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# Endophytic *Metarhizium brunneum* mitigates nutrient deficits in potato and improves plant productivity and vitality

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ABSTRACT

There is growing evidence that entomopathogenic fungi play a role in plant growth promotion as colonizers of internal plant tissues. However, little is known about their potential to mitigate plant abiotic stress. Here, we investigated the influence of soil fertility on *Metarhizium brunneum* strain CB15 endophytism levels in potato plants linked to plant productivity and vitality. By application of encapsulated *M. brunneum*, the endophytism level, i.e. % presence of fungus in plant tissue, increased significantly under nutrient poor conditions. This correlated with significantly improved quantum yields of photosystem II. In addition, water use efficiency was increased with higher levels of *M. brunneum* endophytism. Furthermore, biomass, leaf area, nitrogen and phosphorus contents were enhanced indicating fungal nutrient mobilization and transfer. Our results provide first evidence for the role of entomopathogenic fungi in mitigating nutrient deficits in soil by improving plant productivity and vitality which may increase their use in plant protection strategies.



## 1. Introduction

Crop productivity is impaired by a variety of abiotic and biotic stresses, such as low nutrient availability, drought, diseases or insect herbivores (Pandey et al., 2017). To meet the demands of a growing population, the resilience of crops to these stresses has to be increased to ensure high productivity in less favorable environments (Pereira, 2016).

Limitations of nitrogen and phosphorus are widely observed in crop ecosystems worldwide (Elser et al., 2007). Nowadays, high-yield agriculture, therefore, depends on nitrogen- and phosphorus-rich fertilizers which are commonly applied in large amounts (Leitch et al., 2017). A thus improved nutrient status of crops not only enhances productivity but can also increase plant resilience to other stresses, by e.g. enhancing their water use efficiency (Sheriff et al., 1986) which is beneficial considering dwindling fresh water supplies and high susceptibility of many crops to drought (Hatfield et al., 2001). However, nutrient-use efficiencies in fertilized crops may be low with only 30–50% of nitrogen and about 45% of phosphorus being metabolized by the plants with excess nutrients having negative impacts on ecosystems and economy (Tilman et al., 2002).

Plant root associations with soil microorganisms, such as mycorrhizal fungi, are often observed in natural environments. These typically mutualistic relationships can improve the nutrient status of plants by fungal transfer of limiting soil nutrients, such as nitrogen and phosphorus, to the plant (Parniske, 2008; Smith and Read, 2008). This has often been shown to increase plant growth and resilience to abiotic and biotic stresses such as drought, soil pathogens or herbivores (Gange and West, 1994; Augé, 2001; Sikes et al., 2009). Particularly in nutrient poor soils, mycorrhizal fungi can substantially improve the nutritional status of plants by extending their mycelial network to a widespread surface area and by mobilizing inaccessible nutrients to plant roots (Goltapeh et al., 2008).

While the symbiosis between mycorrhizal fungi and plants has been investigated extensively, only a few studies provide insights

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into symbiotic relationships between entomopathogenic fungi and plants. Generally known as insect pathogens and used in biological pest control (Lacey, 2016) fungi, such as *Metarhizium* spp., can be naturally occurring endophytes in plants (Behie et al., 2015; Murphy et al., 2015), colonizing internal plant tissues without causing visible plant symptoms (Schulz and Boyle, 2005). It has been shown that endophytic *Metarhizium* spp. can transfer insect-derived nitrogen to host plants (Behie et al., 2012; Behie and Bidochka, 2014). In exchange for nitrogen, the plant seems to provide *Metarhizium* spp. with carbon from plant photosynthates (Behie et al., 2017). As a result of these associations, plants showed increased growth in several studies (Kabaluk and Ericsson, 2007; García et al., 2011; Sasan and Bidochka, 2012; Liao et al., 2014; Greenfield et al., 2016; Jaber and Enkerli, 2016, 2017). In addition, *Metarhizium* spp. have been implicated in mitigating salt stress on soybeans (Khan et al., 2012) and iron deficiency in sorghum (Raya-Díaz et al., 2017), and these effects may be linked to the level of endophytism. Recently, we provided evidence that *Metarhizium brunneum* endophytism may be improved by fungal encapsulation in polymer beads (Krell et al., 2017) and by incorporation of enzymes (Krell et al., 2018).

In the present study, we created different levels of *M. brunneum* strain CB15 endophytism in potato plants by application of non-formulated and encapsulated mycelium. We looked into the influence of soil fertility on endophytism levels linked to plant productivity and vitality reflected by data on biomass, leaf surface area, quantum yields of photosystem II, $CO_2$ assimilation, stomatal conductance, water use efficiency and nutritional status of plants. We hypothesized that *M. brunneum* endophytism would be enhanced in plants grown in nutrient poor conditions resulting in improved plant beneficial effects by fungal-mediated nutrient transfer to plants.

## 2. Materials and methods

### 2.1. Materials

All chemical compounds used in this study were acquired from Carl Roth GmbH (Karlsruhe, Germany) and concentrations are given as (w/w), unless stated otherwise.

### 2.2. Fungal biomass and plant material

*M. brunneum* strain CB15 was obtained from Prof. Dr. Stefan Vidal (Agricultural Entomology, Department for Crop Science, Georg-August-University Goettingen, Germany). Aerial conidia were produced on Sabouraud dextrose agar (SDA, 2.0% glucose, 1.0% peptone from casein and 1.8% agar) in the dark at 25 °C. Plates were sealed with Parafilm M (Pechiney Plastic Packaging Inc., IL, USA) to avoid moisture loss. *M. brunneum* was cultured in liquid medium at 25 °C to give rise to finely dispersed mycelium as previously described (Krell et al., 2017). After 48 h, mycelium was harvested and sterile filtrated through a 400 μm sieve and rinsed with 0.9% NaCl solution to remove blastospores and residual cultivation medium.

Plant experiments were conducted with non-treated seed potatoes ranging from 40 to 55 g fresh weight (*Solanum tuberosum* cv. Bintje grown in Groningen, the Netherlands, Ellenberg's Kartoffelvielfalt GbR, Barum, Germany). Prior to planting, tubers were washed with tap water and surface-sterilized for 2 min in 7% sodium hypochlorite followed by 2 min in 70% ethanol and three subsequent rinses in ultrapure water (Elix Advantage Water Purification System, Merck Millipore, Darmstadt, Germany). Samples from the final rinse were plated on SDA to verify the effectiveness of surface sterilization.

### 2.3. Encapsulation

Beads were prepared according to Krell et al. (2018). Briefly, a suspension of 2% amidated pectin (1% Amid AU-L, batch No. 01702147 and 1% Amid CU-L, batch No. 01702148, Herbstreith & Fox KG, Neuenbuerg/Wuertt, Germany), 20% sterile native corn starch (Maisita, Agrana Beteiligungs-AG, Vienna, Austria), 2% cellulose (Avicel RC-591 NF, IMCD, Cologne, Germany, batch No. 110240) and 2% heat-inactivated baker's yeast (*Saccharomyces cerevisiae* strain H205, VITAL-AROM, Deutsche Hefewerke GmbH, Nuremberg, Germany) were mixed on ice. Prior to bead formation, 1 $Ug^{-1}$ cellulase (from *Aspergillus niger*, Sigma-Aldrich, Taufkirchen, Germany) and 1.5% *M. brunneum* mycelium were added to the suspension which was afterwards gently stirred for 1 min. Beads were prepared by dripping the suspension into a cold sterile 2% $CaCl_2$ solution using a syringe with cannula (2.1 × 0.8 mm Sterican, B. Braun Melsungen AG, Melsungen, Germany). After 20 min, beads were separated and washed with ultrapure water. Moist beads were dried at 30 °C for 3 d over silica gel to a final water activity of ≤0.2. Water activities were controlled with a water activity meter (LabMASTER-aw, Novasina AG, Lachen, Switzerland) at 25 °C.

### 2.4. Plant inoculation and growth conditions

Potato tubers were pre-sprouted in the dark until shoots reached a length of approx. 3 mm. Sand was sterilized at 120 °C for 1.5 h and filled into pots with a volume of 3 L (Nitsch & Sohn GmbH & Co. KG, Kreuztal, Germany). The following treatments were applied to pots using ten replicates each: (1) non-treated control tubers, (2) 0.14 g moist non-formulated *M. brunneum* mycelium in 500 μL 0.9% NaCl, (3) 3 g dry beads without mycelium, and (4) 3 g dry beads with 1.5% mycelium. Tubers were placed on top of the applied treatment. Moist mycelium was chosen for application of non-formulated mycelium since drying without encapsulation may cause substantial loss in fungal viability (Krell et al., 2017).

Plant experiments were conducted in nutrient poor and nutrient rich conditions with ten replicates per treatment. Pots in the nutrient poor treatment were watered daily with 50 mL deionized water and did not receive fertilization. Pots in the nutrient rich treatment received 50 mL of a modified Hoagland fertilizer solution (Hoagland and Arnon, 1950) three times a week after first shoots were visible above sand surface. When not being treated with fertilizer solution, pots received 50 mL deionized water instead. Hence, daily watering of all pots was ensured. The full concentration of the fertilizer solution contained 5 mM $KNO_3$, 2.5 mM $Ca(NO_3)_2$, 1 mM $(NH_4)_2SO_4$, 2 mM $NH_4HPO_4$, 2 mM $MgSO_4$, 1 mM KCl, 2 mM NaCl, 2 mM $Na_2SO_4$, 1 mM $FeC_6H_5O_7$, 0.025 μM $H_3BO_3$, 0.002 μM $MnSO_4$, 0.002 μM $ZnSO_4$, 0.005 μM $CuSO_4$, 0.005 μM $MoO_3$ (pH 6.5). Fertilizer application was adapted according to the growth stage of the plants. For the first four fertilizer applications, the solution was diluted 4-fold followed by a 2-fold dilution for the subsequent three applications. Afterwards, the full concentration was applied until the end of the experiment. Homogeneous distribution of water and fertilizer solution was ensured.

Plants were grown in a greenhouse at 18–23 °C with a light-dark cycle of 16 h:8 h and a relative humidity of ~60% for 35 days thereby reaching BBCH 14–16 (Hack et al., 1992). Photosynthetically active photon flux density (PPFD) ranged between 150 and 160 μmol $m^{-2}$ $s^{-1}$. To avoid effects of microclimatic variations due to pot position, plants were randomly rearranged once a week.

### 2.5. Assessment of endophytism

Endophytism was assessed as described in Krell et al. (2018). Briefly, *M. brunneum* was re-isolated from plant tissues and further