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Effects of herbivory and mistletoe infection by *Psittacanthus calyculatus* on nutritional quality and chemical defense of *Quercus deserticola* along Mexican forest fragments

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Abstract Mistletoes are parasitic plants that show effects that can parallel or contrast with those caused by herbivores to their host plants, particularly on aspects such as host biomass, resource allocation patterns, and interspecific interactions at the community level. In this study, we evaluated the potential synergistic effects of herbivory and infection by the mistletoe *Psittacanthus calyculatus* on nutritional quality and chemical defense of the white oak *Quercus deserticola* along forest fragments in Mexico. For this, we sampled leaves of parasitized oaks, unparasitized oaks and mistletoes at four forest fragments in the Cuitzeo basin, Michoacán state, Mexico, and measured herbivory levels and foliar water content, total nonstructural carbohydrates, phenols, flavonoids, and hydrolyzable tannins in each sample. Higher levels of infection by *P. calyculatus* were found in the smaller forest fragments, while foliar damage by herbivores was higher in larger forest fragments. At all sites, levels of herbivory were lower in the mistletoe than in both parasitized and unparasitized oaks. However, there was a positive relationship between herbivory levels in parasitized oaks and their mistletoes. Also, foliar water content and total phenol concentration were positively correlated between the oaks and the mistletoes. The results suggest that herbivory levels in parasitized hosts and mistletoes depend on the close physiological interaction between the nutritional quality and the chemical defense of the two plants involved. This is one of the few studies analyzing the chemical ecology of the interaction between plant hosts and plant parasites.

Keywords Mistletoes · Herbivory · Chemical defense · Nutritional quality · Forest fragmentation · Plant–plant interactions

Introduction

A considerable number of studies on plant–insect interactions have assessed the potential effects of

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chemical defense and plant nutritional quality on performance and host selection by insect herbivores (e.g., Pascual-Alvarado et al. 2008; Gripenberg et al. 2010). However, despite the long-recognized parallels between parasitic plants and herbivores (Govier and Harper 1965), which include similar effects on host biomass and resource allocation patterns, as well as on interspecific interactions at the community level (Pennings and Callaway 2002), few studies have focused on analyzing the chemical ecology of the interaction between plant hosts and plant parasites (Runyon et al. 2008; Smith et al. 2008). It has been suggested that plant secondary metabolites maintain a co-evolutionary interaction with herbivores and pathogens (Ehrlich and Raven 1964; Rausher 2010), resulting in a great variation of combinations, mixtures, and concentrations of these compounds in individuals of the same species (Thrall et al. 2001; García-Brugger et al. 2006), as a result of genetic and environmental factors (Agrell et al. 2000; Underwood 2009). Therefore, plant secondary compounds may induce resistance against both herbivores and parasitic plants (Forkner et al. 2004; Runyon et al. 2008). For example, plants have the ability to perceive herbivore attack and respond actively inducing physical (Lucas et al. 2000) and chemical defenses (Kursar and Coley 2003; Runyon et al. 2008). Nevertheless, defensive responses of plants to the attack of other plants still require considerable research (Smith et al. 2008).

There are about 4100 species of angiosperms that are parasites on other plant species (Nickrent and Musselman 2004), of these, 40% are associated to stems and 60% to roots (Musselman and Press 1995). Particularly, lorantaceous mistletoes obtain all or most of their carbohydrates as a result of their own photosynthetic activity but acquire water and nutrients from the host xylem (i.e., hemiparasitism) (Marshall et al. 1994; Bickford et al. 2005). These mistletoes represent a main group of parasitic plants that colonize branches and roots of tropical and temperate host plants (Calvin and Wilson 2006; Bell and Adams 2011). Parasitism by mistletoes has sublethal effects that reduce host growth, photosynthesis, and respiration rates, modifying both host architecture and nutrient balance (Watling and Press 2001; Meinzer et al. 2004) and also indirectly affecting biotic interactions associated with their host plants (Press and Phoenix 2005; Cuevas-Reyes et al. 2011). However, some of these indirect effects are not necessarily negative, since mistletoes may increase the attraction of seed dispersers and facilitate seedling recruitment of their hosts (e.g., Candia et al. 2014).

Mistletoes, in turn, do not escape from herbivore attack (Sessions and Kelly 2001). The defense mechanisms of mistletoes involve chemical compounds obtained from their host plants such as nutritional compounds and secondary metabolites (Glatzel and Geils 2009). Therefore, chemical compounds of the host plants are important to the successful establishment and development of mistletoes (Pennings and Callaway 2002). Similarly, the selection of host plant tissues by herbivores is related to the content of nutrient compounds and secondary metabolites (Grotewold 2005; Bustamante et al. 2006).

However, forest fragmentation may influence the nutritional quality and plant defense as a result of abiotic changes such as light intensity, wind speed, temperature, humidity, and soil fertility, especially at fragment edges and in small fragments (Valladares et al. 2006). These changes may affect the plant vigor or health as a consequence of stress conditions (Saunders et al. 1991; Prada et al. 1995), which in turn can influence plant–insect and plant–plant interactions (Araùjo et al. 2011; Maldonado-López et al. 2015). For example, parasitic plants are usually more abundant in fragmented landscapes, and mistletoe individuals exposed to sunlight usually have larger flower and fruit displays, rapid fruit ripening, and bigger and sweetener fruits, being more likely to be visited by mutualist animals and hence to recruit (López de Buen et al. 2002).

Most studies have focused on evaluating the incidence levels in correlation with plant defense or nutritional quality in plant–insect and plant–mistletoe interactions independently, without considering the simultaneous relationships among host plants, mistletoes, and insect herbivores in terms of leaf area removed, nutritional quality, and chemical defense. In this study, we evaluated these relationships using as study system the host tree *Quercus deserticola* (Fagaceae) and the mistletoe *Psittacanthus calyculatus* (Loranthaceae) along forest fragments. We addressed the following questions: (i) Is there variation in the levels of herbivory, nutritional quality, and chemical defenses among parasitized and unparasitized hosts and mistletoes? (ii) What is the relationship between the herbivory levels, nutritional quality, and concentration of secondary compounds in parasitized and unparasitized hosts? (iii) Is there a correlation in the nutritional quality and the concentration of secondary compounds between host plants and mistletoes?

Materials and methods

This study was conducted in the Cuitzeo basin, a hydrological unit with an area of 4026 km^2, located in western Mexico in the state of Michoacán, with an average altitude of approximately 2000 m. It is located in the physiographic province of the Trans-Mexican Volcanic Belt. The basin is representative of the environmental and socioeconomic conditions of central Mexico and has experienced strong fragmentation of natural vegetation in the last decades. Around 20% of the basin is covered by temperate pine-oak forests, the subtropical underbrush occupies about 15%, induced grasslands less than 15%, and crops occupy about 40% of the basin (López et al. 2001).

Study system

Quercus deserticola (Fagaceae) belongs to the white oak section (*Quercus*) of the genus. It is a deciduous tree, growing up to 10 m that occurs in semitropical bushes and temperate forests of Mexico (Arizaga 2009). The species is particularly abundant in the study region where it is commonly parasitized by the mistletoe *Psittacanthus calyculatus* (Loranthaceae), which is a generalist hemiparasitic shrub, growing up to 1.5 m and parasitizing different species of *Pinus*, *Quercus*, *Abies*, *Salix*, and *Bursera*, among other hosts (Bello and Gutiérrez 1985).

Sampling design and herbivory patterns

Oak forests of the Cuitzeo basin have been characterized into 1241 fragments of different sizes and shapes covering ca. 20% of the basin (López et al. 2001). On the basis of this information, we selected 4 permanent sites where *Q. deserticola* and *P. calyculatus* occur. The sites were classified in two categories according to their size: i) two small fragments ($\leq$4 ha) (Capula and El Tigre) and two larger fragments (>12 ha) (Lagunillas and Zimpanio).

In order to estimate the herbivory patterns, we first determined the proportion of parasitized hosts in each forest fragment and then sampled leaves from between 6 and 15 (depending on availability) parasitized *Q. deserticola* individuals and an equivalent number of unparasitized trees. Sampling extended from the upper canopy to the lower branches, collecting 30 fully expanded leaves at the top, intermediate, and bottom of the crown for each oak tree (i.e., 90 leaves per plant) (Cuevas-Reyes et al. 2011). In each parasitized host plant, we randomly selected between 1 and 3 mistletoes and randomly collected 20 leaves per individual. To estimate the leaf area removed by folivores, we obtained a digital image of each leaf and then estimated the total area of the leaf and the area removed using Sigma Scan Pro software (Cuevas-Reyes et al. 2004, 2011).

Leaf chemical analysis

To determine the differences in nutritional quality and secondary metabolites among parasitized and unparasitized host plants and mistletoes, we collected 30 additional fully expanded intact leaves per individual from the same trees and mistletoes of the herbivory analyses.

Nutritional quality

Water content Two grams of fresh sample were weighted in an analytical balance and then placed in an oven at 105 °C. The samples were dried to a constant weight and then allowed to cool for weight register. Water content was calculated as fresh weight–dry weight/fresh weight (Marquis et al. 1997).

Total nonstructural carbohydrates (TNC) We performed the phenol–sulfuric method for TNC extraction from plant tissues (Marquis et al. 1997). The concentration of soluble sugars was determined colorimetrically with an UV–Visible spectrophotometer (EZ301 UV/VIS Spectrometer, Perkin-Elmer).

Secondary metabolites

Total content of soluble phenolics A modification of the Folin–Ciocalteu method was used (Torres et al. 1987), extracting the samples with 80% ethanol. One milliliter supernatant of the centrifuged sample was mixed with 0.5 ml of 2 N Folin–Ciocalteu's phenol reagent, and then 1 ml of 20% Na_2CO_3 was added.

After 30 min of incubation at room temperature, the absorbance was measured at 730 nm on a UV–Visible spectrophotometer. The standard curve was created with known concentrations of tannic acid.

Flavonoids The content of total flavonoids was measured using a modification of the method of Zhuang et al. (1992). The samples were extracted with 80% ethanol and 0.1 ml of supernatant was diluted in 0.9 ml of 80% ethanol. A 0.5-ml aliquot was placed in a tube, and 0.3 ml $NaNO_2$ (1:20) was added. After 5 min, 3 ml $AlCl_3$ (1:10) was added. Six minutes later, the solution was mixed and the absorbance was measured at 510 nm. The content of total flavonoids was calculated from a standard curve of quercetin.

Total tannins We used a radial diffusion method with agarose gel added with bovine serum albumin (Hagerman 1987). The samples were extracted with 70% aqueous acetone, allowed to stand for 1 h at room temperature with continuous stirring, and centrifuged for 10 min at 3000 rpm. We punched 8 wells in each plate. Each sample was suspended in 200 µl of 70% acetone and 50 µl of each sample was placed in the wells. The plates were covered and placed in an incubator at 30 °C for 96 h. We used a digital Vernier to estimate the diameter of the ring that formed. The area of the disk is linearly related to the amount of tannins placed in the well.

Statistical analyses

To determine the effects of fragment size (small vs. larger fragments) on the proportion of parasitized hosts, we conducted a generalized linear model applying the GENMOD procedure. The model used a Poisson error distribution and a log-link function (SAS 2000).

A two-way ANOVA test (SAS 2000) was performed to determine the effects of forest fragmentation (small fragments vs. large fragments) and plant status (parasitized vs. unparasitized plants vs. mistletoes) and the interaction (forest fragmentation*plant status) on the leaf area removed by folivores (after an arcsine-square-root transformation). The model used forest fragmentation, plant status, and the interaction of these terms as the independent variables and leaf area removed was used as the dependent variable. An LSMeans test was used for a posteriori comparisons (SAS 2000).

The differences in nutritional quality and secondary metabolites between plants that occur in forest fragments (small vs. larger fragments) were tested separately using a one-way ANOVA test (SAS 2000). An LSMeans test was used for a posteriori comparisons. Similarly, we performed separately a one-way ANOVA test to determine the differences in nutritional quality and secondary metabolites between parasitized and unparasitized oaks and mistletoes. An LSMeans test was used for a posteriori comparisons (SAS 2000).

We conducted Spearman's rank correlation analyses to evaluate the relationships between leaf area removed, plant quality, and plant defense separately in unparasitized and parasitized oaks and in mistletoes. Finally, we used Spearman's rank correlation analysis to determine the relationship among leaf area removed, plant quality, and plant chemical defense between parasitized plants of *Q. deserticola* and mistletoes. Since these tests involved multiple comparisons, a Bonferroni correction was applied to adjust *P* values.

Results

Herbivory patterns

The proportion of parasitized hosts was higher in small than in larger fragments ($\chi^2 = 39.8$; d. f. = 3; $P < 0.0001$) (Fig. 1a). In contrast, we found higher levels of leaf area removed in larger than in small fragments ($F_{153} = 30.7$; $P < 0.0001$). The mean leaf area removed in larger fragments was 6.4% ± 1.1 (Zimpanio >12 ha) and 7.4% ± 1.0 (Lagunillas >12 ha), while that in the small fragments was 4.8% ± 0.6 (El Tigre <4 ha) and 2.2% ± 0.5 (Capula <4 ha) (Fig. 1b). In addition, the leaf area removed by folivores was higher in parasitized than in unparasitized hosts and lower in mistletoes in all forest fragments ($F_{2128} = 36.5$; $P < 0.001$) (Fig. 1b).

Leaf chemical quality and plant defense

We found differences among forest fragments in the means of water content ($F = 12.2$; d. f. = 3; $P < 0.0001$) and nonstructural carbohydrates

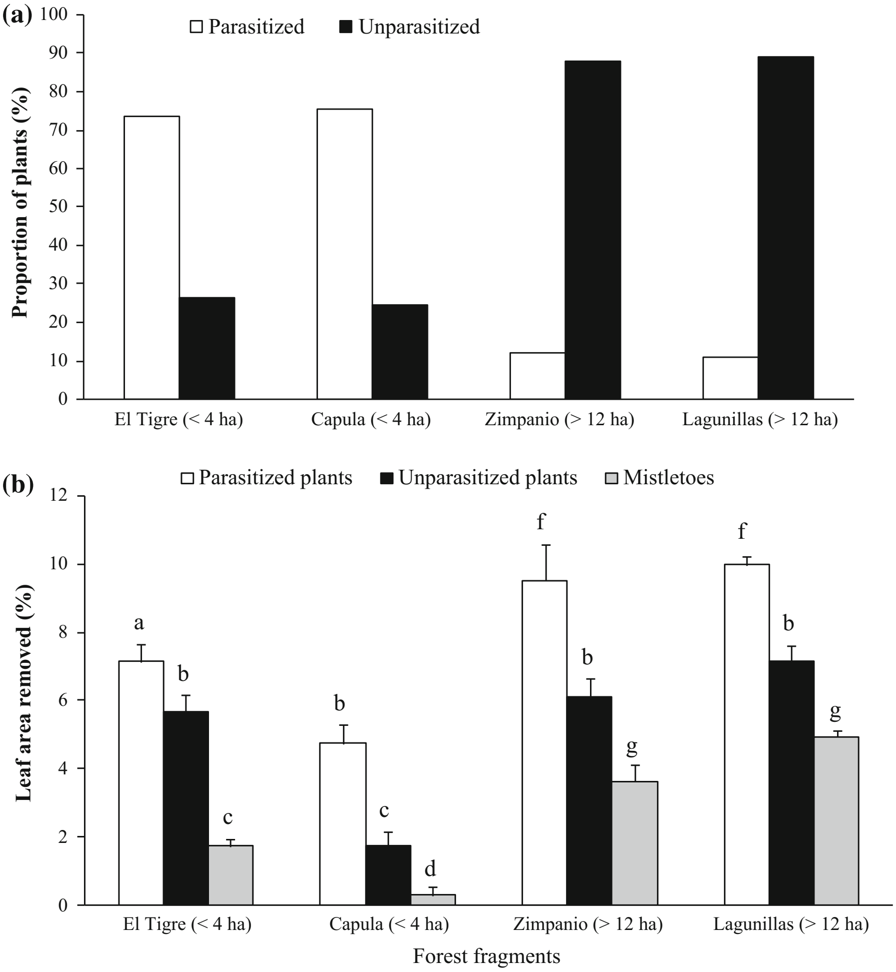

Fig. 1 Patterns of mistletoe incidence and herbivory. **a** Proportion of parasitized hosts in each forest fragment. **b** Comparison of leaf area removed by folivores between both forest fragments and conditions (parasitized plants, unparasitized plants, and mistletoes). Untransformed data are shown. Values with the same letter identify means within treatments that were not significantly different after an LSMeans multiple comparison test ($P < 0.05$)

($F = 7.6$; d. f. = 3; $P < 0.0003$) (Table 1). These response variables were higher in small fragments. Similarly, means of the analyzed variables differed according to plant status (parasitized and unparasitized hosts and mistletoes) (Table 2). Water content was higher in parasitized than in unparasitized hosts and lower in mistletoes ($F = 85.5$; d. f. = 2; $P < 0.0001$). In contrast, mistletoes had the higher concentration of nonstructural carbohydrates, followed by parasitized hosts and finally by unparasitized trees ($F = 56.3$; d. f. = 2; $P < 0.0001$) (Table 2).

In the case of the secondary metabolites, means for all compound classes differed between forest fragments (Table 3). The concentrations of soluble phenolics ($F = 13.5$; d. f. = 3; $P < 0.001$), total flavonoids ($F = 34.7$; d. f. = 3; $P < 0.0001$), and hydrolyzable tannins ($F = 26.8$; d. f. = 3;

Table 1 Variation in means and ANOVA results for effects of forest fragments on leaf nutritional quality

Nutritional quality variables	El tigre (<4 ha)	Capula (<4 ha)	Lagunillas (>12 ha)	Zimpanio (>12 ha)
Water (%)	48.1 ± 2^{a}	49.3 ± 1.2^{a}	41.5 ± 1.5^{b}	43.4 ± 2.2^{b}
Nonstructural carbohydrates (mg/g)	169.5 ± 6.9^{a}	167.3 ± 4.5^{a}	132.1 ± 2.5^{b}	130.4 ± 2.6^{b}

Same letters identify means that were not significantly different according to the LSMeans test

Table 2 Variation in means and ANOVA results for condition (parasitized plants, unparasitized plants, and mistletoes) effect on leaf nutritional quality

Nutritional compounds	Parasitized plants	Unparasitized plants	Mistletoes
Water (%)	49.1 ± 0.9^{a}	45.5 ± 1.1^{b}	33.3 ± 1.2^{c}
Nonstructural carbohydrates (mg/g)	144.1 ± 7.8^{a}	130.9 ± 5.1^{b}	170.6 ± 5.3^{c}

Same letters identify means that were not significantly different according to LSMeans test

Table 3 Differences in concentration of foliar secondary metabolites (mg g^{-1}) between forest fragments according to ANOVA test

Secondary metabolites	El tigre (<4 ha)	Capula (<4 ha)	Lagunillas (>12 ha)	Zimpanio (>12 ha)
Soluble phenolics	3.2 ± 0.2^{a}	2.9 ± 0.1^{a}	4.9 ± 0.3^{b}	5.4 ± 0.2^{c}
Total flavonoids	0.7 ± 0.02^{a}	0.8 ± 0.03^{a}	1.8 ± 0.04^{b}	1.7 ± 0.03^{b}
Total tannins	2.2 ± 0.1^{a}	2.4 ± 0.2^{a}	4.6 ± 0.3^{b}	4.9 ± 0.3^{b}

Same letters identify means that were not significantly different according to LSMeans test

$P < 0.0001$) were higher in larger than in small forest fragments (Table 3). In addition, means for all secondary metabolites varied according to plant status (parasitized and unparasitized hosts and mistletoes) (Table 4). Soluble phenolics were higher in mistletoes, intermediate in parasitized hosts, and lower in unparasitized oaks ($F = 33.2$; d. f. = 2; $P < 0.0001$). In contrast, we found that parasitized hosts had a higher concentration of total flavonoids, followed by unparasitized plants and finally by mistletoes ($F = 13.1$; d. f. = 2; $P < 0.001$). The concentration of total tannins did not differ between parasitized and unparasitized trees but was considerably higher in the mistletoes ($F = 26.1$; d. f. = 2; $P < 0.0001$).

Relationships between herbivory, plant quality, and plant defense within each plant group

In the case of unparasitized *Q. deserticola* individuals, after a Bonferroni correction Spearman's rank correlation showed that the leaf area removed was negatively correlated with the concentration of soluble phenolics, while positive relationships were found between water content and nonstructural carbohydrates, soluble phenolics, and total flavonoids, as well as between soluble phenolics and total tannins (Table 5a).

In parasitized individuals, a similar set of relationships was found, except that there was no correlation between water content and nonstructural carbohydrates and that the leaf area removed was negatively correlated with both water content and the concentration of soluble phenolics (Table 5b).

Relationships of herbivory, plant quality, and plant defense across hosts and parasites

Leaf area removed in the mistletoe was positively related with leaf area removed in the host and negatively correlated with soluble phenolics and

Table 4 Variation in means and ANOVA results for condition (parasitized plants, unparasitized plants, and mistletoes) effect on the concentration of foliar secondary metabolites

Secondary metabolites	Parasitized plants	Unparasitized plants	Mistletoes
Soluble phenolics	3.1 ± 0.1^{a}	2.8 ± 0.2^{a}	5.8 ± 0.2^{b}
Total flavonoids	0.9 ± 0.2^{a}	0.4 ± 0.1^{b}	0.2 ± 0.3^{c}
Total tannins	1.9 ± 0.3^{a}	1.4 ± 0.4^{a}	6.6 ± 0.5^{b}

Same letters identify means that were not significantly different according to LSMeans test

Table 5 Spearman's correlation coefficients of herbivory, plant quality, and plant defense in unparasitized (A) and parasitized plants (B) and mistletoes (C)

	Water	Nonstructural carbohydrates	Soluble phenolics	Total flavonoids	Total tannins	Leaf area removed
(A) Unparasitized plants						
Water	–	0.56*	0.51*	0.53*	−0.17	0.11
Nonstructural carbohydrates	–	–	0.28	0.01	0.008	0.15
Soluble phenolics	–	–	–	0.48	0.49*	−0.55*
Total flavonoids	–	–	–	–	−0.12	−0.11
Total tannins	–	–	–	–	–	−0.16
Leaf area removed	–	–	–	–	–	–
(B) Parasitized plants						
Water	–	−0.09	0.61*	0.50*	−0.06	−0.48*
Nonstructural carbohydrates	–	–	−0.07	−0.39	−0.07	0.11
Soluble phenolics	–	–	–	0.41*	0.54*	−0.65*
Total flavonoids	–	–	–	–	−0.0.04	−0.07
Total tannins	–	–	–	–	–	−0.11
Leaf area removed	–	–	–	–	–	–
(C) Mistletoes						
Water	–	0.33	0.66*	−0.05	0.46*	0.08
Nonstructural carbohydrates	–	–	0.12	0.18	0.48	0.06
Soluble phenolics	–	–	–	−0.13	0.17	−0.69*
Total flavonoids	–	–	–	–	0.53*	0.30
Total tannins	–	–	–	–	–	−0.16
Leaf area removed	–	–	–	–	–	–

* $P < 0.003$

hydrolyzable tannins in the host. Water content and the concentration of nonstructural carbohydrates in the host were positively correlated with water content and the concentrations of total flavonoids and hydrolyzable tannins in the mistletoe. Finally, the concentrations of soluble phenolics and total tannins in *Q. deserticola* were positively correlated with those of the same compounds in *P. calyculatus* (Table 6).

Discussion

Our study shows that forest fragmentation affects the distribution and incidence levels of *Psittacanthus calyculatus* on *Quercus deserticola*. The proportion of parasitized hosts by mistletoes was higher in small than in larger fragments. This result can be explained because mistletoes tend to have a clustered distribution on their host plants as result of the behavior patterns of their seed dispersers such as birds (Aukema 2004; Roxburgh and Nicolson 2008). Because habitat fragmentation modifies abiotic conditions, increasing light intensity, wind speed, and temperature and decreasing humidity and soil fertility especially in fragment edges and small fragments (Gascon et al. 2000), it is possible to expect that the timing and frequency of seed production by mistletoes can be altered, indirectly affecting the preference and incidence of seed dispersers (López de Buen et al. 2002). For example, several studies of the effects of forest fragmentation on interspecific interactions have indicated the presence of compensatory responses of plants, such as demographic changes (Bruna and Noguera-Ribeiro 2005), regrowth rates (Agrawal 2000), and seed production (Herrerías-Diego et al. 2006). In addition, some studies have indicated that the percentage of germination, growth, and fecundity of mistletoes depends on the light incidence.

Table 6 Spearman's correlation coefficients of herbivory, plant quality, and plant defense between parasitized plants of *Q. deserticola* and mistletoes

Parasitized plants	Mistletoes					
	Water	Nonstructural carbohydrates	Soluble phenolics	Total flavonoids	Total tannins	Leaf area removed
Water	0.64*	−0.02	0.45	−0.18	0.39**	0.08
Nonstructural carbohydrates	−0.02	0.43**	0.49	0.57*	0.56	0.11
Soluble phenolics	–	–	0.55*	0.01	0.59*	−0.74*
Total flavonoids	–	–	–	0.16	−0.12	−0.11
Total tannins	–	–	–	–	0.66*	−0.64*
Leaf area removed	–	–	–	–	–	0.78*

* $P < 0.002$

Therefore, environments with high light availability such as forest fragments and edges of fragments represent potential sites to be colonized by mistletoes (López de Buen et al. 2002; Valladares et al. 2006).

On the other hand, the response of insect communities to habitat fragmentation is highly variable because fragmentation may increase resources for some species or reduce resources for others (Barberena-Arias and Aide 2002). For example, some studies have indicated that biotic interactions are destabilized and collapsed as a result of forest fragmentation, increasing herbivore density and decreasing the abundance of their natural enemies such as parasitoids ("top-down" forces) (Kruess and Tscharntke 2000; Arnold and Asquith 2002). In contrast, according to other studies, herbivore abundance and leaf area removed decrease in small forest fragments due to fragment isolation and by changes in environmental conditions (i.e., higher temperature and lower humidity), which in turn reduce insect herbivore survival and performance (Valladares et al. 2006; Ruíz-Guerra et al. 2010). Our results agree with this idea because larger fragments had higher herbivory levels than small fragments.

Interestingly, we found a significant relationship between mistletoe infection and herbivory levels in the host tree, *Q. deserticola*. Across sites, herbivory was higher in parasitized plants than in unparasitized plants and lowest in the mistletoes. Contrary to our results, Canyon and Hill (1997) found higher levels of herbivory in mistletoes than in their hosts because mistletoes have a greater abundance of specialized herbivores. One explanation for our results is that variations in chemical defense and nutritional quality can affect the incidence of herbivorous insects (Lill et al. 2002). Overall, parasitized hosts had higher water content and higher concentration of nonstructural carbohydrates in comparison with unparasitized plants, but similar concentrations of soluble phenolics and total tannins. In turn, mistletoes presented the lowest water content and the highest concentration of total nonstructural carbohydrates, soluble phenolics, and total tannins. It has been reported that in several species of the genus *Quercus*, chemical compounds such as tannins represent an efficient anti-herbivore defense affecting physiological attributes of insects (Herms and Mattson 1992; Forkner et al. 2004). In this study, the negative correlations between leaf area removed and the concentration of soluble phenolics within the three groups of plants (parasitized and unparasitized oaks and mistletoes) support the defensive effect of these secondary metabolites.

The fact that unparasitized oaks had higher water content and concentration of nonstructural carbohydrates may suggest two hypotheses. The first one is that the probability of mistletoe infection is higher in trees with these characteristics. The second is that mistletoes may affect different components of growth and some metabolic functions of their host plants because they obtain nutrients, water, and some amounts of carbon from the xylem or both the xylem and phloem of their host plants (Puustinen and Salonen 1999; Press and Phoenix 2005). These changes in turn may make trees more susceptible to the attack of insect herbivores and other natural enemies (Press and Phoenix 2005; Qasem 2009). However, further observational and experimental studies are necessary to discriminate between the two hypotheses.

The intimate physiological relationship between the nutritional quality and the chemical defense of the host tree and the mistletoe was further evidenced by the observed positive correlations between nutritional quality and the concentration of secondary compounds between the two plants (Table 6). Furthermore, the leaf area removed in the mistletoe was not only positively correlated with the leaf area removed in the host, but was also negatively correlated with the concentrations of soluble phenolics and total tannins in the oak. These results suggest that *P. calyculatus* mistletoes have the capacity to sequester chemical defensive compounds of their hosts, reducing the levels of herbivory. Previous studies have found that mistletoes can sequester and accumulate high amounts of nutritional and chemical compounds such as phenolics and tannins (Glatzel and Geils 2009), which in turn affect biotic interactions, reducing the incidence of herbivores.

The water content and the amount of carbohydrates in the leaves may affect (Brodbeck et al. 1990) the synthesis of chemical compounds associated with plant defense as phenolics and tannins (Shure et al. 1998; Glatzel and Geils 2009). Several studies have indicated that an increment of water availability might cause an increase in the content of defense compounds in plants (Gutbrodt et al. 2011). Our results agree with this idea because water content was positively related with soluble phenolics and flavonoids in both parasitized and unparasitized hosts. Additionally, we also found a positive relationship among water content, soluble phenolics, and total tannins in mistletoes. These results indicate that water content in host plants influences indirectly the production of defense and nutritional compounds, which, in turn, affect the nutritional quality and defense of mistletoes and their interactions with insect herbivores (Huberty and Denno 2004). In addition, it is well known that water plays a key role in host plant–mistletoe interactions because mistletoes are characterized by faster rates of transpiration, deficiency in the water potential, and poorer water use efficiency in comparison with their hosts (Bell and Adams 2011).

In conclusion, our results indicate that forest fragmentation is related to an increase in the incidence of the mistletoe *P. calyculatus* on the host *Q. deserticola*. In turn, the infection is related to nutritional and chemical differences among oak individuals that are also reflected in higher herbivory levels. The mistletoe, in turn, is able to benefit from the interaction since it shows higher concentrations of carbohydrates and secondary metabolites and lower herbivory levels than the infected and even uninfected oaks.

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References

Agrawal A (2000) Overcompensation of plants in response to herbivory and the by-product benefits of mutualism. Trends Plant Sci 5:309–313

Agrell J, McDonald EP, Lindroth RL (2000) Effects of CO_2 and light on tree phytochemistry and insect performance. Oikos 88:259–272

Araùjo WS, Julião GR, Ribeiro BA, Portes I, Silva A, Dos Santos BB (2011) Diversity of galling insects in *Styrax pohlii* (Styracaceae): edge effect and use as bioindicators. Rev Biol Trop 59:1589–1597

Arizaga S (2009) Manual de la biodiversidad de encinos michoacanos. Instituto Nacional de Ecología, México D.F

Arnold AE, Asquith NM (2002) Herbivory in a fragmented tropical forest: patterns from islands at Lago Gatun, Panama. Biodivers Conserv 11:1663–1680

Aukema JE (2004) Distribution and dispersal of desert mistletoe is scale-dependent, hierarchically nested. Ecography 27:137–144

Barberena-Arias MF, Aide TM (2002) Variation in species and trophic composition of insect communities in Puerto Rico. Biotropica 34:357–367

Bell TL, Adams MA (2011) Attack on all fronts: functional relationships between aerial and root parasitic plants and their woody hosts and consequences for ecosystems. Tree Physiol 31:3–15

Bello GMA, Gutierrez GM (1985) Clave para la identificación de la familia Loranthaceae en la porción del Eje Neovolcánico localizado dentro del estado de Michoacán. Cienc Forestal 10:4–66

Bickford CP, Kolb TE, Geils BW (2005) Host physiological condition regulates parasitic plant performance: *Arceuthobium vaginatum* subsp. cryptopodum on *Pinus ponderosa*. Oecologia 146:179–189

Brodbeck BV, Mizell RF, French WJ, Andersen PC, Aldrich JH (1990) Amino acids as determinants of host preference for the xylem feeding leafhopper, *Homalodisca coagulata* (Homoptera: Cicadellidae). Oecologia 83:338–345

Bruna EM, Noguera-Ribeiro MB (2005) The compensatory responses of an understory herb to experimental damage are habitat-dependent. Am J Bot 92:2101–2106

Bustamante RO, Chacón P, Niemeyer HM (2006) Patterns of chemical defences in plants: an analysis of the vascular flora of Chile. Chemoecology 16:145–151

Calvin CL, Wilson CA (2006) Comparative morphology of epicortical roots in Old and New World Loranthaceae with reference to root types, origin, patterns of longitudinal extension and potential for clonal growth. Flora 201:345–353

Candia AB, Medel R, Fontúrbel FE (2014) Indirect positive effects of a parasitic plant on host pollination and seed dispersal. Oikos 123:1371–1376

Canyon DV, Hill CJ (1997) Mistletoe host-resemblance: a study of herbivory, nitrogen and moisture in two Australian mistletoes and their host trees. Austral J Ecol 22:395–403

Cuevas-Reyes P, Quesada M, Hanson P, Dirzo R, Oyama K (2004) Diversity of gall-inducing insects in a Mexican tropical dry forest: the importance of plant species richness, life-forms, host plant age and plant density. J Ecol 92:707–716

Cuevas-Reyes P, Fernandes GW, González-Rodríguez A, Pimenta M (2011) Effects of generalist and specialist parasitic plants (Loranthaceae) on the fluctuating asymmetry patterns of ruprestrian host plants. Basic Appl Ecol 12:449–455

Ehrlich PR, Raven PH (1964) Butterflies and plants: a study in coevolution. Evolution 18:586–608

Forkner RE, Marquis RJ, Lill JT (2004) Feeny revisited: condensed tannins as anti-herbivore defences in leaf-chewing herbivore communities of *Quercus*. Ecol Entomol 29:174–187

García-Brugger A, Lamotte O, Vandelle E, Bourque S, Lecourieux D, Poinssot B, Wendehenne D, Pugin A (2006) Early signaling events induced by elicitors of plant defense. Mol Plant Microb Interact 19:711–724

Gascon C, Williamson GB, da Fonseca GAB (2000) Receding forest edges and vanishing reserves. Science 26:1356–1358

Glatzel G, Geils BW (2009) Mistletoe ecophysiology: host–parasite interactions. Botany 87:10–15

Govier RN, Harper JL (1965) Angiospermous hemiparasites. Nature 205:722–723

Gripenberg S, Mayhew PJ, Parnell M, Roslin T (2010) A meta-analysis of preference-performance relationships in phytophagous insects. Ecol Lett 13:383–393

Grotewold E (2005) Plant metabolic diversity: a regulatory perspective. Trends Plant Sci 10:57–62

Gutbrodt B, Mody K, Dorn S (2011) Drought changes plant chemistry and causes contrasting responses in lepidopteran herbivores. Oikos 120:1732–1740

Hagerman AE (1987) Radial diffusion method for determining tannin in plant extracts. J Chem Ecol 13:437–449

Herms DA, Mattson WJ (1992) The dilemma of plants: to grow or defend. Q Rev Biol 67:283–335

Herrerías-Diego Y, Quesada M, Stoner K, Lobo JA (2006) Effects of forest fragmentation on phenological patterns and reproductive success of the tropical dry forest tree *Ceiba aesculifolia*. Conserv Biol 20:1111–1120

Huberty AF, Denno RF (2004) Plant water stress and its consequences for herbivorous insects: a new synthesis. Ecology 85:1383–1398

Kruess A, Tscharntke T (2000) Species richness and parasitism in a fragmented landscape: experiments and field studies with insects on *Vicia sepium*. Oecologia 122:129–137

Kursar TA, Coley PD (2003) Convergence in defense syndromes of young leaves in tropical rainforests. Biochem Syst Ecol 31:929–949

Lill JT, Marquis RJ, Ricklefs RE (2002) Host plants influence parasitism of forest caterpillars. Nature 417:170–173

López de Buen L, Ornelas JF, García-Franco JG (2002) Mistletoe infection of trees located at fragmented forest edges in the cloud forests of Central Veracruz, Mexico. For Ecol Manag 164:293–302

López E, Bocco G, Mendoza M, Duhau E (2001) Predicting land-cover and land-use change in the urban fringe. A case in Morelia city Mexico. Landsc Urban Plann 55:271–285

Lucas PW, Turner IM, Dominy NJ, Yamashita N (2000) Mechanical defenses to herbivory. Ann Bot 86:913–920

Maldonado-López Y, Cuevas-Reyes P, Stone GN, Nieves-Aldrey JL, Oyama K (2015) Gall wasp community response to fragmentation of oak tree species: importance of fragment size and isolated trees. Ecosphere 6:1–15

Marquis RJ, Newell EA, Villegas AC (1997) Nonstructural carbohydrates accumulation and use in understory rain forest shrub and relevance for the impact of leaf herbivory. Funct Ecol 5:636–643

Marshall JD, Dawson TE, Ehleringer JR (1994) Integrated nitrogen, carbon, and water relations of a xylem-tapping mistletoe following nitrogen fertilization of the host. Oecologia 12:430–438

Meinzer FC, Woodruff DR, Shaw DC (2004) Integrated responses of hydraulic architecture, water and carbon relations of western hemlock to dwarf mistletoe infection. Plant Cell Environ 27:937–946

Musselman LJ, Press MC (1995) Introduction to parasitic plants. In: Press MC, Graves JD (eds) Parasitic flowering plants. Chapman and Hall, London, pp 1–13

Nickrent DL, Musselman LJ (2004) Introduction to parasitic flowering plants. Plant Health Instruct. doi:10.1094/PHI-I-2004-0330-01

Pascual-Alvarado E, Cuevas-Reyes P, Quesada M, Oyama K (2008) Interactions between galling insects and leaf-feeding insects: the role of plant phenolic compounds and their possible interference with herbivores. J Trop Ecol 24:329–336

Pennings SC, Callaway RM (2002) Parasitic plants: parallels and contrasts with herbivores. Oecologia 131:479–489

Prada MOJ, Filho M, Price PW (1995) Insects in flower heads as *Aspilia Foliacea* (Asteraceae) after a fire in a central Brazilian savanna: evidence for the plant vigor hypothesis. Biotropica 27:513–518

Press MC, Phoenix GK (2005) Tansley review: impacts of parasitic plants on natural communities. New Phytol 166:737–751

Puustinen S, Salonen V (1999) The effect of host defoliation on hemiparasitic-host interactions between *Rhinanthus serotinus* and two Poa species. Can J Bot 77:523–530

Qasem JR (2009) An updated inventory of mistletoe (*Plicosepalus acaciae* and *Viscum cruciatum*) Distribution in

Jordan, hosts, and severity of infestation. Weed Technol 23:465–469

Rausher D (2010) Co-evolution and plant resistance to natural enemies. Nature 411:857–864

Roxburgh L, Nicolson SW (2008) Differential dispersal and survival of an African mistletoe: does host size matter? Plant Ecol 195:21–31

Ruíz-Guerra B, Guevara R, Mariano NA, Dirzo R (2010) Insect herbivory declines with forest fragmentation and covaries with plant regeneration mode: evidence from a Mexican tropical rain forest. Oikos 119:317–325

Runyon JB, Mescher MC, De Moraes CM (2008) Parasitism by *Cuscuta pentagona* attenuates host plant defenses against insect herbivores. Plant Physiol 146:987–995

SAS (2000) Categorical data analysis using the SAS system. SAS Institute, Cary

Saunders DA, Hobbs HJ, Margules CR (1991) Biological consequences of ecosystem fragmentation: a review. Conserv Biol 5:18–27

Sessions LA, Kelly D (2001) Heterogeneity in vertebrate and invertebrate herbivory and its consequences for New Zealand mistletoes. Austral Ecol 26:571–581

Shure DJ, Mooreside PD, Ogle SM (1998) Rainfall effects on plant-herbivore processes in an upland oak forest. Ecology 79:604–617

Smith LJ, De Morales CM, Mescher MC (2008) Jasmonate- and salicylate-mediated plant defense responses to insect herbivores, pathogens and parasitic plants. Pest Manag Sci 65:497–503

Thrall PH, Burdon JJ, Young A (2001) Variation in resistance and virulence among demes of a plant host–pathogen metapopulation. J Ecol 89:736–748

Torres AM, Mau-Lastovicka T, Rezaaiyan R (1987) Total phenolics and high-performance liquid chromatography of phenolic acids of avocado. J Agr Food Chem 35:921–925

Underwood N (2009) Effect of genetic variance in plant quality on the population dynamics of a herbivorous insect. J Anim Ecol 78:839–847

Valladares G, Salvo A, Cagnolo L (2006) Habitat fragmentation effects on trophic processes of insect plant food webs. Conserv Biol 20:212–217

Watling JR, Press MC (2001) Impacts of infection by parasitic angiosperms on host photosynthesis. Plant Biol 3:244–250

Zhuang XP, Lu YY, Yang GS (1992) Extraction and determination of flavonoids in ginkgo. Chin Herb Med 23:122–124