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DEGRADATION OF SOME TECHNOLOGICAL FEATURES IN THE WOOD OF ORNAMENTAL SPECIES CAUSED BY *INONOTUS RICKII* (PAT.) REID

Inonotus rickii (Pat.) Reid is a pathogenic wood-decaying fungus that causes severe decay in several ornamental urban trees in Europe. It has been known to occur on different hosts in Sicily (Italy) since 1985, and in Rome (Italy) since 2003.

Some physical and mechanical wood features were studied according to the standards in order to propose an investigation methodology to set a deterioration ranking of urban trees. In this phase of the study, Celtis australis L., Acer negundo L., Acer campestre L., Robinia pseudoacacia L., Tilia × vulgaris Hayne, Ulmus minor Mill., Platanus × acerifolia (Aiton) Willd. and Quercus ilex L. were studied.

The data were analyzed using ANOVA and M-ANOVA tests to check the differences among the specimens. A risk matrix was created in order to combine the features that showed statistical differences between the control specimens and the inoculated specimens, in order to establish, in vitro, a degradation ranking among the wood species.

The wood species which showed no in vitro durability to I. rickii, were the same ones that displayed susceptibility in the living trees.

Keywords: *Inonotus rickii* (Pat.) Reid; wood physical features; axial compression strength; wood decay; urban wood species.

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Introduction

In urban areas, trees offer a number of benefits [Nowak, Dwyer 2007; Robles et al. 2011]. Unfortunately, such trees are also exposed to both biotic and abiotic stress factors, which can cause phytopathological and physiological problems. Pollution, insufficient space for growth, a lack of nutrients, mechanical injury [Marchi et al. 2013] and the proximity of structures (roads, sidewalks, retaining walls...) contribute to a decrease in the vigour of trees and to an increase in the possibility of fungal attacks. All these factors can cause damage to the xylem and can reduce stability and resistance to breakage [Schwarze et al. 2000; Sæbø et al. 2005; Robles et al. 2011]. Dead or decayed wood has a wide range of ecological values in forest ecosystems, it is also considered a relevant indicator in the National Forest Inventories and in an ecologically sustainable forest management [Tavankar et al. 2014], however, in the urban environment it cannot be accepted for the high risk of harm to people. Decay does not necessarily mean the immediate death of trees, as the process may extend over several years. This happens particularly when the damage is done to the heartwood, which does not affect the basic life processes of the tree. In urban areas, there is also the risk of accidents involving people or properties [Terho, Hallaksella 2005; Robles et al. 2011].

Inonotus rickii (Pat.) Reid is a basidiomycete (*Hymenomycetes*) that can cause severe damage to trees in urban areas, producing cankers and white rot in several ornamental trees.

The anamorph state *Ptychogaster cubensis* Pat. has been described by different authors [Davidson et al. 1942; Gilbertson, Ryvarden 1986; Intini 1988; Stalpers 1978, 2000]. Recently, phylogenetic studies have been carried out on isolates of this fungus from different geographic areas. These studies seem to suggest that *P. cubensis* from Florida and the anamorphic form present in Europe, South America, and China may not be related to the same species [De Simone et al. 2011; Cui et al. 2014], but further investigations with a larger number of isolates from the Caribbean are necessary.

In Italy, Jaquenoud [1985] first observed the anamorph, *P. cubensis*, on *Parkinsonia* L. spp. (Sicily) and, in the same place, the author discover later the teleomorph *I. rickii* [Jaquenoud 1987].

Other authors have reported the fungus on *Schinus molle* L. in Catania (Sicily, Italy) [Intini 1988]; on *Celtis australis* L. in Montenegro [Kotlaba, Pouzar 1994]; on *Sambucus nigra* L. in Greece [Kotlaba, Pouzar 1994]. Intini [2002] and Intini and Tello [2003] observed *P. cubensis* attacking *Acer negundo* L., *C. australis*, *Platanus* × *hybrida* Brot. and *S. molle* in Spain. *I. rickii* was noted also on *Albizia* Durazz. spp. in France [Pieri, Rivoire 1996]. In Portugal Ramos et al. [2008] and Melo et al. [2002] studied it on *C. australis* and on *Sapindus saponaria* L.

Since 2003, *I. rickii* has been found on some boulevards in Rome (Italy) on different tree species [Annesi et al. 2003; Annesi et al. 2005; Annesi et al. 2010]. Data concerning fungal isolates collected in Rome [De Simone et al. 2011] indicate that they have thermal requirements similar to subtropical and tropical American isolates [Davidson et al. 1942]. Due to these factors, this species could play an increasing role in the Mediterranean area affected by climate change [Pautasso et al. 2012].

I. rickii is a polyphagous pathogen and it produces annual basidiomes and conspicuous brown powdery masses of chlamydospores, (anamorphic stage). This behaviour favours the spread of the infection on adjacent trees [Annesi et al. 2010].

Contaminated trees can show reduced vegetative vigour. Advanced infection manifests itself by overall decline and death as reported by Ramos et al. [2008] on a boulevard of *C. australis* in Portugal and by Mazza et al. [2008] on tree lines of *A. negundo* and *A. julibbrissin* in Rome [Mazza et al. 2008; Annesi et al. 2010].

The knowledge of the potential occurrence of the pathogen on trees species commonly used in urban trees may provide useful information for tree management (tree planting, tree maintenance). The evaluation of the residual technological properties of wood, after fungal activity, is a key factor in understanding the break risk of a species.

The research aims to assess the physical and mechanical property changes in inoculated wood specimens of 8 ornamental tree species, hosts and non-hosts of *I. rickii*, and to evaluate *in vitro* the potential resistance or vulnerability of each tested species, by means of a new methodology.

Materials and methods

Test *in vitro*: specimen preparation

The wood specimens were obtained from healthy trees mostly grown on the CRA-PAV experimental farm, located in Monterotondo near Rome. *Quercus ilex* was grown in Bultei (Sassari, Italy) and *Quercus cerris* in Vetralla (Viterbo, Italy). Test specimens were prepared from the timber according to the general requirements for physical and mechanical tests [ISO 3129:2012].

The timber was processed to obtain boards, stored in a room at 65 ± 5% relative humidity and at a temperature of 20°C so as to reach 12% moisture content. The boards were further processed to obtain clear wood specimens (with dimensions 20 × 20 × 30 mm).

***In vitro* inoculation of specimens**

The specimens were soaked in H_2O for 48 hours, after which they were sterilized twice for 2 hours at an interval of 24 hours. Before inoculation, the specimens were dried in an oven at 50°C for 4 days.

The wood specimens were infected utilizing three isolates of *I. rickii* obtained from anamorphic fructifications, which were collected in Rome from diseased trees of *R. pseudoacacia* (PF62-1), *A. negundo* (PF76-2) and *C. australis* (PF217-3). Twenty-six sterile transparent polyethylene containers (with dimensions 63 × 80 mm), containing a thin layer of malt agar (MA), were prepared for each fungal isolate and tree species to be tested. Each one was inoculated with one fungal isolate using a mycelium disc grown on a potato dextrose agar (PDA). These were incubated at 28 ± 1°C in the dark. When the mycelium had covered the surface of the agar, a wood specimen was introduced into each pot. The control wood specimens received identical treatment, but they were not inoculated by *I. rickii* isolates before incubation.

After 5 and /or 10 months of incubation at 28°C, the specimens were removed from the containers and the surface mycelium was gently cleaned off. The specimens were dried in an oven at 50°C for 96 hours (table 1).

Table 1. Wood species and incubation period

Wood species	Duration of inoculation
C. australis	5 and 10 months
A. negundo	5 and 10 months
A. campestre	5 and 10 months
R. pseudoacacia	10 months
T. vulgaris	10 months
U. minor	10 months
P. acerifolia	10 months
Q. ilex	10 months

Wood properties

The physical features examined were: wood density (ρ), shrinkage (β) and the related coefficient.

Wood density (g cm^{-3}) at 12% moisture content (MC) was determined according to the UNI/ISO 3131 standard [1985a] [Lo Monaco et al. 2011; Lo Monaco et al. 2015].

Tangential (β_t), radial (β_r) and volumetric (β_v) shrinkage was calculated according to the UNI/ISO 4469 [1985c] and UNI/ISO 4858 [1988] standards, considering the total amount of dimensional variation from fully swollen to

oven-dry. In addition, for each variable measured, the coefficient of shrinkage was calculated (coefficient of tangential shrinkage ($C\beta_t$), coefficient of radial shrinkage ($C\beta_r$) and coefficient of volumetric shrinkage ($C\beta_v$)). These parameters constitute the shrinkage value when the MC decreases by 1% below the fibre saturation point, and under the assumption of a linear relationship, as according to Ferreira et al. [2012]. The coefficient of shrinkage anisotropy (β_t/β_r) is the tangential and radial shrinkage ratio [Giordano 1981].

Four edges and the middle of the surface in the radial and tangential directions, and 4 edges for the axial direction were measured to obtain more accuracy in the evaluation of shrinkage.

The axial compression strength (σ) was determined on specimens at 12% moisture content according to UNI/ISO 3787 [1985b].

The number of the specimens for each feature and set is reported in tables 2 and 3.

Table 2. Number of samples tested 5 months after inoculation

Treatment	Shrinkage		
	C. australis	*A. campestre*	*A. negundo*
Control	13	13	13
PF62-1	13	13	13
PF76-2	13	13	12
PF217-3	13	13	13
Density and axial compression strength			
Treatment	*C. australis*	*A. campestre*	*A. negundo*
Control	13	13	13
PF62-1	15	13	13
PF76-2	15	13	14
PF217-3	7	13	13

Table 3. Number of samples tested 10 months after inoculation

Wood species	Shrinkage				Density and axial compression strength			
	PF62-1	PF76-2	PF217-3	Control	PF62-1	PF76-2	PF217-3	Control
C. australis	9	9	10	10	12	16	15	15
A. campestre	10	10	10	10	15	15	15	13
A. negundo	10	9	9	10	15	14	16	15
R. pseudacacia	9	10	10	9	15	16	16	17
T. X vulgaris	9	10	9	9	17	16	17	17
P. acerifolia	9	8	9	8	17	18	17	17
Q. ilex	9	9	9	10	17	17	17	15

Statistical analysis

The data were analyzed using Statistica 2010 advanced statistics software. As a first step, a data distribution was plotted and visually checked for normality. Differences between the inoculated specimens and the control wood specimens were checked using the standard paired t-test, with ANOVA and MANOVA analysis. Post-hoc tests were conducted using the Tukey HSD test method [Sheskin 2000].

Risk matrix of wood vulnerability

A risk matrix was used to assess the vulnerability of the wood and to evaluate species behaviour. It was created in order to combine the density and axial compression strength.

The percentage range of differences in density and axial compression were divided into 4 classes (minimum 1, maximum 4) and, for each wood species, the class of density was multiplied by class of axial compression strength. In this way, an index was calculated to describe the degradation in terms of density and compression strength.

The minimum value of the matrix was 1 (lower degradation) and the maximum value was 16 (highest degradation). The risk matrix values were divided into three groups: in the first group (values 1–3), the effect of the fungal agent was negligible; in the second group (values 4–6), the effect became evident; in the third group (values 8–16), the effect was considerable (table 4) [Ni et al. 2010].

Table 4. Risk Matrix

	Axial compression strength				
Density	Classes	1	2	3	4
	1	1	2	3	4
	2	2	4	6	8
	3	3	6	9	12
	4	4	8	12	16

Results and discussion

The results, after 5 months' treatment, highlighted the fact that the physical and mechanical features occasionally showed statistically-significant differences (table 5). ANOVA and MANOVA analysis and the *post hoc* Tukey test were performed in order to underline the differences between the inoculated specimens and the control specimens.

Table 5. Analysis of variance of physical and mechanical features after 5 months' treatment

Wood species	Shrinkage							Density and axial compression strength	
	β_r	β_t	β_v	$C\beta_r$	$C\beta_t$	$C\beta_v$	β_t/β_r	σ	P
C. australis	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	< 0.01	n.s.
A. campestre	< 0.05	n.s.	n.s.	< 0.01	n.s.	< 0.01	n.s.	n.s.	n.s.
A. negundo	n.s.	n.s.	n.s.	n.s.	< 0.05	< 0.01	n.s.	< 0.05	n.s.
T. vulgaris	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

In *C. australis*, there was a large statistically-significant differencebetween the inoculated specimens and control specimens, in terms of the axial compression strength.

In *A. campestre*, radial shrinkage and the radial coefficient of shrinkage displayed statistically-significant differences.

In *A. negundo*, the tangential coefficient of shrinkage, volumetric coefficient of shrinkage and axial compression strength showed statistically-significant differences. Whereas *T.* × *vulgaris* did not present these differences.

After 10 months' treatment (table 6), the physical features showed statistically-significant differences, between the inoculated and control specimens for some species only, and not for all the parameters.

Table 6. Analysis of variance of physical and mechanical features after 10 months' treatment

Wood species	Shrinkage							Density and axial compression strength	
	β_r	β_t	β_v	$C\beta_r$	$C\beta_t$	$C\beta_v$	β_t/β_r	σ	P
C. australis	< 0.05	n.s.	n.s.	< 0.05	n.s.	n.s.	<0.05	< 0.01	< 0.01
A. campestre	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	n.s.	< 0.01	< 0.01
A. negundo	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	< 0.01	< 0.01
R. pseudacacia	< 0.05	< 0.01	<0.01	< 0.05	< 0.01	< 0.01	n.s.	n.s.	n.s.
T. vulgaris	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	< 0.01	< 0.01
U. minor	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	< 0.01
P. acerifolia	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	< 0.01	< 0.01
Q. ilex.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Specifically, all kinds of shrinkage, and the relative coefficients, showed statistically-significant differences in *R. pseudacacia* and in *A. campestre*. In *R. pseudacacia*, statistically-significant differences, between the inoculated specimens and control specimens, were large regarding tangential and volumetric shrinkage, and the relative coefficients. In *A. campestre*, the differences were large in terms of radial, tangential and volumetric shrinkage, and relative coefficients.

In *C. australis*, the statistically-significant differences were large with regards to radial shrinkage, the relative coefficient, and the coefficient of shrinkage anisotropy.

Regarding density and axial compression strength, large statistically-significant differences between the inoculated specimens and control specimens were found, with the exception of *R. pseudacacia*, *Q. ilex* and *U. minor*. *R pseudacacia* and *Q. ilex* did not show statistically-significant differences and *U. minor* only showed large statistically-significant differences for density.

In table 7, the percentage differences between the inoculated and control specimens are shown.

Table 7. Percentage reduction of density and axial compression strength

Parameter	*C. australis*	*A.ampe-stre*	*A.negundo*	*R.pseud.*	*T. vulgaris*	*U.minor*	*P. acerifolia*	*Q.ilex*
σ	21%	11%	20%	0%	13%	0%	15%	0%
ρ	16%	10%	9%	0%	9%	7%	11%	0%

After 10 months' treatment, the changes in density and axial compression strength gave interesting information. With the exception of *R. pseudoacacia* and *Q. ilex*, the investigated species displayed a marked decrease. The minimum value of the percentage differences of the axial compression strength was 11% (*A. campestre*) and the maximum value was 21% (*C. australis*); as for density, the minimum value was 7% (*U. minor*) and the maximum 16% (*C. australis*).

The density and the axial compression strength were combined in the risk matrix: the values of the risk matrix are shown in figure 1.

C. australis, *A. campestre*, *A. negundo*, *T. vulgaris* and *P. acerifolia* were in the highest risk group (8–16 points) and *U. minor*, *R. pseudacacia* and *Q. ilex* were in the lowest risk group (1–3 points).

In the specimens after 10 months' treatment, the density and the axial compression strength were analyzed to understand if and how the three isolates showed a different form of wood degradation. In table 8, the results of the Tukey test are shown.

In the majority of the species studied, the three isolates showed a decrease in density and axial compression strength in relation to the control. Different

behaviour was sometimes noted among the three isolates: this was evident in *C. australis*, *A. campestris*, *A. negundo*, *U. minor* and *T. vulgaris*.

Table 8. Tukey test on axial compression strength and density

C. Australis						
Parameter	Units of measurement	Control	PF62-1	PF76-2	PF217-3	P-value
σ	MPa	70.30 c	56.72 b	59.65 b	49.43 a	< 0.01
ρ	g/cm^3	0.769 c	0.659 b	0.662 b	0.619 a	< 0.01
A. campestris						
Parameter	Units of measurement	Control	PF62-1	PF76-2	PF217-3	P-value
σ	MPa	64.28 a	60.57 a.b	54.23 c	56.27 b.c	<0.01
ρ	g/cm^3	0.671 c	0.635 b	0.592 a	0.595 a	< 0.01
A. negundo						
Parameter	Units of measurement	Control	PF62-1	PF76-2	PF217-3	P-value
σ	MPa	59.34 c	44.51 a	48.48 b	48.58 b	< 0.01
ρ	g/cm^3	0.611 b	0.558 a	0.569 a	0.536 a	< 0.01
R. pseudoacacia						
Parameter	Units of measurement	Control	PF62-1	PF76-2	PF217-3	P-value
σ	MPa	90.38				> 0.05
ρ	g/cm^3	0.838				> 0.05
T. vulgaris						
Parameter	Units of measurement	Control	PF62-1	PF76-2	PF217-3	P-value
σ	MPa	42.78 a	35.29 c	36.53 b.c	38.97 b	< 0.01
ρ	g/cm^3	0.439 c	0.383 a	0.398 a.b	0.414 b	< 0.01
U. minor						
Parameter	Units of measurement	Control	PF62-1	PF76-2	PF217-3	P-value
σ	MPa	61.32 ± 7.20				> 0.05
ρ	g/cm^3	0.710 b	0.672 b	0.689 b	0.627 a	< 0.01
P. acerifolia						
Parameter	Units of measurement	Control	PF62-1	PF76-2	PF217-3	P-value
σ	MPa	49.15 b	40.03 a	43.09 a	41.75 a	< 0.01
ρ	g/cm^3	0.652 b	0.587 a	0.588 a	0.575 a	< 0.01
Q. ilex						
Parameter	Units of measurement	Control	PF62-1	PF76-2	PF217-3	P-value
σ	MPa	74.28 ± 6.18				> 0.05
ρ	g/cm^3	0.783 ± 0.04				

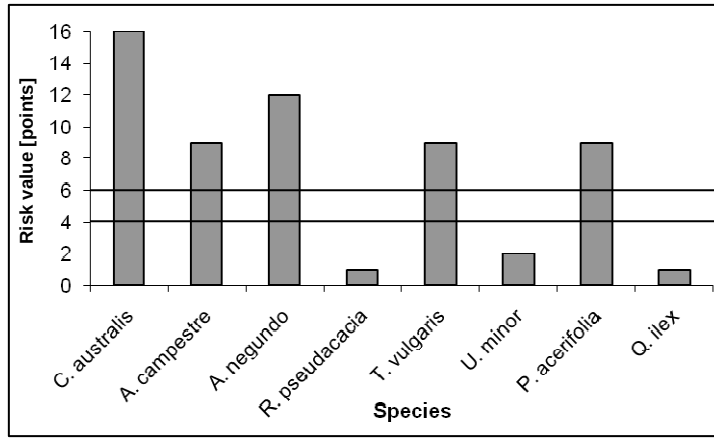

Fig 1. Risk matrix results

Concerning *U. minor*, only the isolate PF217-3 showed statistically significant differences for density.

The parameters taken into account were different from those generally used because the focus of the study was to evaluate the residual strength of the wood after an attack by *I. rickii*.

The shrinkages and the relative coefficients were relevant features between the inoculated and control specimens only for some species, while the density and axial compression strength efficaciously showed the action of *I. rickii* on all the tested species. The *Basidiomycetes* as *I. rickii*, are characterized by their ability to degrade lignin, hemicellulose and cellulose [Martínez et al. 2005] concurrently.

These polymers influence the mechanical characteristics of the wood: the lignin influences the axial compression strength [Giordano 1981]. The considerable statistically-significant decrease in density and in compressive strength is confirmed in the literature concerning various species of brown and white rot fungal agents found in various tree species. The physical, chemical and morphological wood changes, caused by the agents of caries, may be accompanied by a decrease in mechanical properties [Smith, Graham 1983; Green III, Highley 1997; Curling et al. 2002; Clausen, Kartal 2003; Yang et al. 2010; Bouslimi et al. 2014]. Brown rot has been extensively investigated in the literature and in all cases, the studied fungi were found to cause a loss of resistance to axial compression and a decrease in density [Winandy, Morrell 1993; Curling et al. 2002; Clausen, Kartal 2003; Silva Pereira et al. 2006; Silva et al. 2007]. The decrease in mechanical features was mainly due to an alteration in the polymers of the cell wall. Winandy and Morrell [1993] demonstrated the relationship between a degradation of hemicellulose and a decrease in compression strength.

Even weight loss appears to be related to a decrease in compressive strength: Smith and Graham [1983] demonstrated this statement studying weight loss in *Pseudotsuga mennziesii* Franco wood, caused by *Postia placenta* (Fr.) MJ Larsen & Lombard. This agent of white rot only occasionally led to a significant change in shrinkage, in relation to the control. Tsoumis [1991] confirmed that white rot did not cause considerable variation in shrinkage in relation to the control.

Density, in place of a decrease in weight, allows a rapid comparison of the behaviour of fungi which alter wood: this comparison is quantitative as well as qualitative. The density and axial compression strength were parameters that showed statistically the destructive activity of *I. rickii* more clearly than shrinkages and the related parameters.

The tests made it possible to understand that density and resistance to axial compression indicated large statistically-significant differences between the inoculated specimens and control specimens.

Density and axial compression strength were features which displayed large statistically-significant differences: they were the parameters indicating wood degradation due to *Inonotus rickii* (Pat.) Reid. Therefore, a risk matrix was created to establish, *in vitro*, a degradation ranking among the wood species, combining the more significant parameters.

The data showed that *C. australis*, *A. negundo*, *P. acerifolia*, *A. campestre*, e *T. vulgaris* were in the highest risk group. Although *in vitro* wood decay tests do not give definitive evidence of the degradative action of the fungus on living trees, they are useful in defining the potential risk [Baietto, Wilson 2010]. In any case, the results of surveys carried out in this study, and in previous studies in Italy [Annesi et al. 2005; Mazza et al. 2008], in Europe [Intini 2002; Intini, Tello 2003; Ramos et al. 2008] and in Argentina [Robles et al. 2011] support these results. In fact, *A. negundo*, *C. australis* e *P. acerifolia* are the most frequently recorded and most damaged hosts of *I. rickii* in urban boulevards.

Conclusions

- The wood of some species showed high residual density and strength.
- The three isolates exhibited different behaviour towards the species.
- The risk matrix made it possible to evaluate the wood of different wood species using the two statistically significant parameters.
- The wood species which did not show *in vitro* durability to *I. rickii* were the same that displayed susceptibility in living trees.
- The results showed that it is possible to evaluate the relative risk of degradation by *I. rickii* before the planting of trees in urban environments, thereby aiding tree management.

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List of standards

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UNI/ISO 3787:1985b – Wood. Test methods. Determination of ultimate stress in compression parallel to grain

UNI/ISO 4469:1985c – Wood. Determination of radial and tangential shrinkage

UNI/ISO 4858:1988 – Wood. Determination of volumetric shrinkage

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