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

Lara Reinbacher a,b,c, Sven Bacher b, Fionna Knecht a, Christian Schweizer a, Tanja Sostizzo a, Giselher Grabenweger a

**A R T I C L E   I N F O**

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

**A B S T R A C T**

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 and Ericsson, 2007a; Reddy et al., 2014), the importance of temperature (Antwi et al., 2018) and high soil moisture (Kabaluk and Ericsson, 2007a; 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

© 2021 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license
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 1×10⁶ 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) (Ribea 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
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 potato planting. Abundance of the fungus in the soil was assessed by CFU counts in the soil were assessed before and two months after application, as well as eight months after application shortly before planting dates October, 2016. Soil samples of 100 g were collected from each plot, pooled per location to quantify mycosis over 9 weeks.

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., 2006). Petri dishes (volume 90 cm³) were filled with 30 g dry weight of the inoculum (2.7 × 10⁶ conidia 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.

2.6. 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 × 10⁶ 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.
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 (ΔCFU) 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. ΔCFU 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 × 0, 1 to 2 holes × 1.5, 3 to 5 holes × 4 and more than 5 holes × 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 factors.

![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).](image-url)
factor.

3. Results

3.1. Occurrence of Metarhizium at field sites

Natural Metarhizium populations were found in all fields in August except the Geschenen 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 Geschenen 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 ($\Delta$CFU$_{October}$) 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. $\Delta$CFU$_{October}$ was cube root-transformed to fit the assumptions of normality of residuals.

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

### Table 3

Determinants for difference in total numbers of Metarhizium CFUs in soil eight months after fungus application ($\Delta$CFU$_{April}$) 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. $\Delta$CFU$_{April}$ was cube root-transformed to fit the assumptions of normality of residuals of the linear model.

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

### 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.

<table>
<thead>
<tr>
<th></th>
<th>2017/18</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>SE</td>
<td>t</td>
<td>95% CI</td>
</tr>
<tr>
<td>Fixed effects</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>5</td>
<td>1.58</td>
<td>3.15</td>
<td>1.55, 8.43</td>
</tr>
<tr>
<td>Fungus application</td>
<td>9.74</td>
<td>0.76</td>
<td>12.824</td>
<td>8.25, 11.22</td>
</tr>
<tr>
<td>Sampling Time</td>
<td>-0.13</td>
<td>0.62</td>
<td>-0.211</td>
<td>-1.35, 1.09</td>
</tr>
<tr>
<td>Interaction</td>
<td>-2.93</td>
<td>1.08</td>
<td>-2.72</td>
<td>-5.03, -0.82</td>
</tr>
<tr>
<td>Random effect</td>
<td>Variance</td>
<td>SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Site</td>
<td>Intercept</td>
<td>9.26</td>
<td>3.043</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Residual</td>
<td>11.53</td>
<td>3.395</td>
<td></td>
</tr>
<tr>
<td>2018/19</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fixed effects</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>8.17</td>
<td>1.55</td>
<td>5.26</td>
<td>4.87, 11.49</td>
</tr>
<tr>
<td>Fungus application</td>
<td>19.60</td>
<td>1</td>
<td>19.6</td>
<td>17.66, 21.57</td>
</tr>
<tr>
<td>Sampling Time</td>
<td>1.11</td>
<td>0.9</td>
<td>1.24</td>
<td>-0.65, 2.47</td>
</tr>
<tr>
<td>Interaction</td>
<td>-4.41</td>
<td>1.4</td>
<td>-3.14</td>
<td>-7.16, -1.67</td>
</tr>
<tr>
<td>Random effect</td>
<td>Variance</td>
<td>SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Site</td>
<td>Intercept</td>
<td>8</td>
<td>2.83</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Residual</td>
<td>18.62</td>
<td>4.32</td>
<td></td>
</tr>
</tbody>
</table>

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).
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 $2.6 \times 10^3$ and $2.3 \times 10^4$ 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 rhizoplane (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

---

**Table 5**

<table>
<thead>
<tr>
<th>Site</th>
<th>Total WW found</th>
<th>A. obscurus</th>
<th>A. sputator</th>
<th>A. lineatus</th>
<th>Hemipircedius niger</th>
<th>Unidentified</th>
<th>Mycosed WW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geschinen</td>
<td>112</td>
<td>75</td>
<td>1</td>
<td>8</td>
<td>3</td>
<td>25</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>67%</td>
<td>1%</td>
<td>7%</td>
<td>3%</td>
<td>22%</td>
<td>9.5%</td>
</tr>
<tr>
<td>Mülini</td>
<td>169</td>
<td>17</td>
<td>117</td>
<td>6</td>
<td>2%</td>
<td>69%</td>
<td>10%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10%</td>
<td>69%</td>
<td>4%</td>
<td>4%</td>
<td>69%</td>
<td>10%</td>
</tr>
<tr>
<td>Rüterswil</td>
<td>102</td>
<td>69</td>
<td>2</td>
<td>2</td>
<td>1%</td>
<td>27%</td>
<td>34.2%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>68%</td>
<td>2%</td>
<td>2%</td>
<td>2%</td>
<td>68%</td>
<td>2%</td>
</tr>
</tbody>
</table>

*Percentage of wireworms found in FCBK plots.*
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 $1.54 \times 10^4$) 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 Glauninger, 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 (Maistrou 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 Rüetterswil, 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 Rüetterswil (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 compatibility for growth and sporation 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 (Kabuluk 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 Poggendörfer 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.cropred.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

MetroSuisse, 2020b. Climate Norms HorniL.


