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Water potential affects *Coniothyrium minitans* growth, germination and parasitism of *Sclerotinia sclerotiorum* sclerotia

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ABSTRACT

Water availability is an important environmental factor which has major effects on fungal activity. The effects of osmotic (KCl amended agar) and matric Polyethylene glycol ((PEG) 8000 amended agar) potentials over the range −0.1 to −5.0 MPa on mycelial growth and conidial germination of eight isolates of the sclerotial parasite *Coniothyrium minitans* was assessed. The influence of soil water potential on the ability of three selected isolates (LU112, LU545, and T5R42i) to parasitise sclerotia of the plant pathogen *Sclerotinia sclerotiorum* was determined. For all eight *C. minitans* isolates, decreasing osmotic and matric potentials caused a reduction in mycelial growth and conidial germination. Isolates were more sensitive to decreasing matric potential than osmotic potential. Across the isolates, growth at an osmotic potential of −5.0 MPa was 30–70 % of the growth seen in the control, whereas less than 20 % of the control growth was seen at the corresponding matric potential. Across all isolates no conidial germination was seen at matric potential of −5.0 MPa. The *C. minitans* isolates varied in their sensitivity to decreasing water potentials. Mycelial growth and conidial germination of three isolates (LU112, Conio, and CH1) were more tolerant of low osmotic potential and matric potential with respect to mycelial growth. Isolates T5R42i and LU430 were least tolerant. In contrast, conidial germination of isolates Conio, LU545, and T5R42i were less sensitive to decreasing matric potential. Soil water potential was seen to affect infection and viability of sclerotia by the three *C. minitans* isolates. Isolate LU545 reduced sclerotial viability over a wider water potential range (−0.01 to −1.5 MPa) compared with LU112 (−0.01 to −1.0 MPa), with isolate T5R42i being intermediate. Indigenous soil fungi (*Trichoderma* spp. and *Clonostachys rosea*) were recovered from sclerotia but did not result in reduction in sclerotial viability. The relevance of these results in relation to biocontrol activity of *C. minitans* in soil is discussed.

Introduction

Coniothyrium minitans Campbell is a well-documented sclerotial parasite being first identified associated with sclerotia of *Sclerotinia sclerotiorum* (Lib.) de Bary by Campbell (1947) and has since been shown to have a worldwide distribution (de Vrije *et al.* 2001). Its potential as a biocontrol agent of *S. sclerotiorum* was identified by Turner & Tribe (1976). *Coniothyrium minitans* has been shown to infect and reduce sclerotial viability in *in vitro* assays (Whipps & Budge 1990; Jones & Stewart 2000) and to reduce *S. sclerotiorum* disease in glasshouse and field trials (Budge *et al.* 1995; Huang *et al.* 2000; Jones *et al.*

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2004; Li et al. 2005, 2006; McQuilken & Chalton 2009). Different isolates of *C. minitans* have been shown to vary in their ability to reduce sclerotial viability in soil assays (Jones & Stewart 2000). As the activity of fungi can be markedly affected by environmental factors such as pH, temperature, and soil water potential, it is possible that the variability in effectiveness of isolates is in part related to different optima for environmental conditions, with the incubation conditions of the *in vitro* soil assay being conducive for some isolates while restricting the activity of others.

One important environmental constraint with major effects on fungal activity is water availability (Ayres & Boddy 1986). Water potential is a measure of how much energy is required to extract water from a substrate. Total soil water potential is a sum of many components including matric, osmotic, pressure, and gravitational potentials (Cook & Duniway 1980). With regards to soil systems, the most important governing water flow and availability for physiological processes are osmotic and matric. Osmotic potential is due to solutes in soil water and is important in saline soils or soils amended with fertilisers and organic waste. It is also relevant to growth within tissues, and therefore of major importance to both pathogens and sclerotial parasites alike. Matric potential includes both adsorption and capillary effects and is most relevant to growth in soil or on root surfaces.

Water potential has been shown to have significant effects on the activities of plant pathogens (Cook & Baker 1983) such as *S. sclerotiorum* and *Sclerotinia minor* (Hao et al. 2003), *Rhizoctonia solani* (Ritchie et al. 2006), *Fusarium graminearum* (Ramirez et al. 2004), and *Fusarium pseudograminearum* (Singh et al. 2009). However, the effect of water potential on fungal biocontrol agents has not been widely studied. Douglas & Deacon (1994) reported different tolerance to water stress by three randomly selected strains of the biocontrol agent *Idriella bolleyi* (syn. *Microdochium bolleyi*) with one strain having equivalent stress tolerance to the cereal pathogen *Fusarium culmorum* whilst another showed low tolerance similar to the take all fungus *Gaeumannomyces graminis*. The researchers suggested that water stress tolerance could be used to select strains for biocontrol of different root pathogens. *Fusarium equiseti* was reported by Hussain et al. (2005) to reduce *Verticillium dahliae* infection of potatoes at soil water potential of −0.15 MPa but not at −0.03 MPa.

Although McQuilken et al. (1997) demonstrated in laboratory studies that temperature and pH optima for four *C. minitans* isolates were similar, there is little information on the effect of water potential. Jones et al. (1999) showed that mycelial growth of a *C. minitans* isolate (A69) was inhibited at low water potentials (osmotic and matric). However, information on the effect of water potential on spore germination, survival, and biocontrol efficacy of different isolates is limited. *Coniothyrium minitans* was shown to inhibit the mycelial growth of *S. sclerotiorum* in dual plate cultures on osmotically modified agar in the range −0.7 to −7.0 MPa (Whipps & Magan 1987). However, mycelium–mycelium interactions between these two fungi may only be relevant for biocontrol of ascospore infections on leaf surfaces, with little information on the response of sclerotial parasites to osmotic and matric potential with regards to the different growth stages which is relevant to colonisation of sclerotia. Huang & Erickson (2008) reported that two soil moisture treatments, dry at 9 % and wet at 24 % moisture levels, had no effect on sclerotial parasitism by a *C. minitans* isolate. Similarly, Teo et al. (1992) demonstrated that a *C. minitans* isolate completely inhibited carpogenic germination of *S. sclerotiorum* sclerotia incubated in soil at water potentials between 0 and −1.5 MPa, but since no viability assessments were carried out it was unclear whether this was due to reduction in viability of sclerotia or inhibition of germination by *C. minitans*. Infection of sclerotia of the related pathogen, *S. minor*, by a *C. minitans* isolate was reported to increase as soil moisture level decreased from 0 to −100 kPa (0 to −0.1 MPa) (Partridge et al. 2006). Soil moisture level of −1500 kPa (−1.5 MPa) however inhibited *C. minitans* infection. Whether different *C. minitans* isolates vary in their response to water potential is not known.

Inconsistent control by fungal biocontrol agents when applied in the field has been reported as being a limiting factor for the uptake of biocontrol by growers (Stewart 2010). Given the importance of the influence of water potential on fungal activity, information regarding its effect on growth and sclerotial parasitism by fungal biocontrol agents such as *C. minitans* is essential to provide information on the conditions that would restrict biocontrol activity. It is hypothesised that the relative activity of fungal biocontrol strains in soil and hence biocontrol activity are affected by soil water potentials, which can be predicted from their growth response on osmotically and matrically-adjusted media. Further, it is hypothesised that *C. minitans* isolates vary in their ability to parasitise sclerotia of *S. sclerotiorum* at different water potentials, information that would facilitate the selection of isolates or isolate mixtures to enable the control of *S. sclerotiorum* across a broader range of soil water potentials. The aim of this study was therefore to investigate the effect of osmotic and matric potential on mycelial growth and conidial germination of eight different *C. minitans* isolates. Two isolates which have been extensively tested in field and glasshouse studies, Conio (Budge et al. 1995; Jones et al. 2004) and LU112 (Rabeendran et al. 2006), were included. Three *C. minitans* isolates were selected to determine the effect of soil water potential on parasitism of *S. sclerotiorum* sclerotia in soil.

Methods

Fungal cultures

Sclerotinia sclerotiorum isolate G18, originally isolated from diseased carrot, Christchurch, New Zealand (Jones & Stewart 2000), was obtained from the Lincoln University culture collection. *Coniothyrium minitans* isolates LU112 (A69), LU420 (L1E3), LU421 (Le2A4), LU430 (T5R42b), LU545 (T5R42g), and T5R42i and were all originally isolated from *S. sclerotiorum* sclerotia in New Zealand (Jones & Stewart 2000). *Coniothyrium minitans* isolates Conio and CH1 were obtained from the HRI-Warwick culture collection. Fungal isolates were stored in 20 % glycerol at −80 °C and routinely cultured on potato dextrose agar (PDA; Oxoid Ltd, Basingstoke, UK) at 20 °C.

Production of sclerotia

Sclerotia were produced on sterilised wheat grain (Jones & Whipps 2002). Twenty-five grams of wheat seeds (Australian