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Fungi associated to *Platanus x acerifolia* in Uruguay and failure indicators

Hongos asociados a *Platanus x acerifolia* en Uruguay e indicadores de probabilidad de falla

Fungos associados a *Platanus x acerifolia* no Uruguai e indicadores de probabilidade de falha

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Abstract

In the last few years, the interest in keeping the city trees healthy has increased in order to improve their survival and minimize claims due to potential accidents. The pest and diseases, the pollution, and the climate change together with the little genetic diversity of trees in urban areas are some of the factors that contribute to increase the likelihood of death and/or failure of trees in the cities. This work is part of a sanitary and risk of failure assessment of plane street trees (*Platanus x acerifolia)* carried out between 2019 and 2020. A random sample of 10 city blocks and their 193 plane trees was selected. In these, the presence of cankers, abnormal bark colorations, deformations, and a series of structural attributes that determine likelihood of failure variables were registered. The proportion of individuals with each symptom and the severity main index (SMI) were calculated as a weighted average of the different severity (SEV) levels in the total of evaluated plants. The severity indices were determined according to trunk or branches circumference and the portion of the tree affected (1st, 2nd or 3rd portion from the base). Deformations presented the main incidence (0.6), SMI (1.68) and a correlation with the presence of damages and human injuries. The presence of cankers and reddish bark were the symptoms that most affected the density of the crowns.

Keywords: cankers, hypertrophy, urban trees, tree defects

Resumen

En los últimos años el interés por el mantenimiento de la salud de los árboles de la ciudad ha ido incrementando para mejorar su sobrevivencia, así como para minimizar reclamaciones por posibles accidentes. Las enfermedades y las plagas, la polución y la aceleración del cambio climático junto con la poca diversidad genética de los árboles en zonas urbanas son algunos de los factores que contribuyen a aumentar la probabilidad de muerte y/o falla de árboles en las ciudades. Este trabajo es parte de una evaluación sanitaria y de riesgo de plátanos (*Platanus x acerifolia)* ubicados en veredas, realizada entre 2019 y 2020. Se seleccionó una muestra al azar de 10 manzanas con 193 plátanos. En estos, se registró la presencia de cancros, coloraciones anormales de corteza, deformaciones y una serie de variables estructurales indicadoras de probabilidad de falla. Se calculó la proporción de individuos con cada síntoma y el índice medio de

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severidad (IMS) como un promedio ponderado de los diferentes niveles de severidad (SEV) en el total de las plantas evaluadas. Los índices de severidad se determinaron en función de la circunferencia del tronco o las ramas y de los tercios afectados del árbol (1.er, 2.do y 3.er tercio desde la base). Las deformaciones presentaron los valores máximos de incidencia (0,6), de IMS (1,68) y correlación con la presencia de heridas y daños antrópicos. La presencia de cancros y corteza rojiza fueron los síntomas que más afectaron la densidad de las copas.

Palabras clave: cancros, hipertrofia, arbolado urbano, defectos en los árboles

Resumo

Nos últimos anos tem aumentado o interesse na manutenção da arborização urbana a fim de melhorar sua sobrevivência e minimizar reclamações devido a potenciais acidentes. Pragas e doenças, poluição, y mudanças climáticas junto com a pouca diversidade genética das árvores em áreas urbanas são alguns dos fatores que contribuem para aumentar a probabilidade de morte e ou falha de árvores nas cidades. Este trabalho faz parte de uma avaliação sanitária e de risco de falha de plátanos localizados em calçadas (*Platanus x acerifolia*), realizada entre 2019 e 2020. Foi selecionada uma amostra aleatória de 10 quarteirões e seus 193 plátanos. Para cada indivíduo, foi registrada a presença de cancros, colorações anormais da casca, deformações e também uma série de atributos estruturais indicadores de probabilidade de falha. Calculou-se a proporção de indivíduos com cada sintoma e o índice principal de severidade (SMI) como média ponderada dos diferentes níveis de severidade (SEV), no total de plantas avaliadas. Os índices de severidade foram determinados de acordo com a circunferência do tronco ou galhos e a porção da árvore afetada (1ª, 2ª ou 3ª porção da base). As deformações apresentaram os valores máximos de incidência (0,6), SMI (1,68) e correlação com presença de danos e lesões humanas. A presença de cancro e casca avermelhada foram os sintomas que mais afetaram a densidade das copas.

Palavras chave: cancro, hipertrofias, árvores urbanas, defeitos em árvores

1. Introduction

The green areas and urban natural elements are no strangers to the effects of global climate change, pollutants, biological invasion, and pests and diseases[1]. Although inserted in an environment in which they are not the predominant element, sharing the space with abundant buildings and other services typical of cities, the city trees integrate an ecosystem with its own peculiarities[2]. The urban forestry offers numerous benefits to the population, such as reduction of atmospheric pollution, regulation of air temperature, mitigation of city noise, regulation of water cycles, generation of recreational spaces, and the positive impact on human health in addition to the beautification of the streets[3-4]. The interaction between the different components of the urban system —trees, pedestrians, urban infrastructure, utility services, vehicular traffic and streets— must keep a delicate balance to its better use and maintenance[2]. Otherwise, successive interference between trees and other urban components can lead to risky situations due to the intensive management of these to adapt to the urban space[5]. In this sense Coelho-Duarte and others[5] researched the attributes correlated to the likelihood of failure of trees in urban parks of Montevideo (Uruguay), where visual risk of failure and sanitary assessment were included in the studied methods.

In a review paper about pests and pathogens of *Platanus* spp. in urban forestry in Europe, Tubby & Pérez-Sierra reported symptoms attributed to *Ceratocystis platani*, and *Splanchnonema platani* as some of the most noteworthy pathogens affecting plane trees in Europe, as well as *Fimotiporia punctata* and *Inonotus rickii*, that also could cause canker and decay on trees[6]. However, no references were found for deformations manifested as bark fissures and hypertrophies.

Ceratocystis platani is a fungus that causes stain canker that was reported for the first time in the United States by Jackson and Sleeth, cited by Panconessi in 1999[7] and by Tsopelas and others in 2017[8], and was introduced in Europe during the Second World War in infected wooden crates. This fungus penetrates host tissue through wounds, causing canker and staining the surrounding bark with a bluish coloration. As it colonizes the xylem, it causes defoliation and branch die-back. The disease is present in many states of the United States and in most of European countries[6].

Splanchnonema platani is a fungal pathogen that produces damages in the upper surface of main branches and that evolves towards progressive death of tissues with the appearance of cracks. It results in branch death and increases the likelihood of branch failure[6]. Until now, neither *Ceratocystis* nor *Splanchnonema* have been reported in our

country, although they have been investigated in recent works[9]. To the best of our knowledge no studies on plane tree diseases have been done in South America.

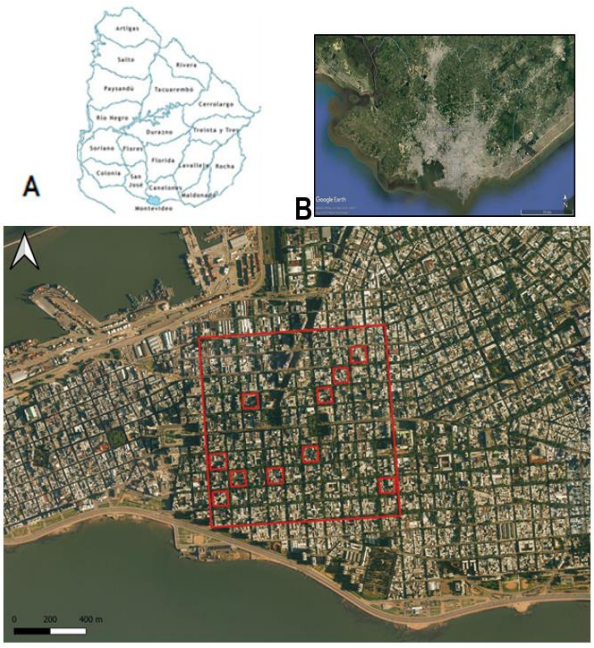
Frequent symptoms present in *Platanus x acerifolia* in Montevideo are trunk hypertrophies, generally associated with bark fissures[9], and appearing with different severity degrees (SEV). However, they can compromise the entire trunk. No references were found regarding these symptoms in *Platanus* spp.

The objectives of this work were: i) to quantify the symptoms of disease present on trunks, ii) to identify the fungi associated with them, and iii) to evaluate the relationship between observed symptoms and attributes correlated to the likelihood of failure of *Platanus* x *acerifolia* street trees in Montevideo, Uruguay.

2. Materials and methods

All the plane trees of 10 randomly selected city blocks among 100 city blocks at the B municipality of Montevideo, Uruguay, were selected for evaluation (Figure 1).

Figure 1. Map of Uruguay (A); Montevideo aerial view (B), and location of the area under study where the blocks analyzed are indicated (C)



For each *Platanus* x *acerifolia* tree, diameter at breast height (DBH), tree height, presence of symptoms and likelihood of failure attributes were evaluated according to Pokorny[10] (Table 1). Additionally, information about the phytosanitary status according to observed symptoms[11], crown size, density, and presence of epicormic shoots (ES)[12] sidewalk lift (SL), and defects such as tree lean (TL), cracks, cavities, dead and hanging branches, pruning wounds, man-made damages, and severed, damaged or girdling roots (Table 1) were also registered. Presence of cankers, bark with reddish patches (reddish bark), or with black patches (black bark), sap oozing, hypertrophies (H) and bark fissures (BF) were registered. The incidences of each symptom separately, of the syndrome of bark fissures and hypertrophies, and of the cankers and reddish bark were calculated as the proportion of plants that showed them (number of trees with symptoms/total number of trees). The severity (SEV) was estimated from the affected proportion of trunk or branches and the position of the tree where symptoms were observed. For each symptom four levels of affected circumference (0%,<25%, 25-50% and >50%) and three different heights (tree divided in thirds: from the bottom to the first half of the trunk is the 1st portion, the second half the 2nd portion, and the crown the 3rd portion) were combined to build four levels of SEV (healthy, slight, moderate, and serious) (Table 2). The Severity Main Index (SMI) was calculated according to the following formula:

$$\mathbf{SMI} = (0*N^{o}_{0} + 1*N^{o}_{1} + 2*N^{o}_{2} + 3*N^{o}_{3}) / N^{o}_{0}+N^{o}_{1}+N^{o}_{2}+N^{o}_{3}$$

were N^{o}_{0} is the number of trees without symptoms; N^{o}_{1} is the number of trees with slight SEV; N^{o}_{2} is the number of trees with moderate SEV; N^{o}_{3} is the number of trees with serious SEV; 0, 1, 2 and 3 are the SEV levels (Table 2).

The relationship between symptoms SEV and the severity categories (SCAT) of defects correlated to those likelihood of failure attributes was studied by estimating the Spearman correlation ($p<0.05$) to determine which of the former should be prioritized to treat and prevent.

Samples of vegetal tissue were taken from the transition zone between the healthy and the symptomatic parts of cankers. Samples were taken at three depths: superficially at the bark level, intermediate from phloem under the bark, and from heartwood (60-100 mm inside the trunk), by using a Pressler drill with a 4-mm-diameter bit, and also from a wooden roll of an already died tree. Each sample was carefully inspected to evaluate the presence of signs. Fragments of approximately 9 mm^2 of tissue were sterilized one minute in alcohol 96°, rinsed in

distilled sterilized water and left to dry on sterilized paper in flux before being sown in potato dextrose agar growing medium (PDA, Oxoid Ltd., Hampshire, England). After a week of incubation at 24°C, the developed fungal colonies were examined: morphotypes and frequency of appearance (number of records of each morphotype in relation to the number of colonies developed) were registered. Some of the morphotypes were incubated under 12 hours of UV light A with 350 nm of wavelength to stimulate the development of reproductive structures that allow identifying the genus. For morphological identification at the genus level, fungal identification keys were used[13-14].

Table 1. Severity categories (SCAT) of factors associated with likelihood of failure

	Categories			
Variables	**0**	**1**	**2**	**3**
Phytosanitary status(*)		Good	Regular	Poor
Crown size(**)		Full	Moderate	Very sparse
Crown density(**)		Very dense	Average	Very sparse
Epicormic shoots(**) (ES)	without ES	Slight	Moderate	Severe
Sildwalk lift (SL) and Lean tree (LT) (***)	Without SL neither LT	with SL without LT	without SL with LT	with SL with LT
Severed, damaged or Girdling roots(***)	Without	up to 25%	between 26 and 50%	more than 50%
Cracks(***)	Without	up to 25%	between 26 and 50%	more than 50%
Cavities(***)	Without	up to 25%	between 26 and 50%	more than 50%
Dead and hanging branches(***)	Without	up to 25%	between 26 and 50%	more than 50%
Pruning Wounds(***)	Without	up to 25%	between 26 and 50%	more than 50%
Man-made damages(***)	Without	up to 25%	between 26 and 50%	more than 50%

Adapted from: (*)Vallejos[11]; (**)Sepúlveda and Johnstone[12]; (***)Pokorny[10]

Table 2. Severity (SEV) levels to evaluate symptoms

% of affected circumference	Position of the symptom	level of SEV
0: healthy	0	Attribute absent
1: <25%	1: 1st third	1: mild
1: <25%	2: 2nd third	1: mild
1: <25%	3: 3rd third	2: moderate
2: 25-50%	1	2: moderate
2: 25-50%	2	2: moderate
2: 25-50%	3	3: serious
3: > 50%	1	3: serious
3: > 50%	2	3: serious
3: > 50%	3	3: serious

After identification, the isolates were included in the collection of the Plant Protection Department of the Agronomy School, University of the Republic.

For representative colonies of the more frequent morphotypes a DNA extraction from 10-days-old mycelia was performed following the protocol described by Paolocci and others[15] with some modifications. They were macerated with lysis buffer (100ul/ml TRIS, HCl 200 mM pH7, 100ul/ml NaCl 250 mM, 100 ul/ml SDS 0,5%, 100ul/ml EDTA 25 mM + NaOH 10%, 600ul bidistilled water) and placed for 2 hours at -20°C. After that, mycelia were crushed with sterilized micropestle and treated with the lysis buffer, sodium chloride and isopropanol (-20°C) with the corresponding homogenizations, temperature incubation and centrifugations. Subsequently, the supernatant was discarded, and the pellet containing was rinsed with 70% alcohol, treated with a buffer TE (10 mM TRIS, HCl pH7.4 + 1mM EDTA pH8) and stored at -20°C.

The DNA was amplified by performing polymerase-chain reaction (PCR) using a PTC-100 Peltier Thermal Cycler. Based on the morphological identification to genus level, different DNA regions were selected for amplifications. The internal transcribed spacer of the ribosomal DNA (ITS) was amplified for some isolates that could not be identified by morphology, since this region is universal for the Fungi kingdom[16]. For those isolates for which the genus could be identified by morphology, other primers recommended in the literature were used. For example, amplification of the region corresponding to glyceraldehyde-3-phosphate dehydrogenase genes (GAPDH) was performed for isolates within *Colletotrichum* genus[17], and the elongation factor 1-α gene (EF-1α) was amplified for isolates within *Botryosphaeria*[18-19] and *Pestalotiopsis* genus[20]. Details of the specific primers and amplification cycles can be found in Table 3. PCR products were analyzed on 1.5% agarose gels stained with GelRedTM and visualized in a transilluminator under UV light. Gene Ruler plus 100 bp DNA (Thermo, Lithuania) was used as a molecular marker. PCR products were purified and sequenced using only forward primers at Macrogen Inc., Seoul, Korea. The sequences were aligned with the ClustalW program with sequences of the gen bank. For species identifications the sequences were compared with the

subjected to BLAST, those deposited in NCBI GenBank on the basis of percent identity values and query coverage.

Table 3. Primers and cycles used for fungal DNA amplification

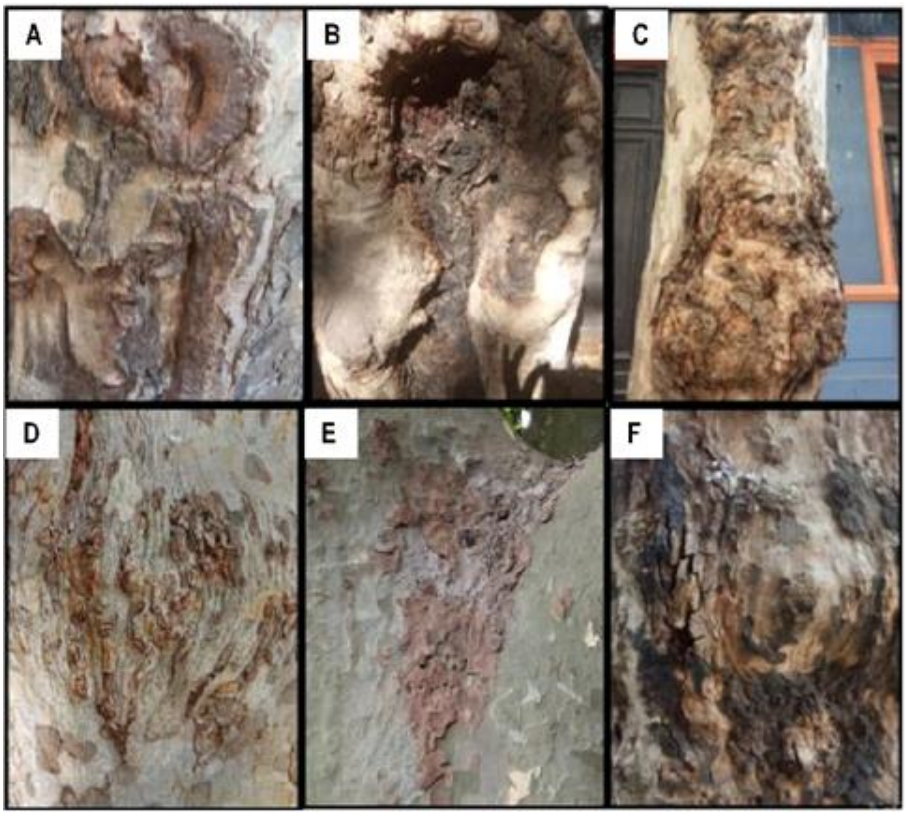
Genus	Locus	Primers	DNA primer sequence	ThermocyclerAmplification cycle Cycle
No identified	ITS (12)	ITS1	TCCGTAGGTGAACCTGCGG	94 ° C for 3 min, 35 cycles at 94 ° C for 30 s, 57 ° C for 50 s and 72 ° C for 45 s
		ITS4	TCCTCCGCTTATTGATATGC	Final extension of 72 ° C for 10 min.
Colletotrichum	GAPDH (13)	GDF	GCCGTCAACGACCCCTTCATTGA	94 °C for 5 min, 35 cycles at 94 °C for 45 s, 52 °C for 30 s and 72 °C for 45 s.
		GDR	GGGTGGAGTCGTACTTGAGCATGT	Final extension of 72 °C for 10 min.
Botryosphaeria	EF 1-α (14)	EF728	CATCGAGAAGTTCGAGAAGG	94 °C for 5 min, 35 cycles at 95°C for 30 s, 55 °C for 45 s,and 72 °C for 1 min.
		EF986	TACTTGAAGGAACCCTTACC	Final extension of 72 °C for 10 min.
Pestalotiopsis	EF 1-α (15)	EF526F	GTCGTYGTYATYGGHCAYGT	94 °C for 5 min, 35 cycles at 95°C for 30 s, 55 °C for 45 s, and 72 °C for 1min.
		EF1567R	ACHGTRCCRATACCACCRATCTT	Final extension of 72 °C for 10 min.

3. Results

From the 202 randomly selected trees, 197 were evaluated, because some specimens were located in construction areas where complete observations were not possible.

The incidence and SEV main index (SMI) of each symptom and of the association between cankers and reddish bark, and between bark fissure and hypertrophy are shown in Table 4; and it may be noticed that the highest values of incidence and SEV main index correspond to bark fissures and hypertrophy. The appearance of some mentioned symptoms is shown in Figure 2.

Figure 2. Recorded symptoms. Cankers (A, B); stretch marks and hypertrophies (C); stretch marks (D) reddish bark (E); black bark (F)



The highest correlation was observed between bark fissures and hypertrophies; then these symptoms were also considered together (Table 5).

The overall phytosanitary status[13] had the highest significant ($p<0.05$) correlation with SEV of bark fissures, hypertrophies and both symptoms considered together (Table 5). That variable also presented positive and significant correlation with cavities, man-made damage, dead branches, reddish bark, and the symptom of reddish bark and cankers considered together. The reddish bark only had significant correlation with dead branches, while the reddish bark and cankers considered together also had a significant relationship with crown density. The SEV of black bark showed a significant and positive correlation with the SCAT of pruning wounds, SCAT cavities, SCAT man-made damages and SEV bark fissures.

Table 4. Incidence and severity main index of the symptoms recorded

Symptoms	Incidence	SMI
Cankers (C)	0,15	0,31
Reddish bark	0,15	0,25
Reddish bark and C	0,25	0,51
Black bark	0,34	0,59
Sap oozing	0,04	0,04
Hypertrophies (H)	0,54	0,98
Bark fissures (BF)	0,46	0,96
H and BF	0,61	1,17

Table 5. Significant correlations between recorded variables

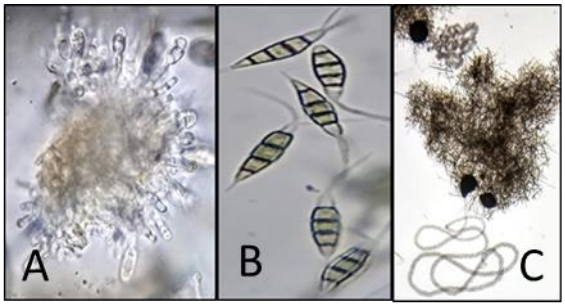
Variable(1)	Variable(2)	N	Spearman	p-value
SCAT Phytosanitary status	SCAT Crown density	191	0,22	0,00195
	SCAT Girdling roots (%)	187	-0,18	0,01162
	SEV reddish bark	193	0,2	0,00428
	SEV canker and reddish bark	193	0,2	0,00541
	SEV BF	193	0,45	<0,0001
	SEV H	193	0,35	<0,0001
	SEV BF and H	193	0,38	<0,0001
	SCAT cavity	184	0,27	0,00024
	SCAT dead branches	185	0,18	0,01572
	SCAT man-made damage	184	0,2	0,00764
	SCAT Roots severed / damaged (%)	187	-0,16	0,02509
SEV canker	SCAT Crown size	191	0,17	0,02233
	SCAT Crown density	191	0,22	0,0028
SEV reddish bark	SCAT dead branches	185	0,18	0,01718
SEV canker and reddish bark	SCAT Crown density	191	0,21	0,00374
SEV black bark	SCAT bark fissures	193	0,21	0,00369
	SCAT cavity	184	0,19	0,00843
	SCAT pruning wounds	185	0,24	0,00107
	SCAT man-made damage	184	0,15	0,04262
SEV BF1	SEV black bark	193	0,21	0,00369
	SEV hypertrophy	193	0,68	<0,0001
	SCAT pruning wounds	185	0,16	0,02458
	SCAT man-made damage	184	0,22	0,00297
	Roots cut / damaged (%)	187	-0,17	0,02184
SEV H2	Strangling roots (%)	187	-0,17	0,0196
	SCAT pruning wounds	185	0,16	0,02588
	SCAT man-made damage	184	0,3	<0,0001
SEV BF and H	SCAT pruning wounds	185	0,15	0,0488
	SCAT man-made damage	184	0,24	0,00128

[1]BF: bark fissures;[2] H: hypertrophies. Non-significant correlations are not shown.

The SEV of the bark fissures, hypertrophies and of both considered together had significant and positive correlations with the category of severity (SCAT) of pruning wounds and man-made damages. Neither significant correlation with the sidewalk lift was not found, nor in the case of the epicormic shoots with none of the recorded symptoms.

From the tissue samples obtained from cankers and reddish bark, 59 fungal colonies and 10 morphotypes were obtained. Among them, the 3 most frequent were 42 colonies out of the 59. Based on morphological characters (mycelium colour and texture, type of spores, presence/absence of fruiting bodies, etc.) genera such as *Pestalotiopsis, Diplodia, Epicoccum* and *Phomopsis* were identified (Figure 3).

Figure 3. Reproductive structures observed. *Diplodia* sp. sporodochium (A); conidia of *Pestalotiopsis* sp. (B); pycnidium and tendrils of *Phomopsis* sp. (C)



Samples from heartwood showed rots with pink and grey coloration (Figure 4). From these samples, 12 of the obtained colonies were identified as *Colletotrichum acutatum*, and the remaining 3 to the genera *Ophiostoma, Nigrospora* and *Aureobasidium*.

From the analysis of sequences several genera were confirmed, and the following species were identified*: Neofusicoccum parvum*, *Diplodia mutila*, *Diplodia pseudoseriata*, *Pestalotiopsis biciliata*, *Pestalotiopsis rhodomyrtus* and *Colletotrichum acutatum* (Table 6). The most frequent morphotypes corresponded to *Pestalotiopsis* spp., *Diplodia* spp. and *Neofusicoccum* spp. The genera *Phomopsis, Phoma, Nigrospora, Aureobasidium, Epicoccum* and *Torula* were identified in a very low frequency.

Figure 4. Wood samples with rot with pinkish and greyish coloration

Table 6. Identified species with sequence analysis

Isolation	Identified species	Gen Bank reference	% indentity
XXVI	Colletotrichum acutatum	JQ948695.1	99,45
XXX	Colletotrichum acutatum	JQ948695.1	99,49
XXXIV	Diplodia pseudoseriata	EU863179.1	100,00
XXXIII	Diplodia pseudoseriata	EU863181.1	98,81
XXXII	Neofusicoccum parvum	MG192343.1	100,00
II	Neofusicoccum parvum	MG192343.1	100,00
IV	Diplodia mutila	AY573219.1	100,00
VI	Pestalotiopsis rhodomyrtus	KX895198.1	99,74
X	Diplodia mutila	MT587356.1	99,33
XI	Pestalotiopsis biciliata	MH554019.1	100,00
XVI	Pestalotiopsis grevilleae	NR_147548.1	100,00
XVII	Neofusicoccum parvum	MT509799.1	100,00

Table 7. Frequency of identified fungi from bark and wood

Sample method	Genus and species	Frecuency
Reddish cankers	Neofusicoccum parvum	15
59 colonies	Diplodia mutila	14
	Pestalotiopsis spp	13
	Epicoccum sp	2
	Torula sp	1
	5 morphotypes NI	14
Wood (drill)	Colletotrichum acutatum	12
15 colonies	Aureobasidium sp	1
	Nigrospora sp	1
	1 morphotype NI	1
Wood (rolo)	Neofusicoccum parvum	6
6 colonias		

4. Discussion

Although some of the symptoms evaluated in this study resemble those previously described as being caused by *Ceratocystis platani* or by *Splanchnonema platani*[6-9][21-23], none of these pathogens were previously reported in our region neither isolated from the samples processed in this work. Similarly, Pelleret and others[24], studying plane trees with symptoms like those caused by *C. platani* (cankers and dieback, trunk, and branch necrosis), mainly isolated *Neofusicoccum parvum* and *Diplodia pseudoseriata* among others *Botryosphaeriaceae*, and isolated *Ceratocystis platani* only from 2 trees (out of 6 symptomatic trees).

Considering the results of Turco and others[25] and Kurbetli and others[26], it can be observed that the mentioned symptoms can also be caused by other genera, those ones belonging to the *Botryosphaeriaceae* family. These include many genera that are also related to branch and trunk canker, necrosis, wood discoloration, branch dieback on fruit trees[19][27-28] and forest trees[29-34]. Species belonging to this family are also reported as endophytes[35-36]. Pelleret and others[24] hypothesize that these *Botryosphaeriaceae* species could be responsible for the observed cankers on plane trees, while other fungal species could contribute to the dieback symptoms. Similarly to Pelleret and others[24], during this study *Diplodia mutila* and *Neofusicoccum parvum* were isolated from symptomatic tissue of *Platanus x acerifolia*. *D. mutila* was reported causing die-back, cankers, necrosis, dead branches and twigs in *Quercus*[32], dieback in grapevine[28], cankers and dieback in araucarias[30], and cankers, dieback, and internal necrosis in walnut[33]. *Diplodia pseudoseriata* also was reported causing cankers, branch dieback and fruit rots in apples[18], and cankers and branch dieback in citrus[37].

Neofusicoccum parvum was reported as associated to cankers and dieback in eucalyptus in Spain and in Mexico[32-33], to cankers and die back in grapevine and sequoia[28][34], and was also reported together with *D. pseudoseriata* causing cankers and branch dieback in citrus[27].

Regarding the isolation of *Pestalotiopsis* spp. and of *Colletotrichum* spp., no previous report has been found about their pathogenicity on *Platanus* spp.[38]; however, Leite and others[39] found *Pestalotia* spp., the closest genus related to *Pestalotiopsis* and that belongs to the *Pestalotia-Pestalotiopsis* complex[40], as epiphytes in *Platanus orientalis* bark in very low frequency, but working with different culture media than the one used in the present work.

Among *Colletotrichum*, there are species considered nonpathogenic endophytes, and other that are considered to host specific necrotrophic pathogens. Several fungal lineages change from endophytes to pathogens on short time scales, including Colletotrichum species[41-42]. Although the species *C. gloeosporioides* is the most mentioned as pathogenic associated to senescent tissues, at present, probably due to advances in identification techniques, it is *C. acutatum* the one that appears to predominate as pathogenic, and in some cases, as in citrus and coffee, associated to symptoms in flowers causing fruit drop[42]. More recently, *C. gloeosporioides* and *C. karstii* associated to dieback of citrus branches have been reported[43].

Regarding the genus *Pestalotiopsis*, belonging to the *Pestalotiopsidaceae* class, it includes species recognized as pathogens[44-45], but also endophytes, and, lately, species that produce metabolites for medicinal use are being studied[46-47].

The possibility that several of the species identified in this work were endophytes[35-36] raises questions regarding their role: so, it is necessary to accomplish Koch postulates, and to analyse healthy bark and wood tissue to determine if they performed as endophytes or as pathogens.

The bark fissures and hypertrophy are associated to each other and to the black bark (Table 5). At the same time, they have positive correlation with the pruning wounds and with the man-made damages, suggesting that it is an urban disorder. Although the cause of bark fissures and hypertrophy considered together is not known yet, the tree workers and managers from Montevideo do not associate it with failure of branches or trees. This coincides with the fact that no significant correlation has been found between these symptoms and the presence of dead or hanging branches, cracks and cavities, indicators

associated to the likelihood of failure of the tree or parts of it[5][12]. No pathogen sign was observed, but the symptoms are very similar to those caused by *Xanthomonas populi* pv. *Populi*[48] in *Populus*, so more studies must be undertaken to determine if the ones recorded in the trees studied in this work could have been generated by any *Xanthomonas* spp.

Although the phytosanitary status was correlated with bark fissures and hypertrophy, in the present work insufficient evidence was found to prove that these deformations affect the health of the specimens and could not be a further damage than aesthetic.

The significant correlation of the cankers and reddish bark with the reduction of crown density, as well as of the reddish bark with the greater presence of dead branches could indicate that these symptoms may be the cause of dieback, an indicator associated to the likelihood of failure of branches[5]. However, considering the correlation values (Table 5), it is necessary to deepen the study of this relationship.

5. Conclusions

The fungal genera more frequently isolated were *Pestalotiopsis*, *Diplodia*, *Neofusicoccum* and *Colletotrichum*.

The cankers and reddish bark affect the urban trees shadow; however they are not associated to each other.

The symptoms attributed in scientific literature to *Ceratocystis platani* also can be caused by other fungal pathogens.

The black bark symptom, and bark fissures and hypertrophy considered together appeared associated to the presence of pruning wounds and man-made damages, so the latter may be the cause of the formers or act predisposing the trees to express them.

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Author contribution statement

Agueda Claudia Scattolini Rimada: conceived, designed, collected information, performed phytopathological analyzes, analyzed and wrote the article.

Ana Paula Coelho Duarte: conceived, designed, collected information, performed statistical analyzes, analyzed and wrote the article.

Caracé Torrano: collected information, performed phytopathological analyzes.

Valeria Cazzola: conceived, designed, collected information, performed phytopathological analyzes.

Pedro Larramendy: conceived, designed, collected information, performed phytopathological analyzes.

Allison Silvera: conceived, designed, collected information, performed phytopathological analyzes.

LizandraParins: conceived, designed, collected information, performed phytopathological analyzes.

Victoria Moreira: carried out molecular analysis and contributed in writing the article.

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