



Fitness components of *Monosporascus cannonballus* isolates from northeastern Brazilian melon fields

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ABSTRACT

Monosporascus root rot and vine decline caused by *Monosporascus cannonballus* is one of the most important melon yield-limiting diseases in northeastern Brazil. This study investigated the fitness components of 57 isolates of *M. cannonballus* obtained from Brazilian melon fields by evaluating: i) their mycelial growth rate (MGR), and perithecia and ascospore production (PP and AP) on potato dextrose agar (PDA); ii) their sensitivity to the fungicide fluazinam; and iii) their virulence to melon seedlings. All *M. cannonballus* isolates showed variability in their MGR, PP and AP values. They were sensitive to the fungicide fluazinam, showing some degree of mycelial growth inhibition (MGI), and were pathogenic to melon seedlings, with a mean disease severity index (DSI) of 62.1%. By univariate analysis, the formation of groups of similarity amongst the isolates of *M. cannonballus* within each variable was not limited by the area of origin of each isolate, given that in most situations, different isolates of the same area were distributed into distinct groups of similarity. A multivariate cluster analysis allowed the separation of the 57 *M. cannonballus* isolates in 18 groups of similarity. The fitness variability among *M. cannonballus* isolates found in this study should be considered when possible sources of resistance are evaluated in melon breeding programs.

Key words: *Cucumis melo*, ascospore production, *Monosporascus* root rot, mycelial growth, perithecial virulence, sensitivity to fungicide.

INTRODUCTION

The northeastern region of Brazil accounts for 95% of melon (*Cucumis melo* L.) production in this country, covering approximately 16,300 ha. The main producing areas are located in the states of Rio Grande do Norte with 7,943 ha and a production of 242,303 t, and Ceará with 5,431 ha and a production of 153,161 t (AGRIANUAL, 2013). In recent years, the expansion of the cultivated area, coupled with intensive and continuous cultivation of melon without crop rotation, has contributed to the increased incidence of fungal diseases affecting the root system. *Monosporascus* root rot and vine decline (MRRVD) caused by the soilborne ascomycete *Monosporascus cannonballus* Pollack & Uecker is one of the most important melon yield-limiting diseases in the Brazilian northeast (Silva et al., 2010; Bezerra et al., 2013), as in other melon-production regions located in hot semi-arid to arid regions, as well as subtropical environments worldwide (Martyn & Miller, 1996; Cohen et al., 2000; Cohen et al., 2012; Ben Salem et al., 2013).

Monosporascus cannonballus was first detected in Brazil in 2002, affecting melon plants cultivated in the agricultural areas of Ceará and Rio Grande do Norte states

(Sales Jr. et al., 2004). Symptoms of the disease include the yellowing and death of the crown leaves, which gradually radiates out and kills the vine as the fruit approach maturity. *M. cannonballus* produces ascospores in perithecia formed on diseased roots at the end of the growing season (Martyn & Miller, 1996; Cohen et al., 2012). Ascospores function as the primary survival structure, as well as the primary inoculum of the fungus for root infection (Stanghellini et al., 1996; 2000; 2010).

No melon cultivar resistant to *M. cannonballus* is commercially available (Fita et al., 2009; Cohen et al., 2012) and chemical control of this pathogen in many circumstances has been unsatisfactory and/or uneconomical (Cohen et al., 2000; Fita et al., 2007; Cohen et al., 2012). Currently, in Brazil there are no fungicides registered for the control of MRRVD (AGROFIT, 2013).

Knowledge of the variability in the pathogen population and the levels of host resistance are essential factors for developing strategies for the management of root diseases of melon. The success of breeding programs needs extensive knowledge about the pathogen variability. Consequently, this aspect should be investigated before selecting sources of resistance in the host (Bruton, 1998).

Various phenotypic and genotypic markers can be used to study the variation within and among plant pathogen populations. Nowadays, molecular markers are widely used to investigate the genetic diversity in plant pathogen populations (Linde, 2010). However, the pathogen populations may consist of genotypes with different fitness, defined as the ability of a pathogen to survive and reproduce (Antonovics & Alexander, 1989; Dyakov et al., 2007). Fitness is a relative concept, where individuals are ranked according to their contribution to the population in the subsequent generation. Therefore, individuals with greater fitness leave more descendants in the subsequent generation (Lannou, 2012).

The competitive ability and relative fitness of a pathogen strain are determined by its intrinsic biological properties, the resistance and heterogeneity of the corresponding host population, the population density and genetic relatedness of the competing strains, and the physical environment. Competitive ability can be inferred indirectly from fitness components (Zhan & McDonald, 2013). Because fitness is relative, it must be estimated by measuring characters that constrain some adaptive advantage among individuals. Phenotypic markers, like mycelial growth rate, reproductive potential, sensitivity to fungicides and virulence, are useful for assessment of fitness variability in plant pathogen populations (Antonovics & Alexander 1989; Allen et al., 1999; Brown, 2006; Lannou, 2012).

Despite the importance of MRRVD worldwide, fitness components of *M. cannonballus* populations have not been properly studied. Thus, the objective of this study was to investigate the fitness components of 57 *M. cannonballus* isolates collected in melon growing areas of northeastern Brazil by evaluating: i) their mycelial growth rate, and perithecia and ascospore production at three temperatures, ii) their sensitivity to the fungicide fluazinam, and iii) their virulence to melon seedlings.

MATERIALS AND METHODS

Fungal isolates

Fifty-seven *M. cannonballus* isolates were obtained in 2009 from roots of melon plants exhibiting symptoms

of MRRVD in seven growing areas of the Brazilian northeastern region, located in the agricultural centers of Icapuí and Quixeré (Ceará state), and Mossoró (Rio Grande do Norte state) (Table 1). All isolates were hyphal-tipped and stored at 25°C in darkness in plastic vials containing sterilized peat (Fertalon Fertilizantes Londrina Ltda.). The isolates were identified morphologically as *M. cannonballus* after growing them on potato dextrose agar (PDA) (Acumedia Co.) and V8-juice agar (100 ml of V8 juice, 1 g of CaCO₃, 18 g of agar, and 900 ml of sterile distilled water) for two months at 28°C in darkness (Sivanesan, 1991). Additionally, the internal transcribed spacer (ITS nrDNA) region, fragments of the translation elongation factor 1-alpha (EF-1 α) and the β -tubulin (β -tub) genes were used to confirm the identification of three representative isolates through BLAST searches (<http://www.ncbi.nlm.nih.gov/BLAST/>). The representative sequences of the studied ITS region of isolates CMM-2401, CMM-2429 and CMM-2365 were deposited in GenBank (JQ762362, JQ762366 and JQ762367, respectively). These sequences have 99.00% similarity with the sequence of *M. cannonballus* (AM167936). The isolates were deposited in the Culture Collection of Phytopathogenic Fungi “Prof. Maria Menezes” (CMM) of the Universidade Federal Rural de Pernambuco (Recife, Brazil).

All experiments were conducted twice. Prior to use, a small portion of the colonized peat from each plastic vial was transferred to potato dextrose agar PDA plates and allowed to grow at 25°C in darkness.

Mycelial growth, and perithecia and ascospore production at different temperatures

The mycelial growth, and perithecia and ascospore production of *M. cannonballus* isolates were determined using cultures grown on PDA. Mycelial plugs (6 mm in diameter) obtained from the growing edge of the colonies were transferred to the center of PDA plates, which were then incubated in darkness at temperatures of 22, 28 and 38°C. There were four replicates for each isolate and temperature combination. The diameter of each colony was measured twice perpendicularly when it reached at least two thirds

TABLE 1 - Geographical origin of *Monosporascus cannonballus* isolates used in this study, obtained from melon plants in northeastern Brazil.

Codes (CMM) ¹	State	Agricultural area	Growing site
2362, 2363, 2365, 2369, 2370, 2371, 2372	Ceará	Icapuí	I
2401, 2402, 2403, 2405, 2430, 2431, 2466	Ceará	Quixeré	II
2376, 2379, 2380, 2381, 2382, 2427, 2429	Rio Grande do Norte	Mossoró	III
2386, 2387, 2389, 2390, 2391, 2394, 2395, 2396, 2397	Rio Grande do Norte	Mossoró	IV
2434, 2435, 2436, 2437, 2438, 2439, 2441, 2443, 2444	Rio Grande do Norte	Mossoró	V
2408, 2411, 2412, 2414, 2418, 2421, 2422, 2446	Rio Grande do Norte	Mossoró	VI
2448, 2449, 2450, 2451, 2452, 2453, 2454, 2455, 2457, 2467	Rio Grande do Norte	Mossoró	VII

¹Culture Collection of Phytopathogenic Fungi “Prof. Maria Menezes” (CMM) of the Universidade Federal Rural de Pernambuco (Recife, PE, Brazil).

of the plate and used to calculate the mycelial growth rate (MGR) as mm per day. Colonies were further incubated until 45 days for perithecia formation and quantification as described by Cluck et al. (2009). Four plugs (12 mm diameter) were randomly selected and removed from each colony, and each plug was considered a replicate (16 plugs per isolate). Each plug was placed between two clear glass slides and flattened in order to observe and count the perithecia under a low power stereoscope. Each plug consisted of a volume of 0.395 cm³ and the number of perithecia produced (PP) was expressed as perithecia per cm³. Colonies were further incubated until 50 days, when perithecia were removed and transferred to clear glass slides for ascospore quantification. Ten perithecia were randomly selected and removed from each colony and each of them was considered a replicate (40 perithecia per isolate). Each perithecia was placed between two clear glass slides and flattened in order to observe and count the number of ascospores produced (AP). Ascospores were observed under a microscope at 40× magnification.

Sensitivity to fungicide fluazinam

The sensitivity of *M. cannonballus* isolates to the fungicide fluazinam was determined *in vitro*. Sterile aqueous solution of fluazinam was mixed with PDA to give concentration of 10 µg a.i./L. The selection of this fungicide and the discriminatory concentration was based on preliminary tests (unpublished data), which evaluated the inhibitory effect of five doses of the five active ingredients on seven *M. cannonballus* isolates, as well as in other studies which evaluated the *in vitro* inhibitory effect of different fungicides against this fungi (Cohen et al., 1999; Pivonia et al., 2010). A mycelial plug (6 mm diameter) from a 15-day old *M. cannonballus* culture was placed in the center of the Petri dish containing the test fungicide. The cultures were incubated at 28°C in darkness for four days. The colony diameter was then measured and mycelial growth inhibition (MGI) calculated relative to the untreated control. There were four replicates for each *M. cannonballus* isolate.

Virulence test

The virulence of the *M. cannonballus* isolates was determined using a method described by Stanghellini et al. (2000) with some modifications. Soil (fine sandy loam, pH 6.5) was passed through a 2-mm mesh screen, and allowed to air-dry for 72 h. This soil was autoclaved for 30 min, allowed to air dry for 24 h, and subsequently autoclaved for 30 min. Samples (40 g) of autoclaved soil were dispensed into polypropylene tubes (11.5 cm long and 3.0 cm diameter) and then infested artificially with 2 ml of ascospore suspensions (1.0×10^4 ascospores/mL) that were obtained from 45-day-old V8-juice agar cultures of the *M. cannonballus* isolates. The control consisted of soil tubes with sterile distilled water without the presence of ascospores. A two-day-old melon (yellow type, cv. Mandacaru F1) seedling (pre-germinated on water agar) was transplanted into each tube. Seeded tubes were placed

in test-tube racks, transferred to growth chambers (12-h photoperiod), and incubated at 28°C and 65% relative humidity for 21 days. Subsequent to transplant, all tubes were irrigated with sterile distilled water (10 mL) at three-day intervals. There were four replicates for each isolate, each consisting of eight seeded tubes (32 seeded tubes per isolate).

At the end of the experiment, the seedlings were removed from the tubes, and the root system of each seedling was gently washed in tap water and rated for disease severity based on the following scale adapted from Bruton et al. (2000): 0 = no discoloration or reduction of root mass; 1 = slight discoloration with up to 30% root mass reduction; 2 = moderate discoloration with up to 50% root mass reduction; 3 = severe discoloration with up to 50% root mass reduction; and 4 = root mass reduction greater than 90%. The quantification of root mass reduction was based on the root mass presented by control plants. Small pieces of representative root tissues of each seedling were surface sterilized for 1 min in 1.5% NaOCl, washed twice with sterile distilled water and plated onto PDA amended with 0.5 g/L of streptomycin sulfate. The pathogen was re-isolated from all inoculated seedlings, while no fungus was isolated from controls. The severity data were processed by McKinney's formula (McKinney, 1923), which generates a numeric disease severity index (DSI) for each repetition: $DSI = (\sum vn)/(NV) \times 100$, where v represents the numeric value of the class, n is the number of plants assigned to the class, N is the total number of the plants in the replication and V is the numeric value of the highest class.

Data analyses

One-way analyses of variance (ANOVA) were conducted with data obtained from experiments on mycelial growth, and perithecia and ascospore production at different temperatures, as well as those obtained in sensitivity to fluazinam and virulence experiments to analyze potential trial-treatment interactions. In all cases, ANOVA analyses indicated that the data between the two types of experiments were similar ($P > 0.05$), so data of all variables from both experiments were combined.

Firstly, a univariate analysis was performed to identify groups of isolates. Data on disease severity (DSI), mycelial growth rate (MGR) and mycelial growth inhibition (MGI) were transformed into $(x+0.5)^{1/2}$, while data on perithecia production (PP) and ascospore production (AP) were transformed into $\log(x+1)$ and subjected to ANOVA, and the means were compared by the Scott-Knott grouping test ($P=0.05$). Then, the correlations between the variables of fitness components (MGR, PP, AP, MGI and DSI) were evaluated by the analysis of Pearson correlation ($P=0.01$). The univariate and correlation analyses were performed using SAEG 9.1 software (Universidade Federal de Viçosa). Finally, using all variables, a principal component analysis was performed and followed by nested ANOVA with the values of scores (Bratchell, 1989). The nested model was

fitted to the data: $Y_{ijkl} = \mu + \alpha_i + \beta_{j(i)} + \gamma_{k(ij)} + \epsilon_{l(ijk)}$, where: Y_{ijkl} = dependent variable; μ = mean, α_i = effect of the production area, $\beta_{j(i)}$ = effect of site nested within area, $\gamma_{k(ij)}$ = effect of isolate nested within the site within the area, and $\epsilon_{l(ijk)}$ = experimental error. The analysis was performed with the R package (R Development Core Team, 2012). Expectations of mean square were manually estimated in order to define the proper error terms to test each component of the model (Kuehl, 1994).

RESULTS

Mycelial growth, and perithecia and ascospore production at different temperatures

Monosporascus cannonballus isolates differed in mycelial growth rate (MGR) in PDA, regardless of the temperature considered. Using the Scott-Knott univariate grouping test, three groups of isolates at 22°C and 28°C, and two groups at 38°C were revealed (Table 2). At 22°C the mean MGR was 19.7 mm/day and the values of this variable ranged from 0.0 to 27.1 mm/day. The isolate CMM-2411 showed no mycelial growth at this temperature. MGR values of ≥ 20 mm/day were observed in 56.1% of the isolates incubated at 22°C. At 28°C, the mean MGR was 23.8 mm/day and the values ranged between 3.5 and 33.7 mm/day. MGR ≥ 20 mm/day were observed in 78.9% of the isolates. At 38°C, the mean MGR was 8.8 mm/day, the values ranged between 0.5 and 16.3 mm/day, and 61.4% of the isolates showed MGR up to 10 mm/day. The remaining isolates (38.6%) had growth rates over 10 mm/day.

Most *M. cannonballus* isolates produced perithecia after 45 days of incubation at 22°C and 28°C, while none of them produced perithecia at 38°C (Table 2). Regarding the number of perithecia produced (PP), three distinct groups of isolates were observed at 22°C and 28°C. At 22°C, the mean PP value was 38.7 perithecia/cm³; 24 isolates (42.1%) did not produce perithecia, and 54.4% produced between 50 and 200 perithecia/cm³. At 28°C, only the isolates CMM-2418 and CMM-2435 produced more than 200 perithecia/cm³. At 28°C, the mean PP was 126.5 perithecia/cm³ and the values ranged between 0.5 and 279.7 perithecia/cm³. Only 10.5% of the isolates produced up to 25 perithecia/cm³; 28.1% produced between 50 and 100 perithecia/cm³; 43.9% produced between 101 and 200 perithecia/cm³; and 17.5% produced more than 200 perithecia/cm³.

When evaluating the number of ascospores produced (AP) after 50 days of incubation at 22°C and 28°C, the formation of two and three distinct groups was observed, respectively (Table 2). At 22°C, the mean AP was of 20.0 ascospores/perithecia and the values of this variable ranged between 0.0 and 110.0 ascospores/perithecia. At this temperature, 35.1% of the isolates did not produce ascospores; one isolate (CMM-2451) produced more than 100 ascospores/perithecia; 54.4% produced from 0.1 to 50 ascospores/perithecia and 8.8% produced between 51 and 100 ascospores/perithecia. At 28°C, the mean AP was 39.1

ascospores/perithecia and the values ranged between 0.1 and 165.1 ascospores/perithecia. Only 10.5% of the isolates produced more than 100 ascospores/perithecia, while 24.6% produced between 51 and 100 ascospores/perithecia, and 64.9% produced from 0.1 to 50 ascospores/perithecia.

Sensitivity to the fungicide fluazinam

Inhibition of mycelial growth (MGI) was observed in all 57 isolates of *M. cannonballus* when exposed to 10 μ g a.i./L of fluazinam (Table 2). Seven distinct groups of isolates were observed by Scott-Knott grouping test. The mean MGI was 56.6% and the values ranged from 5.1 to 91.7%; 59.6% of the isolates had MGI levels between 31 and 75%, and 28.1% showed MGI above 75%. Only two isolates (3.5%) showed MGI levels below 10%.

Virulence test

All *M. cannonballus* isolates were pathogenic to melon seedlings cv. Mandacaru F1. There were differences ($P \leq 0.05$) among the isolates regarding disease severity (DSI). The average DSI value was 62.1%. Using Scott-Knott grouping test it was possible to distinguish five groups of isolates. DSI values between 41 and 80% were induced by 64.9% of the isolates, while 21.1% induced DSI values above 80% and only 5.3% of isolates showed values below 20% (Table 2).

Significant correlation was observed only between PP at 28°C and DSI ($r=0.3725$; $P=0.0043$). By univariate analysis, the formation of groups of similarity of the isolates of *M. cannonballus* within each variable was not limited by the site or the production area of origin of each isolate, given that in most situations, different isolates from the same site were distributed into distinct groups of similarity (Table 2).

Using principal components analysis, the first four main components accounted for only 55.6% of total variance and no clustering of isolates was clearly identified. Thus, it was not possible to establish associations or patterns that would allow the isolates to be grouped. The nested ANOVA on the scores of the principal components revealed that the variation was mainly due to differences among isolates ($F = 2.04^{**}$ to 8.82^{**}). There was no effect of production site and/or area.

DISCUSSION

The 57 *M. cannonballus* isolates from northeastern Brazil included in this study showed variability in fitness components. This is in agreement with the observations of Martyn and Miller (1996) and with the results of a study conducted with Spanish and Tunisian isolates of this fungus (Armengol et al., 2011). Univariate and multivariate analyses revealed that this variation was mainly due to differences among isolates and there was no effect of production site and/or area.

The highest rates of mycelial growth at 28°C were agreement with previous reports from Israel (Pivonia

TABLE 2 - Mycelial growth rate (MGR), perithecia production (PP) and ascospore (AP) production at different temperatures, mycelial growth inhibition by fungicide fluazinam (MGI) and disease severity induced to melon seedlings (DSI) by 57 isolates of *Monosporascus cannonballus* from northeastern Brazil.

Code (CMM) ¹	MGR ² (mm/day)			PP ² (perithecia/cm ³)		AP ² (ascospores/perithecia)		MGI ² (%)	DSI ² (%)
	22°C	28°C	38°C	22°C	28°C	22°C	28°C		
	2362	18.9 a	30.7 a	6.2 b	9.9 c	89.4 b	6.5 c		
2363	20.0 a	27.6 a	11.8 a	64.4 b	182.6 a	15.4 c	26.8 b	73.8 b	65.6 b
2365	24.7 a	25.2 a	9.0 a	0.0 c	24.8 c	0.0 c	3.2 b	31.7 e	75.0 b
2369	22.4 a	21.3 a	16.3 a	99.2 a	234.0 a	41.3 c	4.1 b	91.6 a	65.6 b
2370	23.6 a	30.3 a	4.0 b	37.5 b	136.2 a	0.0 c	0.4 b	47.9 d	40.6 c
2371	27.1 a	18.1 a	7.9 b	69.3 b	176.7 a	28.2 c	147.0 a	47.8 d	75.0 b
2372	24.0 a	24.5 a	15.4 a	0.0 c	170.5 a	0.0 c	56.7 b	86.8 a	50.0 c
2376	16.5 a	19.1 a	7.4 b	188.9 a	103.8 a	98.5 a	51.7 b	82.8 a	68.8 b
2379	20.2 a	27.2 a	5.4 b	67.6 b	87.8 b	14.9 c	9.9 b	80.9 a	37.5 c
2380	19.4 a	14.3 b	4.2 b	0.0 c	100.5 a	0.0 c	165.1 a	50.0 d	56.3 b
2381	25.3 a	11.5 b	9.7 a	163.0 a	279.7 a	32.2 c	3.2 b	50.0 d	91.7 a
2382	26.3 a	9.2 b	2.0 b	0.0 c	8.7 c	0.0 c	0.4 b	50.0 d	18.8 d
2386	16.5 a	22.4 a	1.3 b	37.2 b	78.6 b	0.0 c	23.1 b	57.8 d	65.6 b
2387	22.1 a	33.7 a	11.5 a	97.2 a	98.9 a	36.8 c	30.4 b	85.2 a	46.9 c
2389	23.7 a	21.4 a	6.0 b	0.0 c	78.2 b	14.3 c	66.3 a	88.0 a	93.8 a
2390	22.9 a	32.3 a	9.4 a	56.8 b	116.3 a	13.7 c	3.9 b	58.1 d	68.8 b
2391	12.7 a	22.2 a	3.8 b	66.6 b	98.3 a	33.2 c	39.5 b	34.3 e	75.0 b
2394	21.6 a	24.6 a	9.4 a	3.3 c	230.7 a	0.0 c	19.4 b	31.5 e	75.0 b
2395	24.1 a	28.2 a	12.1 a	75.3 b	94.3 a	5.1 c	34.6 b	50.0 d	46.9 c
2396	1.9 c	27.8 a	6.9 b	113.3 a	115.5 a	9.2 c	9.9 b	66.4 c	56.3 b
2397	20.2 a	31.5 a	14.7 a	99.7 a	226.3 a	10.8 c	51.2 b	35.8 e	81.3 a
2401	18.6 a	19.1 a	10.1 a	115.5 a	116.6 a	22.5 c	7.0 b	69.1 c	62.5 b
2402	20.2 a	8.3 b	4.9 b	145.3 a	162.7 a	91.4 a	11.9 b	88.6 a	90.6 a
2403	23.5 a	30.0 a	0.5 b	117.2 a	164.4 a	0.0 c	82.6 a	55.6 d	59.4 b
2405	24.2 a	32.2 a	15.9 a	0.0 c	70.7 a	0.0 c	107.4 a	51.6 d	3.1 e
2408	23.7 a	23.9 a	10.0 a	0.0 c	225.5 a	34.7 c	17.5 b	55.2 d	65.6 b
2411	0.0 c	6.6 b	3.7 b	0.0 c	8.7 c	0.0 c	0.3 b	22.7 f	3.8 e
2412	17.3 a	3.5 c	1.7 b	0.0 c	2.3 c	0.0 c	2.5 b	5.9 g	71.9 b
2414	24.0 a	16.1 b	5.7 b	0.0 c	99.1 a	0.0 c	53.0 b	53.0 d	59.4 b
2418	22.5 a	23.3 a	9.3 a	256.8 a	232.7 a	24.2 c	57.0 b	53.6 d	90.6 a
2421	18.0 a	23.6 a	7.1 b	138.5 a	88.6 b	47.2 b	144.0 a	91.7 a	71.9 b
2422	26.2 a	27.0 a	6.2 b	122.0 a	0.5 c	2.3 c	12.0 b	66.5 c	75.0 b
2427	14.7 a	26.9 a	13.2 a	101.3 a	228.0 a	48.9 b	14.0 b	41.5 d	50.0 c
2429	12.9 a	23.5 a	9.8 a	0.0 c	221.0 a	0.0 c	4.1 b	85.7 a	100.0 a
2430	14.4 a	23.2 a	2.4 b	80.8 b	118.0 a	2.7 c	26.1 b	18.3 f	62.5 b
2431	19.1 a	24.2 a	12.9 a	51.3 b	101.1 a	7.6 c	19.4 b	53.4 d	46.9 c
2434	24.8 a	26.4 a	8.9 a	0.0 c	68.2 b	0.0 c	12.1 b	50.0 d	68.8 b
2435	19.2 a	27.7 a	10.2 a	202.5 a	85.1 b	9.5 c	0.9 b	77.2 b	100.0 a
2436	21.4 a	23.8 a	10.8 a	0.0 c	81.6 b	22.5 c	2.5 b	73.5 b	75.0 b
2437	18.8 a	32.0 a	14.3 a	0.0 c	125.1 a	28.9 c	65.6 a	52.2 d	81.3 a
2438	17.9 a	29.4 a	8.6 a	0.0 c	130.1 a	15.4 c	34.7 b	79.3 a	15.6 d
2439	20.4 a	25.3 a	8.4 a	160.8 a	8.7 c	28.4 c	0.1 b	50.0 d	31.3 c
2441	25.0 a	25.9 a	10.8 a	0.0 c	141.6 a	2.5 c	1.9 b	68.6 c	40.6 c
2443	14.5 a	23.7 a	6.7 b	0.0 c	117.4 a	0.0 c	57.6 b	84.1 a	93.8 a
2444	15.8 a	24.2 a	7.8 b	125.1 a	69.8 b	92.7 a	15.6 b	50.0 d	56.3 b
2446	17.2 a	28.1 a	2.5 b	0.0 c	154.4 a	0.0 c	2.8 b	90.2 a	59.4 b
2448	22.0 a	12.1 b	12.1 a	0.0 c	135.7 a	0.0 c	103.4 a	18.7 f	34.4 c
2449	16.5 a	21.9 a	8.8 a	94.2 a	133.2 a	52.3 b	46.7 b	19.8 f	50.0 c
2450	23.3 a	30.7 a	13.4 a	55.2 b	118.0 a	13.9 c	20.3 b	37.1 e	65.6 b
2451	9.0 b	27.5 a	6.3 b	171.1 a	52.4 b	110.0 a	122.8 a	76.1 b	50.0 c
2452	18.9 a	28.9 a	13.4 a	0.0 c	168.1 a	54.3 b	89.4 a	77.8 b	62.5 b
2453	24.0 a	24.3 a	8.8 a	66.6 b	87.0 b	0.0 c	15.2 b	31.9 e	68.8 b
2454	21.7 a	22.6 a	14.5 a	0.0 c	197.6 a	15.8 c	60.2 b	16.0 f	46.9 c
2455	15.2 a	22.5 a	15.5 a	91.8 a	260.6 a	20.9 c	69.0 a	59.3 d	87.5 a
2457	20.7 a	31.6 a	10.2 a	0.0 c	114.1 a	0.0 c	90.5 a	5.1 g	43.8 c
2466	18.9 a	25.7 a	11.9 a	0.0 c	221.3 a	30.0 c	33.8 b	50.0 d	81.3 a
2467	21.9 a	28.3 a	12.7 a	0.0 c	166.9 a	0.0 c	55.2 b	83.5 a	84.4 a
Mean	19.7	23.8	8.8	58.7	126.5	20.0	39.1	56.6	62.1

¹Culture Collection of Phytopathogenic Fungi "Prof. Maria Menezes" (CMM) of the Universidade Federal Rural de Pernambuco (Recife, PE, Brazil).

²Values are means per treatment from two independent experiments. Values with the same letter within a column are not significantly ($P>0.05$) different according to Scott-Knott test.

et al., 2002), Japan (Uematsu & Sekiyama, 1990), Spain (Armengol et al., 2011) and the USA (Stanghellini et al., 2000), where many isolates had optimal growth between 28 and 32°C. Growth rates of all *M. cannonballus* isolates at 38°C were similar to those obtained by Armengol et al. (2011), but differed from that found by Pivonia et al. (2002), who showed that the hyphal growth of the *M. cannonballus* included in this study ceased at 39°C.

Perithecia production was greater at 28°C, confirming previous observations (Martyn & Miller, 1996; Waugh et al., 2003; Armengol et al., 2011), which revealed that the temperature range between 25 and 30°C is the most suitable temperature for perithecia production. Moreover, ascospore production was also higher at 28°C and we did not observe perithecia at 38°C. All the isolates included in this study were collected from semi-arid regions, characterized by their hot summer temperatures when melon is grown. Thus, according to our results, these environmental conditions, together with the use of plastic mulches, may raise the soil temperatures to levels that favor growth and infection by *M. cannonballus*, and also perithecia production in diseased roots, as indicated by Martyn & Miller (1996) and Waugh et al. (2003).

The results observed for the mycelial growth rate, perithecia production and ascospore production by *M. cannonballus* isolates at different temperatures confirm the observation that the fitness of an individual or class of phenotypes will be influenced not only by the genetically determined attributes of those individuals, but also by the environments in which they were found (Antonovics & Alexander, 1989).

The reproductive potential of *M. cannonballus* is a significant aspect to be considered in disease management (Waugh et al., 2003). The difference between isolates according to this fitness component, as observed in this study, indicates the difference in competitive potential of the isolates. Accordingly, the reproduction rate considers relative growth rate or the number of new progeny produced by a pathogen strain and is the most common measurement of competition among pathogen strains. Pathogens with higher reproduction rates are thought to have a higher competitive ability. Pathogen strains with a higher competitive ability increase in frequency over pathogen generations and eventually dominate the experimental population (Zhan & McDonald, 2013).

The high sensitivity of most *M. cannonballus* isolates to the fungicide fluazinam was expected, since this fungicide has not been used to control the root rot in melon production areas in the states of Ceará and Rio Grande do Norte. In studies conducted in Israel, fluazinam totally inhibited the growth of *M. cannonballus* in culture at concentrations of 10 µg a.i./L (Cohen et al., 1999; Cohen et al., 2000). The lack of correlation between sensitivity to fungicide fluazinam and disease severity in melon indicates that there was no fitness cost in virulence associated with fungicide sensitivity. Fitness costs associated with fungicide

resistance are often predicted but rarely reported (Zhan & McDonald, 2013).

Disease severity values obtained in this study were useful in separating isolates of *M. cannonballus* into different virulence groups. This knowledge is essential for an effective breeding program (Bruton et al., 2000). Differences in virulence among isolates of *M. cannonballus* from melon in Brazil were reported previously by Andrade et al. (2005), although in this study the authors used a low number of isolates obtained from only two growing areas. Studies conducted in Japan (Uematsu & Sekiyama, 1990) and USA (Bruton et al., 2000) also revealed variability of virulence to melon among isolates of *M. cannonballus*. The lack of a significant correlation between disease severity and most of the fitness components of *M. cannonballus* isolates may indicate that these characteristics are determined by different groups of genes, and the population is recombined spontaneously (Caten & Newton, 2000).

The fitness variability among *M. cannonballus* isolates found in this study should be considered when possible sources of resistance are evaluated in melon breeding programs.

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