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Vine mealybugs disrupt biomass allocation in grapevine

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ABSTRACT

Vine mealybug *Planococcus ficus* Signoret (Hemiptera: Pseudococcidae) is an important phloem-feeding pest species in many grapevine producing areas worldwide. The economic damage of *P. ficus* is thought to be mainly caused by sooty mould on infested grape clusters and transmission of plant viruses. Direct damage caused by mealybug feeding to grapevine plants (*Vitis vinifera*, L.) has only been vaguely described or otherwise completely discarded. The present study is the first to give an insight into the direct impacts of *P. ficus* on vegetative growth and biomass dynamics of grapevine plants. In a screenhouse, three-year-old, potted grapevine plants were infested with mealybugs at two different densities, imitating high and low field infestation levels. Mealybug numbers, plant biomass, leaf area, leaf size and leaf number were monitored over six months and compared to a control treatment without mealybugs. High infestation levels reduced leaf and stem biomass by one third, while low levels of *P. ficus* impacted only stem biomass, indicating a higher sensibility of the perennial parts of the plant or a reallocation of biomass. Leaf area, size and number were not affected by mealybug feeding.
In conclusion, grapevine response to *P. ficus* is gradual and involves different plant parts depending on the severity of the attack. Contrary to previous assumptions, this study demonstrates considerable direct impacts of mealybug feeding on temporal and perennial parts of grapevine plants.

KEYWORDS

Planococcus ficus, *Vitis vinifera*, herbivory, vegetative growth

INTRODUCTION

Insect herbivory is often associated with a leaf-chewing feeding mode, but in viticulture and many horticultural systems sap-sucking insects, such as mealybugs, scale insects, aphids and leafhoppers are a much bigger concern. Contrary to folivores that reduce resource capturing tissues, sap-feeders impact translocated resources, such as the phloem sap which moves carbohydrates and amino acids from photosynthetic and storage tissue (sources) to areas of active growth and metabolism (sinks). Sap-feeders utilise the attracted carbohydrates for their own growth while depleting plant resources, hence acting as an additional carbohydrate sink (Zvereva *et al.,* 2010).

The vine mealybug, *Planococcus ficus* Signoret (Hemiptera: Pseudococcidae), is an invasive phloem-feeding insect from the Mediterranean area, which has become a serious pest in many grape-growing regions worldwide (Daane *et al*.,2012; Mansour *et al.,* 2017; Walton and Pringle, 2004). In Argentinian vineyards, *P. ficus* was first detected in 2001, although it might have been present earlier, and it has spread across all grape-growing provinces of the country (Becerra *et al.,* 2006; Viglianco *et al*., 2016). The problem is aggravated by the presence of the invasive Argentine ant *Linepithema humile* Mayr (Schulze-Sylvester *et al*., 2018), a common mutualist of *P. ficus*, which can increase mealybug densities on grapevine (*Vitis vinifera* L.) plants and clusters by 2- to 80-fold (Daane *et al.,* 2007; Mgocheki and Addison, 2010). Mealybug damagehas mainly been attributed to indirect effects. The honeydew excreted by mealybugs promotes the growth of sooty mould on leaves and fruits, reducing leaf photosynthesis and grape marketability (Daane *et al.,* 2012). Mealybug infestation has also been shown to affect wine and must quality, but the underlying mechanism is unclear; it may be linked to the presence of mealybugs in the grape bunches (*i.e.*, the "mealybug flavour"), to the honeydew and fungus associated with their feeding, or to changes in the plant's physiology (Bordeu *et al*., 2012; Chiotta *et al*., 2010; Viglianco *et al.,* 2016). Moreover, viral plant diseases, such as the grapevine leafroll-associated virus (GLRaV) which can be vectored by mealybugs, have also been shown to reduce crop yield and wine quality (Herrbach *et al.,* 2017). Mealybug infestations can develop quickly from hardly detectable to high levels, as mealybugs go through up to six generations per growing season (Becerra *et al.,* 2006), and each female can produce an average of 230 to 360 eggs under field conditions (Schulze-Sylvester and Reineke, 2019; Walton and Pringle, 2004). Literature reports maximum mealybug densities of up to 1800 individuals per leaf in autumn (Charles, 1981). Other studies used 3 or 5 minutes counts of the whole plant during spring, detecting up to 40 mealybugs per plant (Geiger and Daane, 2001) or classified mealybug densities as high (> 10 individuals), moderate (0-10) or low (0) (Walton *et al.,* 2006).

Possible direct impacts of mealybugs are usually briefly described with the rather general term of "weakening of the plants' vigour" (Daane *et al*., 2012; Walton *et al*., 2004). However, these observations refer to grey literature sources, which have not passed peer-review (Kriegler, 1954; Whitehead, 1957). In their review, Walton and Pringle (2004) also mention leaf loss as a possible consequence, but other studies contradict (Charles, 1981) or assume without further validation that mealybug-free foliage will compensate for debilitated infested leaves (Charles, 1982).

The lack of studies on the direct impacts of mealybugs on grapevine and the assumption-based belief in the secondary nature of the damage profile are surprising, especially since sap-feeding insects are known to cause considerable direct damage to grapevine and other crops. Leafhoppers have been shown to affect vegetative growth and photosynthesis of grapevines (Candolfi *et al*., 1993; Lenz *et al.,* 2009, 2012). Grape phylloxera, *Daktulosphaira vitifoliae* Fitch, infestations have been shown to increase defoliation and impact the water and carbon metabolism of grapevines (Botton and Walker, 2009; Savi *et al*., 2019). Simbiken *et al*. (2015) showed that feeding of scale insects impacts chlorophyll content, leaf drop, and other foliar parameters of grapevines.

While many plants increase their intrinsic rate of biomass growth in response to damage (compensatory growth, see McNaughton (1983)), this is not a common feature in woody plants infested with sap-feeders. Rather, these plants show a reduction in photosynthesis and biomass. Other symptoms include leaf-rolling, shoot distortion, growth reduction and a decrease in yield (see Zvereva *et al*. (2010) and references therein). Mealybugs feed on the phloem of grapevine leaves, as well as on perennial plant parts, but it is unclear how vines react to this in terms of biomass, leaf area and leaf number.

The direct effects of mealybug feeding on grapevine vegetative growth have never been studied and studies on other crops are scarce. It is also unclear how the density of mealybugs (*i.e.*, 'sink strength') impacts the plant. While it is reasonable to assume that more mealybugs cause greater damage, the question remains how different plant parts react to varying infestation levels. Moreover, the effects of low infestation levels might not always be reliably quantified in terms of biomass or leaf area, but may still affect other plant variables. Lenz *et al*. (2012) showed that potato leafhopper, *Empoasca fabae* Harris (Hemiptera: Cicadellidae), affected grapevine carbon assimilation and evaporation with only one individual per leaf, while biomass damage only occurred when infestation levels passed a threshold of three nymphs per leaf (Lenz *et al*., 2009).

The present study investigates the effects of different *P. ficus* densities (no mealybugs, low-density infestation and high-density infestation) on vegetative growth parameters of grapevine plants over the growing season. We hypothesised that in comparison to the mealybug-free control treatment, high densities of *P. ficus* would reduce biomass, leaf area and leaf number. Low densities, on the other hand, were predicted to cause less or even no impact on these parameters.

MATERIALS AND METHODS

1. Insect and plant material, and experimental set-up

In December 2016, three screenhouses (3 x 3 x 2 m, length x width x height) were set up in a greenhouse (30 x 15 x 5) at the National University of Salta, Argentina (southern hemisphere). All-purpose garden fabric (Tela antihelada, Marplast SRL, Salta, Argentina, 26.2 g/m^2) was used to cover the screenhouses, allowing free passage of oxygen and light while preventing mealybugs from escaping. *Planococcus ficus* mealybugs were obtained from the National Agricultural Technology Institute (Instituto Nacional de Tecnología Agropecuaria, INTA) in Cafayate. *Planococcus ficus* was reared on sprouted potatoes in plastic containers (20 x 40 cm) covered with cotton fabric at 23 ± 1 °C and ambient light conditions. Each screenhouse was equipped with seven three-year-old vines (*cv.* Torrontes) in 30 x 50 cm containers, which were watered twice a week. The plants did not receive fungicide treatments or fertilisation throughout the duration of the experiment. Five months prior to the experiment, the vines had been transferred from the field to pots, and were pruned to one spur on that occasion. The three screenhouses corresponded to three treatments: 1) High density of *P. ficus*, 2) low density of *P. ficus,* and 3) a control treatment without *P. ficus*. Infestation levels aimed to mimic field data on low and heavily infested vineyards (Daane *et al.,* 2007; Geiger and Daane, 2001; Walton *et al.,* 2006). Vines were infested using leaf disk transport (Hogendorp *et al.,* 2006; Schulze-Sylvester and Reineke, 2019). Vines were randomly assigned to the different treatments and were placed separately inside the screenhouses. In December, each plant in the high-density treatment received approximately 1000 1st instar *P. ficus* nymphs, and the low-density treatment was infected with approximately 100 nymphs per plant, while the control treatment plants received empty leaf disks. Infestations were repeated in early January and early February to ensure high/low infestation levels. The containers were painted with a sticky, non-toxic paint (CeroPestes Hormiga, Sanipro SRL, Buenos Aires, Argentina) to avoid mealybug tending by ants.

2. Mealybug densities, leaf parameters and biomass

Between December 2016 and May 2017 (early summer to late autumn in Argentina), leaf number and leaf area were measured monthly by taking standardised photos of all leaves on all plants, resulting in seven measurements per treatment per sampling date. The surface area of each leaf was determined using the software ImageJ (National Institute of Health, Bethesda, MD, USA). The average leaf size per plant was calculated dividing the plant's total leaf area by its leaf number. Furthermore, mealybug densities in the three treatments were determined at the end of every month (31 December to 31 May) by carrying out three-minute time counts of visible mealybugs on all plants (Geiger and Daane, 2001), resulting in seven biological replicates per treatment. These time-counts were completed by a final, destructive count of all mealybugs on five plants.

At the end of the experiment, plants were cut at the root collar. To study the biomass allocation in the experimental vines, additional morphological measurements were performed on the plant material. Three leaves from five plants per treatment were randomly sampled, measured, oven-dried for three days at 65 °C (SL30C, San Jor, Buenos Aires, Argentina) and weighed.

The specific leaf mass, (leaf mass per area, LMA) calculated as leaf dry mass/leaf area, was then determined (Pérez-Harguindeguy *et al.,* 2013). Five randomly chosen plants of each treatment were used for further analysis of total mealybug number and dry biomass weight. For this, the plant material was cleaned of honeydew and sooty mould with tap water; mealybugs were collected and counted. After that, all above-ground plant parts were oven-dried for one week at 65 °C and the dry weight was recorded for leaves (leaf biomass), stems (woody parts and petioles), and total plant parts (total biomass, all aerial plant parts). The leaf area ratio (LAR), dividing the plant's total leaf area by its weight (stem and leaves) was determined (Pérez-Harguindeguy *et al.,* 2013).

3. Statistical analysis

Due to the prolonged experiment duration (6 months), the advanced plant sizes and the frequent measurements and maintenance activities, individual "bagging" of plants was not a viable option. Also, the number of available screenhouses was limited and the randomisation of plants from different treatments within the screenhouses is not advisable if cross-contamination is to be avoided. For the statistical analysis, individual vine plants were therefore considered as biological replicates.

Data were tested for normality using Shapiro-Wilks normality test. Mean mealybug densities, leaf area, average leaf size and number of leaves were tested for differences using two-way repeated-measures ANOVAs, followed by Holm-Sidak's multiple comparison test. Deviations from sphericity were quantified and corrected using Greenhouse and Geisser's *epsilon*. Treatment differences for the biomass of leaves, woody parts and total plant parts, as well as LMA and LAR, were analysed with one-way ANOVAs and subsequent Tukey tests. Mealybug three-minute counts were checked for correlation with total destructive counts using Pearson's test. Pearson's correlation analysis was also used to understand how total mealybug numbers were related to the biomass of leaves, stems and total biomass. All analyses were performed with GraphPad Prism version 8.00 for Windows (GraphPad Software, La Jolla, CA, USA).

RESULTS

No significant differences between treatments were found for the leaf area, leaf size and leaf number per plant during the course of the experiment at any time (Figure 1). Not surprisingly, the leaf area and number per plant increased in all three treatments over the duration of the experiment (Figure 1A, C; F = 57.96; df = 2.055, 36.98; $P < 0.0001$ and F = 94.29; df = 1.818, 32.72; $P < 0.0001$ respectively). Meanwhile average leaf size per plant remained constant (high-density treatment) or was reduced to a constant level after January (low-density and control treatment) (Figure 1B; F = 28.06; df = 1.673, 30.11; $P < 0.0001$). One-way ANOVAs of the aboveground leaf area ratio (LAR) and the leaf mass per area (LMA) showed significant differences between treatments (Table 1; F = 9.11; df = 2, 12; $P = 0.004$). The LAR was highest for high-density plants, while the LMA showed the lowest leaf mass per area in the high-density treatment (Table 1; F = 5.60; df = 2, 12; $P = 0.02$).

TABLE 1. Leaf area ratio (LAR) and leaf mass per area (LMA) of grapevine plants infested with different *P. ficus* densities (high, low, control without *P. ficus*)

Treatment	LAR [cm^2/g]	LMA [mg/cm^2]
High	$135.4 \pm 14.93^{b*}$	$3.16 \pm 0.19^{b**}$
Low	111.3 ± 11.28^{ab}	3.88 ± 0.42^{a}
Control	104.1 ± 16.59^{a}	4.03 ± 0.37^{a}

Within columns, different letters indicate a significant difference between means. * and ** denote a significant difference between treatments at $P < 0.05$ and $P < 0.01$ respectively.

Leaf biomass was significantly reduced in the high-density treatment compared to the control (-33.59 %), and low-density treatment (-32.73 %; Figure 2), (F = 4.94; df = 2, 12; $P = 0.03$). No differences in foliar biomass were detected between low-density and control treatment. Regarding the biomass of woody plant parts, both the high-density and low-density treatment showed significantly lower dry weight than the control (-27.82 % and -29.31 % respectively; Figure 2), (F = 10.08; df = 2, 12; $P = 0.003$). These results were also reflected in the total dry weight of the plant, for which the control reaches the highest weight, followed by the low-density treatment (-17.29 %) and the high-density treatment (-30.29 %; Figure 2), (F = 6.79; df = 2, 12; $P = 0.01$). Lastly, all plant parts declined linearly in biomass with increasing mealybug densities (Figure 3). Pearson`s correlation showed decreasing biomass of leaves, stems and total plant parts with increasing mealybug density (Pearson's r = -0.60, $P = 0.017$; r = -0.48, $P = 0.07$; r = -0.62, $P = 0.013$ respectively).

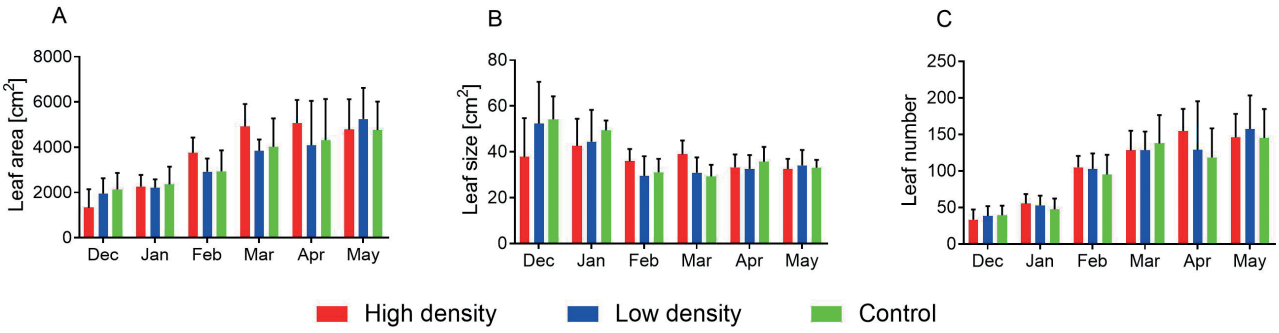

FIGURE 1. Leaf measurements taken between December and May in Argentina (southern hemisphere).

A) Leaf area, B) leaf size, and C) leaf number of grapevine plants infested with different *P. ficus* densities (high, low, control without P. ficus); n = 7 plants, results are expressed as mean values ± SD).

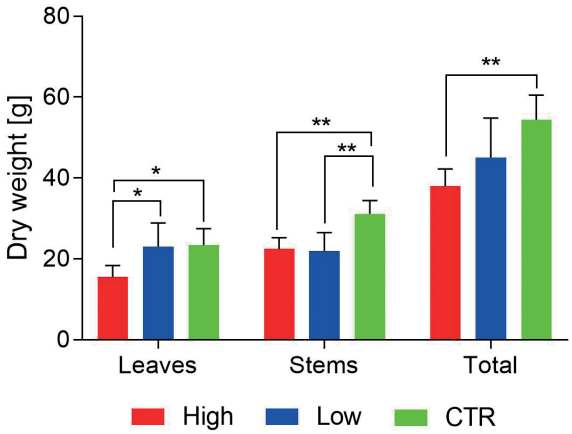

FIGURE 2. Grapevine leaf, stem and total biomass for the high-density treatment (High), low-density treatment (Low), Control treatment (no *P. ficus*) (CTR).

Significant differences between pairs are marked with asterisks: *, $P < 0.05$; **, $P < 0.01$.

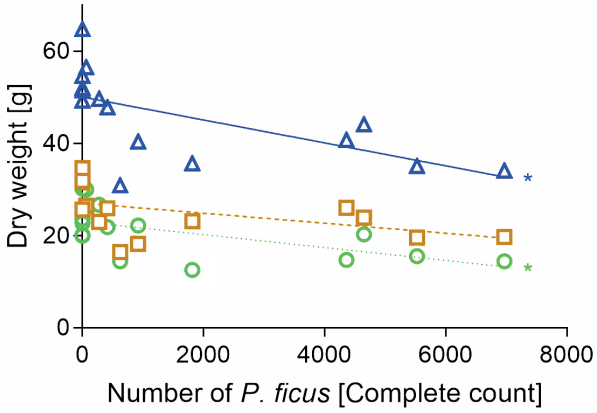

FIGURE 3. Pearson's correlation analysis of grapevine biomass (△ = total; □ = stems; ○ = leaves) and the number of P. *ficus* of all treatments. * indicate significant trends at $P < 0.05$.

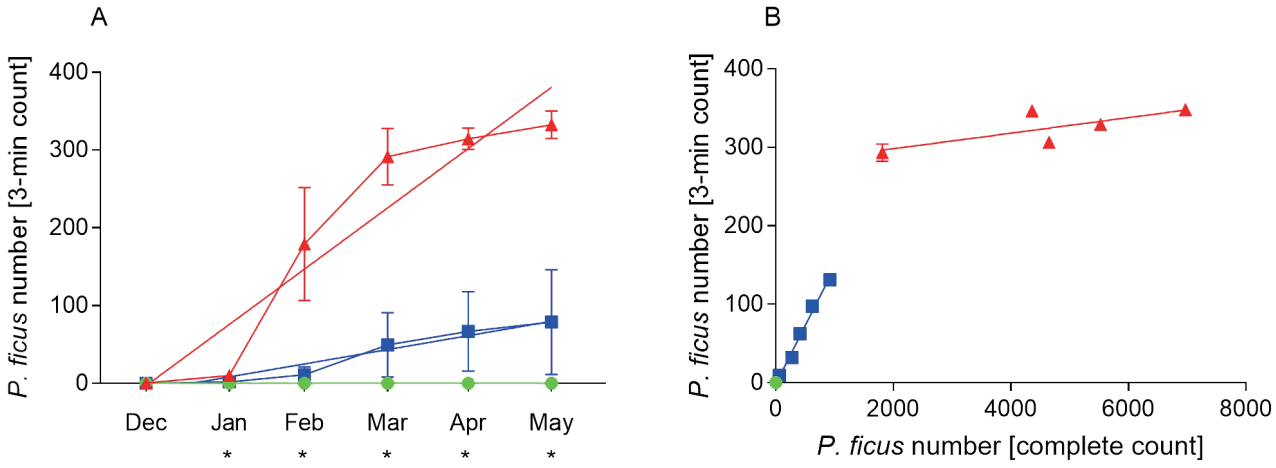

FIGURE 4. Number of mealybugs (*P. ficus*) per vine plant.

red = High-density treatment (High), **blue** = Low-density treatment (Low), **green** = Control treatment (no P. ficus) (CTR).

A = Number of P. ficus determined at the end of each month using 3-min counts, n = 7 plants; B: Pearson's correlation of mealybug numbers determined in the 3-min count (31 May) vs. complete count of all mealybugs of the same plant, n = 5 plants. The study was carried out in Argentina (southern hemisphere). Results are shown as mean values ± SD).

* Treatments were significantly different ($P < 0.05$).

The three-minute counts carried out to evaluate mealybug number per plant showed that mealybug numbers in the different treatments varied over time ($F = 64.69$; $df = 10, 90$; $P < 0.0001$; Figure 4A). As of end-January, *P. ficus* numbers were significantly different among treatments (Holm-Sidak $P < 0.03$; Figure 4A). The correlation between the three-minute counts and the destructive count at the end of the experiment was high for the low-density treatment ($P = 0.0006$, Pearson's $r = 0.99$), but not significant for the high-density treatment ($P = 0.12$, Pearson's $r = 0.77$; Figure 4B). No mealybugs were detected in the control treatment.

DISCUSSION

1. Biomass, leaf area, size and number

While it is not uncommon to use individual vines as replicates (Schulze-Sylvester and Reineke, 2019; Timm and Reineke, 2014), we are aware that our results might be considered pseudoreplicate-based. These results may be limited in their implications and when searching for conclusive general patterns, but they are a valid first step in the search of direct impacts of mealybugs on grapevines.

Mealybug feeding did not alter leaf area, average leaf size or leaf number; however, biomass was strongly affected and it decreased with increasing mealybug densities.

The total leaf area per plant and average leaf size over the growing season are comparable to results from a study on three-year-old Chardonnay and Aíren grapevine plants (Gomez-del-Campo *et al*., 2002). The biomass measured in the present paper is lower than the results from that study, possibly due to different pruning of the experimental plants. We expected biomass, leaf area and leaf number to decline over time due to mealybug feeding, especially in the treatment with high mealybug densities. However, based on the present results, this hypothesis must be partly rejected, as only biomass followed the predicted pattern. While there are no studies on the impacts of *P. ficus* on grapevine biomass, leaf area, size or number, Charles (1981) reported that even very high densities (up to 1800 individuals per leaf) of the long-tailed mealybug *Pseudococcus longispinus* Targioni-Tozzetti did not enhance leaf drop in grapevine. Conversely, a review on *P. ficus* in South Africa reports enhanced leaf drop as part of the damage profile (Walton and Pringle, 2004). While this might be a direct effect of *P. ficus*, it could also be a consequence of mealybug-vectored virus infections as GLRaV, which are known to affect leaves (Walton and Pringle, 2004). The frosted scale *Parthenolecanium pruinosum* (Coquillett), on the other hand, was shown to affect grapevine biomass, leaf area and leaf number, although the effect direction of different vine varieties was inconsistent (Simbiken *et al*., 2015). Other phloem-feeding vineyard pests, leafhoppers and foliar grape phylloxera have been found to reduce leaf area, size or number, which can, in some cases, be compensated for by the plant (Candolfi *et al*., 1993; Lenz *et al*., 2009; McLeod, 1990). Studies on other plants have found that the cassava mealybug *Phenacoccus manihoti* (Matile-Ferrero) reduced leaf production in cassava plants (Schulthess *et al*., 1991), and that biomass and leaf area of colliguay (*Colliguaya odorifera* Molina) seedlings were reduced by *Planococcus citri* Risso feeding (Mills, 1984).

Total biomass of all aboveground plant parts is reduced in the high-density treatment in comparison to the control and the low-density treatment. These biomass differences can be attributed to the reduced dry mass of both leaves and stems of plants. The low-density treatment, on the other hand, only shows a biomass reduction of the stems, while leaf dry mass is comparable to that of the control plants. Surprisingly, the leaf biomass reduction is not related to a reduction in leaf area, size or number, indicating that grapevine plants under mealybug attack maintain leaves and do not diminish their photosynthetic surface and carbohydrate source capacity. Eventually, when mealybug infestation levels become severe, leaves grow thinner, as indicated by the reduced LMA in the high-density treatment. In accordance with our results, woody plants generally react to hemipteran feeding with decreases in biomass (total, stems and leaves), and do not display compensatory growth (Zvereva *et al.,* 2010). Nonetheless, compensatory growth has been described for some grapevine varieties infested with leafhoppers (Candolfi *et al*., 1993; Lenz *et al*., 2009).

Hemipteran feeding usually differs in its consequences from direct leaf herbivory (defoliation) by leaf-chewers, but it is unclear if compensatory mechanisms resemble those observed after defoliation. For example, the reduction of photosynthesis due to defoliation may affect fruit ripening in grapevine (Candolfi-Vasconcelos *et al*., 1994;

Candolfi-Vasconcelos and Koblet, 1990), while reduced photosynthesis caused by sap-feeding leafhoppers did not impact grapevine yield or fruit quality (Candolfi *et al.,* 1993). On the other hand, mealybug feeding in cassava plants reduced leaf growth and strongly affected assimilation and allocation of dry matter to storage roots, causing root yield losses of up to 75 % (Schulthess *et al*., 1991). Grapevines in the present study did not bear fruit; further research is thus needed to evaluate the possible effects of mealybug feeding on this major sink organ of grapevines. For a complete picture, it will also be necessary to include root parameters in future studies.

2. Growth ratios LAR and LMA

Increased aboveground LAR in the mealybug infested treatments reflect a greater leaf surface relative to whole plant biomass. This may be a consequence of reduced stem growth and leaf weight, rather than a stimulation of leaf production, as leaf number, size and area were constant among treatments. Similar biomass allocation patterns were detected for scale insects with a reduction in storage tissue growth in *Eucalyptus* tree seedlings, while leaf production remained unaltered (Vranjic and Ash, 1997).

LMA can be used to study biomass allocation and productivity gradients within the aboveground organs under herbivory stress, since it relates positively to leaf longevity and carbon investments in secondary compounds, such as tannins and lignins (Pérez-Harguindeguy *et al.,* 2013). Typically, the LMA of grapevine plants continuously increases from bud break to harvest. Grapevine leaf thickening continues after leaf expansion, indicating that leaf carbohydrate allocation takes place over a much longer period than lamina expansion, which is usually completed after 30-40 days (Cartechini and Palliotti, 1995; Poni *et al.,* 1994). Mealybugs in the present study did not establish populations until end-January when most leaves were already fully expanded; however, they did affect leaf biomass at high densities, indicating a reduced leaf biomass allocation by the plant, or an increased sink demand for carbohydrates through mealybug feeding on leaf phloem. A reduced LMA indicates weaker leaves, which might eventually lead to increased leaf drop, especially under field conditions in which weather, plant protection activities or harvesting machinery cause mechanical stress.

The LMA of grapevine plants in the present study varied between 3.16 and 4.03 mg/cm^2 for the high-density and the control treatment respectively; this is somewhat lower than the LMA of Riesling grapevines, which showed a reduction from 6.1 to 5.6 mg/cm^2 when leaves were infested with phylloxera (Savi *et al.,* 2019). Similar to that study, *P. ficus* caused stress at the leaf-level and reduced the LMA, suggesting that infested grapevines invest less carbon per unit leaf area compared to the uninfested controls and the low-density treatment. Surprisingly, despite the reduced LMA, phylloxerated grapevines did not show a decline in leaf or stem biomass (Savi *et al.,* 2019), while in our study the reduced LMA translated into reduced leaf biomass. Lower biomass allocation to leaves and stems might be a direct consequence of 1) the carbon export towards the *P. ficus* population, affecting stems at lower infestation levels than leaves, 2) a reallocation of carbohydrates from the stems to the leaves, 3) the redirection of energy to secondary metabolic pathways (defence, repair, signalling, phytohormonal networks) (Timm and Reineke, 2014), or 4) a reduced source availability due to reduced photosynthetic capacity. Photosynthesis reduction is a common response to sap-feeding insect herbivory (Zvereva *et al.,* 2010). It is known that grapevines attacked by leafhoppers, phylloxera and soft scales reduce leaf photosynthesis and/or leaf chlorophyll content (Candolfi *et al.,* 1993; Lenz *et al.,* 2012; McLeod, 1990; Simbiken *et al.,* 2015). Even though the reduced source capacity may be temporarily compensated for by the reallocation of carbohydrates from woody tissues or roots (Candolfi-Vasconcelos *et al.,* 1994; Candolfi-Vasconcelos and Koblet, 1990), such reallocation can affect grapevine performance in the following year (Candolfi-Vasconcelos and Koblet, 1990).

We did not measure photosynthetic parameters, but there is a strong positive relationship between the LMA and photosynthesis (Cartechini and Palliotti, 1995; Poni *et al.,* 1994). Hence, decreases in LMA could indicate reduced photosynthesis, even if the leaf area, leaf number and size remained unaltered by *P. ficus*' feeding. The spread of honeydew and sooty mould on the surface of the leaves might also have hindered photosynthesis, especially in the high infestation treatment. Further studies should elucidate this in more detail.

3. Density-dependent effects of mealybug feeding

The density-dependent effects of *P. ficus* on its host plants have never been considered so far. In congruence with our hypothesis, the present data show a negative relationship between biomass and mealybug numbers. This relationship is significant for the total biomass and the leaf biomass, while the stems show a slight, but not significant, negative trend. The density-dependence of the total biomass reduction is mainly due to the effects on the leaf biomass, while stem biomass decreased equally for both treatments. This suggests that stems are more sensitive to mealybug feeding than leaves. Another explanation could be that the plant redirects stored resources from the stems to maintain leaves (source tissue), but eventually, when a certain infestation threshold is exceeded, leaf biomass also declines. Comparing our results with the few available studies involving sap-feeder densities, the citrus mealybug, *P. citri,* showed a density-dependent effect on the above-ground biomass of the shrub, *C. odorifera*, while the roots did not seem to be affected (Mills, 1984). Frosted scales showed a density-dependent decrease in internodes in grapevine, while effects on the chlorophyll concentration and branch length were not density-related (Simbiken *et al.,* 2015). Scale insects also showed strong density-dependent effects on the root, stem and leaf biomass in eucalyptus plants (Vranjic and Ash, 1997).

4. Insect populations

Mealybug populations are very dynamic and infestation levels can increase dramatically within weeks (Becerra *et al.,* 2006; Geiger and Daane, 2001). Spring populations are typically considered low to moderate when timed counts detect < 10 adult mealybugs, and high when > 10 mealybugs are found (Walton *et al.,* 2006). In autumn, Charles (1981) counted up to 1800 individuals per leaf which corresponded to three generational cycles. In Argentina, six generations of mealybugs are common (Becerra *et al.,* 2006), hence infestation levels might be even higher. Mealybug numbers obtained in the present study resemble infestation levels reported for vineyards in California and Auckland, New Zealand (Charles, 1981; Daane *et al.,* 2007; Geiger and Daane, 2001; Walton *et al.,* 2006) and Salta (Schulze-Sylvester, unpublished data).

Typically, *P. ficus* overwinters in small numbers in refuge areas under bark and on the roots and follow the plant's resources during spring-summer by moving from roots to shoots to leaves, and eventually to fruit clusters (Daane *et al.,* 2012). In local vineyards in Salta, Argentina, mealybugs are usually not detected in the canopy until February (personal observation).

Experimental plants were visibly infested on stems, petioles and leaves with different mealybug densities around mid-February, and time counts had already revealed the first statistically relevant differences between mealybug densities in January. After a steep population growth between February and April, growth rates slowed down, suggesting a density-dependent restriction of mealybug population growth due to limited resources or crowding effects (*e.g.*, reducing size and fecundity, and increasing mortality) (Washburn *et al.,* 1985). Consequently, the per-capita impact of *P. ficus* on plant biomass declined with increasing density. Similar to the study by Geiger and Daane (2001), our results show good correlations between the three-minute counts and the destructive sampling method in the low-density treatment, but not in the high-density treatment. It is possible that there is a maximum number of individuals that can be counted in three minutes and that we reached this limit. We suggest extending the interval for time counts to 5 minutes, especially when mealybug densities are high.

CONCLUSION

In conclusion, the present study showed that mealybugs have considerable direct effects on grapevine plants, limiting vegetative plant growth and disrupting biomass allocation. Grapevine responses to mealybug infestations can be explained by considering mealybugs as additional sinks that compete against plant sinks and reallocate and/or drain resources from the plant. The reduction in leaf and stem biomass may affect plant performance in the present and future growing season. Plant damage might occur with low mealybug numbers, even in the absence of indirect mealybug effects; *i.e.* pathogen transmission and sooty mould growth on grapes. Therefore, we support low action thresholds for plant protection measures against *P. ficus* and recommend commencing control measures early in the season, when mealybug numbers are still low. The results of the present study are not entirely surprising, given that in other plant-pest systems sap-feeders can cause considerable damage. In grapevine, however, it had been assumed that mealybug damage occurs principally through indirect effects. Our results help to complete

this picture and contribute solid data to the damage profile of this important pest organism. Our study contributes to the understanding of plant responses to sap-feeding pests and sheds light on the implications for the future growth of the host plant and the herbivore population.

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