Rev. Fitotec. Mex. Vol. 45 (2): 227-233, 2022

EFFECT OF *Planococcus ficus* (SIGNORET) ON THE GROWTH OF THREE WINE GRAPE CULTIVARS

EFECTO DE *Planoccus ficus* (SIGNORET) SOBRE EL CRECIMIENTO DE TRES VARIEDADES DE UVA DE VINIFICACIÓN

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SUMMARY

Damage caused by herbivores reduces plant survival and growth through its effects on mechanisms involved in tolerance to herbivory. Infestation by sap-sucking insects negatively affects the expression of metabolites related to photosynthesis and growth traits, which in an agricultural context can be useful for designing pest management and crop breeding programs. The effect of infestation by the vine mealybug *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae) on chlorophyll content and plant growth in three varieties of *Vitis vinifera* L. (Garnacha, Tempranillo and Nebbiolo) was evaluated. A common garden experiment was conducted with 60 plants of each variety and two *P. ficus* infestation treatments (infested and non-infested). Plant height, number of leaves, leaf area and chlorophyll content index were measured before and after *P. ficus* infestation for each plant and, additionally, the relative change rate (RCR) for each trait was estimated. The infestation reduced the RCR of the number of leaves (22.57 %) and leaf area (41.65 %). The effect of infestation on plant growth changed between varieties. Nebbiolo plants with infestation showed a reduction of RCR in plant height compared to non-infested plants (57.32 %), while Garnacha and Tempranillo varieties showed similar values in both treatments. A negative effect of *P. ficus* infestation on plant height, number of leaves and leaf area was found. Results suggest that the Nebbiolo variety is less tolerant to *P. ficus* attack. Farmers should adopt more rigorous monitoring and control measures when growing this variety.

Index words: leaf number, leaf size, plant height, Pseudococcidae, tolerance to herbivory.

RESUMEN

El daño causado por los herbívoros reduce la supervivencia y el crecimiento de las plantas mediante sus efectos sobre los mecanismos implicados en la tolerancia a la herbivoría. La infestación por insectos chupadores de savia afecta negativamente la expresión de los metabolitos relacionados con la fotosíntesis y el crecimiento, que en un contexto agrícola pueden ser útiles para diseñar programas de gestión de plagas y de mejora de cultivos. Se evaluó el efecto de la infestación por el piojo harinoso de la vid *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae) sobre el contenido de clorofila y el crecimiento vegetal en tres variedades de *Vitis vinifera* L. (Garnacha, Tempranillo y Nebbiolo). Se realizó un experimento de jardín común con 60 plantas de cada variedad y dos tratamientos de infestación por *P. ficus* (infestado y no infestado). Se midió la altura de la planta, número de hojas, área foliar y el índice de contenido de clorofila antes y después de la infestación para cada planta y adicionalmente se estimó la tasa de cambio relativa (TCR) para cada carácter. La infestación redujo la TCR del número de hojas (22.57 %) y el área foliar (41.65 %). El efecto de la infestación sobre el crecimiento de la planta cambió entre las variedades. Las plantas de Nebbiolo con infestación mostraron una reducción en la TCR de la altura de planta en comparación con las plantas no infestadas (57.32 %), mientras que las variedades Garnacha y Tempranillo mostraron valores similares en ambos tratamientos. Se encontró un efecto negativo de la infestación por *P. ficus* en la altura de planta, número de hojas y área foliar. Los resultados sugieren que la variedad Nebbiolo es menos tolerante al ataque de *P. ficus.* Los agricultores deben adoptar medidas de seguimiento y control más rigurosas cuando cultiven esta variedad.

Palabras clave: Altura de planta, número de hojas, Pseudococcidae, tamaño de las hojas, tolerancia a la herbivoría.

INTRODUCTION

The impact of herbivores on plants is extensive, removing more than 20 % of annual net primary productivity (Agrawal, 2011). Damage caused by herbivores in agricultural systems has meant great economic losses, comparable to the combined effects of drought and frost (Coley *et al.,* 1985; Oerke, 2006). It has been estimated that crop losses due to damage caused by arthropods can exceed 15 % annually (Mitchell *et al.,* 2016). Although global productivity levels increased in agricultural systems during the 20th century, so did economic losses due to herbivore attack, despite extensive use of pesticides (Oerke *et al.,* 2014). This context is a challenge to seek sustainable alternatives for agricultural production that depend much less on chemical inputs such as pesticides. An emerging way to address this challenge is to exploit the standing defensive strategies of plants in a broader way (Mitchell *et al.,* 2016).

Changes in the expression of traits before and after herbivore attack, such as compensatory growth or increased chlorophyll content, are associated with

Recibido: 06 de julio de 2021
Aceptado: 07 de marzo de 2022

DOI: https://doi.org/10.35196/rfm.2022.2.227

tolerance to herbivory (Tiffin, 2000); that is, the defensive strategy that confers plants the ability to reduce the negative effect of herbivores on crop yield once herbivory has occurred (Agrawal, 2011; Simms and Triplett, 1994; Stout, 2013). Theoretical models indicate that since plant tolerance attributes generally have no effect on herbivore fitness, they are unlikely to impose a selective pressure on herbivores. This would limit the evolution of herbivore resistance to plant defenses (Garrido and Fornoni, 2006; Weis and Franks, 2006), pointing out that traits associated with tolerance to herbivory may represent reliable strategies for long-term pest control (Mitchell *et al.,* 2016).

The domestication of plants for agriculture has favored desirable attributes that maximize yield and quality of harvested product in high-input environments (Meyer *et al.,* 2012); however, it has also generated modern varieties with relatively low levels of genetic diversity (Doebley *et al.,* 2006; Khush, 2001; Wright *et al.,* 2005). This reduction in genetic variability could constrain the ability of these varieties to tolerate attack by herbivores (Bello-Bedoy and Núñez-Farfán, 2011; Stout, 2013; Weinig *et al.,* 2003); thus, it is relevant to study the different plant traits and mechanisms associated with tolerance to herbivory in domesticated plants. In the same vein, it is essential to identify potentially tolerant cultivated varieties, with the aim of integrating them into pest management programs and crop improvement strategies (Goggin *et al.,* 2015).

Planococcus ficus (Signoret) (Hemiptera: Pseudococcidae), popularly known as vine mealybug, is a phloem-feeding insect species and has become a key pest in vineyards around the world (Naegele *et al.,* 2020). Recently, it became an important plague in Southern California and Mexico. The infestation of the vines by this mealybug has meant great losses for the wine market in the United States and in the state of Sonora (Mexico), affecting up to 100 % of the yield in the agricultural region of the Coast of Hermosillo (Daane *et al*., 2006; Fu *et al.,* 2004), presents a series of characteristics that make it a serious threat to viticulture. It has a fast life cycle (4-7 generations per year) and a high reproductive rate, with some females laying more than 250 eggs (Daane *et al.,* 2006).

Timm and Reineke (2014) found that grapevine plants respond weakly at the transcriptional level to the attack inflicted by *P. ficus*; however, the damage induced a lower expression of genes and transcripts related to plant defense, suggesting that other phenotypic traits related to tolerance may help the grapevine to defend itself against this pest. Recently, Naegele *et al.* (2020) studied the resistance to *P. ficus* of ten grapevine lines (species, cultivars and rootstocks), finding significant differences between cultivars and rootstocks in the number of *P. ficus* (estimated as juveniles, adults and egg sacs), being Cabernet Sauvignon and Chardonnay the most favorable grape cultivars for mealybug growth; however, the effect of *P. ficus* attack on tolerance-related traits such as plant growth or chlorophyll content of different grapevines cultivars has not been sufficiently studied.

The first year of growth is crucial in the life cycle of the vines since they must develop a strong root system that allows them to sustain their growth in subsequent years. Poor growth during the first year can increase the time required to fully train vines and the time required to achieve full production potential of the vineyard (Bettiga, 2015); thus, this study was carried out to assess the effect of *Planococcus ficus* (Signoret) on plant growth of three different grapevine cultivars for winemaking; specifically, leaf chlorophyll content and plant growth (plant height, number of leaves, and leaf area per plant) were compared between infested and non-infested plants. This assessment will help to understand the role of these traits in tolerance to *P. ficus* infestation of three of the most important varieties of *Vitis vinifera* L. (Garnacha, Tempranillo and Nebbiolo) grown in Ensenada, Baja California, Mexico.

MATERIALS AND METHODS

Experimental site and gentic material

A common garden experiment was performed from April to September 2018 in an experimental vineyard located at Ensenada, Baja California, Mexico. During the experiment the average temperature was 21.4 °C, while precipitation was 5.36 mm. Plants from three of the most important varieties of *Vitis vinifera* in Baja California were used, Garnacha, Tempranillo and Nebbiolo (OEIDRS, 2011). Sixty cuttings per variety were planted in 20-L pots filled with a sterile 70 % Vigoro professional soil + 30 % peat moss mix that were spaced 2 m apart. Plants of each variety belonged to the same clone, which were donated by a certified local farm.

Treatments and experimental design

Two treatments of infestation by *P. ficus* (infested and non-infested) were applied. Plants assigned to the infestation treatment were infested with 20 individuals of *P. ficus* (third instar females) obtained from an experimental colony that was installed in a controlled environment room (Mansour *et al.* 2017). To ensure similar levels of *P. ficus* between varieties of the infestation treatment throughout the experiment, the Infestation with females of *P. ficus* was repeated twice during the experiment, at the third and fourth months after the start of the experiment.

To ensure that the degree of *P. ficus* infestation was the same between varieties, the number of adult females on the abaxial side of five randomly selected mature leaves per plant was counted in a subsample of 15 plants per variety six months after the start of the experiment, which was verified by the absence of significant differences between varieties in the mean number of adult females of *P. ficus* in leaves (F = 1.47, d.f. = 2, P = 0.2302; Nebbiolo = 9.24, SE = 0.47; Garnacha = 8.44, SE = 0.48; Tempranillo = 9.32, SE = 0.15).

Plants assigned to the non-infested treatment were kept free of *P. ficus* by applying 250 mL of the systemic insecticide Imidacloprid at a concentration of 0.3 mL L^{-1} following the manufacturer recommendation. The insecticide solution was applied through the irrigation system three times throughout the experiment (beginning of April, June and August).

To control the effect of water accumulation caused by the slope of the vineyard where the experiment was carried out, plants were arranged in a randomized two-block design; each treatment being replicated 15 times (N = 180 plants). During the experiment the plants showed no signs of any other pest or disease.

Variables measured

Plant height was measured in m, number of leaves were counted and the average area of 20 fully developed leaves per plant in cm^2 was measured using an image analyzer (ImageJ ver. 1.5); in addition, the chlorophyll content index (CCI) was measured in each plant as an indirect estimation of total chlorophyl content in leaves (Cisneros-Silva *et al*., 2017; Filimon *et al*., 2016) with the help of a colorimeter (CCM-200, Opti-Science, Hudson, New Hampshire, USA) from a sample of 10 mature leaves per plant. All measurements were conducted twice after the start of the experiment, before and after *P. ficus* infestation, at the second and sixth months, respectively. To avoid bias due to initial shoot size and sprouting the relative change rate (RCR) for each of the traits was estimated. Following the methodology of Bello-Bedoy and Núñez-Farfán (2011), individual RCR was estimated as $RCR_i = (\ln Pt_2 - \ln Pt_1)/T_{DN}$, where Pt_1 and Pt_2 are, respectively, the first and second measurements carried out; T_{DN} is the number of days elapsed between the first and the second measurements.

The effect of *P. ficus* infestation on RCR of leaf number, leaf area, plant height and CCI of each variety was evaluated by means of an ANOVA mixed-model. Whenever an ANOVA model result was significant, the respective Tukey-Kramer was performed as a *post hoc* test to detect differences between levels of the treatments. Statistical analyses were performed with JMP ver. 14.0.

RESULTS

The ANOVA showed a significant effect of variety and infestation levels on plant height RCR (Table 1A). Garnacha had significantly lower plant height RCR compared to the Nebbiolo and Tempranillo varieties (33.8 and 38.4 % respectively), which did not differ significantly from each other. Infested plants showed 17.9 % reduction in plant height RCR compared to non-infested plants; however, the analysis also detected a significant effect of variety × infestation on plant height RCR (Table 1A), indicating that the effects of infestation on RCR depended on the variety. Nebbiolo showed a significant (57.3 %) reduction in plant height RCR when it was infested by *P. ficus*, whereas Garnacha and Tempranillo maintained similar RCR values (Figure 1A).

Variety and infestation treatment had a significant effect on the RCR of the number of leaves (Table 1B). Garnacha variety had a significantly lower number of leaves RCR compared to Nebbiolo (31.2 %), while Tempranillo and Nebbiolo did not differ significantly from each other (Figure 1B). On the other side, infested plants showed a 22.6 % reduction in the number of leaves RCR compared to non-infested plants (Figure 1C). There was a significant effect of variety factor on CCI RCR (Table 1C). Tempranillo variety had a significantly higher CCI RCR than Nebbiolo and Garnacha, which did not differ from each other (Figure 1D). Finally, the infestation treatment also had a significant effect on the RCR of mean leaf area (Table 1D). Infested plants showed a 41.7 % lower value than non-infested plants (Figure 1E).

DISCUSSION

In general, results showed a negative effect of *P. ficus* infestation on the expression of three plant growth parameters associated with tolerance, plant height, number of leaves and mean leaf area per plant. Interestingly, the effect of *P. ficus* infestation on plant growth differs between grape varieties. Unlike the Garnacha and Tempranillo varieties, infested Nebbiolo plants showed a significant reduction in plant height compared to non-infested plants, pointing that this variety is less tolerant to the attack by *P. ficus*. Only chlorophyll content showed an association to the reduction in relative growth rate of infested Nebbiolo plants. The damage caused by this herbivore might impact mechanisms of photosynthetic rates that influence growth rates. The absence of a negative impact of mealybugs infestation on growth rate of Tempranillo and Garnacha plants indicated that these varieties possess mechanisms of tolerance to sap feeding, enabling them to grow at certain level of damage; thus, the differential effect of sap feeding supports the notion that there should be specific management, depending on the level of tolerance to damage.

Sap-feeders remove assimilates from the phloem or individual cells of their host; thus, the attack by sap-feeders is expected to negatively affect photosynthesis and plant growth (Gonda-King *et al.,* 2014; Strauss and Agrawal, 1999); however, the magnitude of the response of plants to herbivores is complex and it often changes within and between plant species (Walling, 2000). Likewise, variation in the response of domesticated varieties to herbivore damage is common and its responses largely depend on the variety, even when plants are exposed to similar herbivores (Chen *et al.,* 2015). Results of this research reflect this complexity. Significant differences were found between varieties in the relative growth of plant height, number of leaves, and the chlorophyll content index. This is consistent with previous evidence of cultivar variation in shoot physiological and growth parameters in grapevines reported elsewhere (Bica *et al.,* 1997; Borghezan *et al.,* 2012; Gris *et al.,* 2010; Gutiérrez-Gamboa *et al*., 2018; Malinovski

Table 1. Results of Mixed-model ANOVA of A) plant height RCR, B) number of leaves RCR, C) Chlorophyll Content Index RCR, and D) leaf area RCR in response to *Planococcus ficus* infestation treatments (infested *vs*. non-infested), grape variety (Garnacha, Tempranillo and Nebbiolo), infestation × variety interaction and block in a common garden experiment with *Vitis vinifera.* Bold letters indicate significant differences (P ≤ 0.05).

Trait	Source of variation	d.f.	M. S.	F	P	R^2
A) Plant height RCR	Infestation	1	0.00021	6.03	**0.01**	0.243175
	Variety	2	0.0004	11.29	**<0.0001**	
	Infestation × variety	2	0.00026	7.29	**<0.0001**	
	Block (Random)	1	0.00031	8.73	**0.003**	
	Error	155	0.000036			
	Total	160				
B) Number of leaves RCR	Infestation	1	0.00239	6.14	**0.01**	0.097234
	Variety	2	0.00163	4.19	**0.02**	
	Infestation × variety	2	0.00057	1.46	0.2	
	Block (Random)	1	0.00012	0.31	0.5	
	Error	155	0.00039			
`	Total	160				
C) Chlorophyll Content Index RCR	Infestation	1	0.00019	0.2	0.6	0.107164
	Variety	2	0.00588	6.3	**0.002**	
	Infestation × variety	2	0.00167	1.79	0.1	
	Block (Random)	1	0.00333	3.57	0.06	
	Error	155	0.00095			
	Total	160				
D) Leaf area RCR	Infestation	1	0.01124	28.78	**<0.0001**	0.202315
	Variety	2	0.00084	2.16	0.1	
	Infestation × variety	2	0.00035	0.89	0.4	
	Block (Random)	1	0.00158	4.05	**0.046**	
	Error	155	0.00039			
	Total	160				

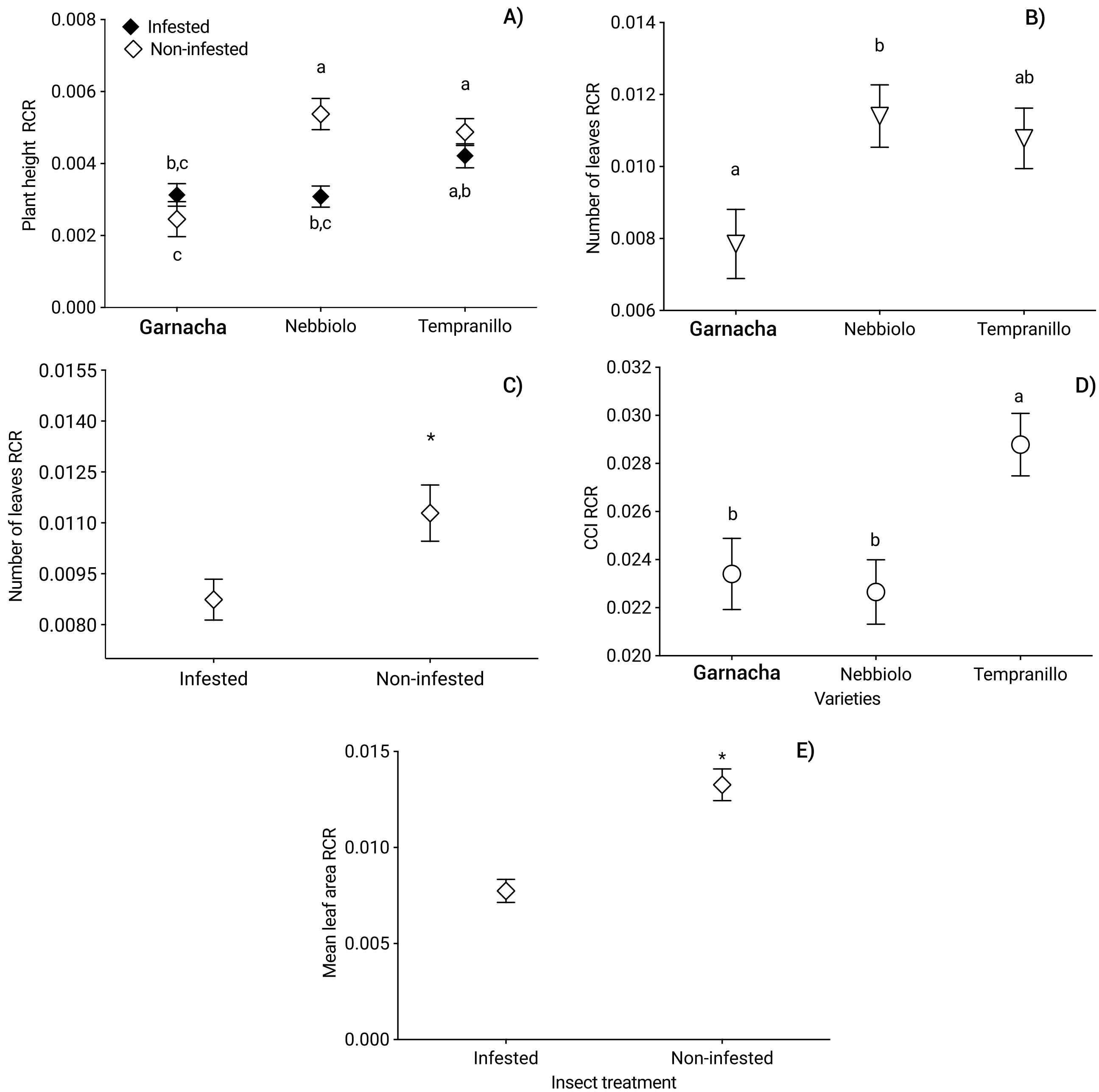

Figure 1. Averages (± S.E.) of A) plant height RCR for infested and non-infested plants of the three varieties of *Vitis vinifera*, B) number of leaves RCR for the three varieties of *V. vinifera*, C) number of leaves RCR of infested and non-infested plants, D) Chlorophyll Content Index RCR and E) mean leaf area RCR of infested and non-infested plants in a common garden experiment with *Planococcus ficus*. Averages not sharing the same letter differ significantly after a Tukey-Kramer LSD *post hoc* test. Asterisk denotes significant differences between levels (P ≤ 0.05).

et al., 2014). In general, results of this study indicate that the exposure of *V. vinifera* to the vine mealybug *P. ficus* reduced the relative change rate of plant height (17.9 %), number of leaves (22.6 %) and leaf mean area (41.7 %), indicating that infestation by this herbivore reduces plant growth of grapevines. This agrees with results of Zvereva *et al.* (2010), who found through a meta-analysis that sap-feeders reduced plant growth (~ 29 %), chlorophyll content (~ 27 %) and leaf biomass (~ 28 %) of woody plants; however, considerable reduction in plant height RCR was primarily observed in the Nebbiolo variety, whereas infested and non-infested plants of Garnacha and Tempranillo retained similar values, which indicates that the damage caused by the mealybug infestation on plant growth differed between varieties, pointing that the studied varieties differ in their ability to tolerate mealybug infestation, and that Nebbiolo is less tolerant to the damage by this sap feeding insect. Similar results were obtained by Khederi *et al.* (2018) who found that the negative effect of infestation by the mite *Colomerus vitis* on shoot length and leaf area differed between five Iranian grapevine varieties: Shahani, Sahebi Uroomie, Khalili Bovanat, Rishbaba and Sezdang Ghalat.

Chlorophyll content is another trait associated with tolerance commonly affected by the infestation of sap-feeding herbivores (Zvereva *et al.,* 2010). It has been reported that sap feeding damage causes a reduction of 27 % in the chlorophyll content in leaves of woody plants as compared to non-damaged plants (Zvereva *et al.,* 2010); however, plants do not always show a reduction in chlorophyll content after herbivory, suggesting that maintaining chlorophyll content levels could be adaptive in terms of tolerance to herbivory (Hodge *et al.,* 2000). In this study, mealybug infestation did not affect variation in the chlorophyll content relative change rate of leaves, even after controlling variation attributable to differences amongst varieties; this agree with previous results from a comparative study between damaged and non-damaged domesticated *Vitis vinifera* plants that found that the damage by this phloem feeder was not enough to induce the expression of transcripts and genes of traits implicated in defense (Timm and Reineke, 2014). Although it might seem counterintuitive, observed absence of a change in chlorophyll levels after leaf damage may have an important role in tolerance to herbivory (Hodge *et al.,* 2000); however, to what extent, sustaining chlorophyll content in the presence of damage by *P. ficus* may sustain the reduction of compensatory growth traits remains to be further studied for the *V. vinifera-P. ficus* interaction.

CONCLUSIONS

A negative effect of *P. ficus* infestation on plant height, number of leaves and leaf area was found. Results of this study contribute to the understanding of the response of domesticated plants to herbivores, providing insights for future studies intended to identify tolerant varieties, as well as potential applications in decision-making for grapevine cultivation in the study region. In vineyards where mealybugs are a persistent problem, farmers must adopt more rigorous monitoring and control measures when growing varieties with low level of tolerance like Nebbiolo. It is also necessary to conduct long-term studies aimed to identify the cultivar-specific physiological mechanisms underlying the response of vines cultivars to the attack by this important herbivore.

ACKNOWLEDGEMENTS

We thank the Plant Physiology course students from the Science Faculty, Universidad Autónoma de Baja California for their help in obtaining data. Financial support was provided to GC by Apoyo a la Incorporación de NPTC Programa para el Desarrollo Profesional Docente, para el Tipo Superior (PRODEP) UABC-PTC-646. GC and RBB used instruments acquired with funding obtained through CONACYT INFRA 2014-226239. GC thanks Vidal Perez for his valuable help in conducting the experiment, data collection, and his comments on the manuscript.

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