

# Pathogenicity evaluation of *Cytospora eucalypticola* isolated from *Eucalyptus* spp. cankers in Uruguay

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## ABSTRACT

*Cytospora eucalypticola* has been frequently associated with twig and stem cankers and as endophyte of *Eucalyptus globulus* and *E. grandis* in Uruguay. Mycelium discs of two *C. eucalypticola* isolates obtained from actively growing colonies were inoculated, both superficially and on experimentally wounded stems of *E. globulus* and *E. grandis*. No inoculated and control plants have shown any discoloration, gumosis or necrosis nor did they display lesions ten months after inoculation. Callus tissue was formed, partially or wholly occluding the wounds. The ability to penetrate healthy tissues and the inability to produce lesions evidenced that the presence of *C. eucalypticola* in twig and stem cankers could result from saprotrophic expansion of the endophytic mycelium in dying tissues, cankers probably being produced by different environmental stress conditions.

**Additional keywords:** endophyte, stem wound, inoculation, saprotrophic expansion, environmental stress.

## RESUMO

### Avaliação da patogenicidade de *Cytospora eucalypticola* isolado de *Eucalyptus* spp. no Uruguai

*Cytospora eucalypticola* tem sido, frequentemente, associado a lesões de pequenos ramos e cancos de caules e como endófito de *Eucalyptus globulus* e *E. grandis* no Uruguai. Discos de micélio de dois isolados do *C. eucalypticola* obtidos de colônias crescendo ativamente foram inoculados superficialmente e em caules de *E. globulus* e *E. grandis* feridos experimentalmente. Não se observou exsudação de goma, descoloração, necrose, nem lesões dez meses depois nos controles. O tecido de calo foi formado, parcialmente ou totalmente fechando as feridas. A capacidade de penetrar tecidos saudáveis e a incapacidade de produzir lesões evidenciou que a presença de *C. eucalypticola* em cancro do caule pode resultar em uma expansão saprofítica do micélio em tecidos enfraquecidos. O cancro provavelmente foi produzido por algum tipo de estresse abiótico

**Palavras-chave adicionais:** endófito, ferida do caule, inoculação, expansão saprofítica, estresse abiótico.

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Fungal diseases of *Eucalyptus* spp. could become a significant problem in Uruguay considering that *c.a.* 370,000 ha have been planted during the last ten years mainly with *Eucalyptus* spp. A species of the genus *Cytospora* has been associated with seedling and tree cankers in several countries (Keane *et al.*, 2000) and also as endophytes on leaves and twigs of *Eucalyptus nitens* (Deane et Maiden) Maiden (Fisher *et al.*, 1993). In Uruguay it has been found in sprouting stumps of *E. globulus* Labillardière (Bettucci & Saravay, 1993) and in healthy and symptomatic twigs of *E. globulus* and *E. grandis* W. Hill ex Maiden (Bettucci & Alonso, 1997; Bettucci *et al.*, 1997; Bettucci *et al.*, 1998). Several isolates obtained from these materials were identified by means of micromorphological and molecular characteristics as *Cytospora eucalypticola* Van der Westh. (Alonso *et al.*, 2002). It had been considered a non pathogenic species by Old *et al.* (1986). This fact led the authors to evaluate if the isolates of this fungus obtained from cankers could be pathogenic to *E. globulus* and *E. grandis*.

Among several isolates of *C. eucalypticola*, it was

possible to distinguish two groups that presented some different cultural characteristics. One group was characterized as having white and abundant aerial mycelium while the other one had olive green and scarce aerial mycelium. One isolate of each group was selected to evaluate its pathogenicity to *Eucalyptus* spp., MVFI 51 was obtained from cankers on twigs of *E. globulus*, and belonging to the first group, while MVHC 7115, isolated from cankers on twigs of *E. grandis* belonged to the second one.

Five, six-month old plants of *E. globulus* and *E. grandis* were inoculated. They were actively growing in an experimental field located in Montevideo, southern Uruguay, and were without apparent environmental stresses. The stems of the five *Eucalyptus* spp. plants were disinfected with 70% ethanol, and 5 mm-diameter disks of bark were taken with a cork borer and replaced with 4 mm diameter disks of mycelium obtained from the margins of a colony and then wrapped in a single layer of Milipore® tape to prevent desiccation and cross contamination. Another five non-wounded *Eucalyptus* spp. plants were inoculated to

evaluate fungal ability for epidermis penetration. In all trees, inoculation with each isolate was performed at three heights of the stem from the ground: 0.15 m (level 1), 1.04 m (level 2) and 1.82 m (level 3). Controls were inoculated with sterile MEA disks. The entire trial was repeated once in the same plantation. The stems were inspected for gummosis, canker or other symptoms at monthly intervals, during ten months after inoculation. Bark and xylem segments from randomly selected plants of *E. globulus* and *E. grandis* were cut off around the inoculation court at each level. Segments were surface sterilized and plated on Petri dishes containing 2% malt-agar. A total of 6000 segments from inoculated and control *Eucalyptus* spp. plants were incubated for three weeks and scored for the presence of *Cytospora eucalypticola*. Frequency of recuperation was calculated as the total number of positive isolations obtained from the segments of each tissue. To detect significant differences between the recuperation frequency of each isolate of *C. eucalypticola* and the success of the inoculations at each level, a *t*-test was performed.

No inoculated or control plants showed signs of discoloration, gummosis or necrosis nor did they display lesions after ten months. Formation of callus around the holes was seen. The fungus *C. eucalypticola* was recovered from xylem and bark of *E. globulus* and *E. grandis*, and was never isolated from the control plants. Although the level of recuperation of both isolates from *E. globulus* and *E. grandis* was low, some differences were observed (Table 1). Significant differences ( $P < 0.05$ ) between the two isolates in their ability to penetrate wounded or superficially inoculated plants were observed. Similarly, significant differences were noted between colonization of bark and xylem in all inoculated plants ( $P < 0.05$ ) by both isolates (Table 1).

At different inoculation levels significant differences ( $P < 0.05$ ) in fungal isolations from both tissues were noted, being lower at level 1 on both *Eucalyptus* spp (Table 1). Bark and xylem were also differentially colonized. The frequency isolation of the isolate MVHC 7115 was higher from xylem than from the bark of *E. globulus* ( $P < 0.05$ ).

The *C. eucalypticola* associated with cankers in twigs and stems of *E. globulus* and *E. grandis* is non pathogenic as shown by Old *et al.* (1986). The recuperation of both isolates from bark and xylem on superficially inoculated plants without any lesion evidenced the ability of this species to penetrate the epidermis without causing any lesions. Differences in colonization ability between the two isolates were evidenced by differences in recuperation frequencies. Thus, the presence of *C. eucalypticola* on symptomatic dying tissues could result from saprotrophic expansion of the endophytic mycelium. Fungal endophytes can live for a certain period as neutralistic symbionts (Stone & Petrini, 1997). Cankers produced by stress environmental conditions, could lead to the disclosure of saprotrophic ability and colonization of exposed tissues (Shoeneweiss, 1981). On the other hand, as both isolates

**TABLE 1** - Recuperation frequencies (number of isolates/100 segments) of *Cytospora eucalypticola* isolates from inoculated plants of *Eucalyptus globulus* e *E. grandis*

<i>Eucalyptus</i> spp. plant	level 1		level 2		level 3		Total isolates
	bark	xylem	bark	xylem	bark	xylem	
<i>Eucalyptus globulus</i>							
7115 (w)	6	12	15	20	11	17	81
7115 (s)	0	0	2	7	0	11	20
51(w)	3	16	34	9	50	15	127
51(s)	4	16	41	4	36	13	114
Control	0	0	0	0	0	0	0
<i>Eucalyptus grandis</i>							
7115 (w)	6	14	21	1	21	9	72
7115 (s)	19	3	79	5	69	0	175
51(w)	2	0	1	7	8	24	42
51(s)	8	2	85	4	11	0	110
Control	0	0	0	0	0	0	0

(s) superficially inoculated; (w): inoculated on experimental wounds

were recovered at lowest frequency near ground level, it is possible that they have lower competitive ability in relation to species that derive from the soil.

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