




Oviposition by herbivorous insects induces changes in optical and mechanical properties of *Prunus avium* leaves

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Abstract

This study of animal–plant interaction focused on the impact of oviposition by an insect on the leaves of *Prunus avium* (cherries). We examined whether the oviposition by *Caliroa cerasi* affects leaf mechanical and spectral traits in *P. avium*. Three cultivars of *P. avium* were studied. Infested leaves had from 1 to 18 eggs and exhibited higher leaf dry mass per area (LMA) than leaves without eggs. Leaf dry weight and LMA were positively correlated with egg number per leaf. Infested leaves tended to have higher number of trichomes. Leaf thickness and material and structural resistance tended to increase in infested leaves. The reflectance across all wavelengths (500–700 nm) in leaves with larger number of eggs was higher compared to leaves without eggs. Photosynthetic performance was reduced and oxidative activity was increased in leaves with eggs. Extrafloral nectaries increased with increasing the number of eggs per leaf and thus play an important role in defense against herbivores by providing nectar rewards that attract their depredators. These responses to oviposition may be beneficial for the plants in terms of resistance to feeding larvae.

Keywords Extrafloral nectaries · Inducible leaf traits · Leaf spectral characteristic · Leaf trichomes

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Introduction

Most studies of induced plant responses by herbivory consider feeding damage as a first interaction between the insect and the plant. However, egg deposition often precedes feeding damage in many herbivores (Pashalidou 2015). Insect eggs when laid on the plant can induce changes in the primary and secondary plant metabolism. The egg-induced effects can be considered at the same time plant defensive responses against egg deposition by herbivorous insects.

Unlike feeding herbivores that can move away from the plant that induces the production of toxins, eggs cannot move. Therefore, plant responses to oviposition can kill them and prevent feeding damage caused by the hatching larvae (Pashalidou 2015). Egg-induced plant defense strategies directly targeting the eggs rather than the egg-laying female include plant-mediated desiccation of eggs, egg dropping, egg crushing, and egg killing (Hilker and Fatouros 2015). Also feeding by herbivorous insects can induce changes in the emission of volatile compounds (Colazza et al. 2004; Heil 2009) or enhance the development of extrafloral nectaries (Pulice and Packer 2008) that act as indirect defenses against herbivores via tritrophic interactions with carnivores

involving three trophic levels: the plant, the herbivore, and its natural enemies.

Some studies have shown that in addition to these egg-induced direct and indirect plant defenses, plants can use insect egg deposition as a warning signal for future larval herbivory. Plants warned by egg deposition start to prepare their defense against feeding larvae even before larval hatching (Hilker and Fatouros 2015) or accelerate their growth and thus begin flowering and reproducing earlier than non-warned plants (Pashalidou et al. 2013).

Egg deposition by herbivorous insects can reduce the expression of photosynthetic genes (Little et al. 2007), and induce the expression of many defense-related genes, including pathogenesis-related proteins (chitinases and glucanases) and genes involved in abiotic stress and phytohormone signaling (Büchel et al. 2012). The mechanisms of egg-induced effects in tissue adjacent to the oviposition site are unknown (Hilker et al. 2002).

Although many studies have evaluated the defenses induced by plant tissue consumption by insects, only recently there has been substantial increases on defense induction by insect egg deposition (Fatouros et al. 2012; Lucas-Barbosa et al. 2012; Pashalidou et al. 2013; Peñaflores et al. 2011; Petzold-Maxwell et al. 2011). Most of these studies on defenses induced by eggs were oriented towards the study of leaf volatile components (Colazza et al. 2004; Fatouros et al. 2012; Lucas-Barbosa et al. 2012; Peñaflores et al. 2011), which play a key role in plant defenses, while leaf mechanical and optical changes as a consequence of egg deposition have been less studied. However, there are reports that herbivorous insects induce morphological resistances in plants when they feed, for example, the mechanical protection on the surface of the plants (e.g., hairs, trichomes, thorns, spines, and thicker leaves) that kill or retard the development of the herbivores (Dalin et al. 2008; Dalin and Björkman 2003; Hanley et al. 2007; Liu et al. 2010; Peschiutta 2015).

Caliroa cerasi (L.) (Hymenoptera: Tenthredinidae), commonly called “pear slug,” is endemic to the Northern Hemisphere, is widespread in South America, and is one of the most important pests of sweet cherry (*Prunus avium* L.) (Mareggiani et al. 2012). *Caliroa cerasi* females are parthenogenetic and they insert their eggs inside the leaves using a serrated ovipositor, and thus they are also known as “sawflies.” Eggs hatch 1 or 2 weeks later and the larvae begin to feed on the upper surface of leaves (Bado 2007; Cittadini and San Martino 2007; Peschiutta et al. 2016; Raffa and Lintereur 1988; Shaw et al. 2004). *C. cerasi* is polyphagous, but prefers to attack trees of the genus *Prunus* (cherry, plum) and *Pyrus* (pear) (Carl 1972). The effects of oviposition by *C. cerasi* on leaves of *Prunus avium* (sweet cherry) are even unknown. The main aim of this study was to evaluate leaf mechanical and spectral traits related to *C. cerasi* oviposition through field studies. Recent studies (Peschiutta et al.

2016, 2018) show that during the leaf expansion phase and before infestation began, leaves of similar age from non-infested trees and chronically infested trees are of similar size. However, at the end of the expansion phase, damaged leaves have lower size (it is considering the area removed by herbivore) than leaves from non-infested trees. In addition, some morpho-physiological traits of non-damaged and damaged leaves from chronically infested trees differ from traits of leaves from trees non-infested for several years. Based on these findings, we hypothesized that most of the mechanical and spectral responses analyzed are induced by the insect oviposition and not merely selected by the insect to enhance its fitness. Two of the variables studied (photosynthesis and oxidative activity), however, should only have detrimental effects on the plant as a consequence of the insect activity. This study was carried out on leaves of three *P. avium* cultivars after 2 weeks of egg depositions, which occur while leaves are in the expansion phase.

Materials and methods

Location and study species

The study was conducted in the Bahia Solano S.A. Ranch, Comodoro Rivadavia, Chubut, Argentina (46°19'S, 71°62'W, altitude 220 m). In the study area, the rains are concentrated in winter. The average annual rainfall is 287 mm (National Weather Service, Comodoro Rivadavia Meteorological Station). The climate of this site is influenced by the proximity to the ocean and the average annual temperature is 12.9 °C (Beeskov et al. 1987).

The study was conducted at the beginning of the infestation by *C. cerasi*, after about 14 days of oviposition and when leaves were in the expansion phase, during December 2012. Three cultivars of sweet cherry (*P. avium*) were selected: Lapins, Bing, and Van. These cultivars are among the most widely used in southern Patagonia, representing a 28.9, 23.7, and 4.8% of sweet cherry cultivars, respectively (Cittadini 2007). Three to seven trees per cultivar affected per *C. cerasi* were randomly selected during the egg-laying period and individual leaves with and without eggs were chosen. Trees were more than 4 years old and drip-line irrigation was used in all trees. The number of *C. cerasi* eggs was counted under a magnifying glass to try to establish a relationship between the leaf properties and oviposition.

Leaf morphological traits

Twenty-five fresh leaf images with and without eggs collected from each tree ($n = 3-7$) were obtained using a scanner. Leaf area (AF) was determined by an image analysis program (ImageJ). Leaf thickness was measured using a

digital caliper (0.01 mm precision). Leaves were then oven dried at 70 °C until constant weight and the dry weight was used to calculate the dry mass per unit leaf area (LMA) (Sack et al. 2003). The extrafloral nectary number (EFN) occurring in each petiole base was counted.

Leaf density (ρ_L) was calculated as $\rho_L = M/V$, where M is the dry mass of the leaf and V is the leaf volume. Volume was estimated by submerging a fresh sample in a container with distilled water resting on a digital balance with a 0.001 g precision using the Arquimides principle. Saturated water content (SWC) was determined as the ratio between saturated mass minus dry mass, and dry mass.

Leaf trichome density (number/mm²) and trichome length were determined in 2–3 sections (disks) on the abaxial surface of three leaves with and without eggs from each plant studied ($n = 3–7$ trees per cultivar). Disks were examined using a magnifying glass (Zeiss, Germany) and images were obtained using a camera (TCA-3C Tucsen, China) mounted on the magnifying glass and acquired with TSVIEW v 6.1.3.9 program. Photographs were taken at 25× to estimate trichome density and size using randomly tissue visual fields. Trichomes associated with the main veins were measured and counted using the ImageJ software.

Foliar nitrogen and carbon

Carbon and nitrogen content of leaves with and without eggs from trees infested by *C. cerasi* (3–4 trees per cultivar) was determined by calcination in a muffle at 550 °C (Schlesinger and Hasey 1981) and by Kjeldahl method (Miller and Miller 1948) at the Estación Experimental Agropecuaria Bariloche—INTA, Argentina. This information was used to calculate the carbon–nitrogen (C/N) ratio.

Leaf mechanical resistance

A penetrometer built in the laboratory (Peschiutta 2015) was used to evaluate the leaf mechanical resistance. Leaves were firmly held between two plates forming the base of the penetrometer and having a central hole. Plates were made of transparent acrylic to allow visualization of the blade position with respect to a metal rod or punch hole (5.03 mm diameter) slightly rounded tip. The upper punch is fixed to a container, which receives water at a constant rate and can increase the mass of the penetrator (= punch + container + water) until a critical value is reached and the metal rod penetrate through the blade. In all cases, the measurements were performed on blades excluding the higher order veins. Penetrator mass is proportional to the leaf hardness. Hardness can be expressed as the critical mass (g) of the penetrator with a standard punch necessary to penetrate the blade. Maximum force can then be expressed per fracture length along the lamina surface (force to punch or structural resistance, F_p , kN m⁻¹) and

per cross-section area of lamina (specific force to punch or material resistance, F_{ps} , MN m⁻²) (Onoda et al. 2011). 20 leaves with eggs and 20 leaves without eggs randomly collected per plant and per cultivar were used.

Optical properties of the leaves

Reflectance of 25 leaves with and without eggs of *C. cerasi* was measured in the spectrum range of 400–900 nm using a spectrometer (Ocean Optics USB 2000) coupled to an integrating sphere designed and built in our laboratory (Peschiutta 2015). A leaf sample was placed between a collimated light source and a leaf sample located on an opening in the integrating sphere wall. A fiber optic was inserted on the opposite side of the wall and connected to the spectrometer port. The inner surface of the sphere was covered with barium sulfate to allow for maximum reflectance. The light was directed towards the adaxial side of the leaf. Darkness adjustments and white point calibrations were regularly performed (using painted with barium sulfate plates) for each leaf. The diffuse reflectance was calculated as a ratio relative to the reference target data. Curves of reflectance were performed for leaves without eggs (no eggs), with 1–3 eggs (low), with 4–6 eggs (medium), and with 7 or more eggs (high) per leaf. Reflectance measurements at 550 nm wavelength were used as an estimate of leaf nitrogen (Blackmer et al. 1994). Photochemical Reflectance Index (PRI)—an estimator of photosynthetic performance—and Brown Pigment Index (BPI)—an index of oxidative stress—both were calculated from the spectral reflectances as (Peñuelas et al. 2004):

$$\text{PRI} = \frac{(R_{531} - R_{570})}{(R_{531} + R_{570})}$$

The BPI index was calculated as the difference between the wavelengths where the second derivative of the reflectance in the far-red region intercepts 0, that is the difference between the wavelength with maximum reflectance slope (λ red edge) and the wavelength with the minimum reflectance slope in the near infrared (Peñuelas et al. 2004).

Statistical analysis

All data were analyzed to assess normality using Shapiro–Wilks and homogeneity of variances using the Levene test before performing the ANOVA. When data were not normal, a non-parametric test was applied as Kruskal Wallis or the χ^2 test. Student t test was used to compare means of leaves with and without eggs in each cultivar. All data were analyzed using the R software (version 2.15.1). Leaf traits were selected assuming that the response of the plant to the insect attack will enhance resistance to herbivory and that

they were not auto-correlated. Linear regressions were used to test the dependence of reflectance (550, 516, 590, and 596 nm) on egg number per leaf.

Results

Number of eggs

Leaves infested by *C. cerasi* had from 1 to 18 eggs. From a total of 482 randomly collected leaves with eggs, the 38.59% of leaves had one egg, 20.95% had two eggs, 12.86% had three eggs, and only 9.75% had 7 or more eggs. There were no differences in the percentage of infested leaves along a

branch and egg number did not differ significantly among cultivars ($F=2.43$; $P=0.12$); however, Bing tended to have more eggs per leaf than Van and Lapins. We observed an average of two eggs per leaf in Lapins and three eggs per leaf in Bing. Despite the higher number of eggs per leaf in Bing, this cultivar exhibited the largest number of necrotic eggs and surrounding leaf tissues (4%) compared to Van and Lapins (2 and 1.8%, respectively).

Morphological characteristics and leaf mechanical properties

Leaves with eggs (Eggs) had a higher leaf dry mass per unit area (LMA) than leaves without eggs (No eggs) in all

Fig. 1 **a** Leaf dry mass per unit area (LMA), **b** leaf thickness, **c** material resistance (F_{ps}), and **d** structural resistance (F_p) of three *Prunus avium* cultivars. Each bar is the mean value + SE ($n=3-7$ trees per cultivar) for leaves without eggs (filled bars) and leaves with eggs (open bars) from infested trees. Significant differences between leaves with and without eggs are indicated as $**P<0.05$ and $***P<0.01$

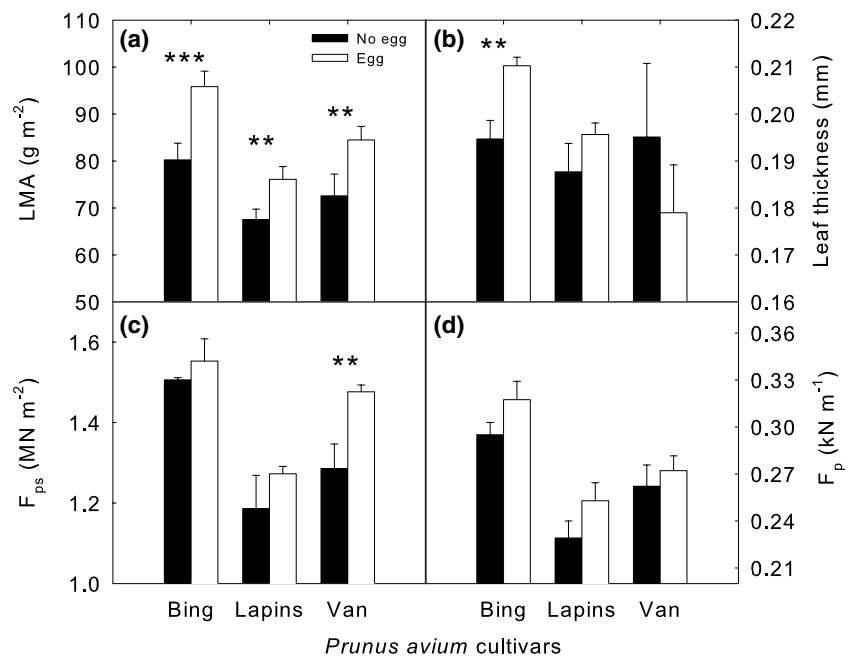


Table 1 LA, ρ_L , saturated water content (SWC), density and length of trichomes, nitrogen content (N), and C/N ratio in leaves without eggs (no eggs) and leaves with eggs (eggs) from infested *Prunus avium* L. trees by *Caliroa cerasi* per cultivar

	LA (cm ²)	ρ_L (g cm ⁻³)	SWC (%)	Leaf trichome density (number mm ⁻²)	Leaf trichome length (mm)	N (%)	C/N ratio
Bing							
No eggs	49.49 ± 3.09 ^a	0.33 ± 0.02 ^a	235.62 ± 11.59 ^a	3.54 ± 0.37 ^a	0.77 ± 0.01 ^a	1.74 ± 0.09 ^a	26.28 ± 1.48 ^a
Eggs	51.67 ± 1.82 ^a	0.36 ± 0.02 ^a	222.48 ± 7.61 ^a	4.43 ± 0.89 ^a	0.79 ± 0.04 ^a	1.77 ± 0.14 ^a	25.68 ± 2.01 ^a
Lapins							
No eggs	69.32 ± 3.71 ^a	0.30 ± 0.01 ^a	261.88 ± 14.74 ^a	1.73 ± 0.18 ^a	0.80 ± 0.05 ^a	2.06 ± 0.08 ^a	21.93 ± 0.67 ^a
Eggs	75.01 ± 5.87 ^a	0.29 ± 0.02 ^a	259.36 ± 21.09 ^a	3.27 ± 0.61 ^b	0.63 ± 0.05 ^b	2.20 ± 0.08 ^a	20.68 ± 0.63 ^a
Van							
No eggs	54.55 ± 3.66 ^a	0.33 ± 0.01 ^a	236.23 ± 2.45 ^a	3.02 ± 0.57 ^a	0.82 ± 0.05 ^a	1.76 ± 0.12 ^a	26.25 ± 1.76 ^a
Eggs	53.63 ± 3.43 ^a	0.36 ± 0.01 ^a	218.22 ± 8.78 ^a	3.72 ± 0.69 ^a	0.76 ± 0.05 ^a	1.63 ± 0.10 ^a	27.92 ± 1.80 ^a

Values are means ± SE ($n=3-7$). Significant differences between leaves with and without eggs within each cultivar are indicated by different letters (t test, $P<0.05$)

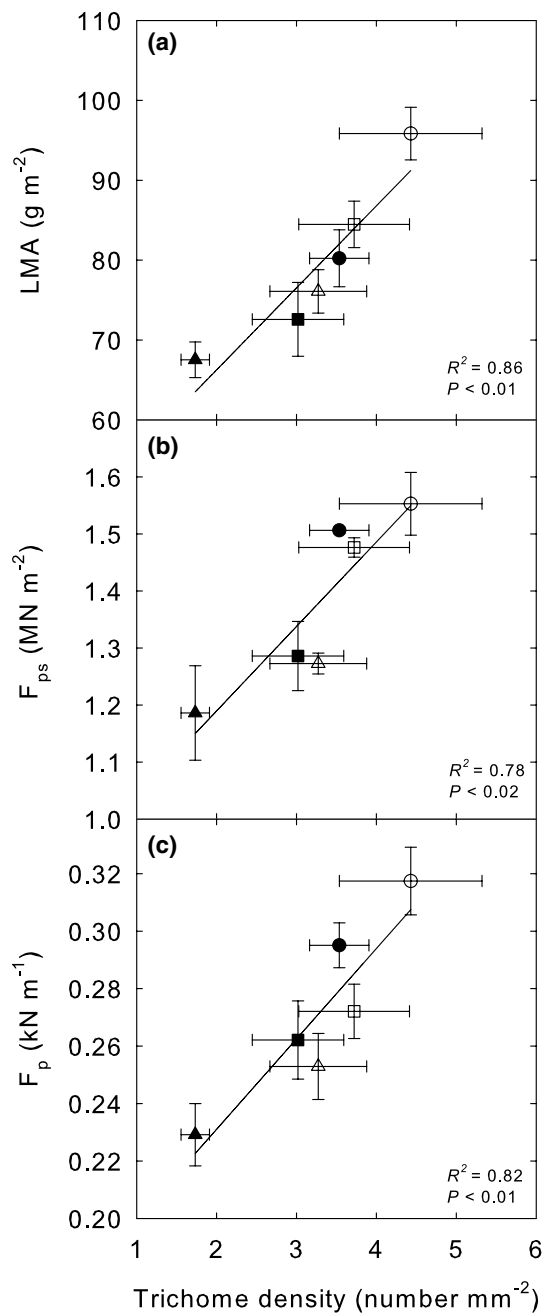


Fig. 2 Relationships between **a** leaf dry mass per unit area (LMA), **b** material resistance (F_{ps}), and structural resistance (F_p) in relation to leaf trichome density associated with major veins. The lines are linear regressions fitted to the data, **a** $y=45.71+10.27\times x$; **b** $y=0.89+0.15\times x$, and **c** $y=0.17+0.03\times x$. Each point represents the mean value of leaves with (open symbols) and without (filled symbols) eggs from three to seven trees per cultivar. Cultivars: Bing (circles), Lapins (triangles), Van (squares)

cultivars (Fig. 1a). Other leaf characteristics such as thickness, F_{ps} , length, and number of foliar trichomes differed depending on the cultivar. For example, leaves with eggs from Bing cultivar were thicker than leaves without eggs

(Fig. 1b), while F_{ps} was higher in leaves with eggs from Van cultivar than leaves without egg (Fig. 1c). The F_p , LA, ρ_L , SWC, N content, and C/N ratio did not differ between leaves with and without eggs across all cultivars (Fig. 1d; Table 1). However, leaves with eggs from Bing and Van cultivars showed a tendency of higher ρ_L and lower SWC (Table 1).

Leaves with eggs from Lapins cultivar had shorter and higher density of trichomes than leaves without eggs (Table 1). Also leaves with eggs from Bing and Van cultivars tended to have higher trichome density than leaves without eggs (Table 1). Trichome density was positively correlated to LMA, F_{ps} and F_p (Fig. 2a–c).

The PRI was lower in the leaves with eggs than in leaves without eggs, with significant differences in Lapins and Van cultivars (Fig. 3a). Leaves with eggs from Van exhibited higher BPI than leaves without eggs (Fig. 3b).

Leaf dry mass per unit area and leaf dry weight were positively and significantly correlated to the egg number per leaf (Fig. 4a, b); however, there was not a significant relationship between leaf size and egg number per leaf (Fig. 4c). The PRI decreased significantly with increasing egg number per leaf (Fig. 4d).

Optical traits

Leaves without eggs of the three *P. avium* cultivars exhibited similar % reflectance across all wavelengths (Fig. 5a). However, leaves with eggs of Van cultivar had higher % reflectance than leaves with eggs from Bing and Lapins cultivar (Fig. 5b). There was a trend to have a higher reflectance across all wavelengths in leaves with larger number of eggs compared to leaves without eggs in all cultivars (Fig. 5c). Reflectance at 550 nm (R_{550}), 516 nm (R_{516}), 592 nm (R_{592}), and 596 nm (R_{596}) wavelengths increased significantly with increasing egg number per leaf (Fig. 6a–d).

Extrafloral nectaries

There were not significant differences in extrafloral nectary number between leaves with and without eggs in all cultivars; however, extrafloral nectaries increased significantly with increasing the number of eggs per leaf (Fig. 7).

Discussion

Plant-induced defenses can be elicited by feeding or oviposition of herbivores (Heong et al. 2015). Plants have evolved responses that target exclusively the insect oviposition, because eggs might serve as reliable cues for plants, since eggs indicate that feeding damage will start as soon as the larvae hatch (Pashalidou 2015). Leaf characteristics

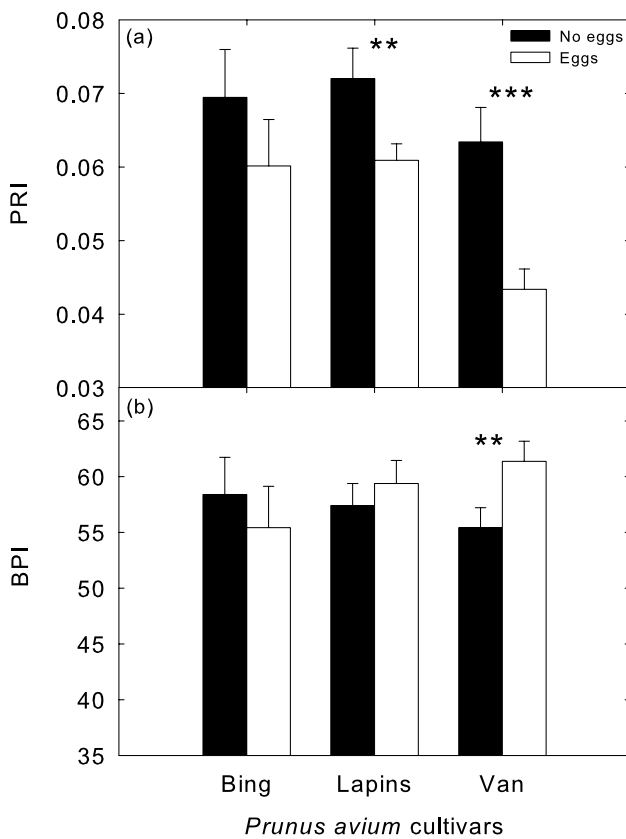
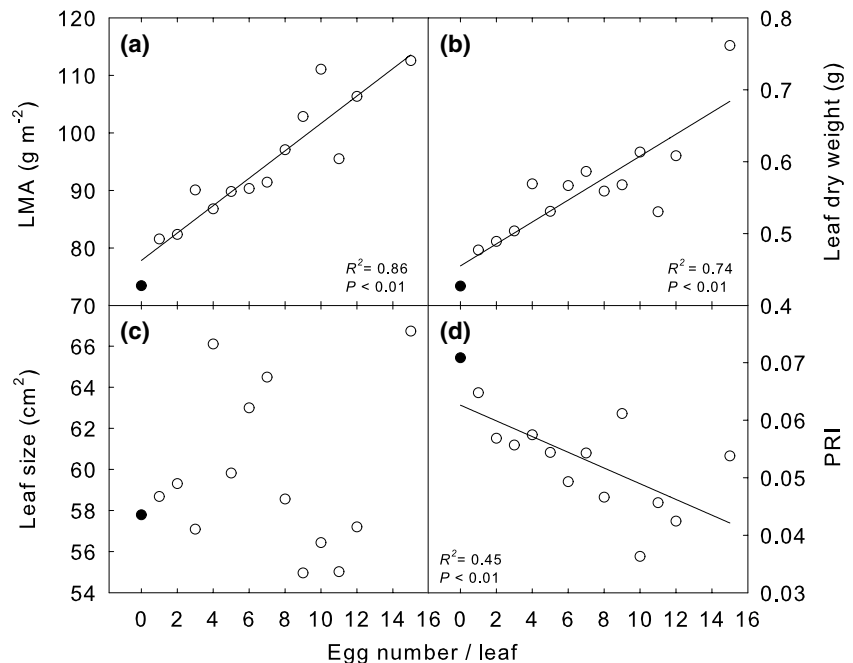


Fig. 3 **a** Photochemical Reflectance Index (PRI) and **b** Brown Pigment Index (BPI) of three *Prunus avium* cultivars. Bars represent the mean value + SE ($n=3-7$ tree per cultivar) for leaves without eggs (filled bars) and leaves with eggs (open bar) from infested trees. Significant differences between leaves with and without eggs are indicated as ** $P < 0.05$ and *** $P < 0.01$

Fig. 4 **a** Leaf dry mass per unit area (LMA), **b** leaf dry weight, **c** leaf size, **d** Photochemical Reflectance Index (PRI) as a function to the egg number per leaf ($n=3-7$ trees, depending on egg number per leaf). Solid lines are linear regressions fitted to the data **a** $y=77.84+2.38 \times x$; **b** $y=0.46+0.02 \times x$, and **d** $y=0.06-0.001 \times x$. Filled symbol represents leaves without eggs



in this study were determined after 14 days since the female laid eggs, close to the emergence of the first larvae (Raffa and Lintereur 1988; Shaw et al. 2004) and when the leaves were still in expansion (Peschiutta 2015). The induction of responses can occur within a few hours or days after the attack (Escobar-Bravo et al. 2017; Karban and Baldwin 1997), and such changes may represent defenses induced by the herbivore. In our study, we observed a tendency to increase the values of leaf traits related to mechanical resistance as response to oviposition. Leaves with higher mechanical resistance may reduce the subsequent impact of larvae on leaf by repelling to larvae or by reducing the eaten leaf area. Consistent with this idea, Bing and Van were the cultivars with higher leaf changes in response to oviposition (higher increase in LMA, thickness, and F_{ps}), and according to the results of Peschiutta et al. (2016) these cultivars exhibit lower removed leaf area by larvae respect to removed area in Lapins (21, 23, and 31%, respectively).

Plants can defend themselves against herbivores by attracting its natural enemies, such as predators or parasitoids. Most studies have focused on volatile organic compounds (VOCs) that attract predators and parasitoids, but herbivory could also change the photosynthetic activity and/or light reflectance of even intact parts of the plant (Amo et al. 2013; Koski et al. 2017; Mäntylä et al. 2004, 2007; Peschiutta 2015; Zangerl et al. 2002). Therefore, changes in photosynthesis and/or other visual properties of the plant may provide visual foraging cues to predators, for example, birds (Amo et al. 2013; Koski et al. 2017; Mäntylä et al. 2004, 2007). In our study, among the cultivars, leaves with eggs of Van exhibited higher reflectance across

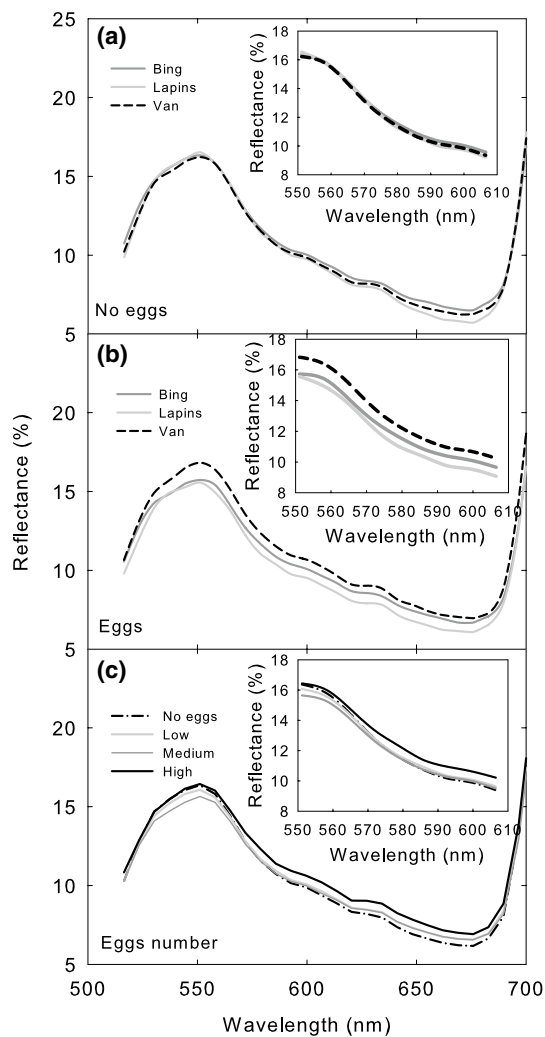


Fig. 5 Spectral reflectance from **a** leaves without eggs, **b** leaves with eggs of the three *P. avium* cultivars, and **c** mean spectral reflectance per cultivar of leaves without eggs, with low egg number (1–3), with medium egg number (4–6), and with high egg number (7 or more) ($n=3-7$ trees per cultivar) from 520 to 700 nm wavelength ($n=7$). Inserts are reflectance spectrum from 550 to 610 nm

all wavelengths analyzed, and this cultivar also tended to have about three eggs per leaf (less than the Lapins cultivar). Leaves with more eggs, regardless of the cultivar, were also those that exhibited higher reflectance in these wavelengths. Leaf characteristics that could be altered during oviposition, as wax layers, cuticle thickness, trichomes, and sclereids, could cause changes in the optical properties of leaves (Baldini et al. 1997), affecting the near-infrared reflectance (750–1350 nm). However, the reflectance in the visible portion of the spectrum (between 400 and 700 nm) could be altered by changes in photosynthetic pigments (Slaton et al. 2001). Egg deposition by chewing and sucking herbivorous insects can lead to a reduction of plant's photosynthetic activity. Egg deposition per se may significantly

affect the plant's primary metabolism and reduce photosynthetic activity (Schröder et al. 2005). We found a decrease in photochemical reflectance index (PRI) of leaves with eggs compared to leaves without eggs, which suggests a reduction in photosynthetic performance. It is known that the photosynthetic efficiency in trees with sawfly eggs is lower than in plants without eggs (Délano-Frier et al. 2012). Negative effects may occur as a result of an egg deposition process, when female with its ovipositor damages plant tissue prior to insertion of eggs on the leaf (Schröder et al. 2005). Reducing diffusion rates of CO_2 in mesophyll cells has been suggested as a possible mechanism which leads to the decrease of photosynthesis during oviposition, in the absence of plant injury (Velikova et al. 2010). Also, oviposition can lead to an increase in water loss in damaged leaf area (Codella and Raffa 2002). Other factors such as production of jasmonic acid can adversely affect photosynthetic rate almost immediately after oviposition (Schröder et al. 2005; Velikova et al. 2010). We found an increased oxidative stress and cell damage—greater BPI—on leaves with eggs in relation to leaves without eggs, principally in Van cultivar. These reactive oxygen species (ROS) constitute an important signaling molecules in defenses (Little et al. 2007) and they may be generated within minutes, with only insect locomotion on foliar surfaces (Bown et al. 2002) or in response to insect eggs (Bittner et al. 2017).

Plant structural traits (e.g., spinescence, trichomes, waxy cuticles, sclerophylly) can act as a physical barrier to herbivores, feeding, and oviposition. Trichomes can prevent insect attachment and limit pest movement on plants (Mitchell et al. 2016). Some studies have shown that plants have the ability to perceive and respond to simple contact (Braam 2005; Telewski 2006) by morphological (Liu et al. 2007), physiological (Chehab et al. 2012), or chemical adjustments (Braam 2005; Markovic et al. 2014). For example, touch in bean leaves causes an increase in leaf weight and a decrease in specific leaf area (inverse of LMA) within 6 days (Markovic et al. 2014). *P. avium* leaves with eggs had higher LMA and thickness, and tended to be more mechanically resistant and have lower water content. Leaf dry mass per unit area changes could be the result of significant increase in leaf dry weight with increasing egg number, but without changes in leaf size, due to a significant increase in trichomes abundance and perhaps in other trait changes. Fornoff and Gross (2014) found that dry matter content increases to 19% with herbivory. Leaves with higher LMA are typically harder and have a lower content of nutrients (Onoda et al. 2004; Poorter et al. 2009) and thus have a greater resistance to herbivory. Wax layers and other structures such as trichomes, thick cell walls, fibers, thick cuticles, and sclereids have been associated with increased LMA (Coley 1983; Onoda et al. 2011; Poorter et al. 2009).

Fig. 6 Relationships between leaf reflectance at **a** 550 nm (R_{550}), **b** 516 nm (R_{516}), **c** 592 nm (R_{592}), and **d** 596 nm (R_{596}) wavelengths in relation to egg number per leaf. Lines are linear functions fitted to the data ($y = 15.72 + 0.12 \times x$; $y = 9.96 + 0.14 \times x$; $y = 9.98 + 0.14 \times x$; $y = 9.69 + 0.14 \times x$, respectively). Values are means of egg number per leaf. Filled symbol represents leaves without eggs

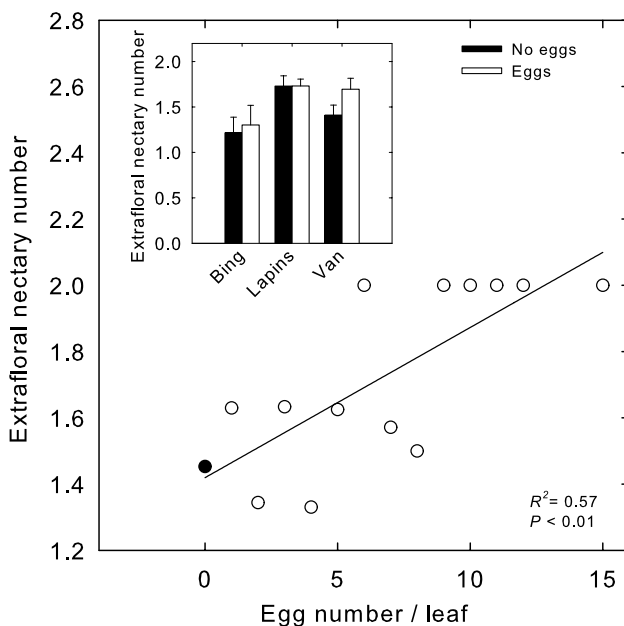
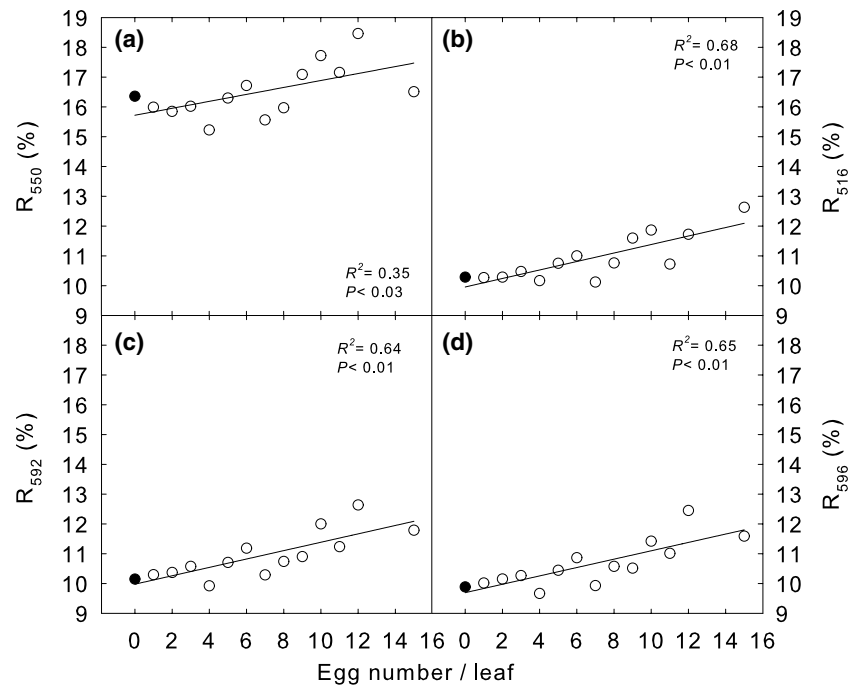


Fig. 7 Extrafloral nectary number in relation to egg number per leaf. Line is the linear function fitted to the data $y = 1.42 + 0.05 \times x$. Values are mean of egg number/leaf from 3 to 7 trees depending on egg number found. Black symbol represents leaves without eggs. Inset represents leaf extrafloral nectary number per leaf with and without eggs from Bing, Lapins, and Van cultivars. Bars represent means + SE of 3–7 trees per cultivar

Feeding by folivorous insects induces an increase in trichomes density within days or weeks after insect attack (Agrawal 1998; Dalin and Björkman 2003). Induction of leaf trichome densities is a common phenotypic response

described in distinct and unrelated plant species when alterations in biotic and abiotic conditions take place (Escobar-Bravo et al. 2017). In the present study, we observed that the production of trichomes tended to increase in all cultivars by the presence of eggs, but with significant changes in Lapins cultivar. This suggests that not only trichome production can be induced by foliar biomass loss after feeding but also by oviposition. This response could protect veins of subsequent larval feeding. The larvae leave intact leaf veins after feeding which contribute to maintain water transport capacity of damage leaves (Peschiutta et al. 2016).

Other defenses observed in this study were the development of a necrotic zone at the site of egg deposition and the production of indirect defenses, such as extrafloral nectaries. Bing showed a tendency to have larger egg number per leaf, but some of them experienced necrosis mortality, similar to the pattern observed by Shapiro and DeVay (1987) in *Brassica nigra*. We found also a positive relationship between egg number and extrafloral nectaries. It is known that *Prunus* has extrafloral nectaries that may be an important indirect defense against herbivores (Bronstein 1998; Gish et al. 2016; Oliveira 1997). Extrafloral nectaries play an important role in plant defense against herbivores by providing nectar rewards that attract ants and other natural enemies such as parasitoid which help diminish the damage by the herbivorous insects.

Our results suggest that *P. avium* have the ability to respond promptly after egg deposition by *C. cerasi* (herbivory early alert; Hilker and Meiners 2006). These early responses may directly affect the larval development by necrotizing eggs or indirectly by attracting egg and larvae predators (Hilker and Fatouros 2016; Koski et al. 2017).

More importantly, *P. avium* may alter negatively the subsequent larval feeding by increasing trichome density (such as in Lapins), leaf thickness, and LMA (as in Bing) or leaf specific force to punch or material resistance (as in Van) as response to oviposition. However, other experiments are required to examine the ecological effects of these changes on resistance of plant to feeding larvae.

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Author contributions MLP, FGS, and GG conceived and designed the experiments. MLP performed the experiments. MLP, FGS, and SJB analyzed the data. MLP, FGS, SJB, and GG wrote the manuscript.

Compliance with ethical standards

Conflict of interest The authors have no conflict of interest to declare.

References

- Agrawal A (1998) Induced responses to herbivory and increased plant performance. *Science* 279:1201–1202
- Amo L, Jansen JJ, Dam NM, Dicke M, Visser ME (2013) Birds exploit herbivore-induced plant volatiles to locate herbivorous prey. *Ecol Lett* 16:1348–1355
- Bado S (2007) Plagas del cultivo de cerezo. *Revista Fruticultura Profesional* 171:14–22
- Baldini E, Facini O, Nerozzi F, Rossi F, Rotondi A (1997) Leaf characteristics and optical properties of different woody species. *Trees* 12:73–81
- Beeskov A, Del Valle H, Rostagno C (1987) Los sistemas fisiográficos de la región árida y semiárida de la provincia del Chubut. SECYT, Puerto Madryn
- Bittner N, Trauer-Kizilelma U, Hilker M (2017) Early plant defence against insect attack: involvement of reactive oxygen species in plant responses to insect egg deposition. *Planta* 245:993–1007
- Blackmer T, Schepers J, Varvel G (1994) Light reflectance compared with other nitrogen stress measurements in corn leaves. *Agron J* 86:934–938
- Bown A, Hall D, MacGregor K (2002) Insect footsteps on leaves stimulate the accumulation of 4-aminobutyrate and can be visualized through increased chlorophyll fluorescence and superoxide production. *Plant Physiol* 129:1430–1434
- Braam J (2005) In touch: plant responses to mechanical stimuli. *New Phytol* 165:373–389. <https://doi.org/10.1111/j.1469-8137.2004.01263.x>
- Bronstein J (1998) The contribution of ant-plant protection studies to our understanding of mutualism. *Biotropica* 30:150–161
- Büchel K, McDowell E, Nelson W, Descour A, Gershenson J, Hilker M, Soderlund C, Gang DR, Fenning T, Meiners T (2012) An elm EST database for identifying leaf beetle egg induced defense genes. *BMC Genom* 13:242
- Carl K (1972) On the biology, ecology and population dynamic of *Caliroa cerasi* (L.) (Hym., Tenthredinidae). *Z Angew Entomol* 71:58–83
- Chehab W, Yao C, Henderson Z, Kim S, Braam J (2012) Arabidopsis touch-induced morphogenesis is jasmonate mediated and protects against pests. *Curr Biol* 22:701–706
- Cittadini E (2007) Sweet cherries from the end of the world: Options and constraints for fruit production systems in South Patagonia, Argentina. PhD thesis. Wageningen University, The Netherlands
- Cittadini E, San Martino L (2007) El cultivo de cerezos en Patagonia Sur Tecnología de manejo, empaque y comercialización. Ediciones INTA 200
- Codella S, Raffa K (2002) Desiccation of *Pinus foliage* induced by conifer sawfly oviposition: effect on egg viability. *Ecol Entomol* 27:618–621
- Colazza S, McElfresh JS, Millar JG (2004) Identification of volatile synomones, induced by *Nezara viridula* feeding and oviposition on *Bean* spp., that attract the egg parasitoid *Trissolcus basalus*. *J Chem Ecol* 30:945–964
- Coley P (1983) Herbivory and defensive characteristics of tree species in a lowland tropical rain forest. *Ecol Monogr* 53:209–233
- Dalin P, Björkman C (2003) Adult beetle grazing induces willow trichome defense against subsequent larval feeding. *Oecologia* 134:112–118
- Dalin P, Ågren J, Björkman C, Huttunen P, Kärkkäinen K (2008) Leaf trichome formation and plant resistance to herbivory. In: Schaller A (ed) *Induced plant resistance to herbivory*. Springer, Dordrecht, pp 89–105
- Délano-Frier J, Sánchez-Hernández C, Tiessen A (2012) Friend or foe? Exploring the factors that determine the difference between positive and negative effects on photosynthesis in response to insect herbivory. In: Najafpour M (ed) *Artificial photosynthesis*. InTech, New York, pp 155–206
- Escobar-Bravo R, Klinkhamer PG, Leiss KA (2017) Induction of jasmonic acid-associated defenses by thrips alters host suitability for conspecifics and correlates with increased trichome densities in tomato. *Plant Cell Physiol* 58:622–634
- Fatouros NE, Lucas-Barbosa D, Weldegergis BT, Pashalidou FG, van Loon JJA, Dicke M, Harvey JA, Gols R, Huigens ME (2012) Plant volatiles induced by herbivore egg deposition affect insects of different trophic levels. *PLoS ONE* 7:e43607. <https://doi.org/10.1371/journal.pone.0043607>
- Fornoff F, Gross E (2014) Induced defense mechanisms in an aquatic angiosperm to insect herbivory. *Oecologia* 175:173–185. <https://doi.org/10.1007/s00442-013-2880-8>
- Gish M, Mescher MC, De Moraes CM (2016) Mechanical defenses of plant extrafloral nectaries against herbivory. *Commun Integr Biol* 9:e1178431
- Hanley ME, Lamont BB, Fairbanks MM, Rafferty CM (2007) Plant structural traits and their role in anti-herbivore defence. *Perspect Plant Ecol Evol Syst* 8:157–178
- Heil M (2009) Damaged-self recognition in plant herbivore defence. *Trends Plant Sci* 14:356–363. <https://doi.org/10.1016/j.tplants.2009.04.002>
- Heong KL, Cheng J, Escalada MM (2015) Rice planthoppers. *Ecology, Management, Socio Economics and Policy. Advanced Topics in Science and Technology in China*. Zhejiang University Press, Hangzhou and Springer, Berlin Heidelberg
- Hilker M, Fatouros NE (2015) Plant responses to insect egg deposition. *Annu Rev Entomol* 60:493–515
- Hilker M, Fatouros NE (2016) Resisting the onset of herbivore attack: plants perceive and respond to insect eggs. *Curr Opin Plant Biol* 32:9–16
- Hilker M, Meiners T (2006) Early herbivore alert: insect eggs induce plant defense. *J Chem Ecol* 32:1379–1397
- Hilker M, Bläske V, Kobs C, Dippel C (2002) Kairomonal effects of sawfly sex pheromones on egg parasitoids. *J Chem Ecol* 26:2591–2601

- Karban R, Baldwin I (1997) Induced responses to herbivory. The University of Chicago Press, Chicago, IL, p. 330
- Koski TM, Lindstedt C, Klemola T, Troschianko J, Mäntylä E, Tyystjärvi E, Stevens M, Helander M, Laaksonen T (2017) Insect herbivory may cause changes in the visual properties of leaves and affect the camouflage of herbivores to avian predators. *Behav Ecol Sociobiol* 71:97
- Little D, Gouhier-Darimont C, Bruessow F, Reymond P (2007) Oviposition by pierid butterflies triggers defense responses in *Arabidopsis*. *Plant Physiol* 143:784–800. <https://doi.org/10.1104/pp.106.090837>
- Liu Y, Schieving F, Stuefer J, Anten N (2007) The effects of mechanical stress and spectral shading on the growth and allocation of ten genotypes of a stoloniferous plant. *Ann Bot* 99:121–130. <https://doi.org/10.1093/aob/mcl230>
- Liu Z, Cai Y, Fang Y, Jing J, Li K (2010) Induced response in *Schima superba*: Effects of early-season herbivory on leaf traits and subsequent insect attack. *Afr J Biotechnol* 9:8731–8738
- Lucas-Barbosa D, van Loon J, Gols R, van Beek T, Dicke M (2012) Reproductive escape: annual plant responds to butterfly eggs by accelerating seed production. *Funct Ecol* 27:245–254
- Mäntylä E, Klemola T, Haukioja E (2004) Attraction of willow warblers to sawfly-damaged mountain birches: novel function of inducible plant defences? *Ecol Lett* 7:915–918
- Mäntylä E, Klemola T, Sirkkiä P, Laaksonen T (2007) Low light reflectance may explain the attraction of birds to defoliated trees. *Behav Ecol* 19:325–330
- Mareggiani G, Bartoloni N, Gorosito N, Laffaye C (2012) Flight detection of *Caliroa cerasi* L. (Hymenoptera: Tenthredinidae) adults in the Andean Region of Parallel 42, Argentina. *Bol San Veg Plagas* 38:233–238
- Markovic D, Glinwood R, Olsson U, Ninkovic V (2014) Plant response to touch affects the behaviour of aphids and ladybirds. *Arth-Plant Int* 8:171–181
- Miller G, Miller E (1948) Determination of nitrogen in biological materials. *Anal Chem* 20:481–488
- Mitchell C, Brennan RM, Graham J, Karley AJ (2016) Plant defense against herbivorous pests: exploiting resistance and tolerance traits for sustainable crop protection. *Front Plant Sci* 7:1132
- Oliveira P (1997) The ecological function of extrafloral nectaries: herbivore deterrence by visiting ants and reproductive output in *Caryocar brasiliense* (Caryocaraceae). *Funct Ecol* 11:323–330
- Onoda Y, Hikosaka K, Hirose T (2004) Allocation of nitrogen to cell walls decreases photosynthetic nitrogen-use efficiency. *Funct Ecol* 18:419–425
- Onoda Y, Westoby M, Adler P, Choong A, Clissold F, Cornelissen J et al (2011) Global patterns of leaf mechanical properties. *Ecol Lett* 14:301–312. <https://doi.org/10.1111/j.1461-0248.2010.01582.x>
- Pashalidou FG (2015) Getting prepared for future attack: induction of plant defences by herbivore egg deposition and consequences for the insect community. Wageningen University
- Pashalidou F, Lucas-Barbosa D, van Loon J, Dicke M, Fatouros N (2013) Phenotypic plasticity of plant response to herbivore eggs: effects on resistance to caterpillars and plant development. *Ecology* 94:702–713
- Peñaflor M, Erb M, Miranda L, Werneburg A, Bento J (2011) Herbivore-induced plant volatiles can serve as host location cues for a generalist and a specialist egg parasitoid. *J Chem Soc* 37:1304–1313
- Peñuelas J, Munné-Bosch S, Llusià J, Filella I (2004) Leaf reflectance and photo- and antioxidant protection in field-grown summer-stressed *Phillyrea angustifolia* Optical signals of oxidative stress? *New Phytol* 162:115–124
- Peschiutta M (2015) El impacto de la herbivoría por *Caliroa cerasi* (Hymenoptera: Tenthredinidae) sobre las relaciones hídricas, intercambio gaseoso, crecimiento y productividad del cerezo (*Prunus avium* L.). Universidad de Buenos Aires
- Peschiutta ML, Bucci SJ, Scholz FG, Goldstein G (2016) Compensatory responses in plant-herbivore interactions: impacts of insects on leaf water relations. *Acta Oecol* 73:71–79
- Peschiutta ML, Scholz FG, Goldstein G, Bucci SJ (2018) Herbivory alters plant carbon assimilation, patterns of biomass allocation and nitrogen use efficiency. *Acta Oecol* 86:9–16
- Petzold-Maxwell J, Wong S, Arellano C, Gould F (2011) Host plant direct defence against eggs of its specialist herbivore, *Heliothis subflexa*. *Ecol Entomol* 36:700–708
- Poorter H, Niinemets Ü, Poorter L, Wright I, Villar R (2009) Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. *New Phytol* 182:565–588
- Pulice C, Packer A (2008) Simulated herbivory induces extrafloral nectary production in *Prunus avium*. *Funct Ecol* 22:801–807
- Raffa K, Lintereur G (1988) New host records and developmental notes on the pear slug *Caliroa cerasi* (Hymenoptera: Tenthredinidae), feeding on *Cotoneaster* and *Chaenomeles* species Great Lakes. *Entomol* 21:75–79
- Sack L, Cowan P, Jaikumar N, Holbrook N (2003) The ‘hydrology’ of leaves: coordination of structure and function in temperate woody species. *Plant Cell Environ* 26:1343–1356
- Schlesinger W, Hasey M (1981) Decomposition of chaparral shrub foliage: losses of organic and inorganic constituents from deciduous and evergreen leaves. *Ecology* 62:762–774
- Schröder R, Forstreuter M, Hilker M (2005) A plant notices insect egg deposition and changes its rate of photosynthesis. *Plant Physiol* 138:470–477
- Shapiro AM, DeVay JE (1987) Hypersensitivity reaction of *Brassica nigra* L. (Cruciferae) kills eggs of *Pieris* butterflies (Lepidoptera: Pieridae). *Oecologia* 71:631–632. <https://doi.org/10.1007/BF00379310>
- Shaw P, Wallis D, Alspach P, Bus V, Brewer L (2004) Pear sawfly (*Caliroa cerasi*) (Hymenoptera: Tenthredinidae) host preference and larval development on six *Pyrus* genotypes. *New Zeal J Crop Hort* 32:257–262
- Slaton M, Hunt E, Smith W (2001) Estimating near-infrared leaf reflectance from leaf structural characteristics. *Am J Bot* 88:278–284
- Telewski F (2006) A unified hypothesis of mechanoperception in plants. *Am J Bot* 93:1466–1476. <https://doi.org/10.3732/ajb.93.10.1466>
- Velikova V, Salerno G, Frati F, Peri E, Conti E, Colazza S, Loretto F (2010) Influence of feeding and oviposition by phytophagous pentatomids on photosynthesis of herbaceous plants. *J Chem Ecol* 36:629–641. <https://doi.org/10.1007/s10886-010-9801-7>
- Zangerl A, Hamilton J, Miller T, Crofts A, Oxborough K, Berenbaum M, de Lucia E (2002) Impact of folivory on photosynthesis is greater than the sum of its holes. *Proc Natl Acad Sci USA* 99:1088–1091