# Endophytic Fungal Diversity of *Cedrela angustifolia* in an Environmental Gradient of Jujuy, Argentina

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**ABSTRACT—** ***Fungal endophytes are a diverse group of microorganisms that colonize plant tissues without causing symptoms or signs. Foliar endophytic fungi were isolated from a tree species distributed in an environmental gradient. Three study sites were selected: urban, peri-urban and rural. Twenty-three taxa were identified using culture-based techniques. The taxa with the highest colonization frequency were: Alternaría, Diaporthe - Phomopsis, Phymatotrichum, Cladosporium and Xylaria. Foliar endophytic fungal diversity was higher in the rural site followed by the peri-urban and urban site.***

**Keywords— Endophyte, Yungas, Meliaceae, forest, community.**

## 1. INTRODUCTION

In Northwest Argentina, *Cedrela angustifolia* is an economically and ecologically important species, due to the quality of its wood, its natural regeneration and for its role in local ecosystem processes [41, 23]. In Jujuy province, this species is distributed naturally in Las Yungas ecoregion, being characteristic of the subtropical montane forest's altitudinal belts [13] while it is also found in forestry and urban plantations.

Endophytic fungi are microorganisms that colonize plant tissues without causing any apparent symptoms. The greatest diversity occurs in tropical or subtropical regions [1, 27, 29]. The distribution and habitat characteristics of host plants have an effect in the endophytic fungi's diversity and their ecological niches [2, 42, 30]. This diverse group of fungi produce secondary metabolites which may confer beneficial effects to the host plants such as competitive capacity [34], resistance to abiotic and biotic stress [21], pathogens, and pests [14, 35].

The potentiality of fungal endophytes in forest ecosystems has been demonstrated [38, 40, 45] and there is a need to study their diversity and environmental dynamics in tree species of economic importance [43]. Based on the significance of *C. angustifolia* in the forests of Yungas and its categorization as an endangered species [49], the aim of the present study was to identify the foliar fungal endophytes of *C. angustifolia* in an environmental gradient of Jujuy province.

## 2. MATERIALS AND METHODS

### Study site and isolation of endophytic fungi

Three sites were selected, distributed in an environmental gradient in the Doctor Manuel Belgrano Department, Jujuy, Argentina: 1. Urban site, located in the downtown area of the city of San Salvador de Jujuy (24°11'45" S - 65°17'62" W), 2. Peri-urban site, with low urbanization, surrounding the urban site and separated from it by the Río Grande de Jujuy (24°09'77" S - 65° 18'90" W), 3. Rural site, without the presence of evident anthropogenic pressures (between 24°02'52" S- 65°15'21" W and 24°14'21" S - 65°04'79" W).

During early fall (April-March), 15 trees of 30 to 70 cm in diameter at breast height (DBH) and 10 to 20 m in height were selected at each site, and 10 asymptomatic adult leaves were collected from each tree. The isolation of endophytic fungi was performed within 48 hours of the collection of leaves.

The chemical treatment was a modification of the method used by Arnold (2007) [2]. The work sequence consisted of washing the *C. angustifolia* leaves with a solution of distilled water and neutral detergent and cutting them into fragments of approximately 0.5 $cm^2$. Subsequently, the fragments were immersed for 1 minute in 70% ethanol and rinsed with sterile distilled water. Then, they were immersed for 2 minutes in 1% sodium hypochlorite and rinsed with sterile distilled water

for 2 minutes. Finally, 20 leaf fragments were plated in carrot agar with 3 repetitions per each tree. In total, 2700 fragments (60 fragments x 45 trees) were used in this study. The plates were sealed with paraffin film and incubated at 26 ± 2°C for seven days.

Identification of the taxa was carried out at the genus level [8, 26, 20, 12], based on cultural characteristics and morphology of fruiting bodies and spores.

### Data analysis

The infection rate was calculated as the total number of plant tissue segments infected by fungi divided by the total number of plant segments incubated [36].

The relative frequency of colonization was calculated as the number of host individuals of a fungal taxon isolated divided by the total number of all host individuals [47].

The diversity of endophytes was estimated using the Shannon-Wiener index. Comparisons between the Means of the Shannon index for each site were calculated with non-parametric Kruskal-Wallis tests [18].

A computer program, EstimateS (version 9.1.1), was used to calculate species accumulation (rarefaction) curves and to estimate the number of potentially undetected taxa with the bootstrap estimator of species richness [16, 25].

The similarity of the endophytic communities between the three sites was evaluated with the Jaccard similarity coefficient [25] .

## 3. RESULTS

### Composition of the endophyte community

A total of 1978 isolates were recovered from 2700 fragments from the host tree species. Of these, 544 isolates were from the urban site, 746 from the peri-urban site and 688 from the rural site. Overall infection rates were: 60% for the urban site, 83% for the peri-urban site, and 76% for the rural site (Table 1).

The Shannon diversity indices of endophytic fungi from high to low were urban site (1.65) > peri-urban site (1.42) > rural site (1.17). There were significant differences between the estimated Shannon indices (H=29; p=<0.0001) for all three sites.

**Table 1.** Overall infection rate, Shannon diversity index (H') and species richness of endophytic fungi isolated from leaves of *C. angustifolia*.

| | Urban | Peri-urban | Rural |
|---|---|---|---|
| N° Host Individuals | 15 | 15 | 15 |
| N° Fragments | 900 | 900 | 900 |
| Richness | 15 | 13 | 16 |
| N° Fragments infected | 544 | 746 | 688 |
| Overall Infection Rate | 0.60 | 0.83 | 0.76 |
| H' (Simpson index) | 1.17 | 1.42 | 1.65 |

*Alternaría* was the dominant genus in all three sites. In the urban site, *Guignardia*, *Penicillium* and *Plenodomus* were sub-dominant. In the peri-urban site *Diaphorte-Phomopsis*, *Cladosporium* and *Phymatotrichum* were sub-dominant, whereas in the rural site *Diaphorte-Phomopsis* and *Alternaría* were co-dominant, and *Phymatotrichum* and *Xylaria* were sub-dominant (Table 2).

**Table 2.** Frequencies of endophytic taxa of *C. angustifolia*, in the environmental gradient.

| Endophytes | Frequency (*F*) per site | | |
|---|---|---|---|
| | Urban | Peri-urban | Rural |
| *Alternaría* | 0.87 | 1 | 0.93 |
| *Armillaria* | 0.13 | - | - |
| *Blastomyces* | 0.13 | - | - |
| *Botrytis* | - | 0.07 | 0.13 |
| *Cercospora* | - | 0.27 | - |
| *Cladosporium* | 0.13 | 0.47 | 0.6 |
| *Colletotrichum* | - | 0.2 | 0.33 |
| *Coniothyrium* | - | - | 0.13 |
| *Lasiodiplodia* | 0.07 | - | - |
| *Glomerella* | 0.07 | 0.13 | 0.33 |
| *Gnomonia* | - | 0.13 | 0.2 |
| *Guignardia* | 0.33 | - | - |
| *Fusicoccum* | - | - | 0.13 |
| *Marsonnina* | - | 0.07 | - |
| *Nigrospora* | 0.13 | - | 0.07 |
| *Penicillium* | 0.27 | 0.13 | 0.27 |
| *Plenodomus* | 0.27 | - | - |
| *Periconia* | 0.07 | - | 0.07 |
| *Diaphorte-Phomopsis* | 0.07 | 0.67 | 0.93 |
| *Phymatotrichum* | 0.13 | 0.47 | 0.8 |
| *Rhizoctonia* | 0.07 | 0.2 | 0.2 |
| *Septoria* | - | - | 0.67 |
| *Xylaria* | 0.13 | 0.4 | 0.67 |

Species accumulation curves demonstrated that for the peri-urban and the rural sites the estimated richness reached an asymptote, whereas for the urban site the curve rose suggesting that for this site further sampling would recover more endophyte taxa (Fig.1). The observed richness values with bootstrap estimated richness values indicated that the 15 samples in each site captured 86 %, 92 %, and 93 % of the estimated endophyte richness of urban, peri-urban and rural sites, respectively.

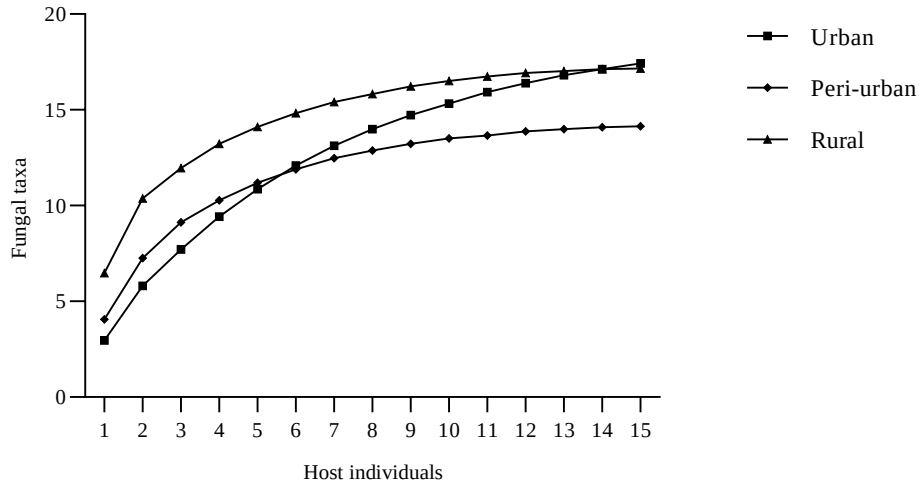


**Figure 1.** Species accumulation curves for fungal endophytes for the three sites.

When analyzing the similarities of the assemblages of the foliar endophyte communities in the environmental gradient with the Jaccard index, it was found that the urban and peri-urban site are the most similar, sharing 61% of the identified taxa, the urban site shares 47% of the taxa with the rural site and 40% of the registered genera with the peri-urban site.

## 4. DISCUSSION

*C. angustifolia* is cited for the first time as a host for foliar entophytic fungi in an environmental gradient in Jujuy province, Argentina. The overall infection rate was high in all three sites. Endophytes such as *Colletotrichum*, *Diaporthe-Phomopsis*, *Xylaria*, *Rhizoctonia*, *Pestalotia*, *Penicillium* and *Nigrospora*, were previously reported in trees of the Meliaceae family, and for tropical and subtropical climates [22, 4, 11, 7, 39].

The observed and estimated species accumulation curves became asymptotic, demonstrating an adequate sampling effort for cultivable endophytes [31]. Observed species accounted for more than 86% of the species calculated by the richness estimators, suggesting that the sample effort was sufficient. Our data coincide with the endophytic range colonization in tropical trees [3, 5].

In concordance with Wolfe (2019) [51], *Alternaría* was the most frequent genus in the case of urbanized sites, probably because it is tolerant to vehicular pollution along with *Cladosporium*.

The presence of *Xylaria* in the environmental gradient may be associated with the temporality of the leaves collected in early Autumn, during which time the decomposition of the cell wall begins, since *Xylaria* plays a primary role in the degradation of organic matter.

The *Diaphorte-Phomopsis* complex was recovered in the three sites, with higher frequencies of colonization in the rural site. In woody plants, *Diaporthe* species produce chemicals implicated in the pest ecology of their hosts [48]. *Colletotrichum* is cited as a dominant foliar endophyte with some pathogenic species, for *C. angustifolia* there is no history of diseases caused by *Colletotrichum*, which suggests that when registered it was in an entophytic state and not latent in a phase of an infection cycle.

Our studies indicate that *Alternaría*, *Diaporthe-Phomopsis*, *Phymatotrichum*, *Cladosporium* and *Xylaria*, would represent generalist endophytic fungi for *C. angustifolia*, in concordance with Govinda (2013) and Stone (2004) [24, 46].

Differences in the composition of the foliar endophytic fungal communities could be observed among the three sites. Ten genera were registered with environmental preference. In the urban site, five exclusive genera were identified: *Blastomyce*s described as a pathogen in humans and *Armillaria*, cited as a pathogen in coniferous and broadleaved trees; *Lasiodiplodia*, *Guignardia* and *Plenodomus*, include plant pathogenic species. For the peri-urban site, two exclusive genera were isolated: *Cercospora* and *Marsonnina*, both with phytopathogenic representatives of higher plants. For the rural site, three exclusive genera were identified: *Coniothyrium* and *Fusicoccum*, both with phytopathogenic or saprophytic representatives of wood, and *Septoria* which is mentioned in the literature as pathogenic for some plants.

According to the Shannon diversity index, the rural site is the most diverse, possibly due to the geographic scale analyzed [50], the characteristics of climate, vegetation and soil types, all factors that would likely affect this assemblage [33, 40]. There is evidence that the surrounding vegetation can favor certain species of fungi, which will later dominate the spore communities in the air and with it the endophytic colonization [6, 15, 17]. The lowest diversity was registered in the urban site. Foliar endophytic fungal communities are likely to be sensitive to air pollution [32, 28, 44], such as accumulation of heavy metals [37].

In Northwest Argentina, different *Guignardia* species were found as endophytes, among them *G. citricarpa*, a pathogenic species in *Citrus.* Its asymptomatic presence may indicate that it is in the incubation stage of the disease it causes [19]. *G. mangiferae*, is another common species in *Citrus* without pathogenic potential [9]. In this study, *Guignardia* was found exclusively in the urban site. This may be due to the origin of these urban trees, the infection by *Guignardia* may have happened during the nursery phase, as it occurs in *Citrus* [10].

This is the first work on foliar endophytic fungi for *C. angustifolia* and it constitutes a basis for future studies of host-microbiome interactions.

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