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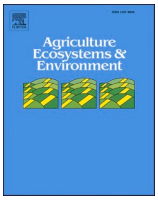

Combining entomopathogenic *Pseudomonas* bacteria, nematodes and fungi for biological control of a below-ground insect pest

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ABSTRACT

Below-ground insect pests are challenging to control because they are hard to target with control measures. Moreover, broad spectrum insecticides are or will soon be banned due to their negative effects on non-target organisms. In this study, we have developed a biological control method for the cabbage maggot *Delia radicum* (Diptera: Anthomyiidae), a significant pest of Brassicacean crops, based on a consortium of three biocontrol agents (BCAs). We chose the bacterium *Pseudomonas chlororaphis* because it can be used in a dual strategy against insect pests and fungal plant diseases, and combined it with the nematode *Steinernema feltiae* and the fungus *Metarhizium brunneum* that have a long history of commercial use against different pest insects. Our aim was to combine BCAs with different modes of action in order to achieve a stable and reliable biocontrol effect. We first tested double combinations of the bacterium with either the nematode or the fungus for improved potential to kill *D. radicum* in laboratory assays. We then evaluated the effect of double and triple combinations on *D. radicum* development and maggot-induced damage on radish bulbs in a series of pot experiments with artificial cabbage maggot infection performed in the greenhouse and outdoors and finally in a field trial with a natural infestation. Our results show that i) insecticidal pseudomonads are highly efficient in *D. radicum* control, ii) the three BCAs are compatible and neither inhibit each other's infectiousness nor survival in the soil or on the roots, iii) synergistic effects of *Pseudomonas*-nematode and *Pseudomonas*-fungus combinations on maggot killing are possible, and iv) the triple combination reduced both pest survival in greenhouse experiments and maggot-induced damage on radish bulbs in the field by 50% each. The strategy we present here is a promising step forward to a reliable and efficient environmentally friendly biological control method for the cabbage maggot, which can also be adapted to other problematic below-ground pests.

1. Introduction

Crop yields are under constant threat from pathogens and pests, both above and below ground (Oerke, 2006; Savary et al., 2019). Monocropping is particularly beneficial for the spread of these pathogens and pests as it provides a high density of suitable host plants within a close range (McDonald and Stukenbrock, 2016). Conventional large-scale agriculture relies heavily on pesticides to protect yields (Panth et al., 2020), but pesticide use is linked to a multitude of problems. The widespread and excessive application of pesticides containing the same

Abbreviations: BCA, biocontrol agent; EPB, entomopathogenic bacteria; EPP, entomopathogenic pseudomonads; EPF, entomopathogenic fungi; EPN, entomopathogenic nematodes; NB, nematode-associated bacteria; Bt, *Bacillus thuringiensis*; Ma, *Metarhizium anisopliae*; Bb, *Beauveria bassiana*; cfu, colony forming units; IJ, infective juveniles.

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group of active ingredients leads inevitably to the development of resistances, rendering the product less- or ineffective (Zhan et al., 2014). Pesticide use also affects the environment and harms non-target organisms, many of which are essential to our food production (Kumar and Kumar, 2019; Islam et al., 2017). Therefore, there is an increasing demand for alternative control measures.

Biological control agents (BCAs) are a promising alternative control measure and BCA products are a rapidly expanding market (van Lenteren et al., 2018). The application of BCAs can reduce pest or pathogen damage below an economic threshold (Babbal et al., 2017; Caltagirone, 1981; Ritika and Utpal, 2014). The most widely used biological control agent (BCA) is the entomopathogenic bacterium (EPB) *Bacillus thuringiensis* (Bt) (Sanchis and Bourguet, 2008). It produces effective small-host-range Cry toxins that are also commonly expressed in transgenic crop plants (Sanchis, 2011). However, the widespread use of Cry toxins either as BCA or in transgenic plants has led to the emergence of insects that are resistant to these toxins (Melo et al., 2016).

One approach to overcome the limitations of a single BCA application is to combine BCAs with other management methods or to combine different biocontrol agents (Malusà et al., 2021). Only a few studies have explored this option so far with mixed results. These involved some lab, greenhouse and field experiments using combinations of nematodes with either fungi or bacteria against different insect pests (Ansari et al., 2010; Bueno-Pallero et al., 2018; Jaffuel et al., 2019; Mc Namara et al., 2018; Shapiro-Ilan et al., 2004). Combinations of BCAs sometimes had improved or neutral effects, sometimes antagonistic effects, and results often varied strongly depending on the year (e.g. for field trials) or the application technique. To increase biocontrol efficacy and consistency, we need to develop effective combinations of BCAs i.e. biocontrol consortia. However, such consortia do not only have to be evaluated for their biocontrol efficacy but also for their compatibility. Thus, the goal of this study was to test three BCAs with different modes of actions for their compatibility and for their combined effect on an important root pest. We chose to test and combine entomopathogenic pseudomonads (EPP), entomopathogenic nematodes (EPN) and entomopathogenic fungi (EPF) for controlling the cabbage maggot *Delia radicum*.

Pseudomonas protegens and *Pseudomonas chlororaphis* are root-colonizing bacteria which are especially interesting for agriculture because they possess plant-growth promoting and disease suppressive capacities (Chin-A-Woeng et al., 1998; Haas and Défago, 2005; Loper et al., 2012; Maurhofer et al., 1994) and additionally have potent oral insecticidal activity (Flury et al., 2016; Rangel et al., 2016; Ruffner et al., 2013). The relationship of entomopathogenic pseudomonads (EPP) with insects and the determinants of their insecticidal activity have been extensively studied over the last 15 years, especially for the model strains *P. protegens* CHA0 (Pronk et al., 2022) and Pf-5 (Loper et al., 2016). EPP rely on multiple factors to infect and kill insects: toxins like the Fit (*P. fluorescens* insecticidal toxin) (Kupferschmied et al., 2014, 2013; Ruffner et al., 2013), rhizoxin (Loper et al., 2016) and TPSA's (two-partner secretion proteins) (Vesga et al., 2020), enzymes such as chitinases and phospholipases (Flury et al., 2016), the Type 6 secretion system (T6SS) (Vacheron et al., 2019), as well as antimicrobial exoproducts (Flury et al., 2017; Jang et al., 2013; Loper et al., 2016). The versatile life-style and multifactorial mode of action of EPP makes them ideal biocontrol agents with little risk of resistance development and dual or even triple use: they can be applied against fungal pathogens and insect pests while at the same time promoting plant growth and vigour in general.

Although pseudomonads have many plant-beneficial activities, there are only a few *Pseudomonas*-based products on the market. Pseudomonads are registered as antifungal agents (e.g. Cerall/Cedomon and Proradix in the EU and in Switzerland) or are ingredients in biofertilizers (Babbal et al., 2017; Ritika and Utpal, 2014). However, the biocontrol potential of these bacteria against insect pests has so far barely been commercially explored. Yet, DuPont Pioneer has inserted an insecticidal protein derived from a *P. chlororaphis* strain into a corn variety, rendering plants resistant to corn rootworms (*Diabrotica* spp.) (Boeckman et al., 2021; Carlson et al., 2019; Schellenberger et al., 2016).

Entomopathogenic fungi and nematodes are widely used to control insect pests and many EPF- and EPN-based biocontrol products are commercially available. The most commonly applied EPF species are *Metarhizium anisopliae* (Ma) and *Beauveria bassiana* (Bb) (van Lenteren et al., 2018). Both Hypocrealean EPF rely on multiple exoproducts to overcome the insect's defence mechanisms and kill it, such as proteases, chitinases, lipases, immunomodulation and transcription factors, as well as beauvericin, bassianin and oosporein for Bb, and cyclosporine and destruxin for Ma (Barelli et al., 2016; Butt et al., 2016; Meyling and Eilenberg, 2007; Schrank and Vainstein, 2010). EPN are associated with specific bacteria (nematode-associated bacteria, NB) that play the most important part in killing the insect, e.g. *Steinernema feltiae* with *Xenorhabdus bovienii* (Campos-Herrera, 2015). EPN enter the insect mainly through natural openings and carry their NB into the haemolymph (Goodrich-Blair and Clarke, 2007; Stock, 2015). In the haemolymph, NB express different insecticidal toxins, e.g. Tc's (toxin complexes) and Mcf (makes caterpillar floppy), and suppress both the hosts immune system as well as other microbes, i.e. using antimicrobials and T6SS, while EPN express venom proteins or ESPs (excreted/secreted products) (Eliáš et al., 2020; ffrench-Constant et al., 2007; ffrench-Constant and Bowen, 2000; Kochanowsky et al., 2020; Lu et al., 2017). As for the pseudomonads, the entomopathogenic activity of EPF and EPN relies on multiple mechanisms. The probability that an insect simultaneously evolves defences against several pathogenicity mechanisms is very low. Thus, the evolution of resistance to EPF, EPN and EPP is unlikely.

The pest used in this study is the cabbage root fly or cabbage maggot *Delia radicum* L. (Diptera: Anthomyiidae) which poses a big challenge for producers of Brassicacean crops. The larvae feed on the below ground parts of several crops such as canola, cabbage, radish, broccoli and cauliflower. Yield losses occur when larvae feed on the produce, e.g. radish or turnip, or reduce plant growth and seed numbers, or cause seedling death due to heavy root damage, e.g. in broccoli or canola. *D. radicum* infestations can be devastating for vegetable and oilseed producers in temperate regions, with estimated annual economic losses of $100 million in Western Europe and Northern America (Sontowski et al., 2022). Only very few insecticides are available for controlling the cabbage maggot and their efficacy is often limited. For example, cyantraniliprole (registered for *D. radicum* control in Canada) is highly toxic to bees (Lewis et al., 2016) and is less efficient than the formerly widely used chlorpyrifos (van Herk et al., 2017) that is now banned in the EU, Canada and the USA (EFSA, 2019; EPA, 2021; PMRA, 2020). As a cultural measure, besides crop rotation and weed management, the use of nets is recommended to keep the flies from laying eggs in the field (Hauenstein and Vieweger, 2021). Though this measure may be very effective, it also complicates field management (Witkowska et al., 2018). There have been several attempts to control the cabbage maggot with entomopathogenic fungi and nematodes. Although laboratory and greenhouse studies identified promising candidates, the efficacy was generally low in field trials (Chen et al., 2003; Herbst et al., 2017; Razinger et al., 2017; Vänninen et al., 1999a).

In order to establish an effective biological control method using multiple BCAs we conducted a series experiments to 1) evaluate the potential of insecticidal *P. chlororaphis* as novel biocontrol agent for controlling *D. radicum*, 2) investigate the compatibility of entomopathogenic pseudomonads with entomopathogenic nematodes and entomopathogenic fungi for joint applications against soil-derived insect pests, and 3) explore BCA consortia for their potential to control *D. radicum* in comparison to each single BCA.

As a first step, different EPP-EPN and EPP-EPF combinations were investigated for synergisms in the killing of *D. radicum* under laboratory conditions. The most promising strains were used to form a tripartite consortium, which was tested against the cabbage maggot on radish in greenhouse pot experiments, outdoor pot experiments and a field trial. The impact of individual BCAs on the survival of the other consortium

members and potential synergistic effects on insect control were monitored at all stages. Using this systematic approach, we have developed an consortium based on three BCAs with different modes of action for controlling the cabbage maggot.

2. Material and methods

2.1. Rearing of organisms

2.1.1. Cabbage maggot Delia radicum

Pupae were obtained from Swiss field sites and from research groups at the Julius Kühn Institute in Braunschweig (Germany) and the University of Rennes (France). Pupae were stored in sand (0.3–0.9 mm) at 3 °C in the dark. To induce fly emergence, the pupae were placed in an insect cage within a climate chamber with the following rearing conditions: 16 h daytime at 20 °C and 15 kLux and 8 h nighttime at 18 °C, and 80% relative humidity. Emerging flies were provided with water by adding wet sand (0.3–0.9 mm) and fed on dry (10 g glucose, 10 g milk powder, 1 g soy flour, 1 g dry yeast) and wet food (5 g honey, 5 g soy flour, 1 g dry yeast, ~6.5 ml ddH_2O) (all ingredients except for glucose were purchased at Coop Supermarket, Switzerland). To induce egg laying, kohlrabi pieces were placed on the wet sand. Eggs were harvested by pouring the sand in an 800 ml beaker, adding water and filtering the water through a ø 185 mm filter paper folded in a funnel. Approx. 80 eggs were transferred on a ø 90 mm filter paper with a brush and placed in an 800 ml beaker on a 2 cm sand layer. Half a kohlrabi was added on top of the eggs and covered with sand. After four weeks, maggots had completed larval development and the pupae were harvested by rinsing the remainder of the kohlrabi and the sand over a 2 mm and then over a 1.5 mm sieve.

2.1.2. Pseudomonas chlororaphis

Bacterial strains (Tables 1 and S1) were stored at − 80 °C in 44% glycerol. Colonies were grown on King's B medium with antibiotics (KB^{+++} with cycloheximide 100 mg/l, chloramphenicol 13 mg/l and ampicillin 40 mg/l; KB^{++G} with gentamycin 10 mg/l instead of ampicillin for *gfp*-tagged strains) (King et al., 1954; Vesga et al., 2021). For experiments, overnight Lysogeny broth (LB; 10 g Bacto Tryptone, 5 g Bacto Yeast Extract, 0.25 g $MgSO_4$ x 7 H_2O, 8 g NaCl dissolved in 1 L ddH_2O) liquid cultures were prepared (Bertani, 1951). These were either used directly or to inoculate KB plates (without antibiotics), where bacteria multiply to high numbers within 24 h. The bacteria were washed in ddH_2O, the optical density at 600 nm (OD_{600}) was measured (Ultrospec 3300 pro, Amersham Biosciences, UK) and suspensions adjusted to the desired concentration with an OD_{600} = 0.125 corresponding to approx. 10^8 cfu/ml.

2.1.3. Steinernema feltiae

The commercially available formulated product of nematode populations (Table 1, S1) was kindly provided by e-nema (Schwentinental, Germany) and Andermatt Biocontrol (Grossdietwil, Switzerland). The powder was dissolved in tap water and suspensions used to infect *Galleria mellonella* (Lepidoptera: Pyralidae) larvae (from Hebeisen Fisher or Andy's Fisher store, Zurich, Switzerland). The other EPN populations were isolated from field studies during 2013–2015 in Switzerland (Campos-Herrera et al., 2015; Imperiali et al., 2017; Jaffuel et al., 2018; Table 1). All nematode populations (also referred to as strains) used in this study were regularly multiplied in *G. mellonella* larvae using the White Trap method (Campos-Herrera et al., 2015; White, 1927). Emerging infective juveniles (IJ) were stored at 500 IJ/ml in tap water in filter cap cell culture flasks (75 cm^2, CELLSTAR®, Greiner Bio-One, Austria) at 15 °C for up to four months. Fresh IJ (no older than three weeks) were used for experiments. The concentration was determined by counting IJs in 20 or 50 µl suspension under a stereomicroscope and suspensions were adjusted with tap water to the desired concentration (1000 IJ/ml for most experiments).

2.1.4. Metarhizium brunneum and Beauveria bassiana

The fungi *M. brunneum* BIPESCO5/F52 (Bip5) and *B. bassiana* ART2587 (Table 1) were stored as conidia on plates with SM medium (Strasser et al., 1996) for up to one year at 3 °C. The infectivity of fungal isolates was maintained by frequent passaging through host insects and subsequent single spore isolation as described by Reinbacher et al. (2021b). For experiments, SM plates were inoculated by transferring fungal spores from a stored culture using an inoculation loop (Strasser et al., 1996) and incubated for two weeks at 24 °C in the dark. Conidiospores were scraped off the plates using sterile inoculation loops or Drigalski spatula and suspended in 0.01% Tween80. For the semi-field trial, sterilized barley kernels were inoculated with Bip5 and incubated for several weeks at 22 °C in the dark as described by Reinbacher et al. (2021a). The spores were dried and harvested from the kernels using a myco-harvester MH5 (VBS Agriculutre Ltd., Beaconsfield, UK). Concentration was measured by counting conidiospores suspended in 0.01% Tween80 under the microscope using KOVA or Thoma chambers and adjusted to the desired concentration with ddH_2O.

2.2. Laboratory sand assay and greenhouse experiments

For investigating basal compatibility and possible synergistic effects of the three BCA, a simple sand-radish bulb test system was used as described by Flury et al. (2019) for pseudomonads and adapted for nematodes and fungi as described in detail in the supplementary methods.

For greenhouse experiments, we used red bulb forming radish *Raphanus sativus* var. *sativus* cultivar 'Riesenbutter' (Samen Mauser or Coop, Switzerland) with the following settings: 21 °C (16 h, day) and 18 °C (night) at 70% humidity. Radish seeds were sown in 11×11×12 cm pots (Lamprecht-Verpackungen, Göttingen, Germany) into a mixture (1:1) of Jiffy peat substrate (Jiffy Products International, Moerdijk, the Netherlands) and Allmig substrate (Trog- und Topferde, Allmig, Baar,

Table 1
Biocontrol agents used in this study.

Species	Strain/ population	Origin	Reference
P. chlororaphis	PCLRT03 (P)	Potato root, CH	Vesga et al. (2021)
P. chlororaphis	PCLRT03-gfp	Derivative of PCLRT03, PCLRT03::miniTn7-gfp2; Gm^R	This study; Provided by Jordan Vacheron, Université Lausanne
P. chlororaphis	PCL1391	Tomato root, ESP	Chin-A-Woeng et al. (1998); Flury et al. (2016)
S. feltiae	RS-5 (RS5) (N)	Soil, wheat field, CH	Campos-Herrera et al. (2015); Jaffuel et al. (2018)
S. feltiae	MG-594 *	Soil, grassland, CH	Jaffuel et al. (2018); this study
S. feltiae	nemaplus	e-nema AG	e-nema AG
M. brunneum	BIPESCO5/F52 (Bip5) (F)	*Cydia pomonella*, AUT	EFSA, 2012
B. bassiana	ART2587	*Meligethes* sp., CH	Meyling et al. (2012); Pilz (2005)

*This population was isolated within the frame of the respective study, but first individually described in this study

Switzerland) and watered by adding water to the trays containing the pots. For each treatment, four trays containing each four pots with three plants per pot were prepared. In general, plants were grown for four weeks, then inoculated with *D. radicum* eggs and subsequently grown for another four weeks until final evaluation. For egg addition, 12–15 freshly harvested eggs were placed onto a small piece of paper and all eggs were washed onto the soil using 1 ml ddH_2O. For final evaluation, all pupae and larvae were collected by sieving soil through a 2 mm sieve. All pupae were stored in a ø 30-mm petri dish for at least one month to allow flies to emerge. For fungal treatments, Bip5 conidia were mixed into the soil, which was taken from freshly opened bags, at 10^6 conidia/g soil immediately before sowing. For bacterial treatments, 20 ml PCLRT03-gfp suspension containing 10^8 cfu/ml were spread around the plants to reach a density of 10^7 cfu/g soil. Bacteria were added twice, first one week after sowing and the second time one week before egg addition. For nematode (RS5) treatments, 5000 IJ in a total volume of 5 ml were pipetted on the soil to reach approx. 50 IJ/cm^2. EPN were added 2 days before egg addition for the triple combination experiments and 3 days after egg addition for the double combination experiments.

2.3. Semi-field trials

Two semi-field trials were performed in 2020 and 2021: trial 1 (April – May) with EPN and EPP single treatments and an EPN/EPP combination and trial 2 (August – September) with EPN, EPP, EPF single treatments, double and triple combinations. The experimental set-ups are shown in Fig. S1. Radish cultivar 'Riesenbutter' seeds were pregerminated in 273-hole Quick-Pots (gvz-rossat, Switzerland) for one week in Jiffy substrate in the glasshouse. Pots (20×20×23 cm, Growland, Germany) were filled with a mixture of field soil and Allmig substrate (1:2 for trial 1 resp. 1:1 trial 2). For EPF treatments, spores of *M. brunneum* Bip5 were mixed into the top third (≈ 1.8 kg) at 10^6 conidia/g soil. Four seedlings were transferred into one pot and pots were placed outside on a 3×20 m seed bed at Agroscope in Zurich, Switzerland (47.250413 N, 8.305810 E). Plants were watered and covered with a shading net (Accura, Germany) according to weather conditions. The day after planting the seedlings, each pot was inoculated with EPP (*P. chlororaphis* PCLRT03) by distributing 50 ml of a suspension containing 4×10^8 cfu/ml on the soil around the radishes. This procedure was repeated after one (trial 2) or two weeks (trial 1). The infestation with *D. radicum* eggs took place one week after the second EPP inoculation. For trial 1 48 eggs and for trial 2 30 eggs were added to each pot except for the *Delia*-free treatment. For EPN (*S. feltiae* RS5) inoculation, 40 ml of a suspension containing 500 IJ/ml were added to each pot three days before egg addition to reach a density of 50 IJ/cm^2. Four weeks after egg infestation, the pots were transferred into the glasshouse to reduce moisture before harvest. Three days later, the radishes were harvested, washed, the roots weighed, and the bulbs rated for *D. radicum* damage. Then, the top two thirds of the soil was mixed and soil samples (approx. 250 g) were taken to monitor BCA populations. Soil samples were stored at 10 °C and root samples at 3 °C until processing the next day (EPP and EPF monitoring) or 2 days later (EPN monitoring) as described below (chapter 2.5). In the days after egg addition in trial 2, soil temperatures reached 30 °C which strongly affected the survival of the insects. As a result, pest pressure was too low to have any effect on plants, therefore only data on BCA populations are presented (Fig. 5A, Table S12).

2.4. Field trial

The field trial was conducted in Windisch (47.476110 N, 8.227799 E; Aargau, Switzerland) in a field sown with radish *Raphanus sativus* L. cultivar 'Andes F1′ (Enza Zaden, Germany) that forms around 35 cm long cylindric white bulbs. The seeds were coated with the fungicide Saphire (active ingredient Fludioxonil; Fenaco, Switzerland). The field was divided into 25 plots of 10 m x 3.2 m; the width of 3.2 m represents eight planting rows. The treatments were distributed according to a Latin square design (Fig. S2). Only the inner area, i.e. four rows (1.6 m) of 5 m length, of each plot was treated with biocontrol agents and sampled. The rest served as buffer zone to avoid cross-contamination. Five different treatments were each applied to five plots: 1) control with no application, 2) EPP *P. chlororaphis* PCLRT03, 3) EPN *S. feltiae* RS5, 4) EPF *M. brunneum* Bip5, and 5) EPP x EPF x EPN with an application of all three agents (PFN). All BCAs were applied using a watering can distributing 2.5 L inoculum suspension for each 5-m planting row. EPF were applied one day after sowing at 2.5×10^{10} spores/5-m row (corresponding to approx. 1.3×10^{14} spores/ha). One week later, first EPN and then EPP were applied at 3.75×10^5 IJ/5-m row (1.9×10^9 IJ/ha) and 2.5×10^{11} cfu/5-m row (1.3×10^{15} cfu/ha), respectively. EPP were applied a second time four weeks after sowing. Two and eight weeks after sowing, soil and root samples were taken to monitor BCA colonization. For this, five samples (three for the control) were taken from each plot. Root samples consisted of one root system and soil samples of three scoops of soil down to 15 cm depth. Samples were taken uniformly over the whole plot. Soil samples were stored at 10 °C and root samples at 3 °C until processing the next day (EPP and EPF monitoring) or two to three days later (EPN monitoring) as described below (chapter 2.5). The final sampling was performed nine weeks after sowing when all radish plants were harvested to evaluate damage. All leaves were cut off and the white bulbs were washed and rated for damage on a scale from 0 to 3 with 0 = no *D. radicum* specific mining; 1 = light damage, 1 mining; 2 = heavy damage, 2–5 minings; 3 = very severe damage, bulb partly or completely destroyed by > 5 minings. We also noted how much each bulb was rotten due to water logging in the field. Heavily rotten bulbs (> 70%) were later excluded from analysis. All treatments were harvested within two days.

2.5. Monitoring of biocontrol agents

For EPP and EPF soil colonization, 10 g soil was suspended in 50 ml sterile 0.9% NaCl solution in a 100 ml Erlenmeyer flask and shaken using a rotary shaker at 200 rpm for 30 min at 3 °C. For root colonization, the roots were weighed and incubated in 50 ml falcon tubes with 40 ml 0.9% NaCl solution, and shaken as described for the soil samples. After shaking, samples were serially diluted in 0.9% NaCl solution and plated on selective agar (SM for EPF, KB^{+++} for EPP). Plates were incubated at 24 °C and colony forming units (cfu) counted after two days (EPP) or two weeks (EPF). *Metarhizium* colonies were identified morphologically, thereby also counting naturally occurring *Metarhizium* species. *P. chlororaphis* PCLRT03 colonies can be distinguished from other pseudomonads since colonies turn green due to phenazine production.

EPN colonization was assessed by qPCR as described in Campos-Herrera et al. (2015). Briefly, 200 g soil samples were suspended in tap water and sieved through a 125 and then through a 25 μm sieve. The EPN collected were sucrose-extracted (Campos-Herrera et al., 2015; Jenkins, 1964) and the samples reduced to 100 μl by centrifugation to allow for DNA extraction. Each sample was disrupted by a pellet pestle motor (KIMBLE®, DWK Life Sciences, Germany) and DNA extracted using the DNeasy PowerSoil Pro Kit (QIAGEN, The Netherlands) according to the manufacturer's instructions. DNA concentration was measured using NanoDrop2000 (Thermo Fisher Scientific, MA, USA) and diluted to 1 ng/μl for qPCR. DNA from 300 RS5 IJ (pure culture) extracted using the same kit was used for the standard curve and miliQ water was used as a negative control. *S. feltiae* specific primers and probes were designed by Campos-Herrera et al. (2011a). The reaction was performed using the TaqMan® polymerase (Thermo Fisher Scientific, MA, USA) in a 7500 Fast Lightcycler (Thermo Fisher Scientific, MA, USA) at the Genetic Diversity Center (GDC, Zurich, Switzerland) and analysed with the 7500 Software (v 2.0.6). Thermal cycling was performed as described in Campos-Herrera et al. (2011b) with 60 °C annealing temperature during 40 cycles. The total reaction volume was

10 µl and two technical replicates were run for each sample.

50 g soil samples were dried for 24 h at 105 °C to calculate soil dry weight.

2.6. Statistical analysis

The analysis was conducted in Rstudio (version 1.4.1717) using R (version 4.1.2). The results from six greenhouse experiments and five to ten supplementary laboratory assays were combined and analysed using a linear mixed-effect model comparing the fly emergence rates across treatments and controlling for experiment and experiment x treatment effects (package lme4 ver. 1.1–27.1). For analysis of single laboratory assays, Kruskal-Wallis and post-hoc Dunn test were performed using the package FSA (ver. 0.9.1). Radish damage ratings were analysed with an ordinal regression model using the function polr (package Mass ver. 7.3–55) and emmeans (package emmeans ver. 1.7.2) was used for post-hoc pairwise testing. Colonization data was log-transformed and tested for normal distribution using a Shapiro-Wilk Normality Test. Since data did not follow a normal distribution, Kruskal-Wallis and post-hoc Dunn test were used for all colonization data sets (field and semi-field trials). Differences were considered to be significant at $P < 0.05$. Boxplots and barplots were created using ggpubr (ver. 0.4.0) and ggplot2 (ver. 3.3.5) packages. Boxplots are standardized with the middle line representing the median, the upper and lower box edge the interquartile range between the 25th and the 75th percentile, the upper and lower line end the maximum resp. minimum values within the 1.5 interquartile range, and dots represent outliers. Biocontrol effect was calculated in excel as difference in mean value compared to the control. These values were used for assessing synergism according to the Bliss independence formula: $E_{12} = E_1 + E_2 - (E_1 \times E_2)$, with $E_{12} > E_{combo}$ = antagonistic, $E_{12} = E_{combo}$ = basic additivity, $E_{12} < E_{combo}$ = synergism (Demidenko and Miller, 2019; Xu et al., 2011).

3. Results and discussion

3.1. The combination of entomopathogenic pseudomonads with nematodes or fungi leads to synergism

In laboratory assays performed at the onset of this study, we had screened different EPP strains and EPN populations and discovered several *P. chlororaphis* strains and *S. feltiae* populations with promising potential to kill larvae of *D. radicum* (Fig. S3, Table S2). From several publications on effects of EPF against *D. radicum*, we know that isolates of *Beauveria bassiana* and *Metarhizium brunneum* and among the latter especially *M. brunneum* strain BIPESCO 5 / F52 (Bip5) have promising activity against the cabbage maggot (Bruck et al., 2005; Myrand et al., 2015; Razinger et al., 2014; Vänninen et al., 1999b). Thus, our first step was to test combinations of EPP and EPN as well as of EPP and EPF for potential synergism. In the first simple radish-sand laboratory experiment, *P. chlororaphis* PCL1391 and *S. feltiae* RS5 and 594 applied alone

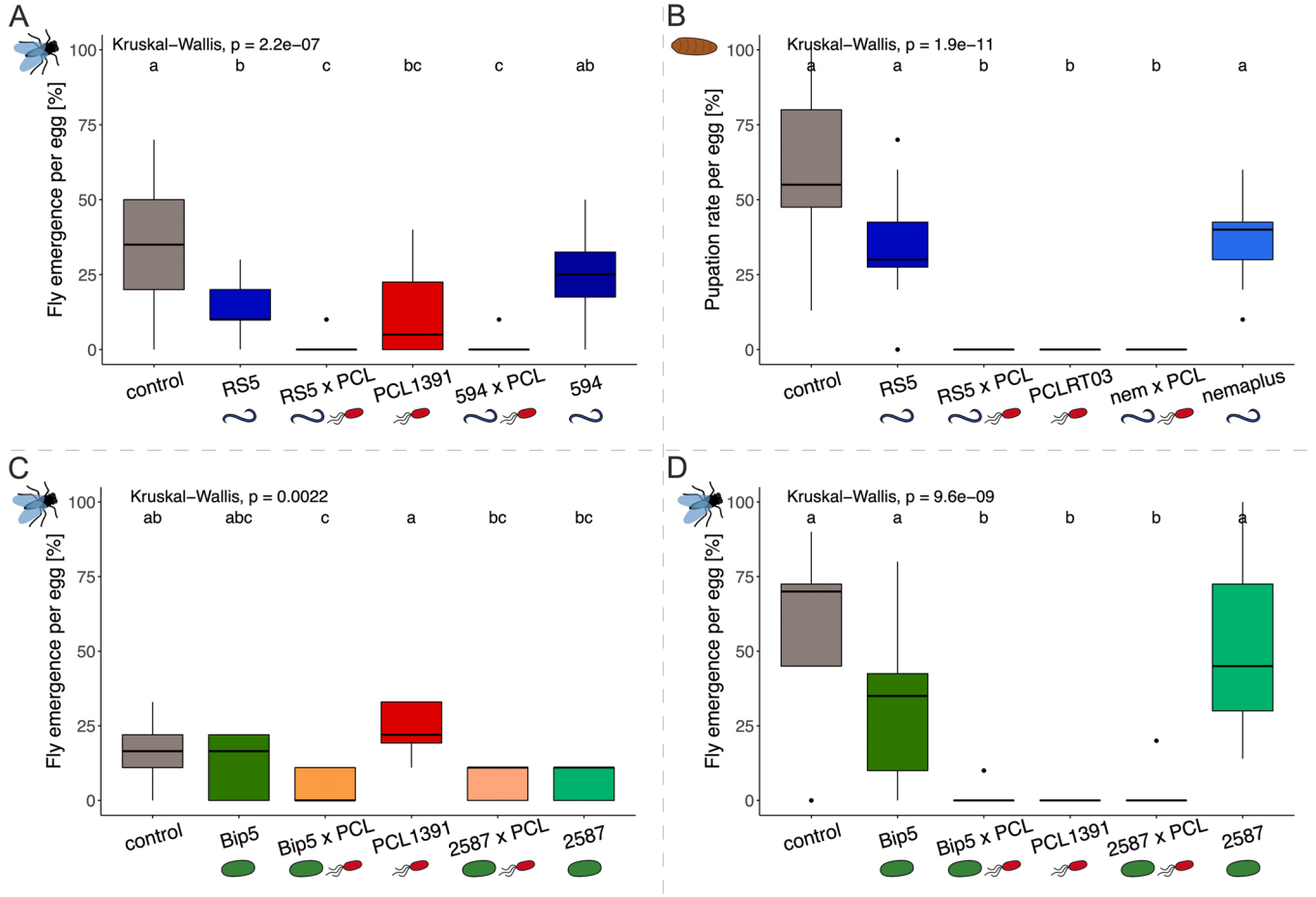

Fig. 1. Effect of combinations of fluorescent pseudomonads with entomopathogenic nematodes or fungi on *D. radicum* development in laboratory assays. *D. radicum* fly emergence and pupation rates per egg, respectively, obtained in a radish-sand laboratory assay. A & B) Two experiments applying different combinations of *P. chlororaphis* and *S. feltiae*. C & D) Two independent repetitions of an experiment applying combinations of *P. chlororaphis* and *M. brunneum* or *B. bassiana*. Strains: *P. chlororaphis* PCL1391 (A, C, D), PCLRT03 (B); *S. feltiae* RS5 (A, B), 594 (A), nemaplus (B); *M. brunneum* Bip5 (C, D), *B. bassiana* 2587 (C, D). Standard boxplots represent the pupation resp. fly emergence rate. Kruskal-Wallis and post-hoc Dunn-test with bh-correction were conducted in Rstudio; different letters indicate significant differences between treatments ($P < 0.05$).

reduced the average fly emergence rate by 65%, 65% and 30%, respectively, compared to the control (Fig. 1A, Table S3). The combination of PCL1391 with either *S. feltiae* population led to a reduction of over 95%. This represents a synergistic interaction according to the Bliss formula (Demidenko and Miller, 2019; Xu et al., 2011; Table S4). In the second experiment, the combinations of *P. chlororaphis* PCLRT03 and two *S. feltiae* populations could not improve efficacy as PCLRT03 alone reached 100% control (Fig. 1B, Table S3).

In the experiments with fungi, fly emergence rate in the control varied strongly between experiments (17% vs 54%; Fig. 1C-D, Table S5). The two EPF strains showed tendencies to reduce fly emergence rates but the effect was not significant in either experiment (Fig. 1C-D). The performance of EPP PCL1391 was highly variable. In the first experiment, the bacteria had no impact at all on fly emergence, in the second, however, all the insects died following PCL1391 application. Therefore, it was not possible to monitor potential effects of combinations in the second experiment. In contrast, in the first experiment, the combination of Bip5 and PCL1391 reduced mean fly emergence by 75% compared to the control (vs. 25% and 0% reduction for respective individual strains), thus resulting in a synergistic effect (Table S4).

The combined application of pseudomonads and nematodes was more promising than that of pseudomonads and fungi and therefore upscaled in a semi-field trial, where radishes were grown in pots under natural weather conditions and inoculated with *D. radicum* eggs. In the treatment without artificial inoculation, a few *D. radicum* larvae were discovered, implying a very small natural infestation. Consequently, only 14% of the plants were affected by the insect (Fig. 2). The artificial inoculation with *D. radicum* eggs, however, created a very high pest pressure, resulting in 67% dead plants in the no BCA control (Table S6). Although single BCA application reduced *D. radicum* damage, these effects were not significantly different to the no BCA control. The high

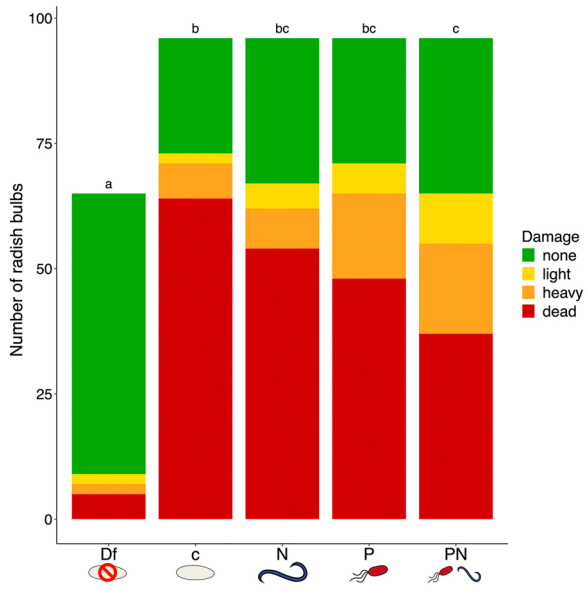

Fig. 2. Impact of insecticidal *Pseudomonas* and entomopathogenic nematodes applied alone and in combination on *Delia radicum* damage on radish bulbs under semi-field conditions. *D. radicum* damage was recorded on radish bulbs grown in pots placed outside under natural weather conditions. In the *Delia*-free (Df) treatment, there was no *D. radicum* inoculation and no BCA application. The remaining treatments were all artificially inoculated with *D. radicum* eggs; c = control with no BCA application; N = EPN population *S. feltiae* RS5; P = EPP strain *P. chlororaphis* PCLRT03; PN = double combination with P and N application. *D. radicum* mining damage on a radish bulb was recorded on the following scale: none = no damage; light = small damage, 1 mining; heavy = large damages, ≥ 2 minings; dead = plant dead. Different letters on top of the barplot refer to significant differences among treatments ($P < 0.05$) according to an ordinal regression model.

efficiency of *P. chlororaphis* PCLRT03 and *S. feltiae* RS5 that was observed under laboratory conditions, was not observed in the upscaling to the semi-field trial. The combination of the two biocontrol agents, however, resulted in a synergistic interaction (Table S4) and significantly reduced *D. radicum* damage by decreasing the number of dead radishes by 42% and increasing healthy radish bulbs by 34% (Fig. 2, Table S6). Thus, the synergistic relationship of the *Pseudomonas*-nematode combination was preserved when upscaling from the laboratory to a semi-field trial.

To the best of our knowledge, this is the first study to demonstrate a synergistic interaction between EPP and EPN against insects under laboratory and semi-field conditions, and of EPP with EPF under laboratory conditions. Previous studies on combinations of nematodes with entomopathogenic bacteria (EPB) have focused predominantly on EPN and *Bacillus thuringiensis* (Bt). Various publications describe additive and synergistic effects of EPN × Bt combinations in laboratory, greenhouse and field experiments against Lepidopteran, Dipteran and Coleopteran pests (Abdolmaleki et al., 2017; Koppenhöfer et al., 1999; Li et al., 2021; Oestergaard et al., 2006). The general conclusion from these studies is that biocontrol efficiency is increased when EPN and EPB are applied together, which is more pronounced in laboratory compared to field studies. Our results support this conclusion although insecticidal pseudomonads and Bt have different modes of action. So far, little is known about the interaction between EPN and EPP. Cambon et al. (2020) discovered pseudomonads in EPN-infected cadavers. Ogier et al. (2020) isolated *P. protegens* and *P. chlororaphis* from infective juveniles of different EPN species and proposed that EPP belong to the EPN pathobiome. The insecticidal toxins produced by EPP and nematode-associated bacteria (NB) show sequence similarity, suggesting a common origin (Ruffner et al., 2015). These findings indicate frequent interactions between EPP and EPN in nature.

Similar to EPN, EPF have already been combined with Bt, which resulted in synergistic effects in laboratory and field studies (Beris and Korkas, 2021; Wraight and Ramos, 2005). Furthermore, EPF have successfully been combined with fluorescent pseudomonads for simultaneous pest and disease control, e.g. of leaf miners and collar rot disease in groundnut (Senthilraja et al., 2010a, 2010b) and leaf folder pest and sheath blight disease of rice (Karthiba et al., 2010). Still, we were surprised to find a synergistic effect in our sand-radish bulb assay because we observed inhibitory effects in a previous in vitro inhibition assay with EPF and EPP (A. Spescha, unpublished data). Fluorescent pseudomonads are well-known for controlling fungal plant diseases and producing a vast array of antifungal exoproducts (Haas and Défago, 2005; Vesga et al., 2021). EPF, on the other hand, also produce antimicrobial compounds that inhibit bacterial growth (Hummadi et al., 2021; Ravindran et al., 2014). The fact that inhibitory effects were visible on agar plates but not observed in the sand-radish bulb assay might be explained by the possibility that the cocktail or amounts of exoproducts produced by the two BCAs might differ between culture medium and our sand system.

In summary, *P. chlororaphis* and *S. feltiae* as well as *P. chlororaphis* and *M. brunneum* seem to be well compatible when applied together against an insect pest. Their different modes of action might be a reason for the synergistic effects we have observed. Combining these organisms might be a promising approach for developing biological control methods.

3.2. Upscaling the application of a tripartite biocontrol consortium from the greenhouse to the field

Our next step was to compare single, dual and triple combinations of EPP, EPN and EPF in greenhouse trials. All three BCAs significantly reduced fly emergence rates compared to the control (Fig. 3, Table S7). *S. feltiae* RS5 reduced the mean emergence by 44% compared to the control, thereby showing a similar efficacy in the greenhouse as in the laboratory sand assay (Fig. S3, Table S2). *P. chlororaphis* PCLRT03 reduced fly emergence in the greenhouse by 59%, which was lower than

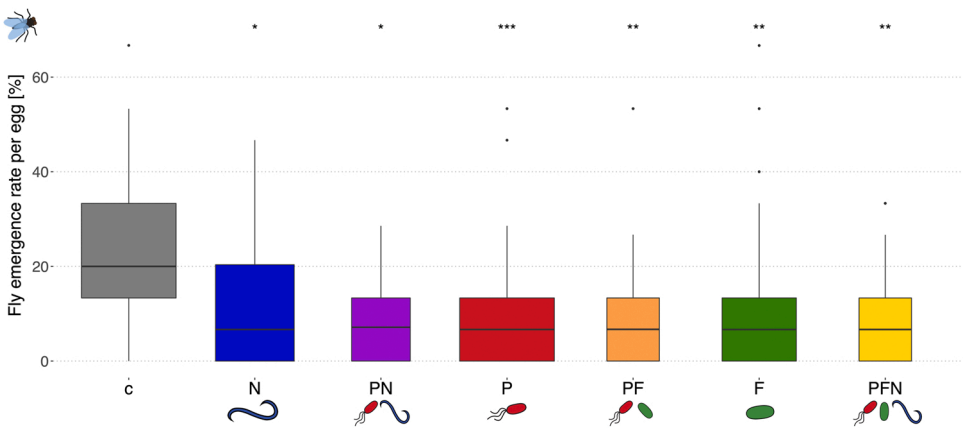

Fig. 3. Effect of single and combined biocontrol agent applications on *D. radicum* development under greenhouse conditions. *D. radicum* fly emergence rate per egg was pooled across six independent greenhouse trials combining EPP, EPN and EPF (two trials for each combination). Treatments: c = control with no BCA application; N = EPN population *S. feltiae* RS5; P = EPP strain *P. chlororaphis* PCLRT03; F = EPF strain *M. brunneum* Bip5; PN, PF and PFN = respective double and triple combinations. Boxplot width indicates the number of experiments in which a treatment was applied in (two trials for each combination, four for F and N, six for P and the control). A lmer model was used to analyse the data; asterisks refer to significant differences compared to the control with *P*-values * = < 0.05, * * < 0.01, * ** <0.001.

in the laboratory assay (Fig. S3, Table S2). *M. brunneum* Bip5 significantly impacted *D. radicum* and lowered mean fly emergence by 54%, thus performing better than in the laboratory assay (Fig. 1C-D, Table S5). Both dual combinations (EPP x EPN and EPP x EPF) significantly reduced *D. radicum* survival compared to the control, lowering the mean fly emergence rate by 60%, but did not differ significantly to the single treatments. The same was observed for the triple combination. The synergistic effects observed for EPP combined with EPN and EPF in the laboratory assays and the semi-field trial could not be verified in the greenhouse. This indicates that synergistic effects are dependent on different conditions. The effects of both dual combinations were very consistent over both experiments. Even though EPP and EPF as well as EPP and EPN combinations did not exhibit a higher efficacy than the single applications, the mean and median values were less variable between experiments (Table S8). The stabilizing effect of combining EPP either with EPF or with EPN was already observed in the laboratory assays (Fig. 1, Tables S3, S5). To verify the biocontrol effect of the consortium under natural conditions, the single BCAs and the triple combination were applied in a field trial.

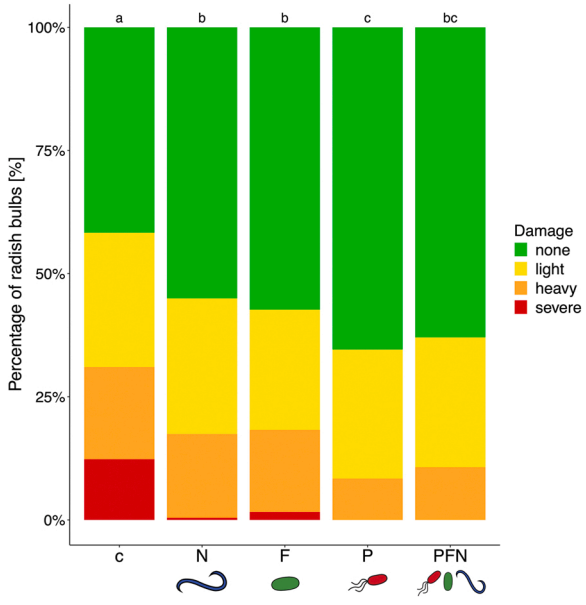

Fig. 4. Reduction of *Delia radicum* damages on radish bulbs by single and combined BCA application observed under field conditions. Treatments: c = control with no BCA application; N = EPN population *S. feltiae* RS5; F = EPF strain *M. brunneum* Bip5; P = EPP strain *P. chlororaphis* PCLRT03; PFN = triple combination with all three BCAs. Bulb damage was scored with the following scale: none = no *D. radicum* damage; light = small damage, 1 larval mining; heavy = large damages, 2–5 minings; severe = severe damages, bulb partly or completely destroyed by > 5 minings. Data was analysed using an ordinal regression model. Significant differences between treatments are indicated by the letters above the barplot.

In the field trial, *D. radicum* damage was significantly reduced compared to the untreated control when BCAs were applied alone and in a triple combination (Fig. 4, Tables S9, S10). However, there was no statistically significant difference between the triple combination and any of the single applications. The percentage of bulbs with no damage was highest in EPP, and second highest in the triple combination. Application of either EPF or EPN increased the percentage of bulbs without *D. radicum* damage (category "none" in Fig. 4) by one third, the application of EPP and the triple combination even by by 50% (Table S9). Bulbs with no damage can be sold to wholesale at standard prices, thus the observed effects of BCA application (either single or in combination) would translate into an increase in marketable plants by 30–50%. The percentage of lightly damaged bulbs, which can, at best, be sold on the farmers market at a lower price, did not differ between treatments. BCA application reduced the proportion of heavily damaged bulbs, which are bulbs that cannot be sold (categories "heavy" and "severe" in Fig. 4). Again, the most efficient treatment was EPP, which reduced heavy damage by 73%. The results of this first field trial are promising, however, more field studies are needed to determine whether reliable *D. radicum* control can be achieved in practice and whether the triple combination is more stable over the long term compared to single applications.

Few published studies on *D. radicum* biocontrol in the field have shown effective control of this pest. For example, Vänninen et al. (1999a) applied different EPF species as well as *B. thuringiensis* and *S. feltiae* in several field trials, but biocontrol effects were scarce and usually observed in one season only, and Chandler and Davidson (2005) reported that *M. anisopliae* reduced *D. radicum* survival under greenhouse conditions, but not in the field. Two recent field studies with application of *M. brunneum* and *B. bassiana* detected a non-significant reduction in pest pressure (Herbst et al., 2017; Razinger et al., 2017). The positive exception was a study by Beck et al. (2014) who observed significantly reduced cauliflower mortality after application of *S. feltiae* in a field trial with a high natural pest pressure. We achieved a significant increase in marketable bulbs upon application of EPF *M. brunneum* and EPN *S. feltiae*. However, the best result was obtained with the entomopathogenic pseudomonads of the species *P. chlororaphis*, which is already used for the biological control of fungal diseases. Although the triple combination did not result in a synergistic effect, our field trial confirmed the results from our greenhouse and semi-field trials: EPP, EPN and EPF are compatible and provide significant *D. radicum* control indicating that their use might contribute to solve this severe problem in the production of Brassicacean crops in organic and conventional agriculture.

In recent studies, EPN and EPP have been combined with arbuscular mycorrhizal fungi (AMF) to improve plant fitness and reduce pest pressure. Imperiali et al. (2017) inoculated wheat fields with *P. protegens* CHA0 and *P. chlororaphis* PCL1391 alone and in combination with different EPN and different AMF. Under a heavy natural frit fly infestation, the combined application of both *Pseudomonas* strains together with *H. bacteriophora* resulted in the highest yield. Jaffuel et al. (2019) treated maize fields over three years with EPP (a mixture of *P. protegens* and *P. chlororaphis*), EPN (a mixture of *H. bacteriophora* and *S. feltiae)* and AMF. Although they did not observe synergistic effects of EPP-EPN combinations, some of the EPP and EPN treatments increased grain yield or reduced damage caused by the western corn rootworm in some of the trials. Taken together, these two studies in combination with our own indicate that EPN-EPP combinations, alone or together with plant-beneficial fungi, can reduce damage caused by different insects on different crops.

3.3. Biocontrol agents do not impact each other's population sizes under semi-field and field conditions

For successful co-application, the biocontrol agents need to persist well together in the soil and on plant roots. We monitored the population levels of EPP, EPF and EPN after single and combined application in two semi-field trials and a field trial. To summarize our results, the EPP, EPN and EPF used in our semi-field trials (Fig. 5A, Tables S11, S12) and the field trial (Fig. 5B, Table S13) established and persisted well in the soil and on the roots. Population sizes of individual BCAs were not altered in dual or triple combinations.

In the second semi-field and the field trial, the mean EPN population levels at harvest ranged between 50 and 100 IJ/100 g soil dry weight, and in the first semi-field trial around 350 IJ/100 g soil fresh weight (Tables S11-S13). In the field trial, EPN numbers decreased slightly but significantly over time (Fig. 5B). The combination with other BCAs did not impact EPN population size neither in field nor semi-field trials.

Mean EPF root colonization was very similar in the semi-field and the field trial, ranging from 7×10^3 to 1×10^4 cfu/g fresh weight (Fig. 5, Tables S12, S13). EPF populations established in the soil were about one order of magnitude higher and means ranged from 1 to 2×10^5 cfu/g dry weight. In the field trial, similar to the EPN populations, EPF levels decreased slightly over the period of the field trial, which was significant in the soil but not on the roots (Fig. 5B). Combining EPF with other BCAs did not influence population sizes.

In the semi-field trials, the mean EPP soil colonization ranged between 3×10^5 and 1×10^6 cfu/g dry weight (Fig. 5A, Tables S11, S12).

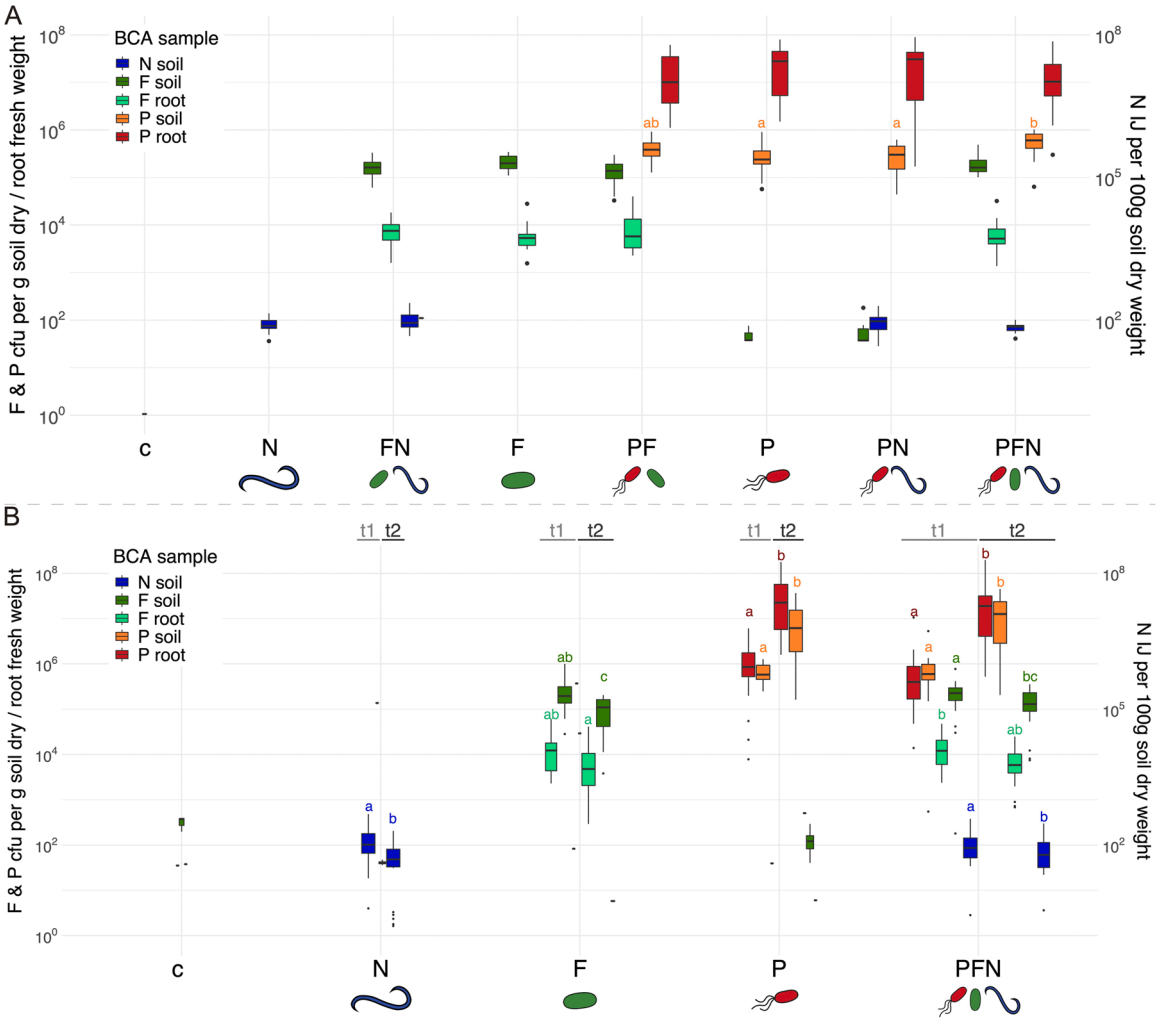

Fig. 5. Soil and root colonization by BCAs applied alone and in combinations under A) semi-field and B) field conditions. A) Soil and root colonization in semi-field trial 2 was assessed for all BCAs during the final evaluation after growing radishes for eight weeks in pots (n = 16) placed outside. B) Soil and root samples (n = 25) were taken two (t1) and eight weeks (t2) after sowing radishes in a field trial. Treatments: c = control with no BCA application; N = EPN population *S. feltiae* RS5; F = EPF strain *M. brunneum* Bip5; P = EPP strain *P. chlororaphis* PCLRT03; FN, PF, PN and PFN = respective double and triple combinations. Letters refer to significant differences among treatments at $P < 0.05$ according to a Dunn-test and may be compared if written in the same colour. Where no letters are shown, no significant differences were detected.

Pseudomonads were enriched on the roots and reached population levels up to 10^8 cfu/g fresh weight. In the field trial, the soil and root colonization levels did not differ much and ranged from 7×10^5 to 1×10^6 cfu/g at the first sampling (Table S13). In contrast to EPF and EPN, EPP population sizes increased more than ten-fold over time and reached levels up to 4×10^7 cfu/g eight weeks after sowing (Fig. 5B, Table S13). It is most likely that the second application after the first sampling has boosted populations. This population boost strongly supports the utility of a second EPP application. As already observed for EPN and EPF, combining EPP with other BCAs did not substantially affect EPP root or soil colonization. BCA colonization was also observed in several greenhouse trials, and neither of the three BCAs had an impact on population sizes of the others in combinations (data not shown).

The soil and root colonization levels established in the semi-field and the field trials exceeded the recommended thresholds for biocontrol activity that were established for all three biocontrol organisms (EPP: 10^5-10^6 cfu/g root, Haas and Défago, 2005; EPF: 10^5-10^6 cfu/g soil, Rogge et al., 2017; EPN: 10^5 IJ/m^2, Campos-Herrera et al., 2015). The EPP and EPN colonisation levels were comparable to those obtained by Imperiali et al. (2017) and Jaffuel et al. (2019).

4. Conclusions

This study has explored the biocontrol potential of *P. chlororaphis* against a root-feeding pest insect using a range of experiments from the greenhouse to the field. Strain PCLRT03 strongly inhibited survival of the cabbage maggot *Delia radicum* under controlled conditions and significantly increased the marketable produce in a first field trial. So far, *P. chlororaphis* is mainly marketed for its plant-growth promoting and disease suppressive traits, but our results indicate that new *P. chlororaphis* products or existing ones could also be developed for use against insects. A novel bacterial and multifactorial BCA for pest control would be most welcome in a future where the use of chemicals is greatly reduced or prohibited.

To our knowledge, this is the first time that a combination of entomopathogenic pseudomonads, nematodes and fungi was successfully used to fight a below-ground insect pest. Taken together, our results from all experiments performed under laboratory, greenhouse, semi-field and field conditions indicate that a combination of compatible BCAs with different modes of action, such as those studied here, can potentially improve *D. radicum* control. The sequential upscaling and the close monitoring of the applied biocontrol agents were essential not only to evaluate the efficiency but also the compatibility of the consortium. When applying our combinations, we observed synergistic effects under semi-field conditions and more stable results in the greenhouse. We propose that unreliable pest control obtained when applying single BCAs may be overcome by the application of multiple BCAs. A successful consortium does not necessarily have to display pronounced synergisms, but should perform better under variable conditions. In case the performance of an individual BCA is hampered by adverse environmental conditions, the other consortium members could compensate and provide effective control. Of course, for our consortium, this will have to be verified in further field trials at different locations and under varying environmental conditions. It will also be interesting to test the consortium against other root feeding pests. Combinations might have a broader activity spectrum than single-organism-based products. This especially applies to the consortium evaluated here, because all its members have demonstrated activity against several insect species. An added value of including *P. chlororaphis* is that these bacteria can also control fungal root pathogens.

Monitoring the three BCA in pot, semi-field and field studies showed clearly that EPN, EPF and EPP do not impact each other's soil and root colonization capacity. This is very promising for the development of combined biocontrol products. However, the interaction between EPP, EPN and EPF needs to be studied more closely, especially regarding the formulation and application. Any negative interactions must be excluded if all three BCAs were applied together (e.g. in one tank mixture) or even included in one single product. It is clear that costs for production, registration and application will be higher for products containing BCA consortia than for single BCA products. This impairs the uptake of combined application strategies of BCAs by farmers for the time being. However, we believe that with improved registration processes, cheaper large-scale production, and more pressure to reduce pesticide application in many countries worldwide, these hurdles can be overcome in the future.

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.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2023.108414.

References

Abdolmaleki, A., Rafiee Dastjerdi, H., Tanha Maafi, Z., Naseri, B., 2017. Virulence of two entomopathogenic nematodes through their interaction with *Beauveria bassiana* and

Bacillus thuringiensis against *Pieris brassicae* (Lepidoptera: Pieridae). J. Crop Prot. 6, 287–299.
Ansari, M.A., Shah, F.A., Butt, T.M., 2010. The entomopathogenic nematode *Steinernema kraussei* and *Metarhizium anisopliae* work synergistically in controlling overwintering larvae of the black vine weevil, *Otiorhynchus sulcatus*, in strawberry growbags. Biocontrol Sci. Technol. 20. https://doi.org/10.1080/09583150903420031.
Babbal, Adivitiya, Khasa, Y.P., 2017. Microbes as biocontrol agents. In: Probiotics and Plant Health. Springer, Singapore, pp. 507–552. https://doi.org/10.1007/978-981-10-3473-2_24.
Barelli, L., Moonjely, S., Behie, S.W., Bidochka, M.J., 2016. Fungi with multifunctional lifestyles: endophytic insect pathogenic fungi. Plant Mol. Biol. 90, 657–664. https://doi.org/10.1007/S11103-015-0413-Z.
Beck, B., Spanoghe, P., Moens, M., Brusselman, E., Temmerman, F., Pollet, S., Nuyttens, D., 2014. Improving the biocontrol potential of *Steinernema feltiae* against *Delia radicum* through dosage, application technique and timing. Pest Manag. Sci. 70, 841–851. https://doi.org/10.1002/ps.3628.
Beris, E., Korkas, E., 2021. Additive and synergistic interactions of entomopathogenic fungi with *Bacillus thuringiensis* for the control of the European grapevine moth *Lobesia botrana* (Denis and Schiffermüller) (Lepidoptera: Tortricidae). Egypt J. Biol. Pest Control 31, 1–7. https://doi.org/10.1186/S41938-021-00455-W.
Bertani, G., 1951. Studies on lysogenesis I: the mode of phage liberation by lysogenic *Escherichia coli*. J. Bacteriol. 62, 293–300.
Boeckman, C.J., Anderson, J.A., Linderblood, C., Olson, T., Roper, J., Sturtz, K., Walker, C., Woods, R., 2021. Environmental risk assessment of the DvSSJ1 dsRNA and the IPD072Aa protein to non-target organisms. GM Crops Food 12, 459–478. https://doi.org/10.1080/21645698.2021.1982348.
Bruck, D.J., Snelling, J.E., Dreves, A.J., Jaronski, S.T., 2005. Laboratory bioassays of entomopathogenic fungi for control of *Delia radicum* (L.) larvae. J. Invertebr. Pathol. 89, 179–183. https://doi.org/10.1016/j.jip.2005.02.007.
Bueno-Pallero, F.A., Blanco-Pérez, R., Dionísio, L., Campos-Herrera, R., 2018. Simultaneous exposure of nematophagous fungi, entomopathogenic nematodes and entomopathogenic fungi can modulate belowground insect pest control. J. Invertebr. Pathol. 154. https://doi.org/10.1016/j.jip.2018.04.004.
Butt, T.M., Coates, C.J., Dubovskiy, I.M., Ratcliffe, N.A., 2016. Entomopathogenic fungi - new insights into host–pathogen interactions. In: Lovett, B., st. Leger, R.J. (Eds.), Adv. Genet. Elsevier Ltd, pp. 307–364. https://doi.org/10.1016/bs.adgen.2016.01.006.
Caltagirone, L.E., 1981. Landmark examples in classical biological control. Annu. Rev. Entomol. 26, 213–232. https://doi.org/10.1146/annurev.en.26.010181.001241.
Cambon, M.C., Lafont, P., Frayssinet, M., Lanois, A., Ogier, J.C., Pagès, S., Parthuisot, N., Ferdy, J.B., Gaudriault, S., 2020. Bacterial community profile after the lethal infection of *Steinernema-Xenorhabdus* pairs into soil-reared *Tenebrio molitor* larvae. FEMS Microbiol. Ecol. 96. https://doi.org/10.1093/femsec/fiaa009.
Campos-Herrera, R., 2015. Nematode Pathogenesis of Insects and Other Pests, Springer. Springer International Publishing,. https://doi.org/10.1007/978-3-319-18266-7.
Campos-Herrera, R., El-Borai, F.E., Stuart, R.J., Graham, J.H., Duncan, L.W., 2011a. Entomopathogenic nematodes, phoretic *Paenibacillus* spp., and the use of real time quantitative PCR to explore soil food webs in Florida citrus groves. J. Invertebr. Pathol. 108, 30–39. https://doi.org/10.1016/j.jip.2011.06.005.
Campos-Herrera, R., Johnson, E.G., EL-Borai, F.E., Stuart, R.J., Graham, J.H., Duncan, L. W., 2011b. Long-term stability of entomopathogenic nematode spatial patterns in soil as measured by sentinel insects and real-time PCR assays. Ann. Appl. Biol. 158, 55–68. https://doi.org/10.1111/j.1744-7348.2010.00433.x.
Campos-Herrera, R., Jaffuel, G., Chiriboga, X., Blanco-Pérez, R., Fesselet, M., Půža, V., Mascher, F., Turlings, T.C.J., 2015. Traditional and molecular detection methods reveal intense interguild competition and other multitrophic interactions associated with native entomopathogenic nematodes in Swiss tillage soils. Plant Soil 389, 237–255. https://doi.org/10.1007/s11104-014-2358-4.
Carlson, A.B., Mathesius, C.A., Ballou, S., Boeckman, C.J., Gunderson, T.A., Mirsky, H.P., Mukerji, P., Roe, J.C., Schmidt, J.M., Zhang, J., Delaney, B., 2019. Safety assessment of coleopteran active IPD072Aa protein from *Pseudomonas chlororaphis*. Food Chem. Toxicol. 129, 376–381. https://doi.org/10.1016/J.FCT.2019.04.055.
Chandler, D., Davidson, G., 2005. Evaluation of Entomopathogenic Fungus *Metarhizium anisopliae* Against Soil-Dwelling Stages of Cabbage Maggot (Diptera: Anthomyiidae) in Glasshouse and Field Experiments and Effect of Fungicides on Fungal Activity. J. Econ. Entomol. 98, 1856–1862. https://doi.org/10.1603/0022-0493-98.6.1856.
Chen, S., Han, X., Moens, M., 2003. Biological control of *Delia radicum* (Diptera: Anthomyiidae) with entomopathogenic nematodes. Appl. Entomol. Zool. 38, 441–448. https://doi.org/10.1303/aez.2003.441.
Chin-A-Woeng, T.F.C., Bloemberg, G. v, van der Bij, A.J., van der Drift, K.M.G.M., Schripsema, J., Kroon, B., Scheffer, R.J., Keel, C., Bakker, P.A.H.M., Tichy, H.-V., de Bruijn, F.J., Thomas-Oates, J.E., Lugtenberg, B.J.J., 1998. Biocontrol by Phenazine-1-carboxamide-Producing *Pseudomonas chlororaphis* PCL1391 of Tomato Root Rot Caused by *Fusarium oxysporum* f. sp. radicis-lycopersici. Mol. Plant-Microbe Inter. 11, 1069–1077. https://doi.org/10.1094/MPMI.1998.11.11.1069.
Demidenko, E., Miller, T.W., 2019. Statistical determination of synergy based on Bliss definition of drugs independence. PLoS One 14. https://doi.org/10.1371/journal.pone.0224137.
Eliáš, S., Hurychová, J., Toubarro, D., Frias, J., Kunc, M., Dobeš, P., Simões, N., Hyršl, P., 2020. Bioactive excreted/secreted products of entomopathogenic nematode Heterorhabditis bacteriophora inhibit the phenoloxidase activity during the infection. Insects 11. https://doi.org/10.3390/insects11060353.
Environmental Protection Agency (EPA), 2021. Tolerance revocations: chlorpyrifos. https://doi.org/www.regulations.gov/document/EPA-HQ-OPP-2021-0523-0001.
European Food Safety Authority (EFSA), 2012. Conclusion on the peer review of the pesticide risk assessment of the active substance *Metarhizium anisopliae* var. *anisopliae* BIPESCO 5/F52. EFSA J. https://doi.org/10.2903/J.EFSA.2012.2498.
European Food Safety Authority (EFSA), 2019. Updated statement on the available outcomes of the human health assessment in the context of the pesticides peer review of the active substance chlorpyrifos-methyl. EFSA J. 17. https://doi.org/10.2903/J.EFSA.2019.5908.
ffrench-Constant, R., Dowling, A., Waterfield, N.R., 2007. Insecticidal toxins from *Photorhabdus* bacteria and their potential use in agriculture. Toxicon 49. https://doi.org/10.1016/j.toxicon.2006.11.019.
ffrench-Constant, R.H., Bowen, D.J., 2000. Novel insecticidal toxins from nematode-symbiotic bacteria. Cell Mol. Life Sci. 57, 828–833. https://doi.org/10.1007/s000180050044.
Flury, P., Vesga, P., Dominguez-Ferreras, A., Tinguely, C., Ullrich, C.I., Kleespies, R.G., Keel, C., Maurhofer, M., 2019. Persistence of root-colonizing *Pseudomonas protegens* in herbivorous insects throughout different developmental stages and dispersal to new host plants. ISME J. 13, 860–872. https://doi.org/10.1038/s41396-018-0317-4.
Flury, P., Vesga, P., Péchy-Tarr, M., Aellen, N., Dennert, F., Hofer, N., Kupferschmied, K. P., Kupferschmied, P., Metla, Z., Ma, Z., Siegfried, S., de Weert, S., Bloemberg, G., Höfte, M., Keel, C.J., Maurhofer, M., 2017. Antimicrobial and insecticidal: Cyclic lipopeptides and hydrogen cyanide produced by plant-beneficial *Pseudomonas* strains CHA0, CMR12a, and PCL1391 contribute to insect killing. Front. Microbiol 8. https://doi.org/10.3389/fmicb.2017.00100.
Flury, P., Aellen, N., Ruffner, B., Péchy-Tarr, M., Fataar, S., Metla, Z., Dominguez-Ferreras, A., Bloemberg, G., Frey, J., Goesmann, A., Raaijmakers, J.M., Duffy, B., Höfte, M., Blom, J., Smits, T.H.M., Keel, C., Maurhofer, M., 2016. Insect pathogenicity in plant-beneficial pseudomonads: phylogenetic distribution and comparative genomics. ISME J. 10. https://doi.org/10.1038/ismej.2016.5.
Goodrich-Blair, H., Clarke, D.J., 2007. Mutualism and pathogenesis in *Xenorhabdus* and *Photorhabdus*: two roads to the same destination. Mol. Microbiol 64, 260–268. https://doi.org/10.1111/J.1365-2958.2007.05671.X.
Haas, D., Défago, G., 2005. Biological control of soil-borne pathogens by fluorescent pseudomonads. Nat. Rev. Microbiol. 3, 307–319. https://doi.org/10.1038/nrmicro1129.
Hauenstein, S., Vieweger, A., 2021, Pflanzenschutz im Integrierten Gemüsebau, 2021st ed. Forschungsinstitut für biologischen Landbau FiBL.
Herbst, M., Razinger, J., Ugrinović, K., Škof, M., Schroers, H.J., Hommes, M., Poehling, H.M., 2017. Evaluation of low risk methods for managing *Delia radicum*, cabbage root fly, in broccoli production. Crop Prot. 96, 273–280. https://doi.org/10.1016/j.cropro.2017.02.023.
van Herk, W.G., Vernon, R.S., Waterer, D.R., Tolman, J.H., Lafontaine, P.J., Prasad, R.P., 2017. Field Evaluation of Insecticides for Control of Cabbage Maggot (Diptera: Anthomyiidae) in Rutabaga in Canada. J. Econ. Entomol. 110. https://doi.org/10.1093/jee/tow238.
Hummadi, E.H., Dearden, A., Generalovic, T., Clunie, B., Harrott, A., Cetin, Y., Demirbek, M., Khoja, S., Eastwood, D., Dudley, E., Hazir, S., Touray, M., Ulug, D., Hazal Gulsen, S., Cimen, H., Butt, T., 2021. Volatile organic compounds of *Metarhizium brunneum* influence the efficacy of entomopathogenic nematodes in insect control. Biol. Control 155. https://doi.org/10.1016/j.biocontrol.2020.104527.
Imperiali, N., Chiriboga, X., Schlaeppi, K., Fesselet, M., Villacrés, D., Jaffuel, G., Bender, S.F., Dennert, F., Blanco-Pérez, R., van der Heijden, M.G.A., Mauhofer, M., Mascher, F., Turlings, T.C.J., Keel, C.J., Campos-Herrera, R., 2017. Combined Field Inoculations of *Pseudomonas* Bacteria, Arbuscular Mycorrhizal Fungi, and Entomopathogenic Nematodes and their Effects on Wheat Performance. Front. Plant Sci. 8. https://doi.org/10.3389/fpls.2017.01809.
Islam, Md.N., Bint-E-Naser, S.F., Khan, M.S., 2017. Pesticide food laws and regulations. In: Pesticide Residue in Foods. Springer International Publishing, Cham, pp. 37–51. https://doi.org/10.1007/978-3-319-52683-6_3.
Jaffuel, G., Blanco-Pérez, R., Hug, A.S., Chiriboga, X., Meuli, R.G., Mascher, F., Turlings, T.C.J., Campos-Herrera, R., 2018. The evaluation of entomopathogenic nematode soil food web assemblages across Switzerland reveals major differences among agricultural, grassland and forest ecosystems. Agric. Ecosyst. Environ. 262, 48–57. https://doi.org/10.1016/j.agee.2018.04.008.
Jaffuel, G., Imperiali, N., Shelby, K., Campos-Herrera, R., Geisert, R., Maurhofer, M., Loper, J., Keel, C., Turlings, T.C.J., Hibbard, B.E., 2019. Protecting maize from rootworm damage with the combined application of arbuscular mycorrhizal fungi, *Pseudomonas* bacteria and entomopathogenic nematodes. Sci. Rep. 9. https://doi.org/10.1038/s41598-019-39753-7.
Jang, J.Y., Yang, S.Y., Kim, Y.C., Lee, C.W., Park, M.S., Kim, J.C., Kim, I.S., 2013. Identification of Orfamide A as an Insecticidal Metabolite Produced by *Pseudomonas protegens* F6. J. Agric. Food Chem. 61 https://doi.org/10.1021/jf401218w.
Jenkins, W.R.B., 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Dis. Report. 48, 492.
Karthiba, L., Saveetha, K., Suresh, S., Raguchander, T., Saravanakumar, D., Samiyappan, R., 2010. PGPR and entomopathogenic fungus bioformulation for the synchronous management of leaffolder pest and sheath blight disease of rice. Pest Manag. Sci. 66. https://doi.org/10.1002/ps.1907.
King, E.O., Ward, M.K., Raney, D.E., 1954. Two simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clin. Med 44, 301–307.
Kochanowsky, R.M., Bradshaw, C., Forlastro, I., Stock, S.P., 2020. *Xenorhabdus bovienii* strain jolietti uses a type 6 secretion system to kill closely related *Xenorhabdus* strains. FEMS Microbiol. Ecol. 96. https://doi.org/10.1093/femsec/fiaa073.
Koppenhöfer, A.M., Choo, H.Y., Kaya, H.K., Lee, D.W., Gelernter, W.D., 1999. Increased field and greenhouse efficacy against Scarab grubs with a combination of an

entomopathogenic Nematode and *Bacillus thuringiensis*. Biol. Control 14. https://doi.org/10.1006/BCON.1998.0663.

Kumar, V., Kumar, P., 2019. Pesticides in agriculture and environment: Impacts on human health. In: Kumar, V., Kumar, R., Singh, J., Kumer, P. (Eds.), Contaminants in Agriculture and Environment: Health Risks and Remediation. Agriculture and Environmental Science Academy, Haridwar, India, pp. 76–95. https://doi.org/10.26832/AESA-2019-CAE-0160-07.

Kupferschmied, P., Maurhofer, M., Keel, C., 2013. Promise for plant pest control: Root-associated pseudomonads with insecticidal activities. Front. Plant Sci. 4. https://doi.org/10.3389/fpls.2013.00287.

Kupferschmied, P., Péchy-Tarr, M., Imperiali, N., Maurhofer, M., Keel, C., 2014. Domain shuffling in a sensor protein contributed to the evolution of insect pathogenicity in plant-beneficial *Pseudomonas protegens*. PLoS Pathog. 10. https://doi.org/10.1371/journal.ppat.1003964.

van Lenteren, J.C., Bolckmans, K., Köhl, J., Ravensberg, W.J., Urbaneja, A., 2018. Biological control using invertebrates and microorganisms: plenty of new opportunities. BioControl 63, 39–59. https://doi.org/10.1007/s10526-017-9801-4.

Lewis, K.A., Tzilivakis, J., Warner, D.J., Green, A., 2016. An international database for pesticide risk assessments and management. Hum. Ecol. Risk Assess. 22, 1050–1064. https://doi.org/10.1080/10807039.2015.1133242.

Li, E.T., Zhang, S., Li, K., bin, Nyamwasaa, I., Li, J.Q., Li, X.F., Qin, J.H., Yin, J., 2021. Efficacy of entomopathogenic nematode and *Bacillus thuringiensis* combinations against *Holotrichia parallela* (Coleoptera: Scarabaeidae) larvae. Biol. Control 152. https://doi.org/10.1016/j.biocontrol.2020.104469.

Loper, J.E., Henkels, M.D., Rangel, L.I., Olcott, M.H., Walker, F.L., Bond, K.L., Kidarsa, T. A., Hesse, C.N., Sneh, B., Stockwell, V.O., Taylor, B.J., 2016. Rhizoxin analogs, orfamide A and chitinase production contribute to the toxicity of *Pseudomonas protegens* strain Pf-5 to *Drosophila melanogaster*. Environ. Microbiol. 18, 3509–3521. https://doi.org/10.1111/1462-2920.13369.

Loper, J.E., Hassan, K.A., Mavrodi, D. v, Davis, E.W., Lim, C.K., Shaffer, B.T., Elbourne, L. D.H., Stockwell, V.O., Hartney, S.L., Breakwell, K., Henkels, M.D., Tetu, S.G., Rangel, L.I., Kidarsa, T.A., Wilson, N.L., van de Mortel, J.E., Song, C., Blumhagen, R., Radune, D., Hostetler, J.B., Brinkac, L.M., Durkin, A.S., Kluepfel, D.A., Wechter, W. P., Anderson, A.J., Kim, Y.C., Pierson, L.S., Pierson, E.A., Lindow, S.E., Kobayashi, D. Y., Raaijmakers, J.M., Weller, D.M., Thomashow, L.S., Allen, A.E., Paulsen, I.T., 2012. Comparative Genomics of Plant-Associated *Pseudomonas* spp.: Insights into Diversity and Inheritance of Traits Involved in Multitrophic Interactions, PLoS Genet 8. Public Libr. Sci. https://doi.org/10.1371/JOURNAL.PGEN.1002784.

Lu, D., Macchietto, M., Chang, D., Barros, M.M., Baldwin, J., Mortazavi, A., Dillman, A. R., 2017. Activated entomopathogenic nematode infective juveniles release lethal venom proteins. PLoS Pathog. 13. https://doi.org/10.1371/journal.ppat.1006302.

Malusà, E., Berg, G., Biere, A., Bohr, A., Canfora, L., Jungblut, A.D., Kepka, W., Kienzle, J., Kusstatscher, P., Masquelier, S., Pugliese, M., Razinger, J., Tommasini, M.G., Vassilev, N., Meyling, N.V., Xu, X., Mocali, S., 2021. A holistic approach for enhancing the efficacy of soil microbial inoculants in agriculture. Glob. J. Agric. Innov. Res. Dev. 8. https://doi.org/10.15377/2409-9813.2021.08.14.

Maurhofer, M., Keel, C., Haas, D., Défago, G., 1994. Pyoluteorin production by *Pseudomonas fluorescens* strain CHA0 is involved in the suppression of *Pythium* damping-off of cress but not of cucumber. Eur. J. Plant Pathol. 100, 221–232. https://doi.org/10.1007/BF01876237.

Mc Namara, L., Kapranas, A., Williams, C.D., O'Tuama, P., Kavanagh, K., Griffin, C.T., 2018. Efficacy of entomopathogenic fungi against large pine weevil, *Hylobius abietis*, and their additive effects when combined with entomopathogenic nematodes. J. Pest Sci. 91. https://doi.org/10.1007/s10340-018-0994-9.

McDonald, B.A., Stukenbrock, E.H., 2016. Rapid emergence of pathogens in agro-ecosystems: global threats to agricultural sustainability and food security. Philos. Trans. R. Soc. B 371. https://doi.org/10.1098/RSTB.2016.0026.

Melo, A.L., de, A., Soccol, V.T., Soccol, C.R., 2016. *Bacillus thuringiensis*: mechanism of action, resistance, and new applications: a review. Crit. Rev. Biotechnol. 36. https://doi.org/10.3109/07388551.2014.960793.

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. Control 43. https://doi.org/10.1016/j.biocontrol.2007.07.007.

Meyling, N. v, Pilz, C., Keller, S., Widmer, F., Enkerli, J., 2012. Diversity of *Beauveria* spp. isolates from pollen beetles *Meligethes aeneus* in Switzerland. J. Invertebr. Pathol. 109, 76–82. https://doi.org/10.1016/j.jip.2011.10.001.

Myrand, V., Buffet, J.P., Guertin, C., 2015. Susceptibility of Cabbage Maggot Larvae (Diptera: Anthomyiidae) to Hypocreales Entomopathogenic Fungi. J. Econ. Entomol. 108, 34–44. https://doi.org/10.1093/jee/tou019.

Oerke, E.C., 2006. Crop losses to pests. J. Agric. Sci. 144, 31–43. https://doi.org/10.1017/S0021859605005708.

Oestergaard, J., Belau, C., Strauch, O., Ester, A., van Rozen, K., Ehlers, R.U., 2006. Biological control of *Tipula paludosa* (Diptera: Nematocera) using entomopathogenic nematodes (*Steinernema* spp.) and *Bacillus thuringiensis* subsp. *israelensis*. Biol. Control 39. https://doi.org/10.1016/j.biocontrol.2006.07.003.

Ogier, J.-C., Pagès, S., Frayssinet, M., Gaudriault, S., 2020. Entomopathogenic nematode-associated microbiota: from monoxenic paradigm to pathobiome. Microbiome 8. https://doi.org/10.1186/s40168-020-00800-5.

Panth, M., Hassler, S.C., Baysal-Gurel, F., 2020. Methods for management of soilborne diseases in crop production. Agriculture 10. https://doi.org/10.3390/agriculture10010016.

Pest Management Regulatory Agency (PMRA), 2020. Re-evaluation decision RVD2020–14, chlorpyrifos and its associated end-use products (environment). https://www.canada.ca/en/health-canada/services/consumer-product-safety/reports-publications/pesticides-pest-management/decisions-updates/reevaluation-decision/2020/chlorpyrifos.html.

Pilz, C., 2005, Natürliches Auftreten insektenpathogener Pilze beim Rapsglanzkäfer (*Meligethes aeneus*) und Versuche der Eignung von *Metarhizium anisopliae* zur mikrobiellen Bekämpfung. M.Sc. thesis, Universität für Bodenkultur (BOKU), Vienna, Austria.

Pronk, L.J.U., Bakker, P.A.H.M., Keel, C., Maurhofer, M., Flury, P., 2022. The secret life of plant-beneficial rhizosphere bacteria: insects as alternative hosts. Environ. Microbiol 24. https://doi.org/10.1111/1462-2920.15968.

Rangel, L.I., Henkels, M.D., Shaffer, B.T., Walker, F.L., Davis, E.W., Stockwell, V.O., Bruck, D.J., Taylor, B.J., Loper, J.E., 2016. Characterization of toxin complex gene clusters and insect toxicity of bacteria representing four subgroups of *Pseudomonas fluorescens*. PLoS One 11. https://doi.org/10.1371/journal.pone.0161120.

Ravindran, K., Sathishkumar, G., Rajkuberan, C., Sivaramakrishnan, S., 2014. Antibacterial activity of indigenous entomopathogenic fungus *Metarhizium anisopliae* against clinically isolated human pathogens. Int. J. Pharm. Pharm. Sci.

Razinger, J., Lutz, M., Schroers, H.-J., Urek, G., Grunder, J., 2014. Evaluation of insect associated and plant growth promoting fungi in the control of cabbage root flies. J. Econ. Entomol. 107. https://doi.org/10.1603/EC14004.

Razinger, J., Žerjav, M., Zemljič-Urbančič, M., Modic, Š., Lutz, M., Schroers, H.J., Grunder, J., Fellous, S., Urek, G., 2017. Comparison of cauliflower–insect–fungus interactions and pesticides for cabbage root fly control. Insect Sci. 24, 1057–1064. https://doi.org/10.1111/1744-7917.12534.

Reinbacher, L., Bacher, S., Praprotnik, E., Grabenweger, G., 2021b. Standard non-target tests for risk assessment of plant protection products are unsuitable for entomopathogenic fungi—a proposal for a new protocol. J. Soils Sediment. 21. https://doi.org/10.1007/s11368-021-02919-w.

Reinbacher, L., Bacher, S., Knecht, F., Schweizer, C., Sostizzo, T., Grabenweger, G., 2021a. Preventive field application of *Metarhizium brunneum* in cover crops for wireworm control. Crop Prot. 150. https://doi.org/10.1016/j.cropro.2021.105811.

Ritika, B., Utpal, D., 2014. An overview of fungal and bacterial biopesticides to control plant pathogens/diseases. Afr. J. Microbiol. Res. 8, 1749–1762. https://doi.org/10.5897/AJMR2013.6356.

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. https://doi.org/10.1007/s10526-017-9816-x.

Ruffner, B., Péchy-Tarr, M., Höfte, M., Bloemberg, G., Grunder, J., Keel, C., Maurhofer, M., 2015. Evolutionary patchwork of an insecticidal toxin shared between plant-associated pseudomonads and the insect pathogens *Photorhabdus* and *Xenorhabdus*. BMC Genom. 16, 609. https://doi.org/10.1186/s12864-015-1763-2.

Ruffner, B., Péchy-Tarr, M., Ryffel, F., Hoegger, P., Obrist, C., Rindlisbacher, A., Keel, C., Maurhofer, M., 2013. Oral insecticidal activity of plant-associated pseudomonads. Environ. Microbiol 15. https://doi.org/10.1111/j.1462-2920.2012.02884.x.

Sanchis, V., 2011. From microbial sprays to insect-resistant transgenic plants: history of the biospesticide *Bacillus thuringiensis*. A review. Agron. Sustain. Dev. 31, 217–231. https://doi.org/10.1051/AGRO/2010027.

Sanchis, V., Bourguet, D., 2008. *Bacillus thuringiensis*: applications in agriculture and insect resistance management. A review. Agron. Sustain. Dev. 28. https://doi.org/10.1051/AGRO:2007054.

Savary, S., Willocquet, L., Pethybridge, S.J., Esker, P., McRoberts, N., Nelson, A., 2019. The global burden of pathogens and pests on major food crops. Nat. Ecol. Evol. 3, 430–439. https://doi.org/10.1038/s41559-018-0793-y.

Schellenberger, U., Oral, J., Rosen, B.A., Wei, J.-Z., Zhu, G., Xie, W., McDonald, M.J., Cerf, D.C., Diehn, S.H., Crane, V.C., Sandahl, G.A., Zhao, J.-Z., Nowatzki, T.M., Sethi, A., Liu, L., Pan, Z., Wang, Y., Lu, A.L., Wu, G., Liu, L., 2016. A selective insecticidal protein from *Pseudomonas* for controlling corn rootworms. Science 354 (1979), 634–637. https://doi.org/10.1126/science.aaf6056.

Schrank, A., Vainstein, M.H., 2010. *Metarhizium anisopliae* enzymes and toxins. Toxicon 56, 1267–1274. https://doi.org/10.1016/j.toxicon.2010.03.008.

Senthilraja, G., Anand, T., Durairaj, C., Raguchander, T., Samiyappan, R., 2010b. Chitin-based bioformulation of *Beauveria bassiana* and *Pseudomonas fluorescens* for improved control of leafminer and collar rot in groundnut. Crop Prot. 29. https://doi.org/10.1016/j.cropro.2010.06.002.

Senthilraja, G., Anand, T., Durairaj, C., Kennedy, J.S., Suresh, S., Raguchander, T., Samiyappan, R., 2010a. A new microbial consortia containing entomopathogenic fungus, *Beauveria bassiana* and plant growth promoting rhizobacteria, *Pseudomonas fluorescens* for simultaneous management of leafminers and collar rot disease in groundnut. Biocontrol Sci. Technol. 20. https://doi.org/10.1080/09583150903576949.

Shapiro-Ilan, D.I., Jackson, M., Reilly, C.C., Hotchkiss, M.W., 2004. Effects of combining an entomopathogenic fungi or bacterium with entomopathogenic nematodes on mortality of *Curculio caryae* (Coleoptera: Curculionidae). Biol. Control 30. https://doi.org/10.1016/J.BIOCONTROL.2003.09.014.

Sontowski, R., Poeschl, Y., Okamura, Y., Vogel, H., 2022. A high-quality functional genome assembly of *Delia radicum* L. (Diptera: Anthomyiidae) annotated from egg to adult (Cervin Guyomar, |, Cortesero, A.-M., van Dam, N.M.). Mol. Ecol. Resour.. https://doi.org/10.1111/1755-0998.13594.

Stock, S.P., 2015. Diversity, Biology and Evolutionary Relationships. In: Campos-Herrera, R.C. (Ed.), Nematode Pathogenesis of Insects and Other Pests. Springer International Publishing, Cham, pp. 3–27. https://doi.org/10.1007/978-3-319-18266-7_1.

Strasser, H., Forer, A., Schinner, F., 1996, Development of media for the selective isolation and maintenance of viruIence of *Beauveria brongniartii*, in: Glare, T., Jackson, T. (Eds.), Proceedings of the 3rd International Workshop on Microbial Control of Soil Dwelling Pests. Lincoln, New Zealand, pp. 125–130.

Vacheron, J., Péchy-Tarr, M., Brochet, S., Heiman, C.M., Stojiljkovic, M., Maurhofer, M., Keel, C., 2019. T6SS contributes to gut microbiome invasion and killing of an herbivorous pest insect by plant-beneficial *Pseudomonas protegens*. ISME J. 13. https://doi.org/10.1038/s41396-019-0353-8.

Vänninen, I., Hokkanen, H., Tyni-Juslin, J., 1999a. Screening of field performance of entomopathogenic fungi and nematodes against cabbage root flies (*Delia radicum* l. and *D. floralis* (fall.); Diptera, Anthomyiidae). Acta Agric. Scand. B Soil Plant Sci. 49, 167–183. https://doi.org/10.1080/09064719909362513.

Vänninen, I., Hokkanen, H., Tyni-Juslin, J., 1999b. Attempts to control cabbage root flies *Delia radicum* L. and *Delia floralis* (Fall.) (Dipt., Anthomyiidae) with entomopathogenic fungi: laboratory and greenhouse tests. J. Appl. Entomol. 123, 107–113. https://doi.org/10.1046/j.1439-0418.1999.00315.x.

Vesga, P., Augustiny, E., Keel, C., Maurhofer, M., Vacheron, J., 2021. Phylogenetically closely related pseudomonads isolated from arthropods exhibit differential insect-killing abilities and genetic variations in insecticidal factors. Environ. Microbiol 23. https://doi.org/10.1111/1462-2920.15623.

Vesga, P., Flury, P., Vacheron, J., Keel, C., Croll, D., Maurhofer, M., 2020. Transcriptome plasticity underlying plant root colonization and insect invasion by *Pseudomonas protegens*. ISME J. 14. https://doi.org/10.1038/s41396-020-0729-9.

White, G.F., 1927. A method for obtaining infective nematode larvae from cultures. Science 66, 302–303. https://doi.org/10.1126/science.66.1709.302.b.

Witkowska, E., Moorhouse, E.R., Jukes, A., Elliott, M.S., Collier, R.H., 2018. Implementing Integrated Pest Management in commercial crops of radish (*Raphanus sativus*). Crop Prot. 114, 148–154. https://doi.org/10.1016/j.cropro.2018.08.008.

Wraight, S.P., Ramos, M.E., 2005. Synergistic interaction between *Beauveria bassiana*- and *Bacillus thuringiensis tenebrionis*-based biopesticides applied against field populations of Colorado potato beetle larvae. J. Invertebr. Pathol. 90, 139–150. https://doi.org/10.1016/J.JIP.2005.09.005.

Xu, X.M., Jeffries, P., Pautasso, M., Jeger, M.J., 2011. Combined use of biocontrol agents to manage plant diseases in theory and practice. Phytopathology 101, 1024-1132. https://doi.org/10.1094/PHYTO-08-10-0216.

Zhan, J., Thrall, P.H., Burdon, J.J., 2014. Achieving sustainable plant disease management through evolutionary principles. Trends Plant Sci. 19, 570–575. https://doi.org/10.1016/J.TPLANTS.2014.04.010.