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From laboratory to field: applying the Fo47 biocontrol strain in potato fields

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Abstract Endophytic microbes conferring biocontrol are an eco-friendly alternative to control diseases in crops. Unfortunately, the use of endophytes to control diseases is not yet widespread as their application in agricultural settings is challenging and the outcome variable. Translating strains that perform well under laboratory conditions to the field poses several challenges. One is large scale inoculum production in a cost-effective manner. Here, we developed a framework to scale up inoculum production of *Fusarium oxysporum* 47 (Fo47), assess inoculum viability and its performance in the field and effects on potato yield and performance. The Fo47 endophyte is a well-described biocontrol agent, isolated from disease suppressive soils in the 1980's. Using mung bean medium, we could routinely produce $\approx 7 \times 10^8$ spores/mL. Using 60 mL of 10^7 spores/mL per tuber we could re-isolate the fungus 79 days after application from 60 to 70% of the inoculated plants in a large-scale potato field trial (Clenze, Germany). Furthermore, this protocol can be used to assess Fo47 biocontrol potential under field conditions. The presence of the fungus did not negatively affect plant yield or starch production and did not increase susceptibility to endemic pathogens.

Keywords Fo47 · Potato · Biocontrol · Field experiment

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Introduction

Many phylogenetically-diverse soil-inhabiting fungi are harmless endophytic colonizers of plant roots. Over the last four decades, endophytes have drawn the attention of the scientific community as some strains enhance plant fitness and increase resilience to pathogens and abiotic stresses, thereby potentially reducing pesticide dependency (Alabouvette 1986; Busby et al. 2016; Ghorbanpour et al. 2018; Latz et al. 2018).

Among the diverse microbiota, *Fusarium oxysporum* (Fo) is ubiquitously present in soils. Fo is infamous for causing vascular wilt diseases in over 100 different crops (Edel-Hermann and Lecomte 2019). In fact, Fusarium wilt diseases rank among the most devastating diseases, constituting a significant agricultural threat (Dean et al. 2012; Fisher et al. 2012). However, most Fo strains are saprotrophs and not able to cause disease. Notably, wilt-disease suppressive soils carry root-

colonizing Fo endophytes that confer biocontrol against pathogenic Fo strains (Alabouvette 1986). Upon sterilization of these soils their biocontrol capacity is lost, but this can be reconstituted by supplementing Fo strains (Tamietti et al. 1993). Biocontrol of Fo pathogens has been reported to be a universal feature of non-pathogenic Fo strains (Bao et al. 2004) and even avirulent Fo pathogens can reduce susceptibility of the host to virulent Fo pathogens (Biles and Martyn 1989; Huertas-Gonzalez et al. 1998; de Lamo et al. 2020). Fo47 is the best studied biocontrol-conferring Fo strain and has originally been isolated from wilt-disease suppressive soils in Châteaurenard (Alabouvette 1986). Fo47 does not promote plant growth, but can reduce susceptibility to vascular fungal pathogens including Fo (Alabouvette et al. 2009; Aimé et al. 2013; de Lamo and Takken 2020) and *Verticillium dahliae* (Veloso and Díaz 2012; Veloso et al. 2016) and to non-vascular pathogens such as root-infecting oomycetes *Pythium ultimum* in cucumber (Benhamou et al. 2002) and *Phytophthora capsici* in pepper (Veloso and Díaz 2012). The mechanisms employed by Fo47 and other Fo to confer biocontrol are proposed to consist of two components; a direct activity against the root pathogen through mycoparasitism, antibiosis and competition for nutrients and root niches (Benhamou et al. 2002; Alabouvette et al. 2009; Le Floch et al. 2009), and an indirect activity by inducing a root-specific plant-mediated resistance response termed endophyte-mediated resistance (EMR) (de Lamo and Takken 2020).

Fo47 has been reported to reduce susceptibility to root pathogens in various Solanaceae such as tomato (Aimé et al. 2013; de Lamo et al. 2018; Constantin et al. 2019), pepper (Veloso and Díaz 2012; Veloso et al. 2016) and eggplant (Pantelides et al. 2009; Zhang et al. 2018). The Solanaceae family embraces plant species of striking relevance to humans as food source (pepper, tomato, eggplant or potato), ornamentals (petunia) or drugs (tobacco) (Kimura and Sinha 2008). Within the Solanaceae, potato is one of the few crops that can be cultivated in open fields in northern Europe. Therefore, a potato field trial was performed in Clenze (Germany) with the ultimate goal of developing a reproducible methodology to test the marketable potential of Fo47 as a biocontrol agent. Traits such as cost-effective spore production, durability and stability upon storage or tolerance to the changing environment of fields are important for biocontrol agents (Spadaro and Gullino 2005). Here, we set out to develop cost-effective large-scale production of Fo47 spore formulations, and to assess resilience of the fungus during storage and in the field. We assessed its ability to colonize potato plants under agricultural conditions in the absence or presence of fertilizer, and we monitored the impact of endophytic colonisation on crop performance.

As a result, we developed a robust method to: (1) cost-effectively mass-produce Fo spores for potato field experiments, (2) assess the viability of the generated spores, (3) inoculate potato mother tubers in the field, (4) monitor successful Fo endophytism under field conditions, and (5) measure tuber yield, starch content and diseases. We propose this method to test and validate the suitability of Fo endophyte use in crops, such as potato. Fo47 successfully colonized field-grown potato without negatively affecting crop performance.

Materials and methods

Media used to assess spore production

Different growth media were tested to assess Fo spore production. As reference NO_3 medium (0.17% Yeast Nitrogen Base without amino acids or $(NH_4)_2SO_4$, 3% sucrose and 100 mM KNO_3) was used for Fo propagation (Gawehns et al. 2014). As alternatives, brown rice, mung bean and polenta were tested and for media preparation 0.4 g or 1 g of the above-mentioned products were suspended in 100 mL miliQ water and autoclaved for 20 min at 120 °C in a 250-ml Erlenmeyer flask sealed with a cotton plug and an aluminium cap. The hence produced medium was used without further treatment.

Inoculum preparation

Fo47 (Alabouvette 1986) was grown on potato dextrose agar (PDA) plates for at least 5 days. From these plates, agar plugs from the edge of the colony containing the youngest mycelium were used to inoculate 11.5 L of 1% mung bean medium. Thereto 250-mLflasks containing 100 mL of medium, two 1-L flasks containing 0.5 L, two 2-L flasks containing 1 L and three 5-L flasks containing 2.5 L were inoculated. The medium containing 10 g of intact mung beans/L was autoclaved at 120 °C for at least 20 min at a pressure > 220 kPa. After 6 days of shake-incubation at 150 rpm at 25 °C the cultures were poured through a sterile filter consisting

of a single layer of Miracloth (Millipore) to remove mycelia. The obtained microconidial spore suspension was spun down at 700×*g* for 10 min. in a Beckman centrifuge with a JA-10 fixed-angle rotor. The pellet containing Fo47 spores was washed with sterile MilliQ water, and after a second centrifugation step re-suspended in 1 L MilliQ water.

Biological materials and inoculation procedure

The above-mentioned concentrated Fo47 spore suspension was quantified by a counting chamber, subsequently gently diluted to 10^9 spores/mL by adding MilliQ water and stored for 1–7 days at 4 °C until field or greenhouse application. For the small-scale greenhouse experiments tubers were planted and inoculated by pouring 60 mL of water (mock) or Fo47 spores (10^7 spores/mL) in the planting hole. The tuber was subsequently covered with soil. After 42 days, samples from the thickest stem (up to the second leaf), mother tuber and root were harvested from each plant to monitor *in planta* presence of the endophyte. For the field trial pathogen-free seed potatoes of the starch potato cv. Jasia (www.saatzucht-niehoff.de/) were planted in agricultural soil in Clenze (Germany). Inoculum was prepared in the field by adding 50 mL of 10^9 spores/mL to 5 L of tap water, which was gently shaken resulting in 10^7 spores/mL inoculum. Each seed potato was inoculated with 60 mL of the latter inoculum by using a ladle.

For tomato assays, Fol007 was inoculated from glycerol stock to PDA plates and grown for at least 5 days at 25 °C. Agar plugs were used to inoculate 100 mL 1% mung bean medium after which the cultures were incubated in the dark at 25 °C and 150 rpm for 5 days. Cultures were filtered through Miracloth (Calbiochem) and diluted to generate a microconidial inoculum of 10^7 spores/mL (de Lamo et al. 2018). Inoculation of 10-days-old tomato seedlings was done by pouring 10, 25 or 50 mL of 10^7 spores/mL in soil-filled pots with a diameter of 12 cm containing the plantlets.

Field fertilization and pesticide application

Some plots were fertilized by adding 120 N kg/ha (7% nitrate, 7% ammonium, 14% urea) and 160 K kg/ha Korn-Kali 40, 40% K_2O, 6% MgO once at planting time. The following pesticides were applied: Monceren® (active compound: pencycuron) against *Rhizoctonia*, Boxer® (active compound: prosulfocarb) against insects such as Colorado Beetles and Infinito® (mixture of fluopicolide and propamocarb-hydrochloride), Banjo forte® (mixture of fluazinam and dimethomorph) and Shirlan® (fluazinam) against *Phytophthora*.

Fungal re-isolation

To check whether Fo47 acted as an endophyte, i.e. was able to colonize inner tissues of potato stem pieces, peels from mother tubers or root slices were surfaced-sterilized by submergence in 70% ethanol for 3 min. Followed by a subsequent wash with sterile water as described (Constantin et al. 2019). The potato pieces were placed on PDA plates supplemented with 200 mg/L streptomycin and 100 mg/L penicillin. These antibiotics were added to prevent bacterial growth without affecting fungal outgrowth. Four days after incubation at 25 °C, mycelia emerging from the plant material that resembled Fusarium was harvested and gDNA was isolated from the mycelium using phenol:chloroform extraction as described (van Dam et al. 2018). To assess whether mycelial outgrowths corresponded to Fo47, PCR was done using Fo-specific *FEM1* (PF: ATGAAGTACACTCTCGCTAC; PR: GGTGAAAG TGAAAGAGTCACC) or Fo47-specific *SCAR* primers (PF: CCTCAACTTCTGATTTAAATATGA; PR: GAGCGAACAACTACAATAAAAG) (Edel-Hermann et al. 2011). When a PCR reaction was positive for both *FEM* and *SCAR* the mycelium was considered to be Fo47.

Mid-term sampling

Seventy-nine days after tuber inoculation with Fo47, one plant per plot was collected to assess Fo47 colonization. From each plot, the plant from the second column and second row was sampled and stored in cool conditions until further analysis (Fig. S1c). Fo47 colonization in the mother tuber, the lateral root and a stem emerging from the mother tuber was monitored. The thickest stem emerging from mother tuber was surface sterilized as described above and two cross-sections of stem sections from the basal (region of contact with the tuber) and crown level were placed on PDA plates to assess Fo47 outgrowth. Additionally, one piece of a lateral root was sterilised and incubated on PDA plates

to assess fungal outgrowth. Confirmation of Fo47 identity was done by PCR as described above.

Experimental layout and harvest

The field was divided into 675 plots (Fig. S1a, b). One part of the field was non-fertilized (plots 1–405) while the other was fertilized by applying N and K (plots 406–675) (Fig. S1a, b). Each plot contained four rows of 10 potato plants each (40 plants in total) (Fig. S1c). A treatment consisted of inoculating nine randomly distributed plots (Fig. S1b) with 60 mL of water (mock) or Fo47 spores (10^7 spores/mL to each potato tuber. Only the two central rows of each plot were harvested. The peripheral two rows were not harvested to avoid edge effects (Fig. S1c). In total, 19 plants/plot were harvested as one was collected during the mid-term sampling to assess Fo47 colonization. The tubers from the two middle rows were used to measure yield, and a subset of those were used to analysed starch content and *Rhizoctonia* disease incidence. For scoring black scurf disease symptoms caused by *Rhizoctonia*, tubers were washed to remove dirt and to enable detection of sclerotia. Starch content was measured according to the commission regulation (EC) No 2235/2003. Data were analysed by performing a Mann-Whitney test through the software PRISM 7.0 (GraphPad).

Results

Mung bean medium yields high concentration of viable Fo microconidia

To identify an easy-to-produce and cost-effective medium that allows high Fo47 microconidia yields, three different media were compared to a refence broth (Fig. 1a). As reference, NO_3 medium was used, as this is the standard medium for Fo propagation in our lab (Gawehns et al. 2014). However, its relatively high cost is prohibitive for mass-scale spore production and field application. As alternative C/N sources to grow the fungus, brown rice, mung beans and polenta were tested, as these products are relatively cheap and readily available in supermarkets. Brown rice retains the bran layer that contains micronutrients such as manganese and iron, which favour sporulation of some fungi (Michal Johnson et al. 2011; Li et al. 2012). Mung beans are well-known as a suitable media for growing Fo such as banana-infecting Fo f.sp. *cubense* strains (Bai and Shaner 1996; Garcia-Bastidas et al. 2019). Polenta was taken along as it has been used to propagate microbes (Kocic-Tanackov et al. 2019).

Six days after inoculation, all 1% *w/v* media were observed to produce a higher number of Fo spores than those containing 0.4% w/v (Fig. 1a). Mung bean broth was found to be the most efficient medium yielding $\approx 7 \times 10^8$ spores/mL, followed by NO_3 ($\approx 4.8 \times 10^8$ spores/mL), 1% brown rice ($\approx 1.6 \times 10^8$ spores/mL) and 1% polenta ($\approx 5.3 \times 10^7$ spores/mL). This result led to selection of mung bean medium for large-scale spore production. Next, viability and infectivity of spores produced in mung bean medium was assessed. Since plant colonisation by an endophyte is laborious to assess and quantify, as it requires isolation of roots and monitoring the amount of fungal biomass, the tomato pathogen Fol007 was used instead. Initial host colonisation by this isolate is similar to that of the Fo47 endophyte (de Lamo and Takken 2020), but the advantage of using a pathogenic isolate is that wilt disease symptoms can be monitored as a proxy for host colonization (Gawehns et al. 2014). Fusarium wilt disease symptoms of 10-days-old tomato seedlings were assessed 3 weeks post inoculation (Fig. 1b). Except for the mock, wilt disease symptoms were observed for all inoculations showing that the Fo inoculum is viable and able to infect tomato (Fig. 1b**)**. In summary, mung bean medium is a cost-effective and high spore-yielding medium allowing large scale production of viable Fo inoculum.

Fo47 colonizes potato plants under green house and field conditions

To determine whether Fo47 spores produced in mung bean medium can colonize potato plants and tubers, a small-scale experiment was performed in the greenhouse. Tubers were inoculated with water (mock) or Fo47 spores (10^7 spores/mL) and after 42 days, samples from the thickest stem (up to the second leaf), mother tuber and root were harvested from each plant to monitor *in planta* presence of the fungus. Samples were surfaced-sterilized and Fusarium resembling mycelia emerging from the plant tissues was harvested. To confirm identify of this mycelium as Fo47, gDNA was isolated followed by PCR with Fo-specific *FEM* primers, and Fo47-specific *SCAR* primers. Four out of seven plants treated with Fo47 spores showed fungal outgrowths that were both *FEM*- and *SCAR*-positive,

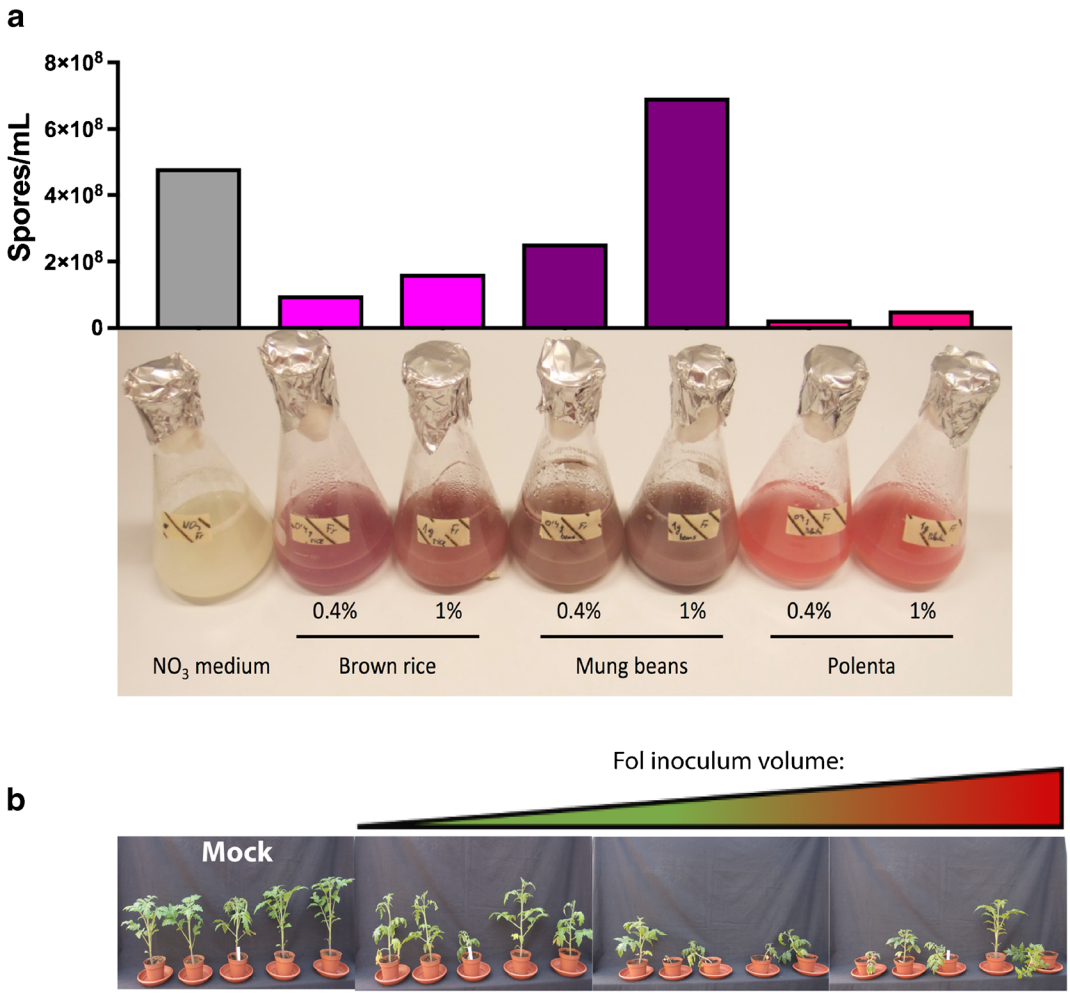

Fig. 1 Mung bean medium yields high concentration of Fo spores. **a** Microconidia concentration of Fo47 inoculated in different media was measured with a counting chamber. Six-days-post-inoculation 1% mung bean medium was found to contain the highest microconidial concentration followed by the commonly used NO_3 medium, brown rice and polenta. **b** Viability and infectivity of Fol spores produced using mung bean medium was assessed using a tomato bioassay. Different volumes (0, 10, 25 and 50 mL of 10^7 spores/mL from left to right) were added to the soil containing 10-day-old tomato plants

showing that they are effectively colonized by Fo47 (Fig. 2a). These data show that pouring Fo47 microconidia (harvested from mung bean medium) onto tubers is an effective method to inoculate potato plants.

Based on these observations, the viability of applying an endophyte at large scale was tested in a field experiment. Common agriculture practices in the region where the field was located include applying fertilization to increase tuber yield. Therefore, to test the influence of fertilization on endophytic performance, part of the field remained unfertilized while the other was treated by applying N and K fertilization (Fig. S1a, b). Each treatment, mock or Fo47 inoculation, was performed in nine randomly selected plots (Fig. S1a, b). From each plot, one potato plant was harvested 79 days after inoculation from the same location in the plot (Fig. S1c). The main root below the thickest stem and one potato tuber per plant were surface-sterilised to determine the presence of the Fo47 inside the plant (Fig. 2b). Unlike in the green-house experiment, mycelium resembling Fo grew out of five of the nine mock-treated tubers. However, PCR analysis showed that it was Fo (*FEM*-positive) but not Fo47 (*SCAR*-negative). In contrast, Fo47 could be re-isolated from seven and six of the nine sampled plants from inoculated non-fertilized and fertilized plots, respectively (Fig. 2b). These data show that

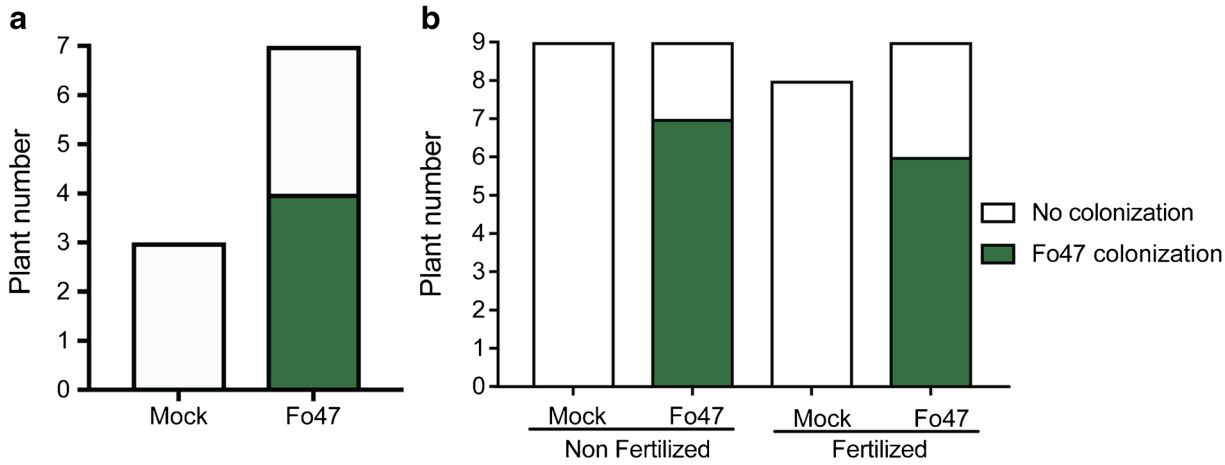

Fig. 2 Fo47 is able to colonize greenhouse- and field-grown potato plants. Each potato tuber was treated with 60 mL inoculum that contained either water (Mock) or Fo47 (10^7 spores/mL). **a** Stem, roots and tuber were collected 42 days after inoculation of greenhouse-grown potato plants or **b**) roots and tubers of field-grown potato were harvested 79 days after inoculation. Samples were surfaced-sterilized for 3 min in 70% ethanol, washed and arranged on PDA plates with antibiotics. Four days after plating, gDNA was isolated from the emerging mycelia and used for PCR reactions with *SCAR* and *FEM* primers. Nine plants per treatment were analysed except for the mock-fertilized treatment where only eight plants were analysed. Fo47 was considered as an endophytic colonizer when mycelia emerging from surface-sterilized root, stem or tuber from one biological sample could be confirmed by PCR with *FEM* and *SCAR* primers

fertilization does not affect endophytic colonization of potato plants by Fo47 and that our laboratory protocol can be efficiently scaled up to field conditions.

Tuber weight and starch content are not affected by Fo47 colonization unlike fertilization treatment

To assess whether Fo47 has an impact on potato production, the yield and starch content of the tubers from the two central rows of every plot were harvested 5 months after inoculation (Fig. S1c). Non-fertilized water (mock)- and Fo47-inoculated plots yielded 28.7 kg and 30.3 kg on average, while applying fertilizer increased the yield significantly to 35.1 kg in the mock plots and 35.0 kg in the Fo47-inoculated plots (Fig. 3a). In contrast, the starch content was significantly reduced in fertilized plots as mock and Fo47-inoculated plots without fertilization yielded 21.2% and 20.8% starch while fertilized plots yielded 20.0% and 19.6%, respectively (Fig. 3b).

Altogether, Fo47 did not negatively affect tuber yield nor the starch content, despite being a potato endophyte (Fig. 3a, b) and regardless of the application of fertilizer. As expected, fertilization significantly increased the tuber yield regardless of a Fo47 treatment (Fig. 3a).

Black scurf disease symptoms on potato tubers are reduced by fertilization but not by Fo47 treatment

To assess whether Fo47 colonisation of roots affects the susceptibility of potato plants to endogenous pathogens, 20 tubers were selected randomly per plot. Visual inspection of the tubers revealed symptoms of scab disease caused by *Streptomyces spp.* and black scurf caused by *Rhizoctonia solani*. Disease symptoms associated with Fusarium spp. where not observed on the tubers. Scab symptoms were omitted for scoring due to the difficulty of consistently assessing the disease level – hence only black scurf symptoms were assessed. A disease index from 0 to 4 was established based on the area covered by sclerotia relative to the total tuber area (Fig. 4a). Fo47 treatment did not cause a significant reduction in black scurf disease symptoms compared to mock treatment in either fertilized or non-fertilized plants (Fig. 4b). However, application of fertilizer reduced black scurf disease symptoms in both mock and Fo47-treated plants (Fig. 4b).

Discussion

Presently, public perspectives and legislation are encouraging the use of alternatives to chemical pesticides

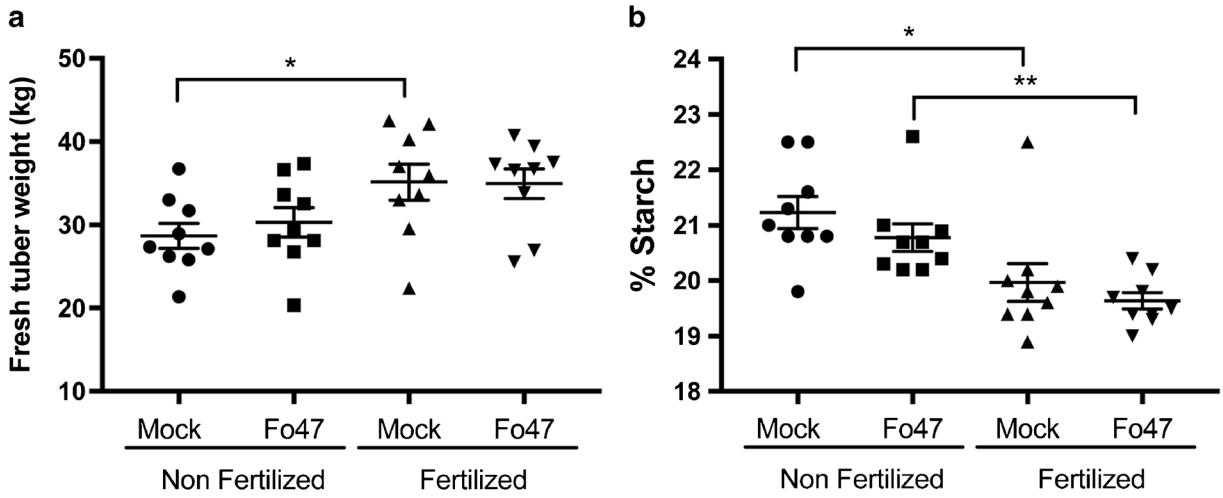

Fig. 3 Potato yield and starch content is affected by fertilization but not by Fo47 application. **a** Average tuber yield per plot in each treatment. Every dot represents the yield of a single plot (19 plants each) **b**) Average percentages of starch accumulated in 5 kg of tubers (Ø > 6.5 cm) per treatment. Each dot represents a plot. Data was analysed using a Mann-Whitney test where $*P < 0.05$ and $**P < 0.01$

and fertilizers in agriculture. Fungal endophytes have been successfully implemented as biocontrol or biostimulant agents in small scale set-ups such as greenhouses (Gill et al. 2016; de Lamo and Takken 2020). However, one stumbling block in field implementation of endophytes as biocontrol agents is a cost-effective protocol for inoculum production and inoculation. Here, we established a cheap, large-scale production method to obtain viable Fo47 microconidia and we confirmed plant colonisation of the endophyte under field conditions.

All media tested (NO_3, mung beans, brown rice and polenta) were suitable for producing Fo47 microconidia, with mung bean being the most effective; yielding ≈7×10^8 spores/mL. This yield is comparable to the ≈9×10^7 Fo spores/mL previously described using this medium, (Garcia-Bastidas et al. 2019), showing that this method is reproducible across different laboratories. Moreover, this medium is not limited to Fo, but can also be used to obtain large amounts of spores from other fungi such as *Rhizopus* (Nout et al. 1987). Additionally, we could show that mung bean medium-produced microconidia are "infective" in bioassays by scoring disease symptoms (Fig. 1a), and by being able to re-isolate the endophytic strain from inoculated potato plants (Fig. 2a, b). When comparing NO_3 medium with mung bean medium, the latter is not only faster and easier to make, but also is approximately 180 times cheaper (1 L of NO_3 costs 8.5 euros while 1 L of mung bean costs 0.0478 euros). Therefore, mung bean medium is a good candidate for scaling-up effective spore production of fungal endophytes with a low production cost.

Another important aspect in our protocol consisted of determining the effectiveness of Fo47 as an endophyte. This is an important step to predict efficiency of a biocontrol agent and pin-point possible negative outcomes (e.g. no protection due to low endophytic colonization levels). In our field experiment, single Fo47 application at planting time resulted in 60–70% of the plants scoring Fo47 positive at mid-harvest. Fertilization treatment which consisted of N and K, did not affect Fo47 colonization of potato plants, showing that, unlike arbuscular mycorrhizal fungi (Ortas 2012), common management practices are compatible with Fo47 field application. Notably, Fo47 could not be re-isolated from newly formed tubers at the final harvest, despite being detectable in 20% of the tubers during mid-harvest (data not shown). This shows that the fungus is not transmitted to the final product and probably has to be re-applied every season.

Three other important parameters for potato tuber marketability were assessed: yield, starch and disease susceptibility in this study. Fo47 treatment did not affect potato tuber yield and starch content showing that there was no penalty of endophytic colonization. When assessing disease symptoms, however, only scab and black scurf disease symptoms could be observed. This low disease incidence is most likely due to the unusually hot and dry conditions during the

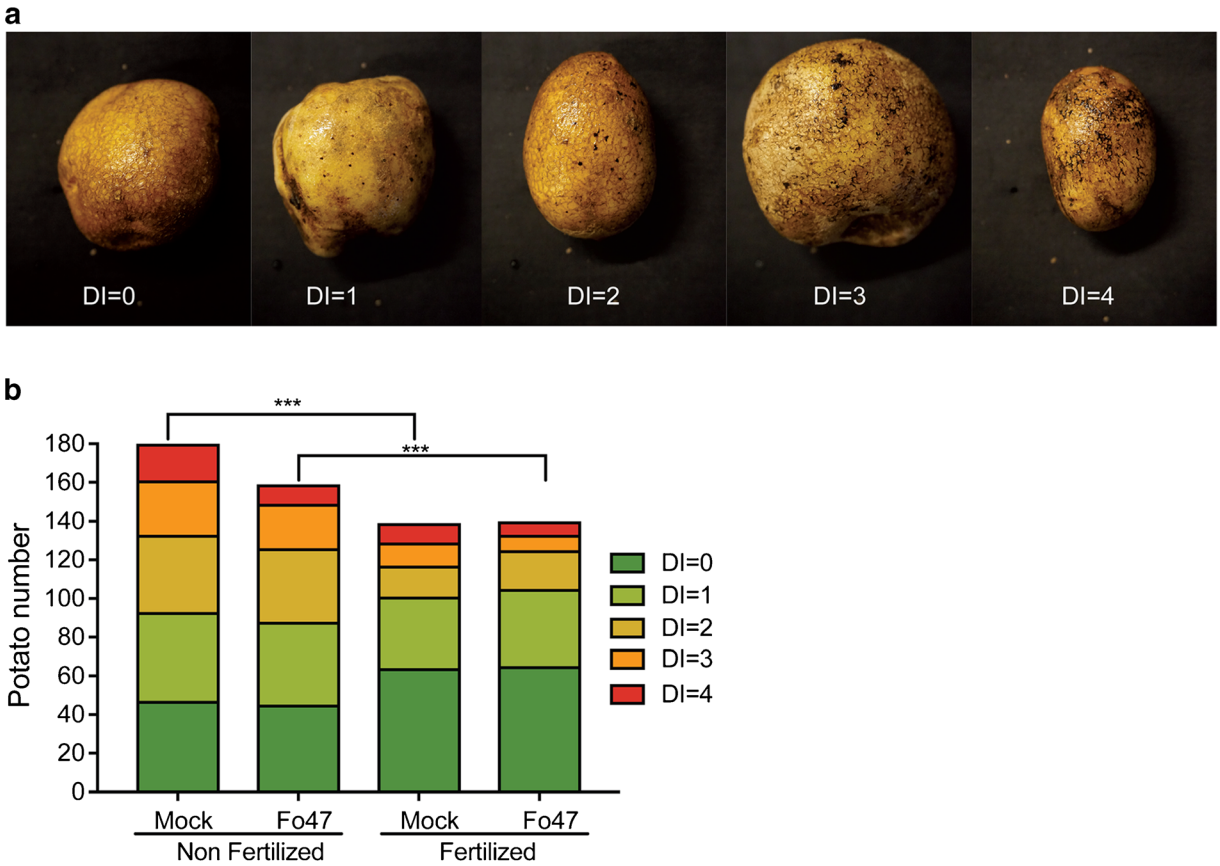

Fig. 4 Fertilization, but not Fo47 treatment, reduces black scurf disease symptoms on potato tubers. **a** Representative pictures of potato tubers showing black scurf disease symptoms from scale 0–4. Based on the percentage of the tuber covered by sclerotia, the following scale was used: DI = 0, less that 1%; DI = 1, 1% of the tuber covered by sclerotia, DI = 2, up to 5% of the tuber covered, DI = 3, up to 10%; DI = 4, sclerotia covers more than 15% of the tuber area. **b** Black scurf disease symptoms on newly formed potato tubers. From each plot 20 tubers were selected randomly. Data was analysed using a Mann-Whitney test where $****P < 0.001$. DI = disease index. Abbreviations: DI: disease index

growing season of the field trial in 2018. Therefore, we could only show that Fo47 did not affect disease susceptibility to black scurf disease.

Fertilization treatment which consisted of addition of N and K, increased potato yield but negatively affected the starch content. K and N treatment have been reported to decrease the starch content of tubers (Westermann et al. 1994) and our data indicate that the yield increase by K and N fertilization is not related to an increase of total starch, but likely due to an increase of the water content in the tuber as also proposed by others (Schippers 1968). Additionally, fertilization reduced black scurf disease symptoms on the assessed tubers. This is in line with previous observations that reported an inverse correlation between black scurf disease symptoms and N concentration (Rêbarz and Borowczak 2007; Klikocka 2009).

In summary, we developed and validated an easily scalable method to produce and apply fungal endophyte spores to crops. This method is of relevance for further exploring the capacity of fungal biocontrol agents in the field. For example, it could be used to test the ability of Fo47 (or other Fo endophytes) to suppress Fusarium wilt disease in crops such as tomato or asparagus, where Fo endophytes were shown to confer protection under laboratory conditions (de Lamo and Takken 2020). For further commercialization of fungal endophytes, parameters such as shelf-life stability should be tested in future research. As a proof of concept, we here applied Fo47 spores to potato tubers and observed no negative affect on yield or starch content of the treated plants. Colonisation of the plants by Fo47 was unaltered by common fertilization practices in the region and did not result in increased susceptibility to endogenous pathogens, such as black scurf disease.

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Author contribution MEC, FJDL, FLWT and MR designed the experiments. MEC, FJDL carried out the experiments and performed the data analysis. MEC, FJDL, FWT wrote the manuscript. MR gave intellectual input and critically revised the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest. The research did not involve Human Participants and/or Animals, hence informed consent is not applicable.

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