Aggressiveness of *Ceratocystis fimbriata* isolates in *Eucalyptus benthamii* and *Eucalyptus grandis*

Agressividade de isolados de *Ceratocystis fimbriata* em *Eucalyptus benthamii* e *Eucalyptus grandis*

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ABSTRACT

*Ceratocystis fimbriata* is the fungus that causes Ceratocystis wilt in tree species, a disease that covers a wide range of hosts, making it of great economic importance. Chemical control is difficult because it is a typical pathogen of the vascular system and is native to the soil. The search for resistant genotypes remains one of the few viable control strategies. The objective of this work was to study the susceptibility of species of the genus Eucalyptus to *Ceratocystis fimbriata*. The pathogenicity of three isolates, obtained from kiwifruit PM08, PP08 and PB01, was tested; in *Eucalyptus benthamii* and *E. grandis* seedlings. The experiment was carried out in a completely randomized design, in a factorial scheme composed of two factors (isolated X clone), with five replications. After a period of two months for the disease to establish itself, the length of the lesion in the wood was evaluated in relation to the height of the plant. It was observed that isolates PB01 and PM08 were more aggressive towards both species.

**Keywords:** phytopathology, ceratocystis wilt, severity.
RESUMO
Ceratocystis fimbriata é o alimento que provoca o avanço de Ceratocystis em espécies arbóreas, causando uma ampla gama de habitantes, o que o torna de grande importância econômica. O controle químico é difícil porque é um patógeno típico do sistema vascular e nativo do solo. A busca de genótipos resistentes segue sendo uma das estratégias de controle viáveis das épocas. O objetivo deste trabalho foi estudar a suscetibilidade de espécies do gênero Eucalyptus a Ceratocystis fimbriata. Se for comprovada a patogenicidade de três isolados, obtidos de kiwi PM08, PP08 e PB01; em plântulas de Eucalyptus benthamii e E. grandis. O experimento foi realizado sob um projeto completamente aleatório, em um esquema fatorial composto por dois fatores (clon X isolado), com cinco repetições. Depois de um período de dois meses para que se estabeleça a enfermidade, avaliou-se a longitude da lesão na madeira em relação à altura da planta. Observou-se que os isoladores PB01 e PM08 foram mais agressivos para ambas as espécies.

Palavras-chave: fitopatologia, marcha por ceratocystis, severidade.

RESUMEN
Ceratocystis fimbriata es el hongo que provoca el marchitamiento por Ceratocystis en especies arbóreas, enfermedad que abarca una amplia gama de huéspedes, lo que la hace de gran importancia económica. El control químico es difícil porque es un patógeno típico del sistema vascular y nativo del suelo. La búsqueda de genotipos resistentes sigue siendo una de las pocas estrategias de control viables. El objetivo de este trabajo fue estudiar la susceptibilidad de especies del género Eucalyptus a Ceratocystis fimbriata. Se probó la patogenicidad de tres aislados, obtenidos de kiwi PM08, PP08 y PB01; en plántulas de Eucalyptus benthamii y E. grandis. El experimento se realizó bajo un diseño completamente al azar, en un esquema factorial compuesto por dos factores (clon X aislado), con cinco repeticiones. Luego de un período de dos meses para que se estableciera la enfermedad, se evaluó la longitud de la lesión en la madera en relación con la altura de la planta. Se observó que los aislados PB01 y PM08 fueron más agresivos hacia ambas especies.

Palabras clave: fitopatología, marchitez por ceratocystis, severidad.

1 INTRODUCTION
Wood has always been a noble product for humanity, and it is estimated that in 2050 demand will be 3 billion m³ year⁻¹, 87% more than current demand (HOEFLICH, 2005). The same author predicts a wood deficit before 2030 in several scenarios.

Brazil is the country with the highest productivity in terms of planted forests. According to the Ministry of Agriculture (2016), in 2015 the figures related to exports of
forest products amounted to ten billion dollars, of which just over 50% corresponded to cellulose sales.

The increase in the production of Eucalyptus spp. in Brazil it presents limitations due to several diseases caused by the attack of fungi and bacteria. One of the main diseases of Eucalyptus is Ceratocystis wilt caused by the fungus *Ceratocystis fimbriata*. This disease is characterized by colonization of the xylem and radial parenchyma cells, culminating in necrosis of infected tissues, wilting and death of the entire plant or its branches (ALFENAS et al., 2009).

The first report of the disease in Brazil was in 1999, in the southeast of Bahia (FERREIRA et al., 1999) and it has already been observed in other countries, such as Uruguay (BARNES et al., 2003) and in countries on the African continent, such as the Republic of Congo and the Republic of Uganda, where the disease caused high mortality in eucalyptus clones (ROUX et al., 2000).

It is a very impactful disease for eucalyptus crops. Losses of 40% are reported in 18-month-old crops and in younger plants, although uncommon, mortality can reach 25% (FERREIRA et al., 2006). Some strategies can be used to prevent the spread of the pathogen, such as disinfection of tools used in pruning and avoiding the transport of contaminated seedlings (FERREIRA et al., 2010). In a symptomatology study carried out by Wingfield (1993), special attention was paid to the possible association of the disease with injuries and punctures by insects in the trunks, since several plants infected by *Ceratocystis* spp. had galleries, however in Brazil there was no interaction between boring beetles and the disease (FERREIRA et al., 1999). Chemical control is difficult because it is a typical pathogen of the vascular and autochthonous soil system, leaving the search for resistant genotypes as one of the few viable control strategies (ALFENAS et al., 2009).

The scarcity of information about the pathosystem *C. fimbriata* in the selection process, the selection of aggressive genotypes is of fundamental importance. The selection of individuals resistant to the pathogen is compromised when a less aggressive isolate is used, causing the result to be misleading (PIVETA, 2013). Therefore, it is necessary to have a greater understanding of the aggressiveness and intensity with which
the disease occurs, the risks it poses to crops and the interaction between the pathogen and different hosts.

The objective of this work was to evaluate the pathogenicity and virulence of Ceratocystis fimbriata isolates obtained from Actinia delicious A. Chev (kiwi) to Eucalyptus benthamii and Eucalyptus grandis and to observe whether E. benthamii and E. grandis present resistance to C. fimbriata isolates.

2 MATERIAL AND METHODS
2.1 EXPERIMENTAL AREA

The tests were carried out in the forest nursery area belonging to the Department of Forestry Engineering (DEF) of the State University of the Center-West (UNICENTRO) located at coordinates 25°32' south and 50°39' west in the municipality of Irati - PR. According to the Köppen climate classification, Irati's climate is type Cfb (temperate).

2.2 OBTAINING ISOLATES AND SEEDLINGS

C. fimbriata isolates PB01, PM01 and PM08 were obtained from the mycothech of the Federal University of Santa Maria-RS (UFSM) (PIVETA, 2013). Seminal seedlings of the species of E. benthamii and E. grandis from Klabin S.A. were used, which were planted in pots with a capacity of five liters, filled with Maxfertil® commercial substrate composed of a mixture of pine bark and biostabilized sawdust, where they remained for 60 days, periodically every 15 days receiving fertilization in solution with NPK 4-14-8 at a concentration of 10 g per liter, 100 mL for each plant.

2.3 PATHOGENICITY TESTS

The selected isolates were cultured in Petri dishes (9 cm diameter) containing approximately 20 mL of MEA culture medium (20 g of Malt Extract and 20 g of Agar) and maintained in an incubator (BOD) with a photoperiod of 12 hours at 25°C for ten days. After this period, 10 mL of distilled and sterilized water was added to the plates and the surface of the colony was scraped with the aid of a Drigalski loop, resulting in a
suspension of spores (conidia, ascospores and aleuriospores), which was filtered in a
double layer of gauze. The concentration of the inoculum suspension was determined in
a hemocytometer under a light microscope and was then calibrated to 2.5 x 10^6 spores.
\text{mL}^{-1} \text{(LAIA et al., 2000)}.

For inoculation, a superficial transverse incision was first made in the stem
approximately two centimeters long, with the aid of a stylus. Then, 500 \text{ \mu L} of the spore
suspension was deposited with an automatic pipette. The wound was covered with PVC
film and for control plants, distilled and sterilized water was used. After this, the plants
were kept in a greenhouse for 60 days to be subsequently evaluated.

2.4 SEVERITY ANALYZES

After the development period, the length of the lesion was evaluated to calculate
severity and, determining it by the following equation according to Oliveira (2010):

\[ S(\%) = \frac{CL}{HP} \times 100 \]

On what:

\( S \) = Severity
\( CL \) = Lesion length
\( HP \) = Plant height

2.5 STATISTICAL ANALYSIS

The trial was set up in a completely randomized design, a factorial scheme
consisting of two factors (host X isolate). Five replications were installed for each of the
three isolates and the control, with each plant representing a sampling unit. The data were
subjected to analysis of variance (ANOVA) and the mean comparison test used was the
Tukey test at 5% significance, using the Assistat 7.6 Beta software (SILVA, 2011).
3 RESULTS AND DISCUSSION

Present the main results achieved, including charts, tables and graphs, and discuss them in order to present to the reader the importance and contribution of the research carried out.

3.1 SYMPTOMS

The symptoms of *C. fimbriata* are characterized by wilting of lateral leaves that evolve into generalized wilting, with necrosis of the wood in both the radial and longitudinal directions, killing a portion of the vascular cambium, phloem and phelloderm (FERREIRA et al., 2006).

In the present study, the most evident characteristic symptom was the necrosis of the wood in the longitudinal direction, evidenced by dark spots resulting from the transport of substances resulting from the oxidation and polymerization of phenolic compounds, which appeared discontinuously, a fact that can be attributed to the low aggressiveness of the isolates used (Figure 1).
Figure 1: Symptoms observed in the inoculation test; A) discontinuous lesion of *Ceratocystis fimbriata* in the longitudinal direction (red arrow); B) Necrosis of the wood evidenced by a dark stain on the tissue (red arrow).

Source: Authors (2024)

No reflex symptoms of the disease were observed in the plants under study. An absence of wilting and drought in eucalyptus plants infected with *Ceratocystis* spp. has already been observed in previous studies (TUMURA, et al., 2010; ZAUZA et al., 2004), unlike the disease in the field, inoculation in seedlings does not always present the expression of wilt.

3.2 SEVERITY

In the present work, there was no effect of the interaction between Eucalyptus species and *C. fimbriata* isolate, and also of the response between *E. benthamii* and *E. grandis*. However, there was an effect on the severity of the pathogen isolates inoculated (Table 2).
Table 1: Calculated severity means (%).

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Control Sample</th>
<th>PP08</th>
<th>PB01</th>
<th>PM08</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. benthamii</td>
<td>20,379</td>
<td>178,350</td>
<td>284,901</td>
<td>540,208</td>
</tr>
<tr>
<td>E. grandis</td>
<td>25,624</td>
<td>175,992</td>
<td>512,229</td>
<td>476,148</td>
</tr>
</tbody>
</table>

Source: Authors (2024)

Table 2: Anova table for the inoculation test of different isolates of Ceratocystis fimbriata in E. grandis and E. benthamii.

<table>
<thead>
<tr>
<th>FV</th>
<th>GL</th>
<th>SQ</th>
<th>QM</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>1</td>
<td>168,30506</td>
<td>168,30506</td>
<td>0.9586 ns</td>
</tr>
<tr>
<td>Isolates</td>
<td>3</td>
<td>14290,306</td>
<td>4763,4353</td>
<td>27,1299 *</td>
</tr>
<tr>
<td>Interaction</td>
<td>3</td>
<td>1227,3074</td>
<td>409,10247</td>
<td>2.3300 ns</td>
</tr>
</tbody>
</table>

* Significant at the 1% error probability level (p < .01). Source: Authors (2024)

There was a statistical difference at the 1% level between the severity of the isolates (Table 2). Isolates PB01 and PM08 demonstrated greater severity than isolate PP08, which did not differ statistically from the control, showing pathogenic variability (Figure 2).

In a study involving the resistance of commercial clones of E. grandis x E. urophylla to C. fimbriata, the materials considered resistant did not have internal lesions progress. Necrosis was restricted to the inoculation region (ZAUZA et al., 2004).
In a study carried out by Oliveira (2010), 10 different isolates of *C. fimbriata* collected in Bahia, Minas Gerais and São Paulo were inoculated into 10 hybrid clones of *E. grandis*. There was a significant interaction between isolated clone X, demonstrating that *C. fimbriata* isolates presented different levels of severity and varied according to the inoculated eucalyptus clone. These results were attributed to variation in aggressiveness among isolates.

In work carried out by Piveta (2013) with the same isolates used in the present study, they were inoculated into clonal seedlings of *E. saligna*; *E. urophylla* X *E. globulus*; *E. urophylla* X *E. grandis* and in seminal seedlings of *E. dunnii*. The author observed that isolate PP08 was the least aggressive, isolate PB01 had a higher mean severity in two of the clones and isolate PM08 had a high mean severity in one of the clones tested. *E. dunnii* showed resistance to the three different isolates. These results
differ from those presented in the present study, in which, firstly, there was no interaction between *C. fimbriata* and *Eucalyptus* spp.

In this study, isolates PB01 and PM08 did not differ from each other. In the study carried out by Piveta (2013) *E. benthamii* was susceptible to isolates PB01 and PM08, thus demonstrating that it is not possible to attribute resistance of this species to the pathogen in question. This fact is important, because even though it is a species closely related to *E. dunnii* in relation to *C. fimbriata*, it is necessary to observe whether the pathogen is present before recommending the cultivation of *E. benthamii*.

From the results observed in the experiment, it can be noted that the genetic variation of the pathogen is decisive in the aggressiveness with which colonization occurs in the plant, as found in previous works (HARRINGTON, 2000; BAKER et al., 2003; FERREIRA et al., 2010; OLIVEIRA, 2010; PIVETA 2013).

Parasitism is a life strategy that involves several organisms in which one survives to the detriment of the other. This life strategy allows the establishment of intimate relationships, at the genetic level, between parasites and hosts, in such a way that genetic modifications in the population of one of the components are accompanied by genetic modifications in the population of the other (CAMARGO, 1995).

Genetic resistance is the main form of control against *C. fimbriata* (ZAUZA et al, 2004, ALFENAS et al., 2009). Therefore, the selection of genotypes resistant to the pathogen is compromised when a less aggressive isolate is used, making the selection of resistant genotypes erroneous. Knowledge of the pathogenic variability of isolates used in eucalyptus breeding programs can minimize the problems of selecting resistant clones in order to obtain isolates with a greater spectrum of action and highly aggressive in the different species and clones of *Eucalyptus* spp. (OLIVEIRA, 2010).
REFERENCES


