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RESEARCH ARTICLE / *ARTIGO*

Biology of *Gaeumannomyces graminis* var. *graminis* isolates from rice and grasses and epidemiological aspects of crown sheath rot of rice

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ABSTRACT

A collection of *Gaeumannomyces graminis* var. *graminis* isolates obtained from symptomatic rice and grass plants in central and northern Brazil were studied in regard to pathogenicity on rice and cultural characteristics. For all isolates, only one type of lobed hyphopodia was observed both in the field and artificially inoculated plants. Perithecia were formed on artificial media and inoculated leaf sheaths. Hyphopodia were formed from ascosporic germ tubes. The hyphae under moist stress conditions produced dark brown chlamydospores that were initially hyaline. Large variation in the number of chlamydospores produced and duration of perithecia formation was observed among the isolates. Pathogenicity assays showed that 60-day old rice plants were more susceptible than 35-day old plants. The isolates from rice and grasses varied significantly in regard to disease severity on both rice seedlings and adult rice plants. In general, the isolates from rice were more aggressive than the isolates from grasses. Spontaneous infection of rice plants by ascosporic inoculum from perithecia on rice stubbles was observed in the greenhouse, suggesting their role as a source of primary inoculum in the field, which deserves further investigation.

Key words: *Oryza sativa*, brown sheath rot, crown sheath rot, perithecia.

INTRODUCTION

The fungus *Gaeumannomyces graminis* (Sacc.) von Arx & D. Olivier var. *graminis*, (Ggg) causing crown sheath rot of rice (*Oryza sativa* L.) was reported first in upland rice and later in irrigated rice in Brazil (Prabhu & Filippi, 2002; Nunes, 2008). Earlier reports refer to the synonyms *Ophiobolus oryzinus* and *Ophiobolus graminis* in Africa, Australia, India, Italy, Japan, Malaysia, New Guinea, North America, South America, Philippines, Sri Lanka and Sweden (Walker, 1981). All species of *Gaeumannomyces* are pathogens that infect roots, crown and lower parts of culm and sheaths of Poaceae and Cyperaceae (Walker, 1981). The two other important varieties of *G. graminis* widely known and studied in different parts of the world, including Brazil, are *G. gramins* var. *tritici* (Ggt), the causal agent of take-all disease of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.), and *G. graminis* var. *avenae (*E.M.Turner) Dennis (Gga), the casual agent of take-all disease of oats (*Avena sativa* L.) (Prestes, 1972; Reis et al., 1982, 1989; Mathre, 1992; Freeman & Ward, 2004). The morphological characteristics that distinguish these varieties are the ascospore size and the form of hyphopodia (Mathre, 1992). The hyphopodia are fixing and penetrating organs and are abundantly produced by *Gaeumannomyces* from mycelium that covers the base of the infected culms or in culture medium (Walker, 1981). While Ggt and Gga produce simple hyphopodia, Ggg produce simple as well as lobed ones, which are hyaline or brown in color, on the tissue surface of the host. The size of ascospores in Gga is 65-176 µm whereas in Ggt it ranges from 27 to 124 µm in length and in Ggg of 75 to 105 µm (Walker, 1981; Datnoff et al., 1993).

Gaeumannomyces graminis var. *graminis* is considered a weakly pathogenic fungus causing no significant damage to its host, which could be used as a bio-control agent against take-all disease caused by Ggt and Gga (Deacon, 1974). In Sweden, Nilsson (1972) observed fungal isolates morphologically similar to Ggg obtained from soil previously cultivated with wheat, which were pathogenic to artificially inoculated wheat. Cross pathogenicity was observed in isolates of Ggg from rice, Bermuda grass and St. Augustine grass in Florida. One of the isolates showed greater disease severity on the sheath base and roots of inoculated rice plant, compared with other rice isolates as well as grasses indicating differential aggressiveness among isolates (Datnoff et al., 1997).

The saprophytic survival of some species within *Gaeumannomyces* is probably related to dormant mycelium

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or resting spores (Garret, 1972). According to Shipton (1981) Ggt may survive in soil as sclerotia. The *in vitro* development of chlamydospores and microsclerotia of Ggt in culture media amended with 5-10% of HCl has been reported, but there is no evidence of their occurrence in the field (Hornby, 1981). Likewise, there are no reports regarding the survival of Ggg in field conditions as resistant structures.

The ascospores of Ggg can be actively released from the ascus in the atmosphere (Gregory & Stedman, 1958), but its role in the epidemics remains controversial (Gerlagh, 1968; Shipton, 1972). There is little information on the epidemiology of crown sheath rot of rice (Webster & Gunell, 1992) and only preliminary data on pathogenicity of Ggg in Brazil (Prabhu & Filippi, 2002).

Crown sheath rot was considered to contribute to considerable losses in grain yield and quality of rice in Texas, USA (Datnoff et al., 1997). In Brazil, the disease is endemic in all major rice growing regions, both in upland and irrigated rice, and its importance as yield-reducing factor is largely overlooked because grain yield losses are more commonly attributed to sheath blight and sheath rot. The pathogen has a wide host range including several grasses commonly found in rice fields and soybean crops in the Midwest United States (Roy et al., 1982). Ggg is a soil borne fungal pathogen and known to survive in rice stubble and alternate hosts (Webster & Gunell, 1992). Even though it is currently considered a minor disease of rice in Brazil, the situation may change with the intensification of cultural practices and the current system of rice-soybean crop rotation. While extensive research on take-all disease of wheat worldwide has led to considerable understanding its various aspects (Hornby, 1981; Brassett & Gilligan, 1989), there is no information on survival mechanisms and differences in aggressiveness of rice and grass isolates, which constitute the basis to improve management of the disease in Brazil. Also, the role of Ggg ascosporic inoculum from rice and several other grasses (*Digitaria* sp., *Panicum* sp. and *Brachiaria* sp.) as a primary source for epidemics is not well understood. This study reports on the aggressiveness, cultural characteristics, perithecia production and role of ascospores on infection of rice plants of Ggg isolates obtained from rice and grasses in Brazil.

MATERIALS AND METHODS

Isolates and their origin

The fungal isolates were obtained from 1) plants exhibiting the typical disease symptoms during visits to commercial fields of rice and grass (*Digitaria* sp., *Panicum* sp. and *Brachiaria* sp.) and 2) the collection of Embrapa Rice and Bean Research Center (Table 1). The symptomatic culms were washed in distilled water, cut into 5 cm fragments and kept in a moist chamber (Petri dishes) for the production of perithecia and ascospore release. Purified colonies from mono ascosporic culturing were maintained on potato dextrose agar (PDA) in tubes and later conserved in sterilized filter paper discs in freezer at temperatures of 25°C. In cases where perithecia were not readily produced, isolations were made directly from symptomatic culms. Small fragments of culms were surface disinfested for 1 min with 70% alcohol followed by 3 to 4 min in sodium

TABLE1 - Isolate, host, location and year of collection of *Gaeumannomyces graminis* var. *graminis* (Ggg)

Isolate[1]	Origin	Year	Host
Ggg-a01	Humaitá (AM), commercial rice	1997	*Oryza sativa*
Ggg-a21	Experimental fields of Embrapa (GO), reisolate of Ggg-a01	2003	*O. sativa*
Ggg-a30	Commercial rice field of cv. Canastra (GO)	2003	*O. sativa*
Ggg-a31	Naturally infected plants in greenhouse of Embrapa (GO)	2003	*O. sativa*
Ggg-a32	Paraúna (GO), commercial rice field of cv. Colosso	2005	*O. sativa*
Ggg-a37	Reisolate of Ggg-a01 from field inoculated plants of rice cv. Bonança	2005	*O. sativa*
Ggg-a38	Experimental irrigated rice field of Embrapa (GO)	2005	*O. sativa*
Ggg-a39	Commercial rice field, culture collection of Embrapa (GO)	2003	*O. sativa*
Ggg-a40	Flores (GO), cv. Bigua, culture collection of Embrapa	2003	*O. sativa*
Ggg-a41	Formoso (TO), cv.Epagri, culture collection of Embrapa	2003	*O. sativa*
Ggg-a42	Formoso (TO), cv. Metica-1, culture collection of Embrapa	2003	*O. sativa*
Ggg-a43	Unaí (MG), culture collection of Embrapa	2003	*O. sativa*
Ggg-a44	Paraúna (GO), cv. Maravilha, culture collection of Embrapa	2003	*O. sativa*
Ggg-a45	Palmital (GO), culture collection of Embrapa	2003	*O. sativa*
Ggg-c34	Experimental field of Embrapa (GO)	2005	*Brachiaria* sp.
Ggg-c18	Experimental field of Embrapa (GO)	2003	*Digitaria horizontalis*
Ggg-c28	Experimental field of Embrapa (GO)	2003	*D. horizontalis*
Ggg-a33	Experimental field of Embrapa (GO)	2005	*Panicum* sp.
Ggg-c36	Experimental field of Embrapa (GO)	2005	*Panicum* sp.
Ggg-c20	Experimental field of Embrapa (GO)	2003	*P. maximum*

[1]Ggg-a = rice isolate; Ggg-c = grass isolate

hypochlorite (0.1%) and transferred to PDA. After the development of colony, agar discs with mycelium were transferred to autoclaved rice leaves on agar-agar in Petri plates for the production and release of ascospores. The cultures showing the lobed hyphopodia typical of Ggg were selected for the pathogenicity tests.

Cultural characteristics and production of perithecia

Observations were made on the mycelial growth characteristics and formation of hyphopodia in naturally infected plants in the field, inoculated plants in the greenhouse and leaf sheath inoculations in the laboratory. Observations were made on 8-day old cultures in PDA plates and the following traits were recorded: colony shape, texture and color. Perithecia development was studied on PDA and disinfested rice leaf sheaths. The leaf sheaths were cut into fragments of approximately 7 cm, surface disinfested with 70% alcohol and 0.83% sodium hypochlorite followed by washing twice in sterilized water. They were incubated in Petri plate humid chamber and inoculated with mycelial discs of each isolate. Similarly, PDA plates were inoculated with mycelial discs and incubated at room temperature (± 25°C) and 12-hour light/dark cycle.

Germination of ascospores and production of chlamydospores

The germination of ascospores was assessed for one isolate (Ggg-a01), which produced perithecia on disinfected sheaths. The ascospore suspension was prepared in sterile distilled water and two drops were transferred to each one of the three cavities of glass slides and on the surface of parafilm on three other glass slides. These slides were incubated in a humid Petri plate chamber at ±25°C. Germination was observed at a 2-hour interval for the first 12 h, and then after 20 and 42 h. The slides were examined for chlamydospore production until 96 h after incubation.

Pathogenicity on seedlings and adult plants

Pathogenicity of all isolates (Table 1) was assessed on both seedlings and adult plants of cultivar BRS Bonança in the greenhouse. Aluminum pots (1 kg) containing autoclaved soil or sand were infested by incorporating 5 g of inoculum, previously grown on autoclaved sorghum seeds, at a depth of 1 cm. The pots were covered with aluminum foil to keep the humidity and incubated for 15 days. Forty seeds of cv. BRS Bonança were sown per pot and the seedlings were thinned to 10 plants after germination. A randomized complete block design with three replications was used. Treatments were the 21 isolates (Table 1) and a non-inoculated check treatment. Disease assessment was made on 33-day old seedlings and it was based on measurement of height of the lesion (cm) above the substrate as indicative of aggressiveness.

The pathogenicity test on adult plants was conducted on plants grown in aluminum pots (2 kg) filled with soil fertilized with NPK (5 g of 5-30-15 + Zn and 2 g of ammonium sulfate for 2 kg of soil). After germination plants were thinned to maintain four plants per pot. A completely randomized block design was used with four replications. At 49 days after planting each potted plant was inoculated with 5 g of infected sorghum seed placed on the soil surface around the plants. The plants were maintained in a greenhouse with temperature ranging from 25 to 30°C and high humidity (> 90%) until evaluation. Disease was assessed 35 days after soil inoculation and it was based on the height of the lesion (cm) in the main tillers of the four plants.

Effect of inoculum quantity and plant age on crown sheath rot

A greenhouse (Figure 1) experiment was conducted to determine the effect of inoculum level (Ggg-a01 isolate) and plant age on the disease severity. The cv. BRS Bonança was grown in aluminum pots (2 kg) containing soil fertilized with NPK (5 g of 5-30-15 + Zn and 2 g of ammonium sulfate for 2 kg of soil). The plants were thinned after germination to maintain one plant per pot. The experiment was in a randomized complete block design with four replications. The treatments consisted of a 4x2 factorial: four inoculum levels (0; 0.5; 1; 2; and 4 g of autoclaved sorghum infested with inoculum) and two plant ages (35 and 60-day old). The inoculum was placed over the substrate around the plant base and covered with plastic sheet for 72 h to keep the soil wet and promote infection. The plants were maintained under high humidity conditions (> 90%), with the use of mist blowers, and at temperatures ranging from 25 to 30°C. The disease was assessed 26 days after inoculation by measuring lesion height (cm) and plant height (cm) on the two main tillers of a plant. The disease severity, in percentage, was based on the height of the lesion in relation to the maximum height of the tiller. The results were presented as disease index once the plants presented different heights.

Role of ascospores in spontaneous infection

The role of ascospores in the infection, from inoculum naturally released from perithecia produced in rice stubbles, was studied in a greenhouse experiment. Non-infected potted plants (35-day old, cultivar BRS Bonança) were arranged in six rows of 12 pots (total 72 plants), being three rows of plants at each side of a central row with 12 pots containing symptomatic rice stubbles. The three plant rows were at 12.5 cm, 40 cm or 67.5 cm distant from the inoculum source. The inoculum consisted of 35 cm rice stubbles (cv. BRS Bonança) containing abundant perithecia (isolate Ggg-a01) from inoculations as described previously. The pots with inoculum were placed inside aluminum trays (1.5 x 0.8 m) containing water (2 cm). Randomized complete block design using three distances and six replications. A high humidity (≥ 90%) was maintained throughout the experiment by using mist blowers. Daily assessments were

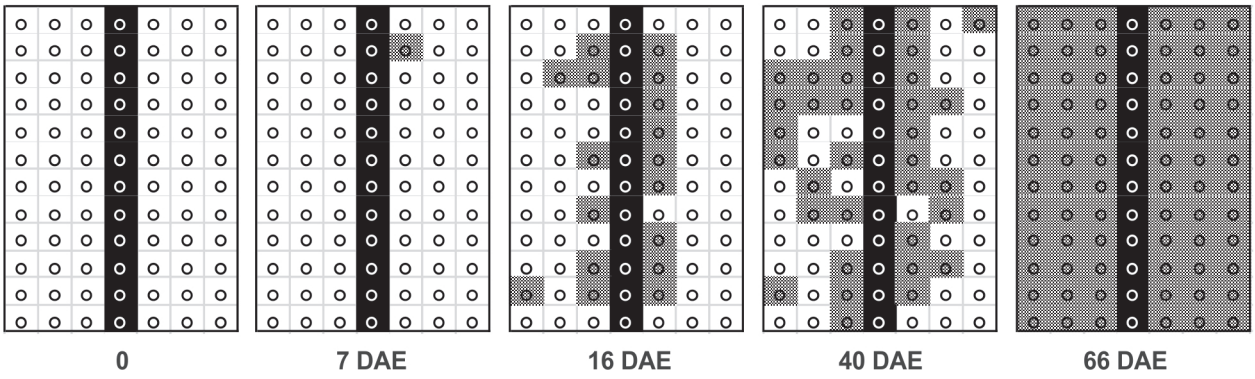

FIGURE 1 - Map depicting the temporal and spatial progress of rice sheath rot incidence on rice, cultivar BRS Bonança, in the greenhouse. The ascosporic infections by *Gaeumannomyces graminis* var. *graminis* were originated from inoculum consisting of rice stubbles with perithecia in 12 pots positioned in a central row between three rows of 12 potted plants in a greenhouse. DAE = days after exposure of healthy plants to inoculum. The dark shaded squares represent infected plants.

made to detect first crown sheath rot symptoms on the culms. The proportion of disease plants was summarized from assessments at weekly intervals until the end of the experiment.

Data analysis

The data of all greenhouse experiments were subjected to the analysis of variance and means were discriminated by Scott-Knot or Tukey`s test at 5% probability.

RESULTS

Morphological observations under field conditions

Both in the field and in inoculated plants, the presence of dark mycelium was observed in sheaths of adult rice plants, 10 to 15 cm above the ground level. The leaf sheaths showing symptoms of dark lesions, in most cases, caused premature drying or death of the leaves. In such cases, tufts of fan shaped mycelium inside the leaf sheath and culm were observed (Figure 2A).

The hyphae of Ggg were brown in color, septate with thick and dark walls characterized as runner or macro hyphae (Figure 2B). Another type of hyphae, hyaline with fine cell walls, characterized as infectious micro hyphae were found in host tissues. The tips of young micro or macrohyphae were hyaline and light brown in color and later turned thick and dark brown with time. Mycelium was not frequently observed on rice roots, contrary to that reported on infected wheat roots. However, in artificially inoculated rice seedlings with Ggg-a01 and incubated for five days in moist filter paper towels, the presence of runner hyphae characteristic of rice pathogen was observed on the surface of roots.

The formation of hyphopodia typical of Ggg was observed on the surface of the rice sheaths and grasses under natural conditions of infection. The hyphopodia were brown, slightly or strongly lobed and formed on lateral ramifications and extremities of hyphae (Figure 2C). The formation of hyphopodia was observed on the leaf sheaths of greenhouse inoculated plants (Figure 2D). In the laboratory, the hyphopodia were formed in 24 to 48 h after the inoculation of disinfected leaf sheaths and incubated in humid chamber of Petri dishes.

There were no marked differences in mycelial growth and colony type except small differences in colony color on PDA. The aerial mycelium was fluffy or cottony with characteristic colony color varying from dirty white to brown and in some cases black. The fungus covered the entire Petri dish, overgrew even up to the lids as well as outside the plate and appeared as rhizomorphs formed by tufts of hyphae.

Perithecia and ascospores production on leaf sheaths

Perithecia were formed singly or in clusters on infected leaf sheaths both under natural conditions (Figure 3A) and in plants infected in greenhouse (Figure 3B). Under natural infection, the perithecia were round to oval in shape with variable neck sizes. Usually, they were found embedded in leaf tissue of sheaths, sometimes superficial, at the base of the culm up to 15 cm height. The ostioles were exposed due to breaking of epidermal cells and turned towards surface. In inoculated leaf sheaths perithecia were formed in 12 days and produced ascus and mature ascospores in five days. They formed superficially and spread along the entire leaf sheath as well as on the filter paper in the Petri dish, differing from naturally infected fields where they were in clusters.

Masses of asci and ascospores were ejected from perithecia (Figure 3C). The asci were hyaline, unitunicated, elongated and clavate, containing eight ascospores and apical ring. The ascospores were liberated in masses of white to yellow in color. They were hyaline, or slightly colored, vacuolated, 3-5 septate, curved or slightly sinuous with round extremities and wide in the middle showing dimensions of 70 to 105 x 2.3 to 3.3 µm (Figure 3D). Both rice and grass isolates of Ggg produced abundant number of ascospores.

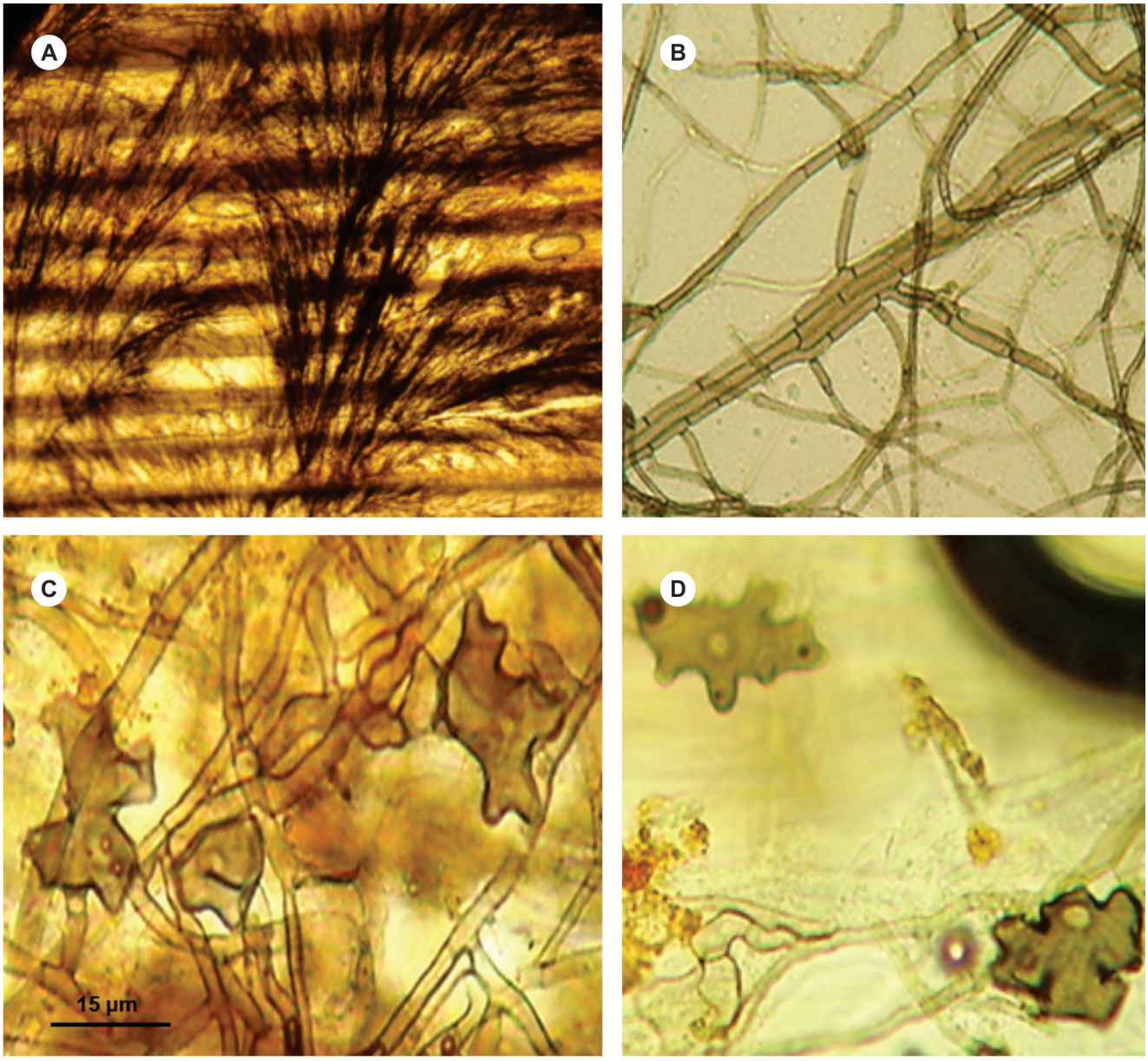

FIGURE 2 - Mycelial characteristics and formation of hyphopodia of *Gaeumannomyces graminis* var. *graminis*. **A.** Fan shaped mycelial strands inside the leaf sheath and culm; **B.** Thick dark walled runner (macro) hyphae; **C.** Hyphopodia on naturally infected; and **D.** Artificially infected rice leaf sheaths.

Germination of ascospores

The ascospores of Ggg-a01 germinated in water after 12 h at 25°C. The germ tubes, with hyphopodia forming on its tip, developed from both extremities of ascospores (Figure 3E) confirming the role of hyphpodia on fixation and penetration of host tissue. In water, the spore suspension produced only mycelium. Mycelial growth and thickening of the hyphae was observed, leading to the formation of chlamydospores, when the ascospore suspension, after the formation of germ tubes, was maintained under high humidity conditions without water. The chlamydospores were unicellular structures composed of granular material, initially hyaline and later became pigmented (Figure 3F).

Production of perithecia in culture medium

The grass isolate Ggg-c28 from *Digitaria horizontalis* produced large number of perithecia in culture medium. On the other hand, one rice isolate Ggg-a38, and three grass isolates Ggg-c20, Ggg-c33 and Ggg-c36 failed to produce perithecia on PDA (Table 2).

In approximately 60 days, the majority of isolates produced fertile perithecia in aggregates, immersed in gelatin matrix or in fine hyaline or white mycelial tufts which are lighter than the mycelium of Ggg. In some isolates, such as Ggg-c28, the production of perithecia was spread all over the plate. A great number of rice isolates produced perithecia with long neck emerging from mycelial tufts on PDA.

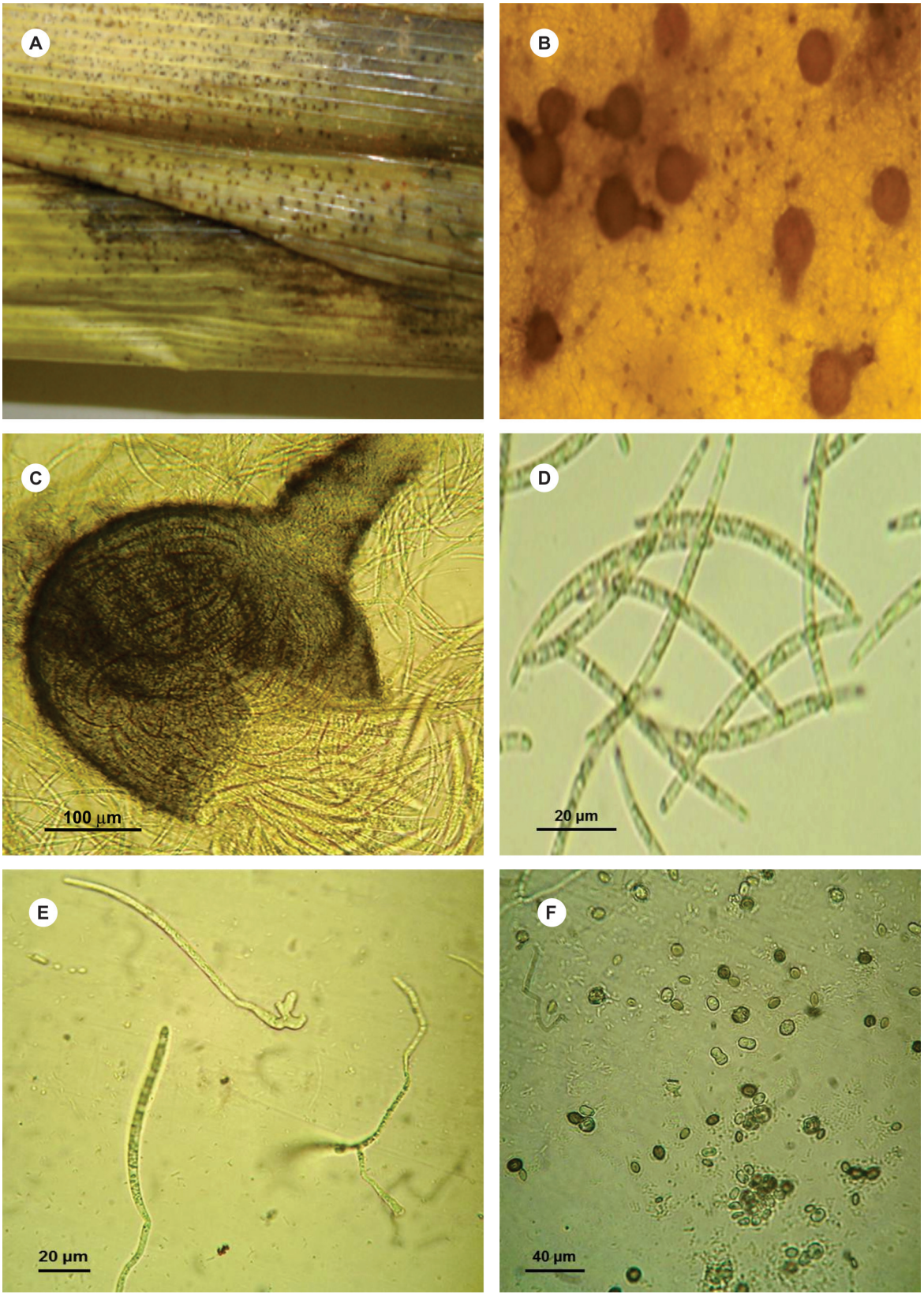

FIGURE 3 - Perithecia of *Gaeumannomyces graminis* var. *graminis*. **A.** Embedded in naturally infected rice leaf sheaths; **B.** Artificially inoculated leaf sheaths; **C.** Masses of ascospores ejecting from the perithecia; **D.** Ascospores showing vacuoles; **E.** Germination of ascospores and formation of hyphopodia; and **F.** Chlamydospores.

TABLE 2 - Production of perithecia and disease intensity measured by lesion height for crown sheath rot in seedlings and adult plants of the rice cultivar BRS Bonança inoculated with *Gaeumannomyces graminis* var. *graminis* isolates originated from rice or grasses

Isolate	Production of perithecia[2]	Lesion height (cm)			
		Seedling		Adult plant[1]	
		Sterilized sand	Sterilized soil	63 DAP[3]	84 DAP
Ggg-a01	++	5.9[4]	4.8	4.9	5.9
Ggg-a43	+++	5.8	4.5	4.8	5.3
Ggg-a45	+++	5.8	4.9	4.7	5.7
Ggg-a37	++++	5.7	4.5	4.6	5.3
Ggg-a30	++++	5.8	4.4	4.6	6.4
Ggg-a44	+++	4.7	4.7	4.2	5.3
Ggg-a32	++++	4.6	5.1	4.2	5.1
Ggg-a31	++++	4.6	5.1	4.1	4.8
Ggg-a21	++	3.8	4.7	4.1	5.0
Ggg-a39	++++	3.6	4.9	3.1	3.6
Ggg-a41	++	2.9	2.1	2.9	3.3
Ggg-a42	+	2.8	3.6	2.0	3.7
Ggg-a40	++++	2.7	2.9	1.4	1.8
Ggg-a38	-	2.2	2.7	0.9	2.1
Ggg-c18	+	0.9	0.9	0.6	1.4
Ggg-c20	-	0.9	1.5	0.5	0.7
Ggg-c28		0.7	1.1	0.5	2.3
Ggg-c34	+	0.6	0.8	0.2	1.4
Ggg-c33	-	0.4	1.2	0.1	1.0
Ggg-c36	-	0.4	1.5	0.1	1.6

[1] Mean lesion height in cm was measured 14 and 35 days after inoculation.
[2] Production of perithecia in culture medium –absent; + = very few; ++ = few; +++ = high; ++++ = very high
[3] DAP - days after planting (soil was inoculated 49 days after planting rice)
[4] Means shaded with the same intensity of gray color in the same column do not differ significantly according to Scott-Knott's test ($p \leq 5\%$).

Aggressiveness on seedlings and adult plants

All Ggg isolates were pathogenic on rice seedlings grown in sterilized soil and sand (Table 2). The typical symptoms of brownish necrosis and black discoloration were observed on basal sheaths. The mean height of the lesions in plants grown in sterilized sand and soil ranged from 0.4 to 5.9 cm and 0.8 to 5.1 cm, respectively, among the isolates. The correlation between lesion height in the two substrates was significant ($r=0.92$, $P\leq0.01$). The grass isolates were significantly less aggressive on seedlings than the rice isolates, for experiments using either sterilized sand or soil as substrate.

Symptoms of crown sheath rot on adult plants of rice cultivar BRS Bonança were induced by all inoculated isolates. Lesion height ranged from 0.7 to 6.4 cm, among the isolates, assessed 35 days after soil inoculation (Table 2). The rice isolates Ggg-a01 and Ggg-a30 were significantly more aggressive compared with all other isolates. These two isolates were also found to be very aggressive on seedlings. The six grass isolates were significantly less aggressive than ten rice isolates. Perithecia were produced in abundance on inoculated plants with all isolates on 84-day old plants. The correlation between lesion height measured in seedlings grown in sterilized soil and lesion height measured in adult plants in non-sterilized soil was significant ($r=0.89$, $P\leq0.01$). For both experiments with inoculations on seedling or adult plant, the grass isolates were less aggressive than most of the rice isolates.

Effect of inoculum level and plant age on crown sheath rot severity

Significant differences in disease severity were observed in relation to inoculum levels and plant age ($P>0.05$) as single factor ($P>0.05$) but not in interaction ($P>0.05$).

Disease severity, at 21 days after inoculation, was higher in 60-day old plants than 35-day old plants (Figure 4a). The disease severity increased with the increase in inoculum level (Figure 5). Soil infestation with 4 g of inoculum was significantly greater than with 0.5 g but did not differ from 1 g of inoculum.

Ascosporic infection from rice-stubble perithecial inoculum

The first symptoms of crown sheath rot symptoms from ascosporic infections were detected seven days after plants were exposed to the inoculum source. The disease was initially detected in the two rows of plants positioned closest to inoculum (12.5 cm) and disease incidence progressed linearly over time with 100% of the plants diseased after 66 days (Figure 1).

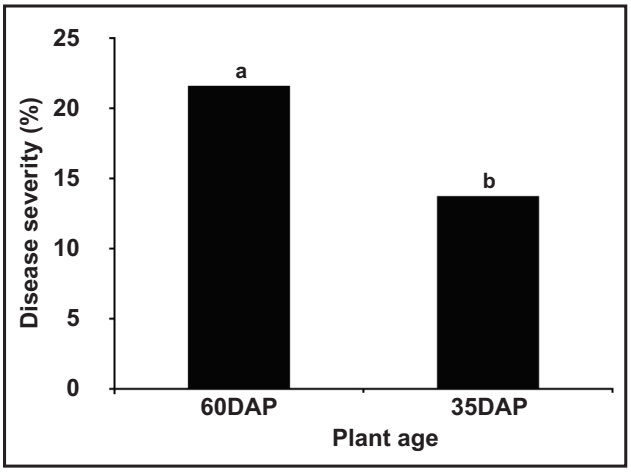

FIGURE 4 - Effect of plant age on crown sheath rot severity (means of five inoculum levels and four replications) in 60 and 35-day old rice plants of cultivar BRS Bonança (DAP = days after planting). Means followed by the same letter are not significantly different according to Tukey´s test (p ≤ 5%).

DISCUSSION

The isolates obtained from rice and grasses exhibited similar cultural characteristics on PDA culture medium and could not be distinguished. According to Cunningham (1981) all three varieties of *G. graminis* have also common colony characteristics, which are insufficient for separating them apart.

The presence of hyphopodia on the surface of the rice sheaths, an exclusive characteristic of Ggg, was observed both in the field and in greenhouse experiments. Two types of hyphopodia varying in size and coloration was reported by Walker (1981). The first type was characterized by brown color, lobed and terminal or lateral ramifications and variable size (15-35 x 10-25 µm). The second type was characterized by pale brown color, almost hyaline, not lobed and simple, terminal or intercalary with size varying from 7-15 x 4-10 µm. The proportion of simple and lobed hyphopodia produced within the same isolate is variable, as well as the structure of lobes and the ability to form such structures (Walker, 1981). Nilsson (1972) observed Ggg isolates that produced both lobed and non-lobed hyphopodia on the base of the culms of cereals and grasses, depending on the temperature; at low temperatures (13 to 14°C) only simple hyphopodia were produced, whereas at high temperatures (20 a 24°C) under natural field conditions, lobed hyphopodia as well as simple one were produced more frequently. In our study, only one type of lobed hyphopodia was observed in culture medium and naturally infected or inoculated leaf sheaths by both the rice and the grass isolates.

Perithecia were formed on infected leaf sheaths both in the field and in the greenhouse experiments, which is in agreement with observations in irrigated rice in Florida (Datnoff et al., 1993). The majority of the rice and the grass isolates of Ggg in our study produced abundant number of mature perithecia and ascospores. Previously in Brazil, Reis (1976) reported the formation of perithecia of *G. graminis* var. *tritici* on PDA culture medium, under continuous light at 20ºC. Only one in twelve isolates produced fertile perithecia, and the others produced dark-brown mycelium. The formation of perithecia by *G. graminis* from wheat and oats was reported in culture medium as well as host tissues only by the highly pathogenic isolates, and not for the weakly ones (Chambers & Flentje,1967). However, the relation between aggressiveness and perithecia production in culture was not explained (Cunningham, 1981).

The germination pattern of ascospores found in our study is similar to observations made by Walker (1981) studying Ggt. According to Cunningham (1981) germination occurred in 2 to 3 days. The ascospores are sensitive to ambient temperature and 20 to 25°C were reported to be

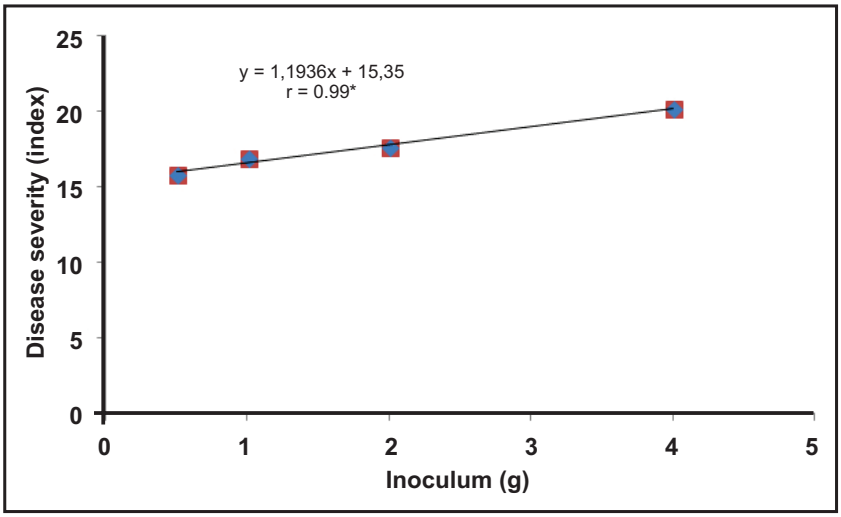

FIGURE 5 - The effect of inoculum level on crown sheath rot severity in rice plants inoculated with the isolate Ggg-a01 of *Gaeumannomyces graminis* var. *graminis* (disease severity index was based on lesion height in relation to the total height of the main tiller; means of 35 and 60-day old plants).

optimum range for germination of Ggt (Cunningham, 1981). Chlamydospore production was observed under water stress conditions, which can induce formation of microsclerotia and chlamydospores in culture medium as is the case of Ggt. The chlamydospores are resistant structures utilized for survival of the fungus in soil or debris (Nilsson, 1972; Sivasithamparam & Parker, 1976).

Pathogencity tests on rice seedlings showed that, in general, the rice isolates were more aggressive than grass isolates on rice seedlings. These results are in agreement with reports by Datnoff et al. (1997) on cross-inoculation tests of rice and grass isolates in Florida. The adult plants were found to be more susceptible than the seedlings. Under natural field conditions crown rot disease manifests after the booting developmental stage of the plant. Inoculation tests under controlled greenhouse conditions with *G. graminis* var. *tritici* and var. *avenae* have been successfully carried out using fungal culture, wheat straw, cereal grains and maize meal, among others, and results varied greatly with the medium used and test conditions (Nilsson, 1969). The unsterilized soil seems to protect wheat plants against the inoculum due to the presence of antagonistic soil microflora and the effect was lost by sterilization (Walker, 1981). In the present investigation there was high correlation between results of inoculation assays in sterilized and non-sterilized soil indicating that the microbial activity was not sufficient to affect disease development. The results also showed severe disease could develop on 60-day old plants inoculated with infected sorghum at levels as high as 2 g or more. The inoculation method proved efficient to provide high disease and should be suitable for pathogenicity and epidemiological studies under controlled conditions.

It was found that the grass isolates were in general less aggressive than most of the rice isolates. In Florida, the isolates of Ggg from rice and two grasses (*Cynadon dactylon* (L.) Pers. x *transvaalensis* Burtt-Davy and *Stenotaphrum secundatum* (Walter) Kuntze) differed in aggressiveness in cross inoculation assays (Datnoff et al., 1997). Polymerase chain reaction comparing isolates of rice, Bermuda grass and St. Augustine grass correlated with the particular grass host from which the isolates were derived (Elliott et al., 1993). According to Datnoff et al. (1997) Ggg isolates from both Florida and Texas tended to be more aggressive on the host from which they were originally isolated, demonstrating host-preference but not host-specificity, which can explain our results. *Digitaria* spp., *Panicum* spp. and *Brachiaria* spp. are grasses commonly found in rice fields from which the grass isolates from our study were originated, and these grasses may possibly have a role in inoculum survival, even though the pathogenic populations from grasses are less aggressive on rice.

Finally, we demonstrated that ascosporic infections of adult plants are likely to occur in the presence of infected rice stubbles with inoculum that was released during the whole period of the experiment and infecting all plants. Ascospores of Ggg are likely to disperse in the atmosphere to long distance between rice and turf grass fields in Florida (Datnoff et al., 1997). Gerlagh (1968) considered ascosporic infection of wheat as less important than in grasses. According to Shipton (1972) the substantial increase of take-all disease in mono culture of wheat and barley was probably due to Ggt ascospores. Gerlagh (1968) reported that the spontaneous infection of healthy wheat plants in the greenhouse and the spread of take-all disease in Netherlands to newly reclaimed polders were caused by wind-dissemination of ascospores of Ggt. Similarly, the unexpected occurrence of take-all of wheat in plants grown in greenhouse in Rothamstead Experimental Station in England was attributed to infection by ascospores (Hornby, 1981). Further studies are needed to confirm and better elucidate the role of ascospores of Ggg under field conditions.

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