Effects of Relative Humidity on the Vector of Rose Rosette Disease, Phyllocoptes fructiphilus (Eriophyidae), and Incidence of Disease Symptoms

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Effects of relative humidity on the vector of rose rosette disease, *Phyllocoptes fructiphilus* (Eriophyidae), and incidence of disease symptoms

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Abstract

The eriophyid mite *Phyllocoptes fructiphilus* Keifer (Acari: Eriophyidae) transmits rose rosette virus to rose (*Rosa* spp.; Rosaceae) while feeding, which causes serious disease referred to as rose rosette disease. Although there is no cure once rose plants are infected with rose rosette virus, understanding the response of *P. fructiphilus* and rose rosette disease to abiotic factors such as relative humidity may help to develop management strategies for the disease. The major objective of the current study was to determine the effect of relative humidity on the abundance of *P. fructiphilus*, as well as the incidence and severity of rose rosette disease symptoms. An experiment was conducted in environmentally controlled chambers where potted pink double knock-out rose plants were maintained at 20, 60, and 95% relative humidity after introducing about 20 *P. fructiphilus* individuals by attaching a 7-cm-long, field-collected terminal to the branches of potted plants. The densities of *P. fructiphilus* were recorded at biweekly intervals for 12 wk. The proportion of terminals with rose rosette disease symptoms (disease incidence) and severity of rose rosette disease symptoms was assessed using the Horsfall-Barratt scale at biweekly intervals for 14 wk. The results show that the number of *P. fructiphilus* individuals was significantly greater under a moderate 60% relative humidity than under a high (95%) or low (20%) relative humidity ($P < 0.05$). However, the incidence and severity of rose rosette disease symptoms were significantly higher under 95% relative humidity relative humidity regimen than under 20% relative humidity regimen ($P < 0.05$). The implications of these results on the breeding program and management of *P. fructiphilus* and the incidence of rose rosette disease are discussed.

Key Words: eriophyid mite; Eriophyidae; *Rosa* spp.; rose rosette virus; Emaravirus; nursery

Resumen

El ácaro eriófido *Phyllocoptes fructiphilus* Keifer (Acari: Eriophyidae) transmite el virus de la roseta a la rosa (*Rosa* spp.; Rosaceae) mientras se alimenta, lo que causa la grave enfermedad conocida como enfermedad de la roseta de la rosa. Aunque no existe cura una vez que las plantas de rosas están infectadas con el virus de la roseta de rosas, al comprender la respuesta de *P. fructiphilus* y la enfermedad de la roseta de rosas a factores abióticos como la humedad relativa puede ayudar a desarrollar estrategias de manejo para la enfermedad de la roseta de rosas. El objetivo principal del presente estudio fue determinar el efecto de la humedad relativa en la abundancia de *P. fructiphilus*, así como la incidencia y gravedad de los síntomas de la enfermedad de la roseta de rosas. Se realizó un experimento en cámaras de control ambiental donde se mantuvieron plantas de rosas rosadas con doble knock-out en macetas a 20, 60 y el 95% de humedad relativa después de introducir aproximadamente 20 individuos de *P. fructiphilus* mediante la colocación de una terminal recolectada en el campo de 7 cm de largo a las ramas de las plantas en macetas. Se registró la densidad de *P. fructiphilus* a intervalos quincenales durante 12 semanas. Se evaluó la proporción de terminales con síntomas de la enfermedad de la roseta de la rosa (incidencia de la enfermedad) y la gravedad de los síntomas de la enfermedad de la roseta de la rosa mediante la escala de Horsfall-Barratt a intervalos quincenales durante 14 semanas. Los resultados muestran que el número de individuos de *P. fructiphilus* fue significativamente mayor bajo una humedad relativa moderada del 60% que bajo una humedad relativa alta (95%) o baja (20%) ($P < 0.05$). Sin embargo, la incidencia y la gravedad de los síntomas de la enfermedad de la roseta de rosas fueron significativamente más altas con el régimen de humedad relativa del 95% que con el régimen de humedad relativa del 20% ($P < 0,05$). Se discuten las implicaciones de estos resultados en el programa de mejoramiento y manejo de *P. fructiphilus* y la incidencia de la enfermedad de la roseta de las rosas.

Palabras Clave: ácaro eriófido; Eriophyidae; *Rosa* spp.; virus de la roseta de la rosa; Emaravirus; guardería

Rose rosette disease is a major disease of rose (*Rosa* spp.; Rosaceae) in the landscape (Jeppson et al. 1975; Crowe 1983; Amrine 1996; Babu et al. 2014; Pemberton et al. 2018) and poses a threat to production in nurseries. Rose rosette disease is caused by the rose rosette virus (Laney et al. 2011), and an eriophyid mite, *Phyllocoptes fructiphilus* Keifer (Acari: Eriophyidae), transmits rose rosette virus (Allington et al. 1968; Amrine et al. 1988). In the US, roses are valued > $203 million, where Florida, California, Texas, North Carolina, Arizona, and Georgia are the top rose-producing states (USDA-NASS 2017). Rose rosette disease and *P. fructiphilus* have been established in western states such as

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Kansas, California, and Arizona for many yr (Wagnon & Nichols 1966, 1970; Jeppson et al. 1975; Crowe 1983), but not until recently was rose rosette disease reported from New England, mid-Atlantic and southeastern states in the US (Tipping & Sindermann 2000; Babu et al. 2014; Grant 2019; RRD EDD 2020), excluding Florida (Hoy 2013).

The rose rosette virus (Emaravirus) infects multiflora and ornamental roses. Rose rosette disease causes foliar mosaic and mottling, flower and leaf malformation, excessive thorniness, increased lateral shoot formation, young shoot reddening, stem thickening, and plant death (Jeppson et al. 1975). To date, once plants exhibit disease symptoms, there is no cure.

Phyllocoptes fructiphilus is approximately 140 to 175 µm long and is tapered at both ends (Otero-Colina et al. 2018). Eggs are oviposited on leaf bracts and sepals of the rose flower, where trichomes are abundant (Otero-Colina et al. 2018). Within 4 d, the eggs hatch, and larvae reach adulthood approximately 1 wk after molting through 2 larval and nymphal stages. Male *P. fructiphilus* release spermatophores on the plant surface, and females gather them from the plant surface through their genital opening and store them (Otero-Colina et al. 2018). The fertilized eggs develop into females, whereas nonfertilized eggs develop into males. Sexually mature adult females oviposit an egg per d and live up to 12 d. The *P. fructiphilus* population builds up from midsummer into Sep (Amrine 1996). Although eriophyid mites have been found on symptomatic roses and healthy plants, the numbers usually are very high on symptomatic roses (Amrine 1996; Otero-Colina et al. 2018). *Phyllocoptes fructiphilus* overwinters inside the ovary of rose fruit (Amrine 1996).

Previous research has shown that biological functions such as oviposition, reproduction, survival, etc., of arthropods are impacted by changes in relative humidity, e.g., western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) on rose (Fatnassi et al. 2015), the wood-boring bamboo beetle, *Dinoderus minutus* Fabricius (Coleoptera: Bostrichidae) (Norhisham et al. 2013), storage mites, *Tyrophagus putrescentiae* (Schrank) (Acari: Acaridae), *Tyrophagus neiswanderi* Johnston and Bruce (Acari: Acaridae), and *Acarus farris* (Oudemans) (Acari: Acaridae) (Sánchez-Ramos et al. 2007), and the spider mite *Tetranychus telarius* L. (Acari: Tetranychidae) (Boubreaux 1958). Relative humidity varies drastically across various regions impacted by rose rosette disease in the US (Barreca 2012). The biology of *P. fructiphilus* under various relative humidity regimes is understood poorly. In 1940, *P. fructiphilus* was first described on *Rosa californica* Cham. & Schldl. (Rosaceae) in California, USA, and it still is not reported from major rose nurseries in the southern San Joaquin Valley in California, USA. Although the exact reason is unclear, perhaps low relative humidity in the southern San Joaquin Valley does not favor population growth of *P. fructiphilus* compared to that in eastern regions of the US. Thus, it is critical to understand the performance of *P. fructiphilus* under varying relative humidity conditions. This information will improve our understanding of the ecology of *P. fructiphilus* to develop an effective, tailored, and region-specific integrated pest management program. The major objective of the current study was to determine the effect of relative humidity on both the abundance of *P. fructiphilus*, as well as on the incidence and severity of rose rosette disease symptoms.

Materials and Methods

The experiment was conducted in environmentally controlled chambers at the University of Georgia, Griffin campus, Georgia, USA, in 2018. Potted 'Pink Double Knock-Out' rose plants (3.7 L) were obtained from a wholesale nursery in Dearing, Georgia, USA. There was no incidence of rose rosette disease in the nursery or on the roses planted in surrounding landscapes. The random sepal samples were collected from rose plants and were devoid of *P. fructiphilus*. The experiment was conducted in 3 environmentally controlled growth chambers and was programmed at 20, 60 and 95% relative humidity. The temperature of all 3 chambers was set at 28 °C with a 16:8 h (L:D) photoperiod. The 4 potted rose plants were introduced into each chamber and were acclimated to the conditions in chambers for 7 d before *P. fructiphilus* was introduced. The relative humidity regimes 20, 60, and 95% were the treatments, and the 4 rose plants were the replications. To prevent desiccation, all the rose plants were monitored daily to ensure sufficient moisture in the potting soil, and were irrigated as needed for the duration of the experiment. Before introducing *P. fructiphilus* into the chamber, rose terminal samples were sampled randomly from the potted rose plants to confirm the absence of any mites on them. There were no rose rosette disease symptoms on rose plants before introducing *P. fructiphilus*.

Phyllocoptes fructiphilus-infested rose terminals (about 7 cm long) were collected from rose rosette disease symptomatic rose shrubs in the landscape within the Griffin campus. The rose rosette virus on the rose shrubs was confirmed using a modified real time reverse transcription PCR (RT-qPCR) using primers developed by Babu et al. (2016) at the plant disease diagnostic laboratory at the University of Florida North Florida Research and Education Center in Quincy, Florida, USA. On 15 Oct 2018, each plant in the chamber was infested with *P. fructiphilus* by attaching field-collected rose terminals on 2 terminals of each rose plant in the chamber using paper clips. Each rose plant received about 20 *P. fructiphilus* individuals. A rose terminal consisted of an opened flower bud and 3 leaves.

After 2 wk of infestation, 2 sepals were collected from each potted rose plant to determine the number of *P. fructiphilus* individuals. The sampling continued at 2-wk intervals for up to 12 wk. The number of *P. fructiphilus* individuals was quantified directly per sepal under 40× magnification using a dissecting microscope (Leica Microsystems, Wetzlar, Germany). *Phyllocoptes fructiphilus* has a subtriangular shield that tapers out to a point approaching the anterior end. This shield, on the mite's ventral side, allows distinction among species (Otero-Colina et al. 2018).

Approximately 10 wk after *P. fructiphilus* infestation, rose plants started to show rose rosette disease symptoms. The number of rose rosette disease symptomatic and total terminals was counted, and then the proportion of symptomatic terminals per plant was determined. The severity of rose rosette disease symptoms was assessed using the Horsfall-Barratt scale starting 10 wk post-infestation (Horsfall & Barratt 1945). To confirm rose rosette virus, the rose rosette disease symptomatic and non-symptomatic leaves were collected randomly from rose plants in the chambers, and leaf tissues were tested using the modified RT-qPCR technique mentioned above at 12 wk post-infestation.

The *P. fructiphilus* data were subjected to ANOVA by wk using the PROC GLM general linear model procedure in SAS (SAS 2012). The data were square-root transformed to establish homogeneity of variance using the PROC Univariate procedure in SAS (2012) before analysis, where relative humidity was the treatment factor. To determine the overall effect of relative humidity treatments, the number of *P. fructiphilus* collected over 12 wk was pooled, square-root transformed, and subjected to ANOVA by wk using the general linear model procedure in SAS (2012). The data on the incidence of rose rosette disease symptoms were log-transformed (ln[x + 1]), whereas the severity of rose rosette disease symptoms assessed using the Horsfall-Barratt scale was arcsine square-root transformed before analysis. The transformed data were subjected to ANOVA by wk using the general lin-

ear model procedure in SAS (2012). The means were separated using Tukey's HSD test for treatment comparisons. All statistical comparisons were considered significant at $\alpha = 0.05$.

Results

The number of *P. fructiphilus* was significantly greater in sepals with 60% relative humidity than in those with 20% relative humidity at 2 wk after infestation ($F = 4.1$; df = 2, 14; $P = 0.040$; Fig. 1A). At 4 wk after infestation, there was no significant difference in the number of *P. fructiphilus* among relative humidity levels ($F = 2.5$; df = 2, 14; $P = 0.116$). A significantly greater number of *P. fructiphilus* occurred on sepals under 60% relative humidity than under 20% or 95% relative humidity at 6 wk after infestation ($F = 6.1$; df = 2, 14; $P = 0.013$; Fig. 1A). The number of *P. fructiphilus* was not significantly different in sepals among relative humidity levels at 8 wk after infestation ($F = 3.1$; df = 2, 14; $P = 0.082$). Overall, the number of *P. fructiphilus* was significantly greater on sepals under 60% relative humidity than under 20 or 95% relative humidity ($F = 5.7$; df = 2, 14; $P = 0.015$; Fig. 1B).

At 10 wk after infestation, there was no difference in the incidence of rose rosette disease ($F = 4.2$; df = 2, 6; $P = 0.073$; Fig. 2A). The Horsfall-Barratt scale showed that the severity of rose rosette disease on plants was significantly greater under 95% relative humidity than under 20% relative humidity ($F = 8.9$; df = 2, 6; $P = 0.016$; Fig. 2B). At 12 wk after infestation, the incidence of rose rosette disease symptoms on plants was significantly greater under 95% relative humidity than under 20 and 60% relative humidity ($F = 8.7$; df = 2, 6; $P = 0.017$; Fig. 2A). The Horsfall-Barratt scale showed that the severity of rose rosette disease on plants was significantly greater under 95% relative humidity than under 20% relative humidity ($F = 10.3$; df = 2, 6; $P = 0.012$; Fig. 2B). At 10 and 12 wk after infestation, the severity of rose rosette disease on plants was not significantly different between 60 and 95% relative humidity. At 14 wk after infestation, the incidence of rose rosette disease was not significantly different on 14 wk after infestation ($F = 3.8$; df = 2, 6; $P = 0.087$). Rose rosette virus was confirmed on symptomatic plant samples under various relative humidity regimes. The severity of rose rosette disease on plants was significantly greater under 95% relative humidity than under 20 and 60% relative humidity ($F = 13.6$; df = 2, 6; $P = 0.005$; Fig. 2B).

Discussion

The results show that the number of *P. fructiphilus* individuals was greater under a moderate relative humidity (60%) than under a high relative humidity (95%) or low relative humidity (20%). Previous studies on a spider mite, *T. telarius*, showed that the total oviposition of female *T. telarius* was reduced by more than half when the relative humidity increased to 95% from 35% (Boubreaux 1958). Variation in relative humidity also can affect biological control activity. The predaceous mite *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) provided greater pest control on the two-spotted spider mite *Tetranychus urticae* (Koch) (Acari: Tetranychidae) under 60 to 85% relative humidity than under lower relative humidity (Stenseth 1979). This suggests that the performance of a specific biological control agent should be studied carefully to match when the abundance of *P. fructiphilus* is high across various stages. The success of biological control could be increased if the agent(s) could reduce the *P. fructiphilus* to very low density, because few individuals could transmit the rose rosette virus through feeding.

In the current study, the incidence and severity of rose rosette disease symptoms were noticeably higher under the high (95%) relative humidity regimen than under the low (20%) relative humidity regimen. This result could impact rose breeding programs. Rose genotypes and cultivars are field-tested for various horticultural attributes and resistance to diseases, including rose rosette disease (Byrne 2015). The data suggest that rose genotypes tested for horticultural attributes and disease resistance to other than rose rosette disease research may not necessarily express rose rosette disease symptoms if infected with rose rosette virus, and viable studies could be conducted. In contrast, rose rosette disease genotype or cultivar screening should be conducted under high relative humidity conditions to ensure reliable results. The rose plants maintained in the greenhouse at 21 °C and about 40% relative humidity with no exposure to rose rosette virus and *P. fructiphilus* did not elicit any rose rosette disease symptoms. In Florida, *P. fructiphi-*

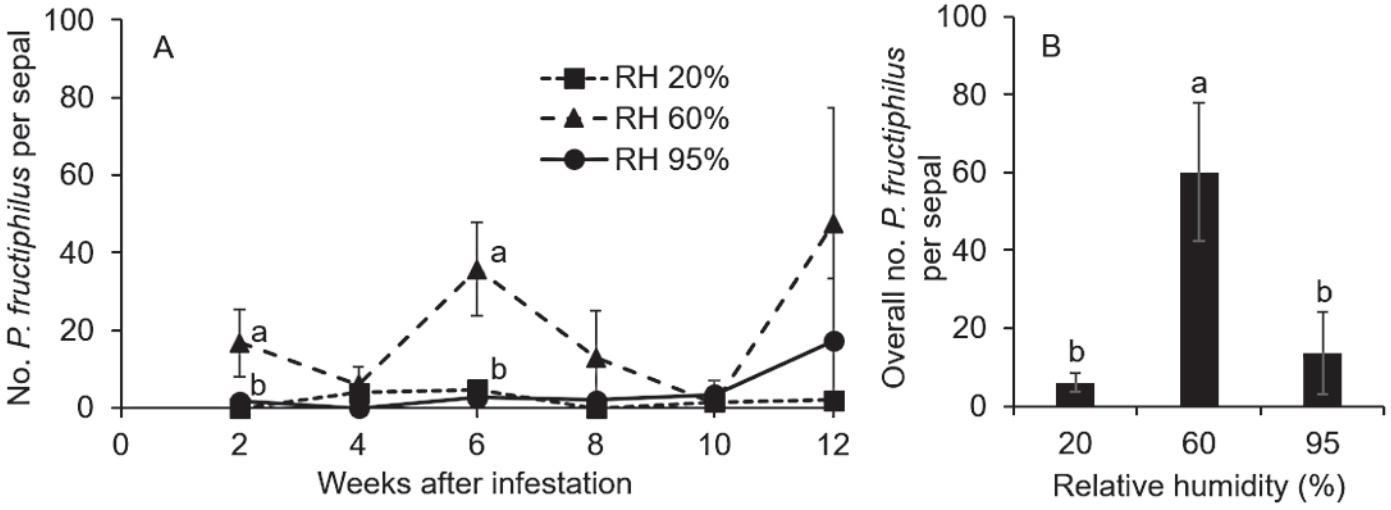

Fig. 1. Mean (± SE) number of *Phyllocoptes fructiphilus* under various relative humidity regimes (A) by wk and (B) for the duration of the experiment. The same letters within a wk after infestation or bars are not significantly different (ANOVA followed by Tukey's HSD test; $\alpha = 0.05$). Where no differences were observed, no letters are included.

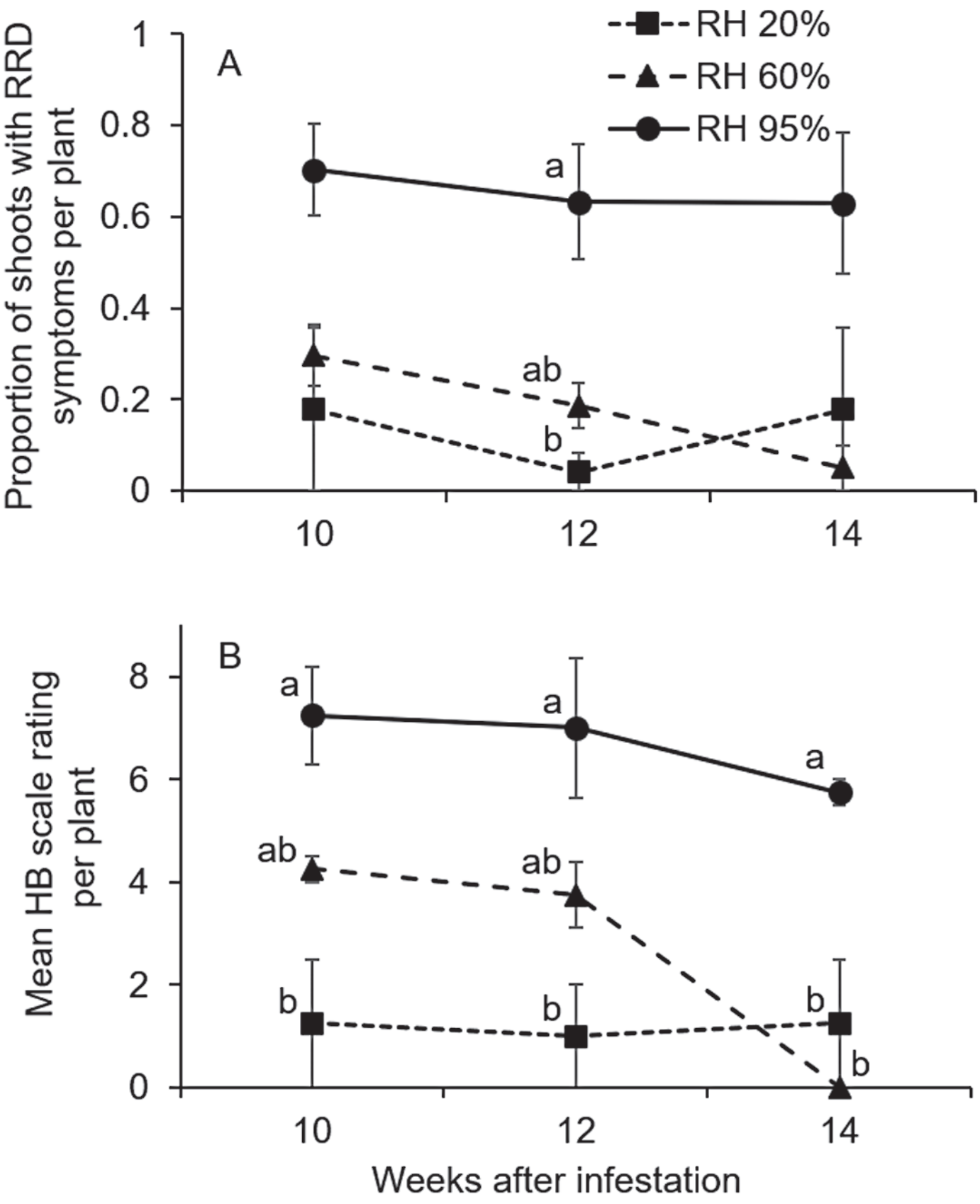

Fig. 2. Mean (± SE) (A) proportion of rose rosette disease symptomatic terminals and (B) value of the Horsfall-Barratt scale on the severity of rose rosette disease. The same letters within a wk after infestation are not significantly different (ANOVA followed by Tukey's HSD test; $\alpha = 0.05$). Where no differences were observed, no letters are included.

lus populations developing on roses without rose rosette virus infection were asymptomatic (Fife et al. 2020).

Relative humidity can vary drastically from western states to eastern states in the US (Barreca 2012). The data suggest that region-specific integrated pest management strategies for *P. fructiphilus* and rose rosette disease should be developed. For example, based on the data, rose rosette virus infected rose plants in a region with high relative humidity ranges have a high probability of rose rosette disease symptom expression. Thus, these rose plants may need comprehensive management to reduce rose rosette virus transmission. In contrast, those plants shipped from the regions with low relative humidity should be subjected to rigorous diagnostic tests, especially before being shipped to regions with high relative humidity. More studies are warranted to determine the effects of relative humidity under low- and high-temperature regimes to improve the management of vector and reduce rose rosette virus transmission.

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