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SHORT COMMUNICATION / *COMUNICAÇÃO*

New cucurbitaceous hosts of *Myrothecium roridum* in Amazonas State, Brazil

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ABSTRACT

Myrothecium roridum is a soil inhabitant and a facultative parasite with a large host range worldwide. However, its importance as a pathogen of vegetable crops, particularly cucurbits, in the humid tropical conditions of the Brazilian Amazon is not known. Seven samples of diseased plants of cucumber (*Cucumis sativus*), gherkin (*C. anguria*) and squash (*Cucurbita moschata*) showing leaf spots were collected in Rio Preto, Silves and Iranduba counties, Amazonas State, Brazil. Five fungal isolates were obtained and identified as *Myrothecium roridum*, with phialide length ranging from 10.3 to 11.2 µm and conidia measuring 6.4-7 x 1.7-2.3 µm. Pathogenicity of these isolates was tested on wounded and non-wounded leaves of gherkin, cucumber, squash, pumpkin, watermelon and melon plants. All isolates fulfilled Koch's postulates and showed a slight variability in their aggressiveness. This is the first record of *M. roridum* causing leaf spots on cucumber, gherkin and squash in Brazil.
Keywords: *Cucumis anguria*, *Cucumis sativus*, *Cucurbita moschata*, Cucurbitaceae, etiology, vegetable crops.

RESUMO

Novas cucurbitáceas hospedeiras de *Myrothecium roridum* no estado do Amazonas, Brasil

Myrothecium roridum é um fungo de solo parasita obrigatório com um grande número de hospedeiras. Entretanto, sua importância como patógeno de hortaliças, em particular cucurbitáceas, cultivadas no trópico úmido, como a da Amazônia brasileira, ainda não foi determinada. Foram analisadas sete amostras de plantas doentes de abóbora, pepino e maxixe com manchas foliares oriundas dos municípios de Rio Preto, Iranduba e Silves, estado do Amazonas. Destas amostras, cinco isolados foram identificados como *Myrothecium roridum*, apresentando comprimento de fiálides variando de 10,3 a 11,2 µm e tamanho de conídios 6,4-7 x 1.7-2,3 µm. Estes isolados foram inoculados em folhas, com e sem ferimentos, de maxixe, pepino, abóbora, moranga, melancia e melão. Todos os isolados completaram os Postulados de Koch, constatando-se variabilidade em sua agressividade. Este é o primeiro registro de *M. roridum* causando manchas foliares em abóbora (*Cucurbita moschata*), maxixe (*Cucumis anguria*) e pepino (*C. sativus*) no Brasil.
Palavras-chave: *Cucumis anguria*, *Cucumis sativus*, *Cucurbita moschata*, etiologia, hortaliças.

Eight species have been described in the genus *Myrothecium* (Tulloch, 1972), most of them being saprophyte soil inhabitants (Costa et al., 2006; Domsch et al., 1980; Souza-Motta et al., 2003). Only *M. roridum* Tode has been considered important as a plant pathogen, affecting more than 200 plant species of distinct botanical families (Tulloch, 1972; Domsch et al., 1980; Ahrazem et al., 2000; Murakami et al., 2005). In Brazil, *M. roridum* was described infecting many plant hosts, such as peanut, soybean, cotton, melon, Antilles cherry, sunflower, rice and common bean (Mendes et al., 1998; Poltronieri et al., 2003; Silva & Meyer, 2006; Quezado-Duval et al., 2010). Potentially, *M. roridum* may affect all cultivated cucurbit species and cause severe losses. After a heavy rain period, an outbreak of *M. roridum* was reported on watermelon cv. Desert King in Georgia State, USA, causing low severity (less than 5% of leaf area affected), and no symptoms were observed on fruits. In Brazil, Myrothecium stem canker is important in the main melon producing areas, such as Mossoró county, Rio Grande do Norte State. Screening for resistance has been carried out in melon (Lima et al., 1997; Noronha et al., 2006). From 150 melon genotypes screened against *M. roridum*, only 26.7% were intermediate resistant, showing the difficulty in obtaining genetic resources with high level of resistance to this pathogen (Noronha et al., 2006).

Leaf spots are the most common symptoms observed on susceptible hosts associated with *M. roridum* infection; however, stem canker and fruit rot may also occur (Domsch et al., 1980). According to Bruton (1996), *M. roridum* causes three distinct phases of disease in cucurbits: fruit rot, crown and stem canker, and leaf spot. As a soilborne fungus with a broad susceptible host range, control of *M. roridum* is difficult (Bruton, 1996). Furthermore, depending on the host-pathogen interaction, *M. roridum* is seedborne, and can be efficiently seed-transmitted (Ellis, 1971; Mendes et al., 1998).

In 2007, seven diseased leaf samples of cucumber (*Cucumis sativus* L.), gherkin (*C. anguria* L.) and squash (*Cucurbita moschata* Duchesne ex Poir.) plants were collected in Silves, Rio Preto, and Iranduba counties, Amazonas State, Northern Brazil. Sample analysis was carried out in the Plant Pathology Laboratory of Embrapa Hortaliças (CNPH) for diagnosis. The objective of this paper was to investigate the etiology and pathogenicity of foliar diseases on cucurbitaceous plants received from Amazonas State, Brazil.

Isolation from cucurbit leaves was performed on PDA plates amended with 50 ppm of rifamycin. For each leaf sample maintained in a moist chamber, a mass of spores was transferred from leaf spots to PDA plates. Monosporic isolates were obtained according to Alfenas & Mafia (2007). Isolates were characterized morphologically by measuring the length of 30 phialides and the length and width of 50 conidia of each isolate. Based on the morphology of sporodochia, phialides and conidia, the fungal species was identified as *Myrothecium roridum* (Ellis, 1971; Tulloch, 1972; Domsch et al., 1980). The isolates were also compared to two standard isolates of *Myrothecium roridum* and *M. verrucaria* belonging to the 'Embrapa Hortaliças' Fungi Collection.

Pathogenicity tests were carried out on cucumber cvs. Caipira and Curumin, melon cvs. Eldorado 300 and Hales Best Jumbo, gherkin, squash cv. Brasileirinha, pumpkin cv. Nirvana and watermelon cvs. Crimson Sweet and Charleston Gray (Table 2). Plants were cultivated in plastic pots with 2L of a mixture of sterilized substrate (1/3 of soil, 1/3 of carbonized rice hulls and 1/3 of sand) kept in greenhouse for 30 days. When plants had two pairs of true leaves, pathogenicity tests were carried out on two plants per pot, with four replications at random. Isolate inoculation was performed by spraying a spore suspension of 1 x 10^5 conidia/ml of each isolate on wounded and non-wounded leaves. Foliar wounds were previously produced by rubbing an abrasive (carborundum, Saint-Gobain) on the leaf surface of the basal and intermediate leaves of each plant. After the inoculation, plants were kept in moist chamber for 48h and afterwards in the greenhouse.

Symptom evaluations were performed seven days after inoculation by checking for the presence of leaf spots similar to those observed in the original host and for the presence of fungal structures on the lesions. The aggressiveness of the isolates was assessed by examining the two lower leaves of each plant. The disease severity was measured according to a grade scale ranging from 1 to 4, where: 1 = asymptomatic plant; 2 = up to 5% of the leaf surface with spots; 3 = from 5.1% to 20% of the leaf covered with spots; 4 = leaf spots on more than 20% of the leaf surface. Symptomatic plants presenting grades between 2 and 4 were considered as hosts of the pathogen. The pathogen's aggressiveness was considered as low, mild, and high according to the grade score 2, 3, and 4, respectively. The pathogen was re-isolated from diseased-leaf tissue to fulfill Koch's postulates.

Five fungal isolates ('Myr.33', 'Myr.34', 'Myr.35', 'Myr.36' and 'Myr.37') were obtained from leaf samples collected in different counties of Amazonas State, Brazil. After seven days, all five isolates showed whitish mycelia, with some light-green points noticed on PDA plates turning to dark-green and black. By using a microscope, the black points were later recognized as typical sporodochia that measured up to 1.4 mm in diameter. Phialides length ranged from 10.3 to 11.2 µm. Conidia were cylindrical with round ends, measuring 6.4 to 7.8 µm length and 1.7 to 2.7 µm width (Table 1). Conidium color varied from hyaline to light-green and the conidia-mass color from green to black. Most of the observed characteristics agreed with those described for *M. roridum* (Ellis, 1971; Tolluch, 1972; Domsch et al., 1980).

M. roridum isolates incited the development of small to large concentric lesions on the leaves of cucumber, squash and gherkin plants (Figure 1). Stereoscopic microscope observations revealed a large number of sporodochia on the edges of necrotic lesions. Often the development of large necrotic lesions of *M. roridum* on plants is correlated to its ability to produce toxins (Murakami et al., 1999). The isolates were able to cause disease on their original plant hosts and also on the majority of other inoculated-plant species (Table 2, Figure 1). Watermelon was not infected by the isolates 'Myr.33' (cv. Charleston Gray) and 'Myr.05' (cv.

TABLE 1- Morphological characteristics and identification of *Myrothecium roridum* isolates collected in Amazonas State, Brazil, causing leaf spots on cucurbit plants

Isolate	Host	Plant Species	Place of Origin	Phialide Length (µm)	Conidia size (µm) (length x width)
Myr.33	Gherkin	*Cucumis anguria*	Silves, AM[1]	9.9-12.0 (10.8)	5.9-7.3(6.5) x 1.7-3.1 (1.7)
Myr.34	Cucumber	*Cucumis sativus*	Silves, AM	9.7-11.8 (10.3)	6.1-7.7(6.4) x 1.4-2.8(1.9)
Myr.35	Cucumber	*Cucumis sativus*	Iranduba, AM	9.2-12.1 (10.5)	6.2-8.2(7.0) x 1.5-2.9(1.9)
Myr.36	Squash	*Cucurbita moschata*	Silves, AM	9.4-11.6 (10.7)	6.2-7.4(6.4) x 1.6-2.9 (2.3)
Myr.37	Squash	*Cucurbita moschata*	Rio Preto, AM	9.5-12.2 (11.2)	5.9-7.4(6.8) x 1.6-2.4 (2.0)

[1]AM: Amazonas State, Brazil.

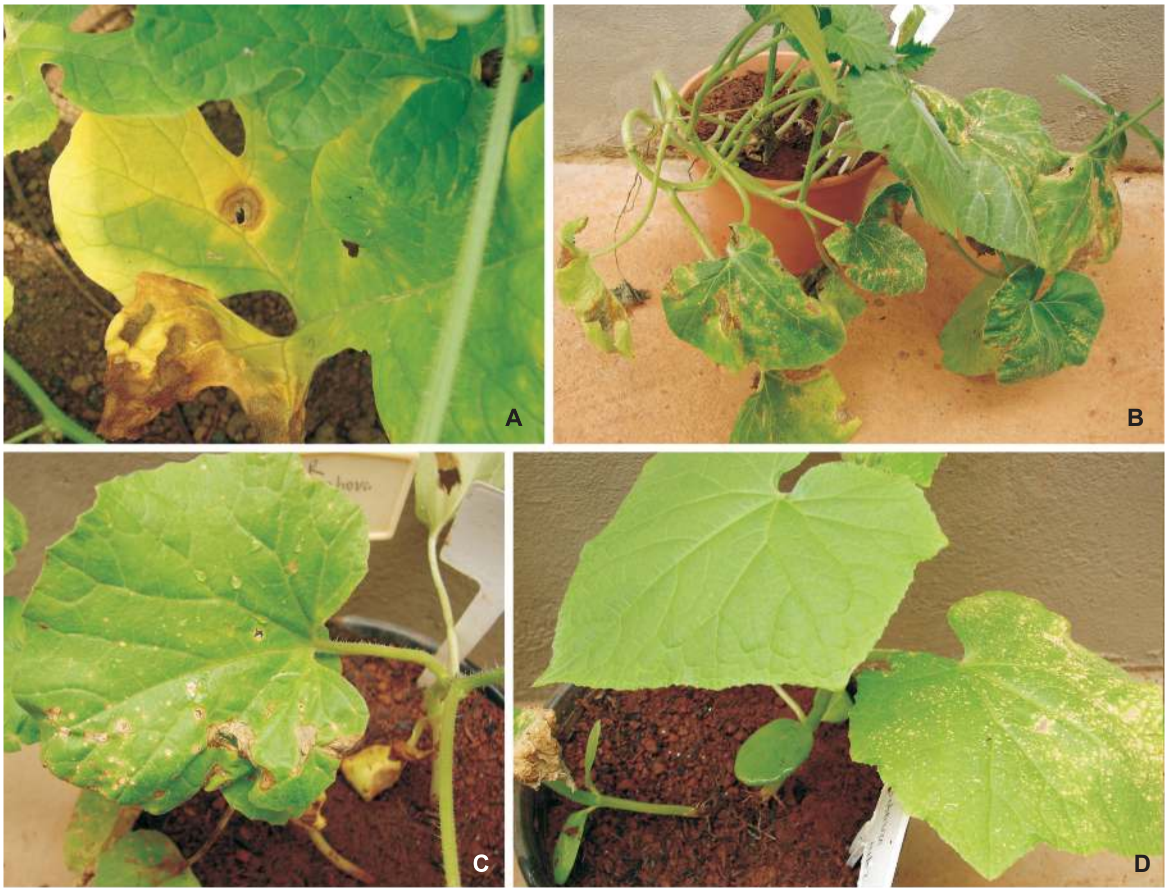

Figure 1- Leaf spots caused by *Myrothecium roridum* on cucurbits: **A.** gherkin (*Cucumis anguria*) in the field; **B.** squash (*Cucurbita moschata*); **C.** melon (*Cucumis melo*) and **D.** cucumber (*C. sativus*) plants inoculated without wounds in greenhouse.

TABLE 2 - Response of cucurbit plants to inoculation with five isolates of *Myrothecium roridum*

Cucurbit species	**Cultivar**	**Disease rate (1~4)***									
		Myr.33		Myr.34		Myr.35		Myr.36		Myr.37	
		Nw[1]	Wo[2]	Nw	Wo	Nw	Wo	Nw	Wo	Nw	Wo
Cucumis sativus	Caipira	3*	4	3	4	3	4	3	4	3	4
Cucumis sativus	Curumin	3	4	2	3	2	4	3	4	3	4
Cucurbita moschata	Brasileirinha	3	4	3	4	3	4	3	4	3	4
Cucurbita maxima	Nirvana	3	4	2	4	3	4	3	4	3	4
Citrullus lanatus	Charleston Gray	1	2	2	2	2	3	2	3	2	3
Citrullus lanatus	Crimson Sweet	2	3	2	3	2	4	2	3	3	4
Cucumis melo	Eldorado 300	3	4	3	4	2	3	2	4	2	4
Cucumis melo	Hales B. Jumbo	2	3	2	2	2	3	3	4	3	4

[1]Nw= non-wounded; [2]Wo= Wounded.
*Disease grade scale (1-4): 1 = asymptomatic plant; 2 = up to 5% of the leaf surface with spots; 3 = from 5.1% to 20% of the leaf covered with spots; 4 = leaf spots on more than 20% of the leaf surface.

Charleston Gray and cv. Crimson Sweet), while cucumber cv. Caipira was highly susceptible to all the isolates of *M. roridum* (Table 2).

The inoculation made on non-wounded plants resulted in less aggressiveness of the isolates on their original hosts. However, the isolates 'Myr.34' and 'Myr.35' were not highly aggressive on cucumber cv. Curumin. On the other hand, when wounded leaves were inoculated, isolates were highly aggressive to their original hosts and also to other plant species. The isolate 'Myr.37' showed the highest aggressiveness when inoculated on wounded leaves (grade 4 on all tested hosts but watermelon cv. Charleston Gray).

The isolates showed some variability in their pathogenicity and aggressiveness to different cucurbit species and cultivars (Table 2). Based on the pathogenicity on distinct plant species and on the variability in aggressiveness of isolates to different hosts, Taneja et al. (1990) suggested the existence of different pathotypes of *M. roridum*. Among the plant hosts in the present work, watermelon was slightly less susceptible to *M. roridum* isolates. It probably indicates some degree specialization in the host-pathogen interaction.

To our knowledge, this is the first report of *M. roridum* causing leaf spots on gherkin, cucumber and squash in Brazil. Overall, these data should be considered in the process of developing strategies for disease management in cucurbits grown in the Northern region due to the potential destructiveness of the pathogen and the susceptibility of some cucurbit species and cultivars (Seebold & Langston, 2005). Furthermore, even though the disease incidence in cucurbits is erratic, the frequent rainfall and warm weather prevalent in the Amazon region is a very conducive to the occurrence of epidemics, which can be a new constraint to growing these crops. Some traditional agricultural practices of Brazilian small-holders, such as producing and planting their own seeds, should be addressed because *M. roridum* is seedborne for some hosts (Ellis, 1971; Mendes et al., 1998).

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