INONOTUS RICKII (PAT.) REID: AN IMPORTANT LEGNICOLOUS BASIDIOMYCETE IN URBAN TREES

INONOTUS RICKII (PAT.) REID: UM IMPORTANTE BASIDOMICETA LENHÍCOLA EM ÁRVORES URBANAS

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ABSTRACT

Inonotus rickii is a basidiomycete that causes cankers and decay in several ornamental trees, and has been reported in Portugal since 2002. The aim of the present work was to evaluate the incidence of the disease caused by *I. rickii* on *Celtis australis*, in the community of Alcântara, Lisbon, where the European hackberry tree is the main sidewalk species. Disease incidence reached 19%, and affected trees showed sparse foliage, death of branches and white rot of heartwood. In some cases, chlamydospores masses and basidiocarps occurred on trunks and branches, especially on trees with more than 40 cm of DBH. Considering the increasingly importance of *I. rickii* and being *C. australis* one of the most important ornamental species used in the green areas of Lisbon, morphological and cultural characteristics of the pathogen are presented and its impact as agent of decline of urban trees is discussed.

Key-words: *Celtis australis*, decay, disease incidence, *Ptychogaster cubensis*

RESUMO

Inonotus rickii é um basidiomiceta que causa cancros, exsudações e podridão branca do cerne em diversas espécies arbóreas, tendo sido identificado em Portugal em 2002. No presente trabalho pretendeu-se avaliar a incidência da doença causada por *I. rickii* em *Celtis australis*, numa zona de Lisboa onde esta espécie é a principal árvore de alinhamento. A incidência da doença ronda os 19%, sendo os sintomas mais frequentes a redução do tamanho das folhas, morte de ramos e podridão branca do lenho. Por vezes, observaram-se a presença de massas de clamidósporos e a formação de basidiomas nos troncos e nas pernadas, essencialmente nas árvores com DAP superior a 40 cm. Considerando a importância crescente de *I. rickii* e sendo o lodão a espécie de eleição da cidade de Lisboa, descrevem-se as características morfológicas e culturais do fungo e discute-se o papel que este patogénio pode assumir no declínio do arvoredo urbano.

Palavras-chave: *Celtis australis*, incidência, podridão, *Ptychogaster cubensis*

INTRODUCTION

Inonotus rickii (Pat.) Reid (anamorph: *Ptychogaster cubensis* Pat.) is classified as a canker rot pathogen that infects branches and trunks of living trees, causes white rot of heartwood and can colonize sapwood and cambium as well. It affects trees stability and results in progressive crown dieback and mortality.

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I. rickii was first misidentified as *Fistulina hepatica* (Huds.) Fr. and later classified as *Ptychogaster cubensis* n. sp. based on its conidial fructifications (Patouillard, 1896). Afterwards, Patouillard reclassified the fungus as *Xanthochrous rickii* n. sp. (Patouillard, 1908), an organism later referred as *Polyporus rickii* (Pat.) Sacc. & Trott. (Saccardo, 1912). More recently, Reid (1957) transferred this fungus to the genus *Inonotus* Karst., where it is recognized as *Inonotus rickii* (Pat.) Reid, having a *Ptychogaster cubensis* Pat. anamorph (Gilbertson & Ryvarden, 1996). Recently, molecular data support the view that *P. cubensis* represents an anamorphic state of *I. rickii* (Gottlieb *et al.*, 2002).

This basidiomycete is widely distributed in the tropics and subtropics, and is found on a large range of broadleaves (Davidson *et al.*, 1942; Jaquenoud-Steinlin, 1985; Farr *et al.*, 1989) being more rare in temperate zones where it seems limited to urban trees (Intini, 2002a).

The first record of *I. rickii* in Europe is owned to Jaquenoud-Steinlin (1985), who observed the anamorph on *Parkinsonia* sp. from Sicily, having described the hymenial elements two years later, from material collected on *Tamarindus indica* L. in Martinica (Jaquenoud, 1987). The ptychogastric stage *P. cubensis* was also reported on *Schinus molle* L., in Catania, by Intini (1988) who have studied the macroscopic, microscopic and cultural characteristics of the fungus. Later, Kotlaba & Pouzar (1994) described the disease on *Celtis australis* L. and *Sambucus nigra* L., in Montenegro and in Greece, respectively, whereas Pieri & Rivoire (1996) found *I. rickii* on decayed *Albizia* sp. in France. Recently, Intini (2002b) and Intini & Tello (2003) reported *P. cubensis* attacking *Acer negundo* L., *C. australis*, *Platanus* x *hybrida* Brot. and *Schinus molle* L., in Spain.

In Portugal specimens of *I. rickii* (anamorph) were detected growing on *C. australis* in 2000 and two years later large fruit bodies (teleomorphs) developed both on european hackberry and on *Sapindus saponaria* L. in the Lisbon area (Melo *et al.*, 2002). Decay and canker rot caused by *I. rickii* was also observed on *A. negundo*, *Parkinsonia* sp. and *S. molle*, both on boulevards and public gardens.

Following a careful survey of several areas of Lisbon it became evident that the pathogen was already spread, resulting in structural damage to the trees and leading to tree decline and mortality, mainly on *C. australis*. Dead branches in the upper part of the crown, sparse foliage, dark jelly exudations, swellings and cracks in the bark of the trunk were the symptoms most frequently found. After the detection of the fungus on this host, the services in charge of the care and management of green areas in Lisbon faced an increasing need of pruning dead branches or even removing declining and dead adult European hackberry trees. As European hackberry is one of the most important ornamental species used in the urban area of Lisbon, representing about 20% of the street trees (Almeida, 2006), the impact of the problem turned out to be noticeable. In fact, *I. rickii* is considered one of the most economically important polypores causing white rot of trees growing in urban settings (Intini, 2002b).

With the purpose of supplying auxiliary information on this pathogen and to better understand its role in damaging *C. australis* in Lisbon city, sign and symptom observations were recorded from 381 street european hackberry trees at the Alcântara community, where 7.1% of the trees of this species can be found, and disease incidence evaluated. *I. rickii* was isolated and diagnostic, morphological and cultural, characteristics were studied.

MATERIAL AND METHODS

The study was performed on 381 trees of *C. australis* along sidewalks of Alcântara, Lisbon. The boulevard where these trees are includes three *A. negundo*, four *Celtis occidentalis* L., 14 *Fraxinus angustifolia* Vahl, one *Koelreuteria paniculata* Laxmann and 14 *Sophora japonica* L..

Observations were conducted during the end of summer and autumn of 2006 and 2007, and most of the material was gathered during autumn and winter; scattered notations were made through the year.

Symptoms such as presence of dead branches, sparse foliage, dark jelly exudations, swellings and cracks in the bark of the trunks were registered. Position and number of sporocarps of *I. rickii* on trees and diameter of the trunks (at 1.3 m above the ground) were recorded. A declining and a dead *C. australis* were felled down and sectioned to examine and describe internal symptoms.

Cultures were obtained from decayed wood and hyphal strands. According to Chang & Fu (1998) single hyphal cultures were grown on potato dextrose agar (PDA, Bacto potato dextrose agar, Difco) and on malt extract agar (MEA, 2% malt-extract, Difco, 2% Bacto agar, Difco) at 30 ºC under permanent dark conditions. Isolates were macroscopically examined after 7-days of incubation for cultural descriptions.

The identification of the fungus was done after macro and microscopical examination. Macroscopic observations were carried out with the aid of a stereo microscope. Free-hand thin sections of the fructifications were prepared for microscopic studies. For observations and measurements of microscopic characters, 3% KOH was used as mounting medium to ensure rehydration. 30 observations were recorded for measurements of characteristic features.

RESULTS

Symptoms and disease incidence

All along the surveyed streets 240 trees (63.0%), out of the 381 *C. australis* observed, showed decline symptoms associated with canker rot disease caused by *I. rickii* (Table 1). Seven years after the first detection of the fungus in the area, 15 trees had died or fallen down. Diseased trees exhibited poor vegetative vigour, shortened branches with few and smaller leaves, abscised twigs, twig dieback and several broken and dead branches. In some trunks inconspicuous elongate sunken cankers were detected. Branch stubs of some infected trees exuded significant quantities of sap, making the surrounding bark to appear wet and stained (Fig. 1).

Some of these trees showed very distinctive fructifications of the *P. cubensis* anamorph both on trunks and on branches (Fig. 2), above ground on standing trunks, inside trunk cavities, at branch stubs and near the insertion point of branches.

Table 1 – Observations on a 381 *Celtis australis* boulevard in Alcântara, Lisbon.

			Presence of sporocarps of *Inonotus rickii*	
	Nº of trees	%	Nº of trees	Relative %
Healthy trees	126	33.0%	0	0.0%
Declining trees	240	63.0%	71	29.6%
Dead trees	9	2.4%	0	0.0%
Butts	6	1.6%	2	33.3%
Total	381		73	19.2%

Figure 1 – Slime flux below an insertion point of branches and associated bark staining.

Figure 2 – Golden-orange masses of fungal tissue of *Ptychogaster cubensis.*

At first, these fructifications had the appearance of golden brown masses of fungal soft and fleshy tissue which exude droplets of slightly coloured to dark liquid. Later, these masses of sterile hyphae assumed a drier and powdery texture, with dark-brown to black colour, due to the formation of chlamydospores. They were cushion-shaped reaching up an extension of 97 cm above the soil.

In total, 73 (19.2%) out of 381 European hackberry trees observed bear anamorphic fructifications of *I. rickii*. The distribution of *I. rickii* related to the diameter of the trees is shown in Figure 3. Only 10.4% of trees with a diameter less than 40 cm had sporophores but the number of infected trees gradually increased with the diameter (or the age of the tree) and among 40-60 cm trees, 57.5% had sporophores. Some trees exhibited one or several sporophores at distinct locations. They appeared single or many together on the same trunk or branches. Fresh fructifications were mostly found on living trunks or living 10-30 cm thick branches, from the ground level up to the crown of the trees. On branches, sporophores were distributed from the basal part to more than 5 m out from the trunk, either on the scaffold branches or on sub-branches. In most of the cases sporophores were located on or close to healing wounds on trunks and branches, on the remaining parts of broken branches, and near broken tops of branches. They often were observed in or close to distinct bark-clad depressed areas and swellings/cracks.

At the end of summer, beginning of autumn, after an unusual rainy period and mild temperatures, several applanate and sessil basidiocarps were detected above the fungal masses attached to the insertion point of scaffold branches and on trunks (Figure 4), sometimes all around the basal part of the trunk or protruding from bark cracks. All the trees where basidiocarps were found exhibited intense defoliation and more than 50% of dead crown.

Stem cross sections of the felled trees showed the decay at first as white or yellow tongues of discoloration, with narrow darkened zones of sound wood at the margins of the decayed wood (Figure 5). These gradually expand and the decayed wood becomes wet and darkener in colour, finally becoming a soft filamentous tissue which gives rise to a hollow. Decay was largely confined to the heartwood, although in some cases the sapwood and the cambium were affected. Anamorphic fruit bodies continued to develop and emerge from stumps, even on those cut very low, at ground level.

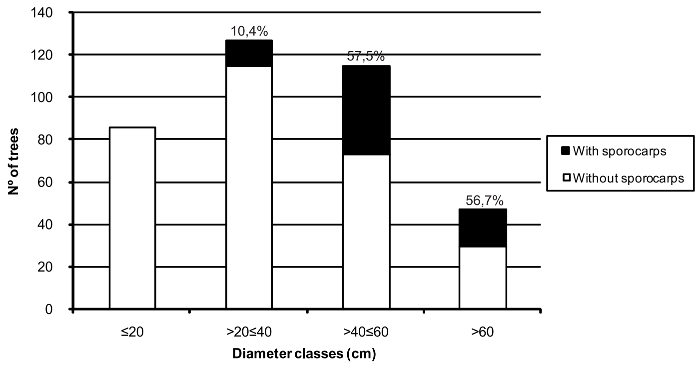

Figure 3 – Investigated *Celtis australis* grouped in four arbitrarily selected diameter classes and percentage of trees with fructifications of *Inonotus rickii*.

Figure 4 – Basidiocarps of *Inonotus rickii* on branches of *Celtis australis*.

Figure 5 – Decayed wood with typical white to yellow-brown colour separated from sound wood by zones of dark-stained wood.

Morphological and Cultural descriptions of *Inonotus rickii*

Basidiome (teleomorph) annual, at first soft and fleshy and then becoming firm, attached to the substrate widely and firmly, sessile, applanate to ungulate, single to imbricate, 23-46 cm wide and 10 cm thick, margin acute or obtuse, entire, undulate. Upper surface golden brown and tomentose in younger parts, oldest parts dark rusty brown and rough, becoming rimose.

Imperfect fruitbody (anamorph) semi-spherical or cushion shaped, soft and fleshy at first, velvety to the touch, yellowish brown to golden brown, later dark brownish and the inner parts totally disintegrating to a mass of chlamydospores, and by age the whole fruitbody is transformed to this kind of spores.

Context up to 3-5 cm thick, dark rusty brown, concentrically zonate, fibrous in texture, watery and spongy when fresh, hard and brittle when dry, with a silky sheen, sometimes crumbling into a mass of chlamydospores. Tube layer non-stratified, distinct from the context, golden brown, brittle when dry, up to 15 mm thick.

Hymenial surface plane to concave, pale brown, often covered by a cream-coloured pruina of secondary hyphae, sometimes reddish brown owing to the large mass of chlamidospores adhering to the surface. Pores medium to large, 2-4 per mm, round to angular, with thin dissepiments, pore mouths entire.

Hyphal system monomitic with setal hyphae. Hyphae simple septate, occasionally branched, yellowish-brown to pale-brownish. Hyphae in context subparallel, thin to slightly thick-walled, 3-8.5 µm in diam., in the transition zone between trama and context narrower and more ramified. Hyphae in hymenial trama subparallel, thin to slightly thick-walled, 3-6 µm in diam.

Setal hyphae frequent, present in the context and in the dissepiments, running parallel to the tubes and sometimes diverging out through the hymenium, up to 15 µm in diam. at the widest point, and more than 250 µm long, subulate, with very thick walls, dark chestnut-brown.

Hymenial setae numerous, 15-40 x 5-11 µm, ventricose to subulate, sometimes with heel, very thick-walled, dark chestnut-brown (Fig. 6).

Basidia broadly clavate to cylindric, 15-25 x 6.5-10 µm, with 4 sterigmata up to 4.5 µm long and oil drops in the citoplasm.

Spores 6-8.5(-9) x 4.5-5.5 µm, broadly ellipsoid, with applanated supra-apicular region, thick-walled, golden-yellow, in KOH reddish brown.

Chlamydospores 10-15 µm in diam., irregular, globose to pyriform, very thick-walled, golden brown to reddish brown (Fig. 7).

Growth rate of isolates 50-70 mm of diameter in 7 days. Colonies were cottony to woolly, white-cream to ochre, dark-brown with age, on PDA, and becoming darker on MEA. In 2-wk-old cultures, presence of abundant brown chlamydospores of varying shape, but usually globose, yellowish-brown with 11 x 10 µm, formed in chains, simply or intercalated; presence of acute, thick-walled, dark brown setal.

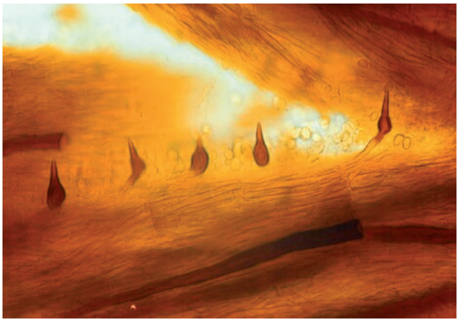

Figure 6 – Setae of *Ptychogaster cubensis* (630 X).

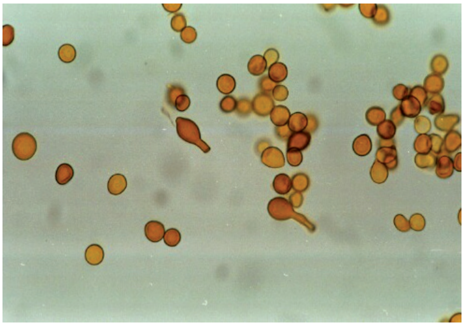

Figure 7 – Chlamydospores of *Ptychogaster cubensis* from a pure culture (400 X).

DISCUSSION

This is the first study on incidence of canker rot disease caused by *I. rickii* in Portugal. Identification was based on fungal morphology and cultural characteristics, according to Gilbertson & Ryvarden (1996), Chang & Fu (1998) and Melo *et al.* (2002). *I. rickii* is recognized by its ptychogaster state and unique chlamydospores. The annual and fleshy basidiocarps, the abundant setal hyphae and hymenial setae ant the relatively broadly ellipsoid, golden-yellow basidiospores are diagnostic characteristics of *I. rickii* (Stalpers, 2000; Gottlieb *et al.*, 2002).

I. rickii causes heartrot in a variety of living hardwood trees, including *C. australis*. Infected trees are deeply affected in their growth, vigour, and mechanical stability. Cankers on the stems are not immediately obvious and slime flux may be intense. The infection seems to be closely connected with bark wounds, although the decay chiefly extends to the inner wood. In the beginning of the infection the detection of the disease may be difficult as crown dieback and sparse foliage are inconspicuous symptoms, also caused by other basidiomycete fungi as *Inonotus hispidus* (Bull.:Fr.) Karst. or *Ganoderma lucidum* (Curtis:Fr.) Karst.. Sometimes the fungus forms hard layers or masses of old mycelial tissue at the stubs and wound areas that become hard, irregular, rough, thick knots of tissue, resembling old sporophores of *I. hispidus* but there is no indication of a basidial layer.

According to Annesi *et al.* (2003) the pathogen is spread through the agamic propagules (chlamydospores) only, which are produced in huge quantity and very easily carried by pruning tools and other agents inflicting accidental wounds. Infections occur when disseminated spores of the fungus are deposited on or near wounds, pruning scars or dead branch stubs of susceptible hosts. Moisture and temperature conditions allow spores germination, and the fungus grows slowly into the vulnerable wood tissues. Following sufficient development in infected tissues, a process sometimes taking years, the decay fungus produces their characteristic fructifications on the tree's external surfaces (Phillips & Burdekin, 1989).

Typically, fresh fruit bodies occur on living trees, where they develop well above ground on standing trunks and branches, but also on dead branches or branch stubs and at or near wounds. Perfect fruit bodies are not regular in their occurrence and several years may elapse between the appearance of consecutive sporophores on an infected tree. According to Jaquenoud-Steinlin (1985) among one hundred anamorphic fructifications on *T. indica*, in Martinica, only two basidiomes were detected. Their occurrence seems to be enhanced by dry and hot summers and mild winters (Annesi *et al.*, 2003). In fact, according to the meteorological data collected at the Meteorological Station of Tapada da Ajuda, since 2000 rain has become concentrated during the months of May and June and October and November, with mild temperatures during autumn and winter (data not shown).

Heartrot fungi are common on old, mature to over-mature trees, especially if they have sustained significant branch or stem injury during their lifetime. A great number of the trees surveyed in this study were more than 50-years old and intensively pruned and subjected to wounds due to automobiles, construction works and excavations.

In the surveyed area of Alcântara, where 7.1% of all sidewalk European hackberry trees of Lisbon are, the incidence of canker rot disease caused by *I. rickii* is around 19% and in the last seven years a significant number of trees had been cut and new ones were planted. According to Annesi *et al.* (2003) the incidence of the disease reached 22% on a boxelder tree boulevard, in the city of Rome, stressing the economic importance of this polypore.

The high incidence of *I. rickii* observed was in fact probably higher as: (1) several hackberry trees without sporocarps showed symptoms characteristic of *I. rickii*, (2) sporocarps positioned high up in some of the trees may have been disregarded, and (3) probably even some still healthy looking trees were also attacked even if no visible sign of infection had yet occurred. Some of the reasons that might explain the high infection incidence in this area of Lisbon are: (1) the old age of the trees have given the fungus a long time for colonisation, and (2) the frequent cutting of branches during long periods of time (for public safety and other reasons) caused additional wounds where infection of the fungus could start. In fact, the possibility of fungal infection rises with life-span. Urban trees are kept much longer than usual in forestry rotation, which itself increases the probability of decay. Additionally, pruning, especially the removal of thick branches from adult trees and trunk and butt injuries caused by automobiles (although the use of guards for newly planted trees) seem to be the main reasons for *I. rickii* infections on sidewalk trees.

In a recent study conducted in Lisbon (data not shown) *I. rickii* was recorded in six out of 25 community areas surveyed, corresponding to an area where 25% of the European hackberry trees of the town are located. Having in consideration the importance of this species as sidewalk tree in Lisbon, and the wide range of hosts of *I. rickii* among broadleaves, canker rot is clearly an emergent phytosanitary problem to be considered by those in charge of care and management of green urban areas.

The disease might become an important constraint to the use of *C. australis* in urban settings, in the centre and the south of Portugal as the fungus was already detected in Beja city (C. Abrantes, personal communication). A similar situation was already stated in Italy (Intini, 2002a), Greece and Montenegro (Kotlaba & Pouzar, 1994). According to Annesi *et al.* (2005) it is evident that the pathogen is going to have an increasing importance, as most of the genera recorded as hosts of *I. rickii* in American countries are present in Europe and are extremely used in urban environments.

In conclusion, *I. rickii* seems to be perfectly acclimatised and widespread in the Lisbon area, causing white rot on various woody species. Diseased trees lose their ornamental value, becoming hazardous elements for the public in urban and peri-urban areas. Once the tree has succumbed to decay, no cure is possible. A survey and detection plan should be implemented: dusty or powdery, red-brown, beard-like fructifications at branch stubs and bark fissures should be looked for; the occurrence of dark red-brown to coffee-brown exudation and bark staining at the same foci are alerting symptoms; examination of internal wood decay provides additional evidence. Finally, microscopic observation of characteristic fungal features and/or laboratory cultural analyses for confirmation should take place. Careful tree maintenance techniques and disease control practices should be implemented; avoidance of wounds in trunks and branches, removal of sporocarps and of dead or seriously affected trees, are measures that prevent the spread of the fungus. All the data show that *I. rickii* should be regarded as one of the most harmful pathogenic polypores in urban trees.

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