

Preventive field application of *Metarhizium brunneum* in cover crops for wireworm control

Lara Reinbacher^{a,b,*}, Sven Bacher^b, Fionna Knecht^a, Christian Schweizer^a, Tanja Sostizzo^a, Giselher Grabenweger^a

^a Agroscope, Ecological Plant Protection in Field Crops, Research Division Plant Protection, 8046, Zurich, Switzerland

^b University of Fribourg, Department of Biology, Unit of Ecology and Evolution, Fribourg, Switzerland

ARTICLE INFO

Keywords:

Metarhizium brunneum
Agriotes
Wireworms
Entomopathogenic fungi
Biological control
Field application
Cover crops

ABSTRACT

Wireworms, the larvae of click beetles (Coleoptera: Elateridae), can cause substantial losses in marketable yield of potatoes, yet control options are limited. The entomopathogenic fungus *Metarhizium brunneum* (Ascomycota; Hypocreales) isolate ART2825 is highly virulent against two of the most detrimental wireworm species, *Agriotes obscurus* L. and *A. lineatus* L., but field application of this isolate during potato cultivation has never succeeded. In this study, we integrated the fungus into the agricultural crop rotation prior to potato cultivation, with the aim of better adapting the application strategy to the fungus' ecological and environmental requirements. Application preceded sowing of cover crops in late summer. We hypothesized that higher temperatures and undisturbed development for several months would support the establishment of the entomopathogen and enhance biocontrol efficiency in the following season. In two subsequent seasons, we quantified (1) fungal establishment in the soil, (2) efficiency of treated soils against wireworms in vitro, and (3) levels of wireworm damage in field potatoes. Spore concentration was enhanced in treated plots and we recovered the released *Metarhizium* isolate from all mycosed, field-collected wireworms. Treated soils increased wireworm mortality in the laboratory, but a statistically significant reduction of potato damage was only achieved in two out of ten field trials. The application strategy shows potential for fungal enhancement and opens new avenues for biological wireworm control.

1. Introduction

Wireworms, (Coleoptera: Elateridae), are common in many habitats worldwide. While the majority of wireworm species spend their larval period mostly unnoticed in the soil or in decaying wood, a few have received attention as pest species feeding on subterranean plant parts of agricultural crops (Traugott et al., 2015). For example, in potatoes, although wireworms do not cause quantitative losses, they can lead to a substantial reduction in potato quality by feeding on and tunneling through the tubers (Parker and Howard, 2001).

Wireworm monitoring and control is particularly challenging. Unlike foliage-feeding insect pests, wireworms have a concealed lifestyle. Their multiannual life cycles and capacity to move vertically in the soil make them highly intractable targets (Vernon and van Herk, 2013). Currently, no reliable chemical or biological plant protection products are available for farmers for wireworm control (Veres et al., 2020). Agronomists are therefore actively seeking innovative solutions that are compatible

with both global health and pest-control standards (Benjamin et al., 2018; Khan and Ahmad, 2019; Poggi et al., 2021).

Entomopathogenic fungi (EPF), particularly *Metarhizium brunneum* (Ascomycota: Hypocreales), have been proposed as a promising tool against wireworms (Milosavljević et al., 2020; Ritter and Richter, 2013). Nevertheless, field efficacy of EPF is often insufficient. Previous studies have identified challenges to field application, including the choice of fungal isolate and its compatibility with environmental conditions (Kabaluk and Ericsson, 2007a; Reddy et al., 2014), the importance of temperature (Antwi et al., 2018) and high soil moisture (Kabaluk et al., 2007; Sharma et al., 2019) linked with soil structure (Brandl et al., 2017; Ensafi et al., 2018), slow speed of kill (Reddy et al., 2014), and the difficulty of reaching wireworms in deeper soil layers (Sufyan et al., 2017). These challenges can be addressed through basic research on fungal isolates to select promising strains and by applied research focusing on formulation and application strategies.

For isolate selection, Ravensberg (2011) provides an overview of

* Corresponding author. Reckenholzstrasse 191, CH, 8046, Zurich, Switzerland.

E-mail address: lara.reinbacher@agroscope.admin.ch (L. Reinbacher).

<https://doi.org/10.1016/j.cropro.2021.105811>

Received 4 June 2021; Received in revised form 23 August 2021; Accepted 26 August 2021

Available online 2 September 2021

0261-2194/© 2021 Published by Elsevier Ltd.

criteria that can guide a structured search for efficient biocontrol agents. These include bioassays regarding the prevalent environmental conditions in the intended application period and area, as well as decision making based on the isolate's speed of kill. To start the selection process, culture collections can be an abundant source for fungal isolates (Humber, 2016). Besides selection, adaptation is another possibility to enhance the tolerance to environmental stress, either by altering conidia production methods (Rangel et al., 2015) or by genetic modification (Lovett and St. Leger, 2018). This approach, however, has not yet been implemented into agricultural practice.

In field trials with wireworms, several authors have tested a range of isolates simultaneously to find suitable candidates (Antwi et al., 2018; Kabaluk et al., 2005; Kölliker et al., 2011; Reddy et al., 2014; Sharma et al., 2019). Some rely on endemic fungal isolates found on the target insect (Hettlage, 2019; Kabaluk et al., 2005, 2007; Kölliker et al., 2011), as they are more likely to grow and cause infections under the relevant conditions (Paixão et al., 2019). The majority of studies include or are restricted to already registered biocontrol agents like *M. brunneum* F52 (Antwi et al., 2018; Brandl et al., 2017; Kabaluk and Ericsson, 2007b; Knodel and Shrestha, 2018; Reddy et al., 2014; Sharma et al., 2019; Tharp et al., 2007), *Beauveria bassiana* ATCC 74040 (Kölliker et al., 2011; Ladurner et al., 2009; Sufyan et al., 2017) or *B. bassiana* GHA (Sharma et al., 2019). While the importance of diligent isolate selection is well known, the inclusion of registered isolates is often not solely based on their high potential, but rather due to the high demands of the registration process for new biocontrol agents (Humber, 2016), possibly leading to lower success rates.

Applied research on application and formulation strategies for wireworm biocontrol has mainly targeted approaches to facilitate contact between pathogen and insect. This has been attempted by focusing application (spot or band applications) on areas close to plant roots where wireworm damage is expected (Brandl et al., 2017; Reddy et al., 2014; Sufyan et al., 2017; Tharp et al., 2007) or by actively luring wireworms with an attract-and-kill approach. Attractive carrier materials such as millet grain (Sharma et al., 2019), carbon dioxide-releasing beads (Brandl et al., 2017) or germinating seeds, in formulations of the fungus as seed coating (Kabaluk et al., 2007), have been tested as bait. Seed treatment has also been used to encourage the fungus to grow as an endophyte to further spread the inoculum to vulnerable plant areas (Kabaluk and Ericsson, 2007b).

A second, more general strategy is to modify environmental conditions to better suit fungal requirements, for example through irrigation (Sharma et al., 2019). In this study, rather than modifying the conditions and generating additional labor or costs, we propose to shift the application time into a period of the agricultural crop rotation that we presume to be more suitable for EPF survival and infection. We therefore elaborated the approach of preventive EPF treatment by Rogge et al. (2017). In a semi-field experiment in pots, they applied EPF during sowing of spring oat, and showed EPF persistence for several months and decreasing levels of wireworm survival with increasing EPF concentration in the soil.

Our study is the first on-farm implementation of this approach. We used a fungal isolate, *Metarhizium brunneum* isolate ART2825, previously recommended for wireworm control (Eckard et al., 2014). We hypothesized that field application of EPF in late summer during the sowing period of the winter cover crop preceding potatoes would provide favorable environmental conditions for fungal persistence. This would increase the prevalence of wireworm infection with the fungal pathogen, establish a fungal population in place and thereby decrease the importance of fast speed of kill, and ultimately enhance potato quality.

2. Material & methods

2.1. Fungal isolate and conidia production

The fungal isolate used in this study, *M. brunneum* isolate ART2825, originates from an infected *Agriotes obscurus* L. (Coleoptera: Elateridae) larva from the rearing facility of Agroscope, Switzerland in 2008 (Kölliker et al., 2011). Conidia were harvested from *A. obscurus* cadavers in the stock culture and single-spore isolates were produced on a selective medium for entomopathogenic fungi (Strasser et al., 1996) to provide starting material for mass production as fungus-colonized barley kernels (FCBK).

In 2017/18, FCBK were produced in autoclavable polyamide bags following a modified protocol based on Aregger (1992). Conidia were collected by rinsing petri dishes with 0.1% Tween®80. The fungus was propagated in sterilized corn steep medium (aqueous solution containing 2.84% sucrose, 1.89% corn steep, 0.36% Na₂HPO₄, 0.21% KH₂PO₄). Husked barley was autoclaved twice before inoculation with the liquid culture. Polyamide bags were sealed and incubated at 25 °C for two weeks. Barley kernels were regularly moved by kneading the bags to prevent clustering. Bags were stored at 4 °C until further use. In 2018/19, FCBK were produced in a solid-state fermenter by the Zurich University of Applied Sciences (ZHAW).

The number of conidia per gram FCBK was determined by shaking samples in 0.1% (v/v) aqueous Tween®80 and counting the resulting conidia densities with a haemocytometer. The suspensions were then adjusted to 1×10^6 spores/ml to evaluate germination rates of the spores. Three 50 µl drops of this suspension were applied on solid complete medium (3.5 mM KH₂PO₄, 10 mM Na₂HPO₄, 13.4 mM KCl, 2.4 mM MgSO₄ 7H₂O, 8.8 mM NH₄NO₃, 0.5% yeast extract and 1% glucose) (Riba and Ravelojoana, 1984) and incubated in darkness at 22 °C, 70% RH. The germination rate was calculated 24 h post inoculation by counting 100 spores for each drop at 400 × magnification. Conidia were considered as germinated if the germ tube was at least the length of the spore itself. Germination rates in all FCBK used were above 95%.

2.2. Wireworms

Wireworms (*A. obscurus*) used for artificial infestation and laboratory trials originated from the laboratory livestock established according to Kölliker et al. (2009). Larvae used in the experiment were approximately one year old. Before the experiment, wireworm fitness was assessed according to van Herk and Vernon (2013) and only larvae that showed normal movement were included.

2.3. Field sites

Field trial sites were selected in Switzerland and Austria for their natural infestation with wireworms. Prior to the experiments, five holes (0.25 m², 40 cm deep) were dug in each field and the soil examined for wireworms. Only sites with a natural infestation of at least two *Agriotes* wireworms/hole were selected for the field trials, which corresponds to an infestation level of about 8 wireworms/m². In Zurich, an artificial infestation was established by releasing 40 (2017/18, Zurich 1) and 100 (2018/19, Zurich 2) wireworms per 27 m² plot, 25 days before starting the experiment. In both years, experiments started in August and lasted a whole year, ending with the potato harvest in late summer.

In total, the study involved 10 field sites, two on the research station Agroscope Reckenholz and eight on working farms, three of which are organic farms that managed fields in accordance with the requirements of the organic farming association Bio Suisse (Table 1).

2.4. Experimental design and application procedure

All trials had a completely randomized design with 6–9 replicates per

Table 1

Overview of wireworm field trials in Switzerland (CH) and Austria (AT). Soil parameters of field sites for texture characterization analyzed by Eric Schweizer AG (Thun). Weather indices for the field trial period (day of FCBK application to day of potato harvest) generated from air temperature (2 m above ground, mean \pm standard deviation) and daily rainfall acquired from neighboring (within 30 km radius) meteorological stations of the SMI (Swiss Meteorological Institute) and the ZAMG (Zentralanstalt für Meteorologie und Geodynamik, Austria). For detailed information on soil and climatic conditions, see supplementary materials.

Location	Year	Plot size (m)	Replicates	Soil texture (FAO, 2006)	Temperature average [°C]	Precipitation sum [mm]	Reference treatment
Colombier (CH)	2017/18	3 × 10	8	Clay loam	11.6 ± 7.9	1044	Ephosin (AI: Chlorpyrifos)
46°33'N 6°28'E Morges (CH)		3 × 10	8	Loam	11.6 ± 7.9	1044	Ephosin (AI: Chlorpyrifos)
46°29'N 6°27'E Tägerig (CH)		3 × 9	8	Loam/Sandy loam	10.7 ± 8.5	884	Ephosin (AI: Chlorpyrifos)
47°24'N 8°16'E Vichtenstein (AT)		9 × 10	8	Sandy loam	11.6 ± 8.6	698	Belem 0.8 MG, (AI: Cypermethrin)
48°32'N 13°39'E Worb (CH)		3 × 10	6	Loam	9.4 ± 8.4	887	Organic farm, no synthetic insecticide
46°56'N 7°34'E Zurich 1 (CH)		3 × 9	6	Clay loam	10.5 ± 8.5	788	Ephosin (AI: Chlorpyrifos)
47°25'N 8°32'E Geschinen (CH)	2018/19	3 × 9	9	Sandy loam	5.3 ± 9.8	1387	Organic farm, no synthetic insecticide
46°30'N 8°17'E Mülchi (CH)		3 × 10	8	Loam	9.1 ± 8.0	705	Ephosin (AI: Chlorpyrifos)
47°10'N 7°47'E Rüeterswil (CH)		3 × 9	9	Loam	7.8 ± 7.9	1329	Organic farm, no synthetic insecticide
47°15'N 8°59'E Zurich 2 (CH)		3 × 9	6	Clay loam	11.2 ± 8.3	1116	Ephosin (AI: Chlorpyrifos)
47°25'N 8°32'E							

site. In addition to the fungus treatment and negative control, a synthetic insecticide treatment was added as a reference except on organic farms. Choice of insecticides depended on the registered product in each country (Table 1). Individual plot size was adapted to the local sites to allow at least 4 potato rows (between row distances 0.75 m) with a length of at least 9 m (see Table 1). Fungus treatments were applied onto the prepared seedbed as FCBK immediately before sowing of the winter cover crop in August. FCBK were manually distributed on the plot at a rate of 5×10^{13} conidia/ha (2017/18) or 10^{14} conidia/ha (2018/19) and immediately incorporated with a rotary harrow (depth 6 cm). The soil was cultivated in the same way on control and insecticide plots. Following EPF application, cover crops were sown on all plots (74% *Avena strigosa*, 16% *Trifolium alexandrinum*, 7% *Guizotia abyssinica*, 3% *Phacelia tanacetifolia*, 32 kg/ha). Insecticides were applied in the following year at the time of potato planting in April according to registered indications.

2.5. Colony forming units (CFU) of *Metarhizium* spp. in soil samples

CFU counts in the soil were assessed before and two months after application, as well as eight months after application shortly before potato planting. Abundance of the fungus in the soil was assessed by counting the number of CFUs per gram of soil as described in Kessler et al. (2003). A composite sample was prepared by taking five randomly positioned soil samples per plot with a soil core borer (diameter 6 cm, depth 6 cm) and mixing thoroughly. The water content of each soil sample was measured gravimetrically. Three sub-samples of 20 g per plot were suspended and dispersed on selective medium (Strasser et al., 1996). Petri dishes were incubated at 22 °C and 70% RH in the dark. CFUs were counted after 2 weeks and numbers of CFUs per gram soil substrate (dry weight = dw) were calculated. For analysis, the median of the three Petri dishes from each plot was used. The effect of fungus application was quantified as the difference in colony forming units/g soil (dw) before (sampling date = August) and after application (sampling dates October, $\Delta\text{CFU}_{\text{October}} = \text{CFU}_{\text{October}} - \text{CFU}_{\text{August}}$, and April, $\Delta\text{CFU}_{\text{April}} = \text{CFU}_{\text{April}} - \text{CFU}_{\text{August}}$). Field sites Vichtenstein and Worb

were ploughed before the third soil sampling and were thus not included in $\Delta\text{CFU}_{\text{April}}$.

2.6. Laboratory tests of wireworm mortality with field soil

Soil samples of 100 g were collected from each plot, pooled per treatment and field site, and thoroughly mixed in a plastic container. Plastic cups (volume 90 cm³) were filled with 30 g dry weight of the mixed soil. Artificial soil containing 74% industrial sand, 20% kaolin clay, 5% peat and 1% calcium carbonate was used as control (OECD, 2016). Artificial soil was either left untreated or enriched with *M. brunneum* ART2825 conidia (3.3×10^6 spores per cup, corresponding to field application rates) and moistened to 50% of its maximum water holding capacity.

Wireworms were kept individually in cups covered with gauze to prevent escape. Carrot slices were provided as food and replaced weekly. Groups of 30 cups were randomly assigned to plastic boxes (20.5 × 30.5 × 9 cm) and stored in a climate chamber in darkness at 22 °C, 70% RH. Wireworm mortality was assessed weekly for a period of 9 weeks.

2.7. Wireworm species identification and damage evaluation

In the 2018/19, wireworm species composition from the three field sites was assessed prior to potato planting. Four soil samples (18.5 × 18.5 × 20 cm) per plot were examined for wireworms. *Agriotes* species were identified morphologically based on mandible angles, presence of setae, granules on thoracic sternites between coxae and characteristics of 9th abdominal segment (Furlan et al., 2021; Lehmus and Niepold, 2015). Soil temperatures at the sampling time ranged from 8 °C to 11 °C and soil moisture content (volume) from 27% to 33% at 15 cm depth. Wireworms were kept individually in moist peat with potato slices as food source at 22 °C, 70% RH to quantify mycosis over 9 weeks. *Metarhizium* mycosis was scored when individuals showed intersegmental outgrowth of white mycelium and formation of green conidia layers. *Metarhizium* genotypes were determined for all mycosed wireworms

using a simple sequence repeat marker analysis following the protocol of Mayerhofer et al. (2019), in comparison with a *M. brunneum* ART2825 reference isolate.

Wireworm damage was evaluated according to the European and Mediterranean Plant Protection Organization (EPPO) standard PP1/46 (EPPO, 2005) by sampling 100 tubers per plot randomly taken from the two inner potato rows (BBCH 99; Hack et al., 1993). Tubers were differentiated into undamaged (no wireworm feeding visible) and damaged (one or more wireworm feeding holes visible).

2.8. Statistical analyses

All statistical analyses were performed using R (version 3.6.1; R Core Team, 2019). Differences in CFUs per gram soil substrate (Δ CFU) after two and eight months were analyzed with a linear mixed model fit by REML using the package “lme4” (version 1.1–23; Bates et al. (2015)) with fungus application (yes/no) as a fixed effect and the field trial site the random effect. Δ CFU was cube root-transformed to meet the assumption of normality of residuals of the model. The two years (2017/18; 2018/19) were analyzed separately. To compare the two sampling dates after application, a linear mixed model was fitted with cube root-transformed total numbers of CFUs as response variable, fungus application, month of sampling and their interaction as fixed

effects and field trial site as random effect.

Effects on mortality (larvae alive vs. dead in week 8) of *A. obscurus* in the laboratory trial were tested with a generalized linear mixed-effects model fitted by the Laplace approximation and assuming a binomial distribution of errors. Fixed effects were fungus treatment (yes/no) and origin of soil (field site/artificial), and the random effect was year.

Effects of treatments on potato quality were also tested with a generalized linear mixed-effect model fitted by the Laplace approximation. Occurrence of wireworm damage on the potatoes ($n = 100$) was the dependent variable and assumed to be binomially distributed with the two groups potatoes damaged and potatoes undamaged as a matrix connected with the function “cbind()”. Treatment (FCBK, insecticide, untreated control), wireworm density and their interaction as well as years were included as fixed factors. Wireworm density was included to control for variation among sites in background wireworm density and was estimated indirectly from the mean damage in control untreated plots of each site. For this, a weighted damage per plot was calculated as the sum of potatoes with no wireworm holes $\times 0$, 1 to 2 holes $\times 1.5$, 3 to 5 holes $\times 4$ and more than 5 holes $\times 8$ from a total number of 100 potatoes. Wireworm density was scaled for the analyses and location of the field trial site was included as a random factor. Additionally, generalized linear models were built for individual field sites with potato damage (binomially distributed) as dependent variable and treatment as fixed

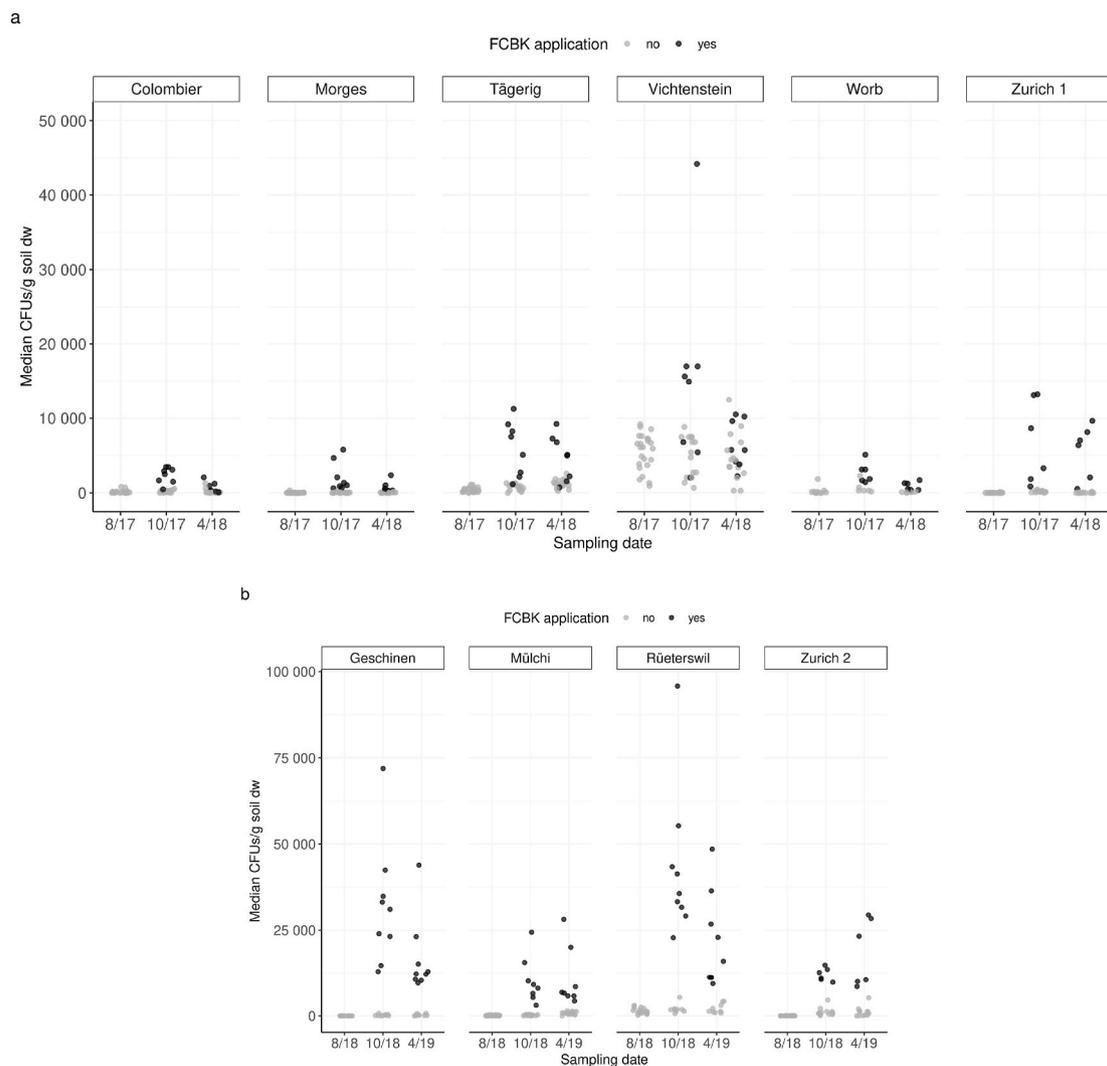


Fig. 1. Median CFUs of *Metarhizium* spp. per gram dry weight soil for fields treated in 2017/18 (a) and 2018/19 (b). Samples were collected in August before FCBK application, and after FCBK application in October, and in April prior to potato planting ($n = 8$ plots at each site and sampling date, except for Worb and Zurich 1/2 $n = 6$, Geschinen and Rüeterswil $n = 9$).

factor.

3. Results

3.1. Occurrence of *Metarhizium* at field sites

Natural *Metarhizium* populations were found in all fields in August except the Geschinen site. The majority of sites showed total *Metarhizium* CFU values between 0 and 3034 CFU/g soil dw (median = 54), with particularly high values in Vichtenstein (948–9162 CFU/g soil dw, median = 6114) (Fig. 1).

Application of *Metarhizium* strongly increased the total numbers of *Metarhizium* CFUs detected in the soil after two months (Table 2) in all year-sites (Fig. 1). Overall, the median increase in treated plots after two months in 2017/18 was 2578 CFU/g dry weight soil (untreated plots increased by 56 CFU/g dry weight soil over the same period). In 2018/19, the application rate was doubled and the median increase after two months was 22522 CFU/g dry weight soil in fungus-treated plots (untreated plots 188 CFU/g dry weight soil).

Eight months after application, fungus-treated plots still showed an increase in *Metarhizium* CFUs (Table 3) for all year-sites. Total amounts of CFUs overall did not substantially differ between the two sampling dates but decreased over winter in the fungus-treated soils (Table 4).

3.2. Laboratory virulence test using field soil

Wireworm mortality showed a strong increase after 63 days (Fig. 2) in soils with FCBK application compared to soils from untreated plots ($z = 8.79, p < 0.001$). Compared to artificial soil, where the influence of other microorganisms should be low and soil texture is standardized, mortality of wireworms was lower in soil from two field sites, Morges ($z = -2.88, p = 0.004$) and Mülchi ($z = -3.26, p = 0.001$).

3.3. Species distribution

The majority of individuals found at all three sites sampled in 2018/19 belonged to the genus *Agriotes* (Table 5); the sites Geschinen and Rüeterswil were dominated by *A. obscurus*, while wireworms found in Mülchi mostly belonged to the species *A. sputator*. At Mülchi, none of the wireworms incubated showed signs of *Metarhizium* infection; this was in contrast to wireworms from the other sites. All mycosed wireworms originated from FCBK plots and belonged to the species *A. obscurus*. All *Metarhizium* spp. recovered from cadavers were identified as the isolate used for the application, *M. brunneum* ART2825.

Table 2

Determinants for difference in total numbers of *Metarhizium* CFUs in soil two months after fungus application ($\Delta\text{CFU}_{\text{October}}$) in treated and untreated plots estimated in a linear mixed effects model. Estimates of coefficients (*B*), standard error (SE), *t*-value and 95% confidence intervals (CI) for fixed effects, and variance and standard deviation for random effects. $\Delta\text{CFU}_{\text{October}}$ was cube root-transformed to fit the assumptions of normality of residuals.

2017/18	B	SE	<i>t</i>	95% CI
Fixed effects				
Intercept	1.64	1.59	1.03	-1.63, 4.96
Fungus application	11.19	1.40	8.00	8.45, 13.95
Random effect				
Site	Intercept	10.81	3.29	
	Fungus Application	55.70	7.46	
2018/19	B	SE	<i>t</i>	95% CI
Fixed effects				
Intercept	5.17	1.40	3.70	2.31, 8.15
Fungus application	22.57	1.45	15.56	19.75, 25.49
Random effect				
Site	Intercept	4.32	2.08	
	Fungus application	38.94	6.24	

Table 3

Determinants for difference in total numbers of *Metarhizium* CFUs in soil eight months after fungus application ($\Delta\text{CFU}_{\text{April}}$) in treated and untreated plots estimated in a linear mixed effects model. Estimates of coefficients (*B*), standard error (SE), *t*-value and 95% confidence intervals (CI) for fixed effects, and variance and standard deviation for random effects. $\Delta\text{CFU}_{\text{April}}$ was cube root-transformed to fit the assumptions of normality of residuals of the linear model.

2017/18	B	SE	<i>t</i>	95% CI
Fixed effects				
Intercept	2.83	2.04	1.38	-1.63, 7.28
Fungus application	8.08	1.05	7.68	6.01, 10.16
Random effect				
Site	Intercept	15.18	3.9	
	Fungus application	22.05	4.7	
2018/19	B	SE	<i>t</i>	95% CI
Fixed effects				
Intercept	6.42	0.87	7.41	4.72, 8.11
Fungus application	17.93	1.35	13.27	15.28, 20.58
Random effect				
Site	Intercept	0	0	
	Fungus application	34.47	5.87	

Table 4

Determinants for total numbers of *Metarhizium* CFUs in soil depending on fungus application (treated or untreated), sampling time (two and eight months after application) and interactions between application and time estimated in a linear mixed effects model. Estimates of coefficients, standard error, *t*-value and 95% confidence intervals for fixed effects, and variance and standard deviation for random effects. Numbers of CFUs was cube root-transformed to fit the assumptions of normality of residuals of the linear model.

2017/18	B	SE	<i>t</i>	95% CI
Fixed effects				
Intercept	5	1.58	3.15	1.55, 8.43
Fungus application	9.74	0.76	12.824	8.25, 11.22
Sampling Time	-0.13	0.62	-0.211	-1.35, 1.09
Interaction	-2.93	1.08	-2.72	-5.03, -0.82
Random effect				
Site	Intercept	9.26	3.043	
	Residual	11.53	3.395	
2018/19	B	SE	<i>t</i>	95% CI
Fixed effects				
Intercept	8.17	1.55	5.26	4.87, 11.49
Fungus application	19.60	1	19.6	17.66, 21.57
Sampling Time	1.11	0.9	1.24	-0.65, 2.87
Interaction	-4.41	1.4	-3.14	-7.16, -1.67
Random effect				
Site	Intercept	8	2.83	
	Residual	18.62	4.32	

3.4. Potato Damage

Overall, FCBK and insecticide treatments both reduced wireworm damage on potatoes compared to controls (FCBK: $z = -2.951, p = 0.003$; insecticide: $z = -6.054, p < 0.001$). There was no conclusive significant interaction between the calculated overall wireworm infestation rates per field (wireworm density across plots) and the treatments (FCBK: $z = 0.274, p = 0.784$; insecticide: $z = 1.832, p = 0.067$). Even though the FCBK application rate was higher in 2018/19, the effect on potato damage did not substantially differ between 2017/18 and 2018/19 ($z = -1.331, p = 0.183$). Looking at individual sites, the treatment effect was not consistent. At the site level, a reduction in potato damage was only apparent in four sites: for the fungus treatment in Worb ($z = -3.284, p = 0.001, 2017/18$) and Rüeterswil ($z = -2.391, p = 0.017, 2018/19$), and for the insecticide treatment in Morges ($z = -6.733, p < 0.001$) and Zurich 1 ($z = -3.982, p < 0.001$) in 2017/18. However, neither the fungus nor the insecticide treatments lowered wireworm damage below 10% at any site (Fig. 3).

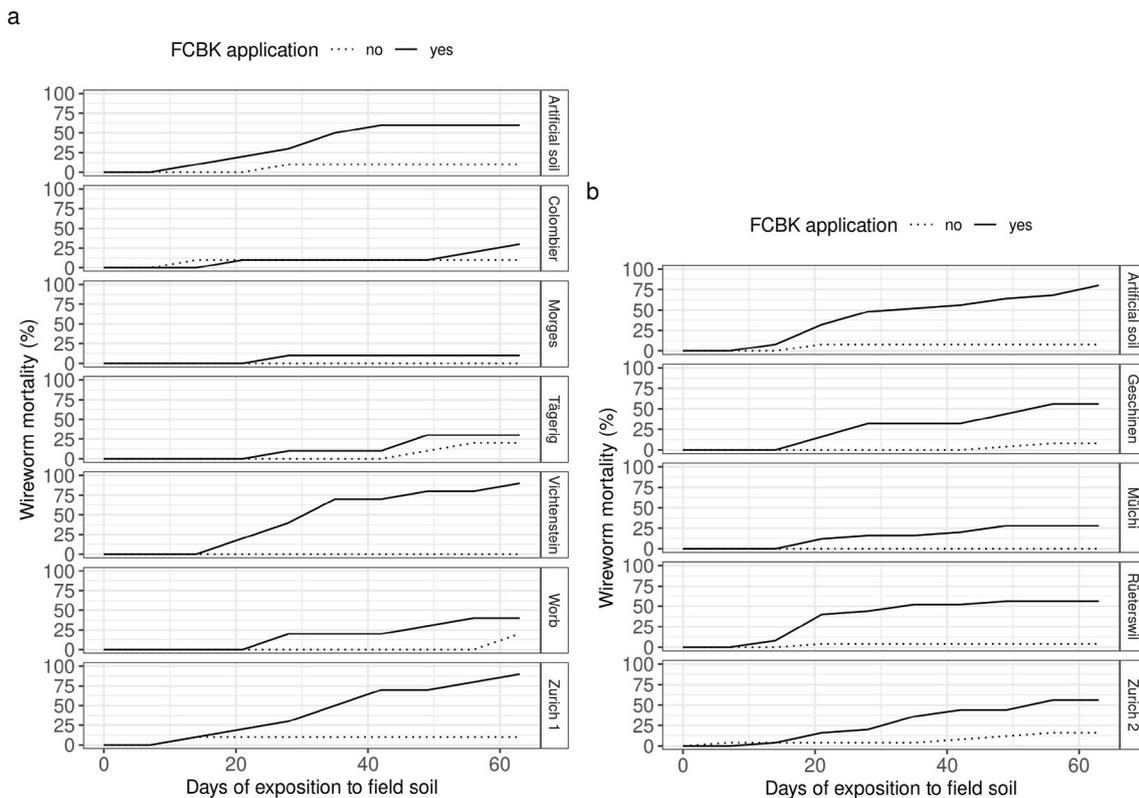


Fig. 2. Mortality of *A. obscurus* larvae over time ($n = 25$) when exposed to soils from field trial sites in 2017/18 (a) and 2018/19 (b), as well as artificial soil (control). Soil samples originated from plots treated with *M. brunneum* ART2825 (FCBK application yes) or untreated plots (FCBK application no).

Table 5

Species distribution in experimental sites with natural wireworm infestation in 2018/19.

Site	Total WW found	<i>A. obscurus</i>	<i>A. sputator</i>	<i>A. lineatus</i>	<i>Hemicrepidius niger</i>	Unidentified	Mycosed WW
Geschinen	112	75	1	8	3	25	4
		67%	1%	7%	3%	22%	9.5% ^a
Mülchi	169	17	117	6	3	26	0
		10%	69%	4%	2%	15%	0% ^a
Rüeterswil	102	69	2	2	1	28	13
		68%	2%	2%	1%	27%	34.2% ^a

^a Percentage of wireworms found in FCBK plots.

4. Discussion

This study aimed to implement a preventive biological plant protection strategy against wireworms in potatoes. In 10 field trials, the entomopathogenic fungus *M. brunneum* ART2825 was incorporated into field soils at the time of cover crop sowing in late summer. We found a strong increase of *Metarhizium* spp. in treated plots with a median of 2.6×10^3 and 2.3×10^4 CFU/g field soil in 2017/18 and 2018/19 respectively. The fungus persisted over a period of eight months until potato planting with only a small decrease in vital propagules over time. The biocontrol agent caused disease in laboratory-reared wireworms when exposed to soil collected from treated fields. Furthermore, it was possible to re-isolate *M. brunneum* ART2825 from wireworm cadavers collected on the experimental sites. Nevertheless, even though there was a statistically significant reduction in potato damage overall, the effect would need to be stronger to provide farmers with a potent tool for wireworm control. The United Nations Agricultural Quality Standards (UNICE) tolerate only 6 per cent by weight of tubers of ware potatoes with external or internal defects, including wireworm feeding holes deeper than 4 mm (UN, 2017). In the following, we will thus discuss our study with respect to the two interlacing elements of effective plant protection strategies: the ability of the preventive application method to

provide a setup for the fungal biocontrol agent to thrive and the suitability of the fungal isolate itself.

4.1. Efficacy of the preventive application method

The main goal of the preventive application of *M. brunneum* was to increase the probability of wireworm infection by raising the level of fungal inoculum in the soil for an extended period of time. The elevated abundances of *Metarhizium* spp. after application and the retrieval of the fungus in treated soils for several months showed that our preventive application method indeed enhanced the persistence of the fungus. The fungus may have benefited from stable soil conditions in the cover crop in contrast to high soil disturbance associated with potato cultivation, as is also seen in conservation tillage regimes (Meyling and Eilenberg, 2007). In general, habitats with little human intervention, like permanent grassland or field margins, often show higher *Metarhizium* densities than arable fields (Botelho et al., 2019; Schneider et al., 2012). Also, *Metarhizium* spp. are commonly found in the rhizosphere (Khan and Ahmad, 2019; Liu et al., 2016) and conditions may have been further improved by the plant cover in reducing soil evaporation and thus increasing soil moisture (Mullan and Reynolds, 2010). Despite these favorable conditions for the biocontrol agent over a period of several

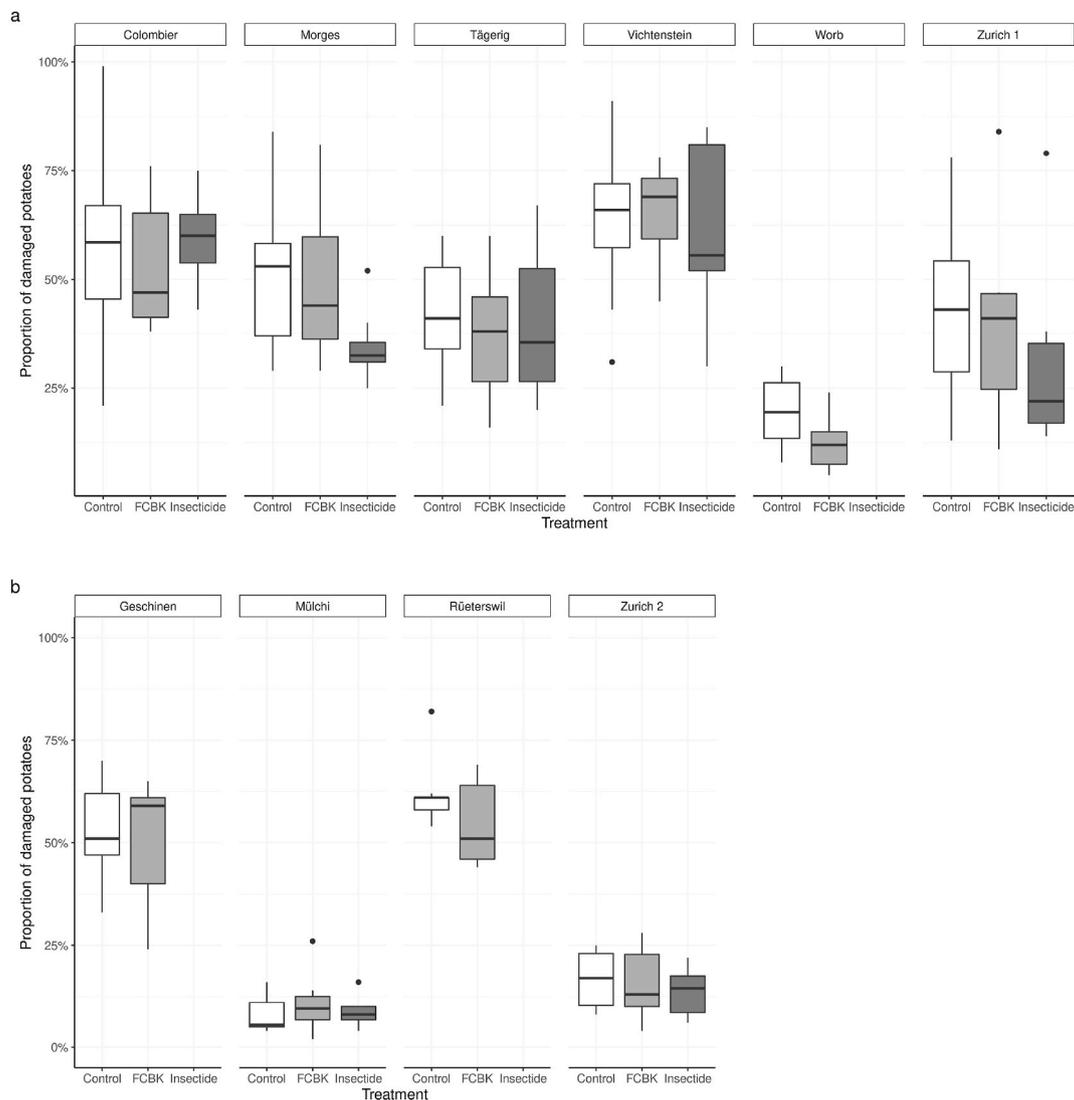


Fig. 3. Proportion of potatoes damaged by wireworm in 2017/18 (a) and 2018/19 (b) from untreated plots (control), plots treated with *M. brunneum* ART2825 (FCBK) and registered insecticide (100 potatoes sampled in each plot; $n = 8$ plots at each site, except for Worb and Zurich 1/2 $n = 6$, Geschinen and Rüeterswil $n = 9$). Sites with no data for insecticide treatment were organic farms where no insecticide plots were created.

months after application, the concentration of the fungus in treated soils may not have been high enough for the infection to spread throughout the wireworm population. To provide an adequate level of wireworm control for this *Metarhizium* isolate, Rogge et al. (2017) suggested a concentration of 10^4 CFUs per gram soil. This concentration was only consistently reached at one site in 2017/18, at the location Vichtenstein (median CFU/g dry weight soil 1.54×10^4) where the natural *Metarhizium* spp. occurrence was already high. In 2018/19, we doubled the amount of inoculum applied, and consequently all sites exceeded the suggested threshold concentration. However, the increase of inoculum did not lead to better damage reduction in the field. This is surprising because exposing laboratory-reared wireworms to FCBK-treated soils from the trial locations caused a significant increase in wireworm mortality in 2017/18 as well as in 2018/19, indicating that the field soils contained enough infective propagules to cause disease outbreak. Thus, it remains unclear if the field infection rates were lower and if so, why.

The first, and perhaps crucial, step for infection is the encounter between wireworm and fungus. Unlike in the laboratory trial where movement was limited by the containers used, the space occupied by wireworms in field soil is much more extensive. The FCBK treatment was incorporated into soil to a depth of 6 cm and while, depending on the soil texture, some conidia may have percolated into deeper soil areas, they

are most commonly retained in the topsoil (Ekesi et al., 2005). Wireworms stay in this upper soil layer for feeding, but migrate into deeper soil layers when environmental conditions are not suitable, for example, when soil moisture decreases (Barnett and Johnson, 2013). This behavior may have reduced contact with the fungus. It is even possible that wireworms sensed and avoided fungus-treated soil layers, a behavior known from other soil-inhabiting insects (Baverstock et al., 2010). Avoidance by *A. obscurus* larvae when exposed to *Metarhizium anisopliae*-contaminated soils has been described, but the effect diminished when a food source was present (Kabaluk and Ericsson 2007a). In our field studies, the growing cover crops offered a diverse range of plant roots as a food source for the wireworms, and it is therefore unlikely that they avoided the upper soil layer containing the fungal inoculum. Furthermore, we collected wireworms from treated plots in three field sites and these developed mycosis without any further exposure to the fungus in the lab, with subsequent genotyping of aerial conidia taken from these cadavers assigning all samples to the applied fungal isolate, ART2825. This clearly demonstrates the concurrence of the fungus in the field, albeit possibly not at the desired abundance. Further research should focus on methods to facilitate transmission of the fungal pathogen to its host. One possibility may be further developing the composition of the cover crop mixture, which could be enriched with

wireworm trap crops (Landl and Glauning, 2013; Sharma et al., 2019) in combination with preventive fungal treatments.

4.2. Efficacy of *M. brunneum* isolate ART2825 as a biocontrol agent against wireworms

Even when pathogen and insect commonly come into direct contact, disease outbreak may be limited. Wireworm mortality can be obstructed by other factors contributing to resistance to mycosis. These limiting factors are often related to the characteristics of the specific fungal isolate (Maistrout et al., 2020; Meyling and Eilenberg, 2007). Of particular importance is host specificity. *M. brunneum* ART2825, the isolate used in this study, is known to be highly virulent against the wireworm species *A. obscurus* and *A. lineatus* in the laboratory (Eckard et al., 2014). However, wireworm species composition in the field is often diverse (Traugott et al., 2015) and not all species are equally susceptible (Eckard et al., 2014). In 2018/19, wireworm samples were collected from three experimental sites, identified to species level and incubated for observation of possible fungal infections. After nine weeks of incubation, all wireworms developing mycosis belonged to the highly susceptible species *A. obscurus*. Consistent with these results, a significant reduction in potato damage following fungus treatment was found at the site Rütterswil, where *A. obscurus* was the dominant species. However, the wireworm species composition at the site in Geschinen was also dominated by *A. obscurus*, and we did not observe a significant effect of our fungus treatment on potato damage, although wireworms collected at Geschinen also developed mycosis in the laboratory. In order to integrate species composition into an application strategy, the development of distribution models or maps could be a useful tool, as wireworm communities vary geographically (Traugott et al., 2015). Recent initiatives, e.g., in Austria (Hann et al., 2019) and Germany (Lehmhus, 2020), are in progress to address this approach and could be extended to other areas.

While our results indicate overall that specificity of the applied fungal isolate might have had an important effect on the efficacy of the treatment, the environmental conditions might have been equally important. Among the sites, there was large environmental variation, for example, in soil parameters (Table 1), and perhaps most pronounced in climatic conditions, with an annual temperature average of 3.7 °C in Geschinen (MeteoSwiss, 2020a) and 6.6 °C in Rütterswil (MeteoSwiss, 2020b). It is well known that soil temperature, texture and moisture can influence the infection process and *M. brunneum* isolates can vary in their response to environmental conditions (Couceiro et al., 2021; Jaronski, 2010). Information on the environmental compatibility, including temperature requirements, is still missing for the isolate used in this study. Previous studies estimated the temperature range for growth and sporulation of most *Metarhizium* isolates between 15 °C and 35 °C (Couceiro et al., 2021). Temperatures above this range, even for short time periods, can lead to deleterious effects, while temperatures below the range may slow or delay the infection process (Keyser et al., 2014). In our study *A. obscurus* collected from treated field plots at soil temperatures between 8 °C and 11 °C developed mycosis only after incubation in the laboratory at 22 °C. Disease outbreak in wireworms that were transferred from the field to the laboratory has already been observed (Kabaluk et al., 2007), and has been attributed to latent infections activated by the temperature increase. However, the *Metarhizium* infection process starts with a non-specific, biophysical attachment mediated by hydrophobic elements on the outer layers of both fungal conidia and the insect cuticle. Germination and growth follow as a second step (St. Leger and Wang, 2020). As wireworms taken from the field sites were not surface sterilized, it is thus also possible that *Metarhizium* conidia were simply attached to the insects and may have germinated after the transfer to more suitable temperature conditions. Regardless of how far the infection process had advanced at the time of sampling, the development of disease in the laboratory indicates that full expression may have been prevented by the colder field

temperatures. Soil temperatures were not recorded on site throughout the trial period. Data available from nearby weather stations, however, showed a restricted time of on average 24 days during which minimum soil temperatures did not fall below the 15 °C threshold (minimum 3 days in Worb, 2017/18) during the application period from August until potato planting in April. In future research, it is thus necessary to evaluate the fungal isolate on its ability to cope with low and fluctuating temperatures so that preventative application not only enables fungal persistence but also germination, infection and growth under field conditions. Furthermore, additional investigations on seasonal movement of wireworms would be valuable, to determine whether the insect pest is likely to be present in the application area during a period suitable for the requirements of the biocontrol agent.

4.3. Reference insecticide treatment

Difficulties in wireworm control not only arise with biological agents but also with synthetic pesticides. Variable efficacy in reducing potato damage has been described for organophosphates such as chlorpyrifos (Vernon and van Herk, 2013), the active ingredient used in our Swiss field trials. In our study, although the insecticide treatment resulted in a significant reduction in wireworm damage, it was not of sufficient magnitude. Similar to fungal agents, varying susceptibility amongst wireworm species (van Herk et al., 2007) and repellency has been observed toward insecticides (van Herk et al., 2015). For crops that are in a vulnerable stage at the time of insecticide application, repellency may provide temporary plant protection (Barsics et al., 2013). In potatoes, however, this is not the case, since insecticide application is typically conducted during planting, whereas formation of the tubers, which are vulnerable to wireworm damage, occurs much later in the season. By this point in time, repellency may already be too low to reduce wireworm numbers and work effectively (Vernon and van Herk, 2013).

5. Conclusions

Preventive EPF application in winter cover crops might prove to be a useful method to increase the abundance of *M. brunneum* in the soil over the entire growing season. We were able to show that the concentration of the fungal inoculum in soils from treated plots was high enough to have an effect on wireworm survival. However, this application did not achieve sufficient reduction of wireworm damage in potatoes, indicating that the presence of the fungus, even at increased densities, is not sufficient for successful wireworm control. Similarly, the synthetic insecticides used in our study did not prevent crop damage. The mechanisms limiting the success of both biological control and chemical control of wireworms are still not sufficiently understood and our study strongly underlines the importance of further research into pest behavior and interaction with control agents.

CRediT authorship contribution statement

Lara Reinbacher: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Validation, Visualization, Writing – original draft. **Sven Bacher:** Methodology, Writing – review & editing, Supervision. **Fionna Knecht:** Investigation, Resources. **Christian Schweizer:** Investigation, Resources. **Tanja Sostizzo:** Investigation. **Giselher Grabenweger:** Conceptualization, Supervision, Funding acquisition, Methodology, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We are grateful to Iris Poggendorf and Yannick Senn, Zurich University of Applied Sciences, and Thomas Held and Matthias Muster, Eric Schweizer AG, Thun, for their support in the project. Special thanks go to the farmers collaborating with us in this study: Steve and Virginie Bugnon, Manuel Kilchenmann, Roger Meier, Jürg Moser, Roland and Sigune Müller, Peter Salzmann, Thomas Schmid and Yves and Angela Staudenmann-Bot. Furthermore, we would like to acknowledge the support from Theodor Ballmer and Christian Vetterli with their potato expertise as well as the technical support of Dany Amstutz, Fritz Käser and Stefan Schwarz. A warm thank you also to Russell Naisbit for proofreading the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cropro.2021.105811>.

Funding

This research was conducted in the course of an Innosuisse Life Science project. The first author received funding from Innosuisse, the Swiss Innovation Agency, via Life Science project no. 19811_PFLS-LS.

References

- Antwi, F.B., Shrestha, G., Reddy, G.V., Jaronski, S.T., 2018. Entomopathogens in conjunction with imidacloprid could be used to manage wireworms (Coleoptera: Elateridae) on spring wheat. *Can. Entomol.* 150, 124–139.
- Aregger, E., 1992. Conidia production of the fungus *Beauveria brongniartii* on barley and quality evaluation during storage at 2 C. *J. Invertebr. Pathol.* 59, 2–10.
- Barnett, K., Johnson, S.N., 2013. Living in the soil matrix: abiotic factors affecting root herbivores. In: *Advances in Insect Physiology*. Elsevier, pp. 1–52.
- Barsics, F., Haubruge, E., Verheggen, F.J., 2013. Wireworms' management: an overview of the existing methods, with particular regards to *Agriotes* spp. (Coleoptera: Elateridae). *Insects* 4, 117–152.
- Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. *J. Stat. Software* 67, 1–48.
- Baverstock, J., Roy, H.E., Pell, J.K., 2010. Entomopathogenic fungi and insect behaviour: from unsuspecting hosts to targeted vectors. In: Roy, H.E., Vega, F.E., Chandler, D., Goettel, M.S., Pell, J., Wajnberg, E. (Eds.), *The Ecology of Fungal Entomopathogens*. Springer Netherlands, Dordrecht, pp. 89–102.
- Benjamin, E.O., Grabenweger, G., Strasser, H., Wesseler, J., 2018. The socioeconomic benefits of biological control of western corn rootworm *Diabrotica virgifera virgifera* and wireworms *Agriotes* spp. in maize and potatoes for selected European countries. *J. Plant Dis. Prot.* 125, 273–285.
- Botelho, A.B.R.Z., Alves-Pereira, A., Prado, R.C., Zucchi, M.I., Júnior, I.D., 2019. *Metarhizium* species in soil from Brazilian biomes: a study of diversity, distribution, and association with natural and agricultural environments. *Fungal Ecology* 41, 289–300.
- Brandl, M.A., Schumann, M., Przyklenk, M., Patel, A., Vidal, S., 2017. Wireworm damage reduction in potatoes with an attract-and-kill strategy using *Metarhizium brunneum*. *J. Pest. Sci.* 90, 479–493.
- Couceiro, J.d.C., Faretto, M.B., Demétrio, C.G.B., Meyling, N.V., Delalibera Júnior, I., 2021. UV-B radiation tolerance and temperature-dependent activity within the entomopathogenic fungal genus *Metarhizium* in Brazil. *Frontiers in Fungal Biology* 2, 6.
- Eckard, S., Ansari, M.A., Bacher, S., Butt, T.M., Enkerli, J., Grabenweger, G., 2014. Virulence of in vivo and in vitro produced conidia of *Metarhizium brunneum* strains for control of wireworms. *Crop Protect.* 64, 137–142.
- Ekesi, S., Maniania, N.K., Mohamed, S.A., Lux, S.A., 2005. Effect of soil application of different formulations of *Metarhizium anisopliae* on African tephritid fruit flies and their associated endoparasitoids. *Biol. Contr.* 35, 83–91.
- Ensaifi, P., Crowder, D.W., Esser, A.D., Zhao, Z., Marshall, J.M., Rashed, A., 2018. Soil type mediates the effectiveness of biological control against *Limoniuss californicus* (Coleoptera: Elateridae). *J. Econ. Entomol.* 111, 2053–2058.
- EPPO, 2005. Wireworms. EPPO Bull. 35, 179–182.
- FAO, 2006. Guidelines for soil description, 4th. FAO, Rome.
- Furlan, L., Benvegnù, I., Bilò, M.F., Lehmus, J., Ruzzier, E., 2021. Species Identification of wireworms (*Agriotes* spp.; Coleoptera: Elateridae) of agricultural importance in Europe: a new “Horizontal Identification table”. *Insects* 12, 534.
- Hack, H., Gall, H., Klemke, T., Klose, R., Meier, U., Strauss, R., Witzinger, A., 1993. Phänologische Entwicklungsstadien der Kartoffel (*Solanum tuberosum* L.). Codierung und Beschreibung nach der erweiterten BBCH-Skala mit Abbildungen. *Nachrichtenbl. Deut. Pflanzenschutzd.* 45, 11–19.
- Hann, P., Kamptner, A., Wechselberger, K., Shala-Mayrhofer, V., 2019. Drahtwurm in Erdäpfel: Erste Monitoring-Ergebnisse im Jahr 2019 in Österreich. *Der Pflanzenschutz* 11–12.
- Hettlage, L., 2019. Use of an Entomopathogenic, Endophytic *Metarhizium brunneum* Isolate (Cb15III) to Manage Wireworm and Colorado Potato Beetle. Georg-August-Universität Göttingen.
- Humber, R.A., 2016. Seeking stability for research and applied uses of entomopathogenic fungi as biological control agents. *J. Asia Pac. Entomol.* 19, 1019–1025.
- Jaronski, S.T., 2010. Ecological factors in the inundative use of fungal entomopathogens. *BioControl* 55, 159–185.
- Kabaluk, J., Goettel, M., Erlandson, M., Ericsson, J., Duke, G., Vernon, R., 2005. *Metarhizium anisopliae* as a biological control for wireworms and a report of some other naturally-occurring parasites. *IOBC/wprs Bull.* 28, 109–115.
- Kabaluk, J., Vernon, R., Goettel, M., 2007. Mortality and infection of wireworm, *Agriotes obscurus* [Coleoptera: Elateridae], with inundative field applications of *Metarhizium anisopliae*. *Phytoprotection* 88, 51–56.
- Kabaluk, J.T., Ericsson, J.D., 2007a. Environmental and behavioral constraints on the infection of wireworms by *Metarhizium anisopliae*. *Environ. Entomol.* 36, 1415–1420.
- Kabaluk, J.T., Ericsson, J.D., 2007b. *Metarhizium anisopliae* seed treatment increases yield of field corn when applied for wireworm control. *Agron. J.* 99, 1377–1381.
- Kessler, P., Matzke, H., Keller, S., 2003. The effect of application time and soil factors on the occurrence of *Beauveria brongniartii* applied as a biological control agent in soil. *J. Invertebr. Pathol.* 84, 15–23.
- Keyser, C.A., Fernandes, É.K., Rangel, D.E., Roberts, D.W., 2014. Heat-induced post-stress growth delay: a biological trait of many *Metarhizium* isolates reducing biocontrol efficacy? *J. Invertebr. Pathol.* 120, 67–73.
- Khan, M.A., Ahmad, W., 2019. *Microbes for Sustainable Insect Pest Management: an Eco-Friendly Approach*. Springer.
- Knodel, J.J., Shrestha, G., 2018. Pulse crops: pest management of wireworms and cutworms in the Northern Great Plains of United States and Canada. *Ann. Entomol. Soc. Am.* 111, 195–204.
- Kölliker, U., Biasio, L., Jossi, W., 2011. Potential control of Swiss wireworms with entomopathogenic fungi. *IOBC/wprs Bull.* 66, 517–520.
- Kölliker, U., Jossi, W., Kuske, S., 2009. Optimised protocol for wireworm rearing. *IOBC/wprs Bull.* 45, 457–460.
- Ladurner, E., Quentin, U., Franceschini, S., Benuzzi, M., Ehlers, R., 2009. Efficacy evaluation of the entomopathogenic fungus *Beauveria bassiana* strain ATCC 74040 against wireworms (*Agriotes* spp.) on potato. *IOBC/wprs Bull.* 45, 445–448.
- Landl, M., Glauning, J., 2013. Preliminary investigations into the use of trap crops to control *Agriotes* spp. (Coleoptera: Elateridae) in potato crops. *J. Pest. Sci.* 86, 85–90.
- Lehmus, J., 2020. Wireworm biology in Middle Europe - what are we facing? *IOBC-WPRS Bull.* 150, 96–99.
- Lehmus, J., Niepold, F., 2015. Identification of *Agriotes* wireworms-are they always what they appear to be. *Journal für Kulturpflanzen* 67, 129–138.
- Liu, X., Nong, X., Wang, Q., Li, X., Wang, G., Cao, G., Zhang, Z., 2016. Persistence and proliferation of a Chinese *Metarhizium anisopliae* s.s. isolate in the peanut plant root zone. *Biocontrol Sci. Technol.* 26, 746–758.
- Lovett, B., St Leger, R.J., 2018. Genetically engineering better fungal biopesticides. *Pest Manag. Sci.* 74, 781–789.
- Maistrou, S., Natsopoulou, M.E., Jensen, A.B., Meyling, N.V., 2020. Virulence traits within a community of the fungal entomopathogen *Beauveria*: Associations with abundance and distribution. *Fungal Ecology* 48, 100992.
- Mayerhofer, J., Rauch, H., Hartmann, M., Widmer, F., Gschwend, F., Strasser, H., Leuchtmann, A., Enkerli, J., 2019. Response of soil microbial communities to the application of a formulated *Metarhizium brunneum* biocontrol strain. *Biocontrol Sci. Technol.* 29, 547–564.
- MeteoSwiss, 2020a. Climate Normals Ulrichen.
- MeteoSwiss, 2020b. Climate Norms Hörnli.
- Meyling, N.V., Eilenberg, J., 2007. Ecology of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in temperate agroecosystems: potential for conservation biological control. *Biol. Contr.* 43, 145–155.
- Milosavljević, I., Esser, A.D., Rashed, A., Crowder, D.W., 2020. The composition of soil-dwelling pathogen communities mediates effects on wireworm herbivores and wheat productivity. *Biol. Contr.* 104317.
- Mullan, D.J., Reynolds, M.P., 2010. Quantifying genetic effects of ground cover on soil water evaporation using digital imaging. *Funct. Plant Biol.* 37, 703–712.
- OECD, 2016. Test no. 226: predatory mite (*Hypoaspis* (*Geolaelaps*) *aculeifer*) reproduction test in soil. OECD Guidelines for the Testing of Chemicals, Section 2. OECD Publishing, Paris. <https://doi.org/10.1787/9789264264557-en>.
- Paixão, F.R.S., Fernandes, É.K.K., Pedrini, N., 2019. Thermotolerance of fungal conidia. In: Khan, M.A., Ahmad, W. (Eds.), *Microbes for Sustainable Insect Pest Management: an Eco-Friendly Approach - Volume 1*. Springer International Publishing, Cham, pp. 185–196.
- Parker, W.E., Howard, J.J., 2001. The biology and management of wireworms (*Agriotes* spp.) on potato with particular reference to the UK. *Agric. For. Entomol.* 3, 85–98.
- Poggi, S., Le Cointe, R., Lehmus, J., Plantegenest, M., Furlan, L., 2021. Alternative strategies for controlling wireworms in field crops: a review. *Agriculture* 11, 436.
- R Core Team, 2019. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.
- Rangel, D.E., Braga, G.U., Fernandes, E.K., Keyser, C.A., Hallsworth, J.E., Roberts, D.W., 2015. Stress tolerance and virulence of insect-pathogenic fungi are determined by environmental conditions during conidial formation. *Curr. Genet.* 61, 383–404.
- Ravensberg, W.J., 2011. Selection of a Microbial Pest Control Agent, A Roadmap to the Successful Development and Commercialization of Microbial Pest Control Products for Control of Arthropods. Springer Netherlands, Dordrecht, pp. 23–57.

- Reddy, G.V., Tangtrakulwanich, K., Wu, S., Miller, J.H., Ophus, V.L., Prewett, J., Jaronski, S.T., 2014. Evaluation of the effectiveness of entomopathogens for the management of wireworms (Coleoptera: Elateridae) on spring wheat. *J. Invertebr. Pathol.* 120, 43–49.
- Riba, G., Ravelojoana, A.M., 1984. The parasexual cycle in the entomopathogenic fungus *Paecilomyces fumoso-roseus* (Wize) Brown and Smith. *Can. J. Microbiol.* 30, 922–926.
- Ritter, C., Richter, E., 2013. Control methods and monitoring of *Agriotes* wireworms (Coleoptera: Elateridae). *J. Plant Dis. Prot.* 120, 4–15.
- Rogge, S.A., Mayerhofer, J., Enkerli, J., Bacher, S., Grabenweger, G., 2017. Preventive application of an entomopathogenic fungus in cover crops for wireworm control. *BioControl* 62, 613–623.
- Schneider, S., Widmer, F., Jacot, K., Kölliker, R., Enkerli, J., 2012. Spatial distribution of *Metarhizium* clade 1 in agricultural landscapes with arable land and different semi-natural habitats. *Appl. Soil Ecol.* 52, 20–28.
- Sharma, A., Jaronski, S., Reddy, G.V., 2019. Impact of granular carriers to improve the efficacy of entomopathogenic fungi against wireworms in spring wheat. *J. Pest. Sci.* 1–16.
- St Leger, R.J., Wang, J.B., 2020. *Metarhizium*: jack of all trades, master of many. *Open Biology* 10, 200307.
- Strasser, H., Forer, A., Schinner, F., 1996. Development of Media for the Selective Isolation and Maintenance of virulence of *Beauveria brongniarii*.
- Sufyan, M., Abbasi, A., Gogi, M.D., Arshad, M., Nawaz, A., Neuhoff, D., 2017. Efficacy of *Beauveria bassiana* for the management of economically important wireworm species (Coleoptera: Elateridae) in organic farming. *Gesunde Pflanz.* 69, 197–202.
- Tharp, C.I., Blodgett, S.L., Jaronski, S., 2007. Control of wireworm (Elateridae) in potatoes with microbial *Metarhizium*. In: 2006. Arthropod Management Tests 32.
- Traugott, M., Benefer, C.M., Blackshaw, R.P., van Herk, W.G., Vernon, R.S., 2015. Biology, ecology, and control of elaterid beetles in agricultural land. *Annu. Rev. Entomol.* 60, 313–334.
- UN, 2017. In: UNECE Standard FFV-52 Concerning the Marketing and Commercial Quality Control of Early and Ware Potatoes, 2017 edition. United Nations, New York and Geneva.
- van Herk, W., Vernon, R., Clodius, M., Harding, C., Tolman, J., 2007. Mortality of five wireworm species (Coleoptera: Elateridae), following topical application of clothianidin and chlorpyrifos. *J. Entomol. Soc. Br. Columbia* 104, 55–64.
- van Herk, W.G., Vernon, R.S., 2013. Categorization and numerical assessment of wireworm mobility over time following exposure to bifenthrin. *J. Pest. Sci.* 86, 115–123.
- van Herk, W.G., Vernon, R.S., Vojtko, B., Snow, S., Fortier, J., Fortin, C., 2015. Contact behaviour and mortality of wireworms exposed to six classes of insecticide applied to wheat seed. *J. Pest. Sci.* 88, 717–739.
- Veres, A., Wyckhuys, K.A., Kiss, J., Tóth, F., Burgio, G., Pons, X., Avilla, C., Vidal, S., Razinger, J., Bazok, R., 2020. An update of the Worldwide Integrated Assessment (WIA) on systemic pesticides. Part 4: Alternatives in major cropping systems. *Environ. Sci. Pollut. Control Ser.* 1–33.
- Vernon, R.S., van Herk, W.G., 2013. Wireworms as Pests of Potato, *Insect Pests of Potato*. Elsevier, pp. 103–164.