Journal of Pest Science
https://doi.org/10.1007/s10340-023-01726-1

ORIGINAL PAPER

Trap crops enhance the control efficacy of *Metarhizium brunneum* against a soil-dwelling pest

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Received: 21 July 2023 / Revised: 17 November 2023 / Accepted: 20 November 2023

Abstract

Soil-dwelling insect pests may cause considerable damage to crops worldwide, and their belowground lifestyle makes them hard to control. Amongst the most promising control agents for subterranean pests are soilborne entomopathogenic fungi (EPF) such as *Metarhizium brunneum*. Albeit EPF can be highly pathogenic to their target pest species under laboratory conditions, their efficacy in the field is often limited due to adverse environmental conditions. Here, we test for the first time if the efficacy of EPF can be improved when they are augmented with trap crops. In a field experiment, the *M. brunneum* strain ART2825 was combined with a trap crops mixture of six plant species and evaluated for its control effect of wireworms (Coleoptera: Elateridae). When both were combined in the main crop, potato damage was lowered on average by 42.5% and wireworm abundance by 50.8%. Single application of trap crops or EPF lowered damage/pest abundance only by 29.9%/15.89% and 34.7%/4.77%, respectively. Importantly, the strength of the synergistic pest control effect between trap crops and EPF increased disproportionately with increasing wireworm abundance. However, DNA-based gut content analysis showed that wireworms' feeding preferences were not shifting toward the trap crops. Our findings demonstrate that combining trap crops with EPF improves the efficacy of the latter and leads to a synergistic control effect which magnifies with increasing wireworm abundance. Hence, the synergistic effect of EPF and trap crops might be a promising control strategy for soil-dwelling pests in general and significantly improve our abilities to manage soil pests environmentally friendly.

Keywords Insect pest · Biological control · Elaterid beetles · *Agriotes spp.* · Wireworm · Entomopathogenic fungus

Key Message

- For sustainable control of soil pests, efficacy of entomopathogenic fungi (EPF) needs improvement.
- Combining trap crops and EPF reduced wireworm damage in potatoes by 43%, and abundance by 51%.
- Trap crops enhanced EPF performance at high pest abundances and resulted in a synergistic control.
- To optimize this approach, the interaction between EPF and trap crop needs further investigation.

Communicated by Antonio Biondi.

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Introduction

Globally, insect soil pests can cause damage in many crops, and may determine the difference between a satisfactory crop, and little or no yield (Blossey and Hunt-Joshi 2003; Lilly 1956; Oerke 2006). However, living in soil protects these pests from many control techniques which can be effectively applied to aboveground pests. In Europe, the ban of neonicotinoids and organophosphates has resulted in insufficient control of soil-dwelling pests at the moment (Ritter and Richter 2013). This lack of control is especially affecting potato production where wireworms, the larvae of click beetles (Coleoptera: Elateridae), are feeding on tubers, consequently lowering potato quality and making them nonmarketable (Keiser 2012). In total, about 5–25% of economic damage in potato production is caused by wireworms (Parker and Howard 2001), and generally they are considered as one of the most important belowground pest insects, damaging a broad variety of crops besides potatoes with species within the genus *Agriotes* being one of the most pestiferous elaterid pests in Europe and Canada (Vernon and van Herk 2013). Therefore, there is a pressing need to generate new and pesticide-free, but still effective control strategies (Pearsons and Tooker 2017).

While adult click beetles are usually not damaging plants, the larvae feed on belowground plant parts (Benefer et al. 2012; Traugott et al. 2015). Young larvae depend on live vegetable material to grow and survive, whereas older larvae can overcome long periods without food (Furlan 1998). Vertical migration to avoid dry summer periods as well as their thick chitinous cuticula makes them very durable insects (Traugott et al. 2015). Adult beetles live aboveground, and reproduce from late spring to late summer according to the species (Furlan 2005). Because of the multiannual belowground larval development, individual control measures are most likely not sufficient, while the combination of measures might be suitable to achieve long-term damage reduction (Traugott et al. 2015).

Current control practices to reduce wireworm damage focus on the larval stage and include chemical, physical or biological methods targeting the pest insect either directly or indirectly (Barsics et al. 2013). Intensive soil cultivation during summer poses the most common physical control method used by farmers, but its efficacy varies and depends on environmental factors as well as the species present (Furlan 2005). At the same time, it negatively affects soil structure, aeration, moisture, organic matter composition and ecological interactions in the subterranean community while increasing the risk of erosion (Furlan 2005; Schwilch et al. 2018). Chemical control practices bare environmental risks due to leaching, toxicity to aquatic species and bees as well as indirect effects on other non-target organisms (Gvozdenac et al. 2022). Environmentally friendly control methods using entomopathogenic fungi, nematodes, bacteria or naturally derived insecticides have already been tested (Poggi et al. 2021). Of these, entomopathogenic fungi (EPF) are promising candidates for the control of wireworms, and many other soil-dwelling pest insects in arable farming (Lata et al. 2018).

The entomopathogenic fungus *Metarhizium brunneum* is one of the most abundant, naturally occurring antagonist and besides its protection against insect herbivores, it was shown to improve plant growth and enhance plant tolerance to abiotic stress in general (Hu and Bidochka 2019). *Metarhizium brunneum* can infect and kill insects either by penetration through the cuticle, or through ingestion (Wang et al. 2019). Under laboratory conditions, a high efficacy against wireworms was proven (Eckard et al. 2014). However, certain *M. brunneum* strains were found to possess sufficient virulence for only a subset of *Agriotes* species and promising results from laboratory experiments could so far not be confirmed under field conditions. Various environmental factors such as soil moisture, soil temperature and UV radiation seem to reduce the establishment and efficacy of EPFs (Brandl et al. 2017; Vega 2018). Soil temperatures above 14 °C are known to promote infection rates of *M. brunneum*, and therefore summer and autumn applications are expected to be more successful for wireworm control (Kabaluk and Ericsson 2007). Solar ultraviolet radiation (UV-A and UV-B) is another environmental factor that negatively affects the viability and virulence of *M. brunneum*, particularly when spores are applied on bare soil (Braga et al. 2007; Rangel et al. 2004). When soil is covered by plant stands, solar radiation is reduced and soil moisture is generally higher, therefore an application in autumn in the preceding cover crop seems to be more favorable for EPF densities than in spring (Reinbacher et al. 2021; Rogge et al. 2017).

Besides the suggested positive effects on EPF densities, the success of insect control could further benefit from additional plant species by using them as trap crops. Trap cropping is another type of companion planting and traditionally used for insect pest management. Pest insects are lured away from the main crop during a critical time period by providing them an alternative, preferred food source (Sarkar et al. 2018). Plant diversification through trap cropping has been shown to promote herbivore suppression through movement patterns, host associations and predation probability (Letourneau et al. 2011). This practice relies on knowledge of insect preferences for certain host plants, based on visual, tactile or olfactory cues. Optimal trap crop species need to be highly attractive to pest species and suitable to be interplanted with susceptible crops (Schoonhoven et al. 2005). The two most important factors influencing the efficacy of trap crops are the (i) attractiveness of the trap crop and its (ii) density in the field (Vernon 2005). The use of trap crops has been proven to reduce wireworm damage on a variety of crops such as wheat, strawberries and maize (Adhikari and Reddy 2017; Staudacher et al. 2013; Vernon 2000). Even though wireworms are polyphagous, previous studies have shown that certain plants are preferred over others (Staudacher et al. 2013). However, trap crops also have disadvantages since they compete on water and nutrients with the main crop, and they lack wireworm population control since wireworms are not killed and stay in the field for the next seasons to come (Ratnadass et al. 2012).

We hypothesize that a combined application of EPF and trap crops adds population control to damage reduction, respectively. Additionally, *M. brunneum* might benefit from trap crops due to its endophytic properties and rhizosphere competence (Hu and Bidochka 2019; Quesada-Moraga et al. 2022). Since autumn application of *M. brunneum* in the previous crop is expected to improve EPF densities, an application in the preceding crop was also investigated for its potential to reduce wireworm abundance and damage. Wireworm's repellency by *M. brunneum* is known to be significantly lower when germinating plants are present (Kabaluk and Ericsson 2007), trap crops are expected to increase wireworm infection rates, resulting in a higher mortality and damage reduction. The main focus of this study was to test the field efficacy of a combined application of *M. brunneum* and trap crops to reduce wireworm damage in potatoes and to test three hypotheses:

1) Wireworm abundance and potato damage is reduced significantly when *M. brunneum* and trap crops are combined, because trap crops support EPF's establishment and enhance its control performance.
2) The presence of trap crops shifts the diet of wireworms away from potatoes since wireworms are attracted by roots of trap crops and preferably feed on these.
3) The combination of EPF and trap crops does not influence potato yield negatively. A possible trap crops' competition for water and nutrients is counterbalanced by plant growth promoting properties of *M. brunneum*.

To test these hypotheses, a field study was performed in Tyrol, Austria where the combination as well as single treatments were implemented in a randomized block design. Wireworm abundance and damage as well as *Metarhizium spp.* densities in the soil were recorded. Additionally, feeding preferences of wireworms were examined through molecular gut content analysis.

Material and methods

Study site and field cultivation

The field study was conducted on an organic field at the research farm of the University Innsbruck in Imst, Austria (N 47.221667, E 10.744361) from 2017 to 2018. This site was chosen because of its high and steady wireworm densities, recorded by a previous monitoring study (Wechselberger et al. 2019). Based on this study, the two *Agriotes* species, *Agriotes sputator* and *Agriotes obscurus,* were known to occur. With its location of 750 m above sea level, a mean temperature of 6.5 °C and an average precipitation of 750–880 mm/year, the climate is considered moderate with sufficient rainfall for potato production. With + 1.8 °C above average and 20% less rainfall than usual, 2018 was an exceptionally warm and dry summer in Tyrol (Proplanta 2018). The average soil temperature during the experimental phase was 19.6 °C (Max.: 30.3 °C; Min.: 11.0 °C) in 15 cm and 17.6 °C (Max.: 24.0 °C; Min.: 12.2 °C) in 30 cm soil depth (Fig. III, Supplementary Information). The soil on the experimental field site contained 6% clay, 43% silt and 51% sand at a pH of 7, and can be described as calcareous alluvial loamy sand. Total organic carbon (elemental analysis after dry combustion) content of the soil was 3.5%.

The study site of 38 m*16.5 m was divided into 36 plots measuring 6 m*2.25 m each, including buffer zones between plots of 1.5 m on the short and 1.5 m on the long sides (Fig. II, Supplementary Information). Each plot was assigned to one of six treatments, following a Latin square distribution resulting in six replications per treatment (Fig. I, Supplementary Information). The grassland was converted to arable land by ploughing on April 18, 2018, followed by harrowing. The potato variety "Anuschka" was planted on April 25, 2018 at a density of 3,500 kg/ha. For weed control, potato ridging was performed twice, once on May 24, 2018 and again on June 12, 2018. The potato crop developed well until mid-July, but afterwards potato beetles (*Leptinotarsa decemlineata*) and leaf blight (*Phytophthora infestans)* weakened the potato plants. The leaf blight stayed untreated, but the potato beetle larvae were collected by hand. At the beginning of August, all crop foliage had withered and the potato tubers were harvested on August 24, 2018 (Fig. 1). During the experimental phase no plant protection products other than EPF, or fertilizers were applied in any treatment, nor was the field irrigated.

Fungus colonized barley kernels (FCBK) production, application and determination of *Metarhizium spp.* colony forming units (CFU)

The entomopathogenic fungus isolate ART2825 of *M. brunneum* was applied as a biocontrol agent, was originally isolated from an infected *A. obscurus* in Switzerland in 2008 (Kölliker et al. 2011). Conidia were formulated on autoclaved barley kernels (FCBK) and used for field application. The FCBK were provided by Agroscope (Zurich, Switzerland), and conidia were cultivated and transferred as described in Reinbacher et al. (2021). FCBK were applied in the corresponding twelve plots in summer 2017 in the grassland at a rate of 1×10^{13} conidia/ha, using a seed drill machine. On April 25, 2018, FCBK were distributed by hand onto the plowed soil at a rate of 5×10^{13} conidia/ha, followed by immediate harrowing to incorporate the kernels into the soil (Fig. 1).

To get an estimate of naturally occurring CFU of *Metarhizium spp.* in the experimental field, as well as the persistence after FCBK application, soil samples were taken and analyzed at four timepoints. The first density determination of

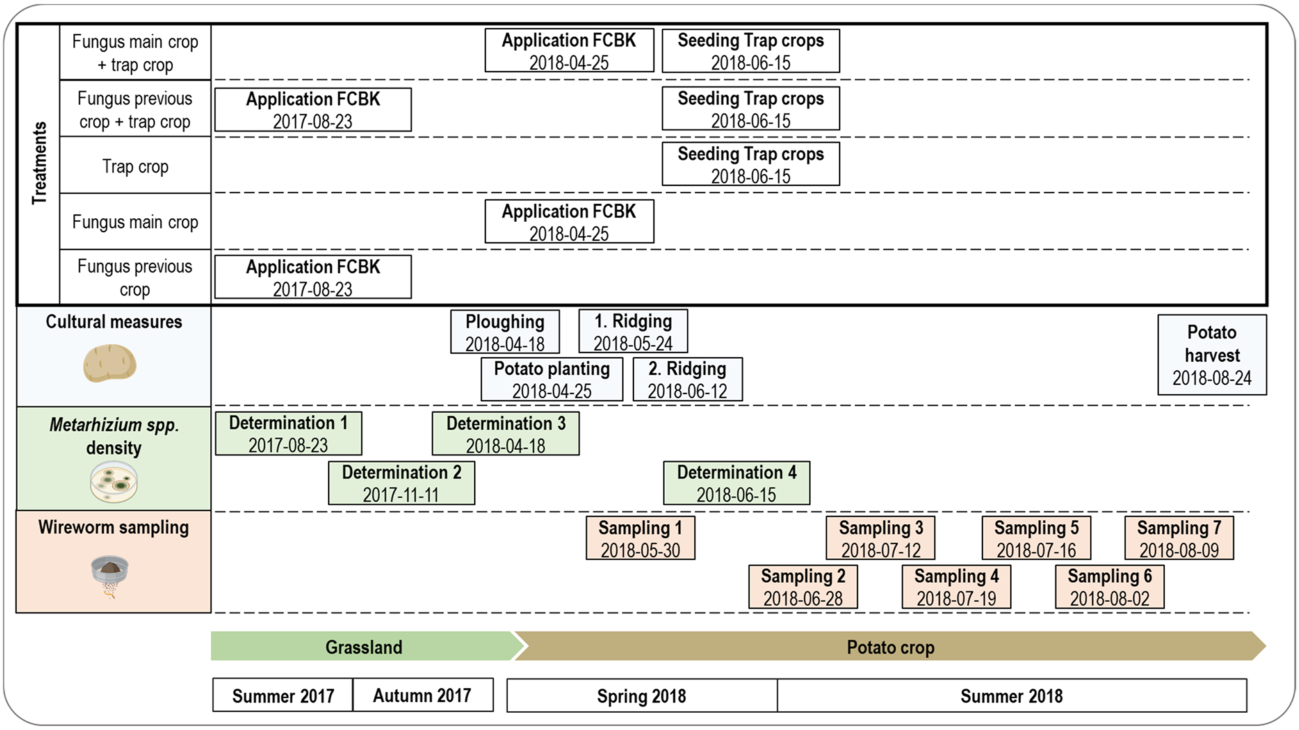


Fig. 1 Timeline and summary of the experimental field management and sampling between August 2017 and August 2018. Fungus colonized barley kernels (FCBK) were applied in the previous crop (grassland) in summer 2017, and in the main crop (potato) in spring 2018. FCBK application was tested singularly or in combination with trap crops, which were sown on the sides of the potato ridges. To monitor the establishment of the entomopathogenic fungus (EPF), *Metarhizium* spp. density was determined as colony forming units (CFU) per g soil at four timepoints

Metarhizium spp. colonies was done before FCBK application. The second determination was done after FCBK application into the grassland and a third density determination was done after winter to monitor the persistence of the applied *M. brunneum* conidia. The final density determination of *Metarhizium spp*. was conducted in June, seven weeks after the second FCBK application. (Fig. 1). For each *Metarhizium spp*. density determination, five soil samples were taken per plot at random, mixed together in a bin and packed into a plastic bag. All soil samples were cooled and taken to Agroscope in Zurich for analysis. CFU of *Metarhizium spp*. in the soil were assessed as the number of CFUs per gram dry soil as described in Reinbacher et al. (2021). The water content of each soil sample was determined gravimetrically. CFUs per gram dry soil were calculated using counted CFUs and gravimetrically measured water content.

Trap crops mixture

Plants for the trap crops mixture were selected based on a previous study conducted by Staudacher (2013). Infrequently consumed plants from this study were discarded and replaced by other more promising species. A mixture of six different plants was selected: (1) *Triticum aestivum*—common wheat (3 g/m^2), (2) *Lupinus angustifolius*—blue lupin (4 g/m^2), (3) *Fagopyrum esculentum*—common buckwheat (3 g/m^2), (4) *Trifolium pratense*—red clover (3 g/m^2), (5) *Trifolium repens*—white clover (3 g/m^2), (6) *Phaseolus coccineus*—runner bean (27 g/m^2). The mixture was seeded at both slopes of the potato ridges after the last weed control on June 15, 2018. First, a 10 cm wide ditch was dug at the bottom between potato ridges with a spade. Then the seed mixture was scattered evenly within the ditch by hand, and covered with soil. The implemented trap crops germinated and grew well, and when the potato crop foliage had already died, the trap crop mixture was well established (Fig. IV, Supplementary Information).

Experimental treatments

The following five experimental treatments, plus a control treatment, were implemented in the field and replicated six-fold (Fig. 1):

(1) *Fungus previous crop (grassland) (FPC):* FCBK were applied into the permanent grassland in summer 2017.

(2) *Fungus main crop (potato) (FMC)*: FCBK were applied to the field in 2018 after ploughing, prior to potato planting.
(3) *Fungus previous crop combined with trap crop (FPC + T):* FCBK were applied into the permanent grassland in summer 2017 and trap crop mixture was sown in summer 2018.
(4) *Fungus main crop combined with trap crop (FMC + T):* FCBK were applied to the field in 2018 after ploughing, prior to potato planting and trap crop mixture was sown in summer 2018.
(5) *Trap crop (T):* Trap crop mixture was sown in summer 2018.
(6) *Control (C):* Neither FCBK were applied, nor was a trap crops mixture sown.

Wireworm sampling

In total, seven wireworm sampling sessions were conducted between May 30, 2018 and August 9, 2018 (Fig. 1). In the beginning of the growing season (May–June), sampling was done once a month, while during the main growing period (July–August), sampling was done once a week. Within the plots only the center row was used for wireworm sampling to exclude cross-over treatment and boundary effects of neighboring plots. Over the entire sampling season, a total of 1008 soil samples were screened for *Agriotes spp.* larvae, and 345 individuals were collected. Sampling for wireworms was done by taking four soil samples per plot using a soil sampler (diameter 7 cm, depth 20 cm). Two samples were taken in the center of the potato ridge, and two on both sides at the ridge slopes. Each sample was examined for wireworms by spreading the soil out in a plastic container. Root and soil clumps were crumbled up and the soil was systematically searched for wireworms by moving it from one side of the container to the other. For each sampling session, the soil samples were taken about 35 cm further down the row from previous sampling locations (Fig. II, Supplementary Information). Each larva was packed separately into a 1.5 ml Eppendorf tube, labelled, and immediately stored in a cold box. At the end of the sampling day, all tubes containing wireworms were stored at -80 °C for later analysis.

Determination of species and larval stage as well as gut content analysis

Prior to DNA extraction for molecular species determination and molecular gut content analysis, all wireworm samples were cleaned from any residual DNA clinging to the wireworm's surface. For this purpose a 1–1.5% solution of sodium hypochlorite, Tween® 20 (AppliChem®, Darmstadt, Germany) and Milli-Q water was used as described in Staudacher et al. (2013). For the larval stage determination, the head capsule of each wireworm was cut off with DNA-free scissors, stored in 80% EtOH, and measured using a stereomicroscope and an objective micrometer with a millimeter scale. The maximal width of the head capsule was measured to the nearest 0.01 mm. Larvae which were too small to behead during lysis in the laboratory, were assigned to the first larval instar. Larval instars were defined by the head capsule widths provided in Klausnitzer (1994).

The body of each larva was cut into pieces and placed in a 2-ml reaction tube filled with 195 µl TES-buffer (0.1 mol/L TRIS, 10 mmol/L EDTA, 2% sodium dodecyl sulfate, pH 8), 5 µl proteinase K (20 mg/ml) and 10 DNA-free glass beads. Samples were homogenized twice for 30 s at 6000 g with a 45 s break in between using a Precellys® 24 Tissue Homogenizer (Bertin Instruments, Montigny-le-Bretonneux, France). The samples were left to rest for 10 min before centrifugation for 1 min at 8,000 g and incubation overnight at 58 °C. DNA extraction was done using the BioSprint® 96 DNA Blood Kit on the automated BioSprint® 96 extraction robotic platform (Qiagen, Hilden, Germany). Manufacturer's instructions were followed, except for the buffers: instead of the ATL- and AE-buffers, TES- and TE-buffers (AppliChem®, Darmstadt, Germany) were used. After extraction, the microplate containing the purified DNA was sealed and stored at − 28 °C. All DNA extractions were carried out in a separate pre-PCR laboratory using a UVC-equipped laminar flow hood for lysis preparations. PCR preparations were done in a cleanroom laboratory, and PCR products were size-separated and visualized using an automated capillary electrophoresis system (QIAxcel®, Qiagen, Venlo, The Netherlands) in a post-PCR laboratory. Thermocycling was carried out on a Mastercycler® Nexus (Eppendorf, Hamburg, Germany). First, all samples were tested in a singleplex PCR using species-specific *A. sputator* primer pairs S212 and A215, (Staudacher et al. 2011; for details on PCR protocols see Supplementary Information). Molecular species identification revealed that 236 (77%) of all collected wireworms were *A. sputator* and 70 (23%) *A. obscurus*.

Second, after molecular species determination, samples were screened for general plant DNA using the trnL primers B49317 from Taberlet et al. (1991) and trnL110R from Borsch et al. (2003). All samples that tested positive for plant DNA were afterwards screened for nine plant species in four multiplex PCRs originally designed by Wallinger et al. (2013), and adapted for this study. The same thermocycling protocols were used. *Triticum aestivum* and *Solanum tuberosum* primers were added to the multiplex system. Ryegrass (*Lolium perenne*) primers were by default part of this multiplex, and were kept since occurrence of ryegrass was expected to happen due to the conversion from grassland to arable land. For *Solanum tuberosum*, species-specific primers were designed and used together with previously published primers targeting *Triticum aestivum* (Table i, Supplementary Information).

Assessment of potato damage and yield

After the last wireworm sampling session, potatoes for damage assessment were harvested by hand on August 24, 2018. In each plot, all potato tubers in the center row within one meter were collected. The fresh biomass of the potatoes was determined and thereafter stored in a cool, dark place. To evaluate larval *Agriotes spp.* damage, 874 potato tubers were evaluated for wireworm damage. Tubers were washed and the wireworm-holes per potato tuber counted. The mass of every single potato was determined to account for different potato sizes, and wireworm-holes per gram potato were calculated. Additionally, other damage such as *Dry core (Rhizoctonia solani)*, scab coverage and other feeding marks (e.g., mice, white grubs or slugs) were recorded. Yield was compared among the treatments based on the fresh biomass per plot (weight/area), mean weight per potato tuber or amount of marketable potatoes based on a minimum mesh size of 35 mm which was converted from the potato mass of each tuber by a correlation factor.

Statistical analysis

Statistical analyses, visualization, and data wrangling were performed using the packages *dplyr, ggplot2, MASS, lme4, effects, scales, lmerTest, reshape2, tidyr, purr, ggeffects, randomForest, vegan* and *SciViews* of the R version 4.1.2 (R Core Team 2021). All statistical tests were performed with an alpha level of 0.05. To take different tuber sizes into account for assessing wireworm damage, number of wireworm holes per gram potato for each tuber was calculated. To exclude extreme sizes, only potatoes between 10 and 150 g were selected and potato weight was log-transformed for normalization. Additionally, potatoes with more than 0.6 wireworm holes/g potato mass and more than 30 holes per tuber were removed (in total four potato tubers). For potato damage rate within each respective plot, central tendency was represented by the median to compensate for skewness. To balance the dataset, all tubers with more than two holes were considered as damaged, this choice was made to generate a balanced dataset with a sufficient number of tubers classified as undamaged. To compensate for low wireworm abundances, wireworm abundance was summarized to monthly catches. For EPF, CFUs in June 2018 were analyzed using the median CFU/g $_{\text{(dry weight)}}$. A few extreme values of subsamples were taken out, by setting a cut off at 25,000 CFU/g $_{\text{(dry weight)}}$. Generalized linear mixed models with a suitable error distribution (either Poisson or binomial) were used to compensate for repeated sampling and non-independence of replicates within the same plot over time, by including time as a random factor. To confirm that model assumptions were met, we examined for each model diagnostic plots according to Zuur et al. (2010).

Results

Metarhizium spp. densities

Colony forming *Metarhizium spp.* units in soil were highest in June 2018 in all plots where fungus colonized barley kernels (FCBK) were applied in the main crop (mean: 8,073 ± 2,145 SD CFUs/g soil) when no trap crop was present (Fig. 2). The presence of trap crops did not influence *Metarhizium spp.* densities in any treatment, but the application timepoint showed a significant effect (Fig. 2). Compared with the control treatment (mean: 1,547 ± 1,203 SD CFUs/g soil), and the application in the previous crop (FPC) (mean: 1755 ± 862 SD CFUs/g soil), the average *Metarhizium spp.* density was higher when FCBK were applied in the main crop in 2018 (mean 8,237 ± 3,134 SD CFUs/g soil, $p < 0.001$). After the first application of FCBK directly into the grassland in the previous summer (FPC), no increase in *Metarhizium spp.* colony forming units could be observed (mean: 1,755 ± 862 SD CFUs/g soil). However, when FCBK were applied in summer 2017 in the FPC treatment, mean colonization was neither in October 2017, nor in spring 2018 significantly higher compared to the other treatments (Fig. V, Supplementary Information). Average *Metarhizium spp.*

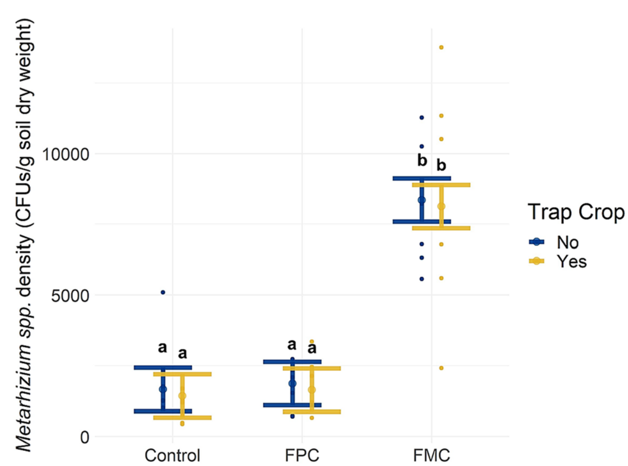


Fig. 2 *Metarhizium* spp. densities in June 2018 on the experimental field, compared among application treatments. The fungal application strategies are displayed on the *x*-axis. Fungus colonized barley kernels (FCBK) were either applied in the previous crop (FPC) or in the main crop (FMC). Average *Metarhizium* spp. colonization, measured as colony forming units (CFU) per g soil dry weight, is displayed on the *y*-axis, and presence or absence of trap crops is indicated in color. Significant differences ($p < 0.05$) between treatments are illustrated by small letters

density was highest when FCBK were applied in the main crop.

Wireworm abundance

Average wireworm abundance per sampling session was significantly reduced when *M. brunneum* conidia were applied in the main crop and combined with trap crops (mean: 1.72 ± 1.36 SD individuals/sampling session, $p = 0.003$, Fig. 3). Compared to the control treatment, the average wireworm abundance was reduced by 50.8%. When EPF were applied in the previous crop, or when only trap crops were implemented in the field, the average wireworm abundance per sampling session was not significantly lower compared to the control. The distribution of wireworms between ridge center and ridge slope showed no significant differences among treatments (Fig. VI, Supplementary Information). *Agriotes sputator* was present in all eight larval instars, while for *A. obscurus* larvae only the instars L3 to L8 were found. For both species, the largest fraction of individuals belonged to L6 (25% for *A. sputator* and 32% for *A. obscurus*), followed by L5 and L7. Species ratio did not significantly differ between treatments or sampling dates (Table i, Supplementary Information).

Wireworm diet

In total, 318 wireworms were analyzed for plant DNA using general primers which resulted in 37.4% of the samples being positive for plant DNA. Over the whole season, between 26.2% (May 30, 2018) and 64.5% (August 2, 2018) of the larvae were tested positive for plant DNA (Fig. VII, Supplementary Information). At the beginning of the sampling season, detection rates of plant DNA were lowest (Fig. 4). Potato DNA was detected significantly more often than the other plants in all treatments at all sampling dates ($p < 0.05$). An increase in plant DNA as well as potato DNA detection rates over time could be observed (Fig. 4). In treatments where trap crops were present, no significant difference of consumed plant species could be detected. Also, no significant species-specific differences for the detection of plant species between *A. sputator* and *A. obscurus* could be found. Runner bean was never detected throughout the season, while lupine was detected only on the 28th of June. Besides potatoes, ryegrass and buckwheat were detected most often.

Potato damage

Potato damage reduction was strongest when trap crops and EPF were combined in the main crop (mean: 0.074 ± 0.03 SD holes/g). Compared to the control treatment (mean: 0.12815 ± 0.06 SD holes/g), wireworm-holes per gram potato were significantly reduced ($p < 0.001$) by this combination by 42.5% on average (Fig. 5). The mean number of wireworm-holes per gram potato was lowered by 34.7% when only EPF were applied in the main crop (mean: 0.084 ± 0.04 SD holes/g), or reduced by 29.9% when only trap crops were present (mean: 0.09 ± 0.04 SD holes/g). When EPF were applied alone in the previous year, potato damage was reduced by 22.3% (mean: 0.1 ± 0.02 SD holes/g). The combination of EPF application in the previous year, combined with trap crops in the main crop, reduced damage by 26.9% (mean: 0.94 ± 0.05 SD holes/g).

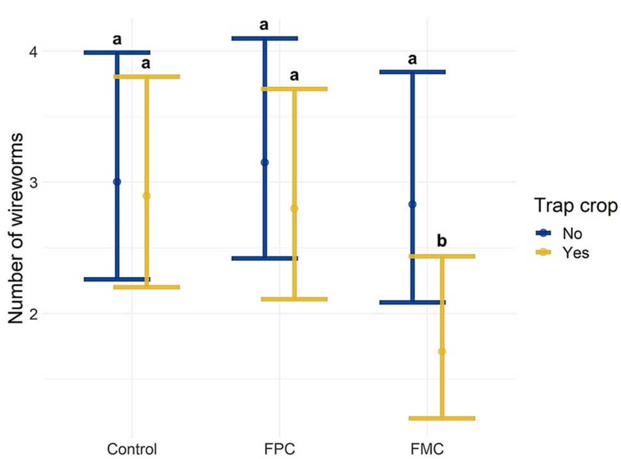


Fig. 3 Average wireworm abundance per sampling session (*y*-axis) compared between treatments (*x*-axis). Within the different treatments, fungus colonized barley kernels were either applied in the previous crop (FPC) or in the main crop (FMC), and combined with or without trap crops (color). Significant differences ($p < 0.05$) between treatments are illustrated by small letters

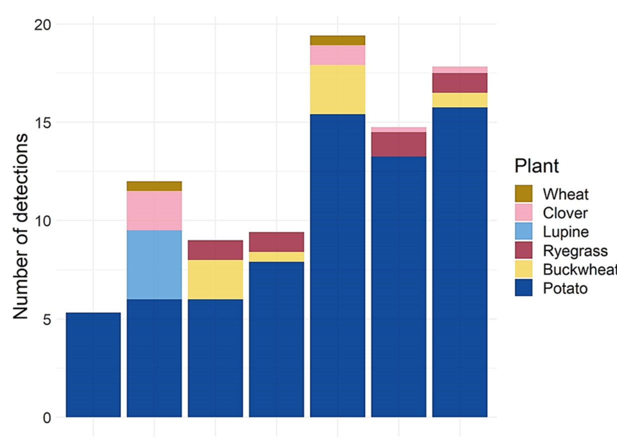


Fig. 4 Absolute number of the detection of DNA from potato and trap crops (*y*-axis) in the gut of field-collected wireworms for all seven sampling sessions (*x*-axis) between May and September 2018. Feeding preferences of wireworms were identified throughout the season by multiplex-PCRs

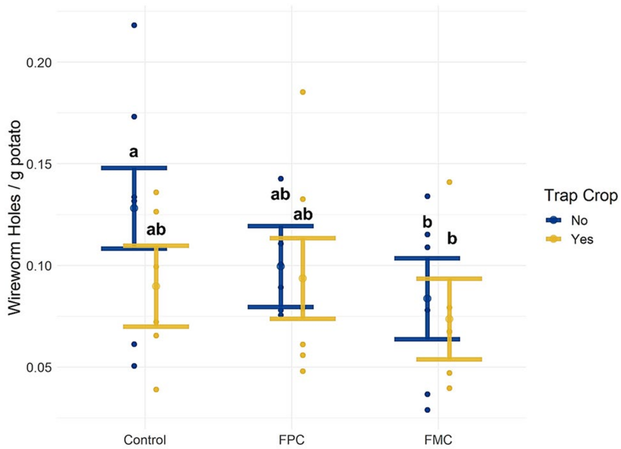


Fig. 5 Wireworm damage assessed as average wireworm holes per g potato mass (*y*-axis) for different treatments (*x*-axis). Within the different treatments, fungus colonized barley kernels were either applied in the previous crop (FPC) or in the main crop (FMC), and combined with or without trap crops (color). Significant differences ($p < 0.05$) between treatments are illustrated by small letters

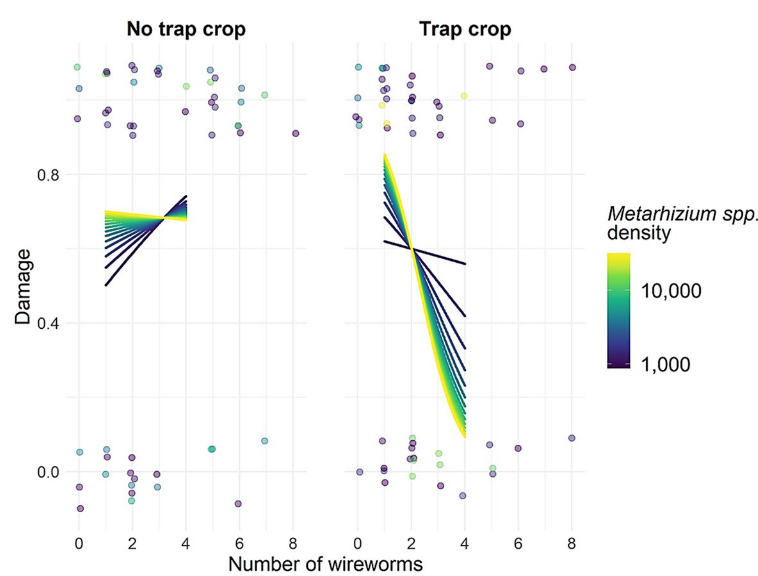
Interaction of EPF and trap crops

The presence of trap crops in combination with high numbers of *Metarhizium spp.* CFUs reduced wireworm damage especially when wireworm abundance was high (Fig. 5). Without trap crops, wireworm damage was reduced only slightly even at high *Metarhizium spp.* densities. In the control treatment, damage was positively correlated to wireworm abundance. The single application of either *M. brunneum* spore application or trap crops reduced wireworm damage, but the size of this effect was not correlated to wireworm abundance. When trap crops were present, damage was reduced drastically with increasing *Metarhizium spp.* densities. Thus, damage reduction was not just linear, but reciprocally correlated to *Metarhizium spp.* densities and pest abundances when trap crops were present. However, trap crops performed better in terms of damage reduction than its combination with EPF when wireworm abundances were smaller than 2 wireworms per sampling session (Fig. 6).

Potato yield

In total, 46.4 kg of potato tubers were harvested for yield estimation within the 36 plots. A comparison of the mean potato tuber weight among treatments showed no significant differences (Fig. VIII, Supplementary Information). In contrast, mean potato mass per tuber indicated differences in tuber size among treatments. Tubers in treatments Trap crop, FPC, FMC and FMC + T had a significantly higher biomass than tubers from the Control treatment and FPC + T (Fig. IX, Supplementary Information). The proportion of potato tubers having at least the required minimal marketable size of 35 mm (31.8 g), was with 57% smallest in the Control treatment followed by the combination of FPC + Trap crops with 60%. The highest relative amount occurred with 73% in the Trap crop treatment. All other treatments reached proportions ranging from 67 to 72% (Table iii, Supplementary Information).

Fig. 6 Simulation of wireworm damage (*y*-axis) on potato tubers as a function of *Metarhizium* spp. density (color), and number of wireworms (*x*-axis), compared between trap crops presence and absence. *Metarhizium* spp. density is measured as colony forming units (CFUs) per g dry soil and number of wireworms refers to average wireworm abundance per sampling session. Dots represent binary wireworm damage (damage = 1/no damage = 0) in relation to the corresponding wireworm abundance, while lines show the fitted trend for damage at different *Metarhizium* spp. levels



Discussion

We here show that combining trap crops with EPF can be highly efficient to reduce potato damage by *Agriotes spp.* and wireworm abundance. We found no statistically detectable suppression of potato damage as well as wireworm abundance by EPF or trap crops when implemented as individual measures. In contrast, combining EPF and trap crops generated a synergistic protection against wireworms without reducing potato yield with disproportionately strong effects at high pest densities.

Synergistic interactions between trap crops and EPF improve control efficacy

We hypothesized that the combination of entomopathogenic fungi and trap crops will reduce wireworm abundance and potato damage the most, because environmental conditions for EPF establishment are improved. Our findings support this hypothesis, as trap crops were able to boost the performance of EPF especially at high pest insect abundances and significantly reduced both, wireworm abundance by 42.5% and damage on potatoes by 50.8% compared to the control treatment.

In the field, establishment of EPF is known to suffer from dry soils and UV-radiation. Plant covers facilitate protection from light, reduce water evaporation, and root material provides an improved habitat for EPF (Quesada-Moraga et al. 2022). Trap crop strips could provide improved conditions for *M. brunneum* to establish by reducing abiotic limiting factors (Jaronski 2010; Vega 2018). Rhizosphere colonization of *M. brunneum* is expected to improve establishment, resulting in increased infection rates on the long term compared to an application on bare soil. Even though *Metarhizium spp.* CFU were not detected to be influenced by the presence of trap crops, wireworm abundance was reduced strongest within the combination. Reduced abundance could be the result of *Metarhizium spp.* densities not being increased in general, but maintained at a high level for a longer time period. Other field experiments suggest that the presence of plants improves the lifetime of EPF spores (Reinbacher et al. 2021; Rogge et al. 2017). For this study the effect on the lifetime of EPF is not known since densities were only measured once when trap crops were present.

Contrary to the expectation that plant covers facilitate improved conditions for EPF establishment, *M. brunneum* was in our study not able to establish well when directly applied into grassland. After FCBK were applied in late summer 2017, EPF might have still been affected by dry conditions and hot temperatures. Additionally, grassland soils possess a diverse, well established soil community of microorganisms and competition between these microorganisms and *M. brunneum* influences its establishment negatively (Mayerhofer et al. 2017). Another factor that putatively restricted the EPF establishment was a decreased aeration when EPF spores were placed beneath the grass sward in summer 2017. *Metarhizium brunneum* was shown to established well when applied into an cover crop in summer after grassland was plowed (Reinbacher et al. 2021). Better aeration of soil after plowing might favor spore germination and establishment.

In addition to an improved establishment or elongated lifetime when EPF and trap crops are combined, the probability of wireworms' infection might be increased when trap crops are present, because wireworms are feeding on, or pass by EPF-colonized roots (Hu and Bidochka 2019; Quesada-Moraga et al. 2022). Repellency of *M. brunneum* toward wireworms is known to constrain infection, except when a food source is available (Kabaluk and Ericsson 2007). In our study, *M. brunneum's* repellency might have been affected by the presence of trap crops roots. The observed synergistic protection against, and reduction of wireworms could be influenced by the rhizosphere colonization of *M. brunneum* and subsequent root feeding of the insect pest. Higher infection rates might occur because spores attach to vulnerable parts of the insect (e.g., thin membranes close to mandibles) more easily due to increased feeding activity close to colonized roots. Wireworm burrowing networks tend to be less complex and shallower when plant roots are present (Booth et al. 2022). This behavior might also favor the infection with *M. brunneum* spores when trap crops are present, since wireworms spend more time in the upper soil layer close to applied conidia. Increased spore attachment on wireworms due to their elevated activity close to trap crops roots, and reduced repellency towards *M. brunneum* when trap crops are present, might be the main drivers of the observed control interaction.

The effect of trap crops on wireworms' diet

Secondly, we hypothesized that the presence of trap crops shifts the diet of wireworms away from potatoes. When trap crops are implemented between maize rows, wireworms can effectively be distracted from the main crop (Staudacher 2013). Due to seasonal changes in feeding preferences, it was suggested that trap crops mixtures should be preferred over the use of a single trap crop. However, within our study no shift in feeding or distraction was observed when trap crops were implemented in between potato ridges. Compared to the study conducted by Staudacher et al. (2013) in maize, trap crops were implemented seven weeks after the main crop. By this time, potato roots were already well developed, resulting in a higher amount of CO^2 emitted compared to the small root systems of trap crops. Since CO^2 is one of the main cues attracting wireworms (Barsics et al. 2014), and CO^2 emission correlates with the mass of belowground plant parts, freshly formed potato tubers might have been the primary CO^2 source. Trap crops will most probably only be successful when food availability is limited close to the main crop while food availability in the trap crop strip is high. Attractiveness of potato tubers compared to trap crops was likely boosted by the high abundance and early presence of potato plants. Since wireworms are known to migrate only if food supply is insufficient on site (Schallhart et al. 2012; Sonnemann et al. 2014), potato roots and tubers might have been attractive enough to avoid wireworm migration already at the beginning of the season. Another reason for the lack of wireworm attraction might be the fact that attraction toward plants is positively correlated to plant density (Sarkar et al. 2018). In this study, trap crops were sown only in small strips on the ridge slopes resulting in low trap crop densities. A denser trap crop strip simulates a perfect habitat for wireworms because they prefer dense root systems and high soil moisture (Traugott et al. 2015).

When soil moisture is low, wireworms are known to damage potatoes stronger in order to take up water from tubers (Langenbuch 1932). Thereby, wireworms might have caused shallow lesions which were too small to be considered as damage, but were sufficient to detect potato DNA within the gut content. This might explain the low tuber damage of the combined treatment, albeit there was a high proportion of potato DNA detected in the gut. The consumption of ryegrass, which was not part of the implemented trap crops, leads to the assumption that weeds also play a role within the wireworms' diet (Traugott et al. 2008). Ryegrass is a common grass species in this region and therefore its occurrence as a weed is very likely. Since wireworm abundance is affected by the density of weeds, knowledge on preferred weeds could help for future wireworm management (Parker and Howard 2001).

Potato yield

Thirdly, we tested, whether trap crops would reduce potato yield, since the main concern with trap crops is their competition on water and nutrients with the main crop (Ratnadass et al. 2012). In our study no effect on potato yield could be observed, but potato size was positively influenced by the presence of EPF as well as trap crops, resulting in a higher ratio of marketable potatoes within these treatments. When applied as a seed treatment, *M. brunneum* was previously shown to improve plant's phosphorus uptake and to possess plant growth promoting properties toward *Vicia faba* in general (Jaber and Enkerli 2016; Krell et al. 2018). *M. brunneum* is able to transfer insect-derived nitrogen to plants while the plant provides in exchange photosynthate to the fungus via mycelia (Lata et al. 2018). These features of *M. brunneum* might compensate for possible yield losses which can occur due to strong competition for light, water, or nutrients when trap crops are implemented (Sastawa et al. 2004). Trap crops and EPF influence nitrogen supply provided by nitrogen fixation bacteria associated with legumes and water availability by plant-plant interactions improving root growth of potatoes through competition and bio irrigation (Singh et al. 2020; Soliveres et al. 2011). Plant growth promoting properties of *M. brunneum* might have positively influenced potato tuber size and accounted for competition for nutrients by trap crops. A concern with trap crops is the increase in food availability for wireworms and its positive effect on their development. Due to a low number of caught wireworms, the influence of trap crops on larval development could not be analyzed within this study. An advantage of trap crops is their ability to limit erosion and nitrate leaching (Mouraux et al. 1992). In addition, the implementation of trap crops increases species richness and biodiversity on field scale. In general, ecosystem function often improves as species richness increases because the chance of species occupying complementary niches, rises (Finke and Snyder 2008; Parker et al. 2016).

Combining trap crops and EPF for practicable control of soil pests

Biocontrol practices as well as integrated pest management (IPM) practices promote the combination of multiple measures for the control of soil-dwelling pests to increase efficacy and reliability, but the compatibility of the measures needs to be evaluated (Poggi et al. 2021; Spescha et al. 2023). Trap crops and EPF seem to be well compatible in the field since neither of them was negatively influenced when applied in combination. To test a potential improved reliability, experiments on multiple fields with varying conditions need to be performed. Before practical implementation, attention has to be drawn on possible negative side effects on non-target organisms as well as crop, and soil health (Nikoukar and Rashed 2022). Considering soil health, the combination of EPF and trap crops has a lower risk of negative impact compared to other IPM practices such as intensive soil tillage or habitat-landscape modifications (Poggi et al. 2021). *Metarhizium brunneum* ART2825 is a naturally occurring entomopathogenic fungus that has already been shown to have no adverse effect on soil microbial communities, and companion planting enhances agroecosystem services and soil health (Mayerhofer et al. 2017; Nikoukar and Rashed 2022). While the damage- and population reduction provided by the combination of EPF and trap crops alone might not be sufficient for commercial potato production, its good compatibility with most other IPM measures, for instance low risk crop rotations or adult biocontrol, might counterbalance this. Therefore, it should be considered as an important and relatively inexpensive practice for IPM and organic farming (Poggi et al. 2021). For large scale implementation, the seeding of trap crops needs to be mechanized, and synchronized with other cultural measures in particular herbicide application and mechanical weed control. Also, the costs for EPF production and application should be optimized to make it economically feasible as a control measure to be adopted by farmers.

Conclusion

The present study revealed positive control interactions of EPF and trap crops for soil pests when applied in combination. *Metarhizium spp.* densities were highest when FCBK were applied in the same year as potatoes. When trap crops and EPF were combined, wireworm damage in potatoes was shown to be reciprocal when high abundances of wireworms occurred, while potato yield was not significantly affected by any of the tested treatments. Plant growth promoting properties of *Metarhizium brunneum* might counterbalance possible yield loss when combined with trap crops (Jaber and Enkerli 2016; Lata et al. 2018). With this study, a three-dimensional interaction between plants, EPF and insect's abundance was shown for the first time to occur under field conditions. These findings play an important role for future strategies of improving soil pest control, but the underlying control mechanism needs to be investigated to further optimize trap crops mixture, its implementation and *M. brunneum* application. Since wireworm diet was not influenced by the presence of trap crops, plant species composition of the trap mixture needs to be improved. Seasonal changes in wireworm's diet are known and have to be considered when candidates for tap crops mixtures are selected (Staudacher et al. 2013). For potatoes, the most relevant damage happens in late summer, and therefore trap crops attractive for this period need to be identified. Combining trap crops and EPF promises to be an important module for future pesticide-free wireworm- and soil pest control in general. An attract-and-kill strategy using trap crops and *M. brunneum* spores should be considered to further improve wireworm control. Wheat, for example, has been shown to increase efficacy of chemical pesticides against wireworms in potatoes (Vernon et al. 2016), or in a field trial performed by Brandl et al. (2017), wireworm damage was reduced significantly by the use of EPF encapsulated together with baker's yeast (*Saccharomyces cerevisiae*) as an attractant. Most probable, the use of individual measures in single years will not be sufficient to control wireworms and a combination of methods should be preferred (Traugott et al. 2015). Further studies are necessary to (i) identify the control mechanism when trap crops and *M. brunneum* spores are combined and (ii) evaluate its control potential when combined with suitable approaches in a crop rotation wide control strategy.

Author contributions

MT and CZ conceived and designed research. GG and LR gave advice on EPF application, provided FCBK and analyzed Metarhizium spp. densities. CZ, DN and CR conducted field experiment and laboratory work. OR and MB analyzed data. MB wrote the manuscript. All authors read and approved the manuscript.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10340-023-01726-1.

Acknowledgements We are grateful to Gerhard Melmer and his team, as well as Christian Partl and his team who maintained the experimental field and gave advice on agricultural practices. We thank Maria Hauser for providing the field for this study. We thank Florian M. Steiner for revising and commenting on the manuscript.

Funding Open access funding provided by University of Innsbruck and Medical University of Innsbruck. This research was conducted in the course of an EIP-AGRI project, 1. Call 2015, project numbers 16.1.1-S2-09/15 and 16.2.1-S2-09/15, funded by the Austrian Federal Ministry of Agriculture, Forestry, Environment and Water Management, the federal states of Austria and the European Union. Additional funding was provided by the Mountain Agricultural Research Unit of the University of Innsbruck and the regional Government of Tyrol.

Data availability The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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