EVALUATION OF *MYCOSPHAERELLA* IMPACT ON EUCALYPTS PLANTATIONS IN PORTUGAL

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ABSTRACT

Mycosphaerella leaf disease (MLD) is one of the most important diseases of eucalypts plantations worldwide. However, only recently it has become relevant in Portugal. Caused by a complex of *Mycosphaerella* species, this disease reduces the photosynthetic area and can cause tree defoliation. In extreme cases it causes reduction in the volume of wood produced. In order to relate the observed symptoms of MLD with the presence of the pathogen and at the same time obtaining an evaluation of eucalypt clones and family susceptibility, two experimental plantations were established in places where the disease has been detected. Data on the percentage of affected crown (necrosis or defoliation) were collected and some of the *Mycosphaerella* species present were identified (*M. africana*, *M. communis*, *M. grandis*, *M. lateralis*, *M. marksii*, *M. nubilosa*, *M. parva*, *M. vespa* and *M. walkeri*).

Key-words: *Eucalyptus globulus*; *Mycosphaerella* leaf disease; MLD; plantation trial.

RESUMO

A doença das manchas das folhas do eucalipto é uma das mais importantes nas plantações de eucalipto, tendo-se só recentemente tornado relevante em Portugal. Esta doença, causada por um complexo de espécies de *Mycosphaerella*, reduz a área fotossintética da árvore, podendo causar desfolha, com consequente redução da taxa de crescimento e do volume de madeira produzido. Com o objectivo de relacionar os sintomas observados com a presença do agente patogénico e avaliar a susceptibilidade de clones e famílias de eucalipto, foram estabelecidas duas plantações experimentais em locais onde foi detectada a doença. Foram recolhidos dados relativos à percentagem de área da copa afectada (por necroses ou desfolha) e identificadas as espécies de *Mycosphaerella* associadas (*M. africana*, *M. communis*, *M. grandis*, *M. lateralis*, *M. marksii*, *M. nubilosa*, *M. parva*, *M. vespa* e *M. walkeri*).

Palavras-chave: *Eucalyptus globulus*; doença foliar; MLD; plantação experimental.

INTRODUCTION

Eucalyptus globulus Labill. is a valuable forestry species due to its rapid growth rate and high quality of wood fibre suited to a wide range of paper products. However, sustainable production from plantations will

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only be possible if major pests and diseases can be avoided or their impact on tree growth and wood quality are kept below economically significant levels. In Portugal, forestry and forest products represent a major industry sector of substantial economic, social and strategic importance. *E. globulus* plantations occupy an area of 646 700 ha, representing 20.6% of the total forest area (DGRF, 2007).

Mycosphaerella leaf disease (MLD - also referred to as *Mycosphaerella* leaf blotch and *Mycosphaerella* leaf spot disease) is one of the most important diseases of plantation-grown eucalypts in the world. It is a very complex and relatively poorly understood disease, mainly because it might better be referred to as a complex of diseases, which are caused by a multiplicity of *Mycosphaerella* species. In fact, nearly 62 species belonging to the *Mycosphaerella* genera have been related to MLD symptoms in *Eucalyptus* spp. worldwide.

The first record of *Mycosphaerella* species on *Eucalyptus* outside its native range comes from Portugal. This species was identified as *Mycosphaerella molleriana* (Thüm.) Lindau in 1881 (Crous & Wingfield, 1997) and afterwards *Mycosphaerella africana* Crous & M. J. Wingfield and *Mycosphaerella walkeri* R. F. Park & Keane (Crous, 1998) were also reported. However, serious damage in industrial plantations of *E. globulus* was only reported in recent years when leaf blotch and serious defoliation of young eucalypt plantations were observed. Some more species were then reported: *Mycosphaerella madeirae* Crous & Denman (Crous *et al.*, 2004), *Mycosphaerella communis* Crous & J.P. Mansilla, *Mycosphaerella heimii* Bouriquet ex Crous, *Mycosphaerella lateralis* Crous & M.J. Wingf., *Mycosphaerella marksii* Carnegie & Keane, *Mycosphaerella nubilosa* (Cooke) Hansf. and *Mycosphaerella parva* R.F. Park & Keane (Crous *et al.*, 2006).

The aim of this work was to relate observed symptoms with the presence of the pathogen and, at the same time, to obtain a first evaluation of the susceptibility of a range of eucalypt clones and families showing resistance to this disease. Two experimental stands were established and parameters for disease evaluation in the field were collected. Symptomatic leaves were collected from the experimental stands and species were identified by their general morphology, type of ascospore germination and cultural characters.

MATERIAL AND METHODS

Experimental stands

During spring 2004 two experimental stands were established in Torres Vedras and Aveiro.

In the Torres Vedras stand, 33 *E. globulus* clones, 4 hybrid clones with *E. globulus* and 13 full-sib families were tested using repetitions of 6 two-tree plots per genotype. Two treatments were applied: a fungicide treated block (bitertanol or tolyfluanid was applied every two weeks until the autumn 2005) and an untreated block. Disease evaluation patterns were recorded every two weeks on a single branch for each tree. The percentage of the affected leaf area was expressed in 4 levels (level 1 – 0 to 24%; level 2 – 25 to 49%; level 3 – 50 to 75% and level 4 – more than 75%).

In the Aveiro stand, 10 *E. globulus* clones and 1 family were tested using repetitions of 10 trees per genotype. Fungicide treatments similar to the Torres Vedras stand were applied. Disease evaluation was based on percentage of crown defoliation.

Morphological characterization of fungi

During spring 2005, as soon as the first lesions on new leaves were reported, 50 leaves were collected randomly from each plantation. For morphologic characterization of *Mycosphaerella* species, lesions were observed under the stereomicroscope and one lesion per leaf was selected. Stereomicroscope photographs of each lesion were obtained and used for description of leaf lesion

dimensions, shape and color. Wherever possible, 30 repeated measurements (600x) were made of ascospore dimensions. Germination patterns of ascospores were determined after 24 h on malt extract agar medium (MEA) 2 % at 24ºC in the dark and single spore cultures obtained. Pure cultures were maintained in MEA 2 %, at 24 ºC in the dark. Linear growth of single spore cultures was assessed after 1 month at 24 ºC in the dark. Colony colours of the surface and reverse were also obtained (Carnegie & Keane, 1998; Crous, 1998 and Crous *et al.*, 2004).

RESULTS AND DISCUSSION

The 50 genotypes tested in the Torres Vedras stand revealed higher levels of disease severity expressed as percentage of leaf area affected on the fungicide untreated plot when compared to the treated plot (figure 1). Analysis of results from the untreated plot permitted to distinguish some *E. globulus* clones (numbers 17, 18 and 25) and hybrid clones with *E. globulus* (numbers 19, 20 and 37) showing resistance to MLD disease. In general, full-sibs families showed sensitivity to disease, with clones 40, 45 and 50 together with some *E. globulus* clones (numbers 11 and 12) the highest sensitive ones. Other *E. globulus* clones and hybrid clones with *E. globulus* presented moderate resistance.

In the Aveiro stand, with exception of clone 4 and 10, the genotypes tested showed significant differences in disease severity, expressed as crown defoliation percentage, between the plot treated with fungicide and the untreated plot (figure 2). Analysis of the results collected from the plot without fungicide application enabled to distinguish different behavior of *E. globulus* clones ranging from highest sensitive (clone 1) to highest resistant (clone 10). Trees of seminal origin showed a median level of defoliation.

In both plantations (Figs. 1 and 2), fungicide application could not completely control the disease development but reduced the severity level.

Results of identification of *Mycosphaerella* species based on general morphology showed no significant differences between plantations concerning species percentage. With the exception of *M. lateralis* that occurred only in Aveiro, all the other species were detected in both plantations (table 1).

M. communis and *M. nubilosa* occurred always alone on the leaf lesions. Although *Mycosphaerella grandis* Carnegie & Keane, *M. parva* and *Mycosphaerella vespa* Carnegie & Keane occurred alone in some lesions, *M. parva* was observed in association with *M. grandis* and *M. vespa*, at a maximum of two species per lesion. *M. lateralis* occurred always in association with *M. parva*.

M. parva was previously reported to colonize the same lesions of *M. cryptica* (Cooke) Hansf., *M. gregaria* Carnegie & Keane, *M. marksii, M. mexicana* Crous and *M. nubilosa* (Park and Keane, 1982; Maxwell, 2004). Also, *M. grandis* was reported to be associated with lesions of *M. gregaria* and *Aulographina eucalypti* (Cooke & Massee) Arx & E. Müll. (Carnegie and Keane, 1994).

Morphologic characteristics of lesions observed in the present study agreed with type descriptions for *M. nubilosa, M. parva* (Park & Keane, 1982), *M. walkeri* (Park & Keane, 1984), *M. grandis, M. marksii* (Carnegie & Keane, 1994), *M. africana, M. lateralis* (Crous & Wingfield, 1996), *M. vespa* (Carnegie & Keane, 1998) and *M. communis* (Crous *et al.*, 2004), except for small differences.

Dimensions of *M. nubilosa* lesions were not given in the type description (Park & Keane, 1982). In this study, lesions were round or irregular with 3-15 mm diameter, sometimes coalescent, forming larger irregular pale brown blotches, frequently surrounded by a raised thin brown margin. Ascospore germination was type C, with germination from both ends, germ tubes parallel to long axis of spore, not darkening or distorting. Culture colonies were 14–18 mm after 1 month, with irregular margins, olivaceous grey at both surfaces.

Leaf spots of *M. parva* were subcircular with 4-15 mm diameter, light brown, surrounded by a raised border and thin, dark brown margin. Ascospore germination was type N. Ascospores and germ tubes became uniformly brown, distorted and verruculose. Cultural features were not given in the type description (Park & Keane, 1982). In this study culture colonies were 15-25 mm after 1 month, olive in surface and green on reverse.

Leaf spots of *M. grandis* were described by Carnegie & Keane (1994) as confined to the margin of the leaf extending from the tip almost back to the petiole. In this study we did not confirm this particular distribution pattern on leaf surface. Ascospores and germ tubes became dark with gross distortion and produced several germ tubes. The lesions of leaves were round and angular with 5-7 mm diameter. The culture colonies were 22-27 mm after 1 month, olive in surface and green on reverse.

Lesions of *M. communis* were sub-circular to circular, 4–12 mm diameter, medium brown, surrounded by a thin, raised, concolorous border. Ascospore germination was type F with ascospores not darkening and germinating from both ends, with germ tubes parallel to the long axis of the spore and distorting prominently upon germination. The culture colonies were 29-36 mm after 1 month (not 20–35 mm as in Crous *et al.*, 2004), irregular, erumpent, uneven, folded, aerial mycelium moderate to sparse, hazel in surface and olivaceous-black on reverse.

Leaf spots of *M. lateralis* were subcircular, 3-12 mm diameter, gray-brown, surrounded by raised borders, medium brown on the adaxial surfaces and concolorous on the lower surfaces. Ascospore germination was type I germinating from both ends, germ tubes parallel to long axis of spore, not darkening, constricted at septum and developing lateral branches. The culture colonies were 32-37 mm after 1 month, not 53 mm as in Crous & Wingfield (1996) or 15–25 mm as in Maxwell (2004), with an even margin, cream aerial mycelium, gray olivaceous on reverse.

Lesions of *M. vespa* were circular to irregular, mostly less than 5 mm diameter, rarely confluent, light-brown to red-brown becoming grey with age, with a red-brown margin that is often raised. Although wasps are often found in cavities within lesions (Carnegie & Keane, 1998) they were not found in this study. Ascospore germinating was type C with germination from the apices of both cells, parallel to the long axis of the spore. Occasionally, a third germ tube grows at an acute angle from the median septum end of one of these cells. Cultures were grey-green to brown, often becoming dendritic towards the outer margins. Aerial hyphae were light grey to white and fluffy, becoming sparser towards the edge of cultures as Carnegie & Keane (1998) and olivaceous grey on reverse.

Lesions of *M. africana* were smaller (1-2 mm) and easy to distinguish because of their dark brown borders frequently surrounded by diffuse red-purple margins. Ascospore germination was type G with irregular germination from both ends, or from different positions in cells, with two or more germ tubes, darkening and distorting.

Lesions of *M. marksii* were bigger than *M. africana* lesions (3-20 mm) with red-purple margins. Ascospore germination was type B with germination from both polar ends with germ tubes parallel to the long axis of spore, not darkening or distorting.

Lesions of *M. walkeri* were very similar to those of *M. marksii*. Ascospore germination was type C, not darkening at germination, germ tubes parallel to the long axis of spore, with the spore becoming swollen and constricted. Conidia of *Sonderhenia eucalypticola* (A.R. Davis) H. Swart & J. Walker were observed in some lesions of *M. walkeri*.

CONCLUSIONS

Differences in disease severity among species of eucalyptus have been described, *E. globulus* being one of the most susceptible (Carnegie *et al.*, 1994; Dungey *et al.*, 1997;

Tejedor, 2004). A comparison of results between the two plantations is difficult because measured parameters were distinct (percentage of leaf area affected / defoliation percentage). Quantity and quality of genotypes tested were also different. Nevertheless, these first field trials enabled important differences to be detected between genotypes (*E. globulus* clones and families) in the susceptibility to MLD. These results show the potential benefits of selecting resistant *E. globulus* material to plantations in areas where MLD is a problem.

Fungicide application could not completely control the disease development but reduced severity levels attained.

The composition of *Mycosphaerella* complex of species recorded in both plantations showed no important differences, except for *M. lateralis* that occurred only in Aveiro. These results should be accepted with some reserves due to difficulties of differentiating *Mycosphaerella* species only on basis of morphologic characteristics increased by the fact that more than one species may occur in the same lesion.

Although leaves were collected only once nine *Mycosphaerella* species were detected in this survey (*M. africana, M. communis, M. grandis, M. lateralis, M. marksii, M. nubilosa, M. parva, M. vespa* and *M. walkeri*). Some of the results were confirmed by work based on molecular methods (Silva *et al*., unpublished).

This study suggests that the number of species present in *E. globulus* plantations is higher, making difficult the attribution of pathogenic/saprophytic role to individual species.

Further research is needed to completely identify species involved in MLD, with comprehension of their biological life cycle and attribution of individual role in MLD development. Additionally, field trials will provide new information on the resistance/susceptibility genotypes to be included in plant production. These tasks will be essential to devising future control strategies preventing economical losses in commercial eucalypts plantations.

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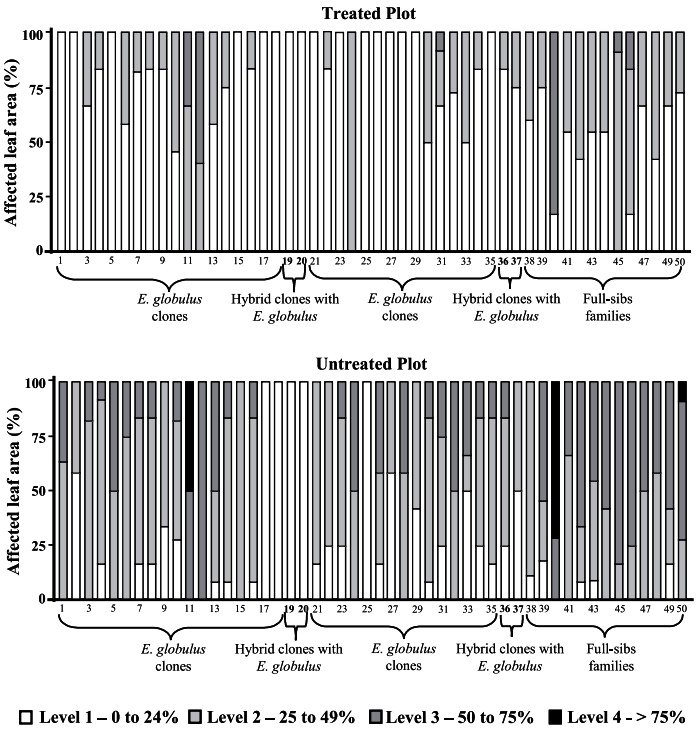

Figure 1 – Distribution of affected leaf area observed in 33 *E. globulus* clones (numbers 1 to 18 and 21 to 35), 4 hybrid clones with *E. globulus* (numbers 19, 20, 36 and 37) and 13 full-sib families (numbers 38 to 50) tested in the Torres Vedras stand. Results in percentage were expressed as 4 levels: 1 – 0 to 24%; 2 – 25 to 49%; 3 – 50 to 75% and 4 – more than 75% and presented per plot, treated and untreated with fungicides.

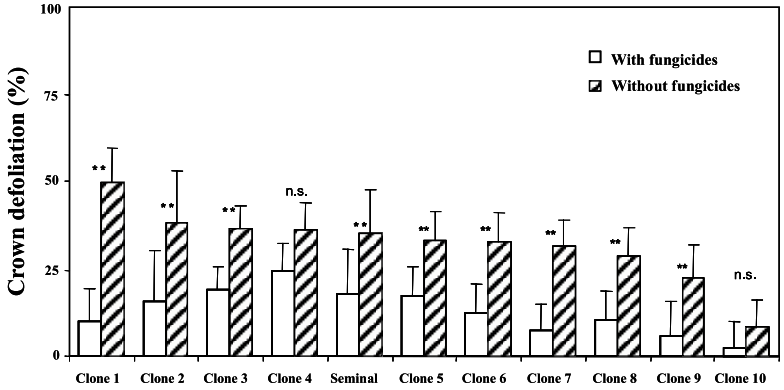

Figure 2 – Percentage of crown defoliation observed in 10 *E. globulus* clones and 1 *E. globulus* family in the Aveiro stand, comparing plots treated and untreated with fungicides. Bars represent the standard deviation of the mean. (n.s.) not significant (p>0,05); (*) p≤0,05; (**) p≤0,01.

Table 1 – Percentage of *Mycosphaerella* species detected in Torres Vedras and Aveiro plantations. Data from 1 lesion per leaf, in 50 leaves collected from each plantation.

***Mycosphaerella* species**	**Torres Vedras (%)**	**Aveiro (%)**
M. nubilosa	22.81	17.46
M. parva	12.28	20.63
M. grandis	28.07	19.05
M. communis	10.53	7.94
M. lateralis	-	6.35
M. vespa	15.79	3.17
Other species (*)	10.53	25.40

(*)Other *Mycosphaerella* species included *M. africana, M. marksii* and *M. walkeri*.