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Evaluation of anamorphic state, wood decay and production of lignin-modifying enzymes for diatrypaceous fungi from Argentina

M.B. Pildain, M.V. Novas and C.C. Carmarán*

Deptmento de Biodiversidad y Biología Experimental, Lab. Micología-PHRIDEB, CONICET, Fac. de Cs. Exactas y Naturales, Universidad de Buenos Aires, Capital Federal EHA14[1]28, Argentina

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Morphological descriptions, wood decay and anatomical criteria, production of ligninolytic and cellulolytic enzymes and melanin production of diatrypaceous species (*Eutypella andicola*, *E. comosa*, *E. leprosa* and *E. scoparia*) from Argentina were investigated. The anamorphic state of *Eutypella andicola* is described for the first time. The morphological characteristic of the anamorphs were similar among all strains. Most isolates were capable of causing mass loss in *Populus deltoides* wood blocks during a 12-week period. Differences among strains were significant. The anatomical observations demonstrated that in a first state of wood colonisation this fungi produced soft rot decay and in later colonisation state the decay type is similar to white rot. Extracellular production of ligninolytic and cellulolytic enzymes were studied by mycelium growing on solid medium supplemented with different dyes (Malachite green, Azure B, Poly R-478, Congo red, tannic and galic acid, and guayacol). Seven of the eight strains analysed decolourised all the dyes except for the Malachite green. Only one strain of *E. scoparia* was able to decolourise Malachite green. Through simple assays we established the production of "extracellular melanins" and its association to growth areas of the fungus and discoloration zones.

Keywords: *Diatrypaceae*, *Eutypella*, ligninolytic enzymes, white rot fungi.

Introduction

The family *Diatrypaceae* (*Xylariales*) includes the genera *Eutypella*, *Eutypa*, *Diatrype*, *Cryptosphaeria*, *Echinomyces*, *Leptoperidida*, *Dothideovalsa* (Rappaz, 1987) with octosporous asci, and the genera *Diatrypella* and *Cryptovalsa* with polysporous asci (Kirk *et al*., 2001). More

*Corresponding author: C.C. Carmarán; e-mail: carmaran@bg.fcen.uba.ar

recently, Chacon (2002) recognised again *Endoxylina* which has been considered as a synonym of *Eutypa* by Rappaz (1987).

Most of *Diatrypaceae* species are predominantly saprobic on angiosperm bark. They are soft rot decay fungi, a decay mechanism characterised anatomically by longitudinal chains of cavities with conical tips in the S2 cell-wall layer (Savory, 1954). Worral *et al*. (1997) have suggested that *Eutypella parasitica* and *Cryptosphaeria lignyota* (Fr.:Fr.) Auersw. have the ability to cause white rot (where all cell-wall constituents are degraded). *Cryptovalsa halosarceicola* has been shown to solubilise significant amounts of lignin, comparable to those of white-rot basidiomycetes (Bucher *et al*., 2004).

White rot fungi are the most efficient ligninolytic organisms described to date (Stalpers, 1978; Seifert, 1983). This capability to degrade lignin is due to their extracellular non-specific and non-stereoselective enzyme system composed by laccases, lignin peroxidases and manganese peroxidases, which function together with H_2O_2-producing oxidases and secondary metabolites (Kirk and Farrell, 1987).

Concepts of wood decay continue to be largely based on a limited range of fungi, primarily in the families *Polyporaceae*, *Hymenochaetaceae* and *Corticiaceae* (Aphyllophorales) (Worrall *et al*., 1997). Other basidiomycetes (Seifert, 1983) and nonbasidiomycetes (Duncan and Eslyn, 1966; Merrill *et al*., 1964; Nilsson, 1973; Nilsson *et al*., 1989; Savory, 1954; Worrall *et al*., 1997, Bucher *et al*., 2004) can also cause degradation of wood, but comprehensive studies on decay by such fungi are rare.

In Argentina, there has been little work on the *Diatrypaceae* in general. All studies have focused on the species descriptions and taxonomy (Carmarán, 2002, 2003 a,b ; Carmarán and Romero, 1992). However, there is no information about the biodegradation potential of these microorganisms.

There have, however, been several studies of anamorphs of diatrypaceous fungi (Croxall, 1950; Glawe 1984; Glawe and Rogers 1986; Rappaz 1987). Glawe and Rogers (1982) have shown that conidia are produced holoblastically from sympodially or percurrently proliferating conidiophores. Conidiogenesis in these fungi have been found to be by one or both modes in each species.

The conidiomata are variable. Glawe and Rogers (1982) have described “disc- shaped sporulating areas” for *Diatrype albopruinosa* and *D. stigma* and “white floccose clumps upon which sporulating areas develop” for *D. viresens*. Glawe and Rogers (1984) have reported “small conidiomata resemble rudimentary pycnidia” for *D. whitmanensis* . and *D. bullata*. Glawe (1984) has described conidiomata in culture of two kinds, discoid to cupulate or globoid with several locules for *Cryptosphaeria pullmanensis*.

A common characteristic the family *Diatrypaceae* is their production of superficial black mycelia in culture and on host tissue. This pigmentation of hyphae is attributed to melanin production (Rappaz, 1997). Fungal melanins are biopolymers synthesized from phenolic compounds and confer certain advantages to fungi such as increasing their pathogenicity (Suryanarayanan *et al*., 2004).

In this work we present the results of the study of the anamorphs of eight strains: one isolate of *Eutypella andicola*, two of *E. leprosa*, one of *E. comosa* Speg. and four of *E. scoparia*.

In a effort to understand the ecology of the *Diatrypaceae* in our country, we aimed to: 1- describe the anamorphs of *E. andicola*, *E. leprosa*, *E. comosa* and *E. scoparia*; 2- study the lignin and cellulose biodegradation potential of these strains; 3- present preliminary results of characterization of the hyphal pigment of this fungus. For this purpose, eight native strains were studied.

Materials and methods

Fungal strains

The study was carried out with eight native Argentinean strains of four different species belonging to the family *Diatrypaceae*: one strain of *Eutypella andicola* (BAFCcult 1754), two of *E. leprosa* (BAFCcult 1744 and BAFCcult 1755), one of *E. comosa* (BAFCcult 393), and four of *E. scoparia* (BAFCcult 146; BAFCcult 357, BAFCcult 1756 and BAFCcult 1758). *Phanerochaete chrysosporium* (ME-446/BAFCcult 232), recognised as a very efficient dye-degrading microorganism (Cripps *et al*., 1990; Spadaro *et al*., 1992; Rodriguez *et al.*, 1999; Podgornik *et al*., 2001), was used to compare decolourising abilities. All strains are deposited in the Culture Collection of the Department of Biological Sciences, Faculty of Exact and Natural Sciences, University of Buenos Aires (BAFC) (herbarium abbreviations follow Holmgren *et al*., 1990). Stock cultures were maintained on malt extract agar slants and stored at 4ºC.

Morphological descriptions

Collected material was air-dried. Specimens were preserved in the BAFC. Observations and measurements were taken from fresh material mounted in distilled water, 5% KOH and phloxine for optical microscopy (M) and Melzer's reagent for the amyloid reaction (I).

Isolates were placed on oatmeal agar (OM) taking ascospores from the perithecia and exposing them to a 12 hour photoperiod. Culture descriptions

are based of two replicates of each strain grown on malt extract agar (MEA), Leonians and malt extract with filter paper (PF), for seven days.

Decay tests

Untreated *Populus deltoides* wood was chosen for its low extractive content and low durability. Wood was cut into 1 × 1 × 2 cm^3 blocks and leached in distilled water for 48 hours to remove water soluble components. Initial dry mass was determined after drying at 80°C for 48 hours. Test blocks were then autoclaved and placed in slants with ME agar that had been previously inoculated with each strain for two weeks. Ten replicate blocks were used per strain and another 10 non-inoculated blocks were used as controls. Incubation was carried out in a growth chamber at the Facultad de Ciencias Exactas y Naturales under controlled environmental conditions.

After 12 weeks, blocks were removed. Observations of the nature and abundance of external mycelium and macroscopic appearance of the decayed wood were made. External mycelium was carefully removed. Blocks were dried at 80°C for 48 hours or until constant weight.

Internal and external colour changes were observed. Cuts on transversal and longitudinal sections of decay and control woods were studied under bright field microscopy mounted in lactophenol/lactic acid, with an epifluorescence microscope (EFM) for lignin autofluorescence and using 5% calcifluor for the observation of β-glucane presence (Romero and Minter, 1988). Principle features recorded were: hyphal presence, cell wall thinning, cell separations and longitudinal cavities in the S2 cell-wall layer.

Loss weight results were expressed as the mean of ten replicates, plus/minus the standard deviation of the mean. Differences in initial and final dry weight were analysed by means of one-way ANOVA for each strain. Loss in dry weight was compared using a one-way ANOVA among strains, and the Least Significant Differences test (LSD) was conducted to compare the means. The assumption for homogeneity of variance and normality were verified.

Enzyme tests

To examine the decolourisation of industrial dyes, all strains were inoculated on agar plates (90 mm in diameter, 20 ml medium/Petri dish) containing malt extract (12.7 g/L), glucose (10 g/L) and agar (20 g/L) (MEA) (unless otherwise noted) supplemented with one of seven different dyes (0.02% of Poly R-478; 50μ M of Azure B, Malachite Green (triphenylmethane) and

Congo Red; 5 g/L of gallic and tannic acid; 0.2% v/v of Guaiacol (also adding 3.66 g/L of citric acid and disodic phosphate 10.72 g/L).

Plates were inoculated in the centre with an 8 mm agar plug of a 6-8 day old culture grown in MEA. Five replicate plates were used for each strain. Two control plates with no addition of dye were also inoculated with each strain. Non-inoculated plates served as controls for abiotic decolourisation. The relative dye decolourisation of each strain was recorder on the basis of clear zones formed around the mycelial disc after incubation at 23°C in darkness for 21-28 days. Growth was followed by measuring radial extension of the mycelium each week.

Diagnostic test for fungal melanin

The method of Gadd (1982) was used for extracting the pigment from the hyphae. Five discs (5mm diam.) were cut from 10-day-old colonies, boiled for 5 minutes in 5 ml distilled water and centrifuged (5000g, 5 minutes). The pellet was washed and centrifuged and the pigment was extracted by autoclavins with 3 ml 1 M NaOH (20 minutes, 120°C). The alkaline pigment extract was analysed with chemical tests that are diagnostic for fungal melanins (Suryanarayanan, *et al*., 2004): insolubility in water, solubility in 1 M KOH (100°C, 2 hours), precipitated with 3 N HCl, decolourised with H_2O_2, producing a brown precipitate with FeCl3 and formation of a grey precipitate with ammoniacal silver nitrate solution.

Results

Taxonomy

Eutypella andicola Speg., Anales Mus. Nac. Buenos Aires 23: 47, 1912.

(Fig.1)

Teleomorph: see Rappaz (1987).

BAFCcult 1754

Anamorph in culture. *Colonies* covering 9 cm diameter Petri plate in 2 weeks in all culture media, with white surface mycelium. In MEA and PF, we observed a blackened floccose clumps producing yellow conidial masses in 3 weeks. In MEA conidiomata consisted of aggregations of conidiogenous cells on blackened mycelial crusts disc-shaped, in Leonians medium they appeared dark, pycnidium-like and with several locules. *Conidiogenous cells* cylindrical, proliferating sympodially. No clear evidence of percurrent proliferation of conidiogenous cells was observed. However, because some

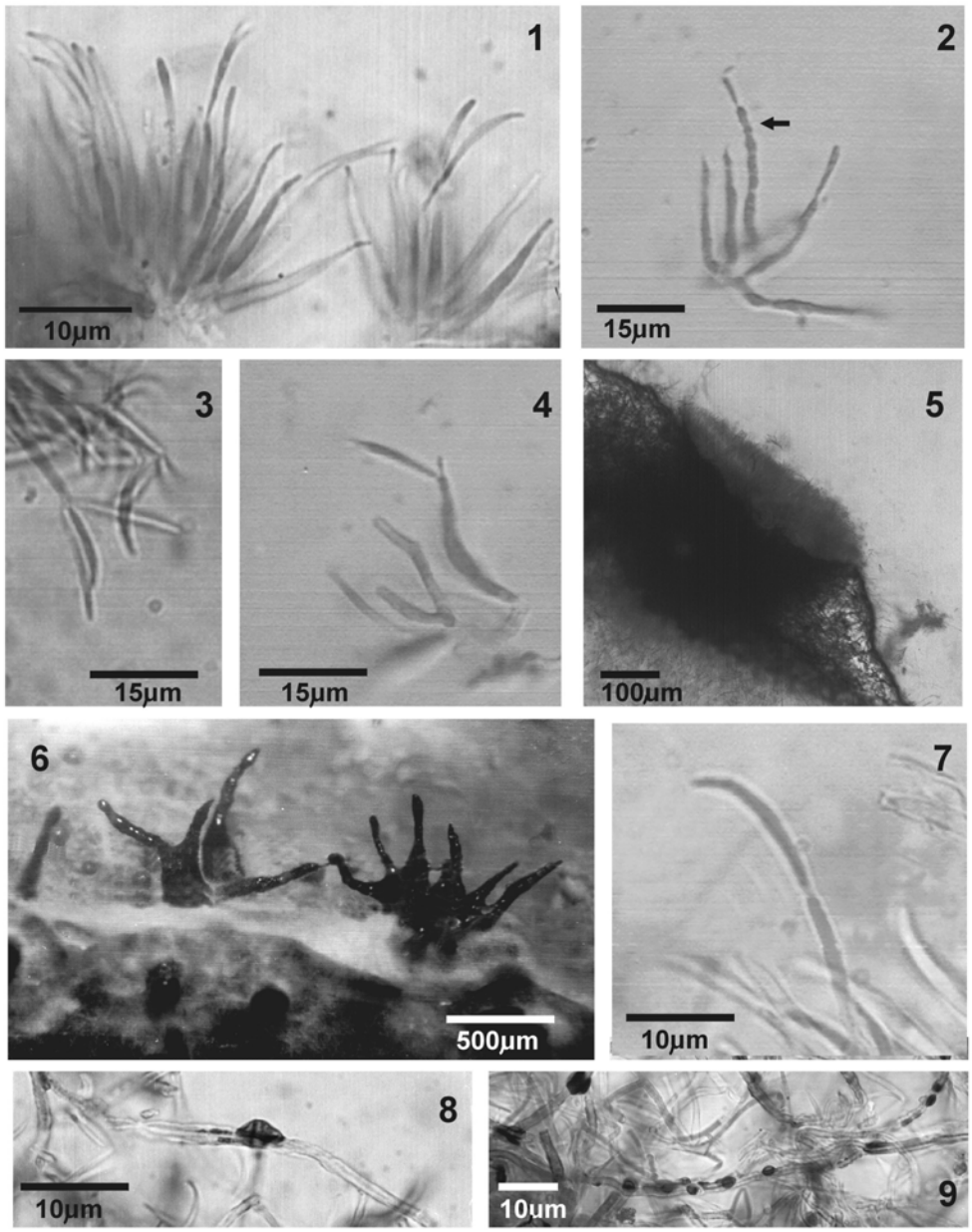


Fig. 1. Conidia and conidiogenous cells of *Eutypella andicola*. **2-4.** Conidia and conidiogenous cells of *Eutypella leprosa*. **4.** Conidiogenous cells with conspicuous proliferation (arrow). **5-7.** *Eutypella scoparia*. **5.** Conidiomata. **6.** Conidium and conidiogenous cell. **7.** Perithecia in culture. **8-9.** Extracellular melanin of mycelia in culture.

diatrypaceous fungi show the two kinds of proliferations in the same culture (Glawe and Rogers, 1984; Glawe and Jacobs, 1987) it would not be surprising to find percurrent and sympodial proliferations in this species. *Conidia* hyaline, smooth, slightly curved, 11-15 × 1.5-2 µm.

Comments: This is the second report of *E. andicola* and the first report of its anamorph. This species is very similar to *E. leprosa* . The anamorphs here described for both species are very similar, thus supporting this relationship.

Eutypella leprosa (Pers. ex Fr.) Berl., Icon. Fung. 3: 74. 1902. (Figs 2-4)

Teleomorph: see Rappaz (1987).

BAFCcult 1744, 1755

Anamorph in culture. *Colonies* covering 9 cm diameter Petri plate in 1-2 weeks in all culture media, with white surface mycelium. In Leonians medium, we observed blackened floccose clumps producing brownish conidial masses in 3 weeks. In MEA and PF, we observed the superficial area deeply blackened. *Conidiomata* observed in 4 weeks for malt extract and in 5 weeks for PF appearing dark, pycnidium-like in the three media. *Conidiogenous cells* cylindrical, proliferating sympodially and percurrently (more conspicuously in BAFC 1755). *Conidia* hyaline, smooth, slightly curved, 13-17 × 1.5-2 µm.

Comments: Prior reports of anamorphs of this species described a conidiomata pycnidium like, with conidia of 15-30 µm.

Eutypella comosa (Speg.) Rappaz. Mycol. Helv. 2: 544. 1987.

BAFCcult 393

Teleomorph: see Carmarán and Romero (1992); Rappaz (1987).

Eutypella scoparia (Schwein: Fr.) Ellis & Everh. (Figs 5-7)

BAFCcult 146, 357, 1758, 1756.

Teleomorph: see Carmarán and Romero (1992); Rappaz (1987).

Anamorph in culture. *Colonies* covering 9 cm diameter Petri plate in 1-2 weeks in MEA and Leonians medium, in 3 weeks in PF. Mycelium with white surface except for a blackened floccose clumps. In BAFCcult 1758 and BAFCcult 146 strains the conidiomata consisted of aggregations of conidiogenous cells on blackened mycelial. In BAFCcult 1756 strain the conidiomata appeared dark, pycnidium-like, and with several locules. In all strains, conidiogenous cells were cylindrical, proliferating sympodially. No clear evidence of percurrent proliferation of conidiogenous cells was observed. *Conidia* hyaline, smooth, slightly curved, 15-22 × 1,5 µm (in BAFCcult 1758 and BAFCcult 146) 12-17 × 2 µm (in BAFCcult 1756).

Comments: Rappaz (1987) showed the great variability in the anamorphs of *E. scoparia*. Both his and our results support the hypothesis that this species is ubiquist and polyphage. In all culture of the species studied we observed large black dense granules out of the wall (Figs 8-9).

Decay tests

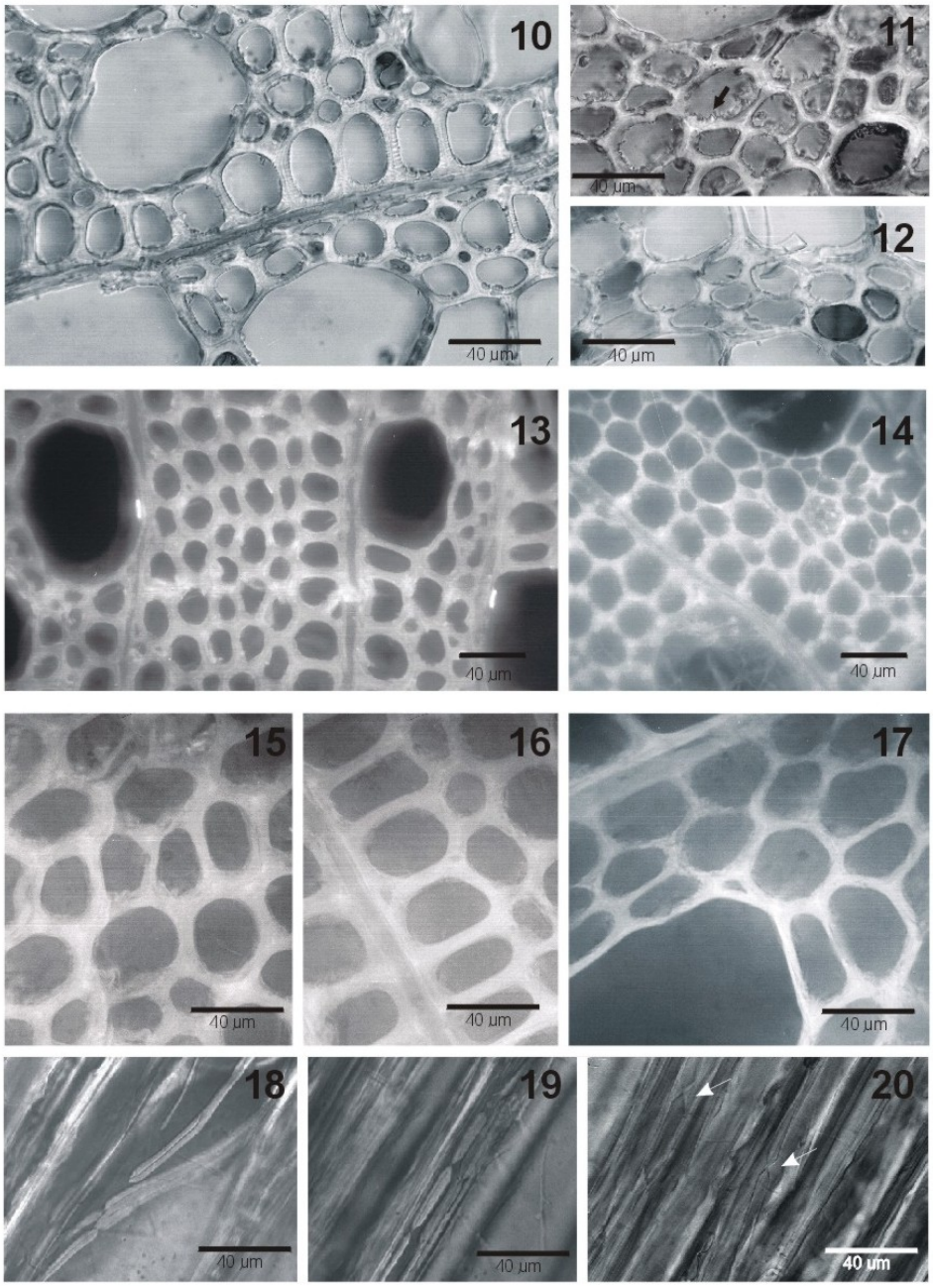
All replicates of inoculated blocks with BAFCcult 1754, BAFCcult 1744 and BAFCcult 1755 strains presented were totally covered with abundant external mycelium; woods with BAFCcult 357, BAFCcult 1756 and BAFCcult 1758 strains presented important blackened areas. In blocks with BAFCcult 146, BAFCcult 1744 and BAFCcult 1758, perithecia were observed (Fig. 6).

The general macroscopic pattern observed in decay wood was the external and internal bleaching. Microscopically, cell wall thinning was observed in wood decay, (Figs 10-17). Caverns in transversal view of wood decay are shown in Figs 18-20. Both lignin autofluorescence evidence that lignin degradation occurs and fluorescence with calcifluor show that cellulose and lignin are simultaneously degraded (Figs 13-17). The longitudinal cavities, which are characteristic of soft-rot type, were observed in longitudinal cuts in the BAFCcult 1754, BAFCcult 1758, BAFCcult 1756, BAFCcult 1755 strains (Figs 18-20).

The analysis of weight loss difference was performed using untransformed and *ln*-transformed dry weight data. But, since the results were similar, only results from the untransformed variable are given. We present actual probability values instead of the traditional arbitrary significance criteria ($P \leq 0.05$) to permit the reader to interpret significance.

Differences between final and initial dry mass were significant for 1744 ($F = 38.3$; $P < 0.0001$), 1755 ($F = 24.7$; $P < 0.0001$), 1758 ($F = 19.7$; $P < 0.0004$), 1756 ($F = 13.96$; $P < 0.0015$), 357 ($F = 8.69$; $P < 0.0085$), 1754 ($F = 0.78$; $P < 0.389$) strains (Fig. 21).

Differences in wood decay among strains were significant ($F = 19.05$; $P < 0.0001$) (Fig. 22). Mean comparisons showed the formation of four groups: one separate group was formed by BAFCcult 393 (*E. comosa*) strain; another group was formed by the four strains of *E. scoparia* (BAFCcult 146, BAFCcult 357, BAFCcult 1756 and BAFCcult 1758); the third group was formed by BAFCcult 1756, BAFCcult 1758 (both *E. scoparia*) and strain BAFCcult 1754 (*E. andicola*) strains; and the fourth group was formed by BAFCcult 1756 (*E. scoparia*), BAFCcult 1754 (*E. andicola*), BAFCcult 1744 and BAFCcult 1755 (both *E. leprosa*) strains.



Figs 10-20. Anatomic observation of wood decay. **10-12.** Wood viewed with Normasky. **10.** Control wood. **11-12.** Wood inoculated with *Eutypella leprosa.* **13-14.** Wood viewed with calcifluor in fluorescence microscopy. **13.** Control wood. **14.** Wood inoculated with *Eutypella leprosa*. **15-17.** Autofluorescence of lignin. **15-16.** Wood control. **17.** Wood inoculated with *Eutypella leprosa*. **18-20.** Longitudinal caverns in decay wood.

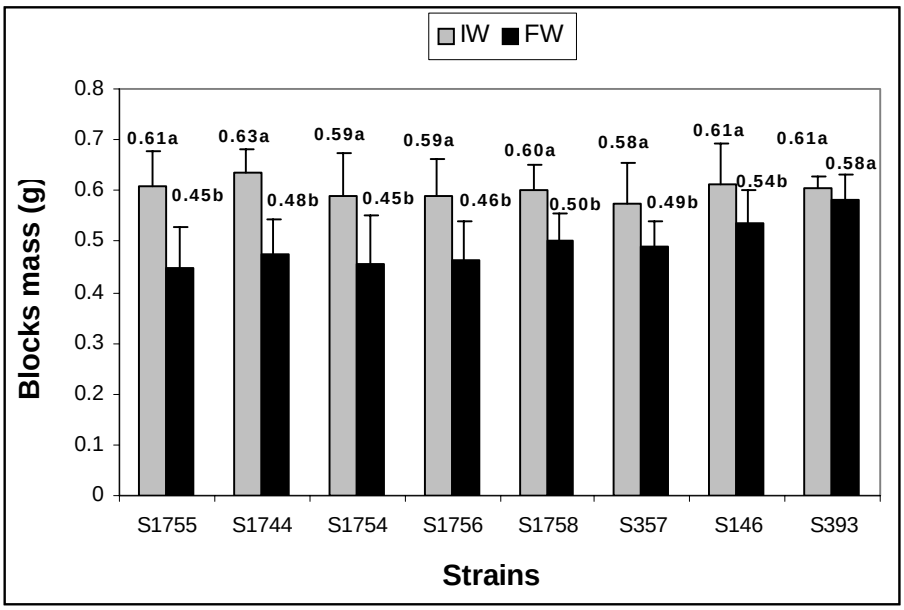


Fig. 21. Differences in initial and final wood blocks dry weight caused by different diatrypaceous species. Species used in the assay: *Eutypella andicola* (S1754), *E. comosa* (S393), *E. leprosa* (S1744, S1755) and *E. scoparia* (S146, S357, S1756, S1758).
IW = Initial block weight; FW = Final block weight.
Differences in IW and FW were analysed using a one-way ANOVA. Different letters above bars indicate significant differences between IW and FW within the same strain ($P<0.05$).

Enzyme tests

The extracellular enzyme production was investigated in solid media containing malt extract/glucose and the different dyes. Table 1 summarises the results of ligninolytic activity (Poly R-478 solid-plate decolourisation rate), lignin peroxidase (LiP) activity (Azure B solid-plate decolourisation rate), Malachite Green (growth and decolourisation rate), cellulases (Congo red solid-plate decolourisation rate), polyphenol oxidases (decolourisation of gallic and tannic acid media), and laccase (guaiacol solid-plate decolourisation rate).

All the strains analysed were cellulolytic. The ability to produce enzymes involved in lignin degradation *in vitro* was greatly uniform among taxa used in this study. The eight *diatrypaceous* strains were able to decolourise six of the seven dyes (Poly R, Azure B, Congo Red, Gallic acid, Tannic acid and

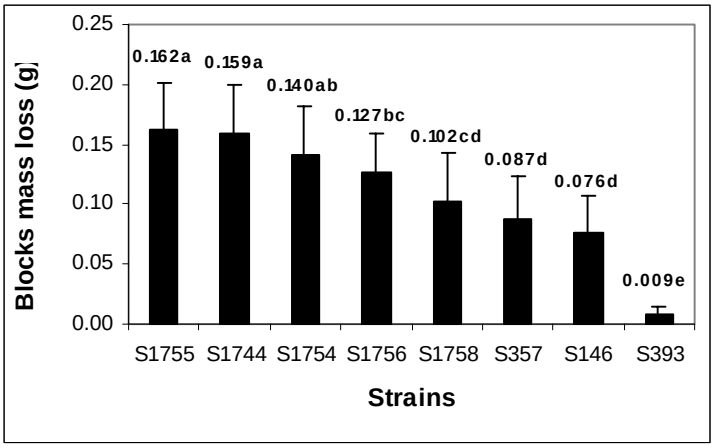


Fig. 22. Wood decay caused by different diatrypaceous species measure as mass loss in *Populus deltoides* wood blocks.
Species used in the wood decay assay: *Eutypella andicola* (S1754), *E. comosa* (S393), *E. leprosa* (S1744, S1755) and *E. scoparia* (S146, S357, S1756, S1758).
Loss in dry weight was compared using a one-way ANOVA. Least Significant Differences test (LSD) was conducted to compare the means. Different letters above bars indicate significant differences between strains (P<0.05).

Guaiacol). Malachite Green was decolourised to one strain, *E. Scoparia* (BAFCcult 1756).

Diagnostic test for fungal melanin

The alcaline pigment extracted showed expected features for melanin in chemical diagnostic tests for fungal melanins: insolubility in water, solubility in 1 M KOH (100%, 2 hours), precipitated with 3 N HCl, decolourised with H_2O_2, producing a brown precipitate with FeCl3 and formation of a grey precipitate with ammoniacal silver nitrate solution.

In the enzyme tests the melanin was observed in the zones where the major decolouration was produced. Microscopic observation (Figs 8-9) showed that this melanin type is extracellular (Bell and Wheeler, 1986).

Table 1. Production of wood decay enzymes *in vitro* by *Diatrypales*.

Species	Strain	Poly R	Azure B	Malachite Green	Congo Red	Gallic acid	Tannic acid	Guaiacol
E. andicola	BAFCcult 1754	+	+	-	+	+	+	+
E. leprosa	BAFCcult 1744	+	+	-	+	+	+	+
	BAFCcult 1755	+	+	-	+	+	+	+
E. comosa	BAFCcult 393	+	+/- a	-	+	+	+	+ b
E. scoparia	BAFCcult 146	+	+	-	+	+	+	+
	BAFCcult 357	+	+	-	+	+	+	-
	BAFCcult 1756	+	+	+	+	+	+	+
	BAFCcult 1758	+	+	-	+	+	+	+

Plates were inoculated for 3-4 weeks at 23°C. Five replicate plates were used for each strain.
BAFCcult = Culture Collection of the Department of Biological Science, Faculty of Exact and Natural Sciences, University of Buenos Aires.
+ or - signs denote whether visible decolouration occurred.
a = decolouration occurred in one replicate after 35 days.
b = decolouration occurred in one replicate after 28 days.

Discussion

Eutypella andicola was isolated for the first time in culture, and a description of its anamorphic stage is given. *Eutypella leprosa* and *E. scoparia* have similar morphology to those described in previous reports (Tiffany and Gilman, 1965; Rappaz, 1997). The MEA was the best media for obtaining anamorphs. The presence of perithecia in *E. scoparia* in the decay test agrees with the observations of Rappaz (1997) who also obtained the teleomorph of this species.

The high incidence of cellulase and ligninase production indicates that at least these members of the *Diatrypaceae* family are physiologically capable of producing wood decay. Our results (Table 1) showed that the ligninolytic and cellulolytic enzyme activity was homogeneous and no obvious differences were observed in the enzyme production among the strains analysed.

The morphology of *E. scoparia* and *E. leprosa* is very different, and studies are needed (Carmarán, 2003 a,b) to determine the phylogenetic relationship of these two species and may warrant placement in different genera.

Enzyme characteristics are similar and extensive throughout the family. However, other studies have reported more variable enzymatic activity than we have. Worral *et al*. (1997) assigned a negative enzyme production pattern to *E. parasitica* and Bucher *et al*. (2004) has observed a cellulolytic profile in a strain of the genus *Eutypa*, with negative results for Azure B and Poly R. In

addition, Bucher *et al*. (2004) have reported the same pattern of enzyme production for *Cryptovalsa halosarceicola* (species with polysporous asci).

The positive correlation between Azure B and Poly R demonstrated that the strains used in this study are physiologically capable to producing ligninolytic and lignin-peroxidase enzymes. The BAFC 1756 strain was the only one capable of growing in Malaquite green. Thus, studies are necessary for the evaluation of its possible utilization in biotransformations and detoxifications.

The results of wood decay agree with the enzyme test: both showing uniform patterns among strains, and it is possible to see a significant difference that allows the strains to be separated into groups. Mean differences in weight loss were significant between the strains. The most remarkable result was that *E*. *scoparia* and *E*. *leprosa* were never within the same group, thus suggesting that even though all the strains are enzyme producers, each species may have a different decay potential. The fact that *E*. *andicola* appeared in the same group as *E*. *leprosa* is correlated with the similar morphologies of both species (Carmarán, 2003 a,b). Fengel and Wegener (1984) consider that fungi that cause a weight loss below 2% may not be decayers. Therefore, since the weight loss observed was between 12 to 44%, our results indicate that this species are decayers. This weight loss obtained is a promising result for the potential application of these strains in the pulp industry.

The type of decay produced is difficult to define and differentiate because the enzyme test reveals white rot decay and the anatomical studies showed a defibration and erosion of the cell wall from the lumen surface (Figs 10-17), both characteristics of white rot. In the anatomical studies, longitudinal chains of cavities with conical tips, a typical characteristic of the soft rot decay, were observed (Figs 18-20). Because features of the decay are apparently not distinctive, Worrall *et al*. (1997) use the taxonomy of the causal agent as a defining character. In this case, the type of decay assigned to the species analysed should be a soft rot.

Chemically, soft rot differs from white rot in that lignin degradation is relatively low in the former. We found a considerable lignin loss demonstrated by the wall thinning and the autofluorescence by the test species and also in the results of enzyme test. Anatomically, we observed a degradation gradient from soft to white rot related to a colonization rate of the blocks. Our data suggest that, in an early state of wood colonization, these Ascomycetes could be considered as soft rot fungi, but that in more advanced colonization states, they show a uniform degradation typical of white rot fungi.

In the present study, we conducted simple assays that show for the first time that these fungi synthesised and deposited melanins over their hyphae

(Figs 8-9). The secretion of ligninases (phenol oxidases) into the external environment to oxidize phenolic compounds has been postulated as a mechanism for the "extracellular melanins" synthesis (Bell and Wheeler, 1986). In the enzyme tests carried out we were able to observe a positive correlation between the discoloration zones and the melanised mycelia, thus supporting Bell and Wheeler's (1986) hypothesis.

No conclusions for *E. comosa* could be obtained from our results because the isolate utilized (BAFC 393) did not produce an anamorphic stage nor wood decay under the culture conditions used, but did show enzymatic activity. This strain was isolated from the teleomorph in 1999 and conserved in slant at 5ºC, a condition that could determine the alteration of the strain (Smith and Onions, 1994).

Based on the magnitude of the decolouration obtained, weight loss and anatomical features, some strains analysed may have potential application in biodegradation. The majority of the previous studies have focused on degrading enzymes of Basidiomycetes. Recently, however, there has been a growing interest in studying enzymes of a wider array of fungi, not only from the standpoint of comparative biology but also with the expectation of finding better degrading systems for use in various biotechnological applications.

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