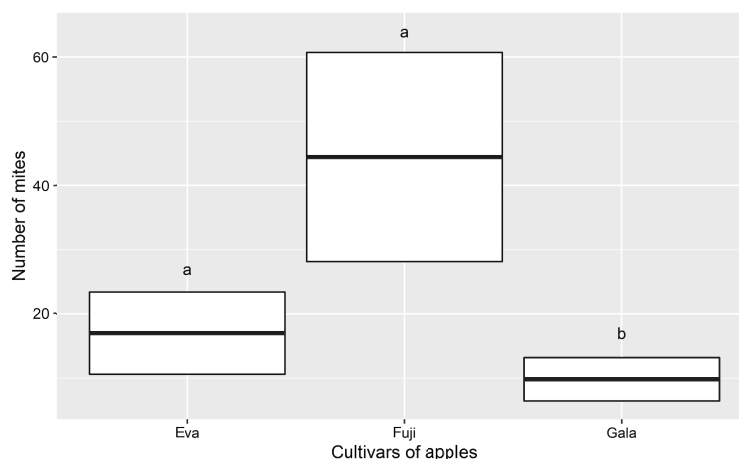
### Analysis of spatial autocorrelation (Moran's I)

Since our variable of interest (number of ARM) could be correlated with geographic distances, for example, higher values of mites if nearer other sampling locations and small values if far from it, a spatial autocorrelation analysis was performed (Global Moran's I). Such analysis evaluates whether the pattern expressed in our data is clustered, dispersed or random. Thus, it was evaluated how related would be the values of the number of ARM based on the locations where they were sampled. Moran's I was calculated after generating a matrix of inverse distance weights (latitude, longitude). On this matrix, entries for pairs of geographic coordinates that are close together are higher than for pairs of geographic coordinates that are far apart. Then, we tested if the quantity of ARM was positively (clustered) or negatively (dispersed) spatially autocorrelated. In other words, it was tested whether municipalities with similar values should be closer to each other or if high values and low values in our data would be more spatially dispersed than random. To estimate the Moran's I, the ‘Moran.I’ function was used in the *ape* library (Paradis *et al.* 2004). Furthermore, the geographic distance matrix (straight-line distance) among pairs of municipalities sampled was calculated using the ‘GeoDistanceInMetresMatrix’ function for R (<https://eurekastatistics.com/calculating-a23distance-matrix-for-geographic-points-using-r/>).

## Results

A total of 1,647 specimens of ARM were found in 66 of the 94 orchards evaluated (70%) and in 17 of the 19 municipalities sampled (89%). The average number (mean  $\pm$  standard deviation) of mites/orchard was of  $24 \pm 55$  in general. Cultivar specific means were  $44 \pm 83$  in Fuji cultivar,  $10 \pm 19$  in Gala and  $17 \pm 21$  in Eva (Figure 2).

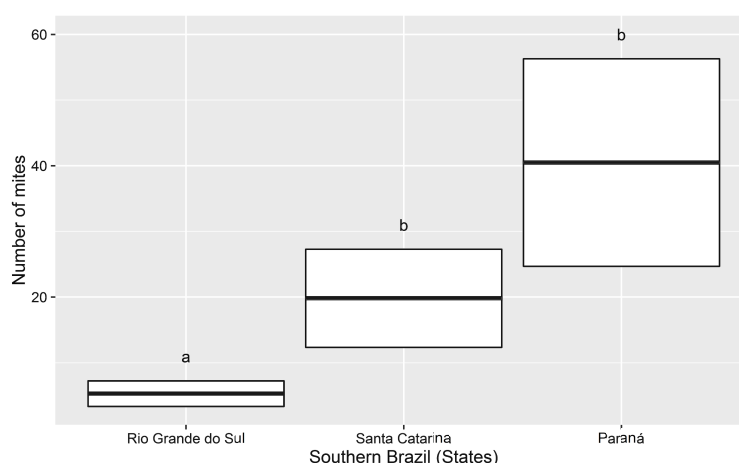
In the state of RS, the average number of mites/orchard was of  $5 \pm 7$ , with  $7 \pm 9$  in Fuji (51%) and  $4 \pm 7$  in Gala (49%). ARM was found in all orchards evaluated in this state, relatively larger abundance was observed in Muitos Capões and smaller in Vacaria. In the state of SC, the average number of mites was of  $20 \pm 39$ , with  $25 \pm 50$  in Fuji (64%) and  $14 \pm 26$  in Gala (36%). Larger abundance was observed in São Joaquim and Ubirici, and smaller in Água Doce and Correia Pinto. No mites were found in orchards located in Lages or Paineira. In the state of PR, a greater abundance of ARM was observed, with averages of  $40 \pm 79$  mites/orchard, with  $127 \pm 131$  in Fuji (75%),  $8 \pm 11$  in Gala (6%) and  $17 \pm 21$  in Eva (19%) (Table S2 – Supplementary material). In all orchards evaluated in this state, the presence of ARM was verified.



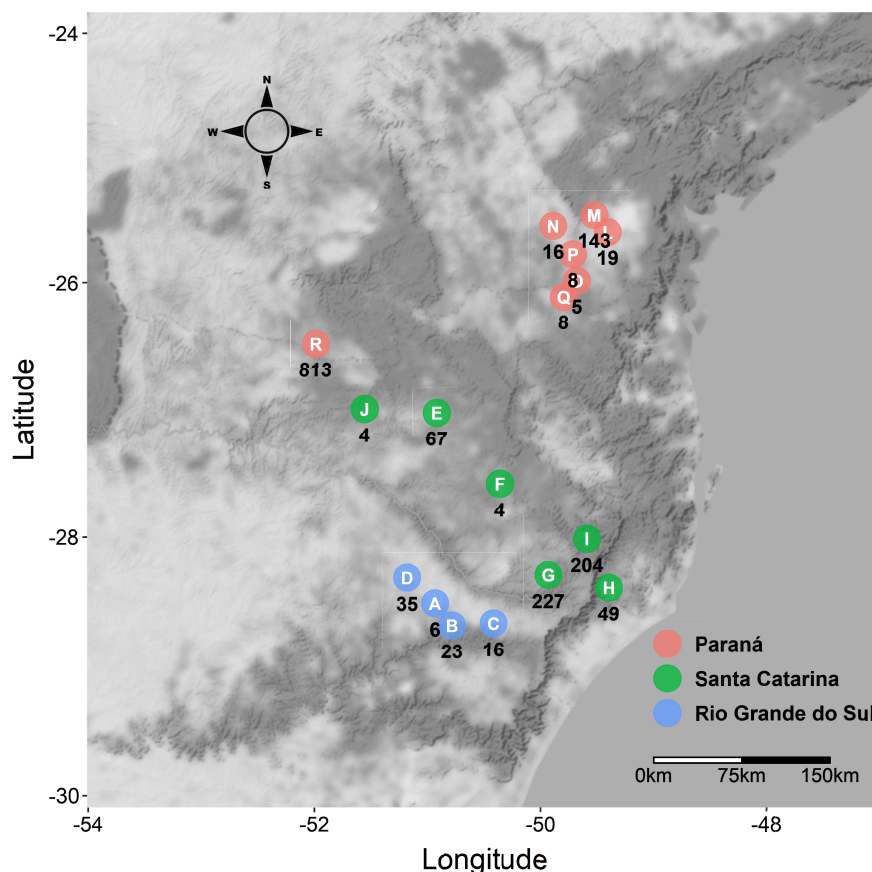
**Figure 2** Crossbar of the pairwise comparisons of the average number of ARM observed on three cultivars of apples. Notes: Different letters indicate a significant difference ( $P < 0.01$ ). Mean estimated via bootstrap (999 repetitions)  $\pm$  standard deviation.

ARM populations were smaller in Gala cultivar (Figure 2) (GLMM Poisson,  $\chi^2 = 9.02$ ,  $p = 0.01$ ) and in the state of Rio Grande do Sul (Figure 3) (GLMM Poisson,  $\chi^2 = 7.46$ ,  $p = 0.02$ ) (Table S3 – Supplementary material).

Regarding the spatial scale, we sampled at municipalities as near as 18,219 Km (Campo do Tenente (PR) – Rio Negro (PR)) and as far as 378,330 Km (Monte Alegre dos Campos (RS) – Campo Largo (PR)), (Table S4 – Supplementary material) and (Figure 4). On the other hand, our analysis of spatial autocorrelation demonstrated that the Moran's I index was -0.04, while the expected index should be -0.06 ( $\pm 0.04$  standard deviation). As a result, we did not find evidence that the number of ARM is spatially autocorrelated ( $p$ -value = 0.68, Figure 5). Therefore, there is no pattern related to the geographic distribution of ARM along their infestation within apple orchards in Southern Brazil, as well as there was no hotspot identified at this time.



**Figure 3** Crossbar of the pairwise comparisons of the average number of ARM observed on the three Southern Brazilian states. Notes: Different letters indicate a significant difference ( $P < 0.01$ ). Mean estimated via bootstrap (999 repetitions)  $\pm$  standard deviation.



**Figure 4** Municipalities and its respective states (see color legend) from Southern Brazil with apple orchards based on geographical coordinates of retrieved from SpeciesLink (<http://splink.cria.org.br/geoloc?criaLANG=pt>) where the specimens of ARM were sampled. This map was generated as “terrain-background” and source=“google” under the key= AIzaSy-CrY3pRXnB7B8Rf9Hb CMrBRu3Qsg96V7E. The letters mean name of municipalities provided in Supplementary Table (Table S4), while the numbers below points are the total quantity of ARM sampled.

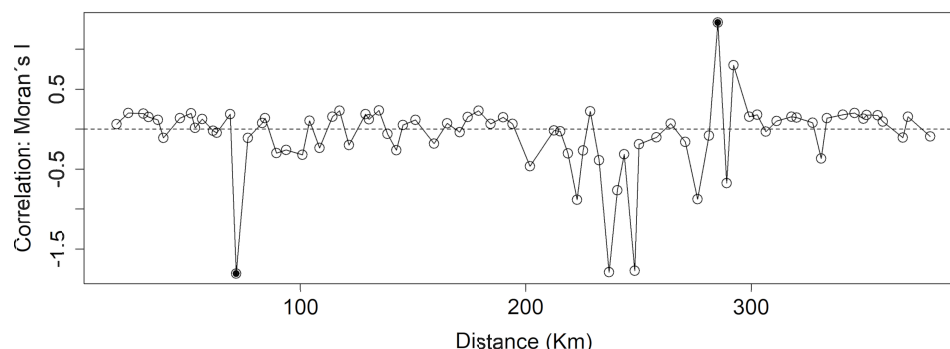
## Discussion

The first report of ARM in Brazil occurred in only one isolated point in the state of Rio Grande do Sul (Ferla *et al.* 2018) and the situation of orchards located in the states of SC and PR was unknown. Our results indicate the presence of ARM in all the producing regions of Southern Brazil, although not in all orchards. These results suggest that this mite can reach other places where apple production occurs. However, so far, there are no reports that this species has reached pest status in the regions or orchards evaluated. We strongly suggest monitoring the infestation and any possible damages, as well as identifying the associated natural enemies, which can carry out population control.

Factors such as cultivars, climatic factors (Danelski *et al.* 2015) and location of the orchard (Denizhan 2011), as well as occurrence of natural enemies (Maula and Khan 2015), may influence ARM populations. Differences among the investigated states were found, with a larger abundance of mites present in Santa Catarina and Paraná and a smaller in Rio Grande do Sul, even though the latter is the second largest producing state in volume of apples produced in Brazil. However, we suggest complementary studies in the evaluated states, in other phenological stages of apple trees, to expand the evaluation performed.

The introduction of this mite in Brazil may have occurred across borders, or due to the intense crossing of fruits from Argentina and Chile to Brazil. However, the possibility of this





**Figure 5** Correlogram. Visualization of Moran's I as a function of distance (Km) among sampling locations. Distances with significant spatial autocorrelation whose values of Moran's I are significantly larger or smaller than expected if no autocorrelated are showed in black points (Moran's I test, p-value = 0.68).

species being associated with traffic of vegetal material can't be excluded, despite the controls established in the Brazilian import system (Ferla *et al.* 2018). Besides that, long distance dispersal through air is an important survival strategy to many organisms, allowing them to swiftly colonize new territories or migrate between habitats (Gage *et al.* 1999). Some eriophyid species can travel short distances within the atmosphere layer close to the ground, while others can travel longer distances, being transported by air currents, reaching cloud stratification of where they are protected from drying or direct light action (Valenzano *et al.* 2019).

There are examples of inadvertent introductions of eriophyid mites in new areas, where they found appropriate conditions to thrive in the absence of efficient natural enemies, resulting in marked damage to infested crops and consequent serious socioeconomic problems (Navia *et al.* 2010). Considering the example of occurrence and dispersal of *Raoiella indica*, the large distance between previously infested areas by RPM in Brazil (North and Southeast) and from this to the Northeast suggests that the propagation of RPM solely by natural dispersal is improbable, and that this mite has reached new regions by transporting host plants or infested vegetal material (Melo *et al.* 2018).

*Aculus schlechtendali* was present in all the evaluated cultivars, with larger populations in the Fuji and Eva, and smaller in Gala, despite being the most representative in Brazilian apple production. Duso *et al.* (2003) also related differences in ARM abundance among the studied cultivars, being larger in Florina and smaller in N.Y. 18491. The knowledge of the difference among cultivars being important to establish the damage threshold and the correct moment for the application of control measures (Angeli *et al.* 2007).

Our hypotheses were confirmed, as there was difference in abundance amongst the cultivars and due to the presence of this mite in the three Southern states. These results may be influenced by the favorable climate for apple cultivation, although no spatial autocorrelation between the sampled locations was found, considering that the distribution in these locations was related not only to geographical proximity, but also to other factors not evaluated in the present study. Also, there was no hotspot identified, confirming that the species is distributed throughout the region.

The presence of this species in the region comes as a warning to the apple production industry, in regards to the occurrence of a species formerly considered of quarantine status. Knowledge about the abundance, places of occurrence of these mites and the cultivars affected are important for the monitoring of the species and future management measures. Additionally, continued monitoring will help in the understanding of the distribution pattern and contribute to

the comprehension of how the dispersal of this mite may have occurred in the evaluated places.

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## Disclosure statement

The authors declare that they have no conflict of interest.

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