EMBOLISM INCREASE AND ANATOMICAL MODIFICATIONS CAUSED BY A PARASITIC PLANT: *PHORADENDRON CRASSIFOLIUM* (SANTALACEAE) ON *TAPIRIRA GUIANENSIS* (ANACARDIACEAE)

Luíza Teixeira-Costa and Gregório Ceccantini*

University of São Paulo, Institute of Biosciences, Dpt Botany, Rua do Matão 277, 05508-090, São Paulo, SP, Brazil

*Corresponding author; e-mail: gregorio@usp.br

ABSTRACT

Parasitic plants are capable of causing a variety of effects to their hosts, including alterations in the process of wood formation. However, the majority of studies dealing with parasitic plant anatomy have focused on the host–parasite interface and the direct action of the haustorium, which is the organ responsible for attaching the parasite to the host. Considering this gap, we studied the anatomical and functional effects caused by a mistletoe species, *Phoradendron crassifolium* (Santalaceae), on the wood anatomy of the host tree *Tapirira guianensis* (Anacardiaceae). Both parasitized and non-parasitized branches were collected from host trees. Traditional wood anatomy procedures were employed, along with functionality experiments using the ascent of safranin solution through the xylem. Prior to the analysis, all sampled branches were divided in “upstream” and “downstream” portions, considering the direction of xylem sap flow inside the plant body. This design was chosen in order to avoid biased results derived from normal ontogeny-related wood anatomical and functional changes. Our results showed that infested wood expressed a higher density of embolized vessels, narrower vessel lumen diameter, higher vessel density, taller and wider rays, and fibers with thinner cell walls. All these responses were most conspicuous in the downstream sections of the parasitized branches. We propose that the wood anatomical and functional alterations were induced by the combination of water stress caused by water use by the parasite and consequent low turgor in differentiating cambial derivates; by unbalanced auxin/cytokinin concentrations originating at the infestation region due to phloem disruptions caused by the parasite’s penetration and action; and by higher than usual ethylene levels. Further analysis of hydraulic conductivity and hormonal changes in host branches are necessary to test this hypothesis.

*Keywords:* Parasitic plants, mistletoe, ecological wood anatomy, wood hydraulics, Santalales, haustorium.
INTRODUCTION

Parasitic plants are angiosperms capable of attaching to and penetrating tissues of other plant species and thereby influence wood formation and various other processes in the host. From the moment of successful seedling attachment to the host, local morphological and anatomical changes take place in the parasitized organ (Heide-Jørgensen 2008). As the parasite grows its influence on the host plant’s body becomes both stronger and more widespread (Press & Graves 1995).

The attachment to the host is performed by an organ known as the haustorium (Kuijt 1969) which acts as a bridge between the parasite and the host, allowing the flux of nutrients, general metabolites, hormones and probably other internal signals (Press & Stewart 1991; Press & Phoenix 2005). Due to the importance of such an organ, studies on parasitic plant anatomy have focused on the development and direct action of the haustorium, e.g., the local anatomical changes it induces in the parasitized host tissue (Cohen 1954; Calvin 1967; Gonzalez & Mauseth 2010 and many others).

An exception to the above mentioned line of research was the work of Srivastava and Esau (1961), which showed alterations in the actual wood anatomy of seven coniferous species infested by Arceuthobium sp. These authors reported that the presence of the parasitic tissue growing within the rays of the host wood caused alterations in the shape and size of the rays and also induced irregularities in tracheid shape.

From an anatomical and ecological perspective, the recent work by do Amaral and Ceccantini (2011) deals with the effect of the endoparasite Pilostyles ulei on the wood anatomy of three Mimosa species. The authors found that parasitized individuals were smaller and had shorter and narrower vessel elements, and also shorter fibers.

Although the above mentioned studies are detailed, both of them dealt with small-bodied and leafless parasites. Large stem parasites present a great biological and ecological diversity and still very little is known about their effects on general features of their host’s wood.

Due to the role of sap conduction by the xylem, changes in wood anatomy, e.g., those caused by the presence and action of a parasitic plant, could also cause alterations in the host’s performance and its water status. Indeed, Calvin (1997) stated that parasitic plants may accelerate the natural process of embolism formation in the host’s xylem. Based on this, our initial hypothesis was that parasitized branches would show a higher degree of embolism when compared to non-parasitized branches.

In order to test this hypothesis, we analyzed a large-bodied parasite, Phoradendron crassifolium (Pohl ex DC.) Eichler (Santalaceae [Viscaceae]) while infesting Tapirira guianensis Aubl. (Anacardiaceae). Both woody species are widespread in South America. The parasite infests trees belonging to more than ten different families (personal observation in herbaria), and develops into a huge shrub with yellowish leaves and fruits attractive to birds.

MATERIALS AND METHODS

The sampling of infected and non-infected host tissues took place in Minas Gerais state in a riparian forest in the Mata Atlântica domain of Southeast Brazil (21°48’30.2”S,
The population from which the samples were collected showed a high degree of infestation, with most of the host trees infested by at least one parasite. Twenty branches from fully developed individuals of *Tapirira guianensis* were collected. Half of the branches were parasitized by *Phoradendron crassifolium* and the other half was not parasitized. Figure 1 illustrates an infested branch. All collected branches bore green leaves and did not show signs of nutritional deficiencies or other health problems.

A previous study was carried out in order to understand the wood structure of the host species *T. guianensis*. Following the methods for length-on-age analysis described by Jono *et al.* (2013), the height of rays and the length of fibers and vessel elements were measured along the radius at each millimeter starting from the cambial zone and ending near the pith, using both radial sections and macerations (Franklin 1945). As a result, we chose to sample only branches thicker than 2 cm in diameter in order to avoid the influence of juvenile wood on the anatomical analysis performed afterwards.

Immediately after cutting, the branches were submerged in water, cut again and then their end surfaces were smoothed with sharp blades. Subsequently they were put in a safranin solution (0.01%) to transpire for about 8 hours (modified from Ellmore & Ewers 1986). Using this procedure we were able to distinguish between functional

Figure 1–4. – 1: Branch of *Tapirira guianensis* parasitized by *Phoradendron crassifolium*. – 2: Cross section of host branch showing safranin-stained (functional) vessels; white squares mark areas where anatomical measurements were taken. – 3: Non-parasitized branch with upstream (U) and downstream (D) sections demarked. – 4: Parasitized branch with upstream (U) and downstream (D) sections demarked; the black square marks the attachment site of the parasite in the host branch.
vessels (stained in safranin-red) and non-functional ones (embolized and therefore not stained) at the time of sampling (Fig. 2).

All branches were divided in two portions considering the direction of water flow inside the plant. We referred to the proximal and distal sections as “upstream” and “downstream”, respectively, following the designation suggested by Zimmermann (1983). This experimental design was chosen in order to avoid biased results influenced by wood anatomical and functional changes in the branches due to tapering (West et al. 1999; Anfodillo et al. 2013).

In the parasitized branches, the upstream and downstream portions were cut at a distance of at least 10 cm from the parasite’s attachment point (Fig. 3) in order to provide samples consisting purely of host wood, free from parasitic tissue within it. Non-parasitized branches were cut at positions with a diameter similar to the upstream and downstream portions of parasitized branches (Fig. 4). All portions were cut clear from nodal regions. The samples were thus grouped in four classes: non-parasitized upstream and downstream; and parasitized upstream and downstream.

All samples were trimmed and sectioned with a sliding microtome (Leica SM 2000R) to produce transverse, radial longitudinal and tangential longitudinal sections. Samples were stained with safranin/astra-blue (Johansen 1940 adapted by Kraus & Arduin 1997). The samples were also macerated following Franklin (1945). The material was photographed using a photomicroscope (Leica DML and camera DFC 310FX).

All measurements were taken at the outermost region of the branch wood area, as indicated by Figure 2. This assures that we have analyzed the host wood under the influence of the parasite, i.e., the wood produced after the infestation had begun or its analogue in the non-infected branches.

The wood anatomical features analyzed were: vessel density (per mm²), vessel diameter and area for both functional and non-functional vessels; vessel element length; fiber lumen diameter, fiber length and fiber cell-wall thickness; ray height and width; radial resin canal density (per mm²); percentage of grouped vessels. Thirty measurements were taken for each anatomical feature in each of the samples. All the measurements were carried out using the free software Image J (Rasband 1997–2011).

Wood measurements were compared among the four groups using a Nested Analysis of Variance (nested ANOVA, Zar 2010). The measurements were nested within the sample they were taken from, the samples were also nested within the groups they belong to, and each group was nested within their respective condition (parasitized or non-parasitized). Measurements were analyzed using the software JMP SAS Institute Inc (SAS Institute Inc., 1998–2014).

RESULTS

The wood of *Tapirira guianensis* has poorly distinct growth rings (Fig. 5), with boundaries marked by relatively thick-walled latewood. The axial parenchyma is scanty paratracheal and tyloses are common (Fig. 6). Rays are 1–3-seriate (Fig. 7), and composed of procumbent body cells, with 1 or 2 rows of square marginal cells (Fig. 9). The wood is also characterized by septate fibers (Fig. 7), radial resin canals

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(Fig. 8), simple perforation plates (Fig. 10) and bordered alternate intervessel pits. The vessel-ray pits have reduced borders (Fig. 10).

The above qualitative features characterized the wood of both parasitized and non-parasitized branches. However, the analysis of quantitative features showed remarkable differences among the four groups we compared. Table 1 summarizes the mean values

Figure 5–10. General wood anatomical features of *Tapirira guianensis*. – 5: Macrograph of transverse surface showing diffuse porosity and poorly marked annual rings. – 6: Transverse section showing axial scanty paratracheal parenchyma and tyloses common. – 7: Tangential section showing 1–3-seriate rays. – 8: Detail of a tangential section showing a radial resin canal. – 9: Radial section showing the cellular composition of rays. – 10: Detail of a radial section showing vessel pitting. — Scale bars in 5 = 1 mm; in 6, 7 & 10 = 100 µm; in 8 = 70 µm; in 9 = 200 µm.
Table 1. Nested ANOVA results for the wood anatomical features analyzed among groups (upstream and downstream) of both parasitized and non-parasitized branches of *Tapirira guianensis*. All bold values indicate significant statistical differences among the groups; letters inside the parentheses indicate the results for the Tukey test (post nested ANOVA).

<table>
<thead>
<tr>
<th></th>
<th>Non-parasitized</th>
<th>Parasitized</th>
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<tbody>
<tr>
<td></td>
<td>Upstream</td>
<td>Downstream</td>
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<tr>
<td><strong>Vessels</strong></td>
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<td>32.3 (C)</td>
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<tr>
<td>Diameter (µm)</td>
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<td>Area (µm²)</td>
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<td>3990 (B)</td>
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<tr>
<td>Vessel-element length (µm)</td>
<td>431.3</td>
<td>424.0</td>
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<td>% Solitary vessels</td>
<td>43.2 (A)</td>
<td>44.5 (A)</td>
</tr>
<tr>
<td>% Vessels grouped in pairs</td>
<td>30.4 (A)</td>
<td>26.9 (A)</td>
</tr>
<tr>
<td>% Vessels grouped in trios</td>
<td>16.2 (A)</td>
<td>22.1 (A)</td>
</tr>
<tr>
<td>% Vessels grouped in 4</td>
<td>10.1 (A)</td>
<td>6.5 (A)</td>
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<td><strong>Functional vessels</strong></td>
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<td>19.9 (B)</td>
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<tr>
<td>Diameter (µm)</td>
<td>78.2 (A)</td>
<td>74.3 (B)</td>
</tr>
<tr>
<td>Area (µm²)</td>
<td>4014 (A)</td>
<td>4009 (A)</td>
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<td><strong>Embolized vessels</strong></td>
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<td>12.3 (C)</td>
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<td>Diameter (µm)</td>
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<td>3767 (B,C)</td>
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<td>Lumen diameter (µm)</td>
<td>10.05 (A)</td>
<td>9.71 (A)</td>
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<tr>
<td>Cell-wall thickness (µm)</td>
<td>1.68 (B)</td>
<td>1.91 (A)</td>
</tr>
<tr>
<td>Length (µm)</td>
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<td><strong>Rays</strong></td>
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<td>Width (µm)</td>
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</tr>
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<td>Height (µm)</td>
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<td>Radial resin canals/mm²</td>
<td>0.49</td>
<td>0.37</td>
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obtained for each group within its respective condition of parasitism and the results of the statistical tests.

The analysis of vessels showed that all branches tend to have a higher vessel density combined with a smaller vessel lumen diameter at the downstream sections. Both upstream and downstream sections of parasitized branches had about 25% more vessels per mm² than the sections of non-parasitized branches. These vessels were also narrower (c. 14%) in both parts of the parasitized branches.

Although vessel lumen diameter decreased from upstream to downstream sections of both infested and non-infested branches, the analysis of mean vessel area showed different results. No statistical difference was detected between the sections of non-infested branches regarding mean vessel area. However, infested branches had a reduction in
Figure 11–18. *Tapirira guianensis*; macroscopic cross sections after safranin infiltration experiments (left column) and stained microscopic cross sections (right column). – 11 & 12: Non-infested branch, upstream portion. – 13 & 14: Non-infested branch, downstream portion. – 15 & 16: Infested branch, upstream portion. – 17 & 18: Infested branch, downstream portion. – Scale bars = 1 mm (macrographs) and 200 µm (micrographs). – Infrared filter used to highlight safranin-stained vessels in the macrographs.
mean vessel area, with narrower vessels in the downstream section (Fig. 15–18). The percentage of grouped vessels and the vessel-element length did not differ statistically among the four groups.

The anatomical changes regarding vessel features were much more pronounced in the host-parasite interface. Firstly, a lower density of functional vessels was observed at the host-parasite interface, with most of these vessels located at the opposite side of the parasite’s attachment (Fig. 19). The expected flow of safranin from host to parasite was also observed (Fig. 19). Regions of host xylem located closer to the parasite’s tissues showed a high density of vessels, which were narrow and grouped both in clusters and radial multiples of 7 or more vessels (Fig. 20).

Indeed, when analyzing the vessels, non-parasitized individuals showed a reduction of 15% in the mean density of functional vessels from upstream to downstream sections (Fig. 11–14), while infested branches showed a larger reduction of 26% (Fig. 15–18). Although non-infested branches had statistically the same density of embolized vessels in upstream and downstream sections, an increase of 20% in the mean density of embolized vessels was also observed in infested branches (Fig. 15–18).

Once again, the proportions – lumen diameter and area – of both functional and embolized vessels showed contrasting results. In non-parasitized individuals the reduction of mean vessel lumen diameter was not followed by a reduction on mean vessel...
area. In parasitized branches, the downstream portion had vessels with smaller mean lumen diameter and also with narrower mean vessel areas.

Considering the wood fibers, in parasitized branches they had a narrower mean lumen diameter and thinner cell walls. A reduction of the mean values of both features was also found when moving from upstream to downstream sections of those branches. In non-parasitized branches, a statistically significant difference between the sections occurred only for mean cell-wall thickness. The fiber length was not significantly different among any of the groups.

Finally, when analyzing the rays, the statistically significant differences were only detected among the infested/non-infested conditions, but not among the upstream and downstream portions. Although the parasitized branches had a smaller average ray density, their rays were about 18% wider and 8% higher on average when compared to both portions of non-parasitized branches. The density of resin canals did not differ among the analyzed groups.

**DISCUSSION**

In order to understand the alterations found in the host wood due to the presence of the parasitic plant, the present study reports a wood anatomy description of the studied host species. Previous studies, such as Record & Hess (1943), Terrazas & Wendt (1995) and Sonsin et al. (2012), have also described the wood of the host species *Tapirira guianensis*. No major differences were found in the qualitative features described here, regardless of the infestation status of the wood. However, considering the quantitative xylem features, the present study shows that changes in the host wood of parasitized branches were evident, especially in their downstream portions.

The first difference analyzed here regards the increased density of vessels and their associated smaller lumen diameter – features also found when comparing upstream vs. downstream portions of the same branches. These are expected results, considering the conduit tapering usually observed in branches of angiosperm trees (West et al. 1999; Anfodillo et al. 2006, 2013). Nevertheless, our work shows that infested branches, particularly at their downstream portions, show even more extreme alterations, which indicates an effect of the parasitic plant on the host wood rather than changes resulting from the conduit tapering effect.

Reduced vessel lumen diameter and increased vessel density have frequently been observed in wood after mechanical wounding (Bauch et al. 1980; Aloni & Zimmermann 1984; Lev-Yadun & Aloni 1993; Lev-Yadun 1994; Arbellay et al. 2010). In such cases the wood-anatomical alterations were proposed to be the outcome of an interruption of auxin flow, which would elevate the local concentrations of auxin, leading to rapid vessel differentiation (Aloni & Zimmermann 1983). Ethylene levels resulting from the wound effects of parasite penetration on the host wood could disturb the polar auxin transport, resulting in the differentiation of vessels with small lumen diameter (Lev-Yadun 1994, 2000, 2002).

Indeed, considering the parasitism relationship established between these plant species, the infestation of host wood by mistletoes could be interpreted as a mechanical wound caused by haustorium penetration and development through host tissues.
In the particular case of *Phoradendron crassifolium*, additional disruptions of the host phloem are observed due to the formation of parasite’s endophytic tissue within the host bark. Such disruptions could lead to “wound ethylene” formation which could provoke an increase in local auxin concentration by blocking its axial polar transport and therefore induce rapid differentiation of narrow vessels.

Mechanical injury has also been associated with an increase in ray dimensions and density (Lev-Yadun & Aloni 1992, 1993, 1995). Moreover, Arbellay *et al.* (2012) stated that broad-leaved trees benefit from ray number and size increase to adjust to mechanical wounding. However, Lev-Yadun (1994) found an increase in ray size in only some of the partially girdled *Ficus sycomorus* trunks. These contrasting observations may indicate the need of more research in this field in order to better understand the differentiation of cambial derivative cells. Further analysis of the infestation by *Phoradendron crassifolium*, as well as other *Phoradendron* species, in different host species should help understanding this matter.

Though less studied, the effect of wounding on xylem fibers seems to be related to an absence or at least a significant reduction of fiber formation (Lev-Yadun & Aloni 1993; Lev-Yadun 1994). In the present study, the density of fibers was not evaluated, but the observed reduction of their lumen diameter and their cell-wall thickness could be related to a hormonal imbalance caused by the parasite penetration of the host tissues.

Do Amaral and Ceccantini (2011) showed similar results regarding vessel density and vessel lumen diameter when analyzing the effects of the endoparasite *Pilostyles ulei* on the host wood of three *Mimosa* species. The authors suggested that such alterations of host wood anatomy could be related to the water deficit commonly caused by a parasitic plant infestation, and also by a local reduction of sugar allocation. As stated by Calvin (1997), parasitic plants may accelerate the process of embolism formation in the host’s wood due to their high transpiration rates and very low water potentials (Press & Graves 1995; Ackroyd & Graves 1997). Therefore, a secondary local water deficit in the host branch could emerge as a result of embolisms.

As for the local sugar deficit, even though mistletoes are capable of photosynthesis and are only observed to tap the xylem of their hosts, Marshall and Ehleringer (1990) have suggested that mistletoes can absorb sugars dissolved in the host’s xylem sap. The authors have estimated *Phoradendron juniperum* to fulfill its carbon requirements using up to 62% of host-derived carbon. This could partly explain the thinner walled fibers observed in infested branches, especially in their downstream portions.

Indeed, the most striking alteration of the host’s secondary xylem observed in the present work was the high degree of embolized vessels present in the wood of parasitized branches, especially at their downstream portions. Although it is usual for angiosperm species to have a loss of hydraulic conductivity due to embolism formation, especially at the downstream portion of branches (Zimmermann 1983; Tyree & Ewers 1991), our results show that more than 50% of the vessels were embolized in parasitized branches. Barão *et al.* (in prep.) also found a high density of embolized vessels when analyzing the host tree *Tipuana tipu* (Fabaceae) infested by *Struthanthus vulgaris* (Loranthaceae), also a large-size leafy mistletoe species.
Actually, most of the features discussed above, which are conspicuously present in the wood of infested branches, are usually related to cavitation resistance. High vessel density, narrower vessels and a high degree of vessel grouping are usually associated with cavitation resistance (Hacke et al. 2006; Sperry et al. 2008; McCulloh et al. 2010; Lens et al. 2011). The increase of ray proportions could be related among other things to the important role of parenchyma cells in the refilling process of embolized vessels (Holbrook et al. 2001; Salleo et al. 2004; Zwieniecki & Holbrook 2009; Nardini et al. 2011).

Therefore, a combination of water stress effects and a local hormone imbalance could, together, explain the results reported in the present work. The wound caused on the host branches could trigger a local accumulation of auxin and wound ethylene which could lead to the production of wood presenting features that provide cavitation-resistance. As recently proposed by Aloni (2014), a local increase in auxin concentrations in the host-parasite interface could also be due to the flow of auxin from the parasite to the host. According to the author, this auxin flow allows the parasite to control the vessel differentiation in the host wood. Further experiments regarding hormone concentrations, especially auxin and ethylene, in different parts of the host branches are fundamental in order to test the hypothesis proposed here. Likewise, measurements of hydraulic conductivity are necessary to test whether the presence of such features could actually enhance the host’s cavitation resistance.

CONCLUSIONS

We have shown that the parasitic plant *Phoradendron crassifolium* causes functional and anatomical changes to the wood of the host tree *Tapirira guianensis*. Alterations include an increase in embolism, reduced vessel lumen diameter, increased vessel density, taller and wider rays, and thinner-walled fibers. All these responses are most evident at the downstream section of the parasitized branches. The functionality loss could be related to the high transpiration rates and the low water potential of parasitic plants which may induce embolism in the host wood. On the other hand, wood-anatomical alterations could be induced by local sugar deficits due to an uptake of host-derived carbon by the parasite, and high auxin and ethylene concentrations due to wound effects and to phloem disruptions caused by the parasite penetration and endophytic spread. Further analysis of hydraulic conductivity and hormone concentration in host branches are necessary to confirm the hypothesis presented here.

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