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Impact of *Rhizophagus irregularis* MUCL 41833 on disease symptoms caused by *Phytophthora infestans* in potato grown under field conditions

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ABSTRACT

In organic potato production in Europe, only copper-based fungicides allow to directly control *Phytophthora infestans* (the causal agent of late blight). Due to environmental concerns caused by the repeated and excessive use of Cu before the nineties, the EU legislation has promoted alternatives approaches such as the use of biocontrol agents (e.g. arbuscular mycorrhizal fungi – AMF). Here, two field trials were conducted over two climatic-contrasting growing seasons. Trial 1 was characterized by a dry and hot cultural season with low pressure of *P. infestans*, while trial 2 was conducted under high humidity and relatively low temperatures with high pressure of the pathogen. In both trials, sprouted potato tubers were inoculated with AMF in the greenhouse before transplanting to the field. A Real-Time quantitative PCR assay was designed to target the inoculant strain *Rhizophagus irregularis* MUCL 41833 as well as the native *Rhizophagus irregularis* strains. In both trials, the inoculated AMF was detected in the roots at harvest, demonstrating the capacity of the inoculated strain to incorporate the microbiome of the potato plants. In the first trial, disease severity in AMF pre-colonized potato plants was markedly decreased and the onset of late blight symptoms was delayed by 10 days. In contrast, in the second trial no differences were noticed between AMF pre-colonized and control plants. In both trials, no mycorrhizal effect was noticed on tuber yield. As a conclusion, disease severity of *P. infestans*, measured by symptoms development on leaves, was decreased in AMF pre-colonized plants under conditions of low pressure of late blight and over a short period of time, while under conditions more adequate to the pathogen, no reduction in symptoms was noticed.

1. Introduction

Potato is the fourth most important crop produced in Europe and the fifth worldwide (FAO, 2014). In Belgium, it occupies 80000 Ha, with Bintje representing around 50% of the surface (FIWAP, 2010). Hundreds of varieties are produced worldwide. However, because of their low genetic base, most varieties are susceptible to many devastating pests and diseases (Consortium, 2011).

Phytophthora infestans, the causal agent of late blight, is the most devastating potato pathogen worldwide (Haas et al., 2009). Damages caused by this Oomycota as well as the measures to control the disease accounts for more than 1 billion € per year in Europe (Vos and Kazan, 2016). This pathogen is a hemibiotroph that behave as a biotroph during the early stages of potato infection turning to a necrotroph in the later stage (Gallou et al., 2011). Genome sequence and analyses showed rapid turnover and extensive expansion of secreted disease effector proteins that alter host physiology (Haas et al., 2009).

Late blight control is mostly achieved via the repeated (10–16 per growing season) applications of fungicides (Haverkort et al., 2009), which may have serious side-effects on the environment and human health (Beketov et al., 2013; Wilson and Tisdell, 2001). Copper application has been used intensively in pest management since the discovery of its effects on diseases by the end of the 19th century. However, the repeated application of Cu at doses 50%–100% higher than the admitted norm have resulted in Cu accumulation in soils with significant impacts on below-ground organisms (Brun et al., 1998; Graham et al., 1986). In 1991, the EU issued a directive (Regulation No. 2092/91 – (Regulation, 1991) limiting the use of Cu in organic farming to

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6 kg/ha per year. In 2009, another directive (the EU directive 2009/128/CE – (directive, 2009) established a framework to achieve a sustainable use of pesticides by reducing the risks associated with their use, particularly on human health and environment. This directive promotes the use of integrated pest management and of alternative approaches or techniques such as the application of nature-based compounds (NBCs) or biological control agents (BCAs). To replace Cu-based fungicides, NBCs including plant extracts or BCAs such as Trichoderma spp., arbuscular mycorrhizal fungi (AMF) or Bacillus spp. have been tested and were shown effective against *P. infestans* or other *Phytophthora* species under laboratory or greenhouse conditions (Dorn et al., 2007).

Arbuscular mycorrhizal fungi are obligate root symbionts that form associations with an estimate of 80% of terrestrial plant species (Smith and Read, 2008), including most crops such as potato, wheat, maize. They improve plant mineral nutrition (especially phosphorus) and thus growth and yield. They also play major roles in protecting the plants against biotic and abiotic stresses (Parniske, 2008; Smith and Read, 2008).

Several studies have reported an increasing resistance of AMF-colonized plants against above and below-ground pathogens (Whipps, 2004). The effects were either local (at the site of infection of the pathogen) (Hayek et al., 2014; Trotta et al., 1996) or systemic (Cordier et al., 1998; Khaosaad et al., 2007) and mostly concerned root pests and diseases. The impact on above-ground pathogens were less numerous and conclusive (Pozo and Azcón-Aguilar, 2007), even if systemic effects have been reported on tomato against *Fusarium sambucinum* or *Alternaria solani* (Ismail and Hijri, 2012; Song et al., 2010) and on potato against *P. infestans* (Gallou et al., 2011). Most studies were conducted under controlled conditions (in the greenhouse or *in vitro*). Results in the field are less abundant and more controversial due among others to the competition with the indigenous microorganisms that could impact the establishment and development of the AMF inoculants (Berruti et al., 2015).

Rhizophagus irregularis is amongst the most widely used AMF in commercial products (Buysens et al., 2017). It is a ubiquitous, generalist symbiont (Berruti et al., 2014) that can colonize a wide variety of plants, including potato (Buysens et al., 2016). Large scale experiments conducted in Canada showed an increase in yield of marketable potato tubers in plants inoculated with *R. irregularis* DAOM 197198 (Hijri, 2016). However, in most studies, the authors could not separate root colonization by the inoculant from the indigenous AMF and thus could not firmly attribute the increased yield to the introduced fungus.

To identify closely related members of the same AMF species, the mitochondrial genome is a promising target (Badri et al., 2016). It was used to relate crop health improvement to inoculants in large scale field experiments (Badri et al., 2016; Börstler et al., 2008; Nadimi et al., 2016). Development of specific molecular markers for field application is particularly interesting when the strain has already shown promising result on *P. infestans* in controlled conditions (Gallou et al., 2011).

Molecular toolkits, based on mitochondrial genome, were developed on spore samples (Badri et al., 2016) and for field assays (Buysens et al., 2017). They are, to the best of our knowledge, the only one-step PCR molecular analysis for AMF strain traceability. During the field experiment of Buysens et al. (2017), *R. irregularis* MUCL 41833 was inoculated on potato and traced via the mitochondrial Large SubUnit (mtLSU). The inoculated AMF strain could be detected but at low levels and no significant difference in total root colonization was noticed between the control (i.e. non-inoculated) and inoculated potato plantlets. This was probably related to various factors among which the mode of inoculation, the viability/infectivity of the introduced isolate, the compatibility of the AMF with the soil environment, host plant or agricultural practices (e.g. frequency of fungicides application) (Buysens et al., 2017; Loján et al., 2017).

The aim of the present study was to evaluate the effects of *R. irregularis* MUCL 41833 on the development of late blight in potatoes grown under field condition. Potato tubers were pre-inoculated in the greenhouse and subsequently transferred to the field. Although, we are aware that this method of inoculation offers few perspectives for potato field production, it allows to ascertain the resilience of the inoculant (i.e. *R. irregularis* MUCL 41833) in the field (i.e. its capacity to develop within roots and to compete with the local AMF community). A Cu-based fungicide was use as positive control. The AMF root colonization was estimated visually and by using mitochondrial markers specific to the inoculated haplotype or to the local *R. irregularis* species in the field, while symptoms of late blight were rated on the leaves. Trials were conducted over two climatic-contrasting growing seasons.

2. Materials and method

2.1. Biological material

Solanum tuberosum cv. Bintje and Nicola (susceptible and semi resistant to foliar late blight, respectively) were provided by the “Station de Haute Belgique”, Libramont (Belgium). Tubers (size 28–35 mm) were placed in a growth chamber set à 20 °C during 15 days to break dormancy.

Seeds of *Medicago truncatula* Gaertn. cv. Jemalong A 17 (SARDI, Australia) and *Zea mays* L.cv. ES. Ballade (Euralis, France) were surface-disinfected following Gallou et al. (2012) and further pre-germinated on wet paper. The maize seedlings were used for mass production of the AMF (IJdo et al., 2011).

The AMF strain *Rhizophagus irregularis* (Błaszk., Wubet, Renker & Buscot) Schüßler and Walker (2010) as [‘irregulare'] MUCL 41833 was provided by the Glomeromycota *in vitro* collection (GINCO: http://www.mycorrhiza.be/ginco-bel) on the Modified Strullu-Romand (MSR) medium (Declerck et al., 1998). Pieces of gel containing AMF-colonized roots and spores were inoculated on tubers of Bintje in 1.4 L pots containing a mix of sterile sand/vermiculite in equal volume, for AMF mass-production. Plants were grown in a greenhouse (25 °C, 75% Relative Humidity (RH) and 16/8 h (day/night) photoperiod under natural light conditions) for 4 months. They received Osmocote® (NPK (Mg): 17/11/10 (2) at regular intervals.

2.2. Pre-mycorrhization of potato tubers

AMF-colonized roots were sampled from the potato plants, chopped in 1 cm fragments and placed in a 4 L container (40 × 60 × 10 cm, ETS Dubois Frère S.A, Belgium) between two layers of sterile sand/vermiculite in equal volume. Pre-germinated seedlings of maize were planted on top of the inoculum layer and covered by 2 cm of sterile sand/vermiculite mix and watered 2 times per week with deionized water. The containers were subsequently placed in the greenhouse (25 °C, 75% RH, 16/8 h (day/night) under natural light conditions) for 1 month. Control containers without AMF were identically set up. Pre-germinated seedlings of *M. truncatula* were transferred in the containers hosting the AMF-colonized or control maize plants for fast and homogenous colonization. The plants received 6.5 g Osmocote® (NPK 17/11/10) and were co-cultured for 6 weeks.

The pre-germinated *M. truncatula* plantlets were colonized within a period of 6 weeks. The plantlets were subsequently transferred into compressed peat pots (6 cm Diam., ref: 306 122 90, Jiffy®, Netherland) filled with sterile mix of sand/vermiculite in equal volumes. Each pot received three AMF-colonized *M. truncatula* plantlets and one sprouted potato tuber (Sup Fig. 1). The systems were maintained 4 weeks under the same greenhouses conditions as above for pre-mycorrhization of the potato plantlets before manual transplantation into the plots. A similar procedure was followed with non AMF-colonized *M. truncatula* plantlets for the control plantlets.