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Article

Susceptibility and prevalence of *Eucalyptus pellita* F.Muell. to the cancer caused by *Chrysoporthe cubensis* (Bruner) Gryzenhout & M. J. Wingf. at the Colombian Orinoquia

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Abstract:

Eucalyptus cancer caused by *Chrysoporthe cubensis* is one of the most limiting diseases for *Eucalyptus* plantations in the tropical region. In order to confirm the cancer causal fungus observed in the commercial plantations of *E. pellita* in the Colombian Orinoquia and explore the possible difference in the susceptibility of some commercial clones of the region, the pathogen was isolated from diseased tree tissue. The strains obtained were inoculated in young plants belonging to ten clones, including one control of *E. urophylla* under greenhouse conditions. The mean wound length was compared for up to 53 days after inoculation, with two isolates of the pathogen. The prevalence of cancer was also estimated in a 7-year-old *E. pellita* plantation. Based on symptoms, and macro and microscopic morphological characters, the isolates were identified as *Chrysoporthe cubensis* and with the use of the molecular markers ITS5 and ITS4 as *C. cubensis* and *C. doradensis*. All clones evaluated showed susceptibility to *Chrysoporthe* attack. Although eucalyptus cancer is a common disease, this is the first record of *C. cubensis* and *C. dorandesis* in *E. pellita* plantations in Colombian Orinoquia. Given the favorable conditions for the development of the disease in the region, it is recommended to continue the selection and evaluation efforts to have the disease tolerant material.

Key words: *Eucalyptus* canker, *Chrysophorte doradensis* Gryzenh. & M.J. Wingf., incidence, Colombian Orinoquia, severity, symptoms.

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Introduction

The disease known as cancer or *Eucalyptus* cancer, caused by *Chrysoporthe cubensis* Brunes Gryzenhout & M. J. Wingf., is one of the most limiting diseases in *Eucalyptus* commercial plantations (Van Heerden and Wingfield, 2001; Gryzenhout *et al*., 2004; Souza, 2008).

Chrysoporthe cubensis infects the trees through wounds, usually at the base of the stem of young trees, causing stunting, wilting, banding and cracking of stems (Gryzenhout *et al.,* 2004; Nakabonge, 2006); besides, produces exudations or enzymatic secretions that can be toxic (Bernal *et al*., 2009), and that in advanced conditions can lead to death, mainly of young plantations in several species of eucalyptus (FAO, 2008).

In Colombia one of the diseases most frequently found in eucalyptus plantations is the cancer caused by *C. cubensis* in *E. grandis* W. Hill ex Maiden and the only effective management measure is the selection and sowing of tolerant clones (Rodas *et al.,* 2005; Mizerit, 2009). In the Colombian *Orinoquia* the selection of species of the genus *Eucalyptus* has been made, from different species and provenances, from which it is concluded that *E. pellita* F.Muell. is the species that reaches the highest volume per unit area with 350 m^3 ha^{-1} (Reforestadora de la Costa, 2009).

Clonal propagation, while maximizing commercial timber production, also has precautionary factors to evaluate, such as the susceptibility of a clone to the attack of pathogens. It is to be expected that for this species, the number of hectares

planted with clones in the medium and short term will increase significantly (Nieto and Gasca, 2010). For this reason, it is important to incorporate the disease resistance variable into the selection programs of *E. pellita* clones suitable for extensive planting in the Colombian *Orinoquia*, even though the species is recognized in the literature because of its low incidence of pests and diseases (Alfenas *et al*., 1983; Borralho and Nieto, 2012). Therefore, the present investigation was aimed at confirming the identity of the causing agent of the cancer of *E. pellita* and to estimate the susceptibility of some clones to the cancer caused by this pathogen in seedlings of 3 months old, as well as the prevalence of the problem in seven-year-old plantations.

Materials and Methods

Study area

The study was carried out in a forest core of 3 000 ha of which 265.21 ha have been established with species of the *Eucalyptus* genus. The plantations are located on the plateau of *San Pedro* in the municipality of *Villanueva*, Department of Casanare, Colombia, at the geographical coordinates 4°57 "N and 73°94" W with an average altitude of 420 m.

The study area is located in the tropical moist forest, with a monomodal precipitation regime which favors the development and dispersal of eucalyptus cancer throughout the year (excepting December, January, February and March), since *C. cubensis* presents optimum growth at elevated temperatures and precipitation of more than 2 000 mm annually (Gryzenhout *et al.,* 2004).

Obtaining of isolates and identification of the pathogen

Samples of plant material with symptoms and signs of *Eucalyptus* cancer were obtained from bark, wood and apical meristems of two-year old *E. pellita* and *E. urophylla* S. T. Blake four-year-old trees. All samples were placed in properly labeled plastic bags and transported in a portable refrigerator at a temperature of about 4 °C (Bernal *et al*., 2009), prior to their analysis in the Health Laboratory of the *Distrito Francisco José de Caldas, Vivero* headquarters, in *Bogotá*.

The thirty-one field-collected samples were individually disinfected in a 2 % sodium hypochlorite (NaCl) solution for two minutes, and rinsed twice with sterile distilled water (Bernal *et al.,* 2009). Two replicates were obtained from each sample, one of which was kept in refrigeration at 4 °C and another was used to obtain reproductive structures of the fungus and the first isolates. Each sample was examined in detail with the help of a Discovery V8 Carl *Zeiss* stereomicroscope with which the ascostroma, perithecial neck and mycelium were identified on the plant tissue. Isolates were obtained from these samples by direct transfer of conidia masses, monosporic culture from suspension of conidia in sterile distilled water (ADE) and seeding sections of infected plant tissue in culture medium. In all cases, Merck-branded 2 % Agar Maltose (Merck) culture medium was used (Gryzenhout *et al.,* 2004; Nakabonge 2006; Bernal *et al.*, 2009, Juárez *et al.,* 2013). The isolates were preserved by the cryopreservation, lyophilization and sterile mineral oil methods.

Corroboration of the causal agent was based on macroscopic characteristics such as color and texture of the mycelium and microscopic morphologies by the observation and identification of conidiophores and conidia. The microscopic characterization of *C. cubensis* and *C. doradensis* Gryzenh. & M.J. Wingf. was performed by direct staining and staining in lactophenol blue. In addition, sections of the 25 µm thick infected material were obtained with a LEICA RM 2255 microtome and added with a non-coagulating fixative with chromic acid and acetic acid (adapted from Reig *et al.,* 2002). Transverse sections of tissues were made with Lactophenol Blue and basic Fushina (González *et al*., 2011). The macroscopic and microscopic characteristics were compared with that described for *C. cubensis* by Gryzenhout *et al.* (2004) and Nakabonge (2006) and *C. doradensis* were compared with those described by Gryzenhout *et al.* (2005). In addition, sequences of the molecular markers ITS4 and ITS5 were obtained using the sequencing and molecular analysis service of the *Instituto de Genética de la Universidad Nacional de Colombia.* (Genetics Institute of the National University of Colombia).

In order to select two virulent isolates, four of the isolates obtained from monosporic cultures were compared and inoculation was performed in 100 branches from ten clones of seven - year - old *E. pellita* following the methodology developed by Juárez *et al.* (2013). The length of the lesion developed from the point of inoculation with *C. cubensis* was used as the response variable and the treatments were determined by the clone and the inoculation strain (strain 17, 26, 29, 31 and control). Additionally, the daily mycelial growth of each isolate in culture medium in the Petri dishes was compared as another parameter equivalent to the invasion capacity that was used as an indicator of virulence (Díaz and Lecuona, 1995; Ochoa, 2004). Based on these tests, isolates 17 and 26 (obtained in *E. pellita*) were selected to perform the assay.

Susceptibility of *Eucayptus pellita* and *Eucalyptus urophylla* seedlings to the inoculation by *C. cubensis* under greenhouse conditions

Seedlings from ten *E. pellita* clones and one from *E. urophylla* (Table 1) *were* displayed in 17 cm diameter plastic pots and kept in an isolated greenhouse built for this study. The second species was also selected for the inoculation in the greenhouse, from its resistance to the attack by *C. cubensis* (Alfenas *et al.,* 1983; Van Heerden and Wingfeld, 2001).

Table1. *Eucayptus pellita* F.Muell. and *Eucalyptus urophylla* S. T. Blake clones used to compare their susceptibility to *Chrysoporthe cubensis* (Bruner) Gryzenhout & M. J. Wingf.

Clone	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**URO1**
Number of seedlings	30	30	30	15	11	8	30	30	27	30	18

The assay was arranged in three completely randomized blocks determined by the isolation (17 and 26) and the other block for the control treatment (inoculation of

seedlings with 2 % AM culture medium). Each block in turn was divided into sub-blocks, which corresponded to the number of replicates, so that in each sub-block all the clones evaluated were represented.

From the subcultures of each isolate of the pathogen, 4 mm diameter mycelial discs were cut with the aid of a pressure cuff and sterile needles. Each disc was stored inside pieces of sterile, non-adherent gauze within sterile Petri dishes, moistened with ADE.

To perform the inoculation, a wound was initially made at the base of the stem of each seedling, using a 4 mm diameter punch. Subsequently, the wet gauze section was placed with the mycelial disk, in direct contact with the wound made in the stem of the seedling and covered with Parafilm.

The development of the lesion was measured weekly for one month with a calibrator (Mitutoyo, CD 6 "CS of 0.01 mm accuracy) for a total of four measurements. At each measurement the precaution was taken to use nitrile gloves and seal again with Parafilm to prevent the entry of any type of contaminant.

Estimation of prevalence of the *Eucalyptus* cancer in a seven-year old *E. pellita* plantation

The prevalence of the disease (incidence and severity) was assessed in a stand made up by 570 seven-year old *E. pellita* individuals. Incidence was considered as the affected trees per cent, while severity was graded with a nominal scale for estimation of this condition of *C. cubensis* in *E. pellita* plantations, with a total number of six categories (adapted from Paredes *et al.,* 2010). (**¡Error! No se encuentra el origen de la referencia.**).

The calculation of the average damage degree, incidence and severity was made as

follows (Parra *et al.,* 1999):

Average damage degree (GMD)

$$GMD=\sum((Ni * CDi)/(N))$$

Where:

i = 1 to 6

Ni = Number of trees in the damage category *i*

CDi = Damage category *i*

N = Total of measured trees

Incidence (INC)

$$INC = (Number\ of\ affected\ trees * 100)/total\ number\ of\ plants$$

Intensity or damage severity

$$I = (\sum((Ni * CDi)/(K * N))*100$$

Where:

i = 1 to 6

Ni = Number of trees in the damage category *i*

CDi = Damage category *i*

N = Total of measured trees

K = Greatest category mayor

Table 2. Scale for estimating *Chrysoporthe cubensis* Brunes Gryzenhout & M. J. Wingf.severity in *Eucayptus pellita* F.Muell. plantations.

Severity	0	1	2	3	4	M
Image				 	 	
Criteria	No swelling of the base of the tree or formation of superficial cracks on the bark.	Presence of fungus structures not visible, only when checked. Presence of very few fissures not deep or presence of reddish-colored exudates.	Deep cracks, with no exudate of reddish color; with swells not so pronounced at the base of the tree.	Deep fissures without detachment of the bark and presence of exudates of reddish tone.	Deep cracks with detachment of bark and presence of exudates of red tone.	Dead

Source: Paredes *et al.,* 2010.

Data processing and analysis

Data obtained from daily mycelial growth of the isolates, lesion size of inoculated branches and inoculum seedling lesion size were organized and processed with descriptive statistics, and the assumptions of normality, homoscedasticity and independence were evaluated by means of the tests of Kolmogorov-Smirnov and Levene, prior to comparison using a variance analysis (ANOVA) and Duncan's post-hoc test. When the data did not present normal distribution, transformations were tested by the Box Cox method, using the SPSS 15.0 statistical program Otherwise, the analysis was performed using non-parametric statistics. The

response variables evaluated were diameter of mycelial growth for the daily evaluation of isolates in laboratory as virulence factor and length of the lesion developed from the point of inoculation for the inoculation in branches and seedlings.

Results

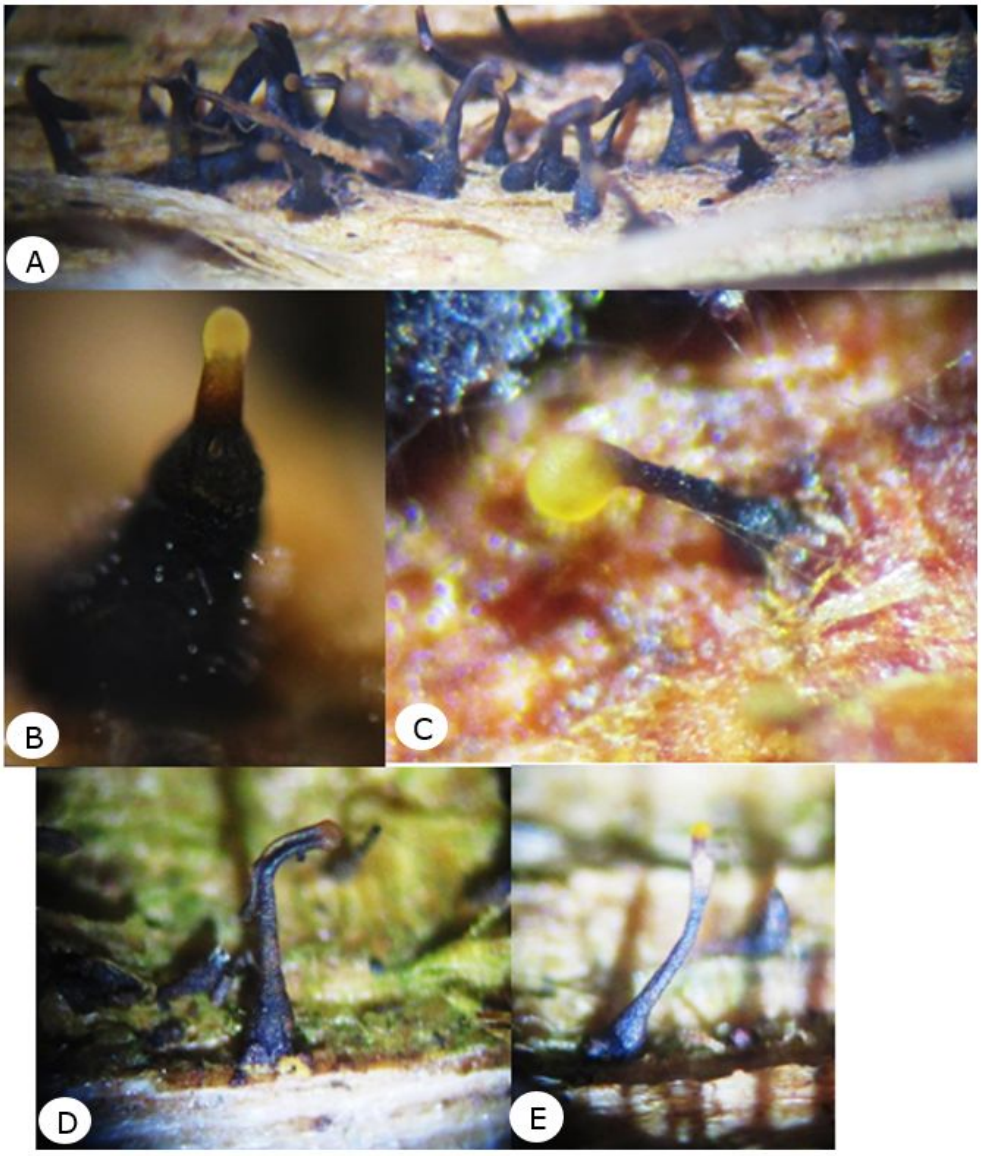
Pathogen agent

The affected trees in the field and from which samples of diseased material were taken showed symptoms and signs characteristic of the cancer caused by *C. cubensis,* such as swelling and fissures in the stem, gum redness associated with cracks on the cortex, presence of pycnidia, long pycnidial neck and globose base with reddish, coffee and black hue. In addition, the release of conidia was observed in mucilaginous masses of yellow tones accumulating on the ostium, composed of conidia (Figure 1). Conidiophors with bifurcations, hyaline with rectangular basal cells and globular appearance (Figure 2B-D). The conidia are unicellular, oval, non-septate hyaline, with bright exudates (Figure 2C), the size of conidia, conidiophores and pycnidia agree with those described in literature (Table 3). Isolates obtained from the field samples produced pycnidia and conidia after approximately 30 days of incubation (27 °C).

Table 1. Size of conidia, conidiophores and pycnidia of *Chrysoporthe cubensis* Brunes Gryzenhout & M. J. Wingf.

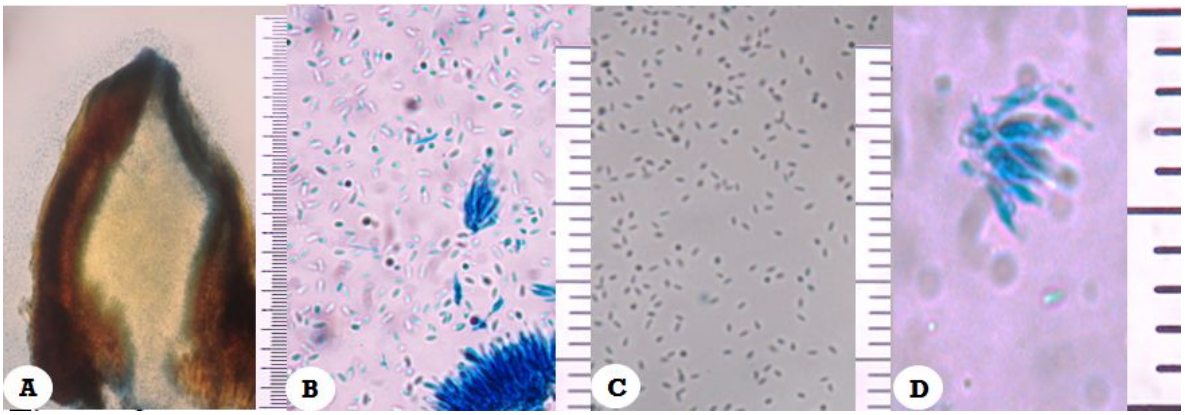
Research	Conidias (µm)	Conidiophores (µm)	Picnidia	
			Length (µm)	Diameter (µm)
Obtained results	2.987 ± 0.106	8.74 ± 0.471	288.86 ± 141.20	195.09 ± 83.17
Gryzenhout *et al.* (2004)	3.5 – 4.5	12-24.5	230	100-950

Identification by DNA extraction and sequencing with the ITS markers are consistent in 99.9 % with sequences published in GenBank as *C. cubensis* for the isolation 17 obtained in *E. urophylla* (Access number JN942342.1) and *C. doradensis* for the strain 26 (Access number JN942330.1) from *E. pellita.*



A. Grouping of pycnidia on wood. B - C. Pycnidia on bark with mass of conidia in the ostium. D-E. Pycnidia on wood with mass of conidia in the ostium.

Figure 1. Pycnidia of *Chrysoporthe cubensis* Brunes Gryzenhout & M. J. Wingf. with mass of conidia ejected through the ostium.



A. Transverse section of pycnidia with conidia (blue-stained by Lactophenol Blue staining (10 X); B, D, Conidiophores (40 X, 100 X); C. Oval-shaped conidia (40 X)

Figure 2. Photomicrographs of *Chrysoporthe cubensis* Brunes Gryzenhout & M. J. Wingf.

The colonies of the four assessed isolates (codes 17, 26, 29 and 31) did not differentiate each other in terms of average mycelial growth (Kolmogorov-Smirnov, for K-S of a sample: 0.365). Strain 17 had the highest average of daily growth (0.75 mm day^{-1}), followed by strain 31 (0.74 mm day^{-1}). Strain 17 exhibited colonies that reached maximum diameter of mycelium (9 cm) at 5 days of measurement, and for strain 31 (9 cm) at 8 days of measurement (Table 4).

Table 4. Growth of *Chrysoporthe* colonies in laboratory conditions.

Isolation	**Host species**	**Diameter (cm)**	**S**
17	*Eucayptus urophylla*	0.75	2.7
26	*Eucayptus pellita*	0.62	1.8
29	*Eucayptus pellita*	0.65	2.3
31	*Eucayptus urophylla*	0.74	2.7

N = Five colonies

The inoculation of eucalyptus branches with the isolates with Chrysoporthe induced the development of typical lesions (Figure 3) and were significantly higher in comparison with the control (inoculated with AM), ($df = 4$, $p > 0.05$, $p = 0.000$). The average length of lesions was 63.4 mm while in the control, 26.3 mm. No significant difference was observed between the strains in the lesion size nor in the lesion induced in the different clones (Table 5).

Figure 3. Injury caused by *Chrysoporthe cubensis* Brunes Gryzenhout & M. J. Wingf. in *Eucayptus pellita* F.Muell. branches in the second week of inoculation. Necrosis in branches of *E. pellita* associated to the inoculation point of *C. cubensis.*

The means for the groups in the homogeneous subsets are shown in Table 5. The sample size of the harmonic mean was 20.00.

Table 5. Sub-sets (1, 2) statistically different from mean length of the lesion caused by four strains of *Chrysoporthe* sp. in eucalyptus branches (Duncan's test).

Strain	**N**	**Subset**	
		1	**2**
Control	20	26.2995	
29	20		58.2363
31	20		65.0500
17	20		65.3700
26	20		66.5165
Significance	20	1.000	0.321

Duncan. Average

Susceptibility of greenhouse seedlings

All clones evaluated showed susceptibility to inoculation with strains 17 and 26 of *Chrysoporthe* (df = 222, p <0.05); however, no statistically significant differences were found between the 11 clones nor between the two assessed *Chrysoporthe* isolates (Table 6).

Seedlings developed progressive and rapidly advanced symptoms of cancer. After 53 days of inoculation with *Chrysoporthe*, they presented increased wound length, forming larger sores. In the inoculated seedlings in turn, exudation of reddish shade of viscous and bright texture was evidenced, which is also characteristic in *Eucalyptus* trees affected by the fungus; also, these seedlings formed additional sores at the inoculation site and which were displayed along the stem (Figure 4).

Table 2. Length (mm) of injury after 53 days of inoculation with *Chrysoporthe cubensis* Brunes Gryzenhout & M. J. Wingf.

Clone	Control			Strain 17			Strain 26		
	Average	**Max.**	**Min.**	**Average**	**Max.**	**Min.**	**Average**	**Max.**	**Min.**
2909	8.86	14.84	4.62	15.09	21.59	6.07	18.82	25.46	13.23
5632	7.53	14.00	4.81	15.83	47.78	7.37	18.49	42.60	9.89
4003	7.47	12.99	4.30	19.24	56.76	7.97	25.02	65.86	8.52
5002	15.50	39.07	6.25	23.44	66.48	10.43	14.90	21.76	8.25
2904	8.06	9.82	6.30	15.59	20.54	9.14	12.14	14.04	7.94
5012	6.70	8.22	5.18	19.03	28.91	11.44	24.61	29.78	20.26
OO16	7.09	12.68	4.33	18.74	41.62	6.84	14.01	28.05	5.20
1110	6.19	8.61	3.94	18.65	29.73	8.14	19.83	40.43	6.62
5026	7.27	11.09	4.53	22.79	52.88	7.41	19.47	37.48	11.22
5013	10.59	37.26	5.04	19.47	38.35	10.67	22.67	82.10	7.20
URO16	7.89	16.83	4.89	16.26	23.89	9.70	12.21	17.99	9.10

A, B, C, D. Seedlings with presence of exudates of reddish hue at the inoculation site. E. Primary bifurcated or dead meristems. F. Galls along seedling stem.

Figure 4. Development of wounds caused by *Chrysoporthe* infection in *Eucayptus pellita* F.Muell. seedlings, 53 days after inoculation.

The disease progressed gradually and caused the death of several of the evaluated seedlings (Table 7). Treatment with strain 26 brought together the largest number of dead individuals (25). Clones 5 026 (20 %) and 5 002 (16 %) achieved the highest mortality rates attributable to treatment.

With strain 17, a 22.2 % mortality rate was verified, corresponding to 20 of the total assessed (90) in this treatment; the clone with the highest number of deaths was 5 013 with 30 % of the seedlings in the treatment described, followed by 5 632 (20 %). Finally, in the control, the death of five seedlings was recorded in the last measurement, which constitutes 5.68 %, of which three were of clone 1 110 (Table 7).

Table 3. Absolute and relative *Eucalyptus* seedling mortality inoculated with *Chrysoporthe* per treatment.

	Days after inoculation				
	7	**14**	**21**	**53**	**Total**
Strain 17	0	3(3.4 %)	3(3.4 %)	14(15.9 %)	20
Strain 26	0	3(3.3 %)	2(2.2 %)	20(22.2 %)	25
Control	0	0	0	5(6.3 %)	5

Prevalence of *Chrysoporthe* sp. in plantations

Of all clones tested (20), 54 % had an incidence of 90 to 100 %. Despite the high values, 51 % had a severity between 50 and 60 %, so the disease was not in advanced stages of development.

The 570 trees evaluated in the plantation of *E. pellita* revealed an average degree of damage (GMD), that between 0.6 and 3.5, that is, with mild to intermediate conditions. However, 51.3 % of the clones had mean damage levels of 2.5 to 3.0, corresponding to deep fissures with exudate of reddish tonality, reproductive

structures (pycnidia) and, in some cases, swelling at the base of the tree.

At the plantation, the ten clones of *E. pellita* evaluated in greenhouse had an average degree of damage between 2.47 and 3.33. They exhibited bark detachment and reddish exudations mainly in clones 1 110 and 5 026. On the other hand, the incidence of *C. cubensis* of these clones in particular, is damaging between 80 and 100 % of the total of individuals, with severity of 49 % and 67 %.

Discussion

The symptoms and signs of the pathogen observed in the E. pellita plantation and those analyzed under laboratory conditions coincide with that reported in previous studies for the attack of *C. cubensis* (Van Heerden and Wingfield, 2001; Gryzenhout *et al*., 2004; Nakabonge, 2006; FAO, 2008; Paredes *et al*., 2010; da Silva *et al*., 2010; Juárez *et al.,* 2013).

The expressions of cancer observed in the field are characteristic of the disease caused by *C. cubensis.* Likewise, the macroscopic morphological study of the fruiting bodies and the fruiting pattern, and the laboratory microscopic study coincided with the descriptions of Van Heerden and Wingfield (2001), Gryzenhout *et al*., (2004) and Nakabonge (2006) for this pathogen.

The symptoms, signs and morphological characteristics of *C. cubensis* and *C. doradensis* are similar and at the microscopic level they are only differentiable by discrete differences in the shape of the conidia and coloration of the conidial mass (Gryzenhout *et al.,* 2004; Gryzenhout *et al*., 2005). For the above, it was initially considered that the two isolates corresponded to two isolates of C. *cubensis.* However, based on the ITS sequence, it was possible to determine the presence of *C. doradensis,* which has also been recorded in *Eucalyptus* plantations in Ecuador

(Gryzenhout *et al.*, 2005). In spite of being a common *occurrence in Eucalyptus,* in this work, *C. cubensis and C. doradensis* are known for the first time in *E. pellita* plantations in Colombian Orinoquia.

The virulence of a pathogen depends, to a large extent, on the capacity for invasion and the production of toxic substances (Ochoa, 2004). In addition, mycelial growth allows the development of the fungus as it absorbs nutrients, which, in turn, make it possible to form new mycelia (Chávez *et al.,* 2011). In this study, colony size as a criterion for selection of the most virulent strains of obtained in the laboratory was not easy to apply, since there were no significant differences in the size of the colony in relation to time.

Probably the strains of *C. cubensis* obtained correspond to sources of inoculum of low genetic variation, since they were collected from hosts within the same batch or nearby; therefore, the branch inoculation response did not show a significant difference. It is possible that the hosts from which the isolates were obtained have been infected by a common primary inoculum, that is, with genetically related.

The *E. pellita* and *E. urophylla* seedlings studied showed susceptibility to the attack of *C. cubensis* and *C. doradensis* isolates, and there were no differences between clones or between isolates of the fungus. Similar results were reported by Juárez *et al.* (2013) in which all clones of *E. urophylla* and *E. grandis* evaluated were attacked by *C. cubensis*, but not consistent with other studies in which *E. pellita* is classified as one of the less susceptible species of the *Eucalyptus* genus To Chrysoporthe (Alfenas *et al.,* 1983), nor with studies in *E. urophylla* and *E. grandis* that showed different levels of tolerance between clones to the attack of *Chrysoporthe cubensis* and, in turn, different degrees of virulence between strains of cancer (Van Heerden and Wingfeld, 2001,;Chen *et al*., 2010; Juárez *et al.,* 2013).

Cloning of C. *cubensis*-resistant genotypes has allowed the control of eucalyptus cancer (Van Heerden and Wingfeld, 2001; da Silva *et al.,* 2010). Although

laboratory and greenhouse tests can be used as indicators of susceptibility of *E. pellita*, they should be interpreted with caution, since in previous studies the behavior of the disease in juveniles does not necessarily represent susceptibility in plantations of greater development (Juárez *et al.,* 2013); so, it is advisable to continue with evaluation of productive clones at different ages of development. The establishment of future plantings of these clones may represent economic and environmental risks, according to the planted area objectives in Orinoquia with *E. pellita* (Mizerit *et al*., 2013).

It is important to emphasize that the study area meets the appropriate climatic conditions for the development of eucalyptus cancer caused by *C. cubensis* and *C. dorandesis* as high temperatures and precipitation. Therefore, it is advisable to continue with research aimed at establishing in more detail the behavior of the fungus in its hosts and, from there, to establish which of them are optimal to carry out a forest plantation of *E. pellita* (da Silva *et al*., 2010). In spite of finding *Chrysoporthe cubensis* in the *Eucalyptus* plantations in Villanueva, the presence of the disease in the native vegetation associated to the plantations was not evident.

Conclusions

Seedlings of the *E. pellita* and *E. urop*hylla clones evaluated showed similar response to inoculation with *C. cubensis* and *C. doradensis* under greenhouse conditions. Laboratory and greenhouse tests should be interpreted with caution so the recommendation is to continue the evaluation of productive clones at different ages.

The susceptibility observed in the assessed clones could represent risks for the establishment of future plantations in the region where there are naturally both inoculum and climatic conditions appropriate for the development of the disease, i.

e., high temperatures and precipitation, for long periods.

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Conflict of interests

The authors declare no conflict of interests.

Contribution by author

Olga Patricia Pinzón Florián: making of the research study proposal, support in the laboratory work, data analysis and writing of the final manuscript; Anagibeth Chocontá López: Sampling and measurement in the field, isolation, characterization and maintenance of strains in the laboratory, analysis of results, document writing work base; Víctor Manuel Nieto Rodríguez: development and direction of the macro-proposal, selection and production of clones to be evaluated, financing of statistical advice, logistics and materials, observations to the results document, and manuscript.