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# Screening of xylophagous fungi associated with *Platanus acerifolia* in urban landscapes: Biodiversity and potential biodeterioration

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ABSTRACT

Trees in urban landscapes provide a wide range of benefits to the environment; however, they are exposed to several stress factors that can make them vulnerable to decay by fungi. The presence and identity of wood decay Basidiomycetes affecting *Platanus acerifolia*, a common tree used in cities, were evaluated in sites with different levels of urban disturbances in order to analyze the relationships between human disturbance level, tree age, fungal pathogens and their degradative potential. We carried out morphological and cultural descriptions of the fungi detected, and studied their decay capacity. Eight species of Basidiomycetes were detected, being *Inonotus rickii* the most frequently isolated and the most widely distributed in the areas sampled. *Bjerkandera adusta*, although rarely detected, caused the greatest loss of dry weight. In some cases phylogenetic analyses were performed under both static and dynamic homologies. The age of the trees (estimated from DBH values) sampled seemed to be more important as a predisposing factor for decay than anthropogenic disturbance of sites. The correlation between tree age, presence and identity of fungi, degradative potential and environment conditions is discussed.



## 1. Introduction

Trees can provide a wide range of benefits to the urban environment and well-being of people, mitigating many of the impacts of the development of cities. They are able to moderate climate, reduce noise levels, improve air quality, lower rainfall runoff and flooding, reduce building heating and cooling energy needs and provide a habitat for many animals, as birds and insects. In addition, they possess an aesthetic value (Nowak and Dwyer, 2007). However, trees in the city are exposed to stress factors that can affect their health. Pollution, lack of space for their growth, lack of availability of nutrients, mechanical injuries and disturbances due to constructions contribute to reduce plant vigor (Sæbø et al., 2005), thus increasing the possibility of entrance of wood-decay fungi in the trees, which can severely decrease their stability and fracture-resistance (Schwarze et al., 2000). Decay does not necessarily mean immediate death of the trees because the process may extend over several years, but in urban areas the risk of accidents involving people or properties could be important (Terho and Hallaksella, 2005).

Taking all these facts into account, inventory works about wood-decay fungi and their putative relationship with the factors mentioned are necessary to estimate the potential hazard of wood damage and thus improve the management and protection of urban trees.

Several studies about wood rot of urban trees reflect the impact of this problem in urban environments (Terho and Hallaksella, 2005, 2008; Terho et al., 2007). In Argentina, Mielnichuk and Lopez (2007), Sede and Lopez (1999a,b) and Wright and Iaconis (1955) have made the first contributions to this subject.

In addition, detection and identification of decay fungi by molecular tools has been used in several recent studies (Adair et al., 2002; Jasalavich et al., 2000; Jellison et al., 2003; Nicolotti et al., 2009). Techniques based on fungal detection are a promising alternative for specific, sensitive and rapid routine diagnoses. PCR-based methods using nuclear or mitochondrial ribosomal DNA (rDNA) loci have proven valuable for fungal detection and identification at different taxonomic levels (Guglielmo et al., 2007, 2010). In Argentina, Gottlieb et al. (2000, 2002) have used PCR methods and RFLP analyses to study species of *Ganoderma* and *Inonotus*. However, there are few studies of these techniques in relation with urban trees (Guglielmo et al., 2007, 2008, 2010).

Ash (*Fraxinus pennsylvanica*) and London plane (*Platanus acerifolia*) are the most frequent urban tree species in Buenos Aires City (Filippini et al., 2000). Whereas the former is not greatly affected

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**Table 1**
Indicators of relative disturbance of each Site sampled. The values represent the percentage of each indicator out of the total number of buildings. SH: shops; PS: petrol stations; SU: supermarkets; V/m: number of vehicles per minute.

| Site | Site A | Site B | Site C |
|---|---|---|---|
| SH | 1.4 | 43.2 | 17.9 |
| PS | 0.7 | 1.2 | 0.8 |
| SU | 0.7 | 1.2 | 0.8 |
| V/m | 4 | 47.6 | 4.1 |

by fungal decay, the latter is one of the most affected by fungal pathogens (Sede and Lopez, 1999b).

The aims of this work were: (i) to evaluate the presence and identity of decay fungi on *P. acerifolia* in relation with different levels of urban disturbances, (ii) to estimate the degradation ability *in vitro* of the isolated decay species and (iii) to test molecular techniques as taxonomic tools to study local populations of wood-rotting fungi.

## 2. Materials and methods

### 2.1. Study area, sampling design

The survey was carried out in Buenos Aires City, Argentina (34°36′43″S; 58°25′02″W), in three sites that showed different levels of human disturbance. A residential area (Site A) in Parque Chas and Agronomía neighbourhoods, a very disturbed site downtown (Site B) in Montserrat, San Telmo and Balvanera neighbourhoods (Perelman et al., 2006) and an industrial and residential area in Mataderos neighbourhood (Site C) (Plan Urbano Ambiental, 2000). The relative disturbance of each site sampled was estimated based on the percentage of an indicator out of the total number of buildings. The indicators used were: shops, petrol stations, supermarkets and number of vehicles per minute (Table 1). Samplings were developed from May to November 2007, and the areas were chosen according to the census of urban trees made in 2001 by the Government of Buenos Aires City.

Forty blocks with London plane trees were inspected in each site. A visual assessment of the trees (VTA) was made and the Diameter at Breast Height (DBH) of each tree was calculated. DBH values were used as indicators of relative age. In municipal street tree inventories, tree age is generally not included. DBH values do not imply the use of high technology and can be easily recorded (Linsen et al., 2005; Maco and McPherson, 2003).

### 2.2. Sampling and culturing

Among the trees inspected, wood samples, basidiomes and conidiomata were removed from standing trees (sampled trees) showing cankers or cavities in their trunks or lower branches by means of an increment hammer. Cavities and cankers are not necessarily related to the presence of wood-rotting fungi but they may constitute ways of entrance for fungi into the tree. The resulting wood, basidiomes and conidiomata samples were carried to the laboratory, where they were processed within 24 h, superficially sterilized (ethanol 50% for 30 s, sodium hypochlorite (55 g/l) 1:3 for 1 min, ethanol 50% for 30 s) and plated onto Malt Extract Agar (MEA) 1.2%. The resulting cultures were examined every 2–3 days in order to detect Basidiomycetes strains. The criteria applied for the selection of potential Basidiomycetes strains were the lack of sporulation, the presence of clamp connections and the appearance of the colony. The sampled trees from which Basidiomycetes strains were obtained were considered as colonized trees. Strains were incorporated to the culture collection of the Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires (BAFCcult). Basidiomes were annotated, dried and deposited in the mycological BAFC Herbarium.

### 2.3. Cultural studies

For the identification, Basidiomycetes strains were grown on MEA 1.25% (Difco Lab.) in the dark at 25 °C (Nobles, 1965), and macro- and micromorphological characters were recorded weekly for 6 weeks. Species were identified by means of keys based on mycelial characters. Oxidase reactions were performed using gallic and tannic acid agar media (Davidson et al., 1938) and tyrosine, paracresol and guaiacol agar media (0.2%) according to Boidin (1954). The relative intensity of the reaction, recorded one week after incubation in the dark at 25 °C, was indicated with plus or minus signs.

### 2.4. Molecular studies

The ITS1 and ITS2 regions from some BAFC cultures were amplified and sequenced. The nucleotide sequences determined in the present study were deposited in the GenBank DNA sequence database. DNA extraction was carried out according to Carmarán et al. (2009). Best amplification results were achieved by adding 6% bovine serum albumin (BSA, Promega Corp.) to the PCR reaction mix.

DNA sequences obtained were compared with sequences from GenBank. Sequences were edited and phylogenetic analyses under static and dynamic homologies were performed according to Carmarán et al. (2009). A sequence of *Amanita muscaria* (EU346871) was chosen as outgroup in all the analyses.

### 2.5. Degradation ability of the xylophagous strains

Loss in dry weight of *P. acerifolia* wood blocks was used to estimate the degradation ability of the decay fungi isolated (Levin and Castro, 1998; Mielnichuk and Lopez, 2007; Schubert et al., 2008). Wood blocks of 1 cm × 2 cm × 0.5 cm, including sapwood and heartwood, were cut from sound *P. acerifolia* branches. Blocks dried at 70 °C for 48 h were conditioned at 30 °C and weighed to determine the initial dry weight. Each block was then saturated by immersion in distilled water for 48 h and sterilized in an autoclave for 20 min at 105 kPa.

MEA 1.25% (Difco Lab.) slopes in 19 cm × 2 cm test tubes were inoculated with mycelial discs (0.6 diam) of one strain of each species (BAFCcult 3297, BAFCcult 3300, BAFCcult 3301, BAFCcult 3306, BAFCcult 3309, BAFCcult 3311, BAFCcult 3317, BAFCcult 3318 and BAFCcult 3319) and then incubated at 25 °C. Wood blocks without inoculum were used as controls and 19 replicates were arranged for each treatment. Once the mycelium covered the surface of the agar, one sterilized wood block was introduced into each test tube. After 3 months of incubation in the dark at 25 °C, wood blocks were removed from the test tubes and the surface mycelium was gently cleaned off. Blocks were dried at 70 °C for 48 h, then at 30 °C and weighed to determine the final dry weight. Initial and final dry weights were used to calculate the weight loss caused by decay according to Mielnichuk and Lopez (2007).

### 2.6. Statistical analysis

One-way ANOVA was performed to determine differences between treatments of dry weight loss (Sokal and Rohlf, 1995), using Statistix for Windows version 2.1. Data were transformed using $y' = y^{1/4}$ to achieve homogeneity and normality prior to statistical analysis. All means were analyzed by Tukey's HSD test.

**Table 2**
Number of inspected trees, sampled trees and colonized trees obtained from survey and sampling in each Site. IT: inspected trees; ST: sampled trees; CT: colonized trees. All percentages were calculated in relation to the total number of inspected trees.

| Sites | IT | ST | | Number of samples | CT | | Strains | Number of species |
|---|---|---|---|---|---|---|---|---|
| | | Number | % | | Number | % | | |
| Site A | 225 | 66 | 29.3 | 134 | 10 | 4.5 | 10 | 5 |
| Site B | 286 | 97 | 33.9 | 146 | 7 | 2.5 | 3 | 2 |
| Site C | 178 | 84 | 47.2 | 130 | 12 | 6.7 | 6 | 3 |
| Total area | 689 | 247 | 35.8 | 410 | 29 | 4.2 | 19 | 8 |

**Table 3**
Percentages of strains obtained from the following sources: wood, basidiomes/conidiomata or wood and basidiomes/conidiomata. Percentages of strains obtained from the following sources: wood, basidiomes/conidiomata or wood and basidiomes/conidiomata. All percentages were calculated in relation to the total number of strains obtained.

| Sites | Basidiomycetes strains (%) | | |
|---|---|---|---|
| | Only from wood | Only from basidiomes/ conidiomata | From wood and basidiomes/ conidiomata |
| Site A | 50 | 40 | 10 |
| Site B | 33.3 | 66.7 | 0 |
| Site C | 66.6 | 16.7 | 16.7 |

For analysis purposes, DBH values were grouped in ranges of 10 cm each and a chi-square test was performed to assess differences between them (Sokal and Rohlf, 1995).

## 3. Results

### 3.1. Survey and sampling

From a total of 689 *P. acerifolia* trees visually inspected across 120 blocks of Buenos Aires City, 36% (247 sampled trees) showed cankers and cavities in their trunks or lower branches. Nineteen Basidiomycetes strains, belonging to eight species, were recovered from wood, basidiomes and conidiomata samples.

In Site A, there were 66 sampled trees from 225 inspected trees, resulting in ten colonized trees. From a total of 134 samples taken (6 basidiomes and 128 wood samples), ten Basidiomycetes strains were obtained. Three of them were identified as *Coprinellus micaceus*, three as *Ganoderma resinaceum*, two as *Inonotus rickii* (*anamorph*: *Ptychogaster cubensis*) and one strain of each of the following species: *Phlebiopsis gigantea* and *Bjerkandera adusta* (Table 2). *C. micaceus* and *P. gigantea* strains were obtained only from wood samples, *G. resinaceum* and *B. adusta* strains were obtained from both wood and basidiomes samples whereas *I. rickii* strains were obtained only from basidiomes samples (Table 3).

In Site B, there were 97 sampled trees from 286 inspected trees, resulting in seven colonized trees. Two colonized trees presented deteriorated basidiomes and it was thus not possible to obtain pure cultures. Two other colonized trees presented conidiomata that seemed to belong to *P. cubensis*. From a total of 146 samples taken (2 conidiomata and 144 wood samples), three pure strains (two identified as *I. rickii* (*P. cubensis*) and one as *Peniophora laxitexta*) were obtained (Table 2). *I. rickii* strains were obtained only from conidiomata samples and *P. laxitexta* strain only from wood samples (Table 3).

Finally, in Site C, there were 84 sampled trees from 178 inspected trees, resulting in 12 colonized trees. Six of the colonized trees presented remains of basidiomes and it was thus not possible to obtain pure cultures. From a total of 130 samples taken (1 basidiome, 3 conidiomata and 126 wood samples), six pure Basidiomycetes strains (three identified as *I. rickii* (*P. cubensis*), two as *C. micaceus* and one as *Trametes trogii*) were obtained (Table 2). One of the strains of *I. rickii* and *T. trogii* strain were detected in the same tree. *I. rickii* strains were recovered only from conidiomata samples and the other strains only from wood samples (Table 3).

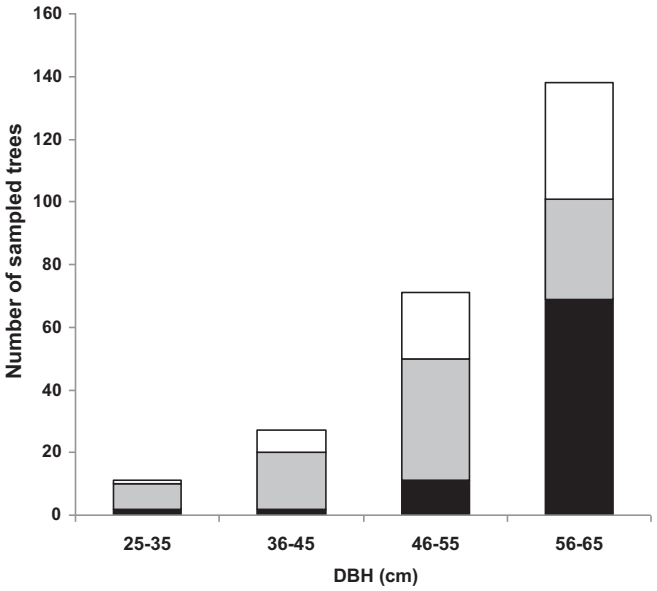


**Fig. 1.** Number of sampled trees at different Diameter Breast Height (DBH) values. White bars: Site A; grey bars: Site B; black bars: Site C.

*I. rickii* was the most frequent species, with a total of seven isolated strains. This was the only species recorded in all the sites sampled. The second most frequent species were *C. micaceus*, which was detected in five colonized trees in Sites B and C, and *G. resinaceum*, which was obtained from three colonized trees, all from Site A. The rest of the species were recorded only once (Table 2).

Most of the inspected trees in Sites A and C showed DBH values of 56–65 cm, corresponding to an estimated age of 70–80 years, according to Linsen et al. (2005). In Site B, on the other hand, we found a greater variation in the sizes of the inspected trees (DBH values between 25 and 65 cm), corresponding to estimated ages between 20 and 80 years (Linsen et al., 2005) (Fig. 1).

The presence of Basidiomycetes, considering basidiomes, conidiomata and those obtained from wood samples, was recorded mostly in large-diameter trees (DBH values of 46-65 cm). Ranges of DBH values differed significantly when analyzed by a chi-square test (ranges of 35–45 cm and 56–65 cm: $\chi^2 = 11.8$, $p < 0.001$, df = 1; ranges of 35–45 cm and 46–55 cm: $\chi^2 = 5.3$, $p < 0.05$, df = 1) (Fig. 2).

During a previous survey to adjust the sampling area, one strain of *Oxyporus latemarginatus* (BAFCcult 3306) and one of *C. micaceus* (BAFCcult 3218) were also detected. Since these strains were not obtained in the sampling area delimited, they were not included in the sites analysis.

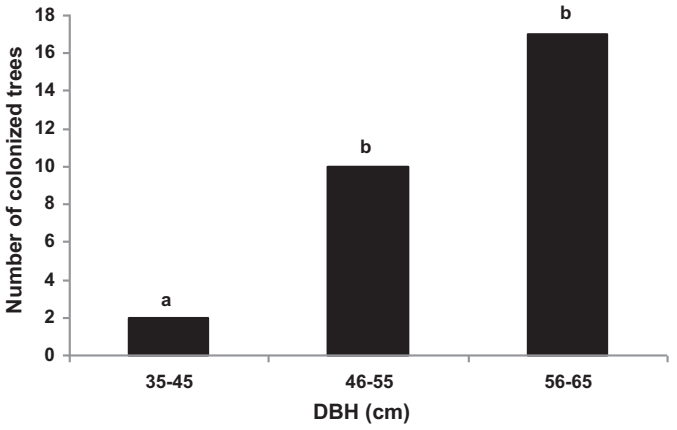


**Fig. 2.** Number of colonized trees at different Diameter Breast Height (DBH) values. Different letters over the bars indicate significant differences ($p < 0.05$).

**Table 4**
Mean (±SD) dry weight loss of wood blocks decayed by fungal species after a three-month treatment. Mean values of results of dry weight loss. SD: standard deviation. Different superscript letters behind mean values indicate significant differences ($p < 0.05$).

| Treatment | Mean (%) | SD (%) |
|---|---|---|
| *B. adusta* | 23.7[a] | 6.8 |
| *T. trogii* | 17.7[ab] | 4.6 |
| *O. latemarginatus* | 17.3[b] | 5.1 |
| *P. laxitexta* | 14.5[b] | 5.6 |
| *G. resinaceum* | 13.5[b] | 2.7 |
| *I. rickii (BAFCcult 3300)* | 8.4[c] | 2.2 |
| *P. gigantea* | 7.5[c] | 3.8 |
| *I. rickii (BAFCcult 3319)* | 6.1[c] | 2.6 |
| *C. micaceus* | 2.9[d] | 1.8 |
| Control | 2.6[d] | 0.9 |

### 3.2. Degradation ability of the xylophagous strains

The assay to test the degradation ability of the wood decay fungi showed that strains produced different dry weight losses after three months (Table 4). *B. adusta* was the most aggressive decay fungus, causing 23.7 ± 6.8% dry weight loss (DWL) in wood. *C. micaceus*, on the other hand, caused the lowest DWL: 2.9 ± 1.8%. Losses in dry weight caused by the other species varied from 17.7% to 6.1%. Treatments differed significantly when analyzed by ANOVA ($F = 76.2$, $p < 0.0001$, df = 9). The comparisons of the means indicated four groups in which the treatments were not significantly different from one another and were related to different degrees of degradative ability of the fungi: the first group was formed by *B. adusta* and *T. trogii*, the second by *T. trogii*, *O. latemarginatus*, *P. laxitexta* and *G. resinaceum*, the third by *I. rickii* (BAFCcult 3309 and 3319) and *P. gigantea* and the fourth by *C. micaceus* and the control.

### 3.3. Considerations on fungal diversity/host

Comments about the occurrence of fungal species in relationship with geographic distribution and reported host are given in the notes below. On the other hand, phylogenetic analyses were performed based on DNA sequences from some fungal strains in order to test the utility of these tools in the identification of fungal biodiversity in local populations. Phylogenetic trees were obtained under static and dynamic homologies (data not shown).

*B. adusta* (Willd.) Karst. Meddn Soc. Fauna Flora fenn. 5: 38, 1879.

Culture descriptions: See Nobles (1965) and Stalpers (1978).

Examined material: Argentina, Buenos Aires City. C. A. Robles, VII 2007. On *P. acerifolia*, BAFC 51667; BAFCcult 3301 (GenBank no. FJ850965).

Notes: Cosmopolitan (Stalpers, 1978) and a common species in forests of Northern Europe (Vasiliauskas et al., 2002). The results of the phylogenetic analyses of the sequences supported the cultural and morphological identification. This species has previously been reported affecting *P. acerifolia* in Buenos Aires City (Sede and Lopez, 1999b) and other urban trees in the Northern Hemisphere (Kotiranta et al., 2007).

*C. micaceus* (Bull.) Vilgalys, Hopple & Jacq. Johnson, Col. Fig. Engl. Fung. Vol. 3, pl. 261, 1800.

Culture description: Colonies covering 9-cm-diameter Petri plates in 2 weeks. Mats white, subfelty then becoming felty with brown woolly aerial mats at 2–3 weeks. In some strains, groups of hyphae formed aerial globose structures, 7–35 μm × 11–37 μm diam., first white then turning dark brown in 5–6 weeks. Odour absent. Advancing zone even. Agar darkened at 6 weeks. Marginal hyphae 2–7 μm diam., thin walled, with simple septa or with single clamp connections, usually branches arising just below or opposite septa. Aerial dark brown hyphae, simple septate with thickened walls, frequently branched (1.5)2–5(6) μm diam. In the last weeks, some hyphae (3–15 μm × 7–21 μm diam.) form a plectenchyma layer, hyaline at first then becoming dark brown. Submerged hyphae contorted and thin walled (1)2–6(7) μm diam., occasionally with swellings. Oxidase reactions: gallic acid: +; tannic acid: +; tyrosine: −; paracresol: −; guaiacol: +. Species code: (1).6.11.16.25.(26).32.37.39.42.53.

Examined material: Argentina, Buenos Aires City. C.A. Robles, I, V, VIII 2007. On *P. acerifolia*. BAFCcult 3218 (GenBank no. FJ850970); BAFCcult 3311 (GenBank no. FJ850971); BAFCcult 3312 (GenBank no. FJ850969); BAFCcult 3313 (GenBank no. FJ850968); BAFCcult 3314-3315.

Notes: This is the first description of *C. micaceus* in culture according to the system of Nobles (1965). Due to the lack of basidiomes and previous culture descriptions, phylogenetic analyses enabled identification and showed that the Argentinean strains were located in the same clade with other members of the species. To our knowledge, this is the first report on *P. acerifolia*.

*T. trogii* Berk., in Trog, Hist. nat. Iles Canar. (Paris) 2: 52, 1850.

Culture descriptions: See Stalpers (1978) and Wright et al. (1973).

Examined material: Argentina, Buenos Aires City. C.A. Robles, XI, 2007. On *P. acerifolia*, BAFCcult 3317.

Notes: The strain studied fits with the description made by Wright et al. (1973) except for the production of larger basidia (10–20 μm). Previously cited for Argentina, mainly on *Populus* and *Salix* (Blumenfeld, 1992; Wright et al., 1973) and reported for Buenos Aires City on *Fraxinus* sp. (Sede and Lopez, 1999b). To our knowledge, this is the first record on *P. acerifolia*. This species has been reported previously on *Platanus orientalis* in Turkey (Gezer et al., 2007; Türkoğlu, 2008).

*G. resinaceum* Boud., in Patouillard, Bull. Soc. mycol. *Fr.* 5: 72, 1890 [1889].

Culture descriptions: See Bazzalo and Wright (1982) and Stalpers (1978).

Examined material: Argentina, Buenos Aires City. C. A. Robles V, XII 2007. On *P. acerifolia*, BAFC 51651, BAFCcult 3296 (GenBank no. FJ850967); BAFCcult3297 (GenBank no. FJ850966); BAFC 51666, BAFCcult3310.

Notes: Results of phylogenetic analyses showed that further population studies are needed on this genus. Already reported for Buenos Aires City on *P. acerifolia* (Sede and Lopez, 1999b).

*I. rickii* (Pat.) D.A. Reid, Kew Bull. 12: 141, 1957.

Culture description: See Wright and Iaconis (1955).

Examined material: Argentina, Buenos Aires City. C. A. Robles, V, IX–XII, 2007. On *P. acerifolia*, BAFCcult 3300, 3304, 3305, 3307, 3308, 3316.

Notes: The description of the strains matches with that given by Wright and Iaconis (1955) for an anamorphic state (*P. cubensis* Pat.) but differs in the growth rate of the culture and the absence of fibre hyphae and pseudoparenchyma. However, the great production of chlamydospores, until there is hardly any mycelium, and the production of numerous setal hyphae suggest that these strains belong to *I. rickii.* A conservative position on the matter should be taken until more molecular and cultural studies are carried out. This is the first record for *P. acerifolia* in Buenos Aires. This species has been reported affecting a wide host range of urban trees, including London plane, in European cities (Annesi et al., 2003; Intini and Tello, 2003; Melo et al., 2002).

An *Inonotus* strain obtained from Site A (BAFC 51672, BAFCcult 3319, GenBank no. GU016326) showed cultural characters similar to those of *I. rickii* but differed in the slower growth rate (3.5–4 cm radium after six weeks) and the texture and colour of the mat, first developing a hyaline mycelium, which later turned yellow, slightly farinaceous in the margins and felty in the rest of the colony. The description of the basidiome fits with that given for *I. rickii* (Gottlieb et al., 2002) but differs in the larger pore size (1–2/mm). Phylogenetic analyses showed that the strain of this study was located in the same clade as the rest of the strains of *I. rickii,* together with two strains of *Inonotus patouillardii*. By means of phylogenetic analysis from ITS sequence data, Gottlieb et al. (2002) obtained evidence that these two species were putative sister taxa. These results lead us to consider this *Inonotus* strain as *Inonotus* aff. *rickii*. Further analysis and studies regarding this strain are needed.

*O. latemarginatus* (Durieu & Mont.) Donk, Persoonia 4(3): 342, 1966.

Culture descriptions: See Lombard et al. (1960) and Stalpers (1978).

Examined material: Argentina, Buenos Aires City. C. A. Robles, X, 2007. On *P. acerifolia*, BAFCcult 3306.

Notes: On angiosperms (Stalpers, 1978). Already cited for Buenos Aires City on *Fraxinus* sp. and *P. acerifolia* (Sede and Lopez, 1999b).

*P. laxitexta* C.E. Gómez, Darwiniana 20(1–2): 195, 1976.

Culture description: See Gómez and Loewenbaum (1976).

Examined material: Argentina, Buenos Aires City. C.A. Robles, IX, 2007. On *P. acerifolia*, BAFCcult 3309 (GenBank no. FJ882040).

Notes: The strain examined fits with the description made by Gómez and Loewenbaum (1976), but differs in the bleached reverse and the absence of hyphae with thickened walls. To our knowledge, there are no previous sequences of *P. laxitexta*. Phylogenetic analyses support the location of the Argentinean strain inside this taxon. Already cited for Argentina, in Buenos Aires province, on *Eucalyptus* sp. and *Ocotea* sp. (Gómez and Loewenbaum, 1976). First report for Buenos Aires City and on *P. acerifolia*.

*P. gigantea* (Fr.) Jülich, Persoonia 10 (1): 137, 1978.

Descriptions: See Deschamps and Wright (1975) and Nakasone (1990).

Examined material: Argentina, Buenos Aires City. C. A. Robles, VIII, 2007. On *P. acerifolia*, BAFCcult 3318 (GenBank no. FJ850972).

Notes: The strain studied matches with a description given by Deschamps and Wright (1975). It also fits with the description given by Nakasone (1990) but differs in the absence of arthrospores. The result of phylogenetic analyses suggests that the Argentinean strain belongs to a species closely related to *P. gigantea*, also suggested by cultural studies. Already cited for Argentina (Deschamps and Wright, 1975). First report for Buenos Aires City. To our knowledge, this is the first report on *P. acerifolia.*

## 4. Discussion and conclusions

This study constitutes the first systematic survey of fungal decay on urban trees in which a potential correlation between the age of trees, the anthropogenic disturbance of their location and the incidence of decay was established. We considered not only the identity of fungal species affecting *P. acerifolia* but also their frequency and distribution.

The most disturbed area sampled, Site B, showed the lowest percentage of colonized trees and trees inspected in this site had a wide range of DBH values. Our results suggest that the environmental differences between sites do not seem to be significantly important on the susceptibility of *P. acerifolia* to wood decay fungi. On the other hand, DBH values, used as indicators of the relative age of trees, could be a relevant factor for decay, as shown in Figs. 1 and 2.

Authors like Lesica et al. (2003) have already observed that certain Basidiomycetes like *Perenniporia fraxinophila* are more frequent on larger and older green ash trees.

Most of the species found in this study, especially those belonging to *Ganoderma* and *Inonotus* genera, have been frequently found affecting urban trees (Sede and Lopez, 1999b; Terho and Hallaksella, 2005; Terho et al., 2007). *P. laxitexta*, a fungus reported only from Argentina (Gómez and Loewenbaum, 1976), has been previously recorded on *Ocotea* sp. (a native plant genus) and *Eucalyptus* sp. (an exotic plant genus). Taking into account both these facts and the present report it is interesting to remark the ability of this fungus to colonize new introduced hosts. This is the first report for *P. laxitexta* and *P. gigantea* in Buenos Aires City. These two species, together with *T. trogii* and *C. micaceus*, are recorded for the first time on *P. acerifolia*.

When we tested the potential degradative ability of the Basidiomycetes isolated, all species caused significant dry weight loss on *P. acerifolia* wood except for *C. micaceus.* These are the first assays of the degradation ability of *P. laxitexta* and *C. micaceus*, as well as the first studies on *P. acerifolia* wood for the rest of the Basidiomycetes tested.

In vitro tests are useful to compare the degradation activity of different species under controlled conditions although they cannot be taken as an absolute evidence of the behaviour of these decay fungi. These results show that in the controlled conditions used here, *B. adusta* was the most aggressive fungus. The percentage of dry weight loss obtained in this study for this fungus is similar to that recorded by Wright and Deschamps (1976) on Salicaceae wood. Other studies, like that by Hakala et al. (2004) on spruce wood (*Picea abies*) and Del Río et al. (2002) on wood from *Eucalyptus globulus*, reported values of weight loss lower than that from this study. *B. adusta* seems to be another potentially important pathogen due to its high degradative ability. Since Sede and Lopez (1999b) noted the high association between *B. adusta* and *P. acerifolia*, it is necessary to highlight the importance of this decay fungus in cities where this tree species is present.

*T. trogii* and *O. latemarginatus* caused a similar weight loss in *P. acerifolia* wood. The high degradation ability of *T. trogii* on other substrates, like Salicaceae wood, has already been noticed (Levin and Castro, 1998; Wright and Deschamps, 1976). There are few studies on the degradative ability of *O. latemarginatus*. Fackler et al. (2007) reported, for this decay fungus, a weight loss on *Picea* sp. wood similar to that obtained in the present work.

The dry weight loss caused by *G. resinaceum* is similar to that observed by Wright and Deschamps (1976) on Salicaceae wood. Schubert et al. (2008) tested the degradation ability of *G. lipsiense* and *G. adspersum* on *P. acerifolia* wood after 6 weeks and obtained values close to 8%. These results indicate that different species of *Ganoderma* seem to have similar degradative activity on *P. acerifolia* wood.

*P. gigantea*, often used as a biocontrol agent against root pathogens such as *Heterobasidion* sp. (Campbell, 1989), showed low degradation ability in comparison with the rest of the species studied. This result is in accordance with those obtained by Hakala et al. (2004), who reported that the degradative capacity of *P. gigan-*

*tea* could be highly variable, even when affecting the same type of wood.

Finally, *I. rickii* showed a regular degradative ability among all the Basidiomycetes tested. However, on other tree species, like box elder (*Acer negundo*), this pathogen can cause a greater loss of weight (12.9%) (Mielnichuk and Lopez, 2007). It is also important to note that *I. rickii* was the most frequently isolated species and the most widely distributed, being the only species found in all the Sites sampled.

Based on these results, *I. rickii* would be the most harmful decay agent in urban trees. The importance of this fungus in urban environments has already been noted by several authors (Annesi et al., 2003; Intini and Tello, 2003; Melo et al., 2002; Ramos et al., 2008). It has been reported in several cities of Europe, has a wide host range and many of its hosts are species of trees used in urban environments. Intini and Tello (2003) have suggested that, in temperate climates, like the one considered in the present study, this species seems to affect mainly urban trees. Our results seem to support this hypothesis.

*G. resinaceum* seems to be the second most dangerous fungal species due to its frequency of appearance and degradative ability. Species of *Ganoderma* are widely distributed throughout the world and are often found in urban areas in Europe (Schwarze et al., 2000).

It is also important to note that *I. rickii* and *B. adusta*, both dangerous agents for urban trees, were isolated from wood samples, basidiomes and conidiomata samples but only from trees with presence of basidiomes and conidiomata. This could indicate a difficulty in detecting these pathogens in early stages of colonization. It would be of great advantage to be able to detect and identify wood-rotting fungi directly from wood samples, extending the use of molecular techniques, already noticed by authors as Jasalavich et al. (2000) and Guglielmo et al. (2010).

Due to the low number of molecular studies carried out on local strains so far, it is essential to adjust these techniques to local populations in order to identify and characterize them clearly.

The biodiversity of Basidiomycetes isolated in this work is likely to be lower than that actually present. Selective media for Basidiomycetes (Baum et al., 2003) and molecular tools to detect fungi directly from wood (Jasalavich et al., 2000) may be used in order to improve these results.

Taking into account the results of this study and the information from previous works, the factors with highest impact when taking management decisions related to landscape planning seem to be: the age of trees (estimated from DBH values), the presence of species belonging to the genera *Inonotus*, *Ganoderma* and *Bjerkandera* and the use of tree species less vulnerable to the action of these xylophagous fungi, as these decay agents showed different degradative capacities depending on the type of wood attacked. On the other hand, the level of anthropogenic disturbance and the extensive knowledge of composition and diversity of fungal communities associated with urban trees would not be relevant factors.

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