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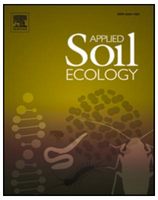

Research paper

Behavioural responses of *Agriotes lineatus* and *Agriotes litigiosus* wireworms to natural substances, synthetic volatile organic compounds and entomopathogenic fungi: Implications for sustainable pest management

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ABSTRACT

This study investigated the behavioural responses of two wireworm species to three selected treatments: compost teas (16 and 19), synthetic 3-octanone, and *Metarhizium brunneum* (strain 1868). Behavioural assays were conducted under controlled conditions using rhizotrons and olfactometers. Results showed that compost tea 16 acted as an attractant, compost tea 19 had no significant effect, while both 3-octanone and *M. brunneum* exhibited consistent repellence in both species. These responses were consistent across experimental systems, indicating robust behavioural patterns. The findings suggest that wireworm behaviour is influenced by chemically mediated belowground cues, with contrasting responses depending on cue origin. These results highlight the potential of these treatments as alternatives for sustainable pest management, including push–pull approaches. Further field validation is required to confirm their effectiveness under practical conditions.

1. Introduction

The functioning of terrestrial ecosystems depends on complex interactions between aboveground and belowground organisms. Wireworms (Coleoptera: Elateridae), the soil-dwelling larvae of click beetles, are among the most damaging agricultural pests worldwide (Veres et al., 2020).In Europe, several *Agriotes* species cause damage to crops, including *A. sordidus* Illiger, *A. ustulatus* Schäller, *A. litigiosus* Rossi, *A. brevis* Candèze, *A. lineatus* Linnaeus, *A. sputator* L., *A. obscurus* L., *A. proximus* Schwarz., and *A. rufipalpis* Brullé (Furlan et al., 2021; Platia, 1994).

Elaterids have a long-life cycle, are polyphagous, capable of burrowing deep into the soil, and are highly fecund; combined with overlapping generations and strong adaptability to soil conditions, these traits make them particularly challenging to control (Sufyan et al., 2014; Furlan, 2005). Wireworms feed on the roots and tubers of numerous crops, including cereals, legumes, potatoes, and sugar cane, and many other economically important crops causing damage that leads to significant yield losses (Vernon and van Herk, 2022; Saguez et al., 2017; Furlan, 2005). Moreover, their impact on plants can influence interactions across multiple trophic levels (Kostenko et al., 2013), from altering the dynamic interplay between belowground and aboveground herbivores, to modifying plant responses to herbivores (Anderson et al., 2011). These changes carry significant consequences for both plants and higher-trophic-level organisms (Johnson et al., 2009). Wireworms are able to move vertically, horizontally, or between crops in search of food when soil conditions are favourable (Sonnemann et al., 2014; Barsics et al., 2013).

Wireworms locate host plants using a behavioural mechanism called klinotaxis, which involves the activation of clusters of sensilla on the maxillary and labial palps (Doane and Klingler, 1978; Klingler, 1957). They use multiple strategies to identify their suitable hosts: they may randomly encounter root signals while foraging (Doane et al., 1975), detect chemosensory cues on the root surface upon direct contact

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(Johnson and Gregory, 2006), or perceive volatile organic compounds (VOCs), as well as CO_2, emitted by plant roots, indicating the presence of living plant tissue (Arce et al., 2021; Johnson and Nielsen, 2012). Several studies have demonstrated wireworms' attraction to CO_2 sources, for instance through the use of baker's yeast (*Saccharomyces cerevisiae*) as a CO_2 generator in attract-and-kill strategy (Brandl et al., 2017).

To mitigate elaterid damage in annual crops, a range of management strategies has been developed, including adult mass trapping (Sufyan et al., 2013), crop rotation (Willis et al., 2010), biofumigation and crop residue removal (Furlan et al., 2010), bioaugmentation with entomopathogenic fungi (EPF) (Razinger et al., 2020), cover crop use (Reinbacher et al., 2021; Rogge et al., 2017), mechanical soil disturbance and biological control (La Forgia and Verheggen, 2019; Reddy et al., 2014), as well as other agricultural practices and insecticides (Furlan et al., 2020). However, the negative environmental and health impacts of insecticides, along with their limited efficacy, have driven the search for sustainable pest management strategies. The introduction of the European Directive 2009/128/EC, made Integrated Pest Management (IPM) mandatory, progressively restricting the use of several synthetic pesticides and encouraging the adoption of agronomic and biological control measures (Pisa et al., 2021; Barzman et al., 2015; Furlan, 2014). Farmers are therefore encouraged to maximize the use of agronomic solutions, while researchers must investigate the most effective and sustainable alternative strategies and equipment (Civolani et al., 2023).

Understanding wireworm behaviour using natural substances (NS), VOCs, and EPF can lead to the development of promising alternative control measures that are environmentally friendly, biodegradable, and nearly residue-free (Civolani et al., 2023; Poggi et al., 2021; Brandl et al., 2017). Compost tea is one such promising natural product. It is a liquid extract derived from high-quality compost of green waste (Zaccardelli et al., 2012), known to suppress plant pathogens and enhance plant growth through enriching microbial diversity and nutrient profile (González-Hernández et al., 2022; Mahnert et al., 2018; Ilangumaran and Smith, 2017). To date, the effects of compost tea on insects remain largely unexplored, particularly with respect to species of *Agriotes*.

Volatile organic compounds, produced naturally by plant roots and microorganisms, can attract or repel wireworms and even promote aggregation behaviour. Several VOCs, such as 2-pentylfuran, hexanal, 1-octen-3-ol, and 3-octanone, have been reported as behaviourally active compounds (La Forgia et al., 2023; La Forgia et al., 2020; Hummadi et al., 2021; Barsics et al., 2017; Barsics et al., 2013). Notably, 3-octanone is also known to possess toxic and defensive properties against nematode infestations (Lee et al., 2023), suggesting potential roles in soil pest management.

Entomopathogenic fungi, particularly *Metarhizium* spp. (Ascomycota: Hypocreales), have shown promising results in infesting wireworms as naturally occurring soil fungi (Kabaluk et al., 2005). Beyond their pathogenic action, EPF also have other beneficial functions as they enhance plant growth and mineral nutrition and exclude phytopathogens from rhizosphere niches (Bourdon et al., 2023; Wood et al., 2023; Ahmad et al., 2020; Razinger et al., 2020; Faria and Wraight, 2007; Rivas-Franco et al., 2019; Herbst et al., 2017). These effects are largely attributed to the VOCs, secondary metabolites, and antimicrobial compounds produced by EPF, many of which possess insecticidal and semiochemical properties (Hummadi et al., 2021; Gibson et al., 2014; Oller-López et al., 2005; Isaka et al., 2003). Beyond their relevance as agricultural pests, wireworms are key soil-dwelling herbivores whose spatial behaviour is shaped by chemical cues produced by plants and soil microorganisms. Understanding how belowground insects perceive and respond to such cues is central to soil ecology, as it influences organism distribution and soil-mediated interactions.

We hypothesize that wireworm behaviour is driven by chemically mediated belowground cues, with compost-derived signals acting as attractants, likely due to microbial activity and associated emissions, whereas fungal and synthetic volatile compounds induce repellence. To test this hypothesis, we evaluated the behavioural responses of two wireworm species, *Agriotes litigiosus* and *A. lineatus*, to compost teas, synthetic 3-octanone, and the entomopathogenic fungus *Metarhizium brunneum* (strain KIS/HJS 1868), previously shown to be pathogenic (Razinger et al., 2018) and potential repellent to wireworms (Razinger et al., 2020), using dual-choice olfactometers and rhizotrons.

2. Materials and methods

2.1. Insect source

Larvae of *Agriotes lineatus* were obtained from a laboratory colony established from adult elaterid beetles collected from field located in Jablje, north of Ljubljana, Slovenia (46°08′29.2”N 14°33′25.0″E; 46°08′31.9”N 14°33′16.1″E) in the early summer of 2023 and reared at the Agricultural Institute of Slovenia according to Kölliker et al. (2009). In February 2024, the larvae reached a suitable size for experimental use (1.6 ± 0.23 cm). They were then transferred to smaller pots containing soil, fed with carrot, and kept at 5 °C to slow development until the start of the experiment. Larvae of *Agriotes litigiosus* were collected using traps filled with vermiculite, wheat, and maize according to Furlan (2014) and Drahun et al. (2022) from fields located in Macerata province, Italy (43°16′30”N 13°32′03″E; 43°16′08”N 13°33′38″E) in September 2024. They were kept in 1 L pots filled with medium loam soil and vermiculite and fed with maize or barley seeds. Seven days prior to the experiments, each larva of both *Agriotes* species was starved and isolated in a single 80 mL plastic rearing cup filled only with commercial planting soil (Potgrond H, Klasmann-Deilmann GmbH, Geeste, Germany). This procedure was applied to avoid cannibalism. The boxes were maintained in glasshouse at controlled temperature (20 ± 5 °C), humidity (58 ± 7%), and natural light condition. Germinated barley or maize was provided as food.

2.2. Larval selection

The larvae undergo three phases in each instar: hardening, feeding, and pre-moulting. The feeding phase is the most active and damaging and represents only approximately 21% of the total larval stage (Furlan, 2004). Starved larvae underwent a mobility test to select individuals 10–15 mm in length that were healthy, and actively foraging, corresponding to the most damaging feeding phase. Only these individuals were used (Furlan, 2004). Larvae in the hardening and pre-moulting phases were discarded, due to their reduced feeding activities (Furlan, 2004).

2.3. Plant material

Seeds of both maize (*Zea mays* L.) and barley (*Hordeum vulgare* L.) were sown in pots and maintained under the same controlled conditions as above. To promote germination, the seeds were soaked daily and thereafter every two days until their use in the experiments.

2.4. Compost tea production and characterization

In this study we used two compost teas, namely Compost 16 (CT16) and Compost 19 (CT19). Each of the two compost teas was prepared using a 1-l airtight glass jar, to produce anaerobic compost teas. The jar was filled with 300 mL of sterile distilled water, to which 75 cc of loose compost was added, resulting in a compost-to-water ratio of 1:5 (*v*/v). The mixture was incubated for six days at 28 °C in a thermostat incubator. After incubation, the liquid was filtered through gauze to remove compost residues. The filtrate was stored at 4 °C until analysis.

The two composts used were both derived from the organic fraction of municipal solid waste (OFMSW), collected through urban waste sorting. CT16 originated from a treatment facility located in Anzio (Rome, Lazio, Italy), while CT19 was obtained from a composting plant in Lovadina di Spresiano (Treviso, Veneto, Italy). Both were supplied by CREA of Pontecagnano (Salerno, Italy).

Moreover, chemical and physical analyses included measurements of pH, electrical conductivity, and C/N ratio, while microbiological analyses comprised total counts of bacteria, fungi, yeasts, and actinomycetes, as well as the detection of *Escherichia coli*.

2.5. *Fungal culture and VOCs analysis*

Fungal strain *Metarhizium brunneum* (KIS/HJS 1868) was subcultured on Potato Dextrose agar (PDA; Biolife, Italy) in Petri dishes and incubated in a cooled incubator (IPPP500, Memmert) at 22 °C for 14 days to obtain sporulating structures (aerial conidia). Three agar plugs (9 mm diameter) were extracted with a cork borer, and each was suspended in 1 mL of 0.1% Tween 80 solution. Conidia were counted using a Neubauer chamber grid, yielding an average of $83.6 \pm 4.1 \times 10^6$ conidia per plug, and this value was used to standardize inoculum across assays.

VOCs released by EPF were subsequently analysed by gas chromatography - mass spectrometry (GC–MS), as described below. Analysis of microbial volatile organic compounds (VOCs) produced by fungal isolates was performed by headspace-solid phase microextraction (HS-SPME) gas chromatography - mass spectrometry (GC–MS) (Cernava et al., 2015; Verginer et al., 2010). Briefly, pure fungal isolates were transferred with an inoculating loop on potato dextrose slope agar (PDA) in 20 mL headspace vials (75.5 × 22.5 mm; Chromtech, Idstein, Germany). The isolate streaked out in 3 parallel lines to ensure similar microbial growth density after incubation. Three replicates of each isolate were processed during the analysis. After 48 h of incubation at room temperature, vials were sealed and incubated for an additional 2 h before analysis. Solid phase microextraction (SPME) was performed with an automated sampler and 50/30 μm Divinylbenzene (DVB)/CarboxenTM/Polydimethylsiloxane (PDMS)/2 cm Stableflex/SS fibre (Supelco, Bellefonte, PA, USA). Volatile compounds were enriched for 30 min at 35 °C and then separated and detected on a GC 7890 A system coupled to a quadrupole MS 5975C (Agilent Technologies, Waldbronn, Germany). Samples were run through a (5%-phenyl) methylpolysiloxane column, 30 m × 0.25 mm i.d., 0.25 μm film thickness (HP-Column: HP-5MS, 30 m × 250 μm × 0.25 μm MS; Agilent Technologies, Waldbronn, Germany), followed by electron ionization (EI; 70 eV) and detection (mass range 25–350 Da). The inlet temperature was adjusted to 250 °C. For the temperature gradient the GC column was kept at 40 °C for 2 min, raised to 100 °C at a rate of 5 °C min^{-1}, then to 280 °C at 10 °C min^{-1} and finally maintained at 280 °C for 3 min. The helium flow rate was set to 1.2 mL min^{-1}. Obtained spectra were compared with NIST Mass Spectra Database 14 entries. Specific compounds were identified based on the relative match of their spectra and the matching of their retention indices.

2.6. *Chemicals*

Preliminary screening experiments involving four chemical compounds selected from the literature, hexanal (Barsics et al., 2017; La Forgia et al., 2020), 2-pentylfuran (La Forgia et al., 2023; Barsics et al., 2013), 1-octen-3-ol, and 3-octanone (Bourdon et al., 2023; Wood et al., 2023) were performed to assess potential effects on wireworm behaviour (data not shown in the manuscript). 3-octanone demonstrated the strongest and most consistent repellent responses and was consequently selected as a positive control for the main experiments. Synthetic 3-Octanone with a purity of ≥98%, was purchased from Sigma-Aldrich (Italy) and used in the experiments, similar to Bourdon et al. (2023) who reported that 3-octanone is also produced by the entomopathogenic fungus *Metarhizium brunneum*.

2.7. *Olfactometer assays*

Olfactometers were constructed based on designs of Gfeller et al. (2013) and Barsics et al. (2017). Each olfactometer consisted of a plastic T-shaped connector with two lateral pipes (Bauhaus, Slovenia) attached at an 87° angle. The entire system measured 30 cm in length, with pipes 63 mm in diameter. Olfactometers were filled with 300 g commercial planting soil (Potgrond H, Klasmann-Deilmann GmbH, Geeste, Germany), adjusted to 70 ± 5% moisture content, and divided into three areas: treated, central (i.e., the wireworm release area), and untreated (Fig. 1). The distance between the wireworm release point and the treatment was 15 cm. Three treatments were tested for behavioural responses of *A. litigiosus* and *A. lineatus*:

(i) Compost teas: 10 mL of compost tea (CT16 or CT19) was injected directly into the treated area to ensure distribution within the targeted area, while 10 mL of tap water was injected into the untreated area as the negative control (Civolani et al., 2023).

(ii) Synthetic 3-octanone: 10 μL (0.1 μL g^{-1} soil) was applied to a dental cotton roll placed beneath maize seedlings in the treated zone; an identical cotton roll with 10 μL of tap water was placed in the untreated area as the negative control (Bourdon et al., 2023).

(iii) Entomopathogenic fungus (*Metarhizium brunneum*, strain KIS/HJS 1868): One agar plug (9 mm) of 14 d old *M. brunneum* PDA culture was placed beneath the seedling in the treated area, while a sterile PDA plug of the same size and age, prepared under identical conditions, was placed in the untreated area as a negative control.

Once prepared, olfactometers were transferred to a dark growth chamber under controlled conditions, temperature (23 ± 3 °C) and humidity (60 ± 5_%), and left for one hour before larval release. Larvae were then placed into the central area, a few centimetres below the soil surface and left for two hours (La Forgia et al., 2023). For each treatment and species, ten replicate experiments were conducted. The number of larvae per replicate (n) varied among treatments due to the limited availability of larvae in the feeding phase at the time of the experiments, with 4–6 larvae per replicate for compost tea and 3-octanone assays, and 6–10 larvae per replicate for *M. brunneum* assays. Consequently, the total number of larvae tested (N) differed among treatments, resulting in $N = 40$ for *Agriotes lineatus* and $N = 60$ for *Agriotes litigiosus*. After two hours, each area was separated and emptied into a plastic box, and the location of the wireworms was determined. The wireworms were counted and classified as responding (those entering the treated or untreated area) or non-responding (those remaining in the central area). To verify the absence of positional bias, control assays were conducted in which both arms of the olfactometer were treated with the negative control (water or PDA plug, depending on the treatment).

2.8. *Rhizotron assays*

The rhizotron consisted of a vertical window made of two non-reflective museum-grade glass panes held together by a wooden frame, enclosing a thin layer of soil (290 × 400 × 4 mm), filled with commercial planting soil (Potgrond H, Klasmann-Deilmann GmbH, Geeste, Germany). Two barley seedlings were positioned 10 cm from the top and the left and right lateral edges. Treatments were applied beneath one of the seedlings, while the other seedling served as an untreated control. A single larva was placed 20 cm from the treatment at the lower central part of the rhizotron before sealing it with the second glass pane, forming the complete sandwich structure (Fig. 1). Rhizotrons were then transferred to a dark growth chamber and maintained under controlled conditions, temperature (23 ± 3 °C) and humidity (57 ± 5%). Larval position was recorded every hour for 8 h by marking their location on a 290 × 400 mm grid sheet.

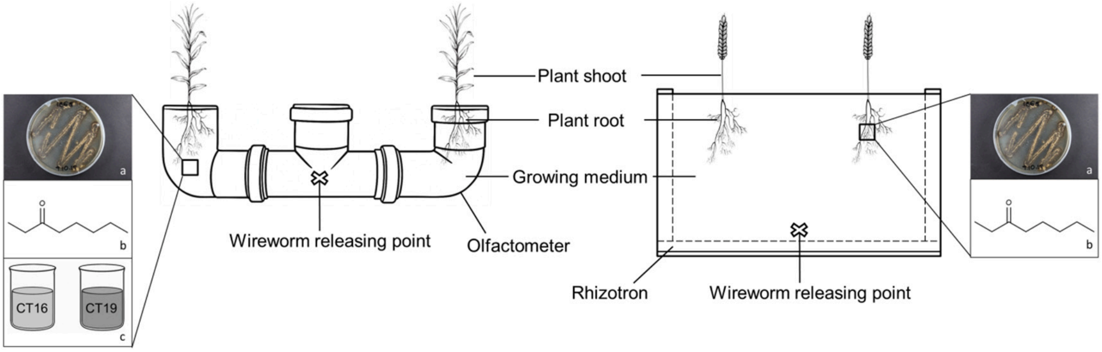

Fig. 1. Olfactometer and Rhizotron designs used for experiments with maize (left) and barley (right), showing the location of wireworms. The figures also indicate the site where treatments were applied on the treated side: *Metarhizium brunneum* 1868 (a), 3-octanone (b) or compost teas (c). *CT16* and *CT19* – two tested compost teas.

Two treatments were tested for behavioural responses of *A. litigiosus* and *A. lineatus*:

(i) Synthetic 3-octanone: 10 μL (0.1 μL g^{-1} soil) was applied to a dental cotton roll placed beneath barley seedlings in the treated zone; an identical cotton roll with 10 μL of water was placed in the untreated area as the negative control (Bourdon et al., 2023).
(ii) Entomopathogenic fungus (*Metarhizium brunneum*, strain KIS/HJS 1868): One agar plug (9 mm) of *M. brunneum* PDA culture was placed beneath the seedling in the treated area, while a sterile PDA plug of the same size and age, prepared under identical conditions, was placed in the untreated area as a negative control.

There were ten separate replicate experiments for each treatment and species, during which an individual larva was observed per replicate ($n = 1$). Therefore, the total number of larvae tested (N) for each treatment and species was 10. To verify the absence of positional bias, control assays were conducted with rhizotrons containing only barley seedlings.

All rhizotron and olfactometer experimental assays were designed to investigate behavioural responses (attraction/repellence) under controlled conditions, rather than to assess control efficacy.

2.9. Statistical analysis

All analyses were carried out using R 4.3.2 (R Core Team, 2023) using the packages *lme4* (Bates et al., 2015) and *rstatix* (Kassambara, 2023). Dual choice olfactometer assays were assessed by counting the number of wireworms on each area of the olfactometer and then analysed using generalized linear models (GLM) with a Poisson distribution, having treatment (control vs. test compound) and species as fixed effects. In case of overdispersion, a quasi-Poisson model was used for this treatment. The fit of the model was tested with the *DHARMa* package (Hartig, 2024). The effects of factors and possible interactions were determined from Anova tables from the *car* package (Fox and Weisberg, 2019). In case of significant terms, we performed pairwise comparisons using estimated marginal means with Tukey-adjusted post-hoc contrasts, with the *emmeans* package (Lenth, 2025).

As an additional measure of larval preference, a Response Index (RI) was calculated for each olfactometer experiment as in Usseglio et al. (2017). An RI of 0 indicates no preference, while positive or negative values indicate attraction or repellence. Shapiro–Wilk tests were performed for each treatment to assess the normality of the RI. If normality was confirmed, one-sample *t*-tests were used to test whether the RI was significantly different from 0. If normality was not met, the Wilcoxon signed-rank test was used. Additionally, it was tested whether the RI differs between species; linear models were used for normally distributed RI data and Wilcoxon rank-sum tests were used for non-normally distributed data.

For rhizotron assays, three spatial models were applied to characterize wireworm preferences and activity patterns in response to treatments, offering complementary perspectives on movement within the rhizotron (please see Fig. 5 for a schematic diagram of the models):

(i) Model A (two-zone model): The rhizotron was divided vertically into untreated and treated zone. The number of hours spent on each side was recorded, providing a basic measure of attraction or repellence.
(ii) Model B (four-zone model): The rhizotron was divided both vertically and horizontally into four quadrants: upper untreated, upper treated, lower untreated, and lower treated. Hours spent in each quadrant zone were recorded, allowing assessment of vertical differences in wireworm behaviour, distinguishing between areas with seedlings and bare soil.
(iii) Model C (bull's-eye model): Three concentric zones were defined around each seedling, with Zone 1 covering a 1 cm radius around the roots and treatment area, Zone 2 extending 1 cm beyond Zone 1, and Zone 3 extending 1 cm beyond Zone 2. Hours spent in each zone around treated and untreated seedling were recorded. This model focused on areas directly associated with roots and treatments, excluding bare soil, and provided the most spatially precise measure of wireworm behaviour.

Rhizotron experiments data were analysed using generalized linear models (GLMs) with a Poisson distribution or linear models (LMs), depending on the type of the response variable. The models assessed the effects of treatment, species, and rhizotron zone (as defined in Models A–C) on two response variables: (i) total time (in hours) spent in each zone of the rhizotron, and (ii) total distance travelled. In cases of overdispersion or zero-inflation, negative binomial models were fitted using the *glmmTMB* package (Brooks et al., 2017). Where appropriate, zero-inflated models were specified with a constant zero-inflation probability to account for excess zeros in the data. The effects of factors and possible interactions were determined from Anova tables from the *car* package (Fox and Weisberg, 2019). In case of significant terms, we performed pairwise comparisons using estimated marginal means with Tukey-adjusted post-hoc contrasts, with the *emmeans* package (Lenth, 2025). To visualize the spatial distribution of wireworms during rhizotron assays, point density heatmaps were generated using the python-based tool *Rhizotron Heatmap Generation* (Lapajne, 2025).

3. Results

3.1. Compost tea analytical assessments

Compost tea 16 (CT16) showed a slightly acidic pH and higher electrical conductivity (EC) compared to Compost tea 19 (CT19). The C/N ratio was also higher in CT16, indicating slightly different levels of organic matter decomposition and nutrient availability. Moreover, CT16 showed notably higher microbial activity, particularly with a greater presence of actinomycetes compared to CT19. *Escherichia coli* was absent of in both compost teas (Table 1).

3.2. Volatile organic compounds produced by *Metarhizium brunneum*

Volatiles produced by fungal isolate *Metarhizium brunneum* KIS/HJS 1868 were classified as amines and arenes. A total of three different molecules were found for *Metarhizium brunneum* KIS/HJS 1868: Ethylenimine (amine), 2-epi-alpha-Funebrene and [3,3′]-Paracyclophane (arenes).

3.3. Olfactometer assays

All models in the dual choice olfactometer assays, except for CT19, showed a significant effect of treatment (Table 2; Fig. 2). 3-octanone (ratio = 3.24, SE = 0.74, z = 5.12, $p < 0.001$) and EPF (ratio = 2.15, SE = 0.3, z = 5.49, p < 0.001) -treated areas attracted significantly fewer wireworms compared to control areas. On the other hand, CT16-treated area attracted significantly more wireworms compared to the control (ratio = 0.33, SE = 0.1, z = −3.647, p < 0.001). There was no effect of CT19 on wireworm choice. Additionally, species significantly influenced the response in EPF and CT19 assays, i.e., *A. lineatus* responded less strongly to the treatment than *A. litigiosus*, with a significantly lower mean count overall (EPF ratio = 0.55, SE = 0.075, z = −4.38, p < 0.001; CT19 ratio = 0.53, SE = 0.14, z = −2.49, $p = 0.013$). The equal distribution of wireworms when both areas were treated with the control (tap water or PDA plug, depending on the treatment), demonstrated that there was no positional bias (not shown in the figure).

Analysis of the Response Index (RI) further supported our results (Table 2; Fig. 3). 3-octanone and EPF showed a significant repellence effect, with a mean RI of −33.7 for 3-octanone and − 23.75 for EPF, indicating a preference for the control area. Contrastingly, CT16 showed a significant attraction effect, with a mean RI of 28.3, indicating a preference for the CT16 side. Furthermore, results showed no significant difference between *A. lineatus* and *A. litigiosus*, suggesting the response of all treatments was consistent in both wireworm species.

3.4. Rhizotron assays

All models analysing the time wireworms spent in each zone of the rhizotron revealed a significant effect of zone (Table 3). In Model A, wireworms spent significantly more time on the untreated zone compared to zones treated with either 3-octanone or EPF (ratio = 0.51, SE = 0.06, z = −6.05, p < 0.001). In Model B, pairwise comparisons showed that wireworms spent significantly more time in the untreated zone compared to the other three zones, regardless of treatment (treated vs. untreated: ratio = 0.32, SE = 0.06, z = −5.76, p < 0.001; treated low vs. untreated: ratio = 0.469, SE = 0.0813, z = −4.37, p < 0.001; untreated vs. untreated low: ratio = 1.63, SE = 0.26, z = 3.02, $p = 0.014$). Additionally, wireworms spent significantly more time in the untreated low zone compared to the treated zone (ratio = 0.52, SE = 0.11, z = −3.14, $p = 0.009$). In Model C, pairwise comparisons showed that wireworms spent significantly more time in untreated zone 1 compared to all other zones, except treated zone 2 in both 3-octanone or EPF assays (treated zone 1 vs. untreated zone 1: ratio = 0.11, SE = 0, z = −3.89, $p = 0.001$; treated zone 3 vs. untreated zone 1: ratio = 0.09, SE = 0, z = −5.65, p < 0.001; untreated zone 1 vs. untreated zone 2: ratio = 8.42, SE = 3, z = 5.52, p < 0.001; untreated zone 1 vs. untreated zone 3: ratio = 7.05, SE = 2, z = 5.56, p < 0.001) (Fig. 4). The equal distribution of wireworms when both seedlings were untreated or mock treated (negative control), demonstrated that there was no positional bias (not shown in the figure).

4. Discussion

The results of our study showed that compost tea CT16 attracted wireworms of both *A. litigiosus* and *A. lineatus*, whereas CT19 had no significant effect on their behaviour. In contrast, 3-octanone and *M. brunneum* exhibited a significant repellent effect on both *Agriotes* species in both olfactometer and rhizotron assays. These findings reveal that wireworm behaviour is highly sensitive to microbially derived volatiles and to the biochemical complexity of compost-based amendments, supporting the concept that soil chemical communication networks strongly influence pest activity and distribution.

The key difference between the CTs used in our experiments is the notably higher presence of actinomycetes in CT16 compared to CT19. Thus, the attraction to CT16 may be linked to enhanced microbial respiration, which could increase local CO_2 emissions and modify the VOC blend in the rhizosphere (Bonanomi et al., 2018). This hypothesis is consistent with evidence that wireworms are attracted to CO_2-rich microenvironments (Johnson and Nielsen, 2012). Such attraction to compost-derived volatiles may suggest that microbial activity within organic amendments indirectly influences pest location behaviour through soil-gas gradients and volatiles emissions. Compost-based material such as CT could, therefore, affect the distribution of soil herbivores beyond their nutritional and physical effects, by altering belowground semiochemical signals. To our knowledge, this is the first study to report the potential use of CTs to modify belowground plant pests' behaviour.

In nature, VOCs and CO_2 emitted by living organisms can influence the behaviour of subterranean insects, either repelling or attracting them (Nikoukar et al., 2025; Arce et al., 2021; Johnson and Nielsen, 2012). Additionally, wireworm responses to VOCs can depend on concentration or mixtures of individual VOCs. Bourdon et al. (2023) demonstrated that *A. lineatus* was attracted to a low dose of 3-octanone ($0.05\ \mu L\ g^{-1}$ of soil) but was repelled at a higher dose ($0.1\ \mu L\ g^{-1}$ of soil). The dose-dependent repellence of 3-octanone supports its potential role in push–pull strategies, as previously demonstrated in other pest systems where repellent VOCs are used to divert pests away from target crops (Khan et al., 2010; Cook et al., 2007). Repellent cues may deter larvae from colonizing newly sown crops, thereby protecting emerging seedlings at critical growth stages. This mechanism could be exploited to spatially displace wireworms toward trap crops or buffer strips, reducing

Table 1
Summary of chemical, physical, and microbiological results. Data are the average of three replicates ± standard deviation.

Sample	pH	EC (μs cm)	C/N (%ss)	Actinomycetes (CFU mL)	Yeasts (CFU mL)	Fungi (CFU mL)	Bacteria (CFU mL)	*Escherichia coli*
CT16	~ 6.71 ± 0.04	~ 7038 ± 74.00	15	20×10^6	0.01×10^6	0.04×10^6	10×10^6	Absent
CT19	~ 6.93 ± 0.06	~ 2403 ± 20.00	11.3	0.01×10^6	0.03×10^6	0.13×10^6	1.0×10^6	Absent

Table 2

Model parameters of count and response index (RI) data, with Wald Chi squared test (χ2) value, t value from One sample *t*-test, F value from linear regression, V value from Wilcoxon signed rank test, W value from Wilcoxon rank sum test, degrees of freedom (df, if applicable) and *p* values. The bold text represents the statistically significant results ($p < 0.05$).

	COUNTS					RESPONSE INDEX				
	Distribution	Fixed effect	χ2	df	p	Test	Effect	t/F/ *V*/*W*	df	p
EPF	quasi-Poisson	Treatment	32.38	1	**<0.001**	One sample t-test	Treatment	−3.93	19	**<0.001**
	quasi-Poisson	Species	20	1	**<0.001**	Linear model	Species	0.90	1	0.354
CT16	Poisson	Treatment	15.47	1	**<0.001**	Wilcoxon signed rank	Treatment	170	Na	**0.003**
	Poisson	Species	2.14	1	0.144	Wilcoxon rank sum	Species	41.5	Na	0.538
CT19	Poisson	Treatment	0.01	1	0.904	One sample t-test	Treatment	0.32	19	0.624
	Poisson	Species	6.49	1	**0.011**	Linear model	Species	0.07	1	0.8
3-octanone	Poisson	Treatment	31.14	1	**<0.001**	One sample t-test	Treatment	−6.27	29	**<0.001**
	Poisson	Species	3.80	1	0.051	Linear model	Species	0.11	1	0.741

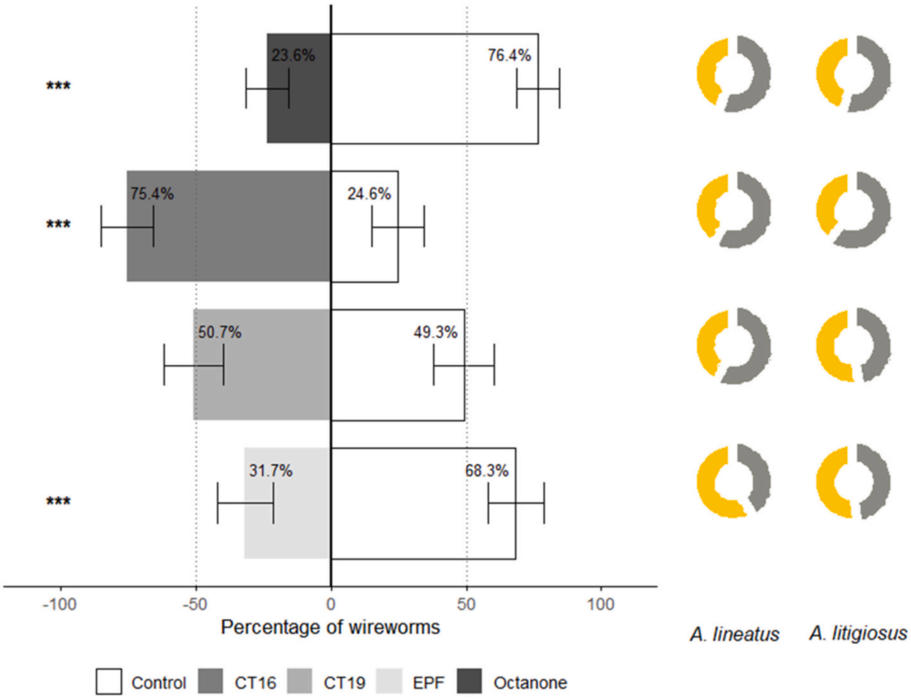

Fig. 2. Percentage (mean ± SE) of wireworms choosing each treatment compared to untreated soil (control) after 2 h in an olfactometer bioassay ($n = 20$). Asterisks (*) indicate significant differences in wireworm response (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Dashed lines indicate the 50% threshold. Donut charts show the percentage of responding (yellow) and nonresponding (grey) wireworms of each species. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

early-season root damage and contributing to integrated, ecologically based soil pest management. Similarly, Barsics et al. (2017) tested a four-aldehyde VOC blend, and it attracted *Agriotes sordidus*. Three fungal VOCs, 1-octene, 3-octanone and 1-octen-3-ol, have been shown to cause mortality in *Cornu aspersum* (O.F.Müller, 1774) and *Deroceras reticulatum* (O.F.Müller, 1774) at high doses, as well as repellent effects at low doses (Khoja et al., 2019). Our findings indicate that, even in the presence of CO_2 emitted by seedlings used in our experiments, 3-octanone at a dose of 0.1 μL g^{-1} of soil repelled both wireworm species, consistent with the repellent effect observed by Bourdon et al. (2023).

Results of gas chromatography–mass spectrometry (GC–MS) analysis of VOCs emitted by *M. brunneum* strain 1868 revealed the presence of three major compounds including Ethylenimine, 2-epi-alpha-Funebrene and [3,3′]-Paracyclophane. These VOCs may be potential candidates with repellent activity against wireworms and could play a defensive ecological role within the rhizosphere. The repellence observed might represent an adaptive mechanism whereby *M. brunneum* VOCs deter root herbivores, indirectly benefiting plants, harbouring *Metarhizium* in their rhizospheres. Such plant–fungus–insect interactions have been reported for other endophytic (Barelli et al., 2020) or rhizosphere competent (Razinger et al., 2020) EPF. In addition to ethylenimine, a compound known for its high reactivity and potential toxicity, the presence of 2-epi-alpha-Funebrene, a sesquiterpenoid structurally related to plant-derived semiochemicals (Gugliuzzo et al., 2023), suggests that it could contribute to below-ground chemical signalling affecting insect behaviour, as sesquiterpenes and other volatile organic compounds are well-established mediators of plant–insect interactions and defence responses (Ali et al., 2023; Zhou and Jander, 2022).

Similarly, although [3,3′]-Paracyclophane is primarily known from synthetic chemistry, related naturally occurring paracyclophane derivatives such as cylindrocyclophane A have demonstrated biological activity in cyanobacterial metabolites (Yamakoshi et al., 2009), indicating that this scaffold could potentially interact with biological systems. Given the detection of ethylenimine and the uncharacterized biological effects of 2-epi-alpha-Funebrene and [3,3′]-Paracyclophane, further characterization of their biological effects and assessment of their ecological safety and functional roles are required before field applications. Other belowground pest studies show that potato tubers

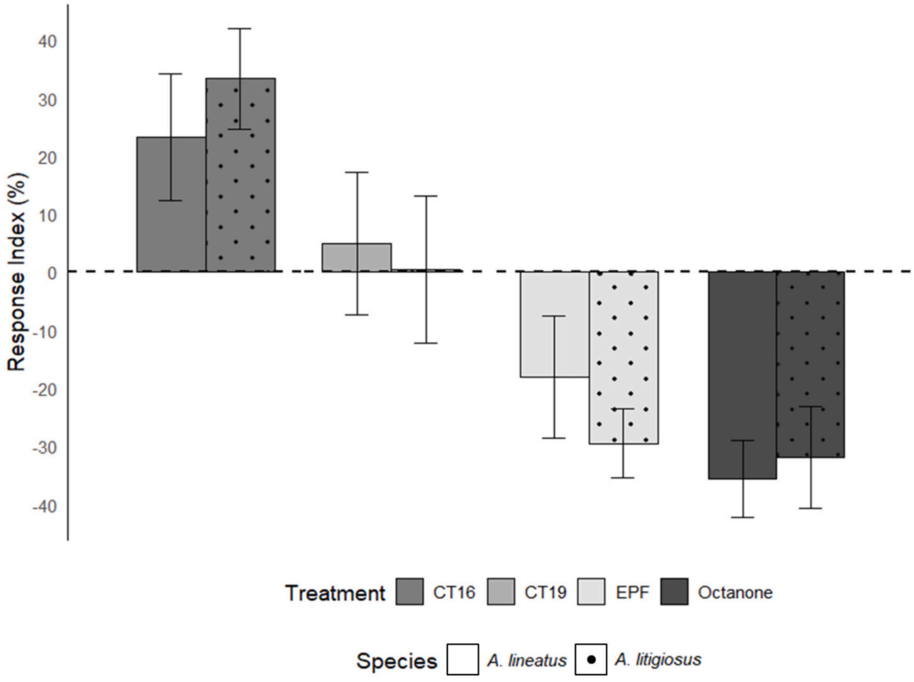

Fig. 3. Response index (mean ± SE) of *Agriotes lineatus* (blank) and *Agriotes litigiosus* (dotted) to different treatments.

Table 3
Model parameters for total time spent by wireworms in each zone of the rhizotron. The table includes model type and distribution, fixed effects, Wald Chi-squared (χ^2) or F-statistics, degrees of freedom (df) and p values. The bold text represents the statistically significant results ($p < 0.05$). *ZINB* – zero inflated negative binomial distribution.

MODEL	TOTAL TIME SPENT IN EACH ZONE				
	Distribution	Fixed effect	χ2	df	p
A	Poisson	Zone	39.40	1	**<0.001**
	Poisson	Treatment	0.00	1	1.00
	Poisson	Species	0.00	1	1.00
	Poisson	Zone*Treatment*Species	2.30	1	0.13
B	Negative binomial	Zone	36.58	3	**<0.001**
	Negative binomial	Treatment	0.00	1	0.977
	Negative binomial	Species	0.01	1	0.91
	Negative binomial	Zone*Treatment*Species	7.16	3	0.067
C	Negative binomial (ZINB)	Zone	70.11	5	**<0.001**
	Negative binomial (ZINB)	Treatment	0.01	1	0.905
	Negative binomial (ZINB)	Species	0.15	1	0.696

colonized by *M. brunneum* are protected from *Agriotes obscurus* larvae (Mayerhofer et al., 2017), while roots of *Picea abies* (L.) H.Karst. treated with *Metarhizium anisopliae* (Metchnikoff) can control nearly 80% of *Otiorhynchus sulcatus* (Fabricius, 1775) larvae (Bruck, 2005). The protective effect, together with the absence of fungal outgrowth, is hypothesized to result from the production and accumulation of fungal metabolites within plant tissues (Gurulingappa et al., 2011).

Both, two-choice olfactometer, and rhizotron assays are valuable tools for studying the behaviour of soil-dwelling insects in response to treatments such as VOCs and EPF. Our results, consistent with previous rhizotron and olfactometer studies (La Forgia et al., 2023; Brandl et al., 2017), confirm that such bioassays can effectively reveal belowground behavioural modulation caused by fungal or microbial volatiles. These experimental approaches have been widely employed to assess insect behavioural changes in soil environments. Previous studies demonstrated that wireworms avoid soil inoculated with *Beauveria bassiana* (Bals.-Criv.) Vuill. and exhibit reduced feeding activity in both olfactometer and rhizotron assays, suggesting that the fungus can influence host behaviour through mechanisms beyond direct infection (Poon, 2023). Similar behavioural alterations have been reported in other insect taxa exposed to *B. bassiana* or *Metarhizium* species, including reduced feeding and avoidance in *Rhynchophorus ferrugineus* (A.G.Olivier, 1791) (Jalinas, 2016), *Coccinella septempunctata* Linnaeus, 1758 (Ormond et al., 2011), and *Dendrolimus pini* (Linnaeus, 1767) (Kovač et al., 2020). Comparable effects were also observed by Kabaluk and Ericsson (2007), who found that wireworms were repelled by *M. anisopliae*-contaminated soil, with repellence intensity increasing alongside fungal spore concentration.

Field applications further support these behavioural observations. For instance, Razinger et al. (2020) demonstrated that maize plants inoculated with *M. brunneum* strain 1868 experienced significantly less wireworm damage at emergence, resulting in a higher proportion of undamaged plants in spring. The authors proposed that *Agriotes* herbivory reduction by *M. brunneum* 1868 could be attributed to larval repellence or other indirect mechanisms mediated by the fungus, an interpretation that aligns with and is further supported by the results of our study. However, one has to keep in mind, that the present study was conducted under controlled conditions. Thus its primary contribution lies in elucidating behaviourally mediated soil interactions, while implications for pest management should be considered prospective rather than immediately applicable.

All these findings support the hypothesis that EPF act not only as pathogens but also as chemical mediators of insect behaviour through the release of VOCs and other semiochemicals (Bourdon et al., 2023; La Forgia et al., 2023; La Forgia et al., 2020; Reddy et al., 2014). This additional role has important ecological implications, as EPF-derived volatiles may actively shape the distribution of soil herbivores and the microbial community structure surrounding plant roots. In line with this, the present study demonstrated that *M. brunneum* strain 1868 and 3-octanone exerted a significant repellent effect on both *A. litigiosus* and *A. lineatus*. From an ecological standpoint, these results suggest that EPF-

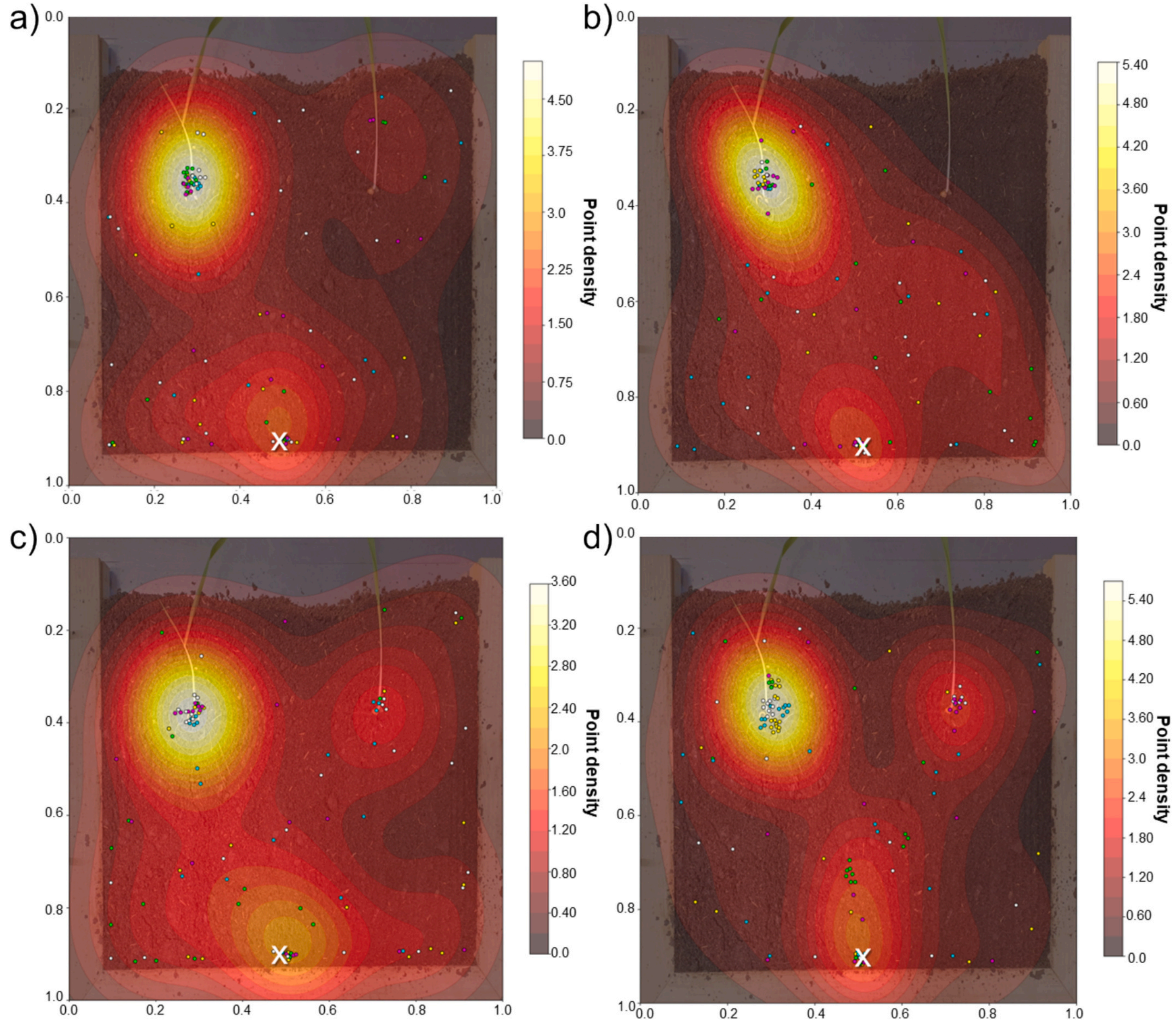

Fig. 4. Heatmaps showing the time-point density of wireworm (*Agriotes* spp.) locations during rhizotron assays, with warmer colours indicating higher locational and temporal densities. In the graphical representation above, the plant on the left side was untreated, while the plant on the right side was treated with either 3-octanone (a, b) or *Metarhizium brunneum* strain 1868 (c, d). Responses to treatments were tested on two wireworm species: *A. lineatus* (a, c) and *A. litigiosus* (b, d). Coloured dots represent individual wireworm positions across different experimental replicates. The "X" indicates the wireworm release point, where initial aggregation was expected.

driven repellence could represent an adaptive interaction where fungi and plants mutually benefit through reduced root herbivory and enhanced rhizosphere stability. This behavioural response indicates that EPF may provide a dual mechanism of pest suppression, combining pathogenic activity with repellence mediated by fungal volatiles. Rather than focusing solely on pest suppression, these findings highlight the functional role of EPF in regulating soil organism interactions and promoting ecologically based approaches to soil health and crop resilience.

Although *Metarhizium brunneum*1868 showed consistent repellent effects at the tested dose, fungal dose may influence behavioural responses (Bourdon et al., 2023; Kabaluk and Ericsson, 2007), and dose dependency therefore warrants further investigation. In addition, the use of a non-sterile commercial substrate was intended to better approximate natural soil conditions; nevertheless, future studies should evaluate how soil texture and resident microbiota could influence VOC diffusion, fungal performance, and wireworm behaviour. These factors should be systematically evaluated across different soil types and under sterile versus non-sterile conditions, particularly in relation to their intended use in plant protection or behavioural-based strategies such as push–pull strategies. Moreover, incorporating multiple wireworm cohorts and repeated assays would further improve the robustness and ecological relevance of behavioural responses. Future studies should assess potential interactions among treatments, as combined applications may result in synergistic or antagonistic effects relevant for push–pull strategies.

5. Conclusion

Taken together, our results provide a novel conceptual framework for integrating attractants and repellents into ecologically based and sustainable wireworm management. CT16 could be exploited as a trap crop stimulant or bait enhancer, whereas 3-octanone and *M. brunneum*-

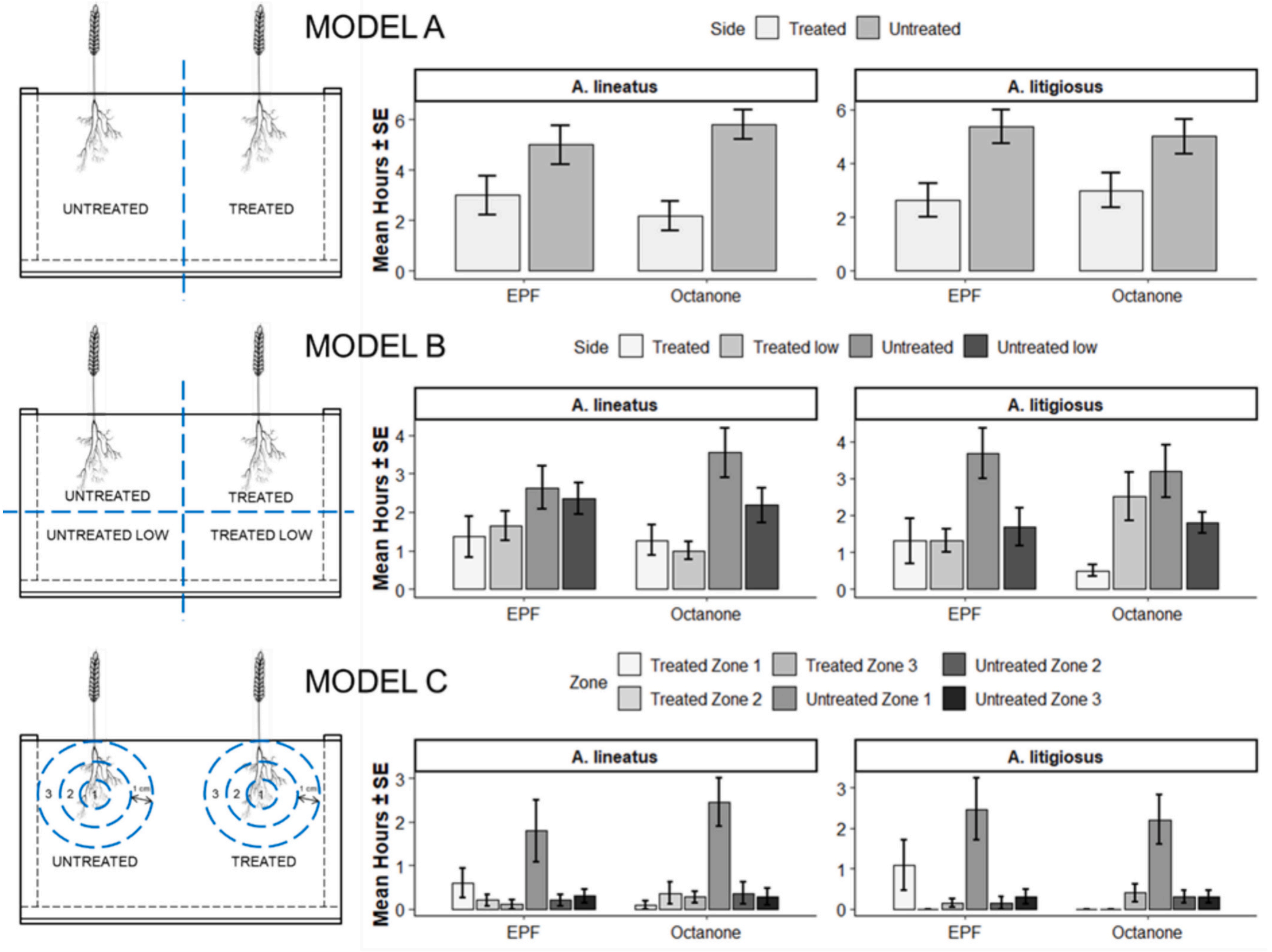

Fig. 5. Schematic representation of the three models (A–C) used to analyse the time spent by *A. lineatus* and *A. litigiosus* wireworms in different zones (left) and the time spent in each zone (right).

derived VOCs could serve as repellent barriers, together forming a push–pull system that reduces reliance on synthetic insecticides. Beyond pest control, these findings highlight the ecological role of microbial volatiles and entomopathogenic fungi as drivers of soil organism interactions and chemical communication in the rhizosphere.

Future research should also focus on field-scale validation, formulation development, and the potential use of microbial consortia that, in addition to pest deterrence, also act as plant biostimulants and modulators of soil microbiome dynamics. Moreover, future studies should include multiple *Metarhizium* strains and species, as well as other entomopathogenic fungi such as *Beauveria*, to assess whether the observed effects are strain-specific or more broadly generalizable. Extending the study to plants from other families, such as Solanaceae (e.g. potato), would further strengthen the ecological and agronomic relevance of this approach. Such strategies could contribute to organic agriculture by simultaneously improving soil health, reducing pesticide inputs, and enhancing crop resilience against subterranean pests.

CRediT authorship contribution statement

Abdalhadi M.A. Abulebda: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Data curation, Conceptualization. **Eva Praprotnik:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sara Ruschioni:** Writing – review & editing, Methodology, Funding acquisition. **Massimo Zaccardelli:** Writing – review & editing, Methodology, Investigation. **Expedito Olimi:** Writing – review & editing, Methodology, Investigation. **Gabriele Berg:** Writing – review & editing. **Paola Riolo:** Writing – review & editing, Funding acquisition. **Jaka Razinger:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

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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.

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Data availability

Data will be made available on request.

References

Ahmad, I., Jiménez-Gasco, M.D.M., Luthe, D.S., Shakeel, S.N., Barbercheck, M.E., 2020. Endophytic Metarhizium robertsii promotes maize growth, suppresses insect growth, and alters plant defense gene expression. Biol. Control 144, 104167. https://doi.org/10.1016/j.biocontrol.2019.104167.

Ali, M.Y., Naseem, T., Holopainen, J.K., Liu, T., Zhang, J., Zhang, F., 2023. Tritrophic interactions among arthropod natural enemies, herbivores and plants considering volatile blends at different scale levels. Cells 12, 251. https://doi.org/10.3390/cells12020251.

Anderson, P., Sadek, M.M., Wäckers, F.L., 2011. Root herbivory affects oviposition and feeding behavior of a foliar herbivore. Behav. Ecol. 22, 1272–1277. https://doi.org/10.1093/beheco/arr124.

Arce, C.C., Theepan, V., Schimmel, B.C., Jaffuel, G., Erb, M., Machado, R.A., 2021. Plant-associated CO2 mediates long-distance host location and foraging behaviour of a root herbivore. eLife 10, e65575. https://doi.org/10.7554/eLife.65575.

Barelli, L., Waller, A.S., Behie, S.W., Bidochka, M.J., 2020. Plant microbiome analysis after Metarhizium amendment reveals increases in abundance of plant growth-promoting organisms and maintenance of disease-suppressive soil. PLoS One 15, e0231150. https://doi.org/10.1371/journal.pone.0231150.

Barsics, F., Delory, B.M., Delaplace, P., Francis, F., Fauconnier, M.-L., Haubruge, É., Verheggen, F.J., 2017. Foraging wireworms are attracted to root-produced volatile aldehydes. J. Pest. Sci. 90, 69–76. https://doi.org/10.1007/s10340-016-0734-y.

Barsics, F., Haubruge, E., Verheggen, F., 2013. Wireworms' management: an overview of the existing methods, with particular regards to *Agriotes* spp. (Coleoptera: Elateridae). Insects 4, 117–152. https://doi.org/10.3390/insects4010117.

Barzman, M., Bàrberi, P., Birch, A.N.E., Boonekamp, P., Dachbrodt-Saaydeh, S., Graf, B., Sattin, M., 2015. Eight principles of integrated pest management. Agron. Sustain. Dev. 35 (4), 1199–1215. https://doi.org/10.1007/s13593-015-0327-9.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67. https://doi.org/10.18637/jss.v067.i01.

Bonanomi, G., Lorito, M., Vinale, F., Woo, S.L., 2018. Organic amendments, beneficial microbes, and soil microbiota: toward a unified framework for disease suppression. Annu. Rev. Phytopathol. 56 (1), 1–20. https://doi.org/10.1146/annurev-phyto-080615-100046.

Bourdon, P.-A., Zottele, M., Zafar, Z., Baxter, I., Midthassel, A., Myrta, A., Wechselberger, K.F., Strasser, H., Butt, T.M., 2023. Behavioral response of three subterranean pests (*Agriotes* lineatus, Diabrotica virgifera virgifera, Phyllopertha horticola) to the fungal volatile organic compounds 1-octen-3-ol and 3-octanone. Arthropod Plant Interact. 17, 473–483. https://doi.org/10.1007/s11829-023-09959-8.

Brandl, M.A., Schumann, M., Przyklenk, M., Patel, A., Vidal, S., 2017. Wireworm damage reduction in potatoes with an attract-and-kill strategy using Metarhizium brunneum. J. Pest. Sci. 90, 479–493. https://doi.org/10.1007/s10340-016-0824-x.

Brooks .E., M., Kristensen, K., Benthem .J.,. van, K., Magnusson, A., Berg .W., C., Nielsen, A., Skaug .J., H., Mächler, M., Bolker .M., B., 2017. GlmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. R J. 9, 378. https://doi.org/10.32614/RJ-2017-066.

Bruck, D.J., 2005. Ecology of Metarhizium anisopliae in soilless potting media and the rhizosphere: implications for pest management. Biol. Control 32, 155–163. https://doi.org/10.1016/j.biocontrol.2004.09.003.

Cernava, T., Aschenbrenner, I.A., Grube, M., Liebminger, S., Berg, G., 2015. A novel assay for the detection of bioactive volatiles evaluated by screening of lichen-associated bacteria. Front. Microbiol. 6. https://doi.org/10.3389/fmicb.2015.00398.

Civolani, S., Benvegnù, I., Accinelli, G., Bruni, M., Bernacchia, G., Parisi, B., Furlan, L., 2023. Evaluation of natural active ingredients for the protection of potato tubers from wireworm damage. Arthropod Plant Interact. 17, 455–463. https://doi.org/10.1007/s11829-023-09975-8.

Cook, S.M., Khan, Z.R., Pickett, J.A., 2007. The use of push-pull strategies in integrated pest management. Annu. Rev. Entomol. 52, 375–400. https://doi.org/10.1146/annurev.ento.52.110405.091407.

Doane, J.F., Lee, Y.W., Klingler, J., Westcott, N.D., 1975. The orientation response of ctemcera destructor and other wireworms (Coleoptera: Elateridae) to germinating grain and to carbon dioxide. Can. Entomol. 107 (12), 1233–1252. https://doi.org/10.4039/Ent1071233-12.

Doane, Klingler, 1978. Location of CO_2-receptive sensilla on larvae of the wireworms *Agriotes lineatus-obscurus* and *Limonius californicus*. Ann. Entomol. Soc. Am. 71 (3), 357–363. https://doi.org/10.1093/aesa/71.3.357.

Drahun, I., Hunt, K.A., Van Herk, W.G., Cassone, B.J., 2022. Evaluation of standardized bait trapping approaches and climatic factors that influence wireworm catch in the canadian prairies. J. Econ. Entomol. 115, 773–782. https://doi.org/10.1093/jee/toac037.

Faria, M.R.D., Wraight, S.P., 2007. Mycoinsecticides and mycoacaricides: a comprehensive list with worldwide coverage and international classification of formulation types. Biol. Control 43, 237–256. https://doi.org/10.1016/j.biocontrol.2007.08.001.

Fox, J., Weisberg, S., 2019. An R Companion to Applied Regression, Third edition. Sage publications.

Furlan, L., 2004. The biology of *Agriotes sordidus* Illiger (Col., Elateridae). J. Appl. Entomol. 128, 696–706. https://doi.org/10.1111/j.1439-0418.2004.00914.x.

Furlan, L., 2005. An IPM approach targeted against wireworms: what has been done and what has to be done. insect pathogens and insect parasitic nematodes: melolontha. IOBC/WPRS Bull 28 (2), 91–100.

Furlan, L., 2014. IPM thresholds for *Agriotes* wireworm species in maize in Southern Europe. J. Pest. Sci. 87, 609–617. https://doi.org/10.1007/s10340-014-0583-5.

Furlan, L., Benvegnù, I., Bilò, M.F., Lehmhus, J., Ruzzier, E., 2021. Species identification of wireworms (*Agriotes* spp.; Coleoptera: Elateridae) of agricultural importance in Europe: a new "Horizontal identification table.". Insects 12, 534. https://doi.org/10.3390/insects12060534.

Furlan, L., Benvegnù, I., Chiarini, F., Loddo, D., Morari, F., 2020. Meadow-ploughing timing as an integrated pest management tactic to prevent soil-pest damage to maize. Eur. J. Agron. 112, 125950. https://doi.org/10.1016/j.eja.2019.125950.

Furlan, L., Bonetto, C., Finotto, A., Lazzeri, L., Malaguti, L., Patalano, G., Parker, W., 2010. The efficacy of biofumigant meals and plants to control wireworm populations. Ind. Crop. Prod. 31, 245–254. https://doi.org/10.1016/j.indcrop.2009.10.012.

Gfeller, A., Laloux, M., Barsics, F., Kati, D.E., Haubruge, E., Du Jardin, P., Verheggen, F. J., Lognay, G., Wathelet, J.-P., Fauconnier, M.L., 2013. Characterization of volatile organic compounds emitted by Barley (*Hordeum vulgare* L.) roots and their attractiveness to wireworms. J. Chem. Ecol. 39, 1129–1139. https://doi.org/10.1007/s10886-013-0302-3.

Gibson, D.M., Donzelli, B.G.G., Krasnoff, S.B., Keyhani, N.O., 2014. Discovering the secondary metabolite potential encoded within entomopathogenic fungi. Nat. Prod. Rep. 31, 1287–1305. https://doi.org/10.1039/C4NP00054D.

González-Hernández, A.I., Pérez-Sánchez, R., Plaza, J., Morales-Corts, M.R., 2022. Compost tea as a sustainable alternative to promote plant growth and resistance against Rhizoctonia solani in potato plants. Scientia Horticulturae. Sci. Hortic. 300, 111090. https://doi.org/10.1016/j.scienta.2022.111090.

Gugliuzzo, A., Francardi, V., Simoni, S., Roversi, P.F., Ferrati, M., Spinozzi, E., Perinelli, D.R., Bonacucina, G., Maggi, F., Tortorici, S., Garzia, G.T., Biondi, A., Rizzo, R., 2023. Role of plant essential oil nanoemulsions on host colonization by the invasive ambrosia beetle Xylosandrus compactus. Ind. Crop. Prod. 195, 116437. https://doi.org/10.1016/j.indcrop.2023.116437.

Gurulingappa, P., McGee, P.A., Sword, G., 2011. Endophytic Lecanicillium lecanii and Beauveria bassiana reduce the survival and fecundity of Aphis gossypii following contact with conidia and secondary metabolites. Crop Prot. 30, 349–353. https://doi.org/10.1016/j.cropro.2010.11.017.

Hartig, F., 2024. DHARMa: residual diagnostics for hierarchical (multi-level/mixed) regression models. In: R package version 0.4.7. Available at: https://CRAN.R-project.org/package=DHARMa.

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.

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, 104527. https://doi.org/10.1016/j.biocontrol.2020.104527.

Ilangumaran, G., Smith, D.L., 2017. Plant growth promoting rhizobacteria in amelioration of salinity stress: a systems biology perspective. Front. Plant Sci. 8, 1768. https://doi.org/10.3389/fpls.2017.01768.

Isaka, M., Kittakoop, P., Thebtaranonth, Y., 2003. Secondary metabolites of Clavicipitalean fungi. In: Clavicipitalean Fungi, pp. 342–383.

Jalinas, J.B., 2016. Evaluating beauveria bassiana on red palm weevil field management and behaviour with acoustics and GIS.

Johnson, S.N., Gregory, P.J., 2006. Chemically-mediated host-plant location and selection by root-feeding insects. Physiol. Entomol. 31, 1–13. https://doi.org/10.1111/j.1365-3032.2005.00487.x.

Johnson, S.N., Hawes, C., Karley, A.J., 2009. Reappraising the role of plant nutrients as mediators of interactions between root-and foliar-feeding insects. Funct. Ecol. 23 (4), 699–706. https://doi.org/10.1111/j.1365-2435.2009.01550.x.

Johnson, S.N., Nielsen, U.N., 2012. Foraging in the dark – chemically mediated host plant location by belowground insect herbivores. J. Chem. Ecol. 38, 604–614. https://doi.org/10.1007/s10886-012-0106-x.

Kabaluk, J.T., Ericsson, J.D., 2007. Environmental and behavioral constraints on the infection of wireworms by Metarhizium anisopliae. Environ. Entomol. 36, 1415–1420. https://doi.org/10.1093/ee/36.6.1415.

Kabaluk, T., Goettel, M., Erlandson, M., Ericsson, J., Duke, G., Vernon, B., 2005. Metarhizium anisopliae as a biological control for wireworms and a report of some other naturally occurring parasites. *IOBC*/wprs Bull 28 (2), 109–115.

Kassambara, A., 2023. rstatix: pipe-friendly framework for basic statistical tests. contributed Packages, CRAN.

Khan, Z.R., Midega, C.A.O., Bruce, T.J.A., Hooper, A.M., Pickett, J.A., 2010. Exploiting phytochemicals for developing a "push-pull" crop protection strategy for cereal farmers in Africa. J. Exp. Bot. 61, 4185–4196. https://doi.org/10.1093/jxb/erq229.

Khoja, S., Eltayef, K.M., Baxter, I., Bull, J.C., Loveridge, E.J., Butt, T., 2019. Fungal volatile organic compounds show promise as potent molluscicides. Pest Manag. Sci. 75, 3392–3404. https://doi.org/10.1002/ps.5578.

Klingler, J., 1957. Über die Bedeutung des Kohlendioxyds für die Orientierung der Larven von Otiorrhynchus sulcatus F., Melolontha und *Agriotes* (Col.) im Boden: (vorläufige Mitteilung). Imprimerie la Concorde.

Kölliker, U., Jossi, W., Kuske, S., 2009. Optimised protocol for wireworm rearing. IOBC/wprs Bull 45, 457–460.

Kostenko, O., Mulder, P.P.J., Bezemer, T.M., 2013. Effects of root herbivory on pyrrolizidine alkaloid content and aboveground plant-herbivore-parasitoid interactions in Jacobaea Vulgaris. J. Chem. Ecol. 39, 109–119. https://doi.org/10.1007/s10886-012-0234-3.

Kovač, M., Lacković, N., Pernek, M., 2020. Effect of *Beauveria bassiana* fungal infection on survival and feeding behavior of pine-tree lappet moth (*Dendrolimus pini* L.). Forests 11, 974. https://doi.org/10.3390/f11090974.

La Forgia, D., Martin, C., Turlings, T.C.J., Verheggen, F., 2023. 2-Pentylfuran: an aggregation attractant for wireworms. Arthropod Plant Interact. 17, 465–472. https://doi.org/10.1007/s11829-023-09958-9.

La Forgia, D., Thibord, J.-B., Larroudé, P., Francis, F., Lognay, G., Verheggen, F., 2020. Linking variety-dependent root volatile organic compounds in maize with differential infestation by wireworms. J. Pest. Sci. 93, 605–614. https://doi.org/10.1007/s10340-019-01190w.

La Forgia, D., Verheggen, F., 2019. Biological alternatives to pesticides to control wireworms (Coleoptera: Elateridae). Agri Gene 11, 100080. https://doi.org/10.1016/j.aggene.2018.100080.

Lapajne, J., 2025. Rhizotron heatmap generation. In: GitHub repository. Available at: https://github.com/Agricultural-institute/Rhizotron-heatmap-generation.

Lee, C.-H., Lee, Y.-Y., Chang, Y.-C., Pon, W.-L., Lee, S.-P., Wali, N., Nakazawa, T., Honda, Y., Shie, J.-J., Hsueh, Y.P., 2023. A carnivorous mushroom paralyzes and kills nematodes via a volatile ketone. Sci. Adv. 9, eade4809. https://doi.org/10.1126/sciadv.ade4809.

Lenth, R., 2025. emmeans: estimated marginal means, aka least-squares means_. R package version 1 (8), 5.

Mahnert, A., Haratani, M., Schmuck, M., Berg, G., 2018. Enriching beneficial microbial diversity of indoor plants and their surrounding built environment with biostimulants. Front. Microbiol. 9, 2985. https://doi.org/10.3389/fmicb.2018.02985.

Mayerhofer, J., Eckard, S., Hartmann, M., Grabenweger, G., Widmer, F., Leuchtmann, A., Enkerli, J., 2017. Assessing effects of the entomopathogenic fungus Metarhizium brunneum on soil microbial communities in *Agriotes* spp. biological pest control. FEMS Microbiol. Ecol. 93. https://doi.org/10.1093/femsec/fix117.

Nikoukar, A., Sadeghi, R., Eigenbrode, S.D., Price, W., Rashed, A., 2025. Wireworms (Coleoptera: Elateridae) use root volatiles and CO2 to discriminate among host plants. Sci. Rep. 15, 10227. https://doi.org/10.1038/s41598-025-93339-0.

Oller-López, J.L., Iranzo, M., Mormeneo, S., Oliver, E., Cuerva, J.M., Oltra, J.E., 2005. Bassianolone: an antimicrobial precursor of cephalosporolides E and F from the entomoparasitic fungus Beauveria bassiana. Org. Biomol. Chem. 3 (7), 1172–1173. https://doi.org/10.1039/B417534D.

Ormond, E.L., Thomas, A.P.M., Pell, J.K., Freeman, S.N., Roy, H.E., 2011. Avoidance of a generalist entomopathogenic fungus by the ladybird, Coccinella septempunctata: predator avoidance of an entomopathogen. FEMS Microbiol. Ecol. 77, 229–237. https://doi.org/10.1111/j.1574-6941.2011.01100.x.

Pisa, L., Goulson, D., Yang, E.C., Gibbons, D., Sánchez-Bayo, F., Mitchell, E., Bonmatin, J. M., 2021. An update of the Worldwide Integrated Assessment (WIA) on systemic insecticides. Part 2: impacts on organisms and ecosystems. Environmental Science and Pollution Research 28 (10), 11749–11797. https://doi.org/10.1007/s11356-017-0341-3.

Platia, G., 1994. Coleoptera: Elateridae. Calderini, Bologna.

Poggi, S., Le Cointe, R., Lehmhus, J., Plantegenest, M., Furlan, L., 2021. Alternative strategies for controlling wireworms in field crops: a rev. Agric 11, 436. https://doi.org/10.3390/agriculture11050436.

Poon, A., 2023. Exploration of factors influencing crop protection against wireworm herbivory (Coleoptera: Elateridae) using Beauveria bassiana.

Razinger, J., Praprotnik, E., Schroers, H.J., 2020. Bioaugmentation of entomopathogenic fungi for sustainable *Agriotes* larvae (Wireworms) management in maize. Front. Plant Sci. 11, 535005. https://doi.org/10.3389/fpls.2020.535005.

Razinger, J., Schroers, H.J., Urek, G., 2018. Virulence of *Metarhizium brunneum* to field collected *Agriotes* spp. wireworms. J. Agric. Sci. Technol. 20 (2), 309–320.

Reddy, G.V.P., Tangtrakulwanich, K., Wu, S., Miller, J.H., Ophus, V.L., Prewett, J., Jaronski, S.T., 2014. Evaluation of the effectiveness of entomopathogens for the management of wireworms (Coleoptera: Elateridae) on spring wheat. J. Invertebr. Pathol. 120, 43–49. https://doi.org/10.1016/j.jip.2014.05.005.

Reinbacher, L., Bacher, S., Knecht, F., Schweizer, C., Sostizzo, T., Grabenweger, G., 2021. Preventive field application of *Metarhizium brunneum* in cover crops for wireworm control. Crop Prot. 150, 105811. https://doi.org/10.1016/j.cropro.2021.105811.

Rivas-Franco, F., Hampton, J.G., Morán-Diez, M.E., Narciso, J., Rostás, M., Wessman, P., Jackson, T.A., Glare, T.R., 2019. Effect of coating maize seed with entomopathogenic fungi on plant growth and resistance against Fusarium graminearum and Costelytra giveni. Biocontrol Sci. Tech. 29, 877–900. https://doi.org/10.1080/09583157.2019.1611736.

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.

Saguez, J., Latraverse, A., De Almeida, J., Van Herk, W.G., Vernon, R.S., Légaré, J.P., Moisan-De Serres, J., Fréchette, M., Labrie, G., 2017. Wireworm in Quebec field crops: specific community composition in North America. Environ. Entomol. 46, 814–825. https://doi.org/10.1093/ee/nvx116.

Sonnemann, I., Grunz, S., Wurst, S., 2014. Horizontal migration of click beetle (*Agriotes* spp.) larvae depends on food availability. Entomol. Exp. Appl. 150, 174–178. https://doi.org/10.1111/eea.12150.

Sufyan, M., Neuhoff, D., Furlan, L., 2013. Effect of male mass trapping of *Agriotes* species on wireworm abundance and potato tuber damage. Bull. Insectol. 66 (1), 135–142.

Sufyan, M., Neuhoff, D., Furlan, L., 2014. Larval development of Agriotes obscurus under laboratory and semi-natural conditions. Bull. Insectol. 67 (2), 227–235.

Team, R. C, 2023. In: R Foundation for Statistical Computing (Ed.), R: A Language and Environment for Statistical Computing. Vienna, Austria. https://www.R-project.org/.

Usseglio, V.L., Pizzolitto, R.P., Rodriguez, C., Zunino, M.P., Zygadlo, J.A., Areco, V.A., Dambolena, J.S., 2017. Volatile organic compounds from the interaction between Fusarium verticillioides and maize kernels as a natural repellents of Sitophilus zeamais. J. Stored Prod. Res. 73, 109–114. https://doi.org/10.1016/j.jspr.2017.08.001.

Veres, A., Wyckhuys, K.A.G., Kiss, J., Tóth, F., Burgio, G., Pons, X., Avilla, C., Vidal, S., Razinger, J., Bazok, R., Matyjaszczyk, E., Milosavljević, I., Le, X.V., Zhou, W., Zhu, Z.-R., Tarno, H., Hadi, B., Lundgren, J., Bonmatin, J.-M., Van Lexmond, M.B., Aebi, A., Rauf, A., Furlan, L., 2020. An update of the worldwide integrated assessment (WIA) on systemic pesticides. Part 4: alternatives in major cropping systems. Environ. Sci. Pollut. Res. 27, 29867–29899. https://doi.org/10.1007/s11356-020-09279-x.

Verginer, M., Leitner, E., Berg, G., 2010. Production of volatile metabolites by grape-associated microorganisms. J. Agric. Food Chem. 58, 8344–8350. https://doi.org/10.1021/jf100393w.

Vernon, van Herk, 2022. Wireworms as pests of potato. In: Insect pests of potato. Academic Press, pp. 103–148.

Willis, R.B., Abney, M.R., Holmes, G.J., Schultheis, J.R., Kennedy, G.G., 2010. Influence of preceding crop on wireworm (Coleoptera: Elateridae) abundance in the coastal plain of North Carolina. J. Econ. Entomol. 103, 2087–2093. https://doi.org/10.1603/EC10184.

Wood, M.J., Kortsinoglou, A.M., Bull, J.C., Eastwood, D.C., Kouvelis, V.N., Bourdon, P. A., Loveridge, E.J., Mathias, S., Meyrick, A., Midthassel, A., Myrta, A., Butt, T., 2023. Evaluation of metarhizium brunneum- and metarhizium-derived VOCs as dual-active biostimulants and pest repellents in a wireworm-infested potato field. J. Fungi 9, 599. https://doi.org/10.3390/jof9060599.

Yamakoshi, H., Ikarashi, F., Minami, M., Shibuya, M., Sugahara, T., Kanoh, N., Ohori, H., Shibata, H., Iwabuchi, Y., 2009. Syntheses of naturally occurring cytotoxic [7.7] paracyclophanes, (−)-cylindrocyclophane A and its enantiomer, and implications for biological activity. Org. Biomol. Chem. 7, 3772. https://doi.org/10.1039/b909646a.

Zaccardelli, M., Pane, C., Scotti, R., Palese, A.M., Celano, G., 2012. Impiego di compost-tea come bioagrofarmaci e biostimolanti in ortofrutticoltura. Italus Hort. 19, 17–28.

Zhou, S., Jander, G., 2022. Molecular ecology of plant volatiles in interactions with insect herbivores. J. Exp. Bot. 73, 449–462. https://doi.org/10.1093/jxb/erab413.