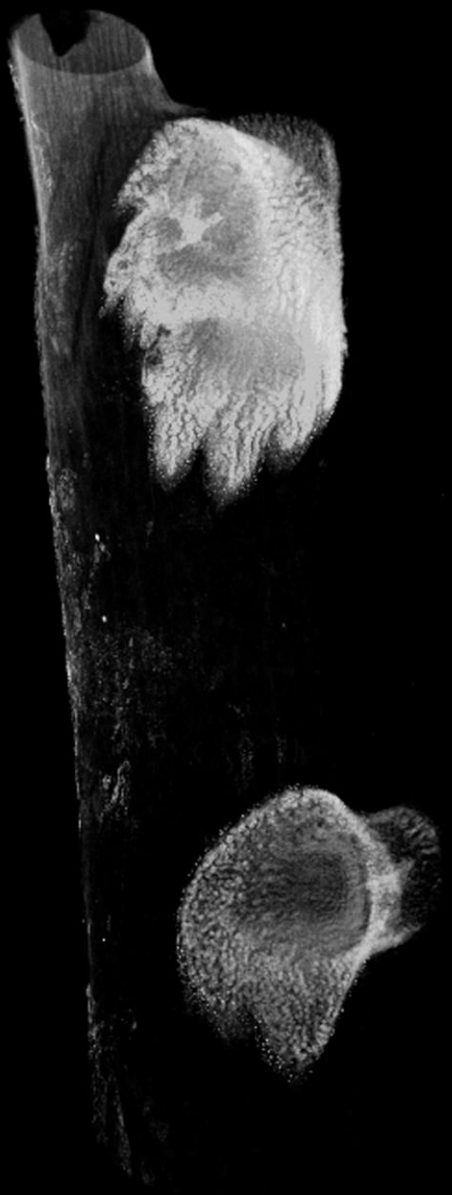

# Aspectos anatômicos e funcionais da interação entre duas espécies do gênero *Phoradendron* (Santalaceae) e suas hospedeiras

# Anatomical and functional aspects of the interaction between two *Phoradendron* (Santalaceae) species and their host trees

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Orientador: Prof. Dr. Gregório Ceccantini

Universidade de São Paulo
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Dissertação apresentada ao Instituto de Biociências da Universidade de São Paulo, para a obtenção de Título de Mestre em Ciências, na Área de Botânica.

Orientador:
Prof. Dr. Gregório Ceccantini

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# Comissão Julgadora:

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Prof(a). Dr(a). Prof(a). Dr(a).

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Prof. Dr. Gregório Cardoso Tápias Ceccantini

Orientador

Às minhas irmãs, Fernanda e Renata,

e ao meu irmão, José Teotônio.

*"Não se aprende Botânica no gabinete;*
*É preciso passear nos jardins ou nos campos*
*e familiarizar-se ali com a própria Natureza,*
*com aquela beleza, regularidade, ordem*
*e inexorável variedade que se encontra*
*na estrutura dos vegetais"*

José Mariano da Conceição Veloso
(Frei Veloso)

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# Índice

# Apresentação

A presente dissertação está organizada na forma de dois capítulos, escritos em língua inglesa, que correspondem aos dois artigos científicos, sendo um já aceito para publicação e o outro em preparação. Os capítulos são precedidos por uma breve introdução geral e sucedidos por considerações gerais, que dizem respeito aos dois capítulos, e, finalmente, por conclusões.

O primeiro capítulo, intitulado "Similar morphology, diverse anatomy: the different infestation patterns presented by two parasitic plants of the genus *Phoradendron* (Santalaceae)" será submetido à revista ***Annals of Botany***.

O segundo capítulo, intitulado "Embolism increase and anatomical modification caused by plant parasitism: *Phoradendron crassifolium* on *Tapirira guianensis*", aceito pela revista ***IAWA Journal*** em 07 de Novembro de 2014, deverá ser publicado no ***IAWA Journal*** em 2015, volume 26, fascículo 1 ou 2.

As considerações gerais retomam os principais resultados e os pontos centrais da discussão expostos nos dois capítulos supracitados, relacionando-os. As conclusões, expostas na forma de tópicos, encerram esta dissertação.

# Introdução Geral

O gênero *Phoradendron*, cujas espécies foram inicialmente descritas como parte de *Viscum*, foi originalmente definido por Nuttall em 1848, que já observara, primeiramente, haver dois grupos de espécies. Atualmente, *Phoradendron* agrupa cerca de 230 espécies, sendo um dos mais numerosos gêneros de plantas parasitas (Kuijt 2003, Heide-Jørgensen 2008). Este número de espécies encontra-se disperso por todo o continente americano, sendo a região da América do Sul o principal centro de diversificação do grupo (Kuijt 2003). No Brasil, suas espécies distribuem-se por todos os biomas do país (Arruda *et al.* 2013).

O posicionamento filogenético, quanto à família a qual o gênero pertence, apresenta certa divergência entre autores. De acordo com a classificação adotada no presente trabalho (APG III 2009 e suas subsequentes atualizações), o gênero *Phoradendron* pertence à família Santalaceae, sendo incluído no clado denominado Viscaceae (Stevens 2014). Por outro lado, de acordo com Nickrent (2011), Viscaceae constitui uma família separada, na qual *Phoradendron* estaria inserido. Os diversos sistemas de classificação historicamente importantes, como os de Hutchinson (1926, 1934), Takhtajan (1969, 1997) e Cronquist (1968, 1988), exibiram também essas visões conflitantes quanto ao posicionamento e circunscrição de Viscaceae.

Em relação às classificações usualmente empregadas para descrever a nutrição de plantas parasitas, as espécies de *Phoradendron* são consideradas parasitas obrigatórias, pois necessitam de uma hospedeira para completarem seu ciclo de vida (Westwood *et al.* 2010), e hemiparasitas de caule, pois se conectam apenas ao tecido

xilemático dos ramos suas hospedeiras, de onde podem obter água, nutrientes e açúcares (Marshall & Ehleringer 1990; Panvini & Eickmeier 1993).

Devido a tais características, diversos estudos abordam os efeitos, frequentemente deletérios à fisiologia de suas hospedeiras, causados por espécies de *Phoradendron* , (Hull & Leonard 1964a, b). Entretanto, pode ser observada uma relação mutualística entre certas espécies parasitas e seus dispersores da avifauna (Aukema 2003), rendendo às espécies de *Phoradendron*, Adicionalmente, o trabalho de van Ommeren & Whitham (2002) aponta a que esta relação mutualística também pode ser observada entre *Phoradendron juniperinum* e sua hospedeira *Juniperus monosperma*, sendo mediada pela avifauna dispersora. Nesse caso, os dispersores tanto da parasita como da hospedeira seriam os mesmos, de forma que a presença da parasita também potencializa a atração de dispersores para hospedeira, incrementando seu *fitness* reprodutivo.

Embora existam casos de relações espécie-específicas, a maioria das espécies do gênero *Phoradendron* pode parasitar uma ampla gama de hospedeiras (Heide-Jørgensen 2008). As hospedeiras mais comuns são angiospermas de porte arbóreo, porém espécies de coníferas também podem ser parasitadas (Ashworth 2000). Até mesmo casos de epiparasitismo, nos quais uma espécie de parasita serve como hospedeira para outra, já foram relatados para o gênero (Calvin & Wilson 2009, Ceccantini com pess.). Em nossas observações no herbário SPF, *Phoradendron crassifolium* ocorria em hospedeiras, de 13 diferentes famílias.

Assim, tendo em vista a diversidade de espécies hospedeiras, bem como a diversidade dentro do próprio gênero *Phoradendron*, as relações estabelecidas entre parasitas e suas hospedeiras também podem variar amplamente. Thoday (1957)

explorou comparativamente a diversidade de modos de infestação de espécies de *Phoradendron*. Entretanto, o trabalho de Thoday (1957) conta apenas com desenhos esquemáticos dos modos de infestação, não permitindo uma comparação detalhada entre as espécies.

Considerando o exposto acima quanto à diversidade de espécies de *Phoradendron* e à variedade de interações entre essas espécies e suas hospedeiras, é possível afirmar que apenas uma pequena parte desta diversidade está contemplada na literatura. Como usualmente ocorre em outros campos da Botânica, as espécies neotropicais são as menos estudadas quanto às relações que estabelecem com suas hospedeiras (Arruda *et al.* 2013) e poucos trabalhos, como o de Thoday (1957) e Ashworth & dos Santos (1997), apresentam uma abordagem comparativa.

Neste sentido, a presente dissertação de mestrado aborda a relação parasita-hospedeira estabelecida entre as espécies *Phoradendron crassifolium* e *Phoradendron* sp[1] e suas respectivas hospedeiras, *Tapirira guianensis* (Anacardiaceae) e *Cedrela fissilis* (Meliaceae). Primeiramente, o Capítulo I traz a comparação ente os sistemas endofíticos formados por estas parasitas, partindo da hipótese inicial de que cada espécie de parasita apresentaria um padrão de infestação particular. A seguir, o Capítulo II aborda efeitos causados por *P. crassifolium* à morfologia e funcionalidade do xilema de *T. guianensis*, considerando a hipótese de que a parasita reduziria a funcionalidade da madeira de sua hospedeira

[1] *Phoradendron* sp parasitando *Cedrela fissilis* não pode ser identificado com segurança, de forma que optou-se, por hora, manter a identificação ao nível de gênero e aguardar a decisão dos especialistas.

Capítulo I

# Similar morphology, diverse anatomy: the different infestation patterns of two parasitic plants of the genus *Phoradendron* (Santalaceae)

***Abstract***

The complex endophytic structure formed by parasitic plant species often represents a difficulty in the study of the host-parasite interface. Even with the large amounts of anatomical slides, the here-dimensional comprehension of the structure may still be difficult to obtain. In the present study we aimed on comparing the patterns of infestation and the morphological/anatomical effects caused by two species of the genus *Phoradendron* on the wood anatomy of their hosts. We applied the new technique of microtomography (MCT) aligned with traditional plant anatomy procedure. Results showed that *Phoradendron crassifolium* forms a small swelling with a concise endophytic system on branches of *Tapirira guianensis*, while *Phoradendron* sp. forms a spread-out endophytic system within the large swelling observed on the branches of *Cedrela fissilis*. The use of MCT analysis proved to be useful for a better understanding of these patterns, given the size of the structures formed at the host-parasite interface. These different infestation patters were associated to the different effects these parasitic plants caused on the wood of their hosts, such as increases in vessel density and grouping index, disruption of the growth-ring pattern and bark tissues, decreases in fibre cell-wall thickness, etc. Altogether, the results indicate that the parasite is not entirely responsible for the morphology/anatomy observed at the interface area. Due to the uniqueness of each pattern and the distinct effects observed, we suggest that the host characteristic are also accountable on the phenotype observed at the host-parasite interface area. Based on this suggestion, we compared the interaction stablished between host and parasitic plants to the interactions between plants and microorganism/insect gall inducers, reinforcing the use of the term "gall". Future studies on the morphological and anatomical aspects of other host-parasite interactions could help confirming the hypothesis regarding the uniqueness of the host-parasite interaction.

## *Introduction*

The complex endophytic structure formed by parasitic plant species renders a rather difficult three dimensional comprehension. Traditional techniques in plant anatomy have been employed to describe the interface formed between parasites and their hosts, usually requiring a long series of sequential anatomical slides. In this context, the use of microtomography (MCT) arises as a new technique that allows three-dimensional analysis of these complex structures, pushing forward the traditional field of plant anatomy.

Still, regarding the studies that employ traditional plant anatomy procedures, the literature is scarce for some of the clades of parasitic plants, given their great diversity and number, which represents around 1% of all extant Angiosperms (Westwood *et al.* 2010). Considering clades of the neotropical region, the lack of studies is even greater (Arruda *et al.* 2013).

This is the case of the genus *Phoradendron*, which comprises ca. 230 species with a great variety of infestation modes, diverse morphology and variable size (Kuijt 2003). Despite this great variety, most of the works dealing with the anatomy of the host-parasite interface have analysed a small number of North American and few Mexican species.

A comparative perspective is even scarcer in the literature, an exception being the paper by Thoday (1957), in which he compared the general features of the haustorial system of seven *Phoradendron* species. However, few details are given about the anatomy of the host-parasite interface and only schematic drawings are presented.

Based on the available studies, the haustorial system formed by different species of *Phoradendron* is observed to share some general characteristics. Following germination, an initial connection with the host is formed at a single attachment site, usually referred to as "primary haustorium", and a local swelling of the host branch is usually formed at the attachment region (Kuijt 1969).

From the initial connection to the host, a system of cortical strands (or cortical haustoria), spreads acropetally and basipetally, running longitudinally within the host tissues (Calvin *et al.* 1991; Kuijt 2003). At certain points of its development, the cortical strands give rise to structures called secondary haustoria or sinkers (Calvin 1967b), which penetrate the host xylem.

The solute transfer from one plant to the other is only partially performed by direct vessel connection between parasite and host (Calvin 1967b; Thoday 1957). The abundant parenchymatic cells play an important role in the solute flux process (Calvin 1967b; Calvin *et al*. 1991; Calvin & Wilson 1995; Kuijt 1964; Schmid *et al.* 2011 and others). Additionally, transfer cells and flange cells were also described for *Phoradendron macrophyllum* (Fineran & Calvin 2000).

Despite this general understanding of the endophytic system of *Phoradendron* species, the diversity center of this clade, the South American region, are still deficient in detailed studies of its species. Considering these matters, we have analyzed the infestation modes and the structure of the endophytic systems of two *Phoradendron* species infesting two different host trees. Traditional plant anatomy techniques were applied together with computer microtomography analysis in order to characterize and compare the infestation patterns, as well as to provide a fully three-dimensional understanding of the studied structures.

## *Material and Methods*

### *Host and parasitic plant material*

The parasitic species *Phoradendron crassifolium* (Santalaceae) was analysed while infesting the host tree *Tapirira guianenis* (Anacardiaceae) (Fig. 1A). It attached to the host branches developing into huge shrubs (up to 1.5 m long) bearing green to yellowish leaves (Fig. 1B). The parasite *Phoradendron* sp.[2] (Santalaceae) infested the host tree *Cedrela fissilis* (Meliaceae) (Fig. 1C) also forming huge shrubs with yellowish leaves (Fig. 1D). The two parasitic plant species show great morphological similarity (Fig. 1C, D) and are both capable of colonizing the host trees, forming moderate to heavy infestations (Fig, 1A, B).

In order to compare these species, parasitized branches of the host tree *T. guianensis* were collected in a riparian forest in Minas Gerais state, Brazil. Parasitized branches of the host tree, *Cedrela fissilis,* were collected in a wooded area inside the main campus of the University of Sao Paulo, Sao Paulo state, Brazil. A total amount of 90 infested branches of *T. guianensis* and 30 infested branches of *C. fissilis* were collected.

Each infested branch was divided in three sections: host branch clear of parasitic endophyte; swollen region corresponding to host-parasite interface (gall); and branches from parasitic plant itself. All material was identified and photographed. Subsequent measuring and analysis were carried out as explained bellow. Several intact galls formed between the aforementioned host-parasite pairs were preserved in

[2] The identification of this species of *Phoradendron* still is doubtful. The authors are waiting on the family experts' decesion.

the Xylarium Nanuza Luíza de Menezes, at Department of Botany of University of São Paulo (SPFw).

***Morphological and anatomical analysis***

Ten samples host branches, parasitic branches and galls of each host-parasite interaction were used for macroscopical analysis. They were initially air-dried, cut in transversal and longitudinal sections, and sanded using sand papers of ascending granulation until a smooth surface was obtained. The material was studied and photographed using a stereo-photomicroscope (Leica DML and camera DFC 310FX).

Regarding wood anatomical analysis, other ten samples of galls, host branches and parasitic branches of each host-parasite interaction were initially fixed in a solution of formaldehyde-ethanol-acetic acid (FAA 50%) and imbedded in polyethylineglicol (PEG). Samples were sectioned in a sliding microtome (Leica SM 2000R) to produce transverse, radial longitudinal and tangential longitudinal sections, which were stained with safranin/astra-blue (Johansen 1940; Bukatsch 1972 adapted by Kraus & Arduin 1997). Non-imbedded samples of each species and samples from different sections of each host-parasite interface were also macerated following Franklin (1945).

***Measurements of morphological and anatomical features***

In order to assess the potential effects caused by the two *Phoradendron* species on the wood of their host trees, morphological and anatomical measurements were taken. The approximated volume of the analyzed branches and swollen regions of the host-parasite interface were calculated by geometrical formula. Branches were considered as regular cylinders and interface regions were considered as ellipsoids.

This approach was chosen due to the deterioration of the host-parasite interface material, which was eventually observed to have hollow areas within the interface region.

Figure 2 illustrates the measurements taken for the material of *T. guianensis* infested by *P. crassifolium* (Fig. 2A, 2B) and *C. fissilis* infested by *Phoradendron* sp. (Fig. 2C, 2D). Using these measurements we calculated the approximated volume of each material following the formulas:

$Vc = \pi . r^2 . h$ $Ve = 4/3 . \pi . a . b . c$ (Wolfram Alpha LLC. 2014).

Where Vc: approximated volume of the cylinder; r: radius of the branch; h: length of the branch; Ve: approximated volume of the ellipsoid; a: smaller diameter of the host-parasite interface region; b: greater diameter of the host-parasite interface region; c: length of the host-parasite interface region.

It is noteworthy that the actual measurement of host and parasitic branches would be unpractical for the intended analysis. Therefore, we only measured the length of the host-parasite interface regions and used this value to calculate the approximated volume of branches as well. The use this approach allowed us to compare the approximated volume of each material regardless of its length.

Anatomical features were measured in order to characterize each species and also to draw comparisons among them. The material was photographed using a photomicroscope (Leica DML and camera DFC 310FX) and measured using the free software Image J (Rasband 1997-2014). Measurements were taken at branches of each analyzed species and also at the gall of both studied host-parasite interactions. Anatomical features measured were: vessel and fibre lumen diameter (µm), vessel

density (vessels/mm²), vessel-element and fibre length (µm) and fibre cell-wall thickness (µm).

Anatomical and morphological features were compared among the host branches, galls and parasitic branches of both host-parasite pairs using a nested analysis of variance (nested ANOVA). The measurements were nested within the correspondent morphological/anatomical features, which were, in turn, nested within their correspondent anatomical position (host branch/gall/parasitic branch); at last, the anatomical positions were nested within the appropriate host-parasite interaction (*P. crassifolium* and *T. guianensis*/*Phoradendron* sp. and *C. fissilis*). Pearson's correlation analysis was also performed among all measured features. The statistical analysis was performed using the software JMP SAS Institute Inc (SAS Institute Inc, 1998-2014).

***Microtomography (MCT) analysis***

Three galls of each host-parasite pair previously fixed in FAA were used for the analysis of the host-parasite interface region employing MCT techniques. In previous trials carried out before the analysis, we compared galls preserved in three different conditions: air-dried, embedded in polyethileneglycol and fixed (wet) ones. As a result, the fixed (wet) material was observed to provide better results and was therefore used in the present work. Also during these trials, parameters were tested and during the analysis we used 62 kv of voltage, 500 µm of wavelength and an aluminum 0.2 mm filter for the scanning of samples.

Data acquisition was carried out using a high performance *in vivo* microtomography scanner (Skyscan 1176). After three-dimensional reconstruction,

images were captured using the free software Dataviewer and CTVox. In order to confirm the observations made with MCT, we compared the images obtained through this technique with morphological and anatomical sections.

## *Results*

### *Wood anatomy of the studied species*

As a means to better understand the morphology and anatomy of the interface between the host trees and the parasitic plant species analyzed here, we begun by studying the wood anatomy of the four species sampled.

Both figures (Fig. 3 and Fig. 4) used for illustrating the wood anatomical features begun with the description of the parasitic plant wood, showing images of the transversal, longitudinal tangential and longitudinal radial sections. Next, sections of the host-parasite interface were described. We chose to present general images in order to provide an initial comparison between the anatomy of interface itself and the wood anatomy of each plant. At the bottom of the figure, sections of the host wood free from parasitic tissues were shown. An image of an infested branch is shown in each figure as a guide to better understand the position from which the sections were taken.

### *Phoradendron crassifolium* **on** *Tapirira guianensis*

The parasite *Phoradendron crassifolium* on branches of the host *Tapirira guianensis* (Fig. 3A) showed a high density of vessels (Fig. 3B), which were mostly grouped in clusters (Fig. 3B) and composed of narrow and short vessel elements (Fig. 3C, 3D). Wood rays were also short and mostly uniseriate (Fig. 3C and 3D). Fibres

were observed to have very thick cell-walls and narrow lumen diameter (Fig. 3B). Rays were composed of upright and square cells (Fig. 3D).

In the cross-section of the host-parasite interface, the endophyte of the parasite *P. crassifolium* was seen in longitudinal view (Fig. 3E). Likewise the vessels in the wood of the parasite, the vessels observed in the endophyte were also grouped and composed of short vessel elements (Fig. 3E). Parenchyma cells and fibres were also present in this region of the endophytic tissue (Fig. 3E). Reduced vessel element size and vessel grouping were observed in the host xylem of *T. guianensis* closer to the endophyte (Fig. 3E).

In longitudinal sections of the interface, the endophyte showed three anatomically distinct regions. The outermost region is composed of parasitic wood, seen in longitudinal view (Fig. 3F region *a*, 3G). Inwards, a parenchyma tissue was observed, eventually containing a group of thick walled fibres (Fig. 3F region *b*, 3G). The innermost region of the endophyte, which was in direct contact with the host xylem, was composed of parenchyma cells, groups of thick walled fibres and short vessel elements that made contact with host vessels (Fig. 3F region *c*, 3G). Analysis of maceration preparations of each of these regions confirmed this cellular composition.

The wood of the host *T. guianensis* showed axial parenchyma scanty paratracheal (Fig. 3H). Rays were uniseriate (Fig. 3I), locally bisseriate, and often containing resin canals. Other features include septate fibres (Fig. 3I), radial resin canals (Fig. 3I) and rays composed by procumbent cells (Fig. 3J).

***Phoradendron* sp. on *Cedrela fissilis***

The parasite *Phoradendron* sp. on branches of *Cedrela fissilis* (Fig. 4A) had very narrow vessels, observed in high density and composed of short vessel elements (Fig. 4B, 4C, 4D). Vessel grouping was also frequent, with the vessels grouped in radial multiples of 4 or more (Fig. 4B). Fibres were very thick-walled (Fig. 4B).

At the host-parasite interface, *C. fissilis* also showed alterations due to the presence of the parasite *Phoradendron* sp. The pattern of annual semi-growth-rings observed at the center-most region of the host wood was interrupted in areas closer to the parasitic endophyte (Fig. 4E). In these areas, vessels were observed to be narrow and eventually grouped in clusters or radial multiples (Fig. 4E). Still, the vessel elements of *C. fissilis* were also shorter in areas closer to the endophyte (Fig. 4F, 4G).

The infested wood of *C. fissilis* clear of parasitic tissues showed a semi-ring-porous pattern (Fig. 4H), marginal parenchyma bands (Fig. 4H), axial parenchyma vasicentric (Fig. 4H), non-septate fibres (Fig. 4I) and heterocelular rays (Fig. 4J).

***Effects of* Phoradendron *species on the host wood***

The analysis of morphological features of branches and parasite galls (Table 1) revealed that branches of the host *C. fissilis* were, on average, 20% larger than those of *T. guianensis*. The galls formed between *Phoradendron* sp. and *C. fissilis* was also larger, showing an approximated mean volume up to 90% bigger than that of structure formed between *P. crassifolium* and *T. guianensis*. The analysis of correlation showed that for both host-parasite interactions the approximated volume of the gall was strongly correlated to the host branch diameter (*P. crassifolium* and *T.*

*guianensis*: p-value < 0.0001, CI = 0.96; *Phoradendron* sp. and *C. fissilis*: p-value < 0.0001, CI = 0.79).

Regarding the anatomical features, branches of *T. guianensis parasitezed* by *P. crassifolium* showed an increased frequency of vessels when comparing the host branch free from parasitic tissues to the interface regions. However, *C. fissilis* parasitized by *Phoradendron* sp. had fewer vessels at the interface region, which were also smaller compared to the non-infested region of the same branch. For *T. guianensis* infested by *P. crassifolium* the difference in vessel lumen diameter was not statistically significant. Results of correlation analysis for both host-parasite pairs showed that vessel density in interface regions was inversely correlated to the volume of the gall (*P. crassifolium* and *T. guianensis*: p-value < 0.0001, CI = -0.44; *Phoradendron* sp. and *C. fissilis*: p-value < 0.0001, CI = -0.84).

In *T. guianensis* the fibres were wider in the branches than in the host-parasite interface region, but the thickness of fibre cell-wall was not statistically different. In *C. fissilis* the fibres were slightly wider and thick-walled in the host-parasite interface region.

The branches of *Phoradendron* sp. were larger than those of *Phoradendron crassifolium*. However, *P. crassifolium* had a greater density of vessels, which were also wider than those of the other parasitic species.

***Anatomy of the host-parasite interface***

Despite their morphological and even anatomical similarities, the two parasitic species showed different infestation patterns (Fig. 5). As seen in transversal section of the infested branch, the primary haustorium of the parasitic species *P. crassifolium* is readily distinguishable from the tissues of the host *T. guianensis*, forming a

continuous interface between them (Fig. 5A). Cortical strands were observed to give rise to short wedge-shaped sinkers that penetrate into the host xylem (Fig. 5B, 5C).

In the case of *C. fissilis* infested by *Phoradendron* sp., the analysis of transverse sections showed a fragmented interface with a diffuse organization of the endophytic system (Fig. 5D). The host-parasite contact is mediated by the multiple cortical strands that spread out through the thick host bark of *C. fissilis* (Fig. 5E) and give rise to a large number of needle-shaped sinkers (Fig. 5F).

***Phoradendron crassifolium* on *Tapirira guianensis***

Although the cortical strands of *P. crassifolium* on *T. guianensis* grew mostly within the host bark, they also caused morphological alterations to surface of host xylem (Fig. 6A). Anatomically, at the beginning of their penetration into the host tissues, the cortical strands were formed by abundant parenchyma cells and few vessel elements (Fig. 6B). Further in their development they were mostly composed of parenchyma, tick-walled fibres and vessels that connected the strand to the sinker (Fig. 6C, 6D).

The sinker was composed of lignified vascular parenchyma cells and vessel elements (Fig. 6D). Only few cortical strands were observed to have secondary growth, which resembled the anatomy of the very wood of *P. crassifolium* (Fig. 6E). Despite the predominance of parenchyma cells at the host-parasite interface, the parasite also made direct vessel connections with the host (Fig. 6F).

***Phoradendron* sp. on *Cedrela fissilis***

In the case of *Phoradendron* sp. in *C. fissilis*, the marks of cortical strands were also seen on the host xylem (Fig. 7A). However, unlike the strands formed by

*P.crassifolium*, the ones formed by *Phoradendron* sp. covered most of the infested branch, forming a sort of chimeric tissue (Fig. 7A). The analysis of infested host barks allowed the observation of cortical strands in different growth stages (Fig. 7B), many of them already showing a secondary structure, which also resembled the wood anatomy of *Phoradendron* sp. branches (Fig. 7C).

The needle-shaped sinkers were composed of lignified vessel parenchyma which encircled the short vessel elements that formed the vessels (Fig. 7D). Few lignified vascular parenchyma cells were also present. Direct vessel contact between host and parasite were observed in this case too (Fig. 7E). In some areas of longitudinal sections of the host-parasite interface, cross-grained wood was observed in the host xylem close to the endophyte (Fig. 7F).

***Microtomography analysis***

The use of the microtomography (MCT) technic was chosen in order to perform a three-dimensional analysis of the host-parasite interface, also allowing us to better observe the whole interface region at one time. In all images obtained from these analyses, the endophytic system of both parasitic plant species was seen in white, while the host wood was seen in black or shades of grey.

Two types of images were captured during the analysis. Using the software Dataviewer, we selected images that were generated during the mictotomography scan. These images were compared to macroscopical sections of the host-parasite interface, in order to better understand the MCT image. The software CTVox was used for volume rendering, creating three-dimensional models from the images captures during the scan. The images selected from the ones generated by this

software were treated in order to remove most of the host tissues, allowing a clear vision of the endophytic system.

In the case of the parasite *Phoradendron crassifolium* infesting *Tapirira guianensis* the cortical strands of the parasite were seen as white blots within the host bark (Fig. 8A). The wedge-shaped sinkers of the parasite appeared on the host wood forming a different pattern from that of the cortical strands on the host bark. With this type of image was not possible to distinguish between the different regions of the sinker, as seen in the macroscopical cross-section (Fig. 8B). On the other hand, these regions were observed in the other type of image acquired (Fig. 8C). Analyzing the image in a closer view (Fig. 8D), it was possible to compare the structures observed to an anatomical cross-section (Fig. 8E), identifying the same regions of the parasitic sinker. The endophytic system of *P. crassifolium* on the host *T. guianensis* was also observed to be concise, stretching only for a few centimeters away from the initial axis (Fig. 8C).

The multiple and wider cortical strands of *Phoradendron* sp. within the bark of *Cedrela fissilis* formed a particular pattern in the MCT images (Fig. 9A) where, for most of the strands, it was possible to distinguish a peripheral area around them (Fig. 9A). In the correspondent macroscopical longitudinal section the cortical strands appeared to be homogenous and no peripheral area was observed (Fig. 9B). The analysis of the detailed image (Fig. 9C) showed the peripheral areas to be correspondent to the phloem of the cortical strands (Fig. 9D). The endophytic tissue as a whole was observed to be more spread out, running farther through the host wood (Fig. 9E).

***Discussion***

Anatomical studies of *Phoradendron* species have focused on the haustorium due to its importance for the very life form of parasitic plants (Kuijt 1969). The wood anatomy of the parasites is seldom described and even when being so, most of the studies have dealt with young stems, which show only the primary structure of the branches (Varela *et al.* 2004, Khan *et al.* 2009, Gómez–Sánchez *et al.* 2011).

An exception is the work of Ashworth & dos Santos (1997), which described the wood anatomy of four Californian species of *Phodradendron*, reporting very short vessel-elements with thick cell-walls, fibres also with thick walled, high vessel density and multiseriate heterocelluar rays. No previous description was found in the literature for the wood anatomy of *Phoradendron crassifolium* and *Phoradendron* sp. Compared to the species described by Ashworth & dos Santos (1997), the parasitic species analyzed here show common wood anatomical features such as the high frequency of vessels, multiple vessels (cluster or radial chains) which were also short and narrow, thin rays, and the thick-walled fibres.

These common features, as well as the elevated vessel grouping seen in wood of *P. crassifolium* and *Phoradendron* sp., can be related to the high transpiration rates and low water potentials observed for parasitic plants (Press & Graves 1995; Ackroyd & Graves 1997; Hollinger 1983; Ehleringer *et al.* 1986), which may lead to cavitation and air seeding in the branches (Zimmerman 1983; Tyree 1997). Nevertheless, the set of wood anatomical features showed by these plants is usually related to cavitation resistance (Hacke *et al.* 2006; Sperry *et al.* 2008; McCulloh *et al.* 2010; Lens *et al.* 2011).

Altogether, these anatomical features endorse the parasitic plants to sustain the high transpiration rates and the low water potentials they need in order to divert the sap flux towards them without compromising their hydraulic system.

At the host-parasite interface, the endophytic tissue of *P. crassifolium* and *Phoradendron* sp. followed the general followed the general description of organization described for other species in the genus, showing n cortical strands that eventually gave rise to sinkers which penetrated the host xylem. Such general structure can be compared to that of a rhizome or rizophore, which gives rise to roots (Bell 2008, Menezes 2008) in a similar manner to that of cortical strands give rise to sinkers.

Actually, this analogy can be applied for all species presenting endophytic tissue formed by cortical strands, i.e. all species in the "Viscaceae" clade within the family Santalaces (Stevens 2014). Some species of *Viscum* have even been observed to form shoot axis from cortical strands (Heide-Jørgensen 2008). In this case the analogy between cortical strands and rhizomes would be even more complete, given the formation of both aerial and "subterranean" axis.

Following this analogy, *P. crassifolium* could be said to have a concise "rhizome" (endophytic tissue), probably homologous to a true stem, restricted to a few centimeters away from the initial infestation point. On the other hand, *Phoradendron* sp. was observed to have cortical strands in different growth stages and to spread its tissue further away from the initial infestation point, forming an actual "rhizome" system (endophytic system).

The concise infestation pattern showed by *P. crassifolium* was circumscribed in the small galls formed between this parasite and its host *T. guianensis*. As for

*Phoradendron* sp. on *C. fissilis*, the spread-out infestation pattern of the endophytic system was associated to the large size of the gall formed. The understanding of the spread of parasitic tissues within the host branches was possible due to the microtomography (MCT) analysis. The use of the MCT technique enabled us to readily analyse the infestation patterns, which otherwise would require a great amount of anatomical slides in order to be understood considering the size of the galls formed.

Once we had a clear visualization of the infestation patterns it was possible to observe they were followed by different effects caused on the host's branch wood anatomy. As the qualitative wood anatomical features of *T. guianensis* and *C. fissilis* free from parasitic tissues did not differ from the descriptions presented by other authors, such as Record & Hess (1943), Terrazas & Wendt (1995) and Sonsin *et al.* (2012), the host's wood anatomy at the host-parasite interface area did showed several alterations.

On the one hand, the wood of *T. guianensis* in direct contact with the endophyte of *P. crassifolium* showed an increase in vessel density, a decrease on vessel lumen diameter and decreases fibre lumen diameter. On the other hand, the wood of *C. fissilis* close to the endophyte of *Phoradendron* sp. had a decrease vessel frequency, a reduction of vessel lumen diameter and increases on the fibre lumen diameter and fibre cell-wall thickness. Additionally, the typical semi-ring porosity of *C. fissilis* is lost at the host-parasite interface, especially close to the sinkers of *Phoradendron* sp.

Such alterations could be related to the mechanical damage caused to the host phloem during the penetration and spread of the endophytic system of the parasites.

Disruptions of phloem could lead to a local increase in auxin concentrations and possibly ethylene liberation which could then result in an increase in vessel frequency and a reduction of vessel lumen diameter (Bauch *et al.* 1980; Aloni & Zimmermann 1983; Lev-Yadun & Aloni 1993; Lev-Yadun 1994; Arbellay *et al.* 2010).

Considering the major disruptions caused by the endophytic system of *Phoradendron* sp. on the bark of *C. fissilis*, the local auxin concentration could reach a point when vessel differentiation is rather inhibited instead of promoted (Doley & Leyton 1968). The reduced vessel frequency and eventual local absence of vessels observed in the wood of *C. fissilis* at the host-parasite interface region corroborates this hypothesis. Additionally, the larger gall formed between *Phoradendron* sp. and *C. fissilis* could be a result of the proliferation of parenchyma cells and/or fibres due to higher local concentrations of auxin (Bauch 1980; Aloni & Zimmermann 1983).

These differences regarding the patterns of infestation and the effects caused on the host wood indicate the unique structure of each gall formed between the host and parasitic species, which are likely to be related to the intrinsic characteristics of the parasites but can also be partially attributed to the characteristics of the hosts. Indeed, a strong and positive correlation was found between the approximated volume of the gall and the host branch diameter, indicating that only larger branches, such as those of *C. fissilis*, could bear the large galls formed by *Phoradendron* sp. Future studies of the relationship between *P. crassifolium* and *Phoradendron* sp. with different host species could corroborate this hypothesis.

Still, these unique interactions are similar to what is observed in plant galls formed between plant species and insect/microorganisms gall inductors. As we have showed here, the interaction between parasitic plants and their hosts can be

considered as plant tumours due to the hyperplasia, hypertrophy and generally altered formation of host tissues, which is possibly promoted by the parasitic species. According to the recent work of Aloni (2014), parasitic plants could be actively responsible for an influx of auxin to the host-parasite interface area and the consequent alterations on the host xylogenesis process.

Based on the data presented here, we reinforce the use of the term "gall" or "woody gall" for the swollen region corresponding to the host-parasite interface area, also considering this structure as a plant tumour.

***Conclusions***

We have shown that *Phoradendron crassifolium* and *Phoradendron* sp. have different patterns of infestation on the wood of their host trees *Tapirira guianensis* and *Cedrela fissilis*, respectively. Each patterns showed particular characteristics, which could be related to the unique interaction stablished by the parasitic plant and the host tree. Similarities were observed between plant galls and the host-parasite interactions analysed here, leading us to reinforce the use of the term "gall" for these interactions as well. The microtomography technique proved to be of great importance for the analysis of the infestation patterns, providing a three-dimensional view of the large structures formed between host and parasite. The different infestation patterns were associated to the different effects the parasites caused on the wood anatomy and morphology of parasitized host branches, which could be related to a local increased in the concentration of auxin within the gall. Future studies on the morphological and anatomical aspects of other host-parasite interactions, as well as studies on the hormone concentration of galls formed by parasitic plants, could help confirming the hypothesis regarding the uniqueness of the host-parasite interaction.

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Table 1: Nested ANOVA results for the morphological and anatomical features analyzed among anatomical positions of the host-parasite interactions between *P. crassifolium* and *T. guianensis* and *Phoradendron* sp. and *C. fissilis*. Letters inside the parenthesis indicate the results for Tukey test (post nested ANOVA).

| | | *Phoradendron crassifolium* on *Tapirira guianensis* | | | *Phoradendron* sp. on *Cedrela fissilis* | | |
|---|---|---|---|---|---|---|---|
| Measured features | | Host branch | Interface region (gall) | Parasite branch | Host branch | Interface region (gall) | Parasite branch |
| Morphological | Greater diameter (mm) | - | 40.3 | - | - | 59.3 | - |
| | Smaller diameter (mm) | 27.3 (B) | 34.8 (A) | 15.2 (C) | 35.3 (B) | 48.3 (A) | 19.1 (C) |
| | Length (mm) | 31.6 | 31.6 | 31.6 | 98.4 | 98.4 | 98.4 |
| | Approximated volume ($cm^3$) | 18.5 (B) | 185.6 (A) | 5.7 (C) | 96.3 (B) | 1180.5 (A) | 18.5 (C) |
| Anatomical | Vessel density (vessels/$mm^2$) | 47.9 (C) | 51.3 (B) | 109.7 (A) | 39.1 (B) | 18.6 (C) | 83.7 (A) |
| | Vessel lumen diameter (µm) | 74.5 (A) | 70.7 (B) | 41.9 (C) | 127.1 (A) | 114.1 (B) | 32.6 (C) |
| | Vessel element length (µm) | 418.1 (A) | 417.3 (A) | 105.8 (B) | 328.6 (A) | 327.5 (A) | 105.8 (B) |
| | Fibre lumen diameter (µm) | 9.4 (A) | 8.0 (B) | 7.0 (C) | 9.5 (A) | 8.1 (B) | 7.1 (C) |
| | Fibre cell-wall thickness (µm) | 1.7 (B) | 1.9 (B) | 2.9 (A) | 2.0 (B) | 2.3 (B) | 2.7 (A) |
| | Fibre length (µm) | 837.3 (B) | 836.4 (B) | 683.9 (A) | 751.2 (A) | 750.3 (A) | 685.5 (B) |

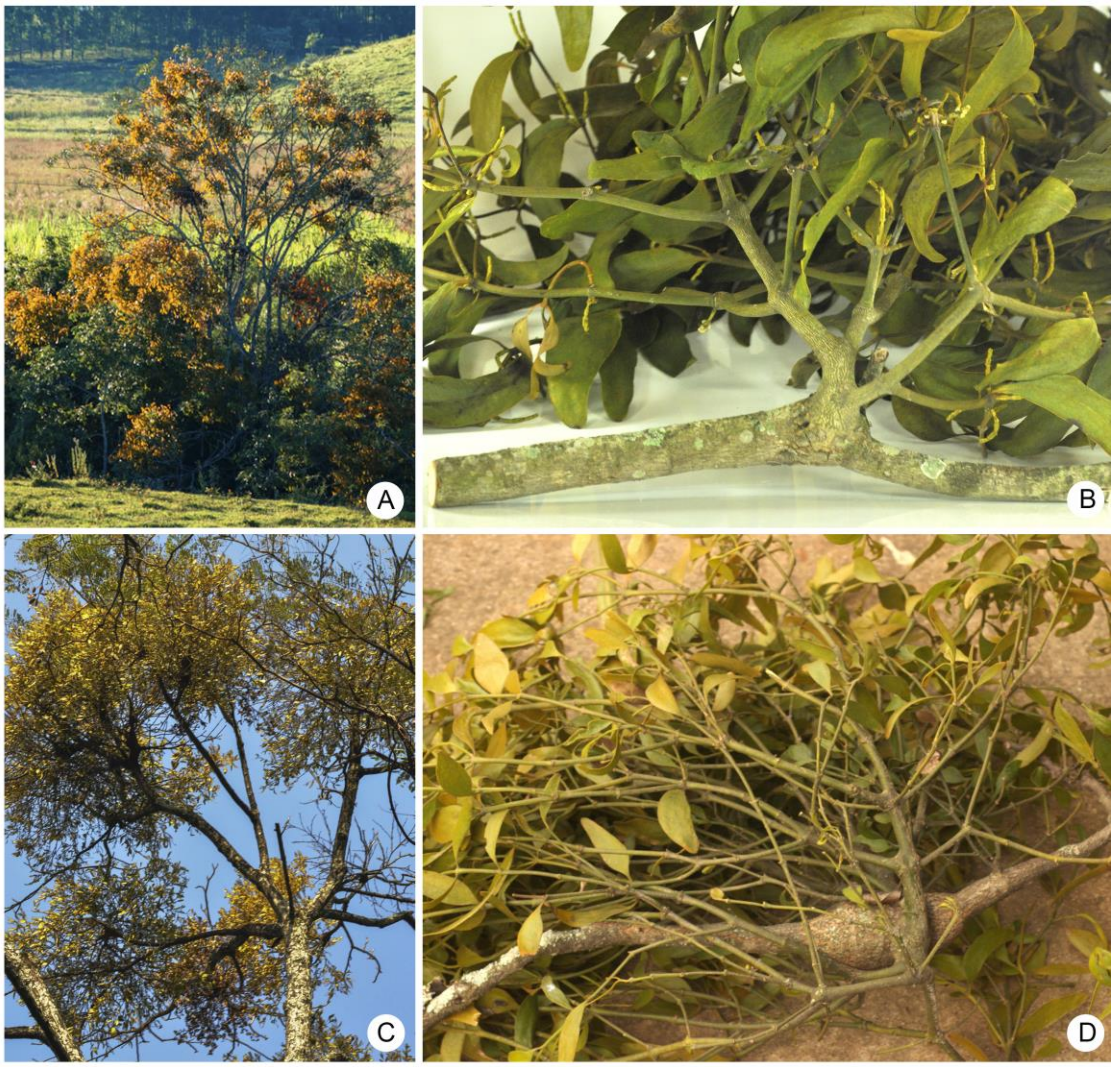



Fig. 1: Two morphologically similar parasites of the genus *Phoradendron* and their respective hosts species. A, B: *Phoradendron crassifolium* on *Tapirira guianensis*. C, D: *Phoradendron* sp. on *Cedrela fissilis*. A, C: Highly infested trees. B, D: Parasitized branches.

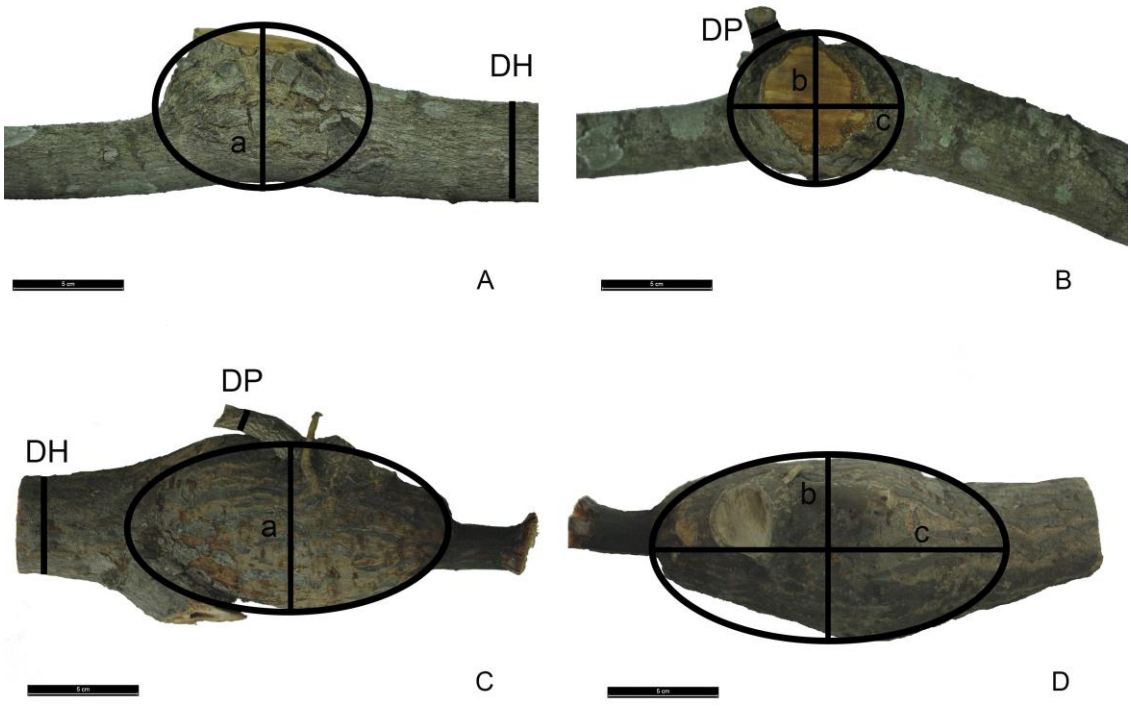



Fig. 2: Indication of the morphological measurements taken at the gall area, host branches and parasitic branches. A, B: *Phoradendron crassifolium* on *Tapirira guianensis*. C, D: *Phoradendron* sp. on *Cedrela fissilis*. DH: diameter of host branch; DP: diameter of parasitic branch; a = smaller diameter of the gall area, b = greater diameter of the gall area; c = length of the gall area. Black spheres indicate the total area of the gall. Scale bars = 5 cm.

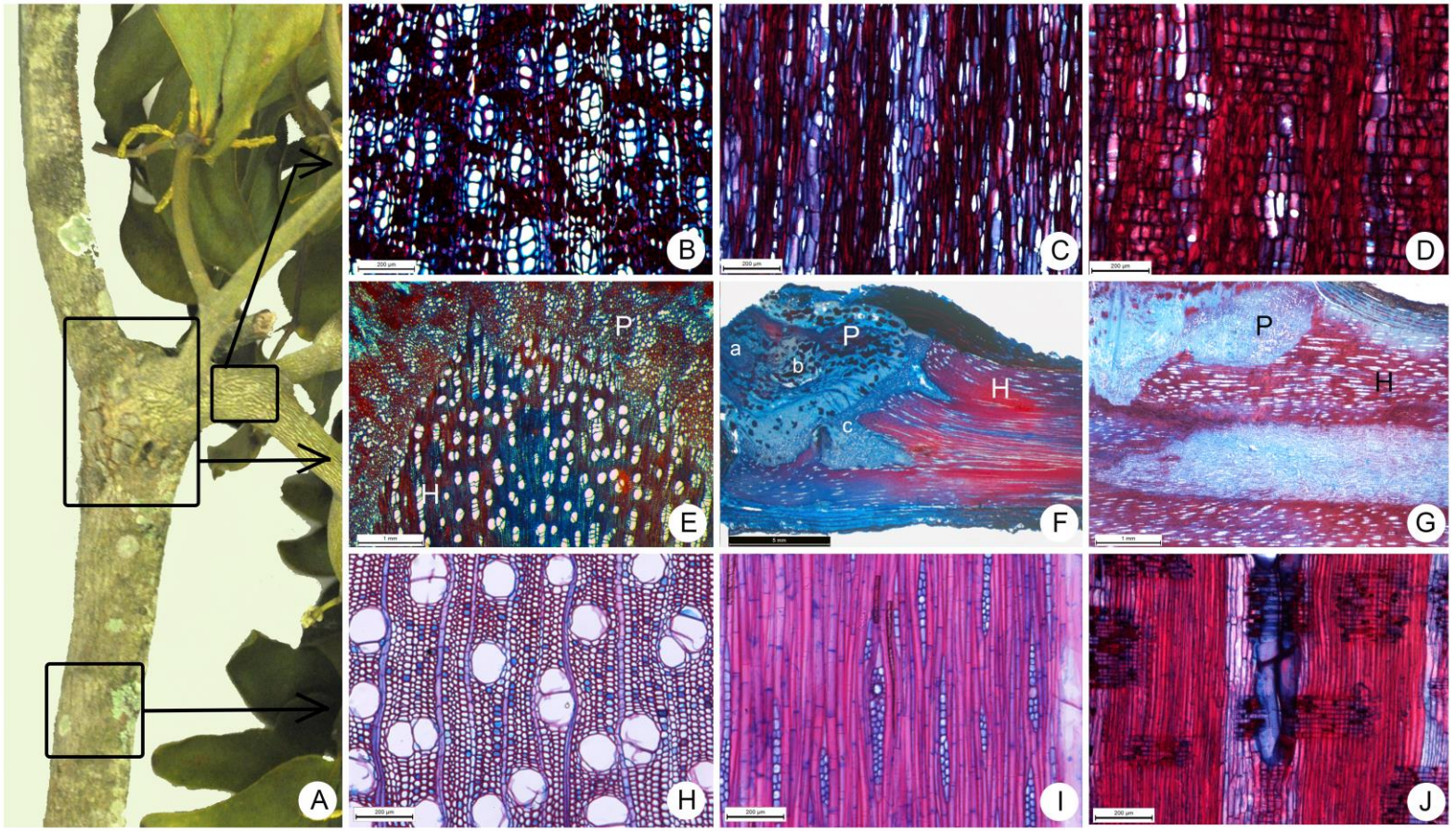



Fig. 3: Anatomy of parasitic branches, host-parasite interface region and host branches for *Tapirira guianensis* and *Phoradendron crassifolium*. A: branch of *Tapirira guianensis* infested by *Phoradendron crassifolium*. B – D: transversal, tangential and radial sections showing the wood anatomy of the parasitic spicies *Phoradendron crassifolium* (scale bars = 200 µm). E – G: transversal, tangential and radial sections showing the host-parasite interface (white scale bars = 1 mm; black scale bar = 5 mm). H – I: transversal, tangential and radial sections showing the wood anatomy of the host species *Tapirira guianensis* (scale bars = 200 µm). H = host tissue; P = parasitic tissue; a, b, c = regions of the parasitic tissue.

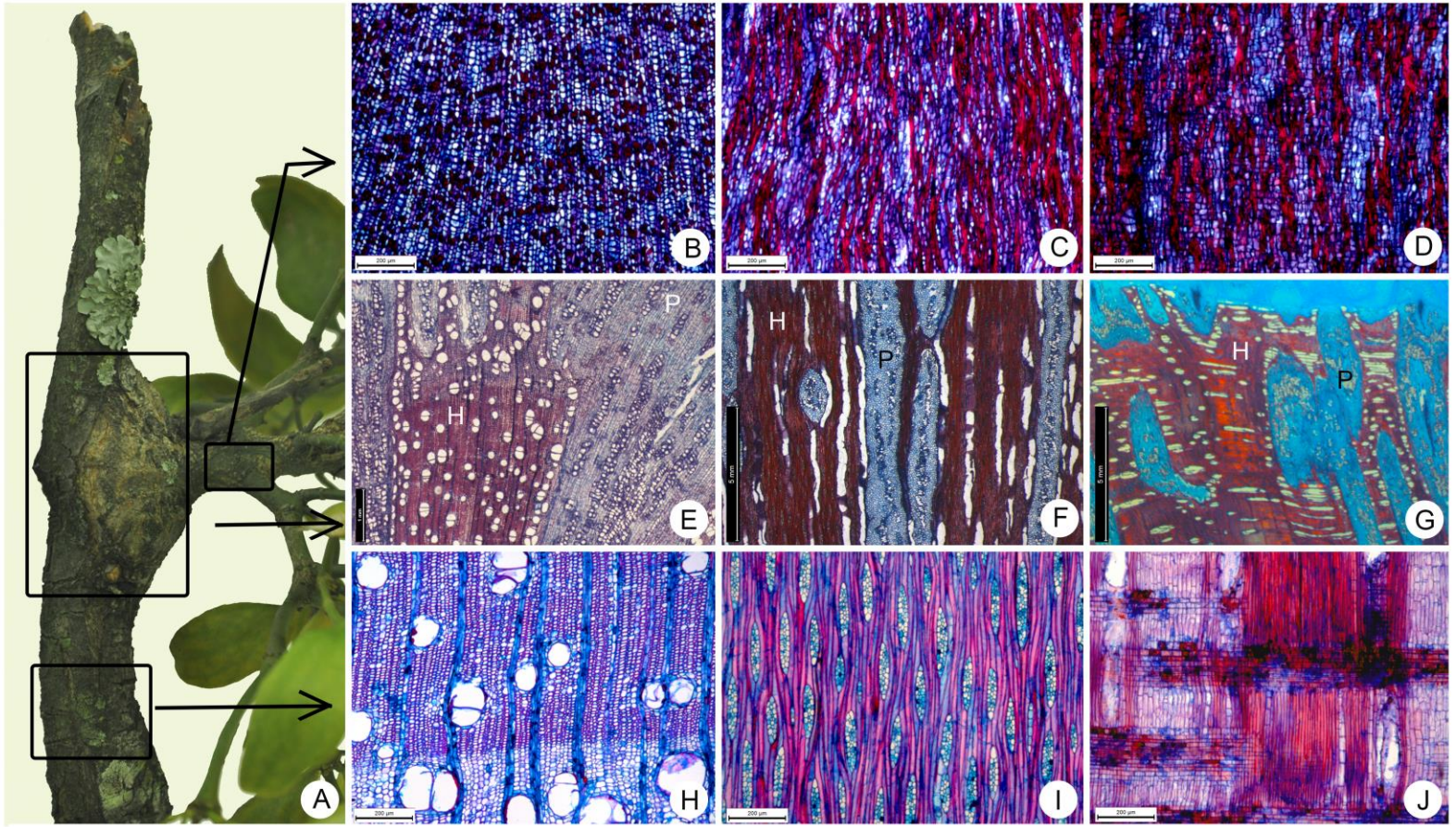



Fig. 4: Anatomy of parasitic branches, host-parasite interface region and host branches for *Cedrela fissilis* and *Phoradendron* sp. A: branch of *Cedrela fissilis* infested by *Phoradendron* sp. B – D: transversal, tangential and radial sections showing the wood anatomy of the parasitic speices *Phoradendron* sp. (scale bars = 200 µm). E – G: transversal, tangential and radial sections showing the host-parasite interface (scale bars = 1 mm and 5 mm). H – I: transversal, tangential and radial sections showing the wood anatomy of the host species *Cedrela fissilis* (scale bars = 200 µm). H = host tissue; P = parasitic tisssue.

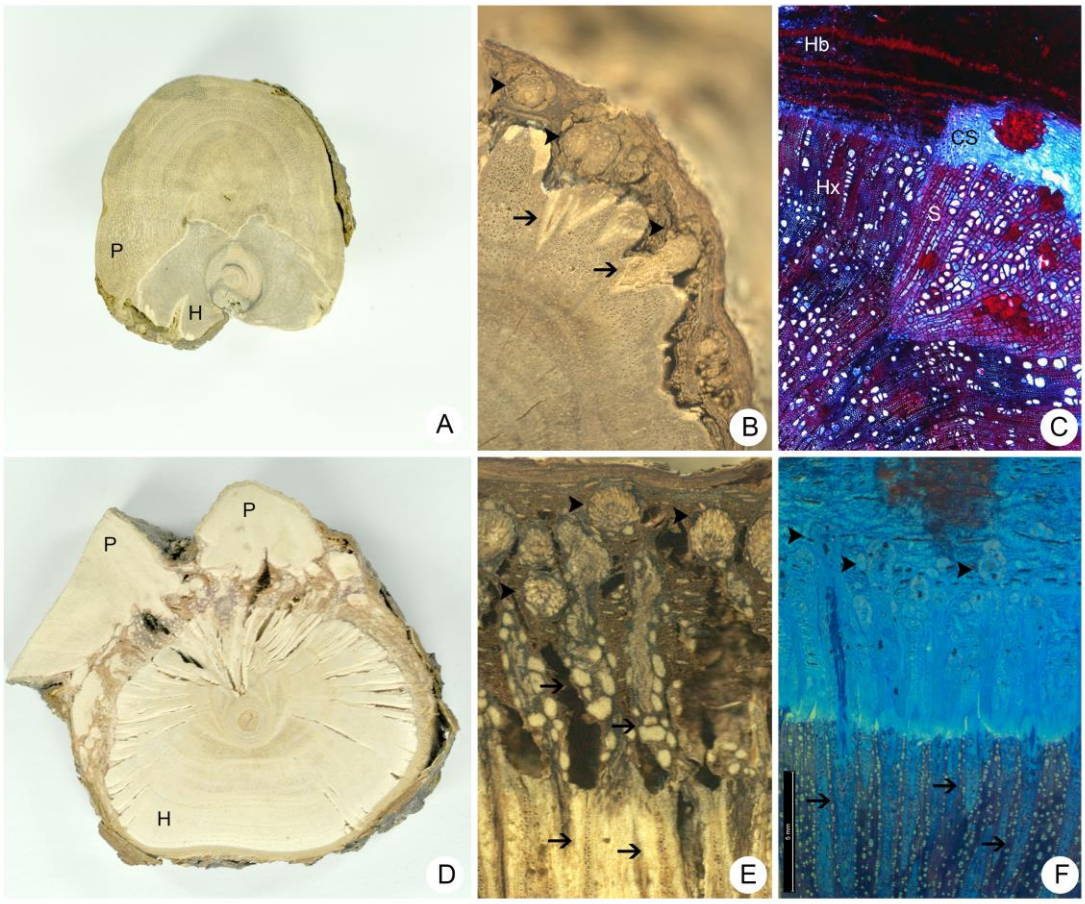



Fig. 5: Comparative cross-sections of the host-parasite interfaces. A – C: *Phoradendron crassifolium* on *Tapirira guianensis*. D – F: *Phoradendron* sp. on *Cedrela fissilis*. A, D: Macroscopical cross-sections showing the maximum size observed for the parasite's primary haustorium. B, E: Detail of the macroscopical cross-section showing sinkers arising from cortical strands. C, F: microscopy cross-section showing the general anatomy of cortical strands and sinkers (white scale bar = 500 mm; black scale bar = 5 mm). P = parasite; H = host; CS = cortical strand; S = sinker; Arrow-heads = cortical strands; Arrows = sinkers.

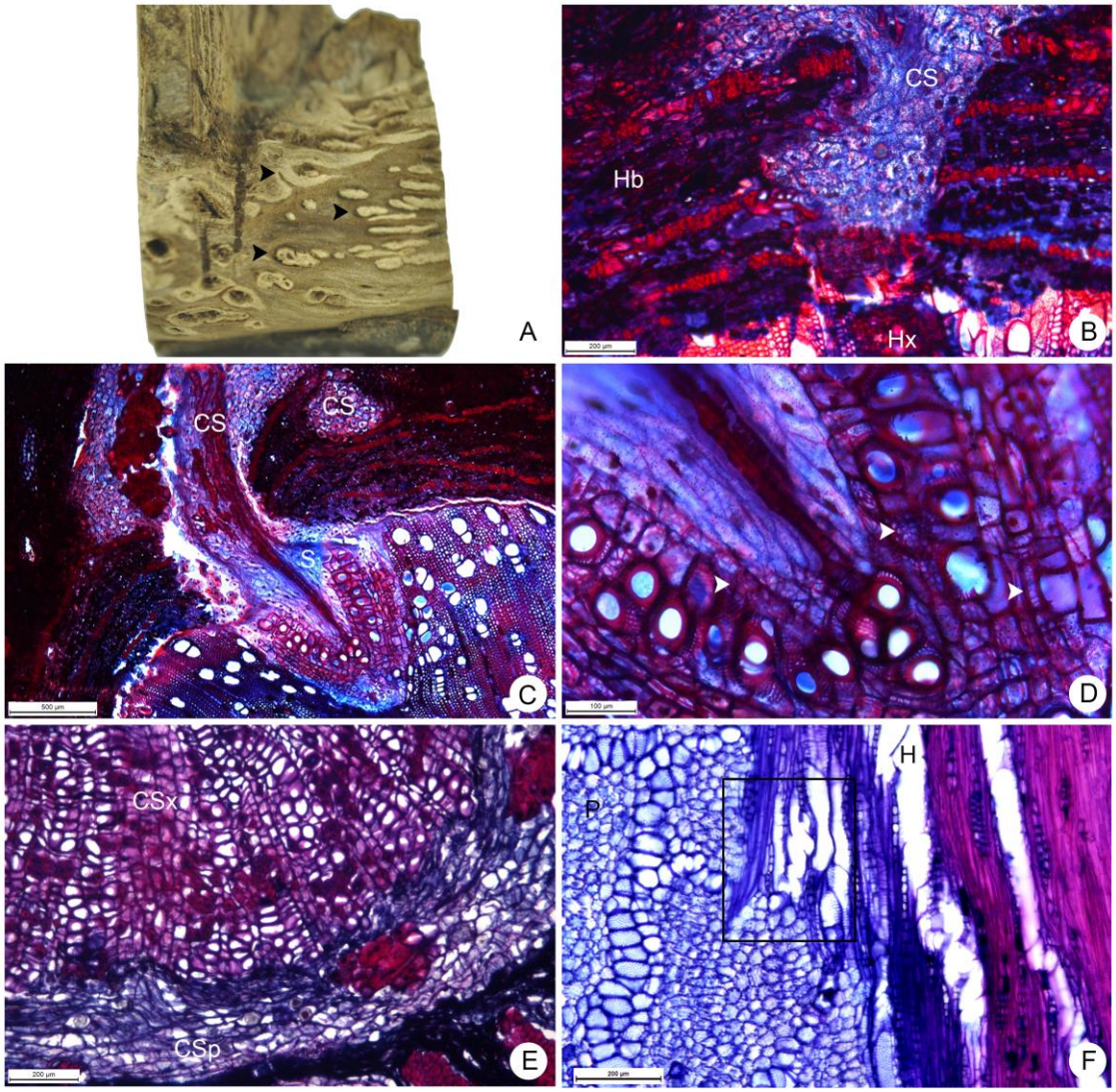



Fig. 6: Aspects of the host-parasite interface between *Tapirira guianensis* and *Phoradendron crassifolium*. A: Macroscopical view of the gall after the removal of the host bark, showing the cortical strands on the host xylem. B: Young cortical strand penetrating the host xylem (scale bar = 200 µm). C: Developed cortical strand giving rise to a sinker (scale bar = 500 µm).D: Detail of the sinker showing the lignified parenchyma cells, (scale bar = 100 µm). E: Cortical strand with secondary growth structure (scale bar = 200 µm). F: Direct vessel connection between parasite and host (scale bar = 200 µm). CS = cortical strand; CSx = cortical strand xylem; CSp = cortical strand phloem; Hb = host bark; Hx = host xylem; S = sinker; P = parasite; H = host; black arrow head = cortical strands; whitearrow heads = lignified parenchyma.

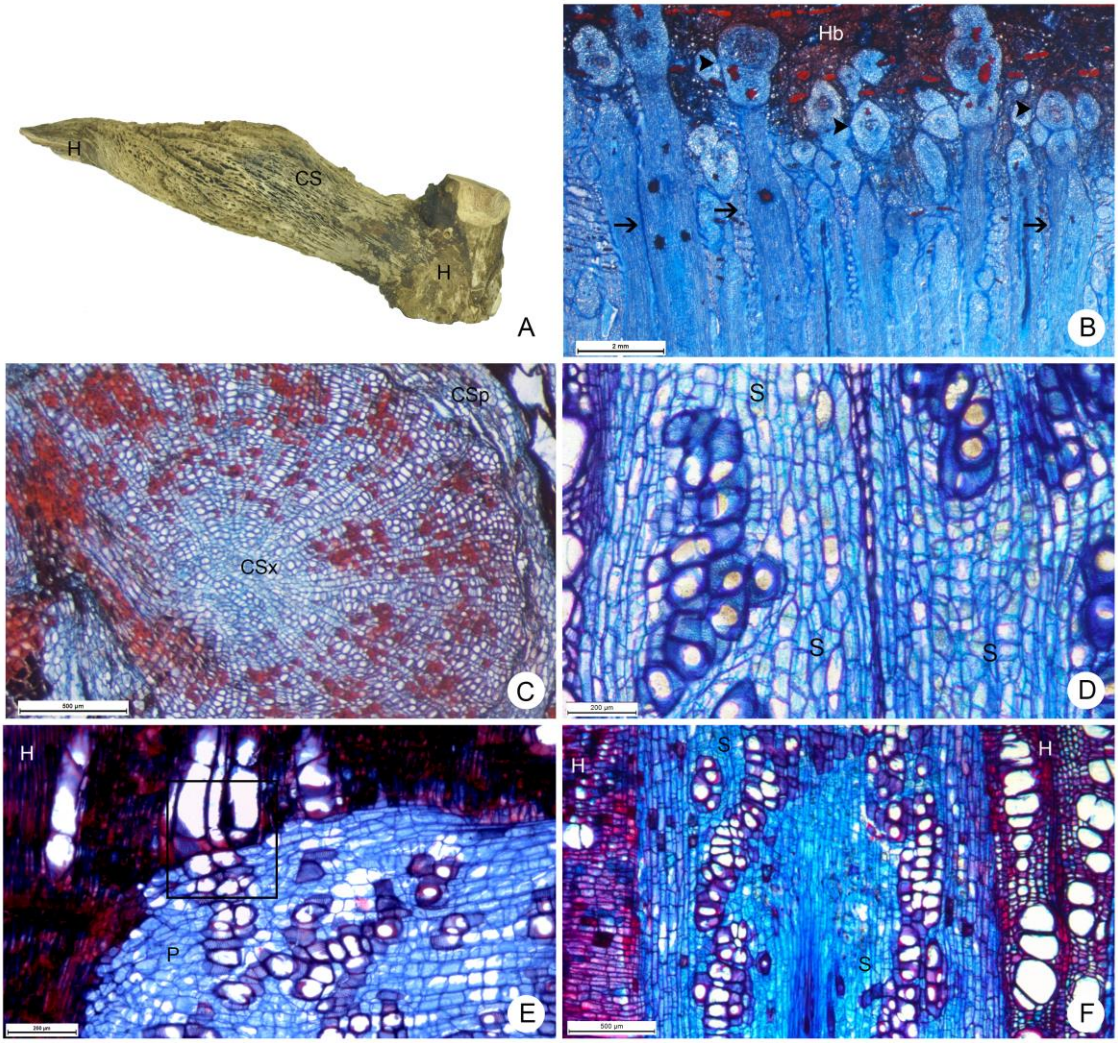



Fig. 7: Host-parasite interface between *Cedrela fissilis* and *Phoradendron* sp. A: Macroscopical view of the gall after the removal of the host bark, showing the cortical strands on the host xylem. B: Multiple cortical strands in different developmental stages within the host bark (scale bar = 2 mm). C: Cortical strand with secondary growth structure (scale bar = 500 µm). D: Detail of the sinker showing the parenchyma cells and vessel elements (scale bar = 200 µm). E: Direct vessel connection between parasite and host (scale bar = 200 µm). F: Region of the host xylem with cross-grained wood close to the sinker of the parasite (scale bar = 500 µm). H = host; P = parasite; CS = cortical strand; CSx = cortical strand xylem; CSp = cortical strand phloem; S = sinker; Arrow-heads = cortical strands; Arrows = sinkers.

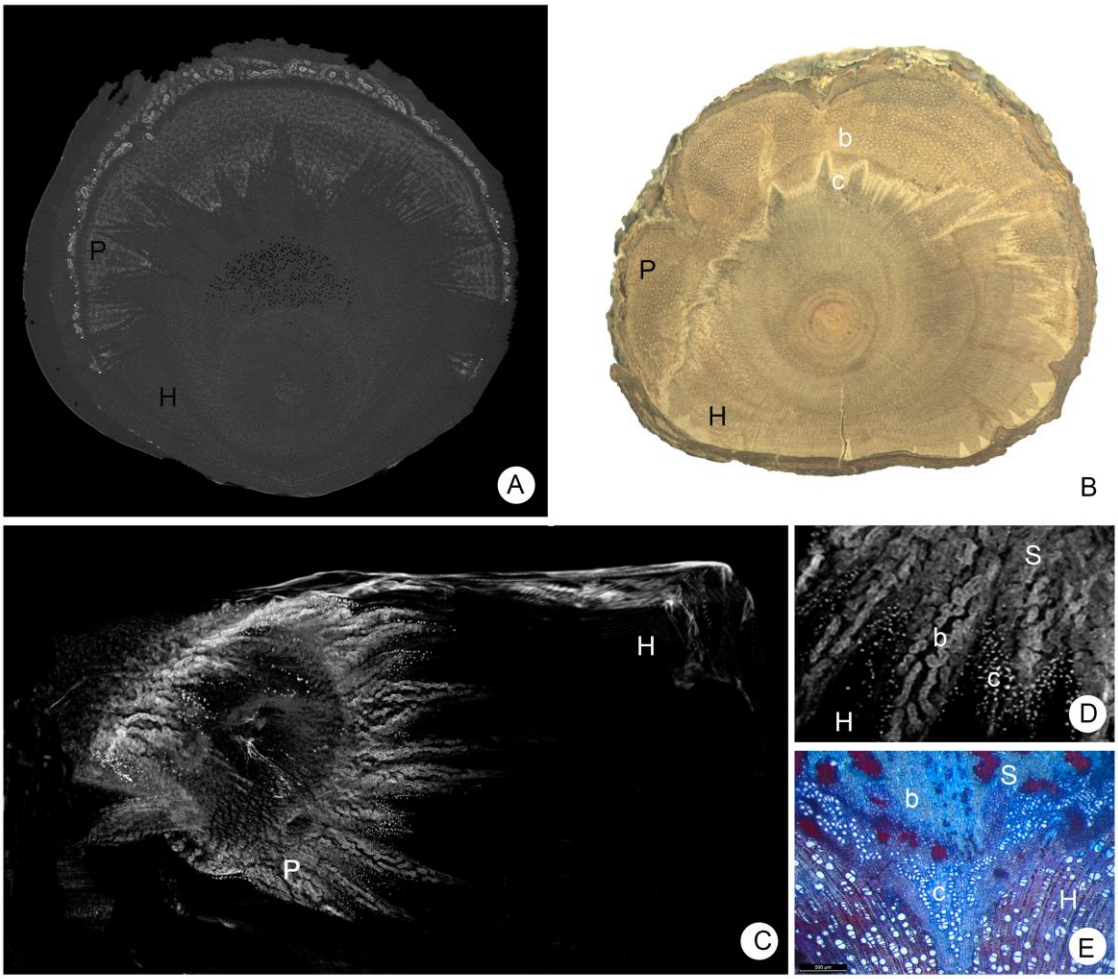



Fig. 8: Mycrotomography (MCT) images of *Tapirira guianensis* parasitized by *Phoradendron crassifolium* compared to anatomical sections. A: Cross-section of the host-parasite interface showing the parasitic endophyte in white and the host wood in grey. B: Macroscopical cross-section of the host-parasite interface showing the same structures as seen in the MCT image. C: Longitudinal section of the host-parasite interface showing the three-dimensional position of the sinkers. D: Detail of the sinker showing two different regions. E: Anatomical cross-section of the sinker showing the same regions as seen in the MCT image (scale bar = 500 µ). H = host; P = parasite; b, c = regions of the parasitic endophyte.

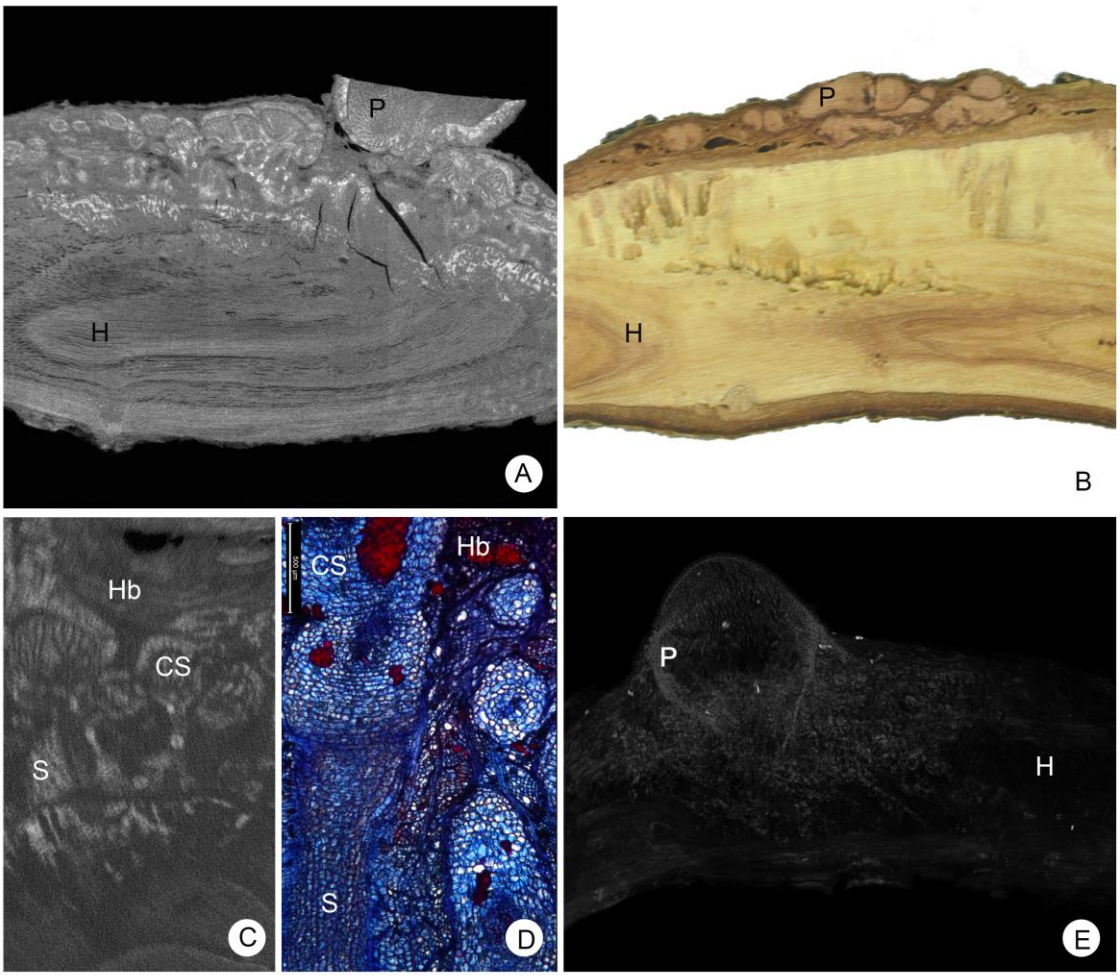
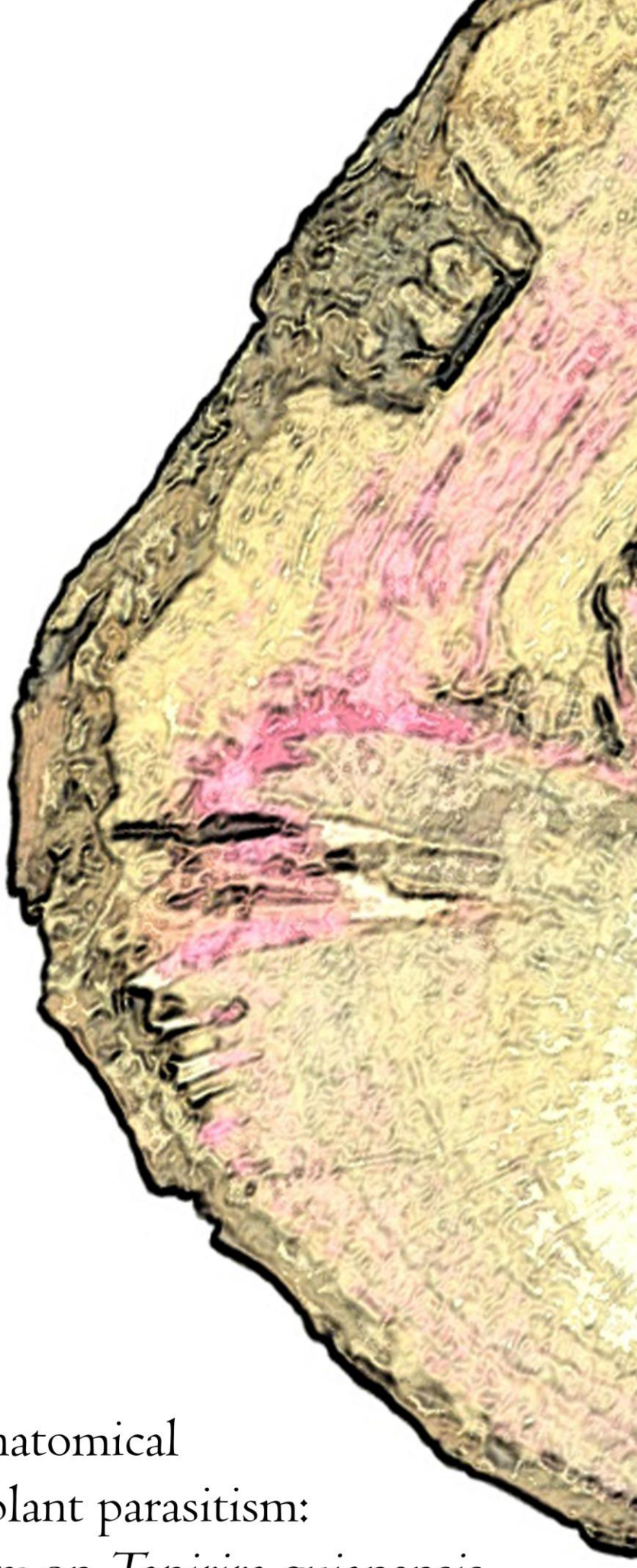



Fig. 9: Mycrotomography (MCT) images of *Cedrela fissilis* parasitized by *Phoradendron* sp. compared to anatomical sections. A: Longitudinal section of the host-parasite interface showing the parasitic endophyte in white and the host wood in grey. B: Macroscopical longitudinal section of the host-parasite interface showing the same structures as seen in the MCT image. C: Detail of the cortical strands and sinkers within the host bark. D: Anatomical cross-section of the cortical strands and sinkers within the host bark as seen in the MCT image (scale bar = 500 µ). E: Longitudinal section of the host-parasite interface showing the three-dimensional spread of the parasitic endophyte thourgh the host branch. H = host; P = parasite; Hb = host bark; CS = cortical strand; S = sinker.



Capítulo II

# Embolism increase and anatomical modifications caused by plant parasitism: *Phoradendron crassifolium* on *Tapirira guianensis*

***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, 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 2004). 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 upon the parasitized host tissue (Cohen 1954; Calvin 1967; Gonsalez & Mauseth 2010 and many others).

An exception to the above mentioned line of research was the work of Srivastava & 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 Amaral & 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 fibres.

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 (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 on the Southeast region of Brazil (21˚ 48’ 30.2’’ S, 45˚ 29’ 59.1’’ W). 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 fibres 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 2cm 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 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 10cm 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 Fig. 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 $mm^2$), vessel diameter and area for both functional and non-functional vessels; vessel element length; fibre lumen diameter, fibre length and fibre cell-wall thickness; ray height and width; radial resin canal density (per $mm^2$); 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., 1998-2013).

***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 (Fig. 6) 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 fibres (Fig. 7), radial resin canals (Fig. 8), simple perforation plates (Fig. 10) and bordered alternate intervessel pits. The radiovessel 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 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 presented about 25% more vessels per mm² than the sections of non-parasitized branches. These vessels were also narrower (ca. 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 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 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. 21).

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 fibres, 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 fibre 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 on 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 1983; 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 stablished between these plant species, the infestation of host wood by mistletoes could be interpreted as a mechanical wound caused by haustorium penetration and development trough 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 fibres seems to be related to an absence or at least a significant reduction of fibre formation (Lev-Yadun & Aloni 1993; Lev-Yadun 1994). In the present study, the density of fibres 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.

Amaral & 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 &

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 fibres 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 on 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 unbalance 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 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 fibres. 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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Table 1: Nested ANOVA results for the wood anatomical features analyzed among groups (upstream and downstream) of both parasitized and non-parasitized branches of *T. guianensis*. All bold values indicate significant statistical differences among the groups; letters inside the parenthesis indicate the results for Tukey test (post nested ANOVA).

| | | Non-parasitized | | Parasitized | |
|---|---|---|---|---|---|
| | | Upstream | Downstream | Upstream | Downstream |
| Vessels | Density (vessels/mm²) | **34.4 (C)** | **32.2 (C)** | **44.8 (B)** | **47.1 (A)** |
| | Diameter (µm) | **87.27 (A)** | **71.53 (B)** | **72.8 (B)** | **62.2 (B)** |
| | Area (µm²) | **3904 (B)** | **3990 (B)** | **4458 (A)** | **3648 (B)** |
| | Vessel-element length (µm) | 431.2 | 424.0 | 418.1 | 425.0 |
| | % Solitary vessels | 43.2 (A) | 44.5 (A) | 43.5 (A) | 44.3 (A) |
| | % Vessels grouped in pairs | 30.4 (A) | 26.9 (A) | 27.3 (A) | 27.2 (A) |
| | % Vessels grouped in trios | 16.2 (A) | 22.1 (A) | 17.9 (A) | 16.5 (A) |
| | % Vessels grouped in 4 and more | 10.1 (A) | 6.5 (A) | 11.2 (A) | 11.9 (A) |
| Functional vessels | Density (vessels/mm²) | **23.7 (A)** | **19.9 (B)** | **18.0 (C)** | **13.3 (D)** |
| | Diameter (µm) | **78.2 (A)** | **74.3 (B)** | **67.8 (C)** | **60.2 (D)** |
| | Area (µm²) | **4014 (A)** | **4009 (A)** | **3388 (B)** | **2949 (C)** |
| Embolized vessels | Density (vessels/mm²) | **10.7 (C)** | **12.3 (C)** | **26.9 (B)** | **33.8 (A)** |
| | Diameter (µm) | **75.3 (A)** | **71.5 (B)** | **76.1 (A)** | **65.3 (C)** |
| | Area (µm²) | **3986 (AB)** | **3767 (BC)** | **4142 (A)** | **3464 (C)** |
| Fibres | Lumen diameter (µm) | **10.05 (A)** | **9.71 (A)** | **8.97 (B)** | **8.05 (C)** |
| | Cell-wall thickness (µm) | **1.68 (B)** | **1.91 (A)** | **1.67 (B)** | **1.50 (C)** |
| | Length (µm) | 849.6 | 830.6 | 837.3 | 828.9 |
| Rays | Density (rays/mm²) | **25.1 (B)** | **26.5 (A)** | **24.4 (BC)** | **23.9 (C)** |
| | Width (µm) | **19.0 (B)** | **18.1 (B)** | **23.1 (A)** | **22.6 (A)** |
| | Length (µm) | **251.9 (B)** | **242.8 (B)** | **275.5 (A)** | **266.1 (A)** |
| | Radial resin canals/mm² | 0.49 | 0.37 | 0.44 | 0.46 |

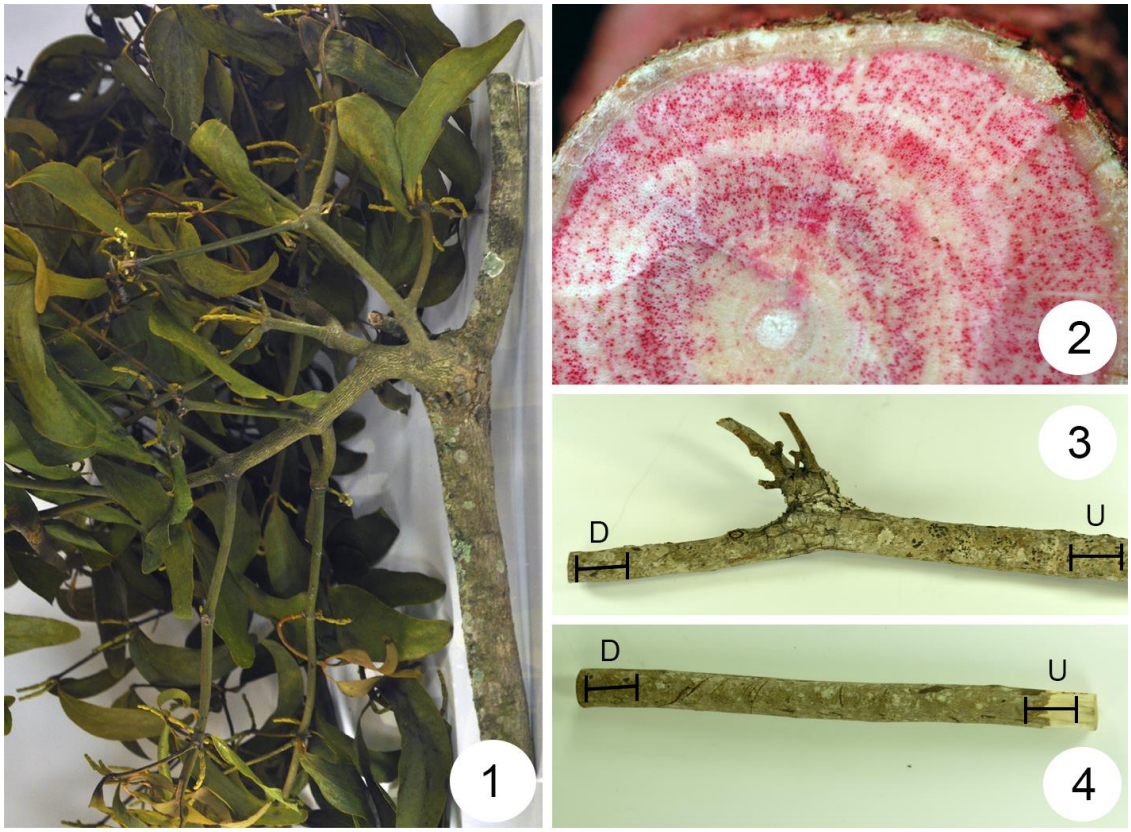
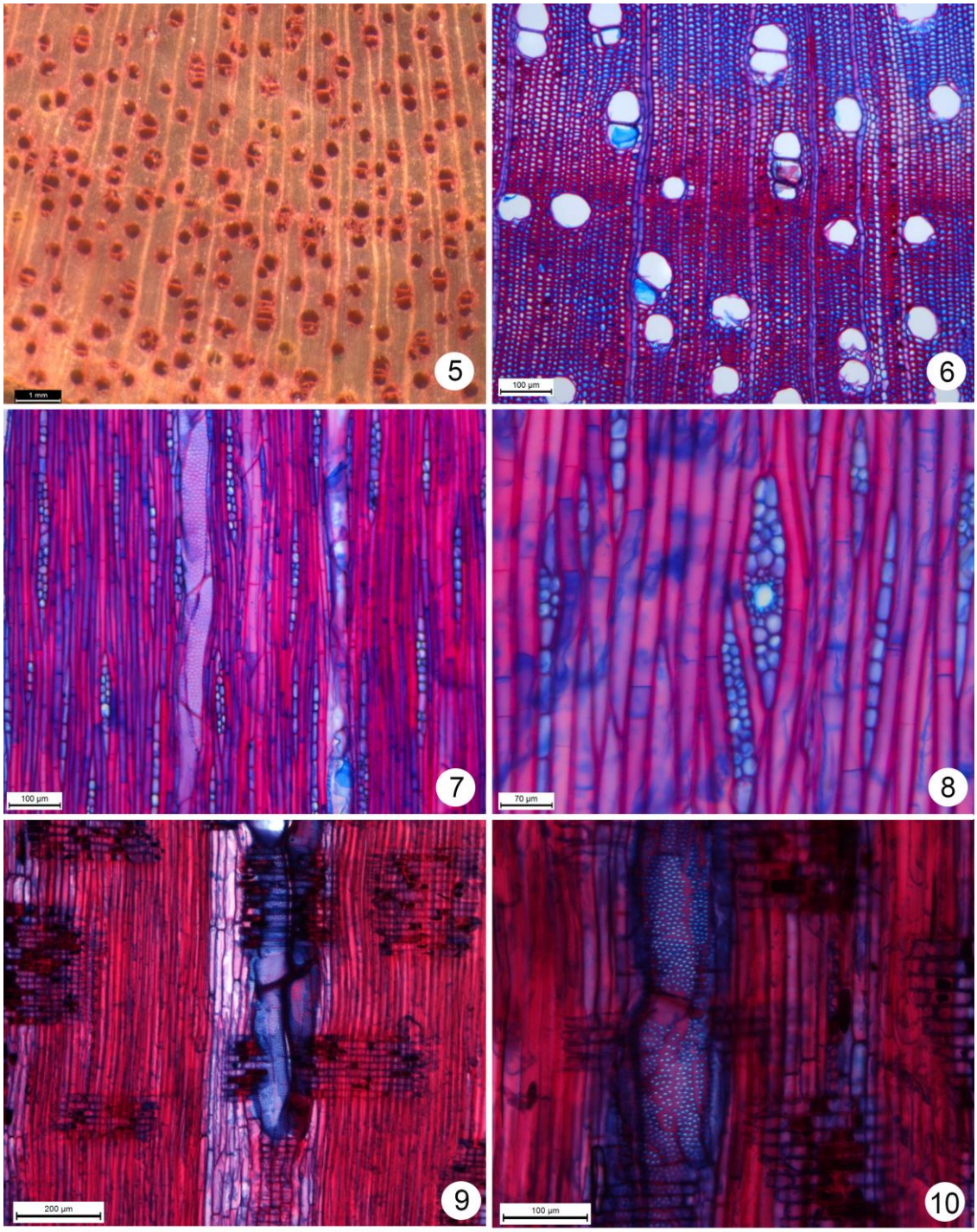



Fig. 1--4: 1: Host tree (Tapirira guianensis) bearing few of its own leaves and highly parasitized by the shrubby mistletoe Phoradendron crassifolium. -- 2: Cross-section of host branch showing safranin-stained (functional) vessels; black squares mark areas where anatomical measurements were taken. -- 3: Non-parasitized branch with upstream (U) and downstream (D) sections demarked. -- 4: Parasitized branch with upstream (U) and downstream (D) sections demarked; black square marks the attachment site of the parasite in the host branch.




Figures 5--10: General wood anatomic features of Tapirira guianensis. -- 5: Macrograph of transverse surface showing diffuse porosity and poorly marked annual rings. -- Scale bar = 1 mm. -- 6: Transverse section showing axial scanty paratracheal parenchyma and tyloses common. -- Scale bar = 100 µm. -- 7: Tangential section showing 1–3 seriate rays. -- Scale bar = 100 µm. -- 8: Detail of a tangential section showing a radial resin canal. -- Scale bar = 70 µm. -- 9: Radial section showing the cellular composition of rays. -- Scale bar = 200 µm. -- 10: Detail of a radial section showing vessel pitting. -- Scale bar = 100 µm.

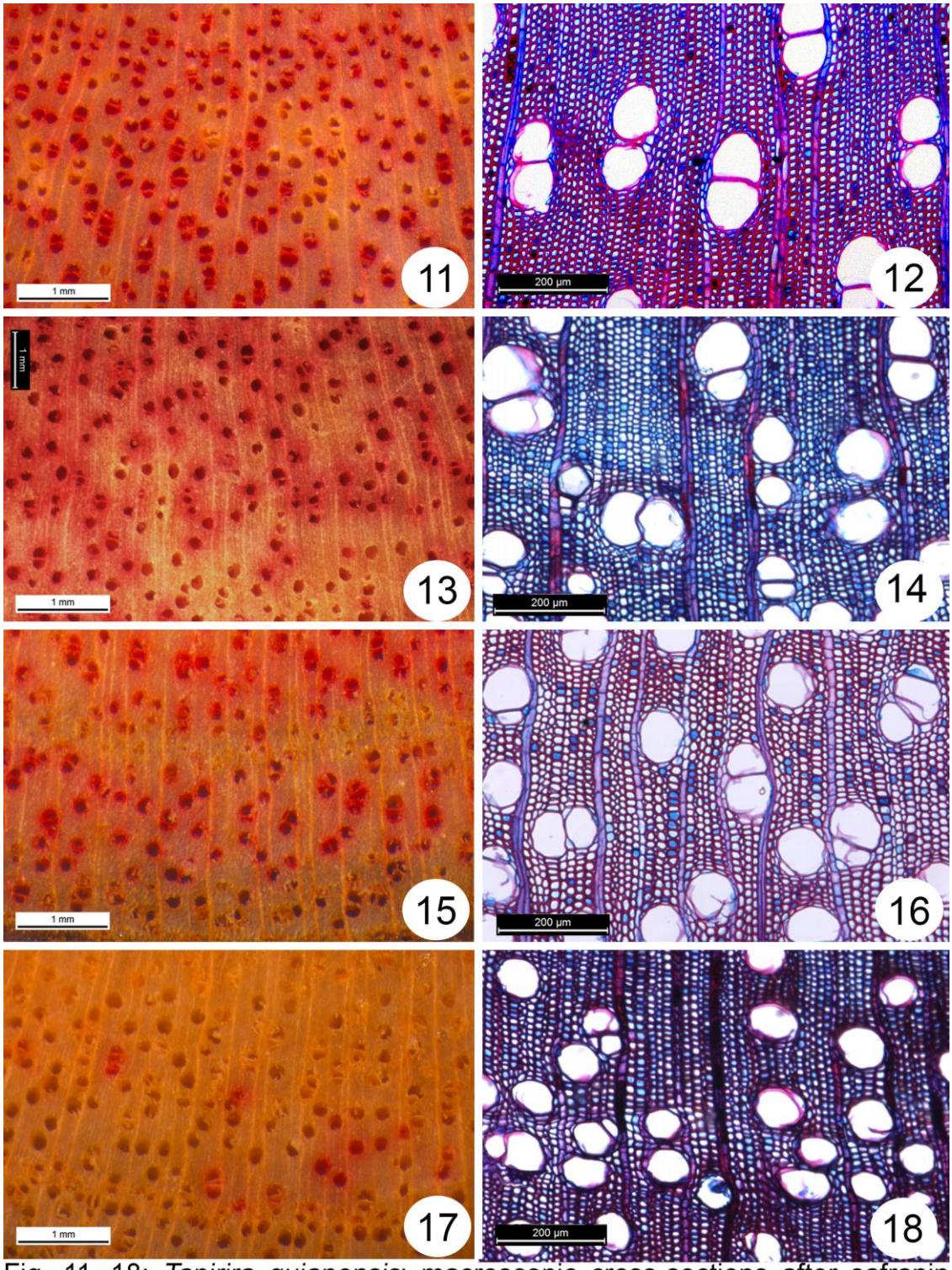



Fig. 11--18: *Tapirira guianensis*; macroscopic cross-sections after safranin infiltration experiments (left column) and microscopic cross-sections after regular wood anatomy cutting and staining procedures (right column). -- 11-12: Non-infested branch, upstream portion. -- 13-14: Non-infested branch, 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.

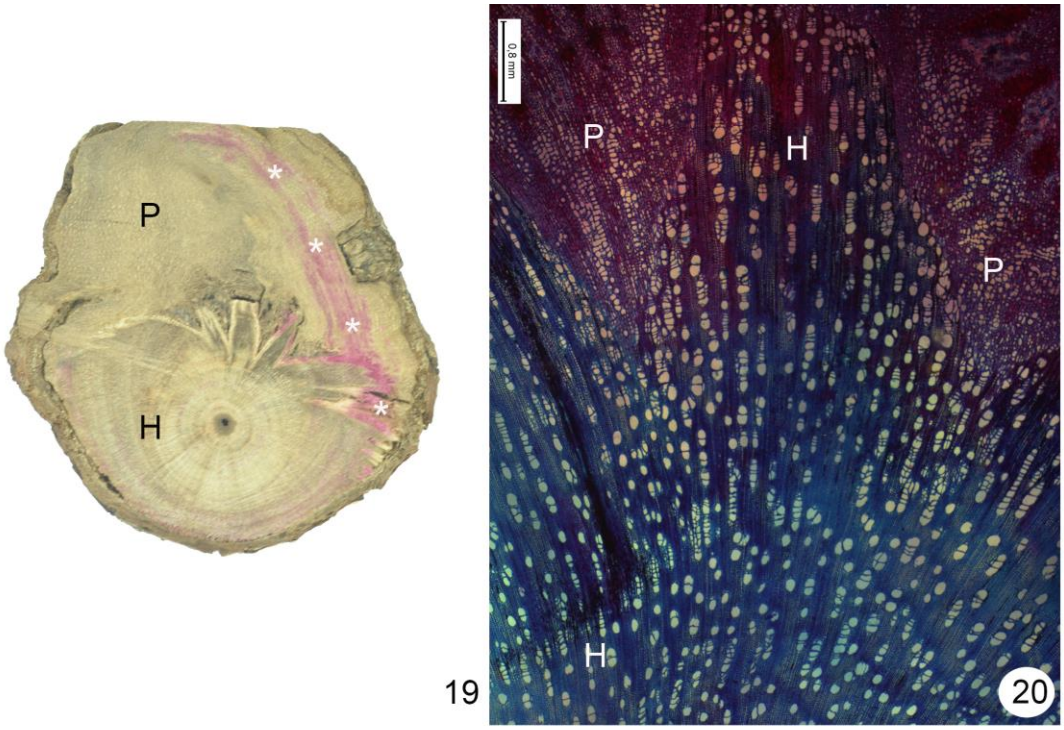



Fig. 19--20: morphology and anatomy of the host-parasite interface between Phoradendron crassifolium and Tapirira guianensis. -- 19: Macroscopic end surface showing a large parasite area (P) compared to the host area (H). -- 20: Microscopic cross-section of the host wood (H) showing narrow vessels and increased vessel density. -- Scale bar = 0.8 mm. -- * indicates the flow of safranin from host to parasite.

# Considerações finais

A presente dissertação apresentou aspectos da relação parasita-hospedeira estabelecida entre duas espécies do gênero *Phoradendron* e suas respectivas hospedeiras. A semelhança morfológica observada entre duas espécies parasitas, que infestam os galhos de suas hospedeiras e se apresentam na forma de grandes arbustos com folhagens amareladas/alaranjadas, não se confirmou no padrão de infestação que tais espécies apresentam. Em decorrência dos diferentes padrões observados, alguns dos efeitos causados às hospedeiras também foram particulares de cada interação estudada.

De maneira simplificada, *Phoradendron crassifolium* apresenta um tecido endofítico circunscrito, mas que é capaz de causar elevação da frequência de vasos embolizados no xilema da hospedeira *Tapirira guianensis*. Por outro lado, *Phoradendron* sp. forma um volumoso sistema endofítico que, embora não provoque alterações sistêmicas quanto à eficiência do uso da água da hospedeira *Cedrela fissilis*, causa notório decréscimo de suas taxas de crescimento[3]. Em ambos os casos, foi observada a morte de galhos de hospedeiras, de árvores inteiras, e consequentemente das parasitas, que apresentaram infestação intensa e prolongada.

Os efeitos relacionados às alterações na xilogênese da hospedeira foram semelhantes em ambos os casos. Ainda assim, na interação ente *Phoradendron* sp. e *C. fissilis* as alterações foram observadas como sendo mais intensas, em comparação às interações entre *P. crassifolium* e *T. guianensis*. Estas diferenças ressaltam o aspecto único de

[3]Dados preliminares obtidos em parceria com Prof. Dr. Roel Brienen, não apresentados nesta versão final.

cada interação parasita-hospedeira. De fato, conforme discutido no primeiro capítulo, os diferentes padrões de infestação observados podem ser interpretados como produto da interação particular estabelecidas entre cada uma das espécies de *Phoradendron* estudadas e sua hospedeira.

Entretanto, em ambos os modelos de interação parasita-hospedeira estudados, os diferentes efeitos observados nas hospedeiras podem ter mediadores comuns. Por um lado, as alterações anatômicas na madeira da hospedeira podem ser causadas por déficit hídrico associado às altas taxas de transpiração de plantas parasitas, incluindo espécies de *Phoradendron* (Hollinger 1983; Ehleringer *et al.* 1986; Press & Graves 1995; Ackroyd & Graves 1997). Isto poderia levar à baixa pressão de turgor nas células do câmbio em diferenciação, alterando as características anatômicas da madeira subsequentemente formada (do Amaral & Ceccantini 2011).

Adicionalmente, um desbalanço local na concentração dos hormônios auxina e citocinina poderia também ser responsável pelas alterações observadas (Aloni & Zimmermann 1983; Lev-Yadun 1994, 2000, 2002). A penetração do sistema endófitico da parasita através corpo de sua respectiva hospedeira, causando distúrbios mecânicos e eventuais rompimentos do tecido floemático, poderiam causar tal desbalanço. Neste sentido, como já comentado anteriormente, alterações anatômicas mais dramáticas foram observadas na madeira de *Cedrela fissilis* parasitada por *Phoradendron* sp., espécie que causa maiores distúrbios no tecido floemático do caule parasitado.

Ainda, de acordo com o recente trabalho de Aloni (2014), plantas parasitas poderiam promover influxo de auxina para a região da galha, causando alterações sobre a madeira de sua hospedeira. Deste modo, é possível considerar que a planta

parasita exerce algum nível de controle sobre xilogênese de sua hospedeira, além de também afetar aspectos de sua fisiologia.

Além da auxina, é possível também que material genético possa ser transferido da parasita para sua hospedeira (Nickrent *et al.* 2004; Richardson & Palmer 2007), o que aumentaria as possibilidade de controle da parasita sobre a hospedeira, semelhante ao que se observa no caso de galhas formadas através de interações de plantas com insetos e microorganismos (Dawkins 1982). Nestes casos, a galha poderia até mesmo ser considerada como fenótipo estendido da parasita sobre sua hospedeira, de modo semelhante ao que é discutido para outros tipos de galhas (Dawkins 1982).

Em conclusão, a presente dissertação ressalta os aspectos únicos das relações estabelecidas entre cada espécie de planta parasita e sua hospedeira, analisando a galha como produto da interação entre duas espécies. Decorrente das particularidades de cada relação estabelecida discutiu-se também os diferentes efeitos causados por duas espécies de *Phoradendron* a suas respectivas hospedeiras.

# Conclusões gerais

I. De acordo com a hipótese inicial, *Phoradendron crassifolium* e *Phoradendron* sp. apresentam diferentes padrões de infestação e causam diferentes efeitos sobre suas hospedeiras;

II. A relação parasita-hospedeira estabelecida em cada modelo estudado é única, sendo que o padrão de infestação, as características anatômicas das galhas e os efeitos observados sobre as diferentes hospedeiras podem ser vistos como produto da interação entre parasita e hospedeira;

III. A região de interface parasita-hospedeira assemelha-se às galhas formadas, por exemplo, em interações inseto-planta, de modo que se propõem a utilização do termo "galha" para também designar o tumor formado na interação parasítica entre duas plantas;

IV. O uso da metodologia que envolve a microtomografia computadorizada (MCT) representa avanço considerável para interpretação da ocupação promovida por plantas parasitas em suas hospedeiras.

V. *Phoradendron crassifolium* causa aumento na frequência de vasos embolisados em galhos da hospedeira *Tapirira guianensis*, o que corrobora a hipótese inicial;

VI. *Phoradendron crassifolium* causa também alterações na anatomia da madeira desta hospedeira com aumento da densidade de vasos, aumento em altura e largura dos raios e redução da espessura da parede celular de fibras;

VII. Plantas parasitas do gênero *Phoradendron* são capazes de alterar a xilogênese de sua hospedeira.

# Resumo

O gênero *Phoradendron* é um dos mais diversos entre as plantas parasitas, agrupando cerca de 230 espécies, que apresentam grande variedade quanto à morfologia e padrões de infestação. A presente dissertação comparou os padrões de infestação de duas espécies de *Phoradendron* parasitando diferentes hospedeiras: *Tapirira guianensis* e *Cedrela fissilis*, além de analisar os efeitos causados por tais parasitas na funcionalidade e na anatomia da madeira destas hospedeiras. Foram realizadas análises tradicionais de anatomia da madeira, análises de microtomografia e experimentos de anatomia funcional com infiltração de corante através da madeira da hospedeira. Os resultados mostraram que, enquanto *P. crassifolium* forma uma galha concisa sobre os ramos de *T. guianensis*, *Phoradendron* sp. é mais agressivo ao espalhar seu sistema endofítico através da madeira de *C. fissilis*, causando maiores rupturas dos tecidos xilemático e floemático da hospedeira. Sugere-se que tais rupturas poderiam levar a uma alteração local do balanço auxina/citocinina e à liberação de etileno. Esta hipótese é reforçada pelas alterações anatômicas observadas em ambos os casos na interface parasita-hospedeira, tais como hiperplasia e/ou hipertrofia, maior densidade de vasos, alterações no agrupamento dos vasos e redução da espessura da parede celular das fibras. *P. crassifolium* também provocou severo aumento da densidade de vasos embolisados na madeira de *T. guianensis*, aumentando também a densidade de vasos e o tamanho de raios, além de reduzir o diâmetro transversal dos vasos e a espessura da parede celular de fibras. Tais efeitos também podem estar relacionados às altas taxas de transpiração e aos potenciais hídricos extremamente baixos apresentados por plantas parasitas, o que pode culminar no aumento da transpiração total da hospedeira, elevando a formação de embolismos, causando estresse hídrico e consequente baixa pressão de turgor nas células derivadas do câmbio. Conclui-se que cada uma das espécies de parasita aqui analisada estabeleceu uma relação única com sua hospedeira, formando diferentes padrões de infestação e alterando de modo particular a xilogênese da hospedeira.

# Abstract

*Phoradendron* is one of the most diverse genera among parasitic plants, comprising ca. 230 species and showing a great variety of morphologies and infestation patterns. The present work compared the infestation patterns of two *Phoradendron* species on different hosts: *Tapirira guianensis* and *Cedrela fissilis*, and also analyzed their effects on the hosts' functionality and wood anatomy. Traditional wood anatomy procedures were used, along with the technique of microtomography analysis and functional anatomy experiments using a dye ascent through host wood. Results showed that while *P. crassifolium* forms a defined gall in branches of *T. guianensis*, *Phoradendron* sp. is more aggressive when spreading its endophytic tissue trough the wood of *C. fissilis*, causing major disruptions on both xylem and phloem tissues of the host. Such disruptions could lead to a local change of the auxin/cytokinin balance and probably ethylene liberation. This hypothesis is reinforced by the anatomical alterations observed in both cases at the host-parasite interface, such as hyperplasia and/or hypertrophy, higher vessel density, alterations of vessel grouping and thin cell-walled fibres. *P. crassifolium* also provoked a severe increase in embolism density on the wood of *T. guianensis*, along with increases on vessel density and ray size, besides reductions in vessel lumen diameter and fibre cell-wall thickness. These effects could also be related to the high transpiration rates and extremely low water potential presented by parasitic plants, which may increase the host's total transpiration, enhancing embolism formation, causing water stress and consequent low turgor in differentiating cambium derivates. In conclusion, each parasitic plant species analyzed established a unique relationship with its host, forming different infestation patterns and differently altering its host xylogenesis.

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