

Herbivory alters plant carbon assimilation, patterns of biomass allocation and nitrogen use efficiency

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ABSTRACT

Herbivory can trigger physiological processes resulting in leaf and whole plant functional changes. The effects of chronic infestation by an insect on leaf traits related to carbon and nitrogen economy in three *Prunus avium* cultivars were assessed. Leaves from non-infested trees (control) and damaged leaves from infested trees were selected. The insect larvae produce skeletonization of the leaves leaving relatively intact the vein network of the eaten leaves and the abaxial epidermal tissue. At the leaf level, nitrogen content per mass (N_{mass}) and per area (N_{area}), net photosynthesis per mass (A_{mass}) and per area (A_{area}), photosynthetic nitrogen-use efficiency ($PNUE$), leaf mass per area (LMA) and total leaf phenols content were measured in the three cultivars. All cultivars responded to herbivory in a similar fashion. The N_{mass} , A_{mass} , and $PNUE$ decreased, while LMA and total content of phenols increased in partially damaged leaves. Increases in herbivore pressure resulted in lower leaf size and total leaf area per plant across cultivars. Despite this, stem cumulative growth tended to increase in infested plants suggesting a change in the patterns of biomass allocation and in resources sequestration elicited by herbivory. A larger N investment in defenses instead of photosynthetic structures may explain the lower $PNUE$ and A_{mass} observed in damaged leaves. Some physiological changes due to herbivory partially compensate for the cost of leaf removal buffering the carbon economy at the whole plant level.

1. Introduction

Herbivory by insects can induce a wide range of metabolic and physical changes in host-plant functioning. Disruption of leaf tissue by herbivore pressure includes changes in CO_2 assimilation (Aldea et al., 2006; Macedo et al., 2005; Thomson et al., 2003; Zangerl et al., 2002), increase in water loss (Aldea et al., 2005; Ostlie and Pedigo, 1984), changes in nutrient concentration (Stockhoff, 1994), and increase in defense compounds such as phenols (Feeny, 1970). Loss of nutrients and photosynthetic leaf area by herbivory (Casotti and Bradley, 1991; Stockhoff, 1994), often leads to a reduction in plant growth or fitness (Coley and Barone, 1996; Marquis, 1984). However, herbivory does not have a detectable effect in some cases (McNaughton, 1983) or may even increase growth and fitness (Maschinski and Whitham, 1989; Paige, 1999). Recurrent herbivory can improve the protection of the leaf

through increases in compounds such as structural and nonstructural carbohydrates as well as soluble phenolics, proteins, lignin and lipids which may result in higher leaf dry mass per unit area (LMA) (Coley, 1983; Onoda et al., 2004; Poorter et al., 2009). But herbivory can also have the opposite effect and result in lower levels of defenses but higher leaf N (Scogings et al., 2011). The induction of defense compounds can divert carbon and nitrogen away from primary metabolism, thus affecting carbon assimilation. In addition, herbivory can change the patterns of resources allocation within plants (Gómez et al., 2010) by allocating more biomass to root and stem tissues than to leaves (Vanderklein and Reich, 1999). This is a response that may reduce damage by herbivores, and it is known as induced resource sequestration (Orians et al., 2011).

Leaf size can be genetically determined or can be regulated by herbivore consumption, resource limitation and mechanical damage

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such as strong winds or environmental induced stresses such as drought and/or freezing temperatures. Chronic herbivory tends to favour small leaf size (e.g. Brown et al., 1991; Moles and Westoby, 2000; Peschiutta et al., 2016). Partial consumption of expanding or mature leaves not only implies a loss of structural components but also proteins associated with carbon uptake and leaf growth (Kursar and Coley, 1992).

We evaluated the effects of an herbivore insect (*Caliroa cerasi* L. cherry slugworm) on leaf traits related to the carbon economy of three *Prunus avium* L. cultivars. This insect is one of the most common pests in *P. avium* crops and the larvae produce skeletonization of the leaves leaving relatively intact the vein network of the eaten leaves (Peschiutta et al., 2016). Our hypothesis was that herbivory on *P. avium* cultivars reduces the photosynthetic rates, total leaf surface area and nitrogen use efficiency and changes the pattern of biomass allocation within the plants. Leaf nitrogen content associated to the photosynthetic machinery may decrease if it is used to enhance chemical defenses. Some of the responses to herbivory may compensate for the effects of leaf removal, such as changes in biomass allocation patterns, buffering the negative effects of herbivory at whole plant level. Leaf nitrogen content, photosynthetic rates, LMA, chemical defense compounds, resources use efficiency and stem growth in non-infested plants as well as in damaged leaves from infested trees of three cultivars (Bing, Lapins and Van) were determined.

2. Materials and methods

The study was conducted in El Porvenir Ranch located next to Los Antiguos town in northwest Santa Cruz, Argentina (46° 19' S, 71° 62' W, altitude 220 m) during the month of the most intense defoliation by *C. cerasi* (February 2011). The climate of this valley is characterized by mild temperatures due to the close proximity of the second largest lake in South America (Buenos Aires-General Carrera Lake). Climatic conditions are also characterized by very low precipitation, because the study site is in the rain shadow of the Andes, preventing the influence of wet fronts coming from the Pacific Ocean. Mean monthly temperatures range from 2.5 to 14.9 °C and the average annual precipitation is about 192 mm falling mostly in the fall and winter (April–September) (San Martino and Manavella, 2004).

The cherry slugworm *C. cerasi* (Hymenoptera: Tenthredinidae) is widespread throughout the Northern Hemisphere. This insect is also conspicuous in many South America countries and it is one of the most important pests in sweet cherries. The larvae feed in such a way that the larger leaf veins remain intact. They rarely penetrate into the abaxial leaf surface, removing part of the mesophyll tissue, resulting in the characteristic skeletonized appearance. Host plants include various species of *Prunus*, *Pyrus*, *Cydonia*, *Cotoneaster* and *Crataegus* (Carl, 1972; Naumann et al., 2002).

Three cultivars of sweet cherry trees (*P. avium*) were selected for the study: Lapins, Bing and Van. These cultivars are the most commonly used in Southern Patagonia, representing 29%, 24% and 5% of the cultivars under cultivation, respectively (Cittadini, 2007). Three to six non-infested and infested by *C. cerasi* trees per cultivar growing in the same habitat were randomly chosen. The infested trees had more than 50% of damaged leaves, while non-infested trees (control group) had less than 1% of damaged leaves. In each individual intact leaves from non-infested trees and damaged and non-damaged leaves from infested trees were randomly chosen and studied. All the sampled leaves were fully expanded and with the same age (time span after the beginning of leaf expansion). All trees were at least 7 years old, planted as free standing trees (280 trees ha⁻¹) and irrigated by gravity (Muñoz, 2004).

2.1. Gas exchange and leaf nitrogen content

Net photosynthetic capacity (*A*) were measured using a portable photosynthesis system (LI-6400, LI-COR, Lincoln, NE). Ten damaged and ten non-damaged leaves from infested trees and ten intact leaves

from non-infested trees within each cultivar (3 control trees and 4 infested trees per cultivar) were studied. All measurements were done on fully developed sun-exposed leaves during mid-morning (1000–1100 h) on sunny days with 400 μmol mol⁻¹ CO₂ inside the leaf chamber generated by a 12 g CO₂ cylinder connected to the LI-6400. The photon flux density (PPFD) was held constant at 1200 μmol m⁻²s⁻¹ to ensure light saturation but avoiding photoinhibition.

The relationship between net photosynthesis (*A*) and PPFD was determined for damaged leaves from infested and intact leaves from non-infested trees (3–6 trees per cultivar). Gas exchange variables were measured at light levels ranging from 0 to 2000 μmol photons m⁻² s⁻¹. Quantum efficiency of photosynthesis (*A*_{qe}), light compensation point (*L*_{CP}), light saturation point (*L*_{SP}) and dark respiration rate (*R*_d) were obtained from the *A*-PPFD relationships.

Leaf N content (*N*_{mass}) of damaged and non-damaged leaves from infested trees and intact leaves from non-infested trees (3 trees per cultivar) was measured using the Kjeldahl method (Miller and Miller, 1948) and it was expressed as leaf nitrogen per area (*N*_{area} = *N*_{mass} * LMA; Ellsworth and Reich, 1992). Instantaneous photosynthetic nitrogen-use efficiency (PNUE) was determined dividing net photosynthesis by foliar nitrogen content (Ellsworth and Reich, 1992).

2.2. Phenolic content of leaves

The total phenolic contents were determined as gallic acid equivalents (EGA) g⁻¹ using the Folin Ciocalteu reagent according to the procedure described by Dastmalchi et al. (2007). Extract was prepared using 2 g dried leaves macerated in 70% ethyl alcohol and was obtained by a solution prepared from boiling macerated leaves with 400 ml distilled water for one hour. This solution, adjusted to 500 ml, was refrigerated until used (Barua and Roberts, 1940). A 1 ml aliquot of extract was transferred to a test tube containing 6 ml of distilled water. Then, 500 ml of Folin-Ciocalteu reagent were added. After, 1.5 ml of Na₂CO₃ solution (200 g l⁻¹) and water were added to reach a volume of 10 ml. After two hours at room temperature, the absorbance was measured at 760 nm using a spectrophotometer (Spectrum SP 1102) and compared to standard curve of gallic acid (0, 50, 100, 250, 500 μg l⁻¹).

2.3. Leaf size and leaf dry mass per unit area

Twenty damaged and 20 non-damaged leaves from infested trees and 20 intact leaves from non-infested trees were collected within each cultivar (*n* = 3 control trees and 4 infested trees per cultivar). Fresh leaf images were acquired using a scanner. To determine leaf size (LA) whole leaf area was used including the area removed by the herbivore (Fig. 1S). This is possible because the larvae produce skeletonization of the leaves leaving relatively intact the vein network, and thus the edges of the leaves are known. Leaf area used to determine leaf dry mass per area (LMA), did not include the leaf section with skeletonization by the herbivores (Fig. 1S). The image analysis was performed using the ImageJ 1.45 k software (Ferreira and Rasband, 2012). Leaves were oven-dried at 70 °C until constant weight, and dry mass was used for leaf dry mass per area (LMA) calculations.

2.4. Tree growth

The stem growth (main branches) of three to five infested and non-infested trees within each cultivar was determined with dendrometer bands. Dendrometers were manually made and consisted of a stainless steel tape encircling a tree stem, with one end passing through a collar (which was attached to the other end) and connected back to itself with a stainless steel spring, as described by Cattellino et al. (1986). Three months after dendrometer installation (allowing for stem dendrometer adjustment), a permanent mark was made on the metal band next to the collar. As stem diameter increases, the mark moves away from the

collar while the spring is stretched, keeping the dendrometer tight. A digital caliper was used to measure stem diameter changes with an accuracy of 0.01 mm. Readings were taken approximately every 30 days from August 2010 to August 2011. Before installing the dendrometers, the external cortex was partially removed leaving a smooth surface. The growth rate was determined from cumulative growth curves plotted as percentage stem increments against time.

2.5. Statistical analysis

All physiological and morphological trait data were normally distributed (Kolmogorov–Smirnov test). Student's *t*-test was used for mean comparisons between damaged leaves from infested trees and intact leaves from non-infested trees within a cultivar. Regression and correlation analyses were used for leaf traits data. All data was analyzed using the R software (Version 3.4.0, Venables (2006)).

3. Results

Herbivory during leaf expansion impacted the leaf size. Totally expanded damaged leaves were smaller than leaves from non-infested trees across all cultivars. The leaf size in infested cultivars was 17 to 11.5 cm² smaller compared to leaf size in non-infested trees, depending on the cultivar. Leaf size which includes also the area resulting from skeletonization by the larvae, decreased exponentially as a function of percentage of leaf area removed by herbivory across all cultivars (Fig. 1). The larger the amount of leaf removed by the herbivore the lower the final leaf size. This functional relationship was independent of cultivar type because only one mathematical algorithm was able to describe the functional relationship with a data set including all leaf sizes and herbivore removal percentage across cultivars. The inset in Fig. 1 shows a leaf with adaxial epidermis and mesophyll partially removed by *C. cerasi*. The veins and abaxial epidermis were left intact.

Mass-based net photosynthesis (A_{mass}), area-base net photosynthesis (A_{area}) and total photosynthetic leaf area per unit branch length were consistently lower across cultivars in leaves from infested trees compared to non-infested leaves (Fig. 2a,b,c). The A_{mass} exhibited between a 43 to a 57% decrease in damaged leaves of infested trees compared to intact leaves from non-infested trees, depending on cultivar. On the other hand, leaf dry mass per unit area (*LMA*) was consistently higher in infested trees compared to non-infested leaves (Fig. 2d). The differences

between non-infested and infested trees values for all variables were mostly significant at *p*-values of 0.05. The three *P. avium* cultivars, including infested and non-infested trees, within a cultivar did not differ in their leaf life span (results not shown).

The herbivory by *C. cerasi* caused significant decrease in mass-based leaf nitrogen (N_{mass}) in two of the study cultivars (Bing and Lapins) (Fig. 2e). Photosynthetic nitrogen-use efficiency (*PNUE*) substantially decreased in damaged leaves from infested trees by about 37% in relation to leaves from non-infested trees (Fig. 2f), and phenolic compounds content tended to increase in the damaged leaves (Fig. 2g).

Non-damaged leaves from infested trees had intermediate values of N_{mass} , *PNUE*, *LA*, A_{area} , and A_{mass} between the intact leaves from non-infested trees and damaged leaves from infested trees (Table 1).

N_{mass} and *PNUE* were linearly correlated to A_{mass} after log transformation of all variables (Fig. 3). With increasing A_{mass} , both N_{mass} and *PNUE* increased but the leaves from non-infested *P. avium* cultivars had higher values than the leaves from infested cultivars. Since A_{mass} was significantly correlated to *LMA* and N_{mass} across cultivars and leaf condition, a multiple regression was fitted to the data set. Leaf mass per area and *N* on a mass basis explained 64% of the A_{mass} variation (Fig. 4). The three-dimensional plot also shows that damaged leaves had higher *LMA*, lower N_{mass} and lower A_{mass} , while intact leaves had higher A_{mass} and N_{mass} but lower *LMA*.

Light response curves of damaged leaves from infested trees and intact leaves from non-infested trees of all cultivars showed the typical asymptotic relationship with net photosynthesis initially increasing linearly with increasing PPFD, and then reaching a maximum asymptotic value at higher PPFD. Photosynthetic light saturation was observed in all cultivars and plant condition above 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and tended to be lower in infested trees (Fig. 5). Quantum efficiency of photosynthesis (A_{qe}) was 13% higher in non-infested trees compared to the leaves of infested ones ($t = 1.79$, $P = 0.087$) across all cultivars, but there were no significant differences in other light curve parameters (L_{CP} - light compensation point, L_{SP} - light saturation point, R_d - dark respiration rate) (data not shown).

Infested trees had higher stem cumulative growth compared to non-infested trees in Bing and Lapins cultivars (Fig. 6a and b). Bing and Lapins cultivars exhibited about 50% higher cumulative growth in infested trees compared to non-infested trees, although the differences were not significant in Lapins (Fig. 6b).

4. Discussion

Multiple defoliation events are likely to occur throughout the lifetime of *P. avium* cultivars in the study area. *Caliroa cerasi* has two or more generations per year (Bado, 2010), and thus continuous attack over a growing season and fruit production period occur. In this study we did not find differences in the pattern of response to herbivory between cultivars. However, there was variability in the response to herbivory between damaged leaves from infested and leaves from non-infested trees produced during the same season. The responses observed could be a consequence of immediate attack of herbivores as well as of chronic and recurrent infestation.

Our results suggest that herbivory by the *C. cerasi* insect may be an important agent inducing changes in physiological and morphological leaf traits in *P. avium*. Accumulation of secondary compounds such as phenols observed in the damaged leaves of the three cultivars can be effective in avoiding further herbivore attacks (Cornelissen and Fernandes, 2001; Karban and Baldwin, 1997; Peschiutta, 2015; Wang et al., 2012). All cultivars showed a decrease in leaf nitrogen content, from intact leaves to damaged leaves. Different reasons could explain the N decrease in leaves from infested trees. Poor nutritive quality of foliage could have evolved as an adaptation to insect herbivory, delaying the larval development and affecting negatively the insect fitness (Casotti and Bradley, 1991; Cornelissen and Stiling, 2006). In addition, during the insect attack, a rapid export of resources from the leaves to

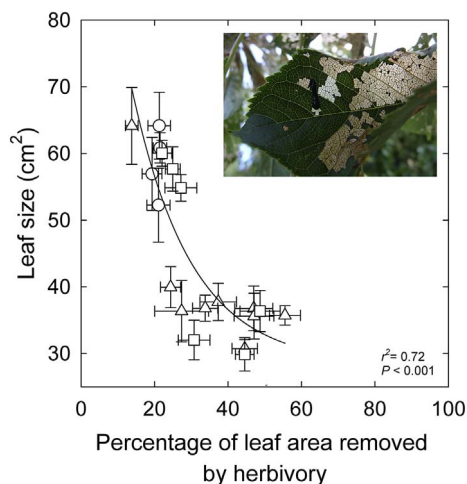


Fig. 1. Leaf size as a function of percentage of leaf area removed by herbivory for all cultivars. The line is the negative exponential function fitted to the data ($y = 28,45 + 97,30 \cdot \exp(-0,06 \cdot x)$). Each symbol represent the mean \pm SE ($n = 10-20$) of both variables in damaged leaves from infested trees. Cultivars: Bing (circles), Lapins (triangle), Van (square). Inset shows a leaf with adaxial epidermis and mesophyll partially removed by herbivory. The veins and abaxial epidermis were left intact.

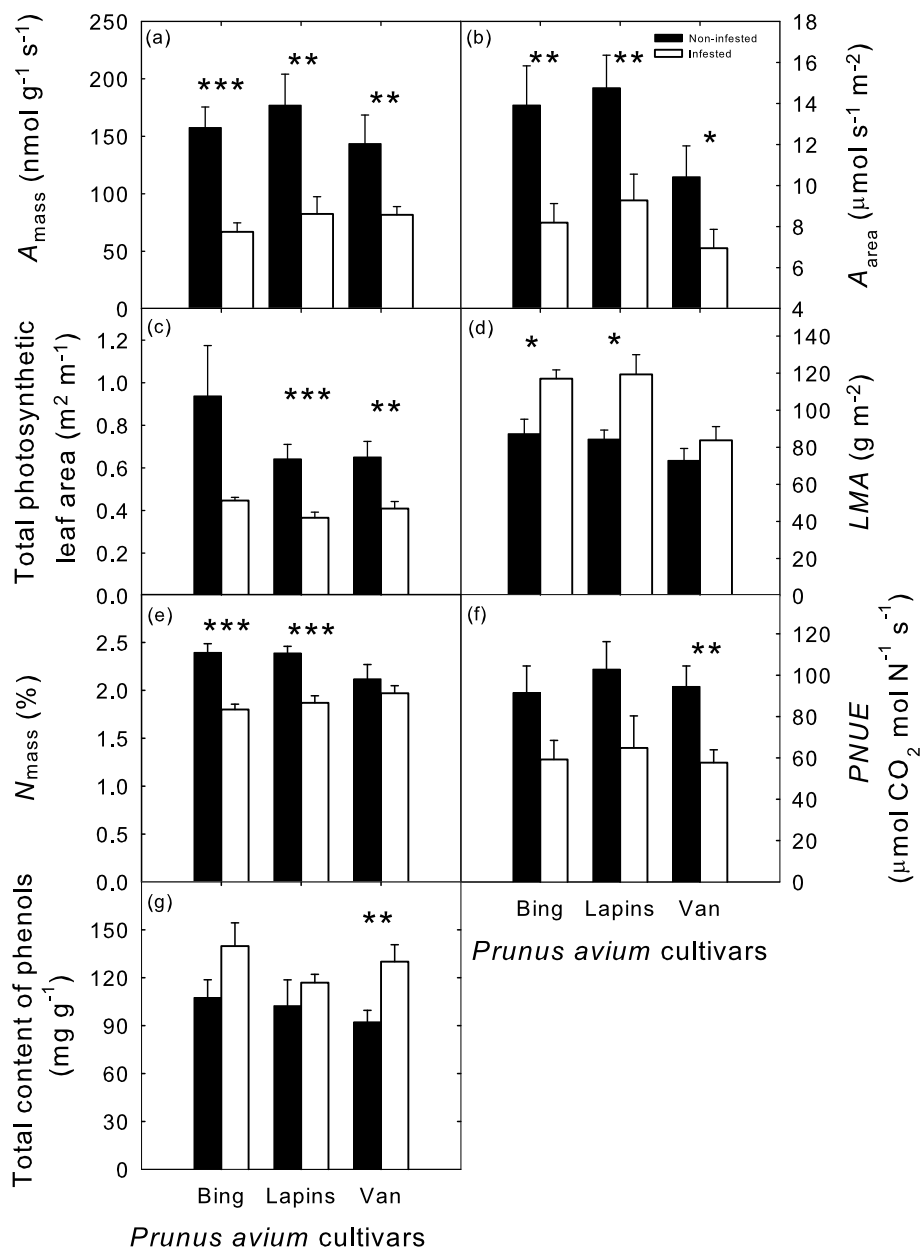


Fig. 2. (a) Mass-based net photosynthesis (A_{mass}), (b) Area-based net photosynthesis, (c) total photosynthetic leaf area per unit branch length and (d) leaf dry mass per unit area (LMA), (e) mass-based leaf nitrogen (N_{mass}), (f) instantaneous photosynthetic nitrogen-use efficiency (PNUE) and (g) total leaf phenols content. Vertical bars represent the mean value + SE ($n = 3$ to 4) of intact leaves from non-infested trees (black bars) and damaged leaves from infested trees (white bars) of the three study *Prunus avium* cultivars. Significant differences between plant condition within each cultivar are indicated as: *** $P < 0.01$, ** $P < 0.05$ and * $P < 0.1$.

Table 1

Area-based leaf nitrogen (N_{area}), mass-based leaf nitrogen (N_{mass}), photosynthetic nitrogen-use efficiency (PNUE), leaf dry mass per unit area (LMA), leaf size (LA), area-based net photosynthesis (A_{area}), mass-based net photosynthesis (A_{mass}), in intact leaves from non-infested trees and in non-damaged and damaged leaves from infested *Prunus avium* trees. Values are means \pm SE ($n = 9$ to 12). Different letters between columns indicate significant differences (P values are included).

	Non-infested trees	Infested trees		P-value
		Non-damaged leaves	Damaged leaves	
N_{area} ($g\ m^{-2}$)	1.90 \pm 0.12 ^a	1.72 \pm 0.07 ^a	1.85 \pm 0.08 ^a	0.355
N_{mass} (%)	2.30 \pm 0.07 ^a	2.10 \pm 0.07 ^{ab}	1.88 \pm 0.04 ^b	0.001***
PNUE ($\mu mol\ CO_2\ mol\ N^{-1}\ s^{-1}$)	96.35 \pm 6.30 ^a	73.97 \pm 4.37 ^b	60.59 \pm 5.61 ^b	0.001***
LMA ($g\ m^{-2}$)	82.43 \pm 4.03 ^a	82.52 \pm 4.43 ^a	99.69 \pm 4.64 ^b	0.011**
LA (cm^2)	74.52 \pm 6.08 ^a	66.69 \pm 3.11 ^{ab}	60.25 \pm 2.03 ^b	0.068*
A_{area} ($\mu mol\ m^{-2}\ s^{-1}$)	13.03 \pm 1.07 ^a	9.15 \pm 0.80 ^b	8.14 \pm 0.62 ^b	0.001***
A_{mass} ($nmol\ g^{-1}\ s^{-1}$)	160.03 \pm 12.81 ^a	111.80 \pm 8.19 ^b	77.03 \pm 5.95 ^c	< 0.001***

other plant organs may occur which can act as a preventive strategy to safeguard valuable leaf resources keeping them away from herbivores (Gómez et al., 2010; Orians et al., 2011). Besides higher N content on leaves relative to roots and stems, loss of leaf area during the successive

defoliation could contribute to further reduction in N reserves (Thornton and Milland, 1997).

Herbivory reduced not only photosynthesis per leaf due to lower leaf nitrogen content, but also total tree photosynthesis as a result of a

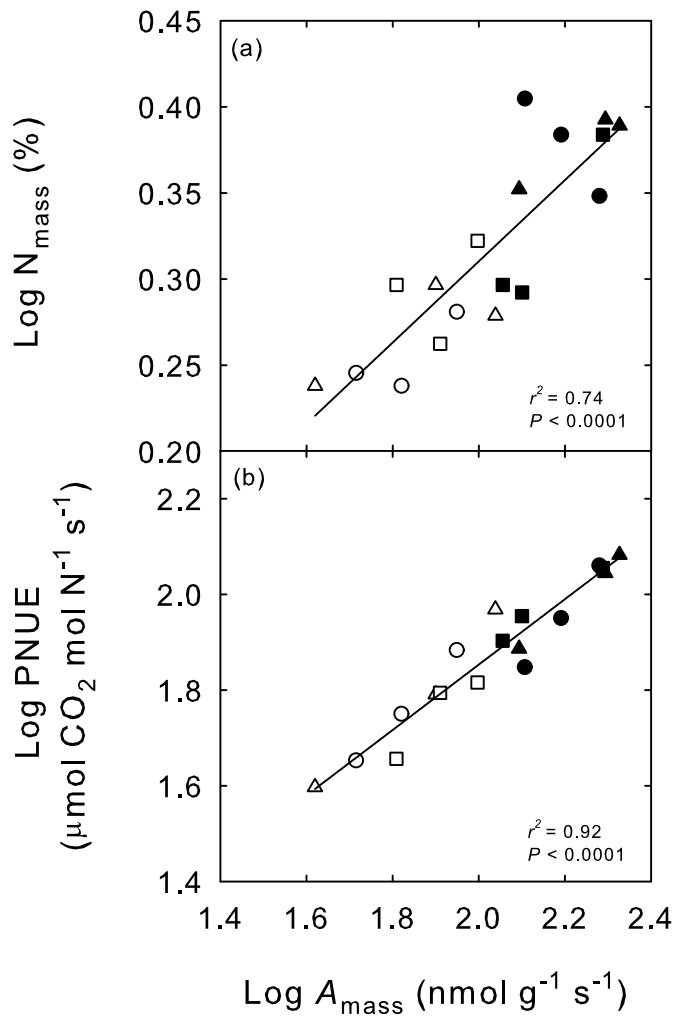


Fig. 3. Relationships between (a) mass-based leaf nitrogen (N_{mass}), (b) photosynthetic nitrogen-use efficiency ($PNUE$) and mass-based net photosynthesis (A_{mass}). The lines are linear regressions fitted to the data, (a) $y = 0.004x + 1.605$, $P < 0.001$ and (b) $y = 0.514x + 16.893$, $P < 0.001$. Each point represents the mean value of damaged (open symbols) and intact leaves (black symbols) from non-infested trees per cultivar ($n = 3$). Cultivars: Bing (circles), Lapins (triangle), Van (square).

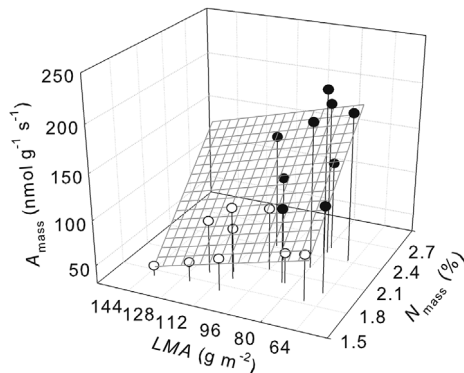


Fig. 4. Three way trait relationship among A_{mass} , N_{mass} and LMA represented in a three dimensional plot. A_{mass} is the predictor variable. The equation describing this relationship is: $A_{mass} = \log(N_{mass}) - 0.41 \cdot \log(LMA)$; $n = 27$, $r^2 = 0.64$, $P \leq 0.0001$.

reduction in total leaf area per plant. We found that the rate of carbon assimilation by a single leaf was highly and positively correlated with leaf organic nitrogen content and $PNUE$, similar to findings observed in other studies (Reich et al., 1994, 1999; Takashima et al., 2004; Wright et al., 2004; Xiang et al., 2013). Leaves from infested trees with low

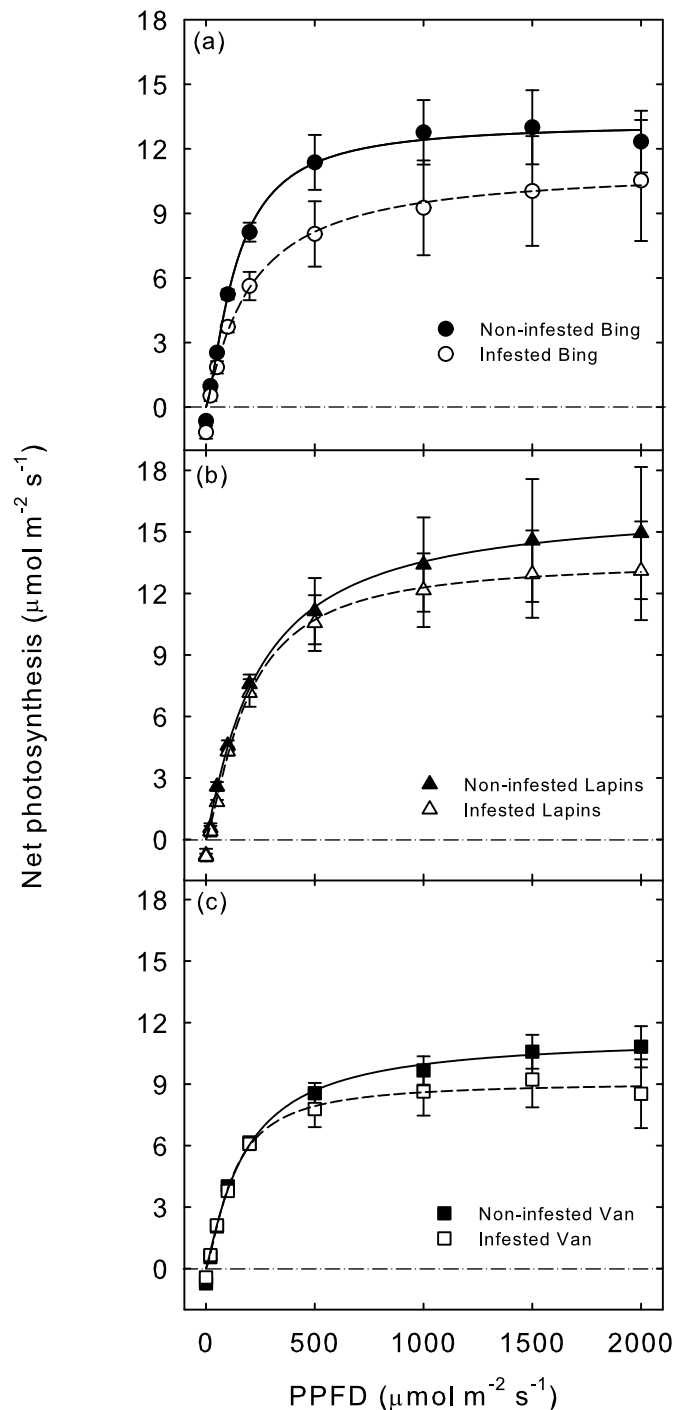


Fig. 5. Light response curves of *Prunus avium* intact leaves from non-infested trees and damaged leaves from infested trees. Data points for assimilation rates for infested (open symbols) and non-infested (filled symbols) trees within each cultivar are means \pm SE ($n = 3$ to 6). The solid (non-infested trees) and short dash (infested trees) lines are the sigmoidal curves fitted to the data ($r^2 > 0.99$, $P < 0.001$). Cultivars: Bing (circles), Lapins (triangle), Van (square).

nitrogen content probably had low $PNUE$ because of the investment of a large proportion of total leaf nitrogen into components involved in processes other than photosynthesis. For example, cell walls (Onoda et al., 2004; Takashima et al., 2004) and defense compounds (Gleadow et al., 1998; Herms and Mattson, 1992) are major N sinks. Some *Prunus* species are known to accumulate N-rich defensive compounds such as cyanogenic glycosides (Miller et al., 2004; Santos Pimenta et al., 2014), which can lead to the decline in $PNUE$ (Field and Mooney, 1986). Leaf

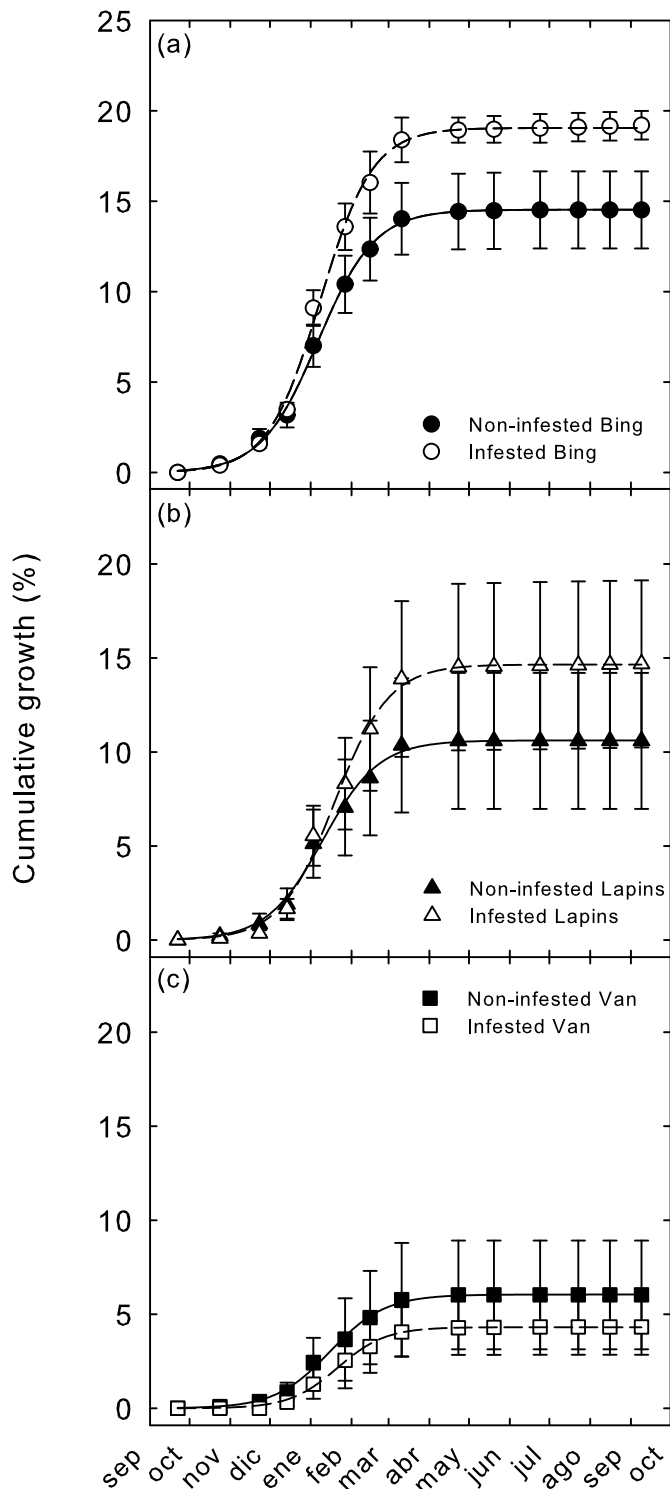


Fig. 6. Stem cumulative growth (%) from August 2010 to August 2011. Each point represents the growth in percentage of the initial stem area (mean \pm ES) for non-infested (closed symbols) and infested (open symbols) trees. The solid (non-infested trees) and dash (infested trees) lines are the sigmoidal curves fitted to the data obtained on three to six trees per cultivar and plant condition (infested and non-infested trees) ($r^2 > 0.99$, $P < 0.001$). Cultivars: Bing (circles), Lapins (triangle), Van (square).

mass per area is often used as an indicator of the structural (cell-wall) component of the biomass (Wright and Cannon, 2001) and was higher in infested trees, possibly due to higher accumulation of carbon compounds in their leaves.

Plants face the dilemma to invest resources in defenses or growth. In

a previous study with the same cultivars (Peschiutta et al., 2016) we observed a reduction in leaf size due to recurrent herbivory. However, other parts of the plants (e.g. stems) showed higher growth despite having lower carbon assimilation. The higher differences in stem cumulative growth were observed just before leaf senescence, which reinforces the idea of an increase in export of existing or newly acquired resources from damaged leaves into storage organs. For a plant, the best investments should be in new leaves for enhancing long-term growth, nevertheless this may be non-advantageous when herbivores are present. In this study we did not evaluate root biomass or total biomass growth, but other studies have shown that defoliation enhances stem growth at the expense of root growth (Yoshizuka and Roach, 2011). The redistribution of resources within the tree may allow for the maintenance of stem growth in infested plants despite of carbon assimilation decreases.

We observed that LMA and N_{area} were positively correlated and LMA and N_{mass} were negatively correlated similar to patterns observed in other studies (e.g. Read et al., 2014). Leaves with a low N_{mass} usually maximize LMA through increased leaf density or thickness, thereby maximizing N_{area} . Negative relationships between LMA and N_{mass} suggest that increasing LMA may result in the dilution of N_{mass} (Ellsworth and Reich, 1992). Differences in photosynthetic capacity between leaves from infested and non-infested trees may arise from the covariation in both LMA and mass-based leaf nitrogen (N_{mass}), both of which contribute to variation in N_{area} (Coste et al., 2005; Ripullone et al., 2003).

Photosynthesis is a physiological process that can be affected not only by environmental factors but also through changes in LMA and leaf nitrogen (Reich et al., 1991). About half of total leaf N is usually allocated to the photosynthetic apparatus (Evans, 1989; Evans and Seemann, 1989; Poorter and Evans, 1998). In this study, the rate of photosynthesis correlated negatively with LMA and positively with $PNUE$, similar to other findings (Mason et al., 2013; Onoda et al., 2004; Xiang et al., 2013).

Infested trees were able to maintain similar timing of leaf activity than the non-infested trees probably by using chemical defenses as phenolics compounds and at the same time developing thicker leaves (higher LMA). Higher LMA could be the result of the incremented vasculature observed in damaged leaves from these same *P. avium* cultivars in a previous study (Peschiutta et al., 2016).

5. Conclusions

Functional leaf traits in the three *P. avium* cultivars differed greatly according to the level of infestation by *C. cerasi*. Decreased photosynthetic capacity in infested trees was not only associated to effects of N decrease by herbivory but also it was associated to a negative feedback mechanism from the leaf carbohydrate pool (increase LMA). In other words, the functional convergence of leaf traits across cultivars and leaf conditions may result from a trade-off in nitrogen and carbon partitioning within the leaf: either for enhancing photosynthetic capacity or for enhancing anti-herbivory defenses. Despite lower A and total leaf area, stem cumulative growth tended to increase in infested plants suggesting a change in the patterns of biomass allocation elicited by herbivory. A larger N investment in defenses instead of photosynthetic structures may explain the lower $PNUE$ and A_{mass} observed in damaged leaves. Biotic pressures change the carbon–nitrogen allocation pattern within the plant. Evidences such as these, emphasize the need to use approaches linking leaf-level ecophysiology with patterns of whole-plant growth and allocation to provide a better understanding of the effects of herbivory at plant level. Some physiological changes due to herbivory partially compensate the cost of leaf removal buffering the carbon economy at the whole plant level.

Contributions of co-authors

ML Peschiutta contributed to the field measurements, the processing and analysis of data and in the manuscript preparation.

FG Scholz supervised the field acquisition and contributed to the manuscript revision.

G Goldstein contributed to the manuscript revision.

SJ Bucci contributed to experimental design of the study, analysis of data and in the manuscript preparation.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.actao.2017.11.007>.

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