Eur J Plant Pathol (2014) 139:393–398
DOI 10.1007/s10658-014-0395-8

Effect of soil matric water potentials on germination of ascospores of *Monosporascus cannonballus* and colonization of melon roots by zoospores of *Olpidium bornovanus*

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Accepted: 5 February 2014 / Published online: 25 February 2014

Abstract Results document, for the first time, the role of soil moisture on a unique, tripartite, host-specific rhizosphere interaction (i.e., *Cucumis melo-Monosporascus cannonballus-Olpidium bornovanus*). Specifically, colonization of cantaloupe roots by zoospores of *O. bornovanus* and germination of ascospores of *M. cannonballus* were highest at a soil matric potential of −0.001 MPa but significantly inhibited at a matric potential of only −0.01 MPa. Matric water potentials of −0.01 MPa or drier are characteristically inhibitory to the motility of zoosporic microbes but not hyphal growth of filamentous fungi like *M. cannonballus*. These results support our previous conclusion that germination of ascospores of *M. cannonballus*, a destructive root pathogen of cantaloupe is mediated by *O. bornovanus,* an obligate, zoosporic fungus.

Keywords *Monosporascus cannonballus* · *Olpidium bornovanus* · Melon · Melon vine decline · Soil matric water potential

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Introduction

Monosporascus cannonballus Pollack & Uecker, a host-specific, root-infecting ascomycete, is the primary species associated with a destructive disease of melons known as Monosporascus vine-decline (Cohen et al. 2012). Most recently, however, another species, i.e., *M. eutypoides,* has been associated with root rot and vine decline of melons in Tunisia (Ben Salem et al. 2013). Ascospores function as the primary inoculum for this soilborne fungus (Stanghellini et al. 1996). Although produced in axenic culture, ascospores germinate only in the rhizosphere of melons growing in field soil (Stanghellini et al. 1996, 2010). However, no ascospore germination, as assessed by observation of ascospore germlings attached to melon roots, occurs in the rhizosphere of melons if the field soil is autoclaved prior to reinfestation with ascospores (Stanghellini et al. 2000). The heat sensitivity suggested that ascospore germination in the rhizosphere of melons is mediated by one or more heat-sensitive members of the soil microflora. Our recent studies demonstrated that ascospore germination is mediated by *Olpidium bornovanus* (Sahtiy.) Karling, an obligate, zoosporic, root-infecting pathogen (Stanghellini and Misaghi 2011). Specifically, ascospore germination occurred in autoclaved field soil only in the rhizosphere of melon seedlings that had been inoculated with zoospores of *O. bornovanus* prior to planting. Our

preliminary studies also indicated that ascospore germination in the rhizosphere of melon roots was more pronounced in soils irrigated daily as opposed to weekly. Wet soil conditions would be expected to favour a zoosporic microbe (i.e., *O. bornovanus*) as opposed to a non-zoosporic microbe (i.e., *M. cannonballus*).

The objective of this research was to assess the effects of specific soil matric water potentials on ascospore germination of *M. cannonballus* and colonization of melon roots by *O. bornovanus*.

Materials and methods

Host plant and soil infestation with *M. cannonballus*

All experiments were conducted using cantaloupe (*Cucumis melo* L. cv. Caravelle) as the susceptible host. A field soil (Meloland sandy loam) naturally-infested with both *M. cannonballus* and *O. bornovanus* was used. The indigenous population of *M. cannonballus* was estimated at 4±1 ascospores g^{-1} soil (Stanghellini et al. 1996). The population of *O. bornovanus* was not determined. However, it was readily isolated from the naturally-infested field soil using melon seedlings as bait (Stanghellini and Misaghi 2011).

Soil infestation procedure

A previously described method for augmenting the field soil with high populations of culturally-produced ascospores of *M. cannonballus* was used (Stanghellini et al. 2000). Briefly, 500 g of field soil were infested with culturally-produced ascospores from 1-month-old 10 % V8 juice agar cultures of *M. cannonballus*. Two single-ascospore isolates of *M. cannonballus,* BH3-19 and Mc14, obtained from infected roots of cantaloupe plants from commercial melon fields in Arizona and California were used. Ascospore population densities after soil infestation were estimated at 2,100±300 and 1,200±150 ascospores g^{-1} soil for BH3-19 and Mc14, respectively. Infested soils were air-dried and stored in plastic bags at 24 °C until used.

Soil moisture effects on ascospore germination in the rhizosphere of melon seedlings

The effect of specific soil moisture levels (matric potential) on ascospore germination was determined using soil moisture tension plates (Griffin 1963) (Fig. 1a). Seven g of ascospore-infested field soil were poured into each of three plastic cylinders (1.7 cm diam. × 3.0 cm long) placed on a sintered glass plate (7 cm in diam., medium porosity). One 3-day-old seedling was

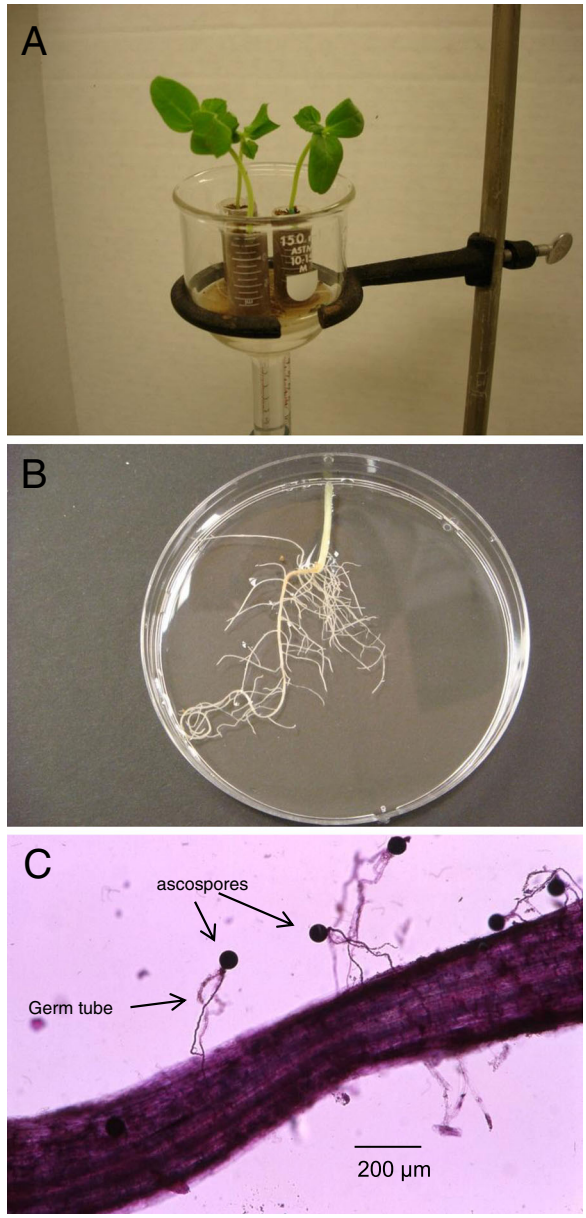

Fig. 1 Assessment of the effect of soil matric water potential on germination of ascospores of *Monosporascus cannonballus*. **a** Moisture tension apparatus used to regulate soil matric water potentials. **b** Root system of a 9-day-old melon seedling. **c** Ascospore germlings of *Monosporascus cannonballus* attached to a melon root. Ascospore diameter = 45 um, root diameter = 300 µm

then placed in each cylinder. Length of radicles on pregerminated seedlings was 2.0 to 2.5 cm. The soil was then wetted to saturation and various suctions were applied by adjusting the height of the water meniscus in the side arm. The time required to saturate and reach equilibrium depended on the suction employed, but never exceeded 15 min. The soil moisture characteristics of the Meloland sandy loam soil are presented in Fig. 2.

Funnels containing the melon seedlings were incubated in a growth chamber (12-h photoperiod) at 30 °C for 9 days. Water was added to the side arm of the apparatus as needed to maintain three matric potentials (i.e., −0.001, −0.005 and −0.01 MPa) over the duration of the experiment. At the end of the incubation period, the cylinders were removed from the funnel and the soil core containing the seedling was removed from the cylinder. The soil was gently rinsed from the root system of each plant, plants were placed separately in 15 ml of water in small Petri dishes, and the number of ascospore germlings attached to the roots of each seedling was recorded as previously described (Stanghellini et al. 2000, 2010; Stanghellini and Misaghi 2011) (Fig. 1b–c). All experiments, which contained three replications, were repeated once except for the experiment involving isolate Mc14 which was conducted only once due to a limited quantity of soil artificially infested with the latter isolate of *M. cannonballus*.

Soil moisture effects on colonization of melon roots by *O. bornovanus*

Although a few sporangia of *O. bornovanus* were microscopically observed in epidermal cells of melon roots colonized by *M. cannonballus* at −0.001 and −0.005 MPa, their populations, although sufficient to mediate ascospore germination, were too low to permit quantitative evaluation of the effect of soil matric potential on the extent of zoospore colonization of epidermal cells. As previously noted (Stanghellini et al. 1996), ascospores of *M. cannonballus* require an incubation period of 6–9 days in the rhizosphere before the onset of germination. Thereafter, lysis of germ tubes occurs rapidly thus precluding accurate enumeration of ascospore germination. The 6–9 day time frame also coincides with the maturation rate of sporangia of *O. bornovanus* following zoospore infection of epidermal cells (Stanghellini et al. 2010). Thus, a separate experiment was designed to permit amplification of colonization of melon roots by zoospores of *O. bornovanus* at soil matric potentials of −0.001, −0.005 and −0.01 MPa. One hundred grams of a field soil naturally infested with *O. bornovanus* was poured into each of three sintered glass funnels (7 cm in diam., medium porosity). Three melon seeds were sown in each funnel. The soil was then wetted to saturation and matric potentials of −0.001, −0.005 and −0.01 were applied as previously described. Water was added to the side arm of the apparatus as needed to maintain the desired matric potential over the duration of the experiment. Melon seedlings in field soil were incubated in a growth chamber (12-h photoperiod) at 30 °C for 28 days. The extended incubation period favored multiple life and infection cycles of *O. bornovanus*. At the end of the incubation period, the soil was gently rinsed from the root system of each plant. Ten root segments, each ca. 30 mm long, were excised from each of the three plants per soil moisture treatment, stained with 0.1 % acid fuchsin and examined at 400X under a microscope. The number of root segments colonized, the percentage root length bearing sporangia of *O. bornovanus* and the level of infestation (based on the number of sporangia per mm of root length) was determined.

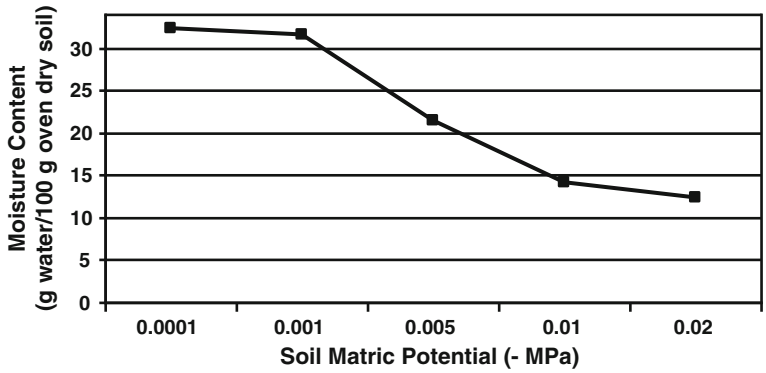

Fig. 2 Moisture characteristics (drying boundary curve) of Meloland sandy loam soil

The rating scale used was as follows: 0=no sporangia, 1=1–70, 2=71–140 and 3=141–600 sporangia/mm of root (Fig. 3). The experiment was repeated once.

Statistical analysis

For the soil moisture effects on ascospore germination, there were three experiments, each with three replications. Experiment 1 and 2 were conducted using *M. cannonballus* isolate BH3-19 and experiment 3 involved *M. cannonballus* isolate Mc14. Analysis of variance and least significant difference (LSD) mean separation procedures were performed for each of the experiments. For studies on melon root colonization by *Olpidium* at various matric potentials, there were two experiments, each with three replications. Percentage data were arcsine transformed. Bartlett's test of homogeneity of variances was performed for repeated experiments. Data sets with homogeneous variances ($P<0.05$) were combined and analysis of variance and least significant difference (LSD) mean separation procedures (SAS ver. 9.2; SAS Institute, Cary, NC) were performed.

Results

Matric water potential effects on ascospore germination

Figure 4 shows the number of ascospore germlings of *M. cannonballus* attached to the root system of melon plants under various soil matric water potentials. Highest numbers of ascospore germlings attached to melon roots were recorded in all treatments in which the soil matric water potentials were maintained at or near saturations levels (−0.001 MPa). As soil matric water potentials were decreased to −0.01 MPa, significant reductions (ca. 80 %) in the number of ascospore germlings attached to roots were recorded in all three experiments.

Matric water potential effects on colonization of melon roots by *O. bornovanus*

Melon root colonization by *O. bornovanus,* as demonstrated by the observation of sporangia in root epidermal cells, at various soil matric water potentials is shown in Table 1 and Fig. 3. A total of 60 root segments, each 30 mm in length, were microscopically examined for sporangia formation at three different matric water potentials in two independent experiments. At −0.001 MPa, sporangia were observed in 77.8 % of the root segments assayed but were observed in only 20 % (a reduction of ca.75 %) of roots maintained at −0.005 MPa. No sporangia were observed in epidermal cell of roots maintained at −0.01 MPa. The total root length assayed in the two separate experiments at each of three different soil matric water potentials was 1,800 mm. At −0.001 MPa, sporangia were observed in 45.6 % of the total root length assayed as opposed to 7.8 % at −0.005 MPa (an 80 % reduction). As pointed out above, no sporangia were observed in epidermal cells of roots maintained at −0.01 MPa. Additionally, the number of sporangia of *O. bornovanus* ranged from 70 to 600 per mm of root at −0.001 MPa (disease severity rating of 2–3) as opposed to populations ranging from 1 to70 per mm of root at −0.005 MPa. No sporangia were observed in roots maintained at −0.01 MPa.

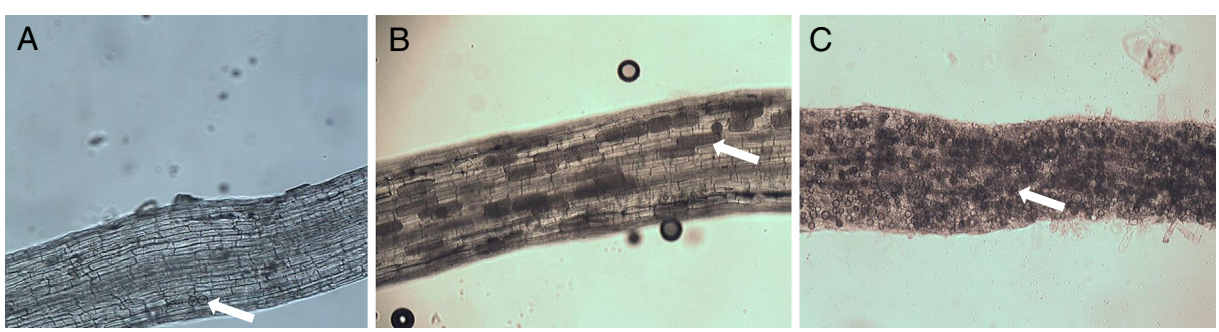

Fig. 3 Colonization index of the population of *Olpidium bornovanus* sporangia in roots of melon plants grown at various soil matric water potentials: Rating scale from 0 to 3: 0=0, 1=1–70, 2=71–140, and 3=141–600 sporangia/mm of root length. A = 1 rating (2 sporangia, arrow), B = 2 rating, C = 3 rating. Root diameters = 210 μm

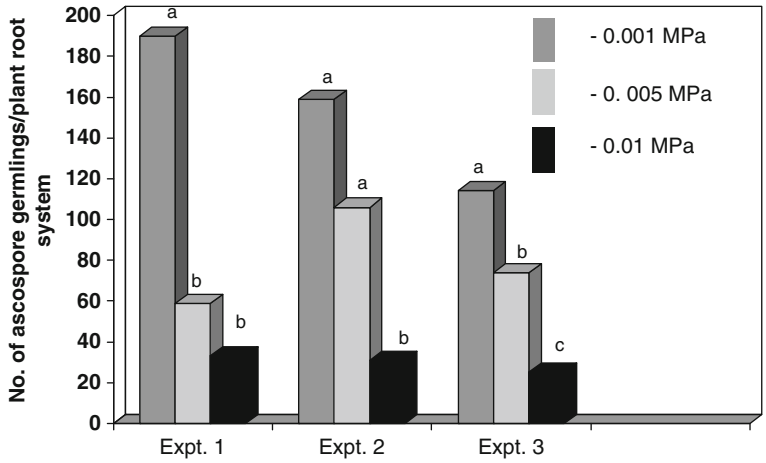

Fig. 4 Effect of various matric water potentials on the germination of *Monosporascus cannonballus* ascospores and germling attachment to melon roots growing in a Meloland sandy loam field soil. Experiments 1, and 2 involved *M. cannonballus* isolate BH3-19, whereas experiment 3 involved *M. cannonballus* isolate Mc14. Bars within each experiment marked with different letters are differ significantly (Anova-SAS)

Discussion

Our results indicate, for the first time, that high soil moisture conditions directly affect root colonization by zoospores of *O. bornovanus* and indirectly affect root colonization by *M. cannonballus*. Specifically, colonization of root epidermal cells by zoospores of O. *bornovanus*, as well as ascospore germination of *M. cannonballus* in the rhizosphere of melon root, were highest at soil moisture levels near saturation (−0.001 MPa) but both were significantly inhibited at a matric potential of −0.01 MPa. These results suggest that the effect of such slight reductions in soil moisture levels are most likely due to restriction on the motility of *O. bornovanus* zoospores. Lack of motility would limit root colonization by this holocarpic zoosporic fungus, which in turn would indirectly limit germination of ascospores of *M. cannonballus*. These results are consistent with previous studies which demonstrated that matric water potentials of −0.005, −0.01, and −0.015 MPa are totally limiting to the movement of motile soil microbes such as zoospores of *Phytophthora cryptogea* (Duniway 1976), *O. brassicae* (Westerlund et al. 1978), and some flagellated bacteria (Wong and Griffin 1976), respectively. Our interpretation that the inhibitory effect of matric water potentials of −0.005 and −0.01 MPa is on *O. bornovanus* and not on *M. cannonballus* is supported by our previous studies which showed that no ascospore germination occurred in the rhizosphere of melon seedlings grown in autoclaved soil (Stanghellini et al. 2000, Stanghellini and Misaghi 2011), and that hyphal growth of *M. cannonballus* is optimal at water potentials between −0.6 to −0.8 MPa (Ferrin and Stanghellini 2005) and decreased significantly only when water potentials approached −3 MPa (Armengol et al. 2011). Soil moisture per se, over the range available to higher plants, (i.e., 0 to –1.4 MPa), is seldom limiting to fungal germination and hyphal growth (Griffin 1963). Cumulatively, these

Table 1 *Olpidium bornovanus* colonization of roots of melon plants grown at various soil matric water potentials

MPa	Number of root segments assayed[a]	Root segments infected (%)	Total root length assayed (mm)	Root length with sporangia (mm)	Total root length colonized (%)	Colonization index[b]
−0.001	60	77.8 a	1,800	820.8	45.6 a	2–3
−0.005	60	20.0 b	1,800	140.4	7.8 b	1
−0.01	60	0 c	1,800	0	0 c	0

[a] 10 root segments, each measuring 30 mm in length were excised from each of three plants per soil moisture level and microscopically (400×) assessed for sporangia of *O. bornovanus* in epidermal cells 28 days after planting in naturally infested field soil. The experiment was repeated once

[b] Number of sporangia per mm of infected root was rated on a scale of 0-3: 0=0, 1=1-70, 2=71-140 and 3=141-600 sporangia/mm root length. Columns with the same letter are significantly different based on ANOVA and least significant difference (LSD) mean separation (P=<0.05). Lack of significant variation, as shown by Bartlett's test of homogeneity, permitted the combining data from the two experiments

results support our previous conclusion that germination of ascospores of *M. cannonballus* is mediated by *O. bornovanus* (Stanghellini and Misaghi 2011).

Although Monosporascus vine-decline of melons has been problematic in Arizona and California since the early 1950s, disease incidence and severity increased dramatically in the late-1980s (Stanghellini et al. 1995). This increase, which also occurred in other major melon production areas of the world (Cohen et al. 2012), coincided with the increased use of hybrid varieties, multiple and consecutive cropping of melons per field, and a shift from furrow to drip irrigation. Enhanced disease development under drip irrigation regimes was attributed primarily to the development of a shallow and dense root system compared to a deep root system of plants irrigated less frequently (Pivonia et al. 2004). In addition to root architecture, the soil depth at which root lesions were first observed in commercial cantaloupe fields was related to the method of irrigation (Stanghellini et al. 1995). In a furrow irrigated field (the driest irrigation practice), all lesions caused by *M. cannonballus* occurred on roots at a soil depth of 6 to 25 cm. No lesions were observed on roots at the 1- to 5- cm soil depth. In a drip-irrigated field, 45 % the lesions occurred on roots at the 1- to 5- cm depth with decreasing lesion numbers at depths of 6 to 25 cm. In a mulch-drip irrigated field (the wettest irrigation practice), all root lesions occurred at the 1- to 3-cm depth. These latter observations indicate that wet soil conditions, in addition to influencing root architecture, is an important environmental factor influencing root infection by *M. cannonballus,* albeit indirectly as we currently understand the biology of this unique tripartite, host-specific rhizosphere interaction (i.e., *Cucumis-M. cannonballus-O. bornovanus)*. Furthermore, results suggest that management of Monosporascus vine decline in the field may benefit from strategies focusing on *O. bornovanus.* Root colonization by this zoosporic fungus, as we demonstrated, is restricted to saturated or near- saturated soils, which would occur only during or immediately after irrigation, particularly in fields employing drip-irrigation. As previously noted, increased incidence and disease severity caused by *M. cannonballus* has been associated with increased use of drip irrigation.

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