WEEDS AS POTENTIAL HOSTS FOR FUNGAL ROOT PATHOGENS OF WATERMELON[1]

RUI SALES JÚNIOR[2]*, ANA PAULA MEDEIROS DOS SANTOS RODRIGUES[2], ANDREIA MITSA PAIVA NEGREIROS[2], MÁRCIA MICHELLE DE QUEIROZ AMBRÓSIO[2], HAILTON DA SILVA BARBOZA[2], ROBERTO BELTRÁN[3]

ABSTRACT - Many watermelon (*Citrullus lanatus*) diseases are caused by soilborne pathogens in Brazil and worldwide. The goal of this study was to identify and quantify the frequency of phytopathogenic fungi associated with watermelon root rot and vine decline that were also present in the roots of weeds in the major watermelon production regions in the state of Rio Grande do Norte, Brazil. We collected root samples from 10 of the most prevalent weed species in 16 watermelon producing areas. The plants were identified and their frequencies in the fields were calculated. The fungi found in the weed roots were isolated and the main genera associated with watermelon vine decline were identified. We identified 13 weed species belonging to nine botanical families. The weed species with the highest frequencies found in the field were *Amaranthus spinosus* (25.0%), *Trianthema portulacastrum* (18.8%), *Commelina* sp. (18.8%), and *Boerhavia diffusa* (12.5%). The fungi *Macrophomina*, *Rhizoctonia,* and *Monosporascus* were isolated from the roots of the weed plants. While *Macrophomina* was isolated from 12 different types of plants, *Rhizoctonia* and *Monosporascus* were isolated from four and two different plant species, respectively.

Keywords: *Citrullus lanatus*. Isolation. *Macrophomina*. *Rhizoctonia*. *Monosporascus*.

PLANTAS DANINHAS POTENCIAIS HOSPEDEIRAS DE PATÓGENOS RADICULARES EM MELANCIA

RESUMO – Considerável número de doenças que acometem a melancia (*Citrullus lanatus*) são causadas por patógenos habitantes do solo no Brasil e no mundo. O objetivo do presente trabalho foi identificar e quantificar a frequência de fungos fitopatógenos associados a "podridão de raízes e declínio de ramas" em melancia, presentes nas raízes de plantas daninhas nas principais áreas produtoras no estado do Rio Grande do Norte. Foram coletadas 10 amostras de raízes de espécies de plantas daninhas de maior prevalência em 16 áreas produtoras de melancia. As plantas foram identificadas e, calculada a respectiva frequência em campo. Foi realizado o isolamento fúngico das raízes, sendo posteriormente identificados os principais fungos associados ao declínio de ramas. Foram identificadas 13 espécies de plantas daninhas, pertencentes a nove famílias botânicas. As espécies de plantas daninhas que apresentaram as maiores frequências em campo foram *Amaranthus spinosus* (25,0%), *Trianthema portulacastrum* (18,8%), *Commelina* sp. (18,8%) e *Boerhavia diffusa* (12,5%). Das raízes das plantas daninhas que apresentaram maior frequência foram isolados os gêneros *Macrophomina*, *Rhizoctonia* e *Monosporascus*. O primeiro foi isolado de 12 espécies de plantas, o segundo de quatro espécies e o terceiro de duas espécies.

Palavras-chave: *Citrullus lanatus*. Isolamento. *Macrophomina*. *Rhizoctonia*. *Monosporascus*.

INTRODUCTION

Watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) is the most produced cucurbit in the world (117 million tons), with Brazil being the fifth largest producer (2,090 million tons) (FAO, 2018).

In Brazil, the northeastern region presents considerable potential for watermelon production due to its adequate edaphoclimatic conditions such as high luminosity, low rainfall, and a dry climate. Currently, the region is responsible for 26.1% of the national production, with Bahia (237.5 tons), Rio Grande do Norte (135.3 tons), and Pernambuco (50.1 tons) as the main producing states (IBGE, 2018).

Considerable number of diseases can affect this crop due to the regional climatic conditions and the period of production, drastically limiting cultivation. Among them, those related to root pathogens have gained increasing importance in recent years (SALES JÚNIOR et al., 2010; 2012), with the emphasis on "root rot and vine decline - RRVD" (MARTIN; MILLER, 1996; BOUGHALLEB et al., 2010; SALES JÚNIOR et al., 2010). Such diseases are treated as complex syndromes and may be caused by a single pathogen or by a simultaneous interaction of pathogens (MARTIN; MILLER, 1996).

Andrade et al. (2005), in a prospective study of pathogens that occur in melon crops (*Cucumis melo* L.) in the states of Rio Grande do Norte (RN) and Ceará (CE), have identified fungi, associated with cucurbit roots, that can cause RRVD, highlighting *Monosporascus cannonballus* Pollack & Uecker, *Macrophomina phaseolina* (Tassi) Goid., *Fusarium solani* (Mart.) Sacc., and *Rhizoctonia solani* Kühn.

Watermelon and melon plants in general, belonging to the Cucurbitaceae family, grown in the same production areas present similar problems caused by root pathogens (SALES JÚNIOR et al., 2010; 2012).

In addition to root pathogens, weeds can also directly interfere with agricultural production as they directly compete with the main crops for water, light, and nutrients; in addition, they often release allelopathic substances that may negatively impact the development of plants (SOARES et al., 2010) and serve as alternative hosts of phytopathogens (SALES JÚNIOR et al., 2012).

Information on the importance of weeds as alternative hosts or multipliers of phytopathogen inoculum in watermelon is scarce. In this sense, it is important to note that during the first semester of each year (rainy season in Rio Grande do Norte), the remaining areas of watermelon cultivation from the second semester of the previous year show abundant growth of weeds, which benefit from the soil nutrients remaining from the previous watermelon crop. Therefore, weeds, if they function as hosts of phytopathogenic fungi, may contribute to their persistence in the production areas and, consequently, to the infection of the following crop.

Sales Júnior et al. (2012) have evaluated the occurrence of weeds as alternative hosts of the main fungi causing RRVD in melon in northeastern Brazil. The same authors have identified the fungus *M. phaseolina* in 100% of the prospected areas, isolated from 13 weed species belonging to 10 different botanical families.

Considering the importance of watermelon cultivation in Brazil, the present research has the objective of identifying and quantifying the frequency of phytopathogenic fungi associated to RRVD present in the weed roots in the main areas of watermelon production in Rio Grande do Norte.

MATERIAL AND METHODS

Characterization of the experimental areas

The study was conducted in 16 watermelon producing areas in the cities of Assú/RN (As) (5º32'20" S and 36º54'44" W), Baraúna/RN (Ba) (5º6'49" S and 37º37'29" W), and Mossoró/RN (Mo) (4º55'10" S and 37º23'57" W) (Table 1)

Weed collection and identification

In each production area, samples of the ten most prevalent weed samples, as proposed by Sales Júnior et al. (2012), were collected, including the root systems).

All roots were rinsed under running water to eliminate debris and observed under a 40x-binocular optical microscope (Nikon E200) for visualization of symptoms and/or fungal structures of root pathogens.

The botanical species were identified using a botanical key (MOREIRA; BRAGANÇA, 2011). The frequency (FE) of each species (%) in the field was calculated as FE = (number of samples containing the species/total number of samples obtained) x 100 (BRANDÃO et al., 1998).

Table 1. Description of sampling areas and genera of isolated fungi from weed roots.

Area	City/RN state	Geographical coordinates	Genus of isolated fungi[1]		
			Ma	*Rh*	*Mo*
A1	Assú	5°24'31" S - 36°50'46" W	X	X	
A2	Assú	5°31'14" S - 36°54'32" W			
A3	Assú	5°32'30" S - 37°10'23" W	X		
A4	Assú	5°31'19" S - 36°54'20" W	X		
A5	Assú	5°31'24" S - 36°54'7,9" W		X	
A6	Assú	5°32'20" S - 36°54'44" W			
A7	Assú	5°32'22" S - 36°54'46" W	X		
A8	Assú	5°32'49" S - 36°54'21" W	X		
A9	Assú	5°31'14" S - 36°54'32" W	X		
A10	Assú	5°32'47" S - 36°54'25" W	X		X
A11	Mossoró	4°54'34" S - 37°24'3,9" W			
A12	Mossoró	4°55'11" S - 37°23'56" W	X		
A13	Mossoró	4°55'6" S - 37°23'51" W	X		
A14	Baraúna	5°6'49" S - 37°37'29" W	X	X	X
A15	Baraúna	5°6'50" S - 37°37'27" W	X		
A16	Baraúna	5°6'50" S - 37°37'25" W	X	X	

[1]*Ma = Macrophomina, Rh = Rhizoctonia, Mo = Monosporascus.*

Fungi isolation

After botanical identification, the collected plants were sent to the laboratory. The aerial plant parts were removed and the root system was rinsed under running water to remove any soil residues. Subsequently, the roots were immersed in 2%-sodium hypochlorite solution (NaClO) for 1 minute and then washed in sterilized water to remove excess chlorine.

Seven root fragments were deposited in Petri dishes containing potato-dextrose-agar medium (BDA), supplemented with streptomycin. For each sample of weed species, five plates were prepared, totaling 35 isolation points per plant. The plates were incubated in an oven-type B.O.D. at 27 ± 1°C and a 12 h photoperiod for 5-7 days. After this period, the points that presented fungal growth were placed onto Petri dishes containing BDA medium to obtain pure cultures, which were later identified and preserved.

Fungi identification

Fungal isolates were identified to the genus level, with emphasis on those that are known as causal agents of watermelon root diseases. For this purpose, slides containing fungal structures, colored with blue-cotton lactophenol, were prepared and compared with the descriptions of the keys for fungi identification (BARNETT; HUNTER, 1998; SEIFERT et al., 2011).

RESULTS AND DISCUSSION

Thirteen weed species belonging to 10 botanical families hosted fungi of the genera *Macrophomina*, *Rhizoctonia*, and *Monosporascus* (Table 1).

The isolates of the genus *Macrophomina* were recorded in 75% (12) of the prospected areas (Table 1), being obtained from roots of 12 weed species distributed in 10 botanical families: *Amaranthus spinosus* L., *A. viridis* L (Amaranthaceae); *Trianthema portulacastrum* L. (Aizoaceae); *Calotropis procera* (Ait.) Ait. (Asclepiadaceae); *Commelina* sp. (Commelinaceae); *Ipomea bahiensis* Willd. Ex Rolan & Schult (Convolvulaceae); *Mimosa modesta* Mart. var. *ursinoides* (Harms) Barneby, *Senna obtusifolia* (L.) Irwin & Barneby (Fabaceae); *Waltheria americana* L. (Malvaceae); *Boerhavia diffusa* L. (Nyctaginaceae); *Portulaca oleracea* L. (Portulacaceae) and *Kallstroemia tribuloides* (Mart) Steud. (Zygophyllaceae) (Table 2).

Rhizoctonia isolates were obtained from roots of four weed species from four botanical families, namely *A. spinosus*, *T. portulacastrum*, *Commelina* sp., and *Herissantia crispa* (L.) Brizicky (Malvaceae) in 25.0% (4) of the prospected areas. At a lower isolation frequency, *Monosporascus* was detected in only two prospective areas (12.5%) and isolated from the two weed species *T. portulacastrum* and *B. diffusa*, belonging to different botanical families (Tables 1, 2).

Table 2. Weed families, species, and frequencies in watermelon production areas of Rio Grande do Norte and associated genera of fungi isolated from the roots of these plants.

Family	Species	Frequency(%)	Fungal genera[1] (area)[2]
Amaranthaceae	*Amaranthus spinosus*	25.0	*Ma* [1, 4, 9, 16]. *Rh* [1, 16]
Amaranthaceae	*Amaranthus viridis*	12.5	*Ma* [7, 13]
Aizoaceae	*Trianthema portulacastrum*	18.8	*Ma* [14]. *Rh* [14]. *Mo* [14]
Asclepiadaceae	*Calotropis procera*	6.5	*Ma* [12]
Commelinaceae	*Commelina* sp.	18.8	*Ma* [15]. *Rh* [14]
Convolvulaceae	*Ipomea bahiensis*	6.25	*Ma* [3]
Fabaceae	*Mimosa modesta* var. *ursinoides*	6.3	*Ma* [10]
Fabaceae	*Senna obtusifolia*	12.5	*Ma* [8, 9]
Malvaceae	*Herissantia crispa*	6.3	*Rh* [5]
Malvaceae	*Waltheria americana*	12.5	*Ma* [3]
Nyctaginaceae	*Boerhavia diffusa*	12.5	*Ma* [10, 12], *Mo* [10]
Portulacaceae	*Portulaca Oleracea*	12.5	*Ma* [8, 13]
Zygophyllaceae	*Kallstroemia tribuloides*	6.3	*Ma* [9]

[1] *Ma = Macrophomina, Rh = Rhizoctonia, Mo = Monosporascus*. [2] Production areas: Assú (1 - 10), Mossoró (11 - 13), Baraúna (12 - 16).

Among the 12 weed species found in association with *Macrophomina*, three have been reported by Sales Júnior et al. (2012) in areas of melon cultivation in the states of RN and CE, namely *A. viridis*, *S. obtusifolia*, and *P. oleracea*. All other species mentioned above are potential new hosts of this phytopathogen (Table 2).

Up to now, two species of the soilborne fungal genus *Macrophomina* have been described, namely *M. phaseolina* and *M. pseudophaseolina* Crous, Sarr & Ndiaye (SARR et al., 2014), of which *M. phaseolina* has a wide range of hosts and is pathogenic to more than 680 botanical species, not including the weed species reported in this study (FARR; ROOSMAN, 2018). In this paper, we refer to the results of studies on the effects of *M. phaseolina* on melon and watermelon in Brazil (ANDRADE et al., 2005) and in several regions of the world, namely Israel (COHEN et al., 2012a), Iran (SALARI et al., 2012), Egypt (EL-KOLALY; ABDEL-SATTAR, 2013), and Chile (JACOB et al., 2013). Due to its genetic variability, as well as its wide host range, *M. phaseolina* is difficult to control.

Studies have shown that *M. phaseolina* can remain in the soil or plant material in the form of sclerotia (resistance structures) for up to 15 years, making it extremely difficult to control this species (LOTFALINEZHAD et al., 2013). Recently, Ambrósio et al. (2015) have tested 97 accessions of melon from different geographic origins and compared them to three isolates of *M. phaseolina*. The results indicated that sources of resistance to *M. phaseolina* are quite limited. The recently reported *M. pseudophaseolina* species is still poorly studied, but it causes charcoal rot in *Abelmoschus esculentus* (L.) Moench, *Arachis hypogaea* L., *Hibiscus sabdarifa* L., and *Vigna unguiculata* (L) Walp in the Senegal (SARR et al., 2014; NDIAYE et al., 2015). Pathogenicity studies comparing the two species of *Macrophomina* have been performed by Ndiaye et al. (2015), who showed that both species had a similar pathogenicity to cowpea (*V. unguiculata*).

Sales Júnior et al. (2012) have identified *R. solani* in roots of *S. obtusifolia* and *H. crispa* in previous studies of weeds in melon fields in RN and CE. In the case of *H. crispa*, the results are in agreement with the data obtained in the present study. *Rhizoctonia solani* is a soilborne fungus with a high saprophytic competition capacity and a wide host range (FARR; ROSSMAN, 2018) and is frequently found parasitizing melon roots, resulting in vine decline (AEGERTER; GORDON; DAVIS, 2000). Some studies highlight the role of *R. solani* as an important pathogen in melon and even as a fungus involved in RRVD (ANDRADE et al., 2005). Messiaen et al. (1994) only considered *R. solani* as an important pathogen in Cucurbitaceae in areas with excess humidity, when the branches or fruits are in contact with the soil; infestations were reduced when the humidity of the soil surface decreased.

Considered as one of the main agents involved in RRVD in Cucurbitaceae, *M. cannonballus* stands out as one of the most aggressive pathogens in melon and watermelon. It is an ascomycete, a natural inhabitant of the soil, and presents thermophilic characteristics, making it an important species in semi-arid regions of Brazil (SALES JÚNIOR et al., 2010; 2012; 2018).

It should be noted that other hosts are also part of the wide range of species subjected by *M. cannonballus* as a root pathogen. In previous reports, cucumber (*Cucumis sativus* L.), zucchini (*Cucurbita pepo* L.), squash (*Cucurbita moschata* (Duchesne) and Duchesne et Poir), gourd (*Cucurbita maxima* Duch.), and luffa (*Luffa aegyptiaca* Mill) (MERTELY et al., 1993; COHEN et al., 2012b) have also been mentioned, as well as sorghum (*Sorghum bicolor* L.), alfalfa (*Medicarpo sativa* L.), beans (*Phaseolus vulgaris* L.), corn (*Zea mays* L.), and cotton (*Gossypium hirsutum* L.) (MERTELY et al., 1993).

It is important to note that in Rio Grande do

Norte, many of the species reported as hosts of *M. cannonballus* are cultivated in the same areas where melon and watermelon are planted. This approach possibly favors the maintenance of the fungus in the field, thus increasing its inoculum potential, especially during the off-season (SALES JÚNIOR et al., 2012; 2018).

Spontaneous vegetation (weeds) is characterized by a high rusticity, growth in inhospitable places, aggressive habits, efficient seed dispersion by wind, high reproductive capacity, resistance to chemical control, large populations, and the occupation of large areas. In this way, these plants become potential sources of phytopathogen inoculants in commercial crops, playing a fundamental role in the epidemiology of diseases as secondary hosts (CHAVES et al., 2003).

The species that presented the highest values of field frequency, namely *A. spinosus* (25.0%), *T. portulacastrum* (18.8%), *Commelina* sp. (18.8%), and *B. diffusa* (12.5%) (Table 2), were also associated with more than one pathogen. Thus, the weeds most commonly found in the growing areas require the special attention of producers, since they may directly contribute to the maintenance of these root pathogens in areas of watermelon production.

Among the species from which *Macrophomina* (Table 2) were isolated, *S. obtusifolia* and *M. modesta* are worth mentioning since they belong to the same family as cowpea (*V. unguiculata*), a species cultivated in the northeastern regions. The gray stem rot caused by *Macrophomina* results in serious losses in cowpea production in Brazil (GOMES et al., 2008). Therefore, crop rotation between these two species (cowpea and watermelon), which is commonly performed in the northeastern regions, may result in an increase in the inoculum of the pathogen in the soil, with consequent damages to the watermelon crop.

In a previous study on pathogens associated with melon roots with symptoms of branch decline, Andrade et al. (2005) have detected the presence of *R. solani* in up to 40% of plants and a reduced frequency of isolation of up to 18% when compared to *M. phaseolina*, which presented a prevalence of 100% and a frequency of isolation of up to 60%. The species *M. phaseolina* is therefore of greater importance than *R. solani* in terms of pathogens that cause root diseases in melon.

Nascimento et al. (2011), surveying weeds in different systems of corn cultivation in the region of Mossoró-RN, verified that *T. portulacastrum* was common in 100% of the conventional pasture areas, indicating that the region is highly propitious for the propagation of this plant; the species is common in crops of several cultivated species (personal observation). Our report is therefore the first report of the species *T. portulacastrum* and *B. diffusa* as hosts of *Monosporascus* (Table 2).

With the importance of *M. cannonballus* as the causal agent of "monosporascus root rot and vine decline – MRRVD" and the lack of an effective control, the weeds commonly found in watermelon and melon cultivation areas in northeastern Brazil are potential hosts of the aforementioned pathogen. This issue is of high importance given that such plants may serve as sources of inoculum, contributing to the maintenance of the pathogen in the off-season.

CONCLUSIONS

Weeds present in watermelon production areas and identified as fungal hosts associated with root rot and branch decline contribute to the maintenance of these soil pathogens.

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