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Potential ecological effects of *Piriformospora indica*, a possible biocontrol agent, in UK agricultural systems

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HIGHLIGHTS

- *Piriformospora indica* can survive UK weather and soil conditions up to 15 months.
- *P. indica* has a substantial effect on soil and root microflora in the first 8 weeks.
- *P. indica* affects two of tested native weeds and alters their relations with wheat.
- *P. indica* wider effects need to be better understood before agricultural deployment.

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ABSTRACT

Piriformospora indica (syn. *Serendipita indica*), a root endophytic fungus, was originally isolated from an arid sub-tropical soil. *P. indica* forms mutualistic symbioses with a broad range of host plants, increases biomass production, resistance and tolerance to fungal pathogens and abiotic stresses. These characteristics make it a very attractive component of more sustainable agriculture. So, it is desirable to understand its wider ecosystem effects. We determined how long *P. indica* could survive in the soil and how it interacts with other soil microorganisms and some important arable weeds.

Survival of *P. indica* in the soil, under winter and summer conditions in the UK was tested by isolating DNA and RNA of *P. indica* from pots of soil which had been left open to winter-summer weather conditions without host plants, followed by PCR and reverse transcription-PCR (RT-PCR) with *P. indica*-specific primers. *P. indica* effects on other soil and root microorganisms were tested by PCR-denaturing gradient gel electrophoresis analysis of DNA extracted from soil and roots from pots in which *P. indica*-infected wheat had been grown. The effect of *P. indica* on growth of black-grass (*Alopecurus myosuroides*), wild-oat (*Avena fatua*) and cleavers (*Galium aparine*) was tested alone and in competition with wheat.

In soil *P. indica*-mRNA and DNA could still be detected after eight months, but not after 15 months. Soils from *P. indica*-inoculated pots had distinct fungal and bacterial species communities which were more diverse than non-inoculated controls. *P. indica* infected *A. myosuroides* and *A. fatua* but was not detected in *G. aparine*. The average above-ground competitiveness of the weeds with wheat was decreased.

If applied to field crops in the UK, *P. indica* would be persistent for up to 15 months and likely to alter competitive relations within vegetation. Increased soil microbial diversity during the first eight weeks after inoculation, although usually desirable, could alter soil composition or functioning.

1. Introduction

Piriformospora indica (Serendipitaceae), a root endophytic fungus (Sebacinales: Basidiomycota), was first found in the Thar dessert of India (Verma et al., 1998; Oberwinkler et al., 2014; Weiß et al., 2016), an arid region which experiences extreme day-time heat and diurnal temperature fluctuations as well as extended drought. *P. indica* promotes plant growth, increases root and above ground biomass and final yield during its mutualistic relationship with a wide variety of plants (Shrivastava and Varma, 2014). It increases resistance of several hosts from diverse families to many biotic stresses under glasshouse and field conditions (Waller et al., 2005; Deshmukh and Kogel, 2007; Ghahfarokhy et al., 2011; Harrach et al., 2013). Tolerance to abiotic stresses is also increased

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in a variety of hosts (Zarea et al., 2012; Alikhani et al., 2013; Ghabooli et al., 2013; Varma et al., 2013). Our previous studies showed that *P. indica* protected wheat from Fusarium crown rot in pot experiments (Rabiey et al., 2015). Our work also suggests that Fusarium head blight and mycotoxin deoxynivalenol contamination are reduced in pot experiments under UK weather conditions (Rabiey and Shaw, 2015). Tests on a field scale are therefore attractive, but as the organism is an alien, it would first be necessary to understand its ecosystem effects and persistence.

How *P. indica* interacts with other soil microorganisms is still unclear. Endophytic fungal symbionts can have profound effects on plant ecology, fitness, and evolution (Brundrett, 2006), shaping plant communities (Clay and Holah, 1999), increasing plant tolerance to abiotic stresses (Murphy et al., 2015), increasing plant resistance to pathogens (Rodriguez et al., 2009; Murphy et al., 2014) and manifesting strong effects on the community structure and diversity of associated organisms (e.g. bacteria, nematodes and insects; Omacini et al. (2001)). Studies on the effects of arbuscular mycorrhizal fungi (AMF) on rhizosphere bacteria have shown variable results, with both negative (decreasing the population of bacteria) (Christensen and Jakobsen, 1993; Amora-Lazcano et al., 1998) and positive (increasing the population of bacteria) (Andrade et al., 1997; Abdel-Fattah and Mohamedin, 2000) effects. The variable results could be due to the fact that some bacteria are being stimulated and others being repressed by AMF (Wamberg et al., 2003). Söderberg et al. (2002) suggested that the effect of AMF differed between plant species; the strength of the effect on the bacterial community in the rizosphere depended more on the plant species than on AMF colonisation. If *P. indica* is going to be applied to crops, a clear picture of how it affects other soil microorganisms would be needed, as the soil microflora plays a major role in the availability of nutrients to plants and has a strong influence on plant health and productivity.

Weed competition can threaten crop quality and quantity and ultimately the farmer's profitability (Bockus et al., 2010); it is usually managed by herbicide application. The key herbicide-resistant weed species of arable crops in the UK are: black-grass (*Alopecurus myosuroides*), wild-oats (*Avena fatua*), cleavers (*Gallium aparine*), Italian rye-grass (*Lolium multiflorum*), common poppy (*Papaver rhoeas*), common chickweed (*Stellaria media*), and scentless mayweed (*Tripleurospermum inodorum*) (Bond et al., 2007; Moss et al., 2011; Hull et al., 2014). These are also important worldwide and in other crops (Yu et al., 2013). Herbicide resistance in the UK is an important and increasing problem, as in other parts of the world including western, central and northern Europe (Mennan and Isik, 2004; Moss et al., 2007; Bertholdsson, 2012). *P. indica* has a wide range of hosts which might include weeds as well. If *P. indica* was as beneficial to weeds as to wheat, it could make weed control more difficult, or increase the damage done by weeds; alternatively, it might increase the competitiveness of wheat against some species or in some settings, which would be useful in managing herbicide resistant weeds. Also, the spread of *P. indica* might have side-effects outside arable fields.

In this study the following hypotheses were tested: *P. indica* would survive the UK weather and soil conditions; *P. indica* would not affect the composition of the bulk soil or root-zone microflora; and *P. indica* would be as beneficial to weeds as to wheat.

2. Materials and methods

2.1. P. indica survival and viability experiment

The utility of mRNA and DNA measurements as indicators of viability of *P. indica* was determined by performing RT-PCR and PCR on heat and cold treated pure cultures of *P. indica*. For this purpose, *P. indica* was obtained from Dr. Patrick Schafer, Warwick University, UK (originally from German Collection of Microorganisms and Cell Culture, strain number DSM 11827) and mycelia was grown on complex modified *Aspergillus* liquid medium (CM medium) (Pham et al., 2004) and incubated on an orbital shaker at 140 rpm at room temperature (21 ± 1 °C) for two weeks. Samples were then kept at 80 °C in a hot water bath for 6 h, then stored at −80 °C for 6 h, one and four weeks. After storage, separate samples of mycelia were transferred to potato dextrose agar to check whether they would grow and used for RNA and DNA extraction followed by RT-PCR and PCR respectively. This experiment was repeated to confirm the results.

P. indica survival in the soil under UK weather conditions was tested in different soil types based on either the soil series or textural classification and each soil was under a different crop management. The soils were collected from the Reading University Farm at Sonning (grid ref: SU76187547). These were (1) a Clay Loam (CL) of the Neville series, from an area under winter barley which had previously been under winter wheat; (2) a Sandy Clay Loam (SCL) of the Sonning series from an area under ryegrass at the time and for the previous two years; (3) a Loamy Sand (LSO) of the Rowland series, under organic management, from an area under faba bean cultivation; (4) a Loamy Sand (LS) of the Rowland series, under non-organic management, from an area under ryegrass cultivation. The experiment was carried out between December 2013 and March 2015 at the University of Reading, under outdoor weather conditions. Six pots (3 L, top diameter: 18 cm, bottom diameter: 14 cm, depth: 15 cm) were filled with each soil. Five out of six pots received 4 g of liquid culture of *P. indica* inoculum containing an unquantified mixture of chlamydospores and mycelium and mixed thoroughly with the soil. The control pot received 4 g of sterilised liquid culture of *P. indica* inoculum. The pots were placed in holes with the tops level with the surrounding soil level to make temperature fluctuations realistic. Around 50 g of each soil type was collected, with a small core (diameter: 12 mm, depth: 8 cm) from the middle of pots, at three and half months (mid-March 2014), 8 months (end of July 2014) and 15 months (end of March 2015) after inoculation with *P. indica*. When collecting the samples, they were kept in a cool box on ice and transferred immediately to −20 °C before DNA and RNA were extracted and PCR or RT-PCR performed. Maximum and minimum temperatures of soil in the pots were recorded every 2 days by a digital thermometer placed in the centre of one of the pots.

2.2. Soil community composition

To examine whether *P. indica* affects other soil microorganisms, wheat was grown in 3 L pots containing one of two soil types, SCL or LSO, as above. Winter wheat seeds, cv. Battalion, were surface disinfected by rinsing for 2 min in 20 mL L^{-1} sodium hypochlorite (Fisher Scientific UK Ltd, UK), followed by three rinses in sterilised distilled water, and germinated on damp filter paper in a Petri dish at room temperature (21 ± 1 °C) under natural indoor light for 48 h. No micro-organisms grew from a sample of seeds so treated and placed on PDA plates for one week. Pre-germinated seeds were planted into 3 L pots (one seed per pot). This experiment had a 2 × 2 × 4 factorial combinations of ±*P. indica* × two soil types × four harvesting points, with two replications completely randomised. The pots were incubated at temperatures ranging between 15 and 25 °C; humidity and light were not controlled. Inoculation with 4 g liquid culture of *P. indica* mixed with soil was done at the time of sowing. Root and soil samples were collected at 2, 4, 6 and 8 weeks after inoculation (wai) for DNA extraction, PCR and DGGE analysis, as below. Samples were transferred and stored as described above.