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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.