

Erika V. de Medeiros¹Katchen J. Pereira Silva²Lenilton A. Oliveira²Hailson A. Ferreira³Rui S. Júnior⁴

Monosporascus cannonballus density in soils cultivated with different crops in Rio Grande do Norte State, Brazil

ABSTRACT

This paper aimed to quantify *Monosporascus cannonballus* ascospores population density in soils cultivated with different crops. The soil samples were collected from 10 plots cultivated with cotton, mango, beans, papaya, chili, watermelon, acerola, banana, coconut and cantaloupe melons, and from one uncultivated area, located in the State of Rio Grande do Norte, Brazil. Ascospores were extracted by means of a modified method of flotation in saccharosis and quantified under stereoscopic microscope at 60X. Ascospores of *M. cannonballus* were detected in all studied soil samples and the means differed significantly ($p < 0.05$) among the sampled areas. Ascospore average density was significantly higher in the soil cultivated with cantaloupe melons (pathogen hosts), reaching 8.09 ascospores g^{-1} , followed by the mean densities quantified in the mango, watermelon and acerola areas, with 2.00; 2.60 and 2.10 ascospores g^{-1} , respectively. These results did not differ significantly among them, but differed significantly from the means quantified in the areas cultivated with beans, chili, papaya, cotton, banana and coconut, which, in turn, did not differ significantly from the results of the uncultivated soil.

Key words: ascospore extraction, inoculum, vine decline

Densidade de *Monosporascus cannonballus* em solos sob diferentes cultivos no Estado do Rio Grande do Norte

RESUMO

Propôs-se, neste trabalho quantificar a densidade populacional de *Monosporascus cannonballus* em solos com diferentes cultivos. As amostras foram coletadas em 10 áreas cultivadas com algodão, manga, feijão, mamão, pimentão, melancia, acerola, banana, coco e melão e em uma de área sem cultivo, localizadas no Estado do Rio Grande do Norte, Brasil. Os ascósporos foram extraídos mediante o método de flotação em sacarose modificado e quantificados em microscópio estereoscópico a 60x. Ascósporos de *M. cannonballus* foram detectados em todas as amostras de solo analisadas e houve diferença significativa ($p < 0,05$) na densidade média de ascósporos entre as áreas analisadas. A densidade média de ascósporos foi significativamente superior no solo cultivado com meloeiro (espécie hospedeira do patógeno), com 8,09 ascósporos g^{-1} de solo, seguido dos solos cultivados com manga, melancia e acerola, apresentando 2,00; 2,60 e 2,10 ascósporos g^{-1} , respectivamente; esses resultados não diferiram significativamente entre si mas sim, em relação às médias quantificadas nas áreas com feijão, pimentão, mamão, banana e coco que, por sua vez, não diferiram significativamente dos resultados apresentados pelo solo não cultivado.

Palavras-chave: extração de ascósporos, inóculo, morte súbita

¹ Doutoranda em Fitotecnia da UFRSA, evmbio@gmail.com

² Graduando em Agronomia UFRSA, katchenjulliany@hotmail.com, leniltonalex@hotmail.com

³ Mestrando em fitopatologia UFRPE, hailson_alves@hotmail.com

⁴ Prof do dep. de Ciências Vegetais, UFRSA, ruisaes@esam.br

INTRODUCTION

The fungus *Monosporascus cannonballus* Pollack & Uecker is a soilborne pathogen which incites root injury and causes a disease known as vine decline, which can take the form of crown rot, root rot, sudden death, sudden wilt, wilt, and vine collapse (Bruton et al., 2000).

Vine decline, caused by *M. cannonballus*, is a destructive disease that affects plant growth, development and reproductive output of melons and watermelon areas mainly in arid and semi-arid regions of the world (Martyn & Miller, 1996; Aegeger et al., 2000). This fungus attacks plant roots and modifies their characteristics and pattern of growth and development. The symptoms include yellowing of older leaves and a general decline and death of plants when fruits approach maturity, following death of the crown leaves and a gradual decline of the vine as the plant approaches maturity. Sometimes, root symptoms include rotting of secondary and feeder roots and reddish or corky lesions on the taproot (Aegeger et al., 2000). This commonly occurs within 2 weeks of harvest. Foliar symptoms are not observed until about 10 to 20 days before harvest (Bruton, 1998).

It has been suggested that some practices, such as introduction of hybrid cultivars, transplants, plastic mulch, drip irrigation and increased plant density in absence of adequate rotation, contribute to the increase in number and severity of soilborne disease (Bruton et al., 2000).

The disease caused by *M. cannonballus* was first reported by Pollack & Uecker (1974). Today is reported in several countries such as India, southern Spain, United States, Saudi Arabia, Central America, Japan, Taiwan and Tunisia (Cohen et al., 2000). This disease was reported on melon for the first time in Brazil in the states of Rio Grande do Norte and Ceará in 2002 (Sales Júnior et al., 2004).

The economic losses caused by this pathogen are estimated at about 10-25% of the crops annually in the United States of America (Martyn & Miller, 1996). In Spain muskmelon production areas decreased in 15 years by more than 40% due mainly to vine decline caused by *M. cannonballus* (García-Jiménez et al., 2000). This fungus is the most aggressive specie involved in the sudden wilt of melons (Bruton, 1998).

Such fungus produce large numbers of distinctive, spherical, thick-walled ascospores, which are produced in perithecia on infected roots, and function as the primary survival structure and *inoculum* for root infection. The ascospores are black, 35 to 50 μm in diameter (Stanghellini et al., 1996; Stanghellini et al., 2000). Waugh et al. (2003) concluded that fields should be considered problematic when the soil presented 2 ascospores g^{-1} of soil, associated with significant crop losses.

Waugh et al. (2003), in a survey about disease dynamics in the field, concluded that *M. cannonballus* is a monocyclic pathogen. The most appropriate strategies used to suppress disease for monocyclic pathogens are those activities that reduce the size of pathogen population (Fry, 1982).

Management *M. cannonballus* caused disease can be made by detection and quantification of the primary *inoculum* (Mertely et al., 1993; Waugh et al., 2003). These ascospores in the soil are extracted through a physical method based

on a sucrose centrifugation technique (Stanghellini & Rasmussen, 1992; Mertely et al., 1993; Beltrán et al., 2005).

However, despite the ascospores of *M. cannonballus* can be extracted from the soil, they can germinate or not in laboratory conditions, making it difficult to evaluate the infectivity of ascospores (Stanghellini et al., 1996). Such infectivity can be influenced by abiotic factors, such as aspect of the ground, temperature, humidity, sources and availability of biotic nutrients (Stanghellini, Kim & Rasmussen, 1996; Pivonia et al., 2002; Waugh et al., 2003; Beltrán et al., 2005).

According to Mertely et al. (1993), the distinct species of the family cucurbitaceae could be distributed according to a scale of susceptibilities in relation to the severity of the symptoms of the collapse, being the species of the cucurbit sort most tolerant to the disease caused for *M. cannonballus*.

Studies carried on in South Korea confirmed the high susceptibilities of cantaloupe melons, watermelon and cucumber to *M. cannonballus* (Heo et al., 2001). Other species of the cucurbitaceae family that are susceptible to *M. cannonballus* are *Cucurbita texana* (Scheele) A. Gray (Martyn et al., 1993) and *B. hispida* (Thunb.) Cogn (Tsay & Tung, 1997). Martyn & Miller (1996), carrying on studies concerning to pathogenicity of *M. cannonballus* in the United States, observed that the pathogen has as cucurbitaceous hosts: *Cucumis melo* L., *C. sativus* L., *Citrullus lanatus* L., *Cucurbita pepo* L., *C. moschata* (Duchesne) Duchesne et Poir., *C. maxima* Duch., *Lagenaria siceraria* (Molina) Standl., and *Luffa aegyptiaca* Mill.

Infantino et al. (2002) showed that vine decline in *Cucumis sativus* L. plants, in Italy, had *M. cannonballus* as one of the main pathogens of this syndrome. In the same way, in Japan, this fungus caused similar symptomatology in *L. siceraria*, used as rootstock against a vascular disease caused by *Fusarium* in watermelon (Uematsu et al., 1992).

The actual damages caused by *M. cannonballus* can also be observed in plants that do not belong to the family cucurbitaceae. Mertely et al. (1993) described *M. cannonballus* in *Zea mays* L., *Sorghum bicolor* L. and *Phaseolus vulgaris* L. This fungus was also found as pathogenic to *Medicago sativa* L. (Pollack & Uecker, 1974), *Triticum aestivum* L. and other species such as *Trifolium pratense* L., *Sesamum indicum* L. (Sivanesan, 1991), *Iris* sp., *Achyranthes aspera* L. in the indian (Hawksworth & Cicarone, 1978), *Lycopersicon esculentum* L., *Gossypium hirsutum* L. and *Brassica oleracea* L.. In other studies, *M. cannonballus* was isolated at low percentages in *Capsicum annuum* L., *Solanum melongena* L., Brassica oleracea var. *italica* and *Brassica oleracea* var. *capitata* (Tsay & Tung, 1997).

The purpose of this study was to quantify the densities of *M. cannonballus* inoculum in soils cultivated with different crops located in the municipalities of Baraúna, Assú and Mossoró, State of Rio Grande do Norte, Brazil.

MATERIAL AND METHODS

Soil samples were collected from ten cultivated areas and from an uncultured one (control) from January to February, of 2006. The crops in cultivated areas were cotton (*Gossypium hirsutum* L.), mango (*Mangifera indica* L.), beans (*Vigna*

unguiculata (L.) Walp.), papaya (*Carica papaya* L.), chili (*Capsicum annum* L.), watermelon (*Citrullus lanatus* (Thumb.) Matsum. et Nakai), acerola (*Malpighia emarginata* DC.), banana (*Musa spp.*), coconut (*Cocos nucifera* L.) and cantaloupe melons (*Cucumis melon* L.).

Each area was represented by five samples, collected in zigzag to the depth of 10-20 cm, where there is the highest concentration of *M. cannonballus* ascospores (Mertely et al., 1993).

Each sample was composed by six repetitions and the *M. cannonballus* ascospores were extracted by the method of flotation in sucrose, according to Sales Júnior et al. (2006), which was adapted from the method of Stanghellini & Rasmussen (1992). Instead of using a 38 µm bolter, the method uses a 30 µm one, in order to capture a higher number of ascospores of *M. cannonballus* in the soil, since the mean diameter of the ascospores is from 35 µm to 50 µm (Martyn & Miller, 1996).

The soil samples were dried in laboratory for later sifting in 2 mm similar meshes to eliminate thicker particles. Afterwards the sieved samples were sifted again in a 250 µm mesh sieve, with 20 g of each sub-sample weighted and mixed to 200 mL of distilled water for 5 minutes, when it was put through 75 µm and 30 µm sieves.

The collected material was washed and centrifuged three times at 2,000 g, approximately 3,000 rotations/minute. The first centrifugation was carried out for four minutes with water only to separate the remaining particles that could reduce the visualization of the ascospores. The two other centrifugations were made from 30-40 mL of 50% sucrose solution to obtain a gradient of density to separate soil and ascospores particles. The last centrifugation was carried out during two minutes and later, the remaining particles were passed through a mesh of 30 µm. The remaining material was washed in water in a Petri dish.

This suspension was diluted in the water at 4 °C and the characteristic ascospores were counted under a stereoscopic 60x microscope. The data were transformed into $\sqrt{(x+1)}$ and submitted to a variance analysis. Means were compared using the Scott-Knott test ($p < 0.05$).

RESULTS AND DISCUSSION

All analyzed soils, including the uncultured soil, contained *M. cannonballus* ascospores, which is in accordance to Stanghellini et al (2004) who have claimed that this fungus is a natural soil inhabitant, mainly in arid and semi-arid regions, as well as in Brazil (Medeiros et al., 2006).

There were significant differences among ascospores average densities. The soil from the area cultivated with cantaloupes had the highest ascospores concentration (8.09 ascospores g⁻¹ of soil), ranging from a maximum of 12.10 to a minimum of 4.55 (Table 1), significantly different from the densities found in the other areas. High indices of *M. cannonballus* ascospores in the soil cultivated with cantaloupes melons were also found in Texas, with as many as 14.4 ascospores g⁻¹ (Mertely et al., 1993).

Table 1. Ascospores population of *Monosporascus cannonballus* in soil samples proceeding from production areas of ten different crops and one uncultivated (Caatinga), located in the cities of Baraúnas, Assú and Mossoró, Rio Grande do Norte, Brazil. 2006

Tabela 1. População de ascósporos de *Monosporascus cannonballus* em amostras de solo provenientes de 10 áreas com diferentes culturas e uma sem cultivo (Caatinga), localizadas nos municípios de Baraúnas, Assú e Mossoró, Rio grande do Norte, Brasil. 2006

Sample	Culture	Place of Collection	Population (ascospores g ⁻¹ of soil)*		
			Means	Maximum	Minimum
LDC-01	Uncultured	Mossoró-RN	0,30 a	0,60	0,10
LDC-02	Watermelon	Mossoró-RN	2,60 b	3,30	2,20
LDC-03	Cantaloups	Mossoró-RN	8,09 c	12,10	4,55
LDC-04	Papaya	Baraúnas-RN	0,58 a	0,80	0,35
LDC-05	Chili	Baraúnas-RN	0,73 a	0,95	0,60
LDC-06	Beans	Baraúnas-RN	1,28 a	1,70	1,05
LDC-07	Mango	Baraúnas-RN	2,00 b	3,50	1,25
LDC-08	Cotton	Baraúnas-RN	0,99 a	1,35	0,65
LDC-09	Banana	Assú-RN	0,64 a	0,85	0,40
LDC-10	Coconut	Assú-RN	1,19 a	2,15	0,50
LDC-11	Acerola	Assú-RN	2,10 b	2,50	1,35
CV (%)=	11,56				

Original data were transformed into $\sqrt{(x+1)}$. Means followed by the same letter in the columns do not differ statistically (Scott-Knott's test; $p > 0.05$).

In Korea, Heo et al. (2001), studying the distribution of ascospores in naturally infested fields, observed that the population density of *M. cannonballus* ascospores in cantaloupes melons varied from 0.115 to 0.73 g⁻¹ soil. These authors also found that the ascospore densities in eastern soils cultivated with cantaloupes ranged from 0.24 to 0.50 g⁻¹ soil.

The relationships between ascospores density and illness severity had not yet been evaluated in Brazil. Waugh et al. (2003) found collapse associated with *M. cannonballus* in cantaloupes plantations, where the ground contained a little more than 2 ascospores g⁻¹ soil. Assuming this threshold level, 36.3% of the samples would be at risk for collapse, since the soils cultivated with mango, watermelon and acerola, which did not show statistical differences among them, presented, respectively, 2.00; 2.60 and 2.10 ascospores g⁻¹ soil. Furthermore, the soils cultivated with cantaloupe melons presented a significantly higher density. The occurrence of *M. cannonballus* in roots of mango and acerola has not been reported, but watermelon is considered to be host to *M. cannonballus* (Sales Júnior et al., 2002)

With respect to ascospores density in soil cultivated with watermelon in two areas of Ichon, it was found an average of 0.26 ascospores g⁻¹ soil, while in nine areas of Kwangju, in Korea, the average rate was 0.27 ascospores g⁻¹ soil (Heo et al., 2001). The cultivated soil with watermelon in Brazil presented higher indices than those found in Korea (Table 1), which can be probably explained by the crop and soil local conditions. With an intensive use, without crop rotation, and with practices such as plastic cover (mulch), drip irrigation,

increase of plant density, that are not always the most indicated techniques to bring a high productivity (Bruton, 1998).

The consequence of this is an inadequate handling that, many times, makes the environment favorable for the development of some phytopathogens, as is the case of *M. cannonballus*. This fungus is thermophilic and develops well with the plastic use that increases the temperature of the microenvironment.

Low levels of *M. cannonballus* ascospores were found in the soils cultivated with beans, chili, papaya, cotton, banana and coconut, with lower population levels for the risk limit (2 ascospores g⁻¹ soil), with no differences to the average level found in the uncultivated environment (control). These data corroborate with Stanghellini et al. (1996), who also observed ascospores in native desert areas, with average densities ranging from 1.11 to 1.72 ascospores g⁻¹ soil, indicating that this fungus is a natural inhabitant of soil.

CONCLUSIONS

The present data show that in the entire analyzed soil samples some level of *M. cannonballus* population was found.

The absence of differences between the ascospores density of the uncultivated soil and those cultivated with plants that are not hostesses of the pathogen, or that are described as hostesses but do not quite frequently, demonstrates that these fungi are natural inhabitants of the soil.

Additional studies are necessary to investigate the factors involving the germination of the ascospores that tend to be the main cause of vine decline in cantaloupes and watermelon in the State of Rio Grande do Norte.

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