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RESEARCH

Field–scale evaluation of irrigation–applied entomopathogens against the truffle pest *Leiodes cinnamomea* in commercial black truffle orchards

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Abstract *Leiodes cinnamomea* is a major pest of black truffle (*Tuber melanosporum*) orchards, where management is constrained by the need to preserve soil biota and ectomycorrhizal symbioses. Field-scale evaluation is complicated by spatial heterogeneity, adult mobility, and reliance on indirect indicators. We evaluated irrigation-applied entomopathogenic agents against *L. cinnamomea* in a commercial orchard, using trap captures as an activity proxy. Field trials were conducted over two seasons in a 4.5-ha orchard with nine irrigation sectors. *Beauveria bassiana* (strain GHA) and *Steinernema carpocapsae* were applied through drip irrigation, with weekly trap monitoring. Laboratory bioassays assessed adult susceptibility to *B. bassiana* at field-relevant temperatures (12°C ± 2 °C). Delivery and viability were verified at dripper outlets. Treatment effects were evaluated using before-after-control-impact analysis and complementary approaches focused on the autumn emergence peak. Adult activity showed pronounced spatial structure, with captures differing by up to an order of magnitude among sectors. Sectors treated with *B. bassiana* consistently exhibited 25%–26% lower captures during peak emergence compared to controls in both seasons, although permutation-based tests provided limited statistical support due to spatial heterogeneity and restricted replication. *S. carpocapsae* showed only a weaker first-season reduction in adult captures and was not evaluated in the second season, whereas halloysite supplementation did not improve the capture response despite higher conidial concentrations at dripper outlets. Irrigation-applied *B. bassiana* was associated with reproducibly lower adult captures during the peak-emergence window, supported by laboratory evidence of susceptibility. While capture responses were moderate and confined to peak emergence, results highlight both the potential and the limitations of soil-applied entomopathogenic fungi as timing-dependent candidates for integrated pest management in truffle orchards.

Keywords Irrigation–applied biocontrol · Soil–insect interactions · Seasonal pest dynamics · Spatial heterogeneity · Drip irrigation delivery

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Introduction

Tuber melanosporum Vittad. is a hypogeous ectomycorrhizal fungus that forms symbiotic associations primarily with oak species (*Quercus* spp.), whose underground fruiting bodies, hereafter referred to as truffles, are highly valued in international markets. As a result, black truffle cultivation has become a strategically important agroforestry activity in several temperate regions (Rubini et al., 2012). Over recent decades, increasing demand and advances in cultivation techniques have promoted the expansion of truffle orchards beyond the species' native range (Coleman et al., 2025). In major producing countries, such as France, Italy, and Spain, most black truffle production now originates from cultivated orchards rather than natural truffle grounds (Oliach et al., 2020; Reyna & Garcia-Barreda, 2014). In contrast to natural truffle ecosystems, production in cultivated orchards is highly dependent on irrigation, which has become essential to stabilize yields under increasing climatic variability (Büntgen et al., 2015; Magarzo et al., 2023; Thomas & Büntgen, 2019).

However, the adoption of irrigated monoculture systems has also altered soil conditions and ecological balances, favoring the emergence of pest species that can compromise truffle quality (Navarro-Llopis et al., 2021). Among these, the mycophagous beetle *Leiodes cinnamomea* (Panzer, 1793) (Coleoptera: Leiodidae) represents the most economically important insect pest of black truffle orchards. Larvae feed directly on truffle tissues and adults use truffles as shelter, bore galleries into fruiting bodies hence reducing quality and market value. In the absence of management measures, yield losses in commercial orchards can reach up to 70% (Julià et al., 2023). *Leiodes cinnamomea* exhibits a univoltine life cycle, with successive cohorts of adults emerging after summer diapause and remaining active from late autumn through early spring, closely synchronized with truffle phenology (Navarro-Llopis et al., 2021). The species is strongly photophobic and spends most of its life cycle underground, which complicates detection and management (Kilian et al., 2022).

Management of *L. cinnamomea* in truffle orchards is constrained by both biological and operational factors. Conventional chemical pesticides are generally discouraged because of their potential effects on ectomycorrhizal symbiosis and soil microbial communities, and because many truffle orchards are managed under organic or low-input schemes (Reyna & Garcia-Barreda, 2014; Varma & Kharkwal, 2009). Mass trapping using food attractants can reduce truffle damage, but only at very high trap densities, which substantially increases labor and operational costs (Navarro-Llopis et al., 2021). Moreover, adult activity is spatially heterogeneous and strongly concentrated during a short autumn emergence period, meaning that trap captures provide a useful but indirect proxy of relative adult activity rather than a direct estimate of population density or crop damage (Araujo et al., 2025; Navarro-Llopis et al., 2021). These constraints highlight the need for complementary management tools that can be deployed during biologically relevant activity windows without disturbing the truffle-producing soil environment.

Within this complex ecological and methodological context, biological control agents represent a potentially valuable complementary tool for the management of *L. cinnamomea*, particularly in high–value production systems where chemical control options are limited. Entomopathogenic nematodes (EPNs) have shown high virulence against different life stages of the beetle under laboratory conditions, especially at low temperatures consistent with autumn and winter activity (Julià et al., 2023). Entomopathogenic fungi (EF), particularly *Beauveria bassiana* (Bals. –Criv.) Vuill., have demonstrated broad efficacy against coleopteran pests and can remain infective under cool soil conditions depending on strain characteristics (Fernandes et al., 2008; Lacey et al., 2015; Quesada-Moraga et al., 2024). However, the translation of laboratory susceptibility into consistent field performance remains uncertain, especially in perennial cropping systems characterized by strong spatial heterogeneity and high pest mobility. Moreover, in irrigated truffle orchards, the efficacy of these agents is further constrained by the delivery system itself.

Drip irrigation—the primary delivery pathway for applying entomopathogens without soil disturbance—concentrates water and inputs within narrow wetted strips, generating heterogeneous distributions of moisture and applied propagules in soil (Clothier & Green, 1997; Keller & Bliesner, 1990). Soil texture and hydraulic properties influence water movement and particle retention around

emitters, potentially limiting uniform exposure of target organisms (Baveye et al., 2018; Bradford et al., 2003). Consequently, field performance may depend as much on the spatial overlap between pest activity and propagule deposition as on intrinsic pathogen traits such as virulence, persistence, and environmental tolerance (Jaronski, 2010; Wraight et al., 2001).

Field-scale evaluations under commercial conditions, even when effects are moderate or temporally restricted, are essential to clarify the realistic roles and limitations of biological control agents in perennial cropping systems with strong spatial heterogeneity and high pest mobility. In this study, we combined laboratory bioassays and field trials conducted over two consecutive seasons to evaluate the performance of irrigation–applied entomopathogenic agents against *L. cinnamomea* in a commercial black truffle orchard. Specifically, we aimed to (i) characterize the seasonal activity pattern of adult *L. cinnamomea*; (ii) assess the susceptibility of adults to *B. bassiana* (strain GHA) under field–relevant temperature conditions; and (iii) assess whether irrigation–applied *B. bassiana* and the commercial EPN *Steinernema carpocapsae* (Weiser, 1955) Wouts, Mráček, Gerdin & Bedding, 1982 are associated with consistent directional changes in adult trap captures during biologically relevant activity windows, while explicitly accounting for strong spatial heterogeneity within the orchard.

Materials and methods

Insects

Adult *L. cinnamomea* used in laboratory bioassays were collected from food–attractant traps deployed in an untreated area of the study orchard (reference plot, SR) during 21–27 October 2024.

After collection, adults were placed in plastic zip-lock bags and kept refrigerated (4°C–6 °C) for a maximum of 48 h before the assays. No food was provided during this holding period. Because *L. cinnamomea* is a subterranean, truffle-associated beetle with a complex life cycle tightly linked to host availability and soil conditions, no standardized laboratory rearing protocol is currently available to obtain adults of known age under controlled conditions. Consequently, bioassays were conducted using field-collected adults. To reduce variability, insects were collected within a narrow temporal window, handled under identical conditions, and only active, undamaged individuals were selected. Adult sex and age were not determined. In each independent pathogenicity bioassay, 40 adults were included (20 per treatment). Two independent assays were conducted under identical conditions (see Online Resource 1).

Entomopathogenic agents

Two entomopathogenic agents were evaluated: the entomopathogenic fungus *B. bassiana* and the EPN *S. carpocapsae*.

Beauveria bassiana

The fungal agent was a commercial formulation of *B. bassiana* (Botanigard®; strain GHA; Certis Belchim, Spain) with a manufacturer–declared concentration of 2.11×10^{10} conidia mL^{-1}. Strain GHA was selected due to its documented capacity to germinate and remain infective under cool conditions representative of autumn–winter truffle orchards (Fernandes et al., 2008).

For laboratory bioassays, *B. bassiana* was cultured on potato dextrose agar (PDA) plates and incubated for 14 days at room temperature until abundant sporulation developed. These cultures were used for direct–contact exposure of adult beetles (see below).

For field applications, the commercial formulation was applied via the drip irrigation system at a dose of 0.5 L per irrigation sector (≈0.5 ha) per application event, equivalent to 1.0 L ha^{-1} per event.

Steinernema carpocapsae

The EPN used in the 2024–2025 field season was a commercial formulation of *S. carpocapsae* (NEMATOcontrol-C; Agrobío S.L., Spain). The product is marketed as infective juveniles of *S. carpocapsae*, although the strain identity is not disclosed by the manufacturer. No laboratory pathogenicity bioassay with S. carpocapsae was conducted in the present study. This decision was based on previous

laboratory evidence showing that *L. cinnamomea* adults and larvae are susceptible to EPNs, including *S. carpocapsae*, under temperature-dependent conditions relevant to truffle orchards (Julià et al., 2023). Therefore, in the present study *S. carpocapsae* was evaluated only under field conditions, with the aim of assessing its performance when applied through drip irrigation at commercial orchard scale. Before application, nematodes were suspended in irrigation water and activated for approximately 30 min. During preparation, infective juveniles were visually observed to be active in the inoculation suspension. Delivery and post-injection viability were verified at dripper outlets by examining collected irrigation-water samples under a laboratory stereomicroscope, confirming the presence of motile infective juveniles. A total of 1×10^8 infective juveniles were applied per sector (≈0.5 ha) per application event, corresponding to 2×10^8 infective juveniles ha^{-1} per event. *S. carpocapsae* was included only during the 2024–2025 season; the rationale for the second-year treatment structure is described in the field experimental layout.

Laboratory bioassays

Pathogenicity bioassay under field–relevant temperature (12 ± 2 °C)

Virulence of *B. bassiana* (strain GHA) against adult *L. cinnamomea* was assessed in two independent laboratory bioassays using a soil–based assay adapted from Julià et al. (2023). Bioassays were conducted in 5.5 cm diameter Petri dishes containing 15 g of soil collected from a truffle plantation (5–20 cm depth), autoclaved (121 °C, 20 min), and moistened with sterile water to 10% (*w/w*). One adult beetle was placed per dish.

For the fungal treatment, each adult was exposed by direct contact to a sporulating *B. bassiana* culture (14–day PDA plate) for 10 min, allowing free movement on the plate surface, with three manual shakings of 5 s to standardize contact. Control insects were handled identically but exposed to non–inoculated PDA plates.

After exposure, insects were transferred to soil–filled Petri dishes and placed inside a sealed plastic container covered with transparent film perforated with five small holes to allow gas exchange. High relative humidity (approx.. 75%) was maintained using an open vessel containing 100 mL of saturated NaCl solution within the container (Hong et al., 2005). Containers were kept in a temperature–controlled chamber at 12°C ± 2 °C for the 7–day observation period. Mortality was recorded every 24 h. Insects surviving to day 7 were treated as right–censored observations. No food was provided during the assay.

Auxiliary estimation of conidial load acquired during direct–contact exposure

To provide an approximate estimate of the exposure intensity under the direct-contact protocol, three additional adults were exposed to sporulating cultures as described above. This measurement was intended as an auxiliary methodological check to document the order of magnitude of conidial acquisition, rather than as a replicated dose–response or inferential experiment. Immediately after exposure, each insect was transferred to a sterile 1.5 mL microcentrifuge tube containing 1 mL of 0.01% (v/v) Tween 80. Tubes were manually shaken twice for 10 s and vortexed for 10 s to detach cuticular conidia. Conidial concentration was quantified using a Neubauer hemocytometer by counting five central squares. Two independent counts were performed per insect and averaged. Conidial load was estimated as conidial concentration multiplied by wash volume (1 mL) and expressed descriptively as mean ± SD (n = 3 insects).

Survival analysis

Time–to–death data were analyzed using Kaplan–Meier survival analysis. Median lethal time (TL_{50}) and 95% confidence intervals (CI) were estimated non–parametrically. Confidence intervals were calculated using Greenwood's method and the Brookmeyer–Crowley estimator. Survival distributions were compared using the log–rank (Mantel–Cox) test. All analyses were conducted in Python 3.11 using the lifelines package (v0.28; Davidson-Pilon & contributors, 2023).

Field study

Study site and experimental layout

Field trials were conducted in a commercial black truffle orchard located in northern Spain, in Burgos province (approximately 41.811° N, 4.066° W; ~ 920 m a.s.l.). The orchard consisted of a 12–year–old *Quercus ilex* L. plantation producing *T. melanosporum* and managed under organic farming practices, with no chemical fertilizers or pesticides applied during the study period.

The orchard covered 4.5 ha and was subdivided into nine irrigation sectors (ST1–ST9), each approximately 0.5 ha (Fig. 1). Sectors were contiguous and arranged according to the drip irrigation layout, with no buffer zones between adjacent sectors. Mean truffle yield prior to the study was approximately 45 kg ha^{-1} $year^{-1}$, although spatial variation within the orchard was not formally quantified. The soil has a sandy clay loam texture (silt: 15.7%, clay: 33.4%, sand: 50.8%).

Treatments were assigned at the sector level. During the 2024–2025 season, sectors ST1, ST4, and ST7 received *B. bassiana*; sectors ST2, ST5, and ST8 received *S. carpocapsae*; and sectors ST3, ST6, and ST9 served as untreated controls.

During the 2025–2026 season, *B. bassiana* was again applied to ST1, ST4, and ST7, while sectors ST2, ST5, and ST8 received *B. bassiana* supplemented with white halloysite, and ST3, ST6, and ST9 remained untreated controls. *Steinernema carpocapsae* was not repeated in the second season because it showed a weaker adult-capture response than *B. bassiana* during the first season, and the second-year trial was therefore designed to further evaluate the fungal treatment and test whether halloysite amendment could improve fungal delivery/performance, while maintaining three treatment groups within the fixed irrigation-sector layout.

Treatments were not re-randomized between seasons because the second-year design aimed to assess whether the directional response observed for *B. bassiana* in the first season was reproducible when the fungal treatment was reapplied under the same spatial context. Maintaining *B. bassiana* and control sectors in fixed positions allowed interannual patterns to be evaluated against a consistent orchard-scale spatial background. This decision was also constrained by the fixed irrigation-sector layout of the commercial orchard and by the need to apply treatments through sector-level irrigation infrastructure. However, this design cannot fully separate treatment effects from persistent sector-level location effects. Therefore, field responses were interpreted conservatively, emphasizing within-sector temporal contrasts, spatially paired comparisons, and consistency of effect direction and magnitude rather than definitive evidence of efficacy.

Monitoring was conducted weekly from 23 September 2024 to 9 February 2025 and from 22 September 2025 to 19 January 2026. No soil disturbance or tillage occurred during monitoring periods beyond routine irrigation management.

An adjacent untreated orchard area (SR plot), located close to the experimental orchard sectors and managed under similar agronomic conditions, was monitored in parallel (Online Resource 2). The SR

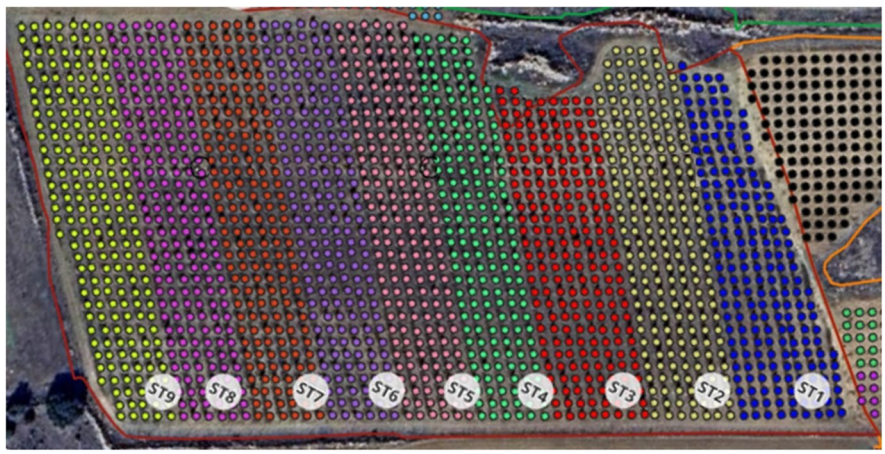

Fig. 1 Irrigation sectors (ST1–ST9) of the experimental orchard

plot was used to characterize background adult activity and seasonal phenology and served as the source of insects for laboratory bioassays.

Environmental conditions

Air temperature and soil temperature at 10, 20, and 30 cm depth were recorded in the center of the orchard at each application event (Table 1). Soil water content at 10 cm depth was monitored throughout the study period. Atmospheric conditions on application days were characterized by using the daily clearness index (Kt) as a proxy for cloudiness (Online Resources 3 and 4). Detailed environmental data are provided in Online Resources 5 and 6.

Trap deployment and monitoring

Adult *L. cinnamomea* activity was monitored using commercial food–attractant traps (Leiodelt®; Prodobelt S.L., Spain). In the experimental orchard, four traps were installed per irrigation sector (36 traps total), placed along the central row of each sector, aligned with irrigation lines, and spaced approximately 5–7 trees apart (Fig. 2). Traps were installed at ground level in contact with the soil surface.

Traps were inspected weekly on a fixed day (Fridays). Captured insects were counted and removed at each inspection. Capture data were aggregated at the sector level. In the SR plot, nine traps were deployed across approximately 2 ha using the same trap type, attractant, and inspection schedule (Fig. 3). The field endpoint was adult trap capture, used as an operational proxy of relative adult activity. Direct assessment of truffle damage, larval infestation, or yield protection was not included in the field protocol.

Field applications and delivery verification

Irrigation system and treated area Field applications were delivered through a drip irrigation system targeting the productive wetted strip where truffles typically develop. Each tree row was supplied by five parallel drip lines spaced 0.5 m apart, providing homogeneous wetting over a 2.5 m–wide strip (≈ 1.25 m on each side of the trunk). Inline emitters (1 $L{\cdot}h^{-1}$) were spaced 0.33 m apart and operated at 3.5

Table 1 Air and soil temperatures (in °C) at 10, 20, and 30 cm depth recorded at each application event

	08/10/2024	04/11/2024	03/10/2025	20/10/2025
Air temperature	14.0	11.0	14.3	13.3
10 cm soil temperature	12.4	9.4	13.6	12.8
20 cm soil temperature	13.3	10.4	15.6	14.8
30 cm soil temperature	14.2	11.3	16.3	15.0

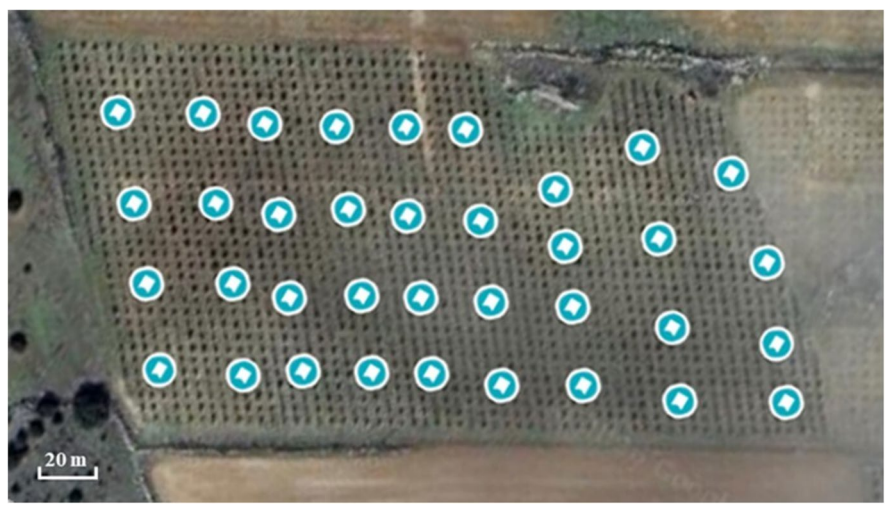

Fig. 2 Spatial distribution of food–attractant traps within the irrigation sectors of the experimental truffle orchard where entomopathogenic treatments were applied. Blue symbols indicate trap locations, arranged along the central tree row of each irrigation sector. Traps were deployed uniformly across sectors to monitor adult *Leiodes cinnamomea* activity during the study period

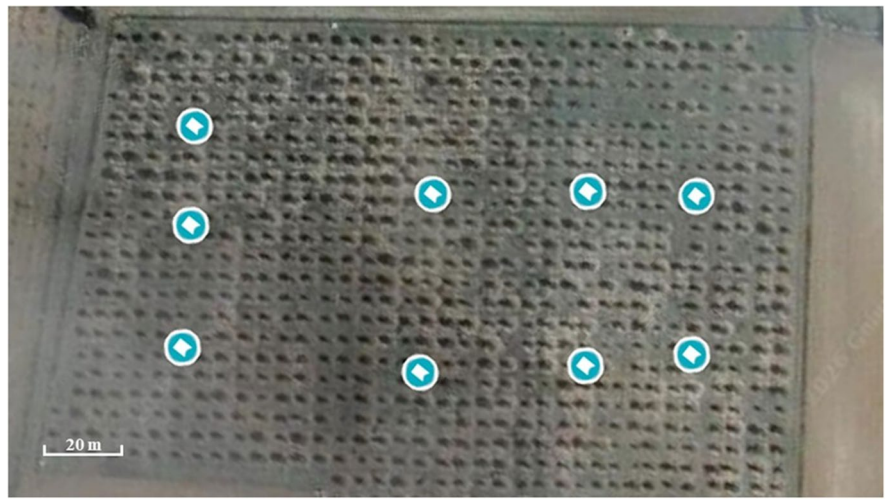

Fig. 3 Spatial distribution of food–attractant traps within the reference (SR) truffle orchard used to characterize the seasonal activity of *L. cinnamomea*. Blue symbols indicate the position of food–attractant traps deployed across the plot

bar. Irrigation intensity within the wetted strip was 5.3 $L \cdot m^{-2} \cdot h^{-1}$. All dose metrics reported below refer exclusively to this wetted productive strip (not to the total sector surface).

Preparation and injection of entomopathogens Two application events were performed per season (8 Oct and 4 Nov 2024; 3 Oct and 20 Oct 2025). Prior to each application, irrigation filters were removed, and products were injected into the main line supplying each sector using a piston–based fertigation injector. For *B. bassiana*, 0.5 L of a commercial formulation was mixed into a 100 L inoculation tank. The tank was maintained under continuous aeration to ensure homogeneous suspension. The inoculum was injected for 30 min after pre–wetting the soil, followed by standard post–application irrigation (~2 h) without product. During 2025–2026, sectors assigned to the amended formulation received *B. bassiana* supplemented with white halloysite (Itaprochim S.r.l., Italy) (external diameter 50–200 nm; tube length 0.1–3.0 µm) (30 g $sector^{-1}$ per event); the halloysite suspension was sonicated for 10 min prior to addition and maintained in suspension by continuous aeration. The suspension was subsequently passed through sterile gauze to remove large aggregates prior to injection, minimizing the risk of emitter obstruction. Halloysite was included as an exploratory inert mineral carrier to test whether a low-dose clay amendment could modify conidial suspension and deposition during drip irrigation delivery, while minimizing the risk of emitter obstruction.

For *S. carpocapsae* (2024–2025 only), 1×10^8 infective juveniles (IJs) $sector^{-1}$ per event were prepared according to the manufacturer's instructions and injected via the same fertigation system; IJ presence and motility were checked at drippers immediately after sampling.

Dripper sampling and CFU quantification During the injection period, irrigation water was sampled at dripper outlets within treated sectors to verify delivery and quantify cultivable propagules. For each treated sector and event, a composite sample (13 mL) was obtained by pooling water from six emitters distributed across different drip lines in the central part of the sector. Samples were kept at ambient temperature and plated within 3–6 h of collection. For each composite sample, 20 µL of undiluted irrigation water were plated on PDA supplemented with antibiotics (streptomycin (0.1 $g \cdot L^{-1}$) and ampicillin (0.05 $g \cdot L^{-1}$)), with three replicate plates, and incubated at 23 °C. Colony–forming units (CFU) were counted after 2–3 days at first clear colony development and expressed as $CFU \cdot mL^{-1}$.

Estimation of the viable dose delivered per unit area To express delivery in agronomically comparable units, the viable dose delivered during the injection window was estimated by combining CFU concentrations measured at dripper outlets with irrigation intensity and injection duration. Dose estimates were expressed as $CFU \cdot m^{-2}$ and refer exclusively to the wetted productive strip along tree rows where truffles

typically develop (not to the total sector surface). Detailed assumptions and calculations are provided in Online Resource 7.

Dose ($CFU \cdot m^{-2}$) = ($CFU \cdot mL^{-1}$ at drippers) × (irrigation volume during injection, $mL \cdot m^{-2}$).

Given an irrigation intensity of 5.3 $L \cdot m^{-2} \cdot h^{-1}$, the 30–min injection period corresponds to 2.65 $L \cdot m^{-2}$ (i.e., 2650 $mL \cdot m^{-2}$). This calculation assumes approximately steady concentration during injection (continuous tank agitation) and treats CFU as a conservative proxy for the cultivable subset of viable propagules.

Operational checks All irrigation lines and emitters were fully operational during application events, and no blockages or pressure irregularities were detected. No additional agronomic operations or soil disturbances were conducted during application periods.

Statistical analysis

Weekly trap captures were analyzed at the irrigation–sector level, using sector–by–week totals as the observational unit. Given the absence of buffer zones, potential adult movement among sectors, and persistent spatial heterogeneity, treatment-associated changes in adult captures were evaluated using a before–after–control–impact (BACI) framework combined with exact permutation testing (Anderson, 2001; Underwood, 1994). Trap density was constant across sectors (four traps per sector throughout the study), and no offset was therefore required.

For each season, mean weekly captures were calculated for a short pre–application baseline and for biologically defined post–application windows (see temporal windows below). Sector–specific changes were quantified as:

$$\Delta = mean(post) - mean(pre)$$

BACI contrasts were computed as differences between mean Δ values of treated and control sectors.

Exact permutation tests were conducted by enumerating all possible reallocations of three "treated" labels among six sectors (20 permutations). Two–sided p–values were calculated as the proportion of permutations producing contrasts as extreme as or more extreme than the observed contrast. Because the number of possible permutations under the 3-versus-3 allocation was small (C(6,3) = 20), all permutations were enumerated exactly. The number of sectors per treatment ($n = 3$) was constrained by the fixed irrigation layout and operational feasibility at commercial scale; consequently, exact permutation tests were used primarily as descriptive diagnostics rather than formal hypothesis tests of efficacy.

Spatial heterogeneity and seasonal dynamics were explored descriptively using sector–by–week heatmaps ordered according to the fixed 3 × 3 orchard layout. Given the limited pre–treatment baseline (two weeks per season) and strongly pulsed phenology, inference was interpreted conservatively, emphasizing effect direction, magnitude during biologically relevant windows, and consistency across seasons rather than reliance on single–threshold statistical significance. Accordingly, the experimental design prioritized ecological realism and operational representativeness over statistical power, reflecting the constraints inherent to field–scale experiments in commercial perennial cropping systems.

All statistical analyses and visualizations were performed in Python 3.11 using the libraries pandas (data handling), NumPy (numerical computation), SciPy (statistical procedures), matplotlib, and seaborn (visualization). Exact permutation tests were implemented by exhaustive enumeration. Confidence intervals for rate ratios were obtained using nonparametric bootstrap resampling (50,000 iterations), resampling sectors within treatment groups (n = 3 per group).

Temporal windows Temporal windows were defined a priori based on application dates and independently characterized seasonal phenology. In the 2024–2025 season, the pre–application baseline comprised the two weeks preceding the first inoculation (23 and 30 September 2024). Two post–application windows were defined: (i) an early post–application window centered on the main emergence peak and the immediately following week (14 October–3 November 2024), occurring after the first inoculation (8 October), and (ii) a late post–application window following the second inoculation (4 November).

In the 2025–2026 season, the pre–application baseline comprised 22 and 29 September 2025. The early post–application window spanned the peak emergence

period following the first inoculation (13 October–2 November 2025).

Complementary effect–size and restricted randomization analyses To complement the conservative BACI framework and explicitly account for strong baseline differences among sectors and the 3 × 3 spatial layout, we conducted a block–restricted analysis focused on phenological windows within each season. Weekly captures were aggregated by sector into three windows: pre–peak (weeks 1–3), peak (weeks 4–6), and post–peak (week ≥ 7), corresponding to the period of maximum adult activity in each season. For each window, treatment-associated differences were summarized as rate ratios (RR), defined as the mean cumulative captures per treated sector divided by the mean cumulative captures per control sector. Uncertainty was quantified using a nonparametric bootstrap (50,000 resamples), resampling sectors within treatment and control groups ($n = 3$ sectors each) to obtain 95% confidence intervals for RR. In addition, to reduce sensitivity to orchard–scale gradients, we performed a spatially restricted paired comparison within the three plot rows (3 blocks), computing within–row differences (treated − control) in peak–window cumulative captures and evaluating evidence using an exact sign–permutation test ($2^3 = 8$ permutations; two–sided p–values). Because only three spatial blocks were available, all possible sign permutations ($2^3 = 8$) were enumerated exactly.

Results

Seasonal activity in the untreated reference plot

Adult activity of *L. cinnamomea* in the untreated reference plot (SR) followed a sharply pulsed seasonal pattern in both monitored seasons (Fig. 4). In the 2024–2025 season, a total of 5,793 adults were captured between late September and mid-January, whereas 3,239 adults were captured over the same standardized period in 2025–2026, representing a 44% reduction in cumulative captures between years.

In both seasons, adult captures increased rapidly during early October and reached a pronounced maximum within a narrow temporal window. Peak activity occurred during the week of 14–20 October 2024 (932 adults) and one week earlier in 2025–2026 (13–19 October; 781 adults). Following peak emergence, captures declined steeply toward winter. The post–peak decline was more abrupt in 2024–2025, whereas in 2025–2026, relatively higher background activity persisted into late autumn and early winter.

Despite marked interannual differences in overall adult capture levels and peak magnitude, the phenological structure was conserved across seasons, characterized by a short and intense emergence peak followed by a rapid decline. This compressed activity window defined the biologically relevant period for subsequent spatial and treatment–related analyses.

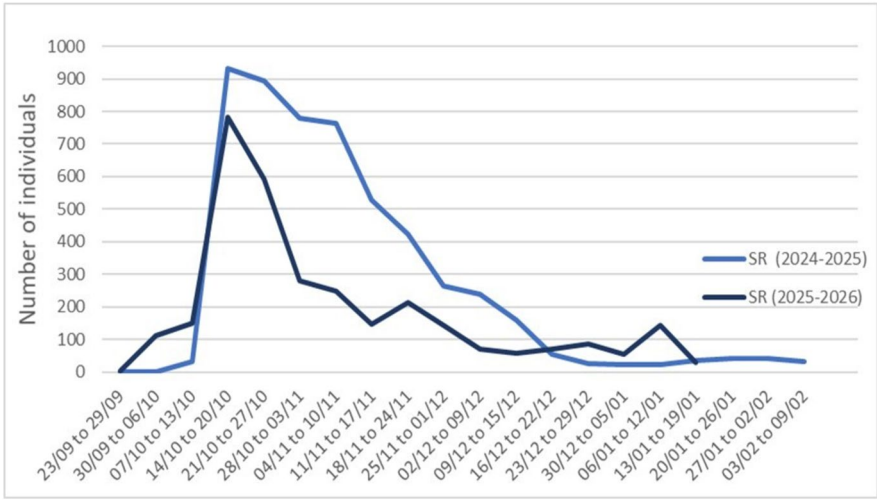

Fig. 4 Weekly trap captures of adult *L. cinnamomea* in the untreated reference plot (SR) during the 2024–2025 and 2025–2026 seasons. Both seasons exhibited a sharply pulsed emergence pattern, with rapid increases in adult activity during October, followed by a steep decline toward winter

Persistent spatial heterogeneity in adult activity within the orchard

Within the experimental orchard, adult trap captures exhibited the same pulsed seasonal dynamics observed in the SR plot (Fig. 5), but capture intensity varied markedly among irrigation sectors.

During the peak emergence week of the 2024–2025 season (14–20 October), total orchard captures reached 2,871 adults, while sector–level counts ranged from 66 to 622 individuals across sectors ST1–ST9, corresponding to a 9.4–fold difference. In the 2025–2026 season, peak activity occurred during 13–19 October, with 2,037 adults captured orchard–wide and sector–level counts ranging from 117 to 415 individuals (3.5–fold difference).

Sector–by–week heatmaps confirmed persistent spatial gradients conserved across seasons (Fig. 6), including within untreated controls (Online Resource 8), indicating that heterogeneity primarily reflected orchard structure rather than treatment effects.

High susceptibility of adult L. cinnamomea to B. bassiana at autumn temperature

Under field–relevant temperature conditions (12°C ± 2 °C), exposure to *B. bassiana* (strain GHA) caused a rapid and pronounced reduction in survival of adult *L. cinnamomea* compared with untreated controls (Fig. 7).

All fungus–exposed insects died within the 7–day observation period, with mortality occurring between days 2 and 6 days. Survival distributions differed significantly between treatments (log–rank test: $\chi^2 = 16.83$, $p = 4.1 \times 10^{-5}$). The median lethal time (TL_{50}) for *B. bassiana*–exposed insects was 4.0 days (95% CI: 4.0–5.0 days), whereas TL_{50} could not be estimated for control insects because survival did not fall below 50%. As an auxiliary estimate of exposure intensity, the mean conidial load acquired per beetle during direct–contact exposure was $(4.47 \pm 0.25) \times 10^7$ conidia insect^{-1} (Online Resource 9). Following death, inoculated insects consistently developed external mycosis, and *B. bassiana* was

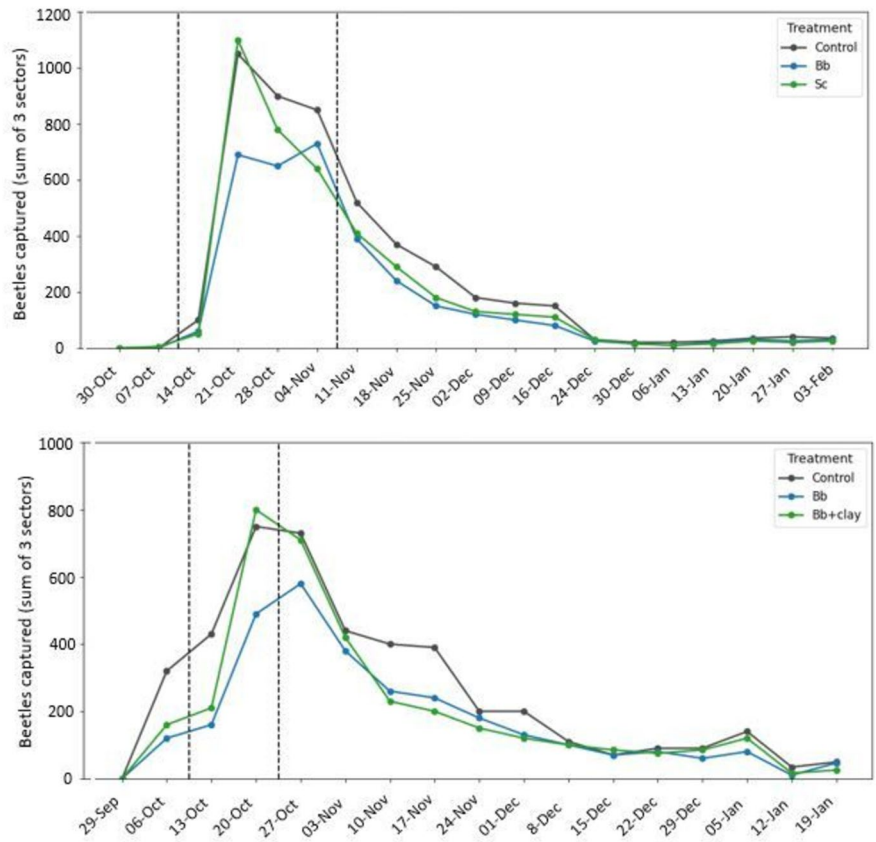

Fig. 5 Weekly adult *L. cinnamomea* captures by treatment. Weekly trap captures aggregated across irrigation sectors for each treatment in the experimental orchard during the 2024–2025 (top panel) and 2025–2026 (bottom panel) seasons. Vertical dashed lines indicate the dates of field applications of entomopathogenic agents (8 October and 4 November 2024; 3 October and 20 October 2025). In both seasons, adult activity showed a sharply pulsed emergence pattern, with peak captures occurring within a narrow temporal window in mid–October, followed by a rapid decline toward winter. Bb: *B. bassiana*, Sc: *S. carpocapsae*, Bb + clay: *B. bassiana* + white halloysite

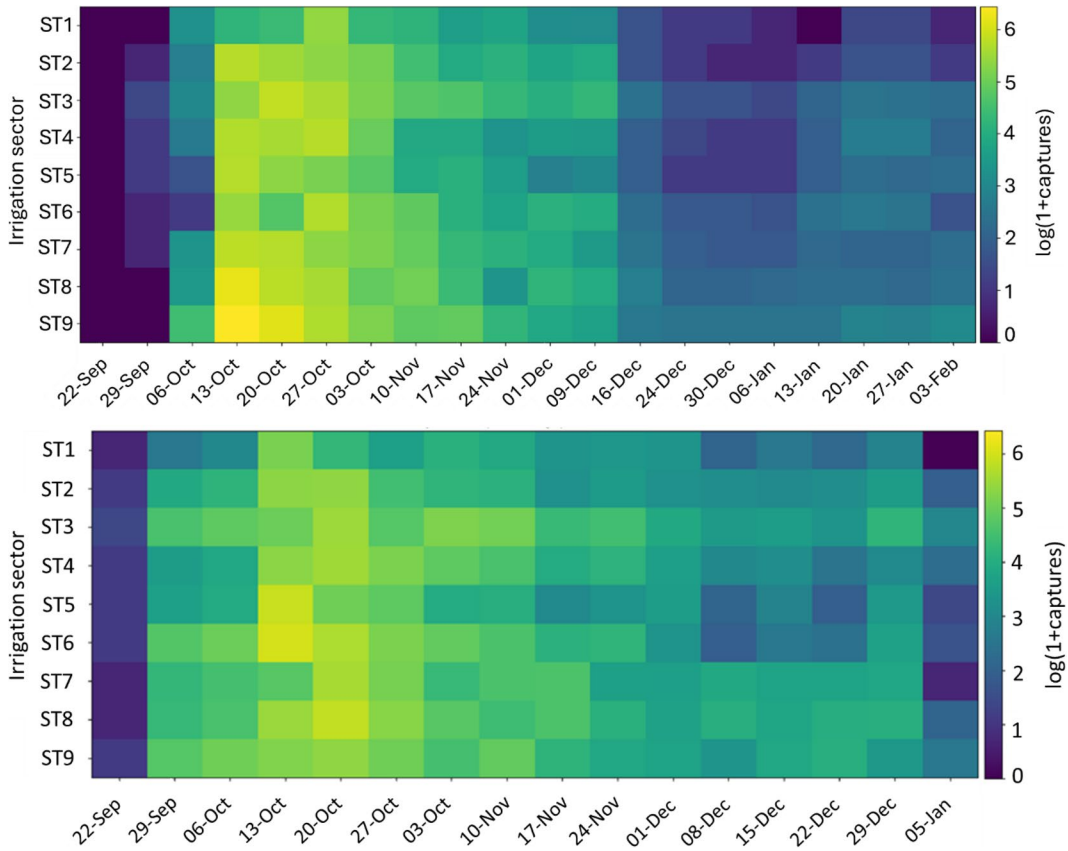

Fig. 6 Spatial and temporal distribution of adult *L. cinnamomea* captures within the experimental orchard. Sector–by–week heatmaps of trap captures ($\log_{10}[1+\text{captures}]$) for the 2024–2025 (top) and 2025–2026 (bottom) seasons. Rows correspond to irrigation sectors (ST1–ST9), ordered according to their fixed spatial position within the orchard, and columns correspond to sampling weeks. Heatmaps illustrate pronounced and persistent spatial heterogeneity in capture intensity, with sectors showing consistently high or low activity across weeks and seasons. These patterns reflect underlying orchard–scale spatial structure and were used as exploratory diagnostics to contextualize treatment–effect analyses

successfully re–isolated from cadavers (Fig. 8), confirming fungal infection as the cause of mortality.

Results were consistent in the independent replicate assay ($TL_{50}=4.0$ days, 95% CI: 3.0–6.0 days; log-rank $\chi^2=36.92$, $p=1.23\times10^{-9}$; Online Resource 1).

Field delivery and viability of entomopathogenic agents

Viable *B. bassiana* propagules were consistently detected at dripper outlets in all sectors receiving fungal treatments during both seasons (Online Resource 10), confirming successful delivery of *B. bassiana* through the drip irrigation system. Dripper–outlet concentrations were typically in the order of 10^3–10^4 CFU·mL^{-1}, with the highest values recorded during the halloysite–amended application on 3 October 2025 (Table 2).

When expressed as estimated viable dose delivered during the 30–min injection window within the wetted productive strip, these concentrations corresponded to approximately 10^6–10^7 CFU·m^{-2} per application event for *B. bassiana* alone, and up to ~10^7 CFU·m^{-2} for the halloysite–amended formulation (Table 2; Online Resource 7). Across events, the estimated CFU-based dose within the wetted strip ranged from 6.57×10^6 to 4.79×10^7 CFU·m^{-2} per application event, depending on season and formulation.

For *S. carpocapsae* applications, active infective juveniles were observed at dripper outlets in

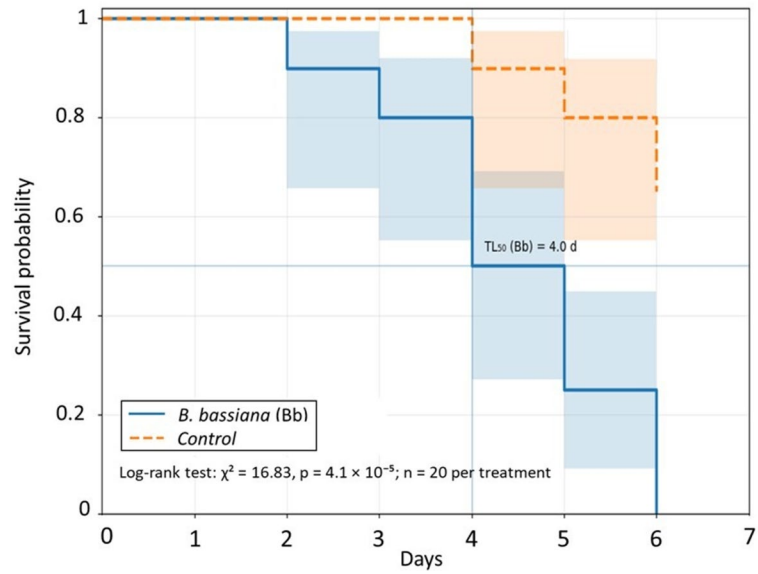

Fig. 7 Kaplan–Meier survival curves of adult *L. cinnamomea* exposed to *B. bassiana* (Bb) and untreated control insects under field–relevant temperature conditions (12°C ± 2 °C). Shaded areas represent 95% confidence intervals. Survival distributions differed significantly between treatments (log–rank test: $\chi^2 = 16.83$, $p = 4.1 \times 10^{-5}$). Individuals surviving to day 7 were treated as right–censored observations

Fig. 8 External mycosis caused by *B. bassiana* on adult L. cinnamomea following inoculation, death, and incubation under humid conditions. The fungus was successfully re-isolated from cadavers, supporting fungal infection as the cause of mortality

Table 2 Dripper–outlet concentration and estimated viable dose during the 30–min injection window

Season	Date	Formulation	CFU·mL^{-1} at drippers (mean ± SD across treated sectors; $n = 3$)	Estimated viable dose (CFU·m^{-2})
2024–2025	8 Oct 2024	*B. bassiana*	$(2.48 \pm 0.77) \times 10^3$	6.6×10^6
	4 Nov 2024	*B. bassiana*	$(5.07 \pm 0.84) \times 10^3$	1.3×10^7
2025–2026	3 Oct 2025	*B. bassiana*	$(5.45 \pm 1.78) \times 10^3$	1.4×10^7
	20 Oct 2025	*B. bassiana*	$(1.96 \pm 0.18) \times 10^3$	5.2×10^6
	3 Oct 2025	*B. bassiana* + halloysite	$(1.81 \pm 0.25) \times 10^4$	4.8×10^7
	20 Oct 2025	*B. bassiana* + halloysite	$(4.99 \pm 1.99) \times 10^3$	1.3×10^7

Values are means ± SD across the three treated sectors per formulation and event ($n = 3$ sectors). CFU·mL^{-1} was calculated from colony counts obtained by plating 20 µL of undiluted dripper water. Estimated dose assumes 2.65 L·m^{-2} applied during the 30–min injection

all sampled sectors, confirming nematode delivery, although slightly reduced motility was noted in one sector during the second application event.

Treatment-associated differences in adult captures were restricted to the peak emergence period

Treatment–related patterns were evaluated within a biologically defined early post–application window encompassing the main adult emergence peak and the immediately following weeks (Fig. 9).

In the 2024–2025 season (14 October–3 November), cumulative captures during this window totaled 2,081 adults in sectors receiving *B. bassiana* compared with 2,829 adults in untreated control sectors, corresponding to 26.4% fewer adult captures relative to untreated controls. Mean captures were 231.2 beetles per sector–week in *B. bassiana*–treated sectors versus 314.3 beetles per sector–week in control sectors. Sectors receiving *S. carpocapsae* showed a smaller difference relative to controls (−10.2%).

In the 2025–2026 season (13 October–2 November), sectors treated with *B. bassiana* again exhibited

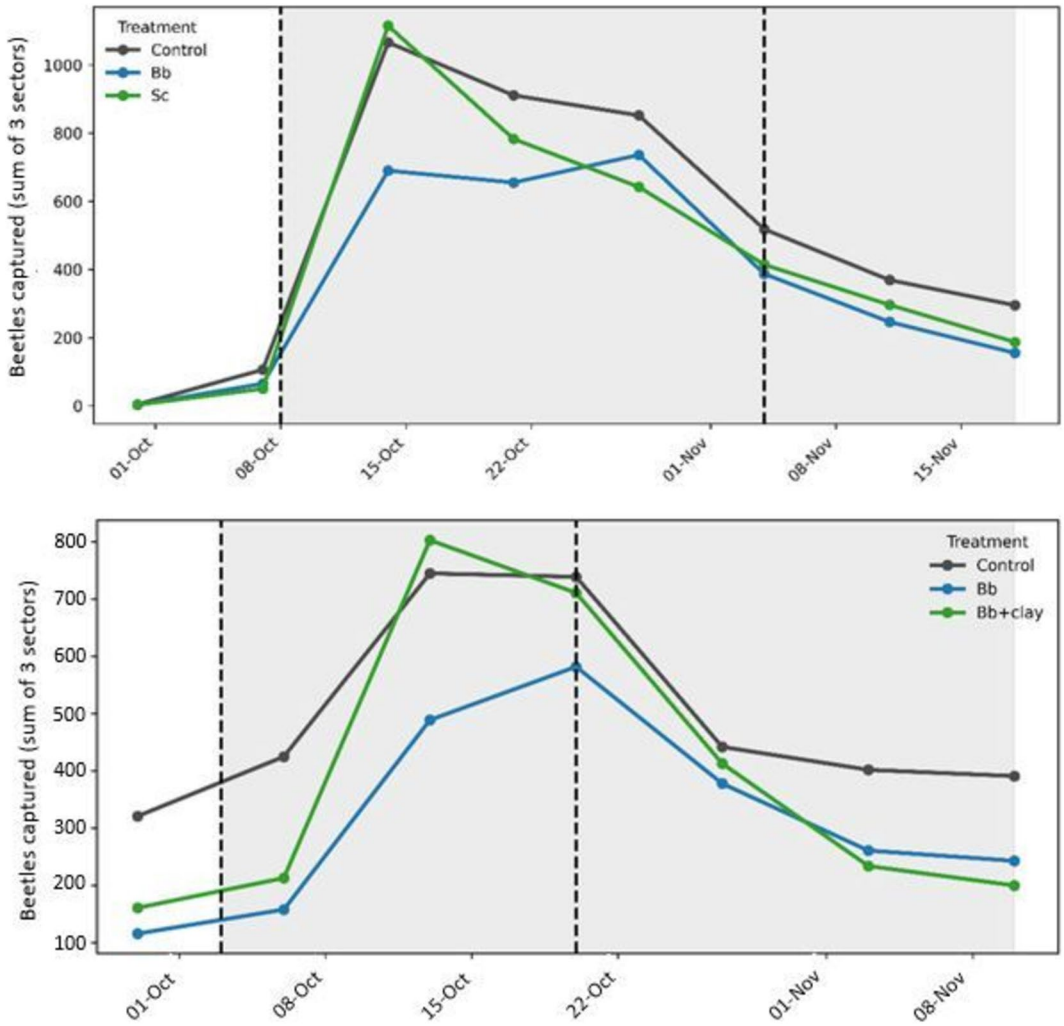

Fig. 9 Weekly adult *L. cinnamomea* captures aggregated by treatment during the central emergence period, including application dates, in the 2024–2025 and 2025–2026 seasons. Lines represent weekly totals summed across the three irrigation sectors corresponding to each treatment. Vertical dashed lines indicate dates of entomopathogen application. Shaded areas highlight the early post-application windows used for treatment comparisons

lower adult captures during the peak window (1,449 beetles) than control sectors (1,926 beetles), corresponding to 24.8% fewer adult captures relative to untreated controls. In contrast, sectors treated with the *B. bassiana* + halloysite formulation did not show a comparable reduction relative to controls, despite higher conidial concentrations detected at dripper outlets.

Spatially paired comparisons within orchard blocks were consistent with these capture patterns. In 2024–2025, *B. bassiana*–treated sectors showed lower captures than their paired control sectors in two of three blocks, while in 2025–2026, treated sectors showed equal or lower captures than paired controls in all three blocks (Online Resource 11).

Complementary window–based analyses yielded directionally consistent capture contrasts. In 2024–2025, the estimated RR for *B. bassiana* during the peak window was 0.736 (95% CI: 0.383–1.240), corresponding to 26.4% fewer adult captures relative to controls, whereas *S. carpocapsae* showed a weaker and less consistent contrast (RR = 0.898; 95% CI: 0.599–1.407). In 2025–2026, *B. bassiana*–treated sectors again showed a comparable directional reduction in adult captures (RR = 0.752; 95% CI: 0.433–1.152), while the *B. bassiana* + halloysite formulation did not differ from the control (RR = 1.001; 95% CI: 0.724–1.408). Spatially restricted paired comparisons within orchard rows yielded conservative exact sign–permutation p–values ($n = 3$ blocks), consistent with limited statistical power under strong spatial heterogeneity, while preserving the directional pattern for *B. bassiana* (Online Resource 12).

Outside the peak window, capture trajectories converged and treatment differences diminished. During late autumn and early winter, when overall adult activity declined sharply, differences among treatments were small and inconsistent, and no sustained separation of capture trajectories was observed. Accordingly, treatment–related differences were smaller following the second application in the 2024–2025 season, coinciding with the declining phase of adult activity, when overall movement and trap captures were already decreasing across all sectors.

Formal BACI contrasts based on exact permutation tests provided limited statistical support for treatment-associated changes in captures. In 2024–2025, the BACI contrast for *B. bassiana* during the early post–application window was negative (−76.1 captures per sector–week) but not statistically significant (two–sided p = 0.60), while the corresponding contrast for *S. carpocapsae* was smaller (−22.6 captures per sector–week; p = 0.70). In 2025–2026, BACI contrasts were sensitive to pre–application differences and the presence of two application dates, yielding a positive contrast for *B. bassiana* (+25.7 captures per sector–week; p = 0.50) and a larger positive contrast for the *B. bassiana* + halloysite formulation (+62.1 captures per sector–week; p = 0.20).

Given the strong spatial heterogeneity and limited replication, permutation-based BACI tests were interpreted conservatively. Nevertheless, across seasons and analytical approaches, sectors receiving *B. bassiana* were consistently associated with lower adult captures during the peak emergence window. A synthesis of peak-window treatment-associated capture differences across seasons is provided in Fig. 10, which summarizes the direction and magnitude of rate ratios relative to untreated controls. This reduction in captures should be interpreted as a change in adult activity or trap encounter probability rather than as direct evidence of population suppression.

Discussion

This study provides a field-scale evaluation of irrigation-applied entomopathogenic agents against *L. cinnamomea* under commercial black truffle orchard conditions characterized by pronounced spatial heterogeneity, compressed phenology, and operational constraints. By combining laboratory bioassays with two consecutive seasons of field monitoring, the study was designed not to demonstrate definitive control efficacy, but to assess whether treatment-associated signals in adult activity could be detected under realistic orchard conditions.

Laboratory bioassays conducted under autumn-relevant temperature confirmed that adult *L. cinnamomea* is highly susceptible to *B. bassiana* strain GHA. Fungal exposure resulted in rapid mortality, with a median lethal time of approximately 4 days at 12 °C ± 2 °C and consistent results across two independent assays. This response is consistent with the known susceptibility of coleopteran pests to *B. bassiana* under direct-contact conditions (Bayindir Erol

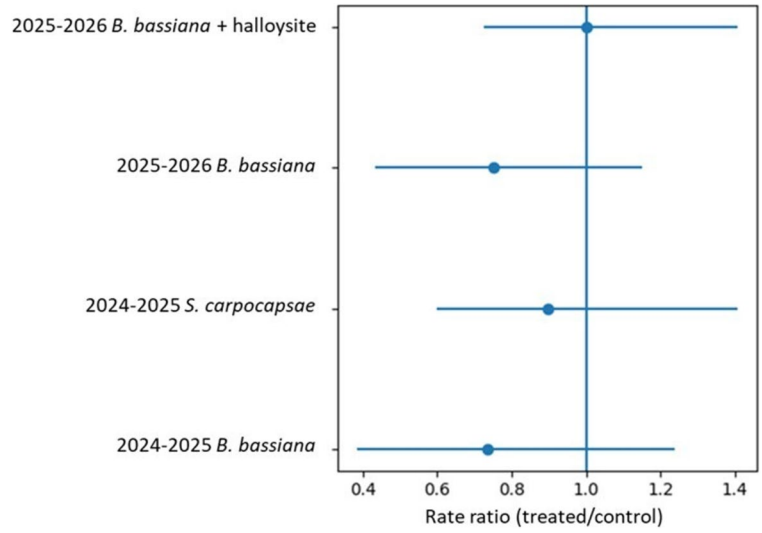

Fig. 10 Direction and magnitude of treatment-associated differences in adult captures during the peak emergence window. Rate ratios (RR) of cumulative adult captures in treated sectors relative to control sectors during the peak emergence window for each treatment and season. Points represent median RR values, and horizontal bars indicate 95% confidence intervals obtained by nonparametric bootstrap resampling. RR < 1 indicates fewer captures than the control during the peak emergence window

et al., 2023; Wang & Chi, 2019), and with the dose- and contact-dependent nature of entomopathogenic fungal infection (Barta, 2010). However, the direct-contact exposure used in the laboratory likely resulted in much higher conidial acquisition than would occur under field irrigation delivery. Therefore, these assays should be interpreted as evidence of intrinsic susceptibility, not as direct evidence of field efficacy.

At field scale, adult activity was strongly pulsed, with most captures concentrated within a narrow autumn emergence window. This phenological pattern was consistent across seasons and agrees with previous observations in truffle orchards, where adult activity and infestation risk are concentrated mainly in autumn (Araujo et al., 2025; Navarro-Llopis et al., 2021). Such temporal compression is important for biological control because contact-based agents depend on encounter probability between the target insect and infective propagules. Applications made before or during the ascending phase of adult emergence are therefore more likely to coincide with host movement than applications made after the peak, when activity is already declining (Alqubori et al., 2025; Cohnstaedt et al., 2012).

Spatial heterogeneity was another dominant feature of the field data. Capture intensity differed strongly among irrigation sectors, and these gradients were persistent across seasons and also evident among untreated controls. This indicates that sector-level differences largely reflected orchard structure, such as microhabitat conditions, soil properties, irrigation patterns, or production history, rather than treatment alone. Similar spatial structuring has been reported in other truffle orchard studies (Araujo et al., 2025; Navarro-Llopis et al., 2021). The absence of buffer zones and possible movement of adults among contiguous sectors further complicate fine-scale attribution of treatment effects and likely diluted contrasts among treatments.

Within this heterogeneous context, sectors receiving *B. bassiana* consistently showed lower adult captures than untreated controls during the biologically relevant peak-emergence window. Across both seasons, reductions in cumulative adult captures were approximately 25%–26% relative to controls. Formal BACI contrasts provided limited statistical support, reflecting short pre-treatment baselines, restricted replication, and strong spatial heterogeneity. Nevertheless, the repeated direction and similar magnitude of the response across seasons, analytical approaches, and spatial comparisons support the interpretation of a reproducible but temporally restricted reduction in adult activity associated with *B. bassiana* application. Importantly, this should be interpreted as a change in adult trap captures or activity, not as direct evidence of population suppression, larval reduction, or crop protection.

The delivery context may help explain why the capture response was moderate despite high laboratory susceptibility. Drip irrigation is a low-disturbance and operationally practical delivery pathway for truffle orchards, particularly during autumn when

truffles are already developing in the soil and mechanical disturbance should be minimized (Reyna & Garcia-Barreda, 2014). However, drip irrigation delivers water and suspended particles into localized wetted zones, and the geometry of these wetting patterns depends on emitter discharge, irrigation duration, and soil hydraulic properties (Al-Ogaidi et al., 2016; Hardie et al., 2018; Kandelous & Šimůnek, 2010). The sandy clay loam soil of the study orchard may further influence particle retention and lateral transport around emitters, as attachment, straining, and retention of colloid-sized particles in porous media depend on pore structure, particle size, and physicochemical interactions with soil surfaces (Baveye et al., 2018; Bradford et al., 2002). Therefore, CFU values measured at dripper outlets should be interpreted as conservative indicators of cultivable propagule delivery, not as direct measurements of uniform soil exposure.

The estimated field-delivered cultivable dose was lower than the nominal conidial input. The nominal formulation input corresponded to approximately 1.06×10^{13} formulated conidia per sector per application. Based on the irrigation intensity during the 30-min injection window and the estimated wetted productive strip area, complete passage and homogeneous dilution of the formulated inoculum would correspond to an approximate nominal concentration of 1.5×10^{6} conidia mL^{-1} at dripper outlets. In contrast, cultivable propagules recovered at dripper outlets were in the order of 10^{3}–10^{4} CFU mL^{-1}, corresponding to approximately 10^{6}–10^{7} CFU m^{-2} during the injection window. This difference should be interpreted cautiously because nominal conidial concentration and CFU recovery are not equivalent measurements: CFU counts reflect only the cultivable fraction recovered under the sampling and plating conditions and are affected by hydraulic dilution, aggregation, recovery efficiency, and delivery heterogeneity (Faria & Wraight, 2007; Jaronski, 2010). Therefore, lower CFU recovery at dripper outlets does not necessarily imply loss of all non-recovered inoculum, but may reflect a combination of dilution, non-culturable or aggregated propagules, and retention or deposition within the irrigation system or around emitters. No pressure irregularities or emitter blockages were observed during application, but partial retention of propagules in irrigation lines or localized deposition near emitters cannot be excluded. Thus, the biological relevance of the applied dose depends less on nominal input alone and more on whether infective propagules overlapped spatially and temporally with adult beetle movement during peak activity.

S. carpocapsae showed only a weak reduction in adult captures during the first season and was not evaluated in the second season. Because the susceptibility of L. cinnamomea to EPNs, including S. carpocapsae, has already been demonstrated under laboratory conditions (Julià et al., 2023), the limited field response observed here is more likely to reflect ecological and delivery-related constraints than a lack of intrinsic susceptibility. Nematode mobility, host-seeking behaviour, and infection success are strongly temperature dependent, and autumn soil temperatures may have reduced infective juvenile activity or host-penetration efficiency under field conditions (Grewal et al., 2005; Kaya & Gaugler, 1993). In addition, S. carpocapsae is generally classified as an ambush forager, relying primarily on host contact in localized zones, whereas species such as Steinernema feltiae (Filipjev, 1934) show a more intermediate or cruiser-like strategy and may search more actively through the substrate. The ambush strategy of S. carpocapsae may therefore be less compatible with a subterranean beetle whose adult movement pathways in soil are poorly characterized and likely heterogeneous, particularly when infective juveniles are delivered through localized drip irrigation (Shapiro et al., 2000). Nematode delivery was verified qualitatively by observing motile infective juveniles at dripper outlets, but infective juvenile concentration at the outlet and recovery efficiency through the irrigation system were not quantified. Therefore, potential losses due to sedimentation, retention in irrigation lines, or localized deposition around emitters could not be estimated. This limitation may be particularly relevant for EPNs because infective juveniles are considerably larger than fungal conidia and may therefore be more susceptible to physical retention or uneven delivery during drip irrigation. Targeted experiments comparing EPN species with contrasting foraging strategies, application timings, and quantitative recovery at dripper outlets would be required to determine whether EPN performance in truffle orchards can be improved under drip-irrigated field conditions.

The halloysite-amended B. bassiana formulation was included as an exploratory carrier-based treatment to test whether a low-dose clay amendment could modify conidial suspension, deposition,

or persistence during drip irrigation delivery. Halloysite was selected because clay minerals, including halloysite, can adsorb microbial particles, promote aggregation, and influence particle retention and mobility in porous media (Baveye et al., 2018; Lvov et al., 2014). However, despite higher CFU concentrations at dripper outlets, the halloysite-amended formulation did not improve the adult-capture response. This indicates that higher outlet concentrations do not necessarily translate into greater biological exposure at the orchard scale. One plausible explanation is that halloysite altered particle aggregation or deposition patterns during drip delivery, modifying the spatial distribution of conidia relative to beetle movement pathways. However, because aggregation state and soil-level conidial distribution were not directly quantified, this interpretation remains speculative and should be tested in future work.

An additional strategy deserving evaluation is the application of entomopathogens earlier in the season, before the autumn adult emergence peak. The present study targeted the period of adult activity, when contact between emerging adults and infective propagules was expected to be most likely. However, applications during late summer or early autumn could potentially target pre-emergent stages, including diapausing larvae, and allow more time for propagule persistence or recycling in soil before adult flight activity begins. This may be particularly relevant for EPNs, as Julià et al. (2023) reported high laboratory efficacy of *Heterorhabditis bacteriophora* against diapausing larvae of *L. cinnamomea*. Whether such earlier applications would improve field performance under drip irrigation remains unresolved and should be tested in future studies comparing application timings, target life stages, and entomopathogen species.

Several limitations should be acknowledged. First, truffle damage, larval infestation, and yield protection were not quantified because standardized assessment of damage in harvested truffles across all sectors would have required sector-level traceability of fruiting bodies, detailed grading of damage severity, and repeated assessments throughout the harvest period, which were not feasible under the operational conditions of this commercial orchard. Consequently, results should be interpreted as changes in adult activity rather than direct estimates of crop protection or effects on larval stages. Second, trap captures represent an indirect proxy of relative adult activity rather than a direct measure of population density or damage. Third, sectors were contiguous and lacked buffer zones, so adult movement among sectors could not be excluded and may have diluted treatment contrasts. The flight capacity of adult *L. cinnamomea* and the use of attractant-baited traps make crossover among neighbouring sectors plausible, particularly near sector boundaries. Fourth, treatments were not re-randomized between seasons because repeated application in the same sectors was used to evaluate whether the directional response to *B. bassiana* was reproducible under a consistent spatial context; nevertheless, this design cannot fully separate treatment effects from persistent sector-level location effects. Finally, laboratory bioassays were conducted with field-collected adults of unknown age and sex because, to our knowledge, no standardized laboratory rearing protocol is currently available for this subterranean species.

Overall, irrigation-applied *B. bassiana* did not produce sustained season-long suppression, but it was consistently associated with lower adult captures during the narrow peak-emergence window across two consecutive seasons. These findings suggest that irrigation-applied *B. bassiana* warrants further evaluation as a tactical, timing-dependent complement within integrated pest management programs for truffle orchards, rather than as a stand-alone control measure. Future studies incorporating stronger spatial replication, buffer zones, direct assessment of truffle damage, and soil-level quantification of propagule distribution will be required to determine whether the observed differences in adult activity translate into commercially relevant crop protection.

Conclusions

This study evaluated irrigation-applied entomopathogenic agents against *L. cinnamomea* under commercial black truffle orchard conditions characterized by strong spatial heterogeneity, compressed adult phenology, and reliance on trap captures as an indirect monitoring endpoint. Laboratory bioassays showed that adult *L. cinnamomea* was highly susceptible to *B. bassiana* strain GHA under direct-contact exposure at autumn-relevant temperatures.

At field scale, viable *B. bassiana* propagules were consistently recovered at dripper outlets, confirming that the fungus could be delivered through the

drip irrigation system. Sectors receiving *B. bassiana* showed lower adult trap captures than untreated controls during the narrow autumn emergence peak in both seasons. However, this pattern was moderate, temporally restricted, and not strongly supported by permutation-based BACI tests, reflecting limited replication, short pre-treatment baselines, and pronounced spatial heterogeneity within the orchard. *S. carpocapsae* showed only a weaker reduction in adult captures during the first season and was not evaluated in the second season, whereas the halloysite-amended *B. bassiana* formulation did not show a comparable reduction relative to untreated controls.

Because truffle damage, larval infestation, and yield protection were not quantified, these findings should be interpreted as treatment-associated changes in adult activity rather than evidence of population suppression or crop protection. The lack of treatment re-randomization, contiguous sectors without buffer zones, and use of attractant-baited trap captures further limit causal inference.

Overall, irrigation-applied *B. bassiana* was associated with a reproducible but limited directional signal during peak adult activity under the conditions tested. Further studies incorporating stronger spatial replication, buffer zones, soil-level quantification of propagule distribution, and direct damage-based endpoints are needed to determine whether this approach can provide practical benefits for integrated management of *L. cinnamomea* in truffle orchards.

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Data availability The datasets generated during this study are available in the online resources. Any other data required will be made available upon request to the corresponding author.

Declarations

Competing interests The authors declare no competing interests.

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