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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 infected 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 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} CO_2 inside the leaf chamber generated by a 12 g CO_2 cylinder connected to the LI-6400. The photon flux density (PPFD) was held constant at 1200 μmol $m^{-2}s^{-1}$ 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 Na_2CO_3 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 mg 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 Cattelino 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