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Is ball moss (*Tillandsia recurvata*) a structural parasite of mesquite (*Prosopis laevigata*)? Anatomical and ecophysiological evidence

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Abstract

Key message **The epiphyte *Tillandsia recurvata* modifies the anatomy of the xylem, phloem and periderm of its host *Prosopis laevigata*, these modifications affect water flux and photosynthetic activity in this host.**

Abstract The relationships between epiphytes and host plants are commensal interactions, where epiphytes obtain support to growth without damaging their hosts. However, some epiphytes may cause the death of branches in their hosts. Ball moss (*Tillandsia recurvata*) is an epiphyte with elevated reproductive success in semiarid zones of México, growing mainly on mesquite trees (*Prosopis laevigata*). Mesquites are of great ecological and economical importance in central and northern Mexico, but the elevated mortality of branches and even whole trees have been associated with high ball moss loads. This study evaluates whether ball moss causes structural damage in the phloem, xylem, and periderm of mesquite branches, also testing whether the physiological performance of these branches was reduced. For this we measure the effective quantum yield of photosystem II (Φ_{PSII}), hydraulic conductivity, water flux, number of vessels and the effective area of conduction in mesquite branches with and without ball moss. The results demonstrated that juvenile ball moss only affected the periderm and the collapsed phloem with no damage to non-collapsed phloem or xylem, but the reproductive individuals modified the anatomy of the xylem, phloem and periderm of mesquite. These structural modifications affected both water flux and hydraulic conductivity and reduced the Φ_{PSII}. Our findings suggest that structural and ecophysiological changes induced by the ball moss are the cause of high branch mortality in mesquite. This is the first study showing anatomical and ecophysiological evidence of an epiphytic plant damaging its host.

Keywords Bromeliaceae · Chihuahuan desert · Mesquite · Phorophytes · Plant anatomy · Plant ecophysiology · Prosopis · Structural parasitism

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Introduction

Prosopis spp. (Fabaceae) are hardwood trees and shrubs widely distributed in the arid and semiarid regions of the World (Burkart 1976). These species are commonly denominated as ''Mesquite'' in USA and "Mezquite" in Mexico (Rzedowski 1988), and they are known for being drought tolerant (Nilsen et al. 1983). They are also important for reducing soil erosion, fix nitrogen and improve soil fertility in arid and semiarid zones (Tiedemann and Klemmedson 1973; Salazar et al. 2019). Mesquites also provide food for many wild and domestic animals which feed on their nectar, pollen, leaves and pods (Ruiz-Nieto et al. In press). Mesquites are also an important source of income for local people for charcoal trade in local and

international markets (Navar et al. 2019). These characteristics make mesquite a very important natural resource.

Prosopis laevigata (Willd.) M.C. Johnst is a mesquite of great ecological and economical importance in central and northern Mexico, where it is used as source of fuel, food for cattle and several other raw materials (Almanza and García-Moya 1986). This species is a conspicuous tree that can reach 6–9 m tall, but shrub growth-forms are also common (Burkart 1976). Populations of this mesquite species in Mexico are thought to be in decline (Aguilar-Rodríguez et al. 2007; Flores-Palacios et al. 2014), as a result of heavy loads of the epiphytic plant *Tillandsia recurvata* (L.) L. (Bromeliaceae) in their branches, averaging up to 0.4 Ton/ha of biomass (Flores-Palacios et al. 2015). Declining of *P. laevigata* populations could affect ecosystem services and also the income of the people inhabiting the Mexican drylands.

Tillandsia recurvata (ball moss) is an atmospheric epiphyte with holdfast-like roots whose main function is anchoring the plant on host branches; besides performing photosynthesis, their leaves are covered by a dense layer trichomes that uptake water and nutrients directly from the atmosphere (Wendt 1999). This species spreads from southern USA to the middle Argentina, inhabiting in dry and semi-dry climates (Caldiz et al. 1993; Bernal et al. 2005; Flores-Palacios et al. 2014). Epiphyte species could be host limited (Vergara-Torres et al. 2010) and individual host tree traits are related to increased epiphyte abundance (Chaves et al. 2016). However, the degree of dependence of the ball moss on hosts varies, and in some cases, this species may completely dispense living hosts, and thrive on artificial substrates such as electrical cables (Puente and Bashan 1994).

Ball moss is considered as an autonomous self-pollination plant that produces a great number of viviparous seedlings in the Southern Chihuahuan Desert (approximately 70 viviparous seeds per fruit; Pérez-Noyola et al. 2020). These viviparous seedlings have the ability to attach on the surface of branches of *P. laevigata* (Pérez-Noyola et al. 2020). Therefore, this epiphyte is able to spread easily in large areas.

It is assumed that host (the phorophyte; sensu Benzing 1990) is not negatively affected by the presence of the epiphyte, while this latter species benefits from physical support and access to light (Sáyago et al. 2013). Indeed, Stanton et al. (2014) found that epiphytes can have positive effects on host plant ecophysiology and forest ecosystem processes, because they improve host plant water use by microenvironment modification. However, in arid and semiarid ecosystems of America, ball moss (*Tillandsia* spp.) can have detrimental effects on several *Prosopis* species, causing abscission of leaves, increased branch mortality, reduced growth and, eventually, partial or complete death of the host tree (Flores-Palacios et al. 2014; Soria et al. 2014).

Ecophysiological and anatomical studies are still necessary to determine how ball moss causes these effects on its hosts. Some previous studies have shown that fixation of *T. recurvata* roots around *P. laevigata* branches leads to deep invaginations of penetrating roots of *T. recurvata* in the bark, interrupting the continuity of the cortex and phloem, and causing damage to the periderm (Aguilar-Rodríguez et al. 2007). The invaginations are characterized by the formation of clefts where the collapsed phloem (in different degrees) is separated, losing its continuity. In the region where deep invaginations form, all the phloem tissue is divided and the distinctive zones of the cortex (non-collapsed phloem, collapsed phloem and cortex) become indistinguishable (Aguilar-Rodríguez et al. 2007).

Deep invaginations also induce the accumulation of phenolic substances in those *P. laevigata* branches with *T. recurvata*, probably as chemical defense against external diseases, and causes the proliferation of parenchyma cells near the vascular cambium (Aguilar-Rodríguez et al. 2007). Further, it was suggested that these bark invaginations may alter the typical arrangement of wood cells if they reach the vascular cambium, which could reduce the density of xylem vessels and their diameter (Aguilar-Rodríguez et al. 2007). Thus, *T. recurvata* can be expected to induce large changes in the anatomy of their hosts, altering the water flow patterns in affected branches. This, in turn, could cause other physiological changes that may reduce the performance of the host. Indeed, as root development in ball mosses is positively related with the age of the plant (Brige 1911), older epiphytes can be expected to have stronger detrimental effects on their hosts.

Taking into account these issues, we analyzed anatomical differences in branches of *P. laevigata* with and without *T. recurvata* in the southern Chihuahuan Desert, and also evaluated changes in the physiology of the tree. We hypothesized that ball moss roots provoke anatomical damage and alter the water flow patterns in mesquite branches, and ultimately affect the physiology of host trees, and that such a damage increases with the size of the epiphytes.

Materials and methods

Study area

This study was conducted in a farm in the municipality of Mexquitic de Carmona, S.L.P., located in the southernmost section of the Chihuahuan Desert, Mexico (22° 16′ N, 101° 07′ W). The vegetation is secondary xerophilous scrub composed by sparse creosotebushes (*Larrea tridentata*), huisaches (*Vachellia farnesiana* and *Vachellia schaffneri*) and mesquites. Annual rainfall in the study site averages 422.7 mm and mean annual temperature is 16.7 °C (Medina

et al. 2005). In the Southern Chihuahuan Desert, *T. recurvata* shows complete vivipary incidence, which is probably adaptive because roots of seedlings may attach to hosts more readily than seeds without roots (Pérez-Noyola et al. 2020).

Anatomical changes in branches caused by ball mosses of different age

To assess the morphoanatomical changes that *T. recurvata* of different age induce on mesquite branches, we selected three mesquite trees (5 m height) in the study area. On each tree, we selected a branch with 1.8 – 2 cm radius containing a single ball moss reproductive adult (7.5 ± 0.8 cm height, 12.8 ± 0.6 cm diameter, 1.2 ± 0.3 cm length of the longest root), a branch supporting juvenile ball moss (3.7 ± 0.5 cm height, 5.3 ± 0.4 cm diameter, 0.6 ± 0.2 cm length of the longest root), and a branch without ball moss (control). These second order branches were similar to the ones used in the water flux experiment. These two sizes (juvenile and reproductive adult) were selected to evaluate the damage caused for only one individual.

The branches were taken from the same section of the tree crown. These branches were collected and taken to the laboratory. On each branch we performed transversal cuts at the section where ball mosses were attached to the rhithydome. These cuts were visually analyzed with a stereomicroscope with integrated digital camera (Leica EZ4D) to assess whether the roots of ball mosses penetrate into the branches. After that, conventional microtechnics were used to assess whether mesquite branches exhibited structural damage. Samples of branches were fixed in FAA (formaldehyde: ethanol:acetic acid). Transversal cuts of samples were performed with sliding microtome. Cuts were stained with bright blue cresyl and analyzed with an optical microscope (Leica 2000) equipped with digital image capture system (LEICA EC3).

Water flux in mesquite branches with and without ball moss

In order to assess the time it takes the water to travel a distance of 30 cm in a mesquite branch without epiphytes at noon, we applied the technique used by Reyes-García et al. (2012), with modifications, in eight branches of second order (four branches of 4.5 cm diameter and four branches of 8 cm diameter) of mesquite trees, as follows: In each branch we drilled a hole of 2 mm in diameter and 10 mm in depth using a steel microcorer (Trephor, University of Padua, Italy) at 10 cm from the point of attachment to the immediate branch. Immediately after perforation, a plastic syringe of 2 mm in diameter filled with 2 ml of 0.1% safranin aqueous solution was inserted into the drilled hole and the dye was injected. As soon as the syringe was removed, the hole

was immediately plugged with silicone sealant and covered with parafilm. The branches were sectioned at 30 cm from the drilled hole after 0.5, 1 and 2 h later for examination in the laboratory, establishing that in two hours the dye traveled this distance. With this procedure is possible to distinguish the functional vessels (red stained) and the effective conduction area in the xylem.

To determine whether roots of ball moss affect water flow on mesquite branches, we performed this experiment at noon using the same procedure as mentioned in the previous paragraph. Twelve mesquite branches of second order were selected during the dry season: four branches without ball moss (control), four branches 4.5 cm in diameter with juvenile ball moss individuals (3.8 ± 0.8 cm height, 5.5 ± 0.9 cm diameter, 0.7 ± 0.2 cm length of the longest root) and four branches 8 cm diameter with ball moss reproductive adults (7.7 ± 0.4 cm height, 13.2 ± 0.8 cm diameter, 1.4 ± 0.2 cm length of the longest root).

Two hours later, the branches were sectioned 15 cm above and below where the ball moss plant was attached, and immediately transported to the laboratory. Subsequently, the cuttings were performed in the laboratory before and after the ball moss attachment point. With this procedure, it was possible to distinguish the functional vessels (red stained) and the effective conduction area in the xylem.

The polished transverse was observed under the stereomicroscope with an integrated digital camera (Leica EZ4D) and digital images were taken. The Image J software was used to count the number of vessels stained in red and measure the effective conduction area as the red stained vessels lumen area in 1 cm^2. The data obtained were standardized to ratio values due to the differences in diameter of the branches and the effect of allometry on the total number of vessels. The following formulas were used: (a) Functional vessels ratio (%) = (number of stained vessels where the dye was injected – number of stained vessels upwards)*100/number of stained vessels where the dye was injected; (b) Effective conduction area (%) = (Effective conduction area where the dye was injected – Effective conduction area upwards)*100/ Effective conduction area where the dye was injected. We also measured the length of vascular secondary tissues (rythidome, phloem and xylem wound) where the ball moss plant was attached and the control branches.

Evaluation of hydraulic conductivity in mesquite branches with and without ball moss

Stem xylem hydraulic measurements provide complete information on the transport of fluids inside the conducting vessels of the plant to supply water to photosynthetic tissues (Melcher et al. 2012). To determine the impact of ball moss in the branch water conduction, we measured hydraulic conductivity (K_h, flux per pressure gradient) and specific

hydraulic conductivity (K_s, K_h/cross-sectional area of the branch) (Sperry et al. 1988) in 10 cm length X 0.8 cm ± 0.1 diameter segment of mesquite branches collected during the dry season (May) as described by Melcher (2001), to evaluate the effect of *T. recurvata* on the hydraulic conductivity in the most critical season for water supply to the foliage. Before this experiment, we checked that 10 cm vessel length segment was enough. We estimated the maximum vessel length with the method of Melcher et al. (2012) and the samples were determined to be 10 cm of branch cut length.

Four branches without ball moss (control) and four branches with ball moss attached, were wrapped in wet towels, stored in plastic bags and sealed for transport to the laboratory in the next 3–5 h. Prior to each measurement, the distilled water was boiled and overnight degassed under laboratory vacuum at 70 kPa (Millipore Sigma) and the branches were hydrated for 120 min (Espino and Schenk 2011). We used the driving flow rate method (Melcher et al. 2012) with a column of water pressurized (70–150 kPa) with a field-portable cylinder of compressed N2 (M-1515D). The perfusion distilled water passing through the segment of the branch was collected in a 0.5 ml pipette, which showed the volume of water passing through it. The pipette carried a syringe, which allows the pipetting to be zeroed at the beginning of the measurement.

Effect of ball moss on the effective quantum efficiency of Photosystem II (Φ_{PSII}) of mesquite

To determinate the effect of ball moss on the effective quantum efficiency of photosystem II (Φ_{PSII}) of mesquite, five trees were selected from each of two categories: juveniles (ca. 2.5 m tall) and adults (> 4 m). In each tree, we selected six branches with leaves, three with one ball moss seedling (4.5–5 cm height) per branch and three without ball moss. Branches with larger ball moss plants did not have leaves and looked dead. Care was taken to ensure that each selected branch had healthy looking leaves. The mesquite leaves are composed of leaflets, from the first day the leaflets where chlorophyll fluorescence measurements would be carried out were marked. Measurements were made in the adaxial surface of the leaflets at 10 cm from the insertion of ball moss plants. These fluorescence measurements were taken in the dry season.

Seven biweekly measurements of the effective quantum efficiency of photosystem II (Φ_{PSII}) were performed using a portable pulse amplitude modulation fluorometer (Mini-PAM; H. Walz, Effeltrich, Germany). The experiment started on November 8, 2014 and ended on February 7, 2015, the last date on which leaves were found to be in good condition. Branches with and without ball moss had similar light levels and leaf temperatures in each measurement time. Because damage to photosystem II (PSII) is the first manifestation of

stress in a leaf, quantification of Φ_{PSII} can provide information about the magnitude to which PSII is using the energy absorbed by chlorophyll (Maxwell and Johnson 2000). The fluorescence was recorded instantaneously, which allowed to know the differences and the variations in the effective quantum efficiency of the photosystem II (Φ_{PSII}).

The Φ_{PSII} was measured at noon, the time of day with the highest temperature and solar radiation. It was obtained with the formula $PSII = (F'_m - F_t)/F'_m$, where F_t is the fluorescence of the chlorophyll emitted by the plants under a stable illumination state (eg, under light conditions in the field) and F'_m is the maximum fluorescence emitted by chlorophyll when a saturating pulse of actinic light is superimposed to environmental levels of light (Genty et al. 1987).

Statistical analysis

For the water flux experiment, a one-way ANOVA was performed to find significant changes between the control and branches with ball moss juveniles and reproductive adults. Differences between hydraulic conductivity in branches with and without ball moss were found with a *t*-test ($\alpha = 0.05$). The anatomical response variables were evaluated by using one-way ANOVA and Tukey tests. Data from the ecophysiological experiment were analyzed with a repeated measures two-way ANOVA, and because the assumption of sphericity was violated, the degrees of freedom were corrected with the Huynd–Feldt correction. The factors were: Tree size (with two levels) and presence of ball moss (two levels). The ANOVA was followed by the multiple comparison test of Bonferroni ($P < 0.05$). The data fulfilled the assumptions of normality and homogeneity of variances.

Results

Anatomical changes in branches caused by adult and juvenile ball mosses

There was evidence of structural damage in the rhytidome, as well as in deeper areas such as the phloem and xylem of mesquite caused by ball moss. The stems of ball moss adhere to mesquite branches, establishing in the fissures of the rhytidome. Through time, changes were observed in the cortex and xylem, changing the common form of the branch.

The tree seals off the wound where the ball moss was attached, and new growth is present around the damage (compartmentalization) (Fig. 1a, b). The structure of the wood is characterized by annular porosity with vasicentric, aliform confluent paratracheal parenchyma, fine uniseriate difusse apotracheal parenchyma, and multiseriate rays. In the bark, four areas were observed: periderm, cortex, collapsed phloem, and non-collapsed phloem (Fig. 1c). The
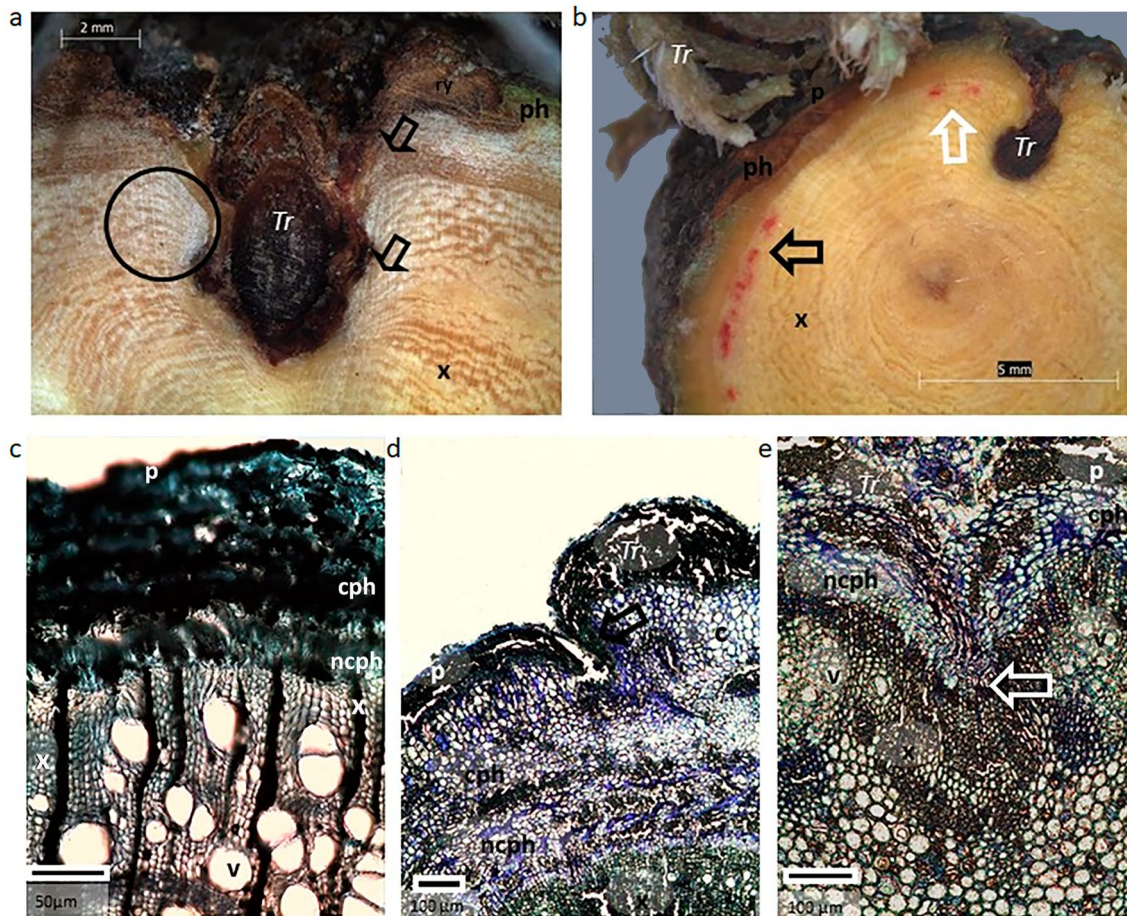
Fig. 1 Transverse section of *P. laevigata* branch. Macroscopic: **a** a large section of the *T. recurvata* axis embedded in the xylem and phloem is observed (arrows), folding them to the sides and losing continuity in the structure, in addition to a decrease of vessels in the xylem (circle). **b** A decrease in the effective conduction area (red safranine stain) and functional vessels density (white arrow) compared to the area without *T. recurvata* (black arrow). Microscopic: **c** without the presence of *T. recurvata*, from the outside inwards is observed the periderm, collapsed phloem, non-collapsed phloem and xylem with annular porosity. **d** The juvenile plant of *T. recurvata* initially established on the surface of the bark, affected the cortex and collapsed phloem folding the outermost tissues of the bark (arrow), leaving the lower immediate of non-collapsed phloem and xylem intact. **e** The wood and bark of *P. laevigata* imbibe over time the lower root and stem portion of the *T. recurvata* adult plant initially established on the surface of the bark while both plants grow, causing damage (arrow) to all tissues, from the phloem that folds along the axis of the *T. recurvata* to the xylem where an absence of vessels in the surrounding tissue is observed. TR = *T. recurvata*, ry = rythidome; p = periderm, ph = secondary phloem, cph = collapsed phloem, ncph = non-collapsed phloem, c = cortex, x = secondary xylem, v = vessel

periderm is formed by pheloderm, phellogen and phellem (suber), organized in radial rows (Fig. 1a, c). A rhytidome may develop with accumulation of numerous peridermis. The collapsed phloem was organized in two layers separated by a discontinuous band of fibers. From the second discontinuous sclerenchyma band, we distinguished the collapsed phloem zone where the sieve tube elements collapse, observing small strata that are separated in their tangential margins by the axial parenchyma organized in tangential rows.

The roots of adult ball moss left a lesion in the cortex of mesquite branches. Retrieval of the cortex and wood (xylem) of the mesquite branches was observed next to roots of adult ball moss (Fig. 1b, d, e). The roots and stem of adult ball moss wounded the bark and wood of mesquite branches.

Initially, juvenile ball moss only affects the periderm and the collapsed phloem with no damage to non-collapsed phloem or xylem (Fig. 1d). But over time, the reproductive ball moss affects the cambium, twisting inwards and heading towards the surface of the wound, and the successively formed growth zones close to the lateral edge of the wound, changed their orientation to perpendicular to the original periderm (Fig. 1b, e). The first traumatic xylem (the first mechanical damage in the xylem caused by *T. recurvata* roots) and the xylem close to the edge of the wound show

high proportion of axial parenchyma and less vessel density (Fig. 1e).

Juvenile ball moss affected mesquite by retracting the periderm and the collapsed phloem (Fig. 1d). No damage in non-collapsed phloem or xylem was observed (Fig. 1d). In contrast, the damage caused by adult ball moss was considered a cleft that induces the folding of the periderm, collapsed and non-collapsed phloem until they become undistinguishable, losing their continuity and forming a zone of suber (Fig. 1e). Wood also appeared retracted with a loss of continuity, less vessels and changes in their orientation were observed (Fig. 1e).

Water flux in mesquite branches with and without adult and juvenile ball mosses

There were functional and anatomical alterations in the mesquite wood caused by adult and juvenile ball moss, particularly where it was fixed, which included a decrease in the effective conduction area (Fig. 1b), implying a decrease in the percentage of functional vessel ($F = 703.14$; $P < 0.00001$; Fig. 2), and a decrease in percentage of effective conduction area ($F = 19.09$; $P = 0.0025$; Fig. 3), without differences between adult and juvenile ball mosses. In the branches marked as control, the effective area of conduction and the vessel percentage were higher than in branches with *T. recurvata* (Figs. 2, 3), the pattern of porosity parallel to the longitudinal axis of the branches did not change direction.

The broader rhytidome was associated with reproductive *T. recurvata* with a significant difference between the branches without *T. recurvata* and with juvenile ($F = 5.245$, $P < 0.009$), but there were no differences between the latter (Table 1). As the reproductive *T. recurvata* remains on the

Fig. 3 Effect of juvenile and reproductive *T. recurvata* on the percentage of effective conduction area in *P. laevigata* branches ($n = 4$). Different letters indicate significant differences by Tukey test at $p < 0.05$

branch, more rhytidome develops on the sides of the wound, probably to contain the expansion of the plant, but more studies would be necessary to test this.

However, the length of the secondary phloem in the branch transverse section in the wound caused by *T. recurvata* was significantly shorter than those of the control ($F = 17.045$, $P < 0.0001$). The branch without *T. recurvata* showed an almost twice longer phloem than the branches with juvenile and reproductive *T. recurvata*, but there was no difference between them (Table 1). Although no damage was observed in the non-collapsed phloem, the roots probably crushed the tissue since the plant was a juvenile.

The depth of the wound caused by the lower part of the stem and the root of *T. recurvata* embedded in the xylem showed significant differences ($F = 461,893$, $P < 0.0001$). Reproductive *T. recurvata* remaining on the branch for a long time forms a wound three times deeper than does the juvenile *T. recurvata* (Table 1). The effective conduction area is affected by the reduction of the frequency of vessels in the area occupied by the plant in the xylem and the area of xylem that surrounds the wound compared to intact branches, with a slight tendency to decrease when *T. recurvata* is larger. However, the reaction of the vascular tissues surrounding *T. recurvata* contain to some extent its expansion, preventing further damage.

Evaluation of hydraulic conductivity in mesquite branches with and without ball moss

Hydraulic conductivity and specific hydraulic conductivity were 63.7% and 59.5% lower in branches with ball moss than without it. Hydraulic conductivity was lower in branches with ball moss (0.05 ± 0.01 kg m MPa^{-1} s^{-1}) than without it (0.138 ± 0.01 kg m MPa^{-1} s^{-1}) ($t = 8.353$,
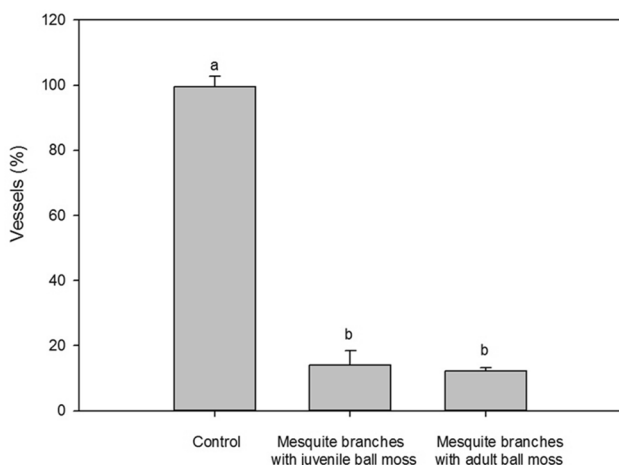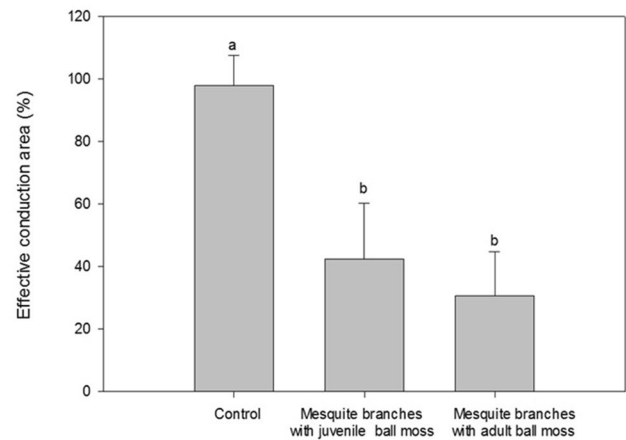

Fig. 2 Effect of juvenile and reproductive *T. recurvata* on the vessels percentage in *P. laevigata* branches ($n = 4$). Different letters indicate significant differences by Tukey test at $p < 0.05$

Table 1 Effect of reproductive and juvenile *T. recurvata* on *P. laevigata* branches. Different letters within the same column indicate significant differences by Tukey test at $p < 0.05$ and $n = 5$

Treatment	Secondary phloem length (μm) (Mean ± SE)	Rhytidome length (μm) (Mean ± SE)	*T. recurvata* depth in secondary xylem (μm) (Mean ± SE)
Branch with reproductive *T. recurvata*	785.8 ± 98.6 a	1795.1 ± 107.3 b	5405.9 ± 156.4 c
Branch with juvenile *T. recurvata*	600.4 ± 51.0 a	1029.1 ± 244.0 a	1691.1 ± 158.8 b
Control	1216.1 ± 72.4 b	1181.9 ± 151.4 a	0 a

Table 2 Results of repeated measures two-way ANOVA from effect of ball moss on effective quantum efficiency of Photosystem II (Φ_{PSII}) of juvenile and adult mesquites

Factor	Degree freedom	*F*	*P*
Presence of ball moss	1	6.28	0.015
Tree size	1	2.20	> 0.05
Ball moss × tree size	1	0.0834	> 0.05
Time	6	36.30	< 0.0001
Ball moss × time	6	5.31	< 0.0001
Time × tree size	6	3.13	0.005
Ball moss × time × tree size	6	1.7438	> 0.05

Fig. 4 Effective quantum yield of photosystem II (Φ_{PSII}) of *P. laevigata* with and without *T. recurvata* ($n = 10$). ANOVA was followed by Bonferroni test. Asterisks (*) imply statistical differences between treatments at time points
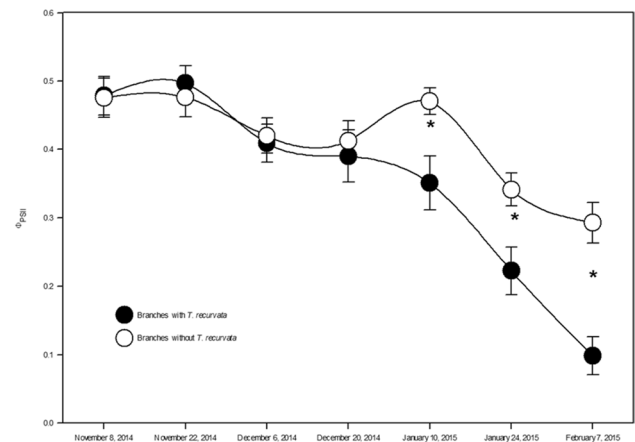
$P = 0.003$). Specific hydraulic conductivity was also lower in branches with ball moss (0.991 ± 0.079 kg m^{-1} MPa^{-1} s^{-1}) than without it (2.452 ± 0.079 kg m^{-1} MPa^{-1} s^{-1}) ($t = 8.353$, $P = 0.003$).

Effect of ball moss on the effective quantum efficiency of Photosystem II (Φ_{PSII}) of mesquite

There were significant effects of the presence of ball moss and time factors, as well as of ball moss X time, and time X tree size interactions (Table 2). The effective quantum efficiency of photosystem II (Φ_{PSII}) of mesquite decreased across time from 0.476 ± 0.02 at the first evaluation to 0.195 ± 0.02 at the last evaluation, after three months. Although the interaction time X tree size was significant, the Φ_{PSII} of branches of small and big mesquites at each evaluation was similar. Φ_{PSII} was also similar (c. 0.4) between branches with ball moss to branches without it during the first four evaluations, but it was lower (≤ 0.35) in branches with ball moss after two months (Fig. 4).

Discussion

Several studies suggest that ball moss causes damage to the branches of mesquite, but they do not show the mechanisms of such damage (Benzing and Seeman 1978; Montaña et al. 1997; Soria et al. 2014; Flores-Palacios et al. 2015;

Cortes-Anzures et al. 2017). We found that the roots and stems of adult ball moss wounded the bark and wood of mesquite branches. Probably this injury originates during the establishment of ball moss seedlings because the plant adheres to the branch on the rhytidome, and over time the branch continues to grow around the stem of the ball moss.

We also found that adult ball moss caused more anatomical changes in mesquite branches than juvenile ones. Juvenile ball mosses affected mesquite by retracting the periderm and the collapsed phloem, but no damage in non-collapsed phloem or xylem was observed. In contrast, there was evidence of structural damage in the periderm, as well as in deeper areas such as the phloem of mesquite caused by adult ball moss. The roots of adult ball moss were observed to adhere to mesquite branches, establishing in the fissures of the cortex. Changes were observed in the cortex and xylem, changing the common form of the branch. Although these negative effects were measured at the individual level of ball moss, they might have an effect at the colony or population level, e.g. Benzing and Seemann (1978) suggested xylem and phloem transport in *Quercus virginiana* Mill. to be obstructed at the junction between host and epiphyte by roots of mature *T. recurvata* colonies.

The attached ball moss also negatively affected the water flow through the branch. We found that, next to the insertion there were fewer conducting vessels in the xylem (less stained) and they deviated their parallel trajectory present in the branches without *T. recurvata*, recovering it later where *T. recurvata* was no longer present. We also found collapsed phloem in the bark, which was organized in two layers separated by a discontinuous band of fibers. The xylem structural modifications also had an impact on hydraulic conductivity because the average K_h and K_s values dropped ca 60%. Although epiphyte is causing damage and not parasitizing water and nutrients, our findings are similar to those found for the holoparasitic endophyte *Bdallophyton americanum* (R. Br.) Harms affecting *Bursera simaruba* (L.) Sarg., where K_s on parasitized roots during the dry season was 61% lower than in non-parasitized roots (García-Franco et al. 2007). We cannot compare our results with epiphytic plants per se, because there is no literature about effects of damage by epiphytes on the vascular system of plants. Parasite plants provoke water stress due to embolisms formation in the host's wood because their higher transpiration rates and low water potential, and alterations in wood structure (Teixeira-Costa and Ceccantini 2015) like those found in this study. Although *T. recurvata* it is not considered a parasite, apparently the wood anatomical alterations by themselves could cause embolisms and the loss of a significant proportion of hydraulic conductivity, affecting photosynthesis and causing eventual death of branches.

Our results of obstruction in water flux, reduced hydraulic conductivity and diminished effective quantum efficiency of photosystem II coincide with the severe anatomical alterations found in mesquite due to the presence of ball moss, which increased with the development of the epiphyte. Our results are similar to those of Aguilar-Rodríguez et al. (2016) that found that changes range from those non-detectable to the naked eye, through slight modifications, to notable changes in the bark and wood, coinciding with the damage founded in other experimental studies on mechanical wound repair (Zajaczkowska 2014; Chano et al. 2015). However, our results do not support Stanton et al. (2014) findings that epiphytes improve host plant water use by microenvironment modification.

We also found a reduction of vessel density due to the modifications that occur in the xylem, without differences between adult and juvenile ball mosses. However, although a well-developed velam was described in ball moss roots, this is not able to absorb substances from its host (Aguilar-Rodríguez et al. 2007). Teixeira-Costa and Ceccantini (2015) found that branches of *Tapirira guianensis* Aubl. parasitized by mistletoe *Phoradendron crassifolium* (Pohl ex DC.) Eichler had approximately 25% greater number of vessels than non-parasitized branches, which contrasts with our results of fewer vessels. In addition, the same authors found that the vessel diameter of the parasitized branches were 14% narrower than the non-parasitized vessels and observed a greater density of the functional vessels at the host-parasite interface. Perhaps these results are due to the nature of *P. crassifolium* which is a parasite of xylem.

Teixeira-Costa and Ceccantini (2015) mention that some parasitic plants can cause a variety of effects to their hosts in the process of wood formation, and that these plants can accelerate the natural process of embolism in the host's xylem, by reducing the diameter of the vessels and making the walls thinner. Ball moss was not found to absorb water or nutrients from its phorophyte (Aguilar-Rodríguez et al. 2016), but it did cause a water flow deviation (xylem) on both sides of the insertion of the root of ball moss in mesquite.

Ball moss was found to affect the ecophysiology of the leaves as they caused a decrease in the effective quantum efficiency of photosystem II after two months. Probably this decreased effective quantum efficiency of photosystem II relates to partial stomatal closure in the branches with epiphytes (due to decreases in water flow to the leaves, accelerated senescence, phloem effects, etc.). These results support the hypothesis of Montaña et al. (1997) of an inhibition of photosynthesis in hosts of ball moss. In this research, however we found that the effect was not due to the shade produced by ball moss on the leaves of the phorophyte, because the leaves selected experimentally were 10 cm away from the insertion and not shaded. In addition, our experiment evaluated the damaged caused by small ball moss plants (< 5 cm) that provide negligible shade.

Conclusions

Our hypothesis that ball moss roots cause anatomical damage and alter water flow patterns in mesquite branches, and ultimately affect the physiology of host trees was corroborated, although the hypothesis that such a damage increases with the size of the epiphytes was only partially supported. Adult ball moss caused more anatomical changes in mesquite branches than juvenile ones. Juvenile epiphytic plants of ball moss affected the rhytidome, cortex and collapsed phloem of mesquite, leaving intact the layers of non-collapsed phloem and xylem. Adult ball moss wounded mechanically the mesquite branches, modifying the xylem, phloem and periderm structure. These structural modifications affected the hydraulic conductivity and the water flow (xylem), decreasing the effective quantum efficiency of photosystem II (Φ_{PSII}). Thus, the anatomical modifications in the branches of mesquite are most likely the cause of the high branch mortality, which could impact growth and productivity of mesquite.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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