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SHORT COMMUNICATION / *COMUNICAÇÃO*

Inoculum density of *Plectosporium alismatis,* a potential mycoherbicide, in relation to control of the aquatic weed *Sagittaria montevidensis*

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ABSTRACT

Sagitaria montevidensis (arrowhead) is one of the worst weeds of irrigated rice. Control of this weed by means of chemical herbicides has become increasingly difficult on account of herbicide resistance in populations of *S. montevidensis*. Mycoherbicides are recognized as potential alternative tools against *S. montevidensis.* In Australia, the fungus *Plectosporium alismatis* was reported as a possible mycoherbicide for *Damansonium minus* and other alismataceous weeds, but Australian isolates of the pathogen did not infect *S. montevidensis*. In contrast, Brazilian isolates of *P. alismatis* obtained from *S. montevidensis* have shown promise in controlling the weed. The present study investigated effects of different inoculum densities of a selected strain of *P. alismatis* on *S. montevidensis.* Respective densities of 2 x 10^6 and 2 x 10^7 conidia.mL^{-1} caused an average of 86 and 93 % leaf blight followed by growth cessation and death of the plants. It was concluded that a density of 2 x 10^6 conidia.mL^{-1} was sufficient to control *S. montevidensis* and that this density should be adopted as standard in future experiments.
Key words: *Rhynchosporium alismatis*, arrowhead, biological control, mycoherbicide.

RESUMO

Densidade de inóculo de *Plectosporium alismatis,* um potencial micoherbicida, em relação ao controle de *Sagittaria montevidensis*

Sagitaria montevidensis (sagitária ou aguapé-flecha) é uma das plantas invasoras mais nocivas na cultura do arroz irrigado. O controle desta invasora tem se tornado difícil devido ao surgimento de populações desta espécie resistentes a herbicidas. O uso de micoherbicidas pode vir a ser uma alternativa para contornar este problema. Na Austrália o fungo *Plectosporium alismatis* já foi detalhadamente investigado como potencial micoherbicida para *Damansonium minus* e outras espécies da família Alismataceae, entretanto os isolados australianos deste patógeno não eram infectivos a *S. montevidensis*. Isolados deste fungo obtidos no Brasil foram promissores no controle de *S. montevidensis.* O presente estudo investigou o efeito de diferentes densidades de inóculo de *P. alismatis* para o controle de *S. montevidensis*. Densidades de 2 x 10^6 e 2 x 10^7 conídios.mL^{-1} causaram em media 86 e 93 % de queima foliar seguido de paralisação do crescimento e morte das plantas. Concluiu-se que uma densidade de inóculo de 2 x 10^6 conídios.mL^{-1} seria suficiente para o controle de *S. montevidensis* e que esta densidade deveria ser adotada como padrão em futuros experimentos.
Palavras-chave: *Rhynchosporium alismatis*, aguapé-flecha, controle biológico, micoherbicida.

Sagittaria montevidensis Cham. & Schltdl. is a South American aquatic plant of the Alismataceae (Lorenzi, 2000); it is considered one of the worst weeds in irrigated rice in the southern Brazilian states of Rio Grande do Sul and Santa Catarina. It is also an important weed in Australia and the USA. ALS-synthesis inhibiting herbicides are widely used for controlling the weed, but herbicide-resistant populations of *S. montevidensis* are increasing worldwide, making chemical control difficult (Pratley et al., 2001; Concenço et al., 2007). In recent years biological control has gained increasing attention as an option for controlling alismataceous weeds, including *S. montevidensis*. Soares et al. (2009) surveyed fungal pathogens of *S. montevidensis* in Brazil, in an effort to identify potential biocontrol agents. Based on field observations, pathogenicity tests, and other available information, *Plectosporium alismatis* (Oudem.) W.M. Pitt, W. Gams & U. Braun was selected for studies as a potential mycoherbicide of *S. montevidensis*.

Plectosporium alismatis is an anamorphic hyphomycete with hyaline, straight to slightly navicular, two-celled phialoconidia, which has been recorded on several alismataceous hosts (Pitt & Gams, 2005; Soares et al., 2009). This pathogen was investigated extensively in Australia for controlling *Damansonium minus*, *Alisma lanceolatum* and other alismataceous weeds in irrigated rice (Cother & Gilbert, 1994a, 1994b; Cother & Van de Ven, 1999; Lanoiselet et al., 2001; Jahromi et al., 2002,

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2004, 2006; Cliquet et al., 2004; Pitt et al., 2004a; Ghajar et al., 2006; Cliquet & Zeeshan, 2008).

Until recently, *P. alismatis* was regarded as not being capable of infecting *S. montevidensis* (Ash et al., 2008; Pitt et al., 2004b); however, Soares et al. (2009) reported *P. alismatis* infections on *S. montevidensis* as being rather common in Brazil. Earlier abstracts published in conference proceedings by R. A. Pitelli and co-workers referred to a fungus attacking *S. montevidensis* as *Cylindrocarpon* sp. and recognized it as having potential for development as a mycoherbicide. From our experience, it is considered likely that fungi isolated from *S. montevidensis* and reported as *Cylindrocarpon* sp. were misidentified and were probably isolates of *P. alismatis*. Re-examination of these isolates appears justified, especially given the similar morphology of the two genera. Soares et al. (2009) considered that a fungus described in Japan as *Cylindrocarpon sagittariae* Negeshi was also misidentified.

Commercial development of a fungus as a mycoherbicide generally requires that inoculum can be mass-produced at low cost under controlled conditions. A preliminary assay was conducted involving 14 liquid media recipes (unpublished data), aimed at finding a medium that might be adequate for mass-producing *P. alismatis* conidia and that meets with the requirements of commercial production. The media included lima bean broth (LBB) as described by Cother and Van de Ven (1999) and thirteen others. Since soybean flour (SF) medium (Vieira, 2008) yielded abundant infective conidia (an average of 10^7 conidia.mL^{-1}), and is of simple composition it was chosen for inoculum production in the present work.

The aim of the present work was to determine the effectiveness of *P. alismatis* against *S. montevidensis* and the minimum inoculum density of the pathogen needed to provoke disease that is sufficiently severe to reduce or stop growth of *S. montevidensis*. The findings would provide a basis to further evaluate the potential of Brazilian strains of the pathogen for use in arrowhead biocontrol.

Among four available isolates of *P. alismatis* (DJS-163b; DJS-166b; DJS-458b and RWB-814a) RWB 814a was the most virulent on *S. montevidensis* (unpublished data) and was selected for use in a bioassay. Plants of *S. montevidensis* used in the assay were grown from seeds collected from plants at the Lagoa da Pampulha (Belo Horizonte, state of Minas Gerais). The seeds were sown in pots containing water-saturated soil. The pots were positioned inside 500 L water tanks such that the top of each pot was 10 cm deep in the water. Each plant had at least four fully-developed sagitate leaves above the water. The tanks were located outdoors at an experimental field on the campus of the Universidade Federal de Viçosa and the experiment was conducted during late winter and early spring (August-September) 2008.

Inoculum of *P. alismatis* for use in the bioassay was produced as follows: 100 mL of SF was dispensed into each of several 250 mL Erlenmeyer flasks, autoclaved at 121ºC for 10 min, allowed to cool, and seeded with discs from 7-day colonies of isolate RWB-814a grown on plates containing Vegetal Broth Agar (Pereira et al., 2003). The flasks were placed on a controlled temperature orbital shaker operated at 150 rpm and maintained with 12 h light/dark cycle at 25 ± 2ºC for 7 days. The colonized liquid medium was filtered through muslin to remove the mycelial mass, and the filtrate containing the conidia and residues of the SF medium was diluted with sterile distilled water to provide inoculum densities of 2 x 10^3, 2 x 10^4, 2 x 10^5, 2 x 10^6 and 2 x 10^7 conidia.mL^{-1}. The initial conidial density in the filtrate was estimated by means of haemocytometer counts.

Healthy leaves of *S. montevidensis* plants in the bioassay were spray-inoculated to run-off with the conidial suspensions. The inoculations were performed late in the afternoon so that no special care for maintaining high humidity levels for inoculated plants was necessary. Inoculated plants were observed daily until the first symptoms appeared.

The experimental design was completely randomized with three replicate plants per treatment. Control plants were not inoculated. Seven days after the inoculations, the largest leaf of each plant was detached and its petiole was removed. Each detached leaf was separately and immediately scanned using an HP Scanjet 6100c scanner (Hewlett Packard Company). The plants, each with at least three remaining inoculated leaves, were left in the water tanks to allow further disease development. Scanned images of the leaves were assessed with the software Quant® (Vale et al., 2004) to determine the percent leaf area that was healthy and the percent area with disease symptoms. The data were submitted to homocedastic test and analysis of variance (ANOVA) using SAS® (version 9.1). The equation and curve adjusted to the data were obtained using a version trial of the Table Curve 2D® v5.01 available at SYSTAT Software Inc. homepage (http://www.systat.com).

The evaluations of the proportion of diseased leaf area, as provided by use of Quant® software, are exemplified in Figure 1. There was a high correlation between the spore density and necrotic leaf area (Figure 2). In this assay the best inoculum density was considered as the lowest density that was sufficient to result in > 80% leaf area blighted.

Moderate to severe symptoms appeared at three to four days after inoculations in leaves inoculated with a high density of inoculum (10^6 and 10^7 conidia.mL^{-1}). Conidial densities of 10^6 and 10^7 conidia.mL^{-1} resulted in severe leaf blight (average 86.1 and 93.3 %, respectively), interruption of plant growth and subsequently (7 to 20 days after application) death of the foliage, including the leaf blades and petioles. At the high inoculum densities, new leaves that emerged up to 10 days after inoculation also died. Mean disease severity on detached leaves inoculated respectively with densities of 10^3, 10^4 or 10^5 conidia.mL^{-1} was 0.85, 17.6 and 57.7%. At these lower densities the inoculated plants maintained growth, produced new leaves and, after about 30 days, also flowered.

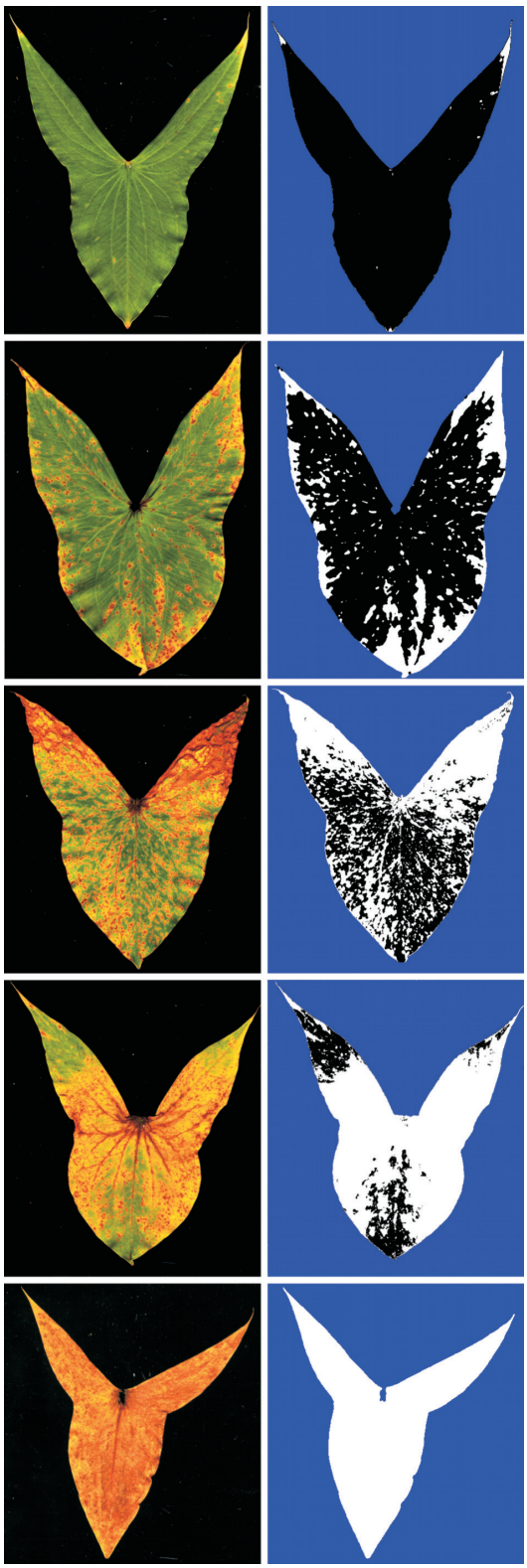

FIGURE 1 - Representative digitized images of leaves of *Sagittaria montevidensis*, with the percent of lesioned areas (left) and its respective Quant® (right) estimation, in white see text for values, seven days after inoculation with *Plectosporium alismatis* at densities of 2 x 10^3, 2 x 10^4, 2 x 10^5, 2 x 10^6, and 2 x 10^7 spores.mL^{-1} (from top to bottom, respectively).

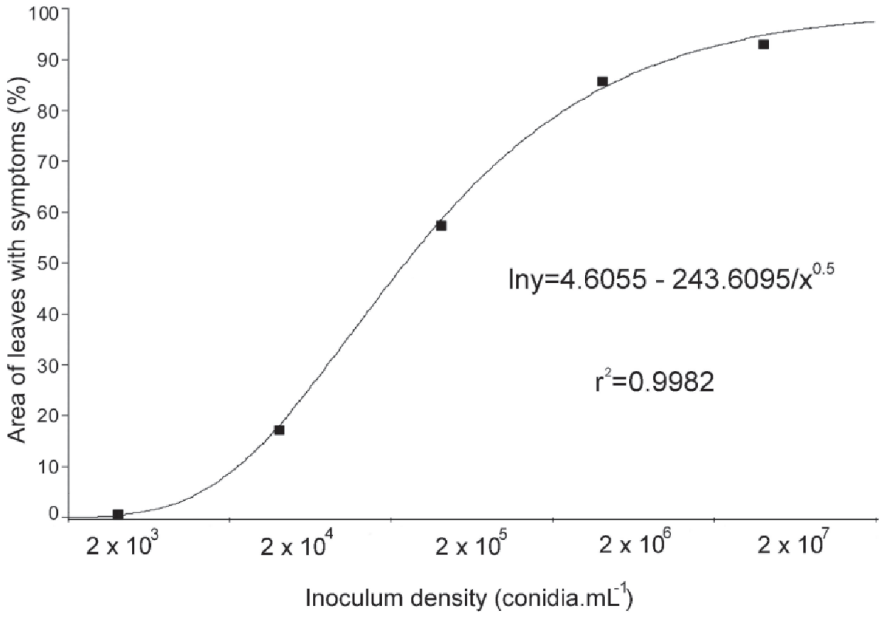

FIGURE 2 - Relationship of inoculum density (conidia.mL^{-1}) of *Plectosporium alismatis* and disease severity (% area of leaves with symptoms) in *Sagittaria montevidensis* at seven days after inoculation. Each data point represents the average of three replicates.

We conclude that an inoculum density of 2 x 10^6 conidia.mL^{-1} is adequate for application of *P. alismatis* aimed at controlling *S. montevidensis* and suggest that this density be used in further studies towards the development of a mycoherbicide for control of *S. montevidensis*. A similar level of inoculum density (10^6 conidia.mL^{-1}) was found by Cother and Gilbert (1993) and Jahromi et al. (2004) to be adequate for controlling *D. minus* and other alismataceous weeds. We have also demonstrated that *S. montevidensis* is a host of *P. alismatis* and that, in contrast to findings of Pitt et al. (2004b) for Australian isolates, that Brazilian isolates of *P. alismatis* are pathogenic to arrowhead. Our findings underscore the opportunity to exploit *P. alismatis* for development as a mycoherbicide against *S. montevidensis*.

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REFERENCES

Ash GJ, Chung YR, McKenzie C, Cother EJ (2008) A phylogenetic and pathogenic comparison of potential biocontrol agents for weeds in the family Alismataceae from Australia and Korea. Australasian Plant Pathology 37:402-405.

Cliquet S, Ash GJ, Cother EJ (2004) Production of chlamydospores and conidia in submerged culture by *Rhynchosporium alismatis*, a mycoherbicide of Alismataceae in rice crops. Biocontrol Science and Technology 14:801–810.

Cliquet S, Zeeshan K (2008) Impact of nutritional conditions on yields, germination rate and shelf-life of *Plectosporium alismatis* conidia and chlamydospores as potential candidates for the development of a mycoherbicide of weeds in rice crops. Biocontrol Science and Technology 18:1–11.

Concenço G, Noldin JA, Lopes NF, Comiotto A (2007) Aspectos da resistência de *Sagittaria montevidensis* ao herbicida pirazosufuron-ethyl inibidor da ALS. Planta Daninha 25:187–194.

Cother EJ, Gilbert RL (1993) Mycoherbicide. Patent WO/1993/022923. Available at http://www.wipo.int/pctdb/images4/PCT-PAGES/1993/281993/93022923/93022923.pdf

Cother EJ, Gilbert RL (1994a) Pathogenicity of *Rhynchosporium alismatis* and its potential as a mycoherbicide on several weed species in the Alismataceae. Australian Journal of Experimental Agriculture 34:1039–1042.

Cother EJ, Gilbert RL (1994b) Efficacy of a potential mycoherbicide for control of *Alisma lanceolatum* and *Damasonium*

minus in Australian rice crops. Australian Journal of Experimental Agriculture 34:1043–1050.

Cother EJ, Van de Ven R (1999) The influence of nutrition on conidial production by *Rhynchosporium alismatis* and on their subsequent infectivity to *Alisma lanceolatum*. Biocontrol Science and Technology 9:395-407.

Ghajar F, Holford P, Cother EJ, Beattie A (2006) Enhancing survival and subsequent infectivity of conidia of potential mycoherbistats using UV protectants. Biocontrol Science and Technology 16:825-839.

Jahromi FG, Ash GJ, Cother EJ (2002) Early infection process of *Damasonium minus* by the potential mycoherbistat *Rhynchosporium alismatis*. Canadian Journal of Plant Pathology 24:131-136.

Jahromi FG, Ash GJ, Cother EJ (2004) Factors affecting disease development by *Rhynchosporium alismatis* in starfruit (*Damasonium minus*), a weed of rice. Biocontrol Science and Technology 14:281-290.

Jahromi FG, Van de Ven RJ, Cother EJ, Ash GJ (2006) The interaction between *Plectosporium alismatis* and sublethal doses of bensulfuron-methyl reduces the growth of starfruit (*Damasonium minus*) in rice. Biocontrol Science and Technology 16:929-940.

Lanoiselet V, Cother EJ, Ash GJ, Van de Ven R (2001) Production, germination and infectivity of chlamydospores of *Rhynchosporium alismatis*. Mycological Research 105:441-446.

Lorenzi H (2000) Plantas daninhas do Brasil: terrestres, aquáticas, parasitas e tóxicas. 3nd Ed. São Paulo SP. Instituto Plantarum.

Pereira JM, Barreto RW, Ellison C, Maffia LA (2003) *Corynespora cassicola* f. sp. *lantanae*: a potential biocontrol agent for *Lantana camara* from Brazil. Biological Control 26:21-31.

Pitt WM, Gams W (2005) A redescription of *Plectosporium alismatis* (hyphomycetes with glomerellaceous affinities). Nova Hedwigia 81:311-323.

Pitt WM, Goodwin SB, Ash GJ, Cother EJ (2004a) *Plectosporium alismatis* comb. nov. a new placement for the Alismataceae pathogen *Rhynchosporium alismatis* Mycological Research 108:775-780.

Pitt WM, Cother EJ, Cother NJ, Ash GJ (2004b) Infection process of *Plectosporium alismatis* on host and non-host species in the Alismataceae. Mycological Research 108:837–845.

Pratley JE, Broster JC, Flower GE, Flower R (2001) Herbicide resistance in rice growing regions of southern Australia. RIRDC Publication no. 01/40.

Soares DJ, Barreto RW, Braun U (2009) Brazilian mycobiota of the aquatic weed *Sagittaria montevidensis*. Mycologia 101:397-412.

Vale FXR, Jesus Junior WC, Liberato JR, Souza CA (2004) Quantificação de doenças e do crescimento do hospedeiro. In: Vale FXR, Jesus Junior WC, Zambolim L (Eds.) Epidemiologia aplicada ao manejo de doenças de plantas. Belo Horizonte MG. Editora Perfil. pp. 91-123.

Vieira BS (2008) *Lewia chlamidosporiformans* como mico-herbicida para *Euphorbia heterophylla*. PhD Thesis. Universidade Federal de Viçosa. Viçosa MG.

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