



Compatibility of soil application of *Metarhizium brunneum* and cover crops against *Ceratitis capitata* soil-dwelling stages

Joaquín Cruz-Miralles¹ · Inmaculada Garrido-Jurado² · Meelad Yousef-Yousef² · M. Victoria Ibáñez-Gual³ · Óscar Dembilio⁴ · Enrique Quesada-Moraga² · Josep A. Jaques¹

Received: 20 June 2023 / Revised: 23 October 2023 / Accepted: 26 October 2023
© The Author(s) 2024

Abstract

Fruit fly ground-dwelling stages (late third instar larvae, pupae, and teneral adults) are susceptible to predation from generalist ground-dwelling predators and to infection by entomopathogenic fungi (EPF). The effect of predators can be enhanced with cover crops and that of EPF by augmentative releases. However, whether these two biological control methods could be combined has not been studied under field conditions yet. Here, we studied in the field whether the enhanced activity of predators against the medfly, *Ceratitis capitata*, already observed in a *Lolium arundinaceum* ground cover could be impaired by a soil application of *Metarhizium brunneum*. Our results show that *C. capitata* adult emergence was reduced by this EPF for up to three months after fungal application, with the combination of the cover and *M. brunneum* being the most effective at reducing *C. capitata* emergence relative to bare soil (92.5% reduction). Although *M. brunneum* reduced the activity density of ground-dwelling predatory beetles up to 93 days after application, it showed no clear negative effects on earwigs, no effects on spiders, and a positive effect on ants up to 65 days after application. Therefore, the combined use of a ground cover of *L. arundinaceum* and *M. brunneum* against the soil-dwelling stages of *C. capitata* seems to work synergistically and appears as a strong and sustainable control tactic against the medfly and other fruit orchard pests.

Keywords Entomopathogenic fungus · Ground predator · *Lolium arundinaceum* · Natural enemies · Conservation biological control · Augmentation

Key message

- Medfly ground-dwelling stages are susceptible to ground predators and entomopathogenic fungi
- Ground covers are used as conservation biological control to enhance the impact of these predators
- Augmentative releases of *M. brunneum* can be used to control *C. capitata*
- A cover of tall fescue and *M. brunneum* was the most effective combination against *C. capitata*
- Activity density of ground beetles but not other predators was temporarily reduced by *M. brunneum*

Introduction

The family Tephritidae is the most species-rich family within Diptera, with more than 5000 described species (EFSA PLH Panel 2020). Among these species, around 35% have been

Communicated by Antonio Biondi.

✉ Josep A. Jaques
jacas@uji.es

¹ Departament de Biologia, Bioquímica i Ciències Naturals, ESTCE, Universitat Jaume I (UJI), Campus del Riu Sec, Av. Vicent Sos Baynat S/N, 12071 Castelló de la Plana, Spain

² Department of Agronomy, María de Maeztu Excellence Unit DAUCO, ETSIAM, University of Cordoba, Campus de Rabanales, Building C4 Celestino Mutis, 14071 Cordoba, Spain

³ Departament de Matemàtiques- IMAC, Universitat Jaume I, ESTCE, Campus del Riu Sec, Av. Vicent Sos Baynat S/N, 12071 Castelló de la Plana, Spain

⁴ Empresa de Transformación Agraria S.A., S.M.E., M.P. (TRAGSA), Av. Industria 26, 46980 Paterna, Spain

documented to attack fruit and are thus considered as true fruit flies (EFSA PLH Panel 2020). The economic importance of these flies is recognized as major worldwide (De Meyer and Ekesi 2016). The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), is a highly invasive agricultural pest, endemic to most sub-Saharan countries and reported on more than 300 plant species including fruits, vegetables, and nuts (CABI 2023). Fruit rotting following oviposition and the consequent larval feeding on mature fruits may result in complete yield loss (Weldon 2020). In temperate areas, most severe damage occurs in stone fruit and citrus, where this species finds a refuge during winter (Jacas et al. 2010). Economic costs include direct crop losses, control and prevention of *C. capitata* infestation (both pre- and post-harvest), and limited or loss of access-free export markets (Cruz-Miralles et al. 2022). Total damage caused by tephritids in crop production, harvesting, packing, and marketing activities worldwide was estimated to amount to more than two billion dollars annually (Enkerlin et al. 2017).

Until recently, the preferred control option for fruit flies in general and especially for *C. capitata* was based on the use of insecticides, often in combination with food attractants targeting adult flies (Dias et al. 2022, CABI 2023). However, negative effects on biodiversity and human health caused by pesticides have prompted National Plant Protection Organizations to progressively implement policies aimed at reducing pesticide use (Mathis et al. 2022). This situation has increased the interest in alternative more sustainable methods to control *C. capitata*, including fruit bagging, sanitation, mass trapping, biological control, or the sterile insect technique, often combined in the context of area-wide control programs (Pla et al. 2021; Cruz-Miralles et al. 2022). Among these methods, biological control is becoming increasingly important. In a recent review of the biological control of fruit flies, Dias et al. (2022) showed that the most studied species was indeed *C. capitata*. These authors also found that 22% ($n=50$) of the studies considered corresponded to natural/conservation biological control, whereas classical and augmentative biological control accounted for 6% ($n=13$) and 5% ($n=11$) of these studies, respectively. Parasitoids were the most studied fruit fly biological control agent, followed by entomopathogenic fungi (EPF), nematodes (EPN), bacteria, and predators. The fruit fly stage targeted by parasitoids is mostly the larva within the fruit, whereas those targeted by EPF, EPN, and predators are mostly those occurring in the soil, namely late third instar larvae (LIII, which drop from the fruit to the ground, burrow in the soil and pupate), pupae, and teneral adults, which remain on the ground until they can fly. These stages are susceptible to predation by generalist ground-dwelling predators (Monzó et al. 2008, 2011) and to infection by EPF (Quesada-Moraga et al. 2006; Garrido-Jurado et al. 2011a; c) and EPN (Kapranas et al. 2023). The effect of

EPF and EPN can be increased by periodical applications of formulated microbials (augmentative biological control) (Scheepmaker and Butt 2010; Köhl et al. 2019), while that of predators, which include beetles, ants, earwigs, and spiders, can be enhanced through habitat manipulation (conservation biological control) (Cruz-Miralles et al. 2022). Whether these two biological control methods could be combined has not been studied yet.

A recent study investigated the association between three ground cover management options (bare soil, a seeded cover of *Lolium arundinaceum* (= *Festuca arundinacea*), and a mulch of straw), the emergence success of *C. capitata*, and the activity density of the most important groups of ground-dwelling predators (Cruz-Miralles et al. 2022). Reduced *C. capitata* emergence in the seeded cover of *L. arundinaceum* relative to bare soil (29% reduction) was related to higher diversity and activity density of ground-dwelling predators in the seeded cover, where more complex relationships among predators and between them and medfly occurred relative to the other options. Three predatory groups (beetles, ants, and earwigs) were negatively related to *C. capitata* emergence in that cover. Although the activity density of the most abundant predator group in that study, spiders, was not negatively related to *C. capitata* emergence, their important role should not be ruled out. That result could be attributed to spiders feeding mostly on either LIII before burial or teneral adult flies but not on buried pupae, two stages which were not properly considered in that study. Indeed, Monzó et al. (2008) proved that *Pardosa cribata* Simon (Araneae, Lycosidae), the most abundant ground-dwelling spider in citrus orchards in eastern Spain, could prey on both LIII and adults but not on pupae, with a preference for adult fruit flies.

The virulence of EPF toward other tephritids such as the olive fruit fly *Bactrocera oleae* (Rossi) adults and preimaginals has been demonstrated both at laboratory and field conditions, with even an existing commercial development of a control strategy based on the ground application of *Metarhizium brunneum* Petch. (Ascomycota: Hypocreales: Clavicipitaceae) beneath the tree canopy targeting preimaginals (Yousef et al. 2017; 2018). However, there is a lack of such developments for other key tephritids such as *C. capitata*. The pathogenicity of EPF has been widely evaluated for preimaginal *C. capitata* control in laboratory conditions during the last 20 years (Ekesi et al. 2002; Quesada-Moraga et al. 2006; Beris et al. 2013), particularly several aspects related to the influence of (1) abiotic factors in the infection ability (Ekesi et al. 2003; Garrido-Jurado et al. 2011a, c; Gava et al. 2021), (2) natural enemies (Gava and Paranhos 2023), (3) associated endoparasitoids (Ekesi et al. 2005), and (4) pesticides (Mochi et al. 2006; Yousef et al. 2015). However, field or semifield experiments evaluating any of these interactions together or individually have not

been undertaken yet (Gava et al. 2021). Moreover, the use of a ground cover in combination with EPF has not been dealt with yet. Considering that the adoption of cover crops is a key agricultural tactic to achieve the EU's ambitions to reduce EU greenhouse gas emissions by 2030 (Riviere et al. 2022), the evaluation of the compatibility of any pest control measure targeting the soil with natural or artificial ground covers is urgently needed. As to date, we are not aware of any study addressing the compatibility of ground cover management and the application of EPF to control *C. capitata*; in this study, we have investigated the effect of bare soil and a seeded cover of *L. arundinaceum* when either treated or not with *M. brunneum* on the emergence of *C. capitata*. To ascertain any side-effect of *M. brunneum* on the most relevant groups of ground-dwelling predators, we have also evaluated the effect of these treatment combinations on the activity density of the main groups of generalist ground-dwelling predators (spiders, beetles, ants, and earwigs). Our objective has been to explore the association between these treatment combinations and (1) the emergence success of *C. capitata*; and (2) the abundance of the most important groups of ground-dwelling predators of *C. capitata*. We challenged the hypothesis that the enhanced activity of predators in the seeded cover of *L. arundinaceum* could be impaired by the application of *M. brunneum*, which could therefore be incompatible.

Materials and methods

Experimental orchard

Experiments were carried out from June to December 2022 in a 1 ha citrus orchard located in Les Alqueries (Spain, 39° 54' N; 00° 06' W), already used in previous studies on the effects of ground cover management on the emergence of *C. capitata* (Cruz-Miralles et al. 2022). Trees were 20-year-old "Clemenules" mandarins [*Citrus clementina* Tanaka (Rutaceae)] grafted on citrange Carrizo (*Poncirus trifoliata* (L.) Rafinesque × *Citrus sinensis* (L.) Osbeck). Rows were 6 m apart and followed a N-S orientation. Within rows, trees were spaced 4 m, drip-irrigated, and maintained weed-free by herbicide treatments (glyphosate). Twenty-four adjacent trees in two consecutive rows (12 trees per row) in the middle of the orchard were removed in 2014 and replanted in late summer 2019 with 2-year-old clementine trees (same scion-rootstock combination as before). These 24 trees were individually enclosed in an aluminum cage (4 × 4 × 4 m) covered with a rigid nylon mesh (10 × 14 threads cm⁻²) on all sides but one (the western side of the western row and the eastern side of the eastern row). These cages received one of the two ground management treatments considered in this study, which were randomly distributed within each

row (i.e., 6 cages per treatment and row; see Fig. 1 Supplementary). The first treatment was bare soil by means of herbicide application (glyphosate + MCPA). The second treatment consisted of a homogeneous cover of *L. arundinaceum*, which was established in early autumn 2019 and subsequently mowed twice per season with grass clippings left in place. In this treatment, the vicinity of the tree was maintained free of *L. arundinaceum* by mechanical means. A datalogger (CEM, model DT-171; www.cem-instruments.com/en/Product/detail/id/980), which continuously monitored temperature and relative humidity values at 1-h intervals, was set in one of the cages. Daily rainfall measurements were obtained from the meteorological station of Vila-Real (<http://riegos.ivia.es/listado-de-estaciones/vila-real>), which is located 5 km NW of the orchard.

Fungal strain, cultivation, and inoculum production

The EAMa 01/58-Su *M. brunneum* strain used in this study was obtained from the culture collection at the Agronomy Department of the University of Cordoba. This strain was originally isolated from soil in a wheat plantation at Hinojosa del Duque, Córdoba, Spain. The strain was deposited in the Spanish collection of culture types (CECT) with accession number CECT 20764. This fungal strain was licensed to Koppert B.V., Netherlands, in 2021. The cultivation and inoculum production for the experiment were performed as described by Yousef et al. (2017).

Soil application of the fungal suspension

For each ground management option (bare soil, BS, and a seeded cover of *L. arundinaceum*, LA), cages were randomly assigned to one of the following three groups: control, one, and two applications of the fungal suspension (C, A1, and A2 in Fig. 1 Suppl., respectively) with four replicates (two per row) for each treatment * application combination. On June 28, 2022, the soil of the cages assigned to A1 and A2 was sprayed with 1 l of a suspension of *M. brunneum* EAMa 01/58-Su strain, which contained 2.5 g of conidia or 1 × 10⁸ viable conidia, using an atomizer (Yousef et al. 2017). In the control cages, the soil was sprayed with 1 l of water. In all the experiments and subsequent to fungal application, the presence of *M. brunneum* in the soil was monitored. For this, soil samples were collected immediately after the treatment and then, once per month, with six completely randomized soil samples collected using a soil corer (5 cm in diameter) to a depth of 15 cm. These samples were transferred to the laboratory to determine the presence of entomopathogenic fungi in the soil using a selective growing medium. Briefly, 1 g of the homogenized soil sample was added to 9 ml of sterile distilled water, and shaken for 60 min. After homogenization, aliquots of 100 µl were spread onto Petri

plates containing Sabouraud glucose agar chloramphenicol medium (0.5 g/l). The plates were incubated at 25 °C for 7–10 days and colony forming units were counted (Garrido-Jurado et al. 2011a). Based on this monitoring and on results of adult emergence (see below), a second treatment was forecasted for half of the treated cages (A2 in Fig. 1 Suppl.) toward the end of summer–early autumn. At that time heavy rains, which could wash the inoculum from the soil, usually take place in the study area.

***Ceratitis capitata* stock colony**

Third instar larvae (LIII) of the Vienna-8^{D53-} genetic sexing strain obtained from a mass-rearing facility located in Caudete de las Fuentes (Valencia, Spain) were used in our assays. These insects were reared on an artificial diet at a temperature of 25.8 ± 0.3 °C and $91.5 \pm 1.5\%$ RH at dark (Pla et al. 2021).

***C. capitata* management**

From June to November 2022, once or twice per month (depending on results), around 3000 mature third instar larvae (LIII) were refrigerated (9 °C) and transferred to UJI facilities, where they were split in groups of 100. Two of these groups were kept in the laboratory and 24 were further processed in the field. One of the two laboratory groups was introduced into a 10 cm in diameter Petri dish, which was wrapped in aluminum foil to keep it dark and transferred to a climatic cabinet set at 25:20 °C and a 12:12 L:D photoperiod. The other laboratory group was set on top of a 21.5 cm diameter and 19 cm high pot containing a mixture of vermiculite and peat (1:3; vol:vol) watered to field capacity and similarly transferred to the same climatic cabinet. Pupation, adult emergence, and sex ratio were scored. The former two values were used as control to correct values recorded for field-released insects (see below) while the sex ratio was used as reference to detect any difference with field-released insects (see below).

The 24 groups of 100 LIII each were eventually released in the field, one per cage. Larvae were released in an emergence trap (see Cruz-Miralles et al. 2022 for details) consisting of a 15-cm long and 12 cm diameter toilet soil pipe fitted to a bottomless transparent PVC bottle of the same diameter and 35 cm long, with a zenithal ventilation hole covered with a mesh to prevent water condensation. The pipe and the bottle were fitted with a rubber band. Prior to the release, a hole of the same dimensions as the soil pipe was dug by carefully extracting a soil cylinder. Then, the pipe was put in place and the previously removed soil cylinder introduced into the pipe. The 100 LIII were distributed on the surface of the trap, which was covered with the bottomless bottle to protect them from

predation. Two days later the bottles were removed and the number of individuals (either larvae or pupae) remaining on the top of the trap were counted and removed. This figure was considered as representative of unsuccessful larval burial (i.e., individuals either dead or fully exposed to predation). Then, the trap was left uncovered to allow full exposure of the buried individuals to natural enemies and abiotic stressors (i.e., rainfall). Based on the temperatures measured in the orchard, the thermal constant and the lower development threshold of *C. capitata* (260 DD and 11 °C, respectively; Escudero-Colomar et al. 2008), the emergence date was estimated. One week before reaching that date, the bottomless bottle was set again in place. At that moment, the bottle included a transparent plastic sheet sprayed with tangle-trap® covering the inner wall of the bottle. Traps were periodically inspected and adults found stuck on the glue were counted, removed, and taken to the laboratory for further processing (see below). The whole trap was removed after seven days with no adult captures. Field unsuccessful larval burial (% of total LIII) and adult emergence (% expressed on both total LIII and those successfully buried) were calculated based on the number of larvae and adults counted. These percentages were corrected with the corresponding laboratory values (Abbott 1925). As soon as the traps were removed (from 15 to 30 days after initial setup), a new set of larvae and traps was put in place.

Specimens stuck on the glue of the emergence trap were taken to the laboratory to ascertain whether they were infected by *M. brunneum*. These flies were immediately surface-sterilized with 1% sodium hypochlorite followed by three rinses with sterile distilled water for 1 min each. They were then placed on sterile wet filter paper in sterile petri plates, sealed with parafilm, and kept at 25 °C to be inspected for fungal outgrowth on the cadavers (Quesada-Moraga et al. 2006).

Pitfall traps

From June to December 2022, to identify potential ground predators of *C. capitata*, a pitfall trap was set in each cage (24 traps in total). Traps consisted of a plastic jar (12.5 cm diam., 12 cm depth), with a plastic funnel fitted to its upper edge. A plastic container (250 ml) half filled with a 3:1 mixture of water and ethanol, and 1.25% antifreeze, was placed inside the plastic cup. Traps were serviced every 15 days. Captures were transported to the laboratory, where they were sorted out. Specimens were determined to family level under a binocular microscope and categorized into the following generalist soil-dwelling predator groups: Coleoptera (Carabidae and Staphylinidae), Dermaptera (Forficulidae), Formicidae, and Araneae (Lycosidae).

Statistical analyses

Corrected unsuccessful larval burial (%), adult emergence (%; relative to the initial 100 LIII and to those LIII successfully buried in the soil), sex ratio (females/total), adult infection (%), and predator counts for Coleoptera, Formicidae, Dermoptera, and Araneae (activity density; number per trap per day) were modeled using generalized linear mixed models (GLMM) with a negative binomial distribution of the error and a logit link function, with treatment (*M. brunneum* or control), cover (BS or LA), and date as fixed factors and replicate (= cage) as a random factor. All interactions (2- and 3-factor) were considered. Once differences along time were established, for each date, (1) unsuccessful larval burial and adult emergence were further modeled using general linear models (GLM) with a binomial distribution of the error and logit link function, and (2) predator counts were modeled using GLM with a quasi-Poisson distribution of the error and a logit link function. In all cases, treatment, cover, and their interaction were considered as fixed factors. Models were refined by progressively removing non-significant ($P > 0.05$) factors. Akaike information criterion (AIC) (Akaike 1974) was used to select the best model. When necessary, pairwise comparisons were made using Tukey post hoc test ($P < 0.05$). The R software (R Core Team 2023) was used to fit the models. The package lme4 (Bates et al. 2015) was used to fit both GLMMs and GLMs, and the package multcomp (Hothorn et al. 2008) was used to perform the pairwise comparisons.

Results

Mean monthly values of temperature and relative humidity ranged from 13.3 to 27.6 °C (mean: 21.9 °C) and 55 to 87.3% (mean: 75.2%), respectively, during the study period. The absolute minimum and maximum temperatures ranged from 5.8 to 20.3 °C and 24.9 to 40.3 °C, respectively. A total of 321.5 mm of rain were registered during this period, with 39.6 mm on June 27, the day prior to the application of the fungus, and two episodes of heavy rain (> 40 mm in 24 h) on November 11 and 12. From the application day to October 5, rain occurred only 8 times for a total of 18.7 mm during this 100-d period (Fig. 1).

Fungal inoculum was found in treated cages only and no *Metarhizium* species were found in the untreated cages. The concentration of *M. brunneum* EAMa 01/58-Su strain ranged from $5.0 \cdot 10^4$ to $8.3 \cdot 10^5$ CFU g^{-1} soil for bare soil, 147 and 14 days after treatment, respectively (Fig. 1). As a consequence, no additional fungal application was made at the end of the summer as initially planned, which eventually made the number of replicates (cages) for the fungus-treated cages double than control (8 versus 4 replicates per cover for fungus-treated and control cages, respectively). Although sentinel LIII were introduced into the cages at monthly intervals, no adults could be recovered for those released beyond day 87 after the fungal application (September 29, 2022). Therefore, the experiment was discontinued in December 2022.

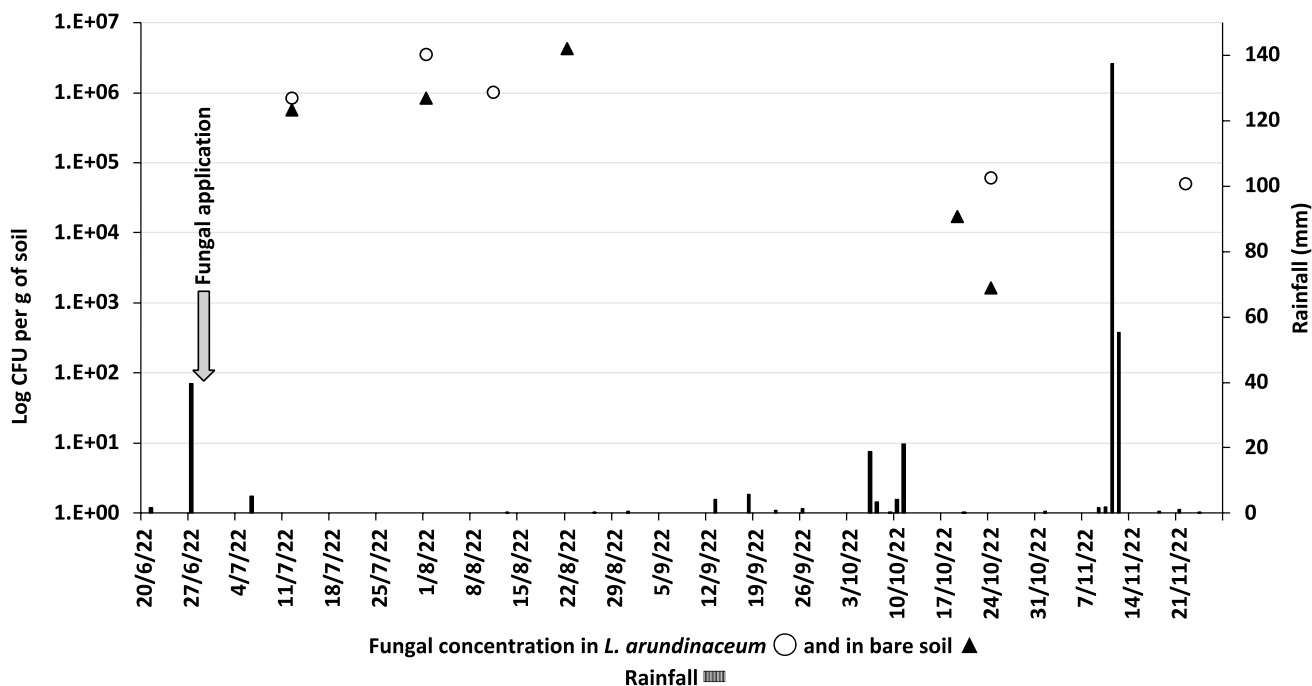


Fig. 1 Rainfall (bars) and fungal concentration (dots) during the assay. The fungal treatment (arrow) was applied on June 28

Larval successful pupation in the laboratory was 100%. Consequently, no correction was needed for field-collected data. Successful larval burial into the soil (Fig. 2) was significantly affected by time since fungal application (z -value = -13.22 ; $P < 0.001$) and its interaction with fungal application (z -value = -5.03 ; $P < 0.001$) and cover (z -value = 13.72 ; $P < 0.001$). All other interactions were not significant (AIC = 2809.3 and $df = 137$ for the model). When each date was separately analyzed (Table 1), with the exception of the first date after application, successful burial was significantly higher in bare soil relative to *L. arundinaceum* with no clear effect of the fungal application (Table 1).

Adult emergence in the laboratory ranged from 90.5 to 94.5%. These values were used to correct field-collected data (Fig. 2). Field adult emergence (irrespective of whether referred to the 100 sentinel LIII released or to those successfully buried into the soil) was affected by fungal application (z -value = -2.16 ; $P = 0.030$ and z -value = -1.91 ; $P = 0.050$, respectively), time since application (z -value = 10.18 ; $P < 0.001$ and z -value = 8.39 ; $P < 0.001$, respectively) and the interactions of time with fungal application (z -value = -2.86 ; $P = 0.004$ and z -value = -3.11 ; $P = 0.002$, respectively) and cover (z -value = -3.73 ; $P < 0.001$ and

z -value = -3.14 ; $P = 0.002$, respectively). All other interactions were not significant (AIC = 652.8 and 629.2, respectively, and $df = 89$ in both models). When each date was separately analyzed (Tables 2 and 3), with the exception of the first date after application, adult emergence was higher in bare soil relative to *L. arundinaceum* and in control relative to *M. brunneum*-treated cages (Tables 2 and 3). As consequence, the efficacy of the different combinations tested relative to bare soil at inhibiting adult emergence ranged from 59.4 to 92.5% in fungus-treated bare soil and fungus-treated *L. arundinaceum*, respectively. The sex ratio of these adults ranged from 0.06 to 0.58 females/total, with differences between dates (AIC = 246.7; z -value = 2.215; $df = 71$; $P = 0.026$) but no significant effects of the fungal treatment, the cover, or their interactions. Adult infection ranged from 5.1% for fungus-treated bare soil 87 days after the treatment to 67.3% for fungus-treated *L. arundinaceum* 64 days after the treatment. However, the low number of adults emerged and recovered from fungus-treated cages (Table 2) precluded the successful modeling of these results.

The results of the GLMM applied to the activity density of the different groups of ground-dwelling predators are shown in Table 4. Time since fungal application was

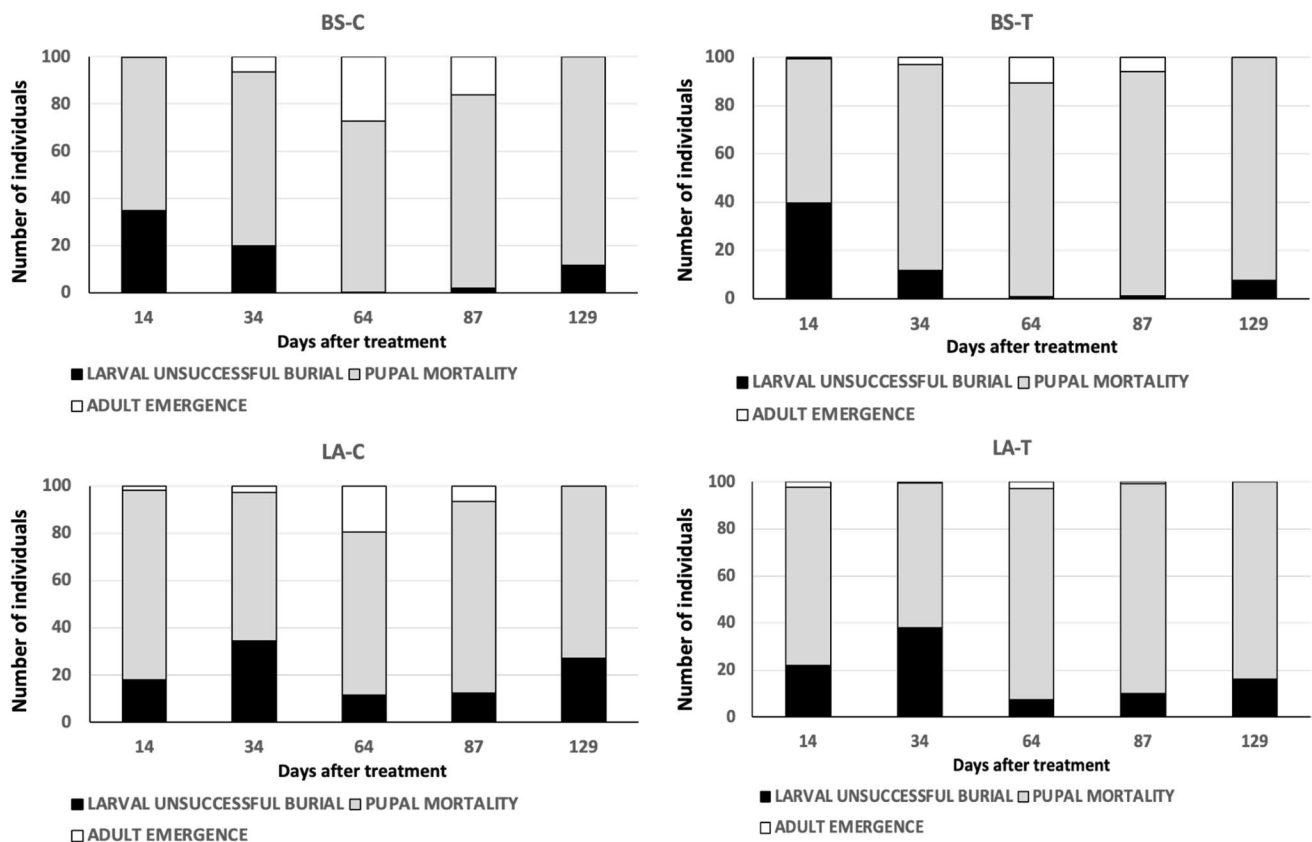


Fig. 2 Fate of the 100 sentinel LIII *C. capitata* released at different times after fungal treatment (14–129 days) on either bare soil (BS) or a seeded cover of *L. arundinaceum* (LA) and subject to an application of *M. brunneum* (BS-T, LA-T) or not (BS-C, LA-C)

Table 1 Statistics (AIC; df; z- and P-values) of the GLM adjusted to larval unsuccessful burial into the soil at different times after fungal treatment and larval unsuccessful burial into the soil (%) and efficacies (%; in brackets) relative to BS-C (Means \pm SE)

Time (d)	AIC	df	Factor/interactions (z-value; P)			Larval unsuccessful burial (%) and efficacy (%; in brackets) relative to BS-C			
			Treatment	Cover	T*C	BS-C	BS-T	LA-C	LA-T
14	727.56	21	3.61; 0.001 C < T	-7.24; <0.001 LA < BS	ns	35.0 \pm 14.0 Aa -	39.8 \pm 8.9 Ab (7.3)	18.0 \pm 12.1 Ba (0)	21.9 \pm 9.9 Bb (0)
34	731.71	20	-3.62; 0.001	4.64; <0.001	3.84; <0.001	19.8 \pm 9.6 b -	11.9 \pm 8.6 c (0)	34.5 \pm 8.4 a (18.4)	37.9 \pm 10.4 a (22.6)
64	143.55	22	ns C = T	7.33; <0.001 LA > BS	ns	0.3 \pm 0.3 B -	0.9 \pm 0.4 B (0)	11.5 \pm 4.1 A (11.3)	7.5 \pm 2.8 A (7.3)
87	185.15	22	ns C = T	8.22; <0.001 LA > BS	ns	2.0 \pm 1.1 B -	1.3 \pm 0.7 B (0)	12.5 \pm 5.0 A (10.7)	10.1 \pm 3.9 A (8.3)
129	315.52	45	-2.62; <0.001 C > T	13.56; <0.001 LA > BS	ns	11.8 \pm 3.7 Ba -	7.5 \pm 1.2 Bb (0)	27.0 \pm 4.4 Aa (17.3)	16.0 \pm 3.0 Ab (4.8)

Within each row, values followed by different letters are significantly different ($P < 0.005$). Except for time = 34, where the interaction between treatment and cover was significant (see Table 2), capital letters refer to the cover as lowercase letters to the fungal treatment

Table 2 Statistics (AIC; df; z- and P-values) of the GLM adjusted to adult emergence (% expressed on 100 LIII released) at different times after fungal treatment and adult emergence (% expressed on 100 LIII released) in bare soil (BS) and a seeded cover of *Lolium arundinaceum* (LA) either treated (BS-T, LA-T) or not (BS-C; LA-C), as well as the corresponding efficacies (%; in brackets) relative to BS-C. Means \pm SE

Time (d)	AIC	df	Factor/interactions (z-value; P)			Adult emergence (% expressed on 100 LIII released) and efficacies (%; in brackets) relative to BS-C			
			Treatment	Cover	T*C	BS-C	BS-T	LA-C	LA-T
14	67.722	22	ns C = T	3.22; 0.001 LA > BS	ns	0.3 \pm 0.3 B -	0.6 \pm 0.9 B (0)	1.9 \pm 0.3 A (0)	2.2 \pm 0.8 A (0)
34	79.031	21	-3.76; <0.001 C > T	-3.97; <0.001 LA < BS	ns	6.4 \pm 0.5 Aa -	2.9 \pm 0.7 Ab (54.7)	2.7 \pm 0.4 Ba (57.8)	0.4 \pm 0.4 Bb (93.8)
64	215.03	20	-6.97; <0.001	-2.57; 0.010	-3.06; 0.002	27.2 \pm 7.8 a -	10.6 \pm 2.4 c (61.0)	19.3 \pm 7.5 b (29.0)	2.9 \pm 1.4 d (89.3)
87	110.78	21	-6.81; <0.001 C > T	-6.08; <0.001 LA < BS	ns	16.0 \pm 3.2 Aa -	6.0 \pm 1.1 Ab (62.5)	6.4 \pm 0.5 Ba (60.0)	0.9 \pm 0.7 Bb (94.4)

Within each row, values followed by different letters are significantly different ($P < 0.005$). Except for time = 64, where the interaction between treatment and cover was significant, capital letters correspond to the cover as lowercase letters to the fungal treatment

significant in all cases, the fungal application was significant for all groups except for spiders, the cover was also significant in all groups except for ants, and the only significant interaction, treatment*time, occurred in earwigs. When each group was separately analyzed by date, for beetles, treatment was significant ($P < 0.05$) only 65 and 93 days after treatment, whereas cover was not significant ($P > 0.05$) 7, 21, 65, and 135 days after treatment. When significant, beetle captures were higher in *L. arundinaceum* relative to bare soil and in those two dates where the treatment was also significant, higher values were observed in control cages (Fig. 3). On average, staphylinids and carabids represented around 50% of total captures each in all four treatment combinations. For ants, treatment was significant only 36 and 65 days after treatment. On both dates, ant captures were

lower in control cages (Fig. 4). For earwigs neither treatment nor cover or their interaction were ever significant (Fig. 5). Finally, for spiders (mostly Lycosidae), cover was significant for all dates up to 107 days after treatment, with higher spider captures in *L. arundinaceum* compared to bare soil. Afterward, there were no differences between covers (Fig. 6).

Discussion

In agreement with previous results (Cruz-Miralles et al. 2022), the *L. arundinaceum* cover alone proved effective at reducing *C. capitata* larval burial and adult emergence compared to bare soil (14.4 and 48.9% mean reductions,

Table 3 Statistics (AIC; df; z- and P-values) of the GLM adjusted to adult emergence (% expressed on LIII successfully buried) at different times after fungal treatment and adult emergence (% expressed on 100 LIII successfully buried) in bare soil (BS) and a seeded cover of*Lolium arundinaceum* (LA) either treated (BS-T,LA-T) or not (BS-C; LA-C), as well as the corresponding and efficacies (%; in brackets) relative to BS-C. Means \pm SE

Time (d)	AIC	df	Factor/interactions (z-value; P)			adult emergence (% expressed on 100 LIII successfully buried) and efficacies (%; in brackets) relative to BS-C			
			Treatment	Cover	T*C	BS-C	BS-T	LA-C	LA-T
14	65.286	22	ns C=T	2.82; 0.004 LA>BS	ns	0.9 \pm 1.0 B –	0.9 \pm 0.5 B (0)	2.4 \pm 0.3 A (0)	2.8 \pm 1.1 A (0)
34	90.024	21	–3.99; <0.001 C>T	–3.07; <0.001 LA<BS	ns	8.3 \pm 1.5 Aa –	3.9 \pm 1.3 Ab (53.3)	4.2 \pm 0.8 Ba (49.6)	2.1 \pm 2.2 Bb (74.8)
64	223.33	20	–6.92; <0.001	–1.64; 0.10	–3.32; <0.001	27.3 \pm 7.8 a –	10.7 \pm 2.5 c (60.9)	22.7 \pm 9.5 b (17.0)	3.1 \pm 1.4 (88.8)
87	110.21	21	–6.89; <0.001 C>T	–5.52; <0.001 LA<BS	ns	16.4 \pm 3.2 Aa –	6.1 \pm 1.1 Ab (62.7)	7.3 \pm 0.5 Ba (55.1)	1.0 \pm 0.7 Bb (93.8)

Within each row, values followed by different letters are significantly different ($P < 0.005$). Except for time = 64, where the interaction between treatment and cover was significant, capital letters correspond to the cover as lowercase letters to the fungal treatment

Table 4 Statistics (AIC; df; z- and P-values) of the GLMMs adjusted to activity density of Coleoptera, Formicidae, Dermaptera, and Araneae. Treatment (control, C, and *M. brunneum*, T) and cover (baresoil-BS, *L. arundinaceum* cover-LA) were considered as a fixed factors and time as a random factor

Predator group	df	Factor/Interactions (z-value; P)						
		Treatment (A)	Cover (B)	Time (C)	A*B	A*C	B*C	A*B*C
Coleoptera AIC=540.1	282	–2.13; 0.033 C>T	8.88; <0.001 LA>BS	3.91; <0.001 ¹	ns	ns	ns	ns
Formicidae AIC=850.9	283	2.40; 0.016 C<T	ns LA=BS	–3.60; <0.001 ¹	ns	ns	ns	ns
Dermaptera AIC=292.1	281	–1.47; 0.143 C<T	5.09; <0.001 LA>BS	–2.81; 0.005 ¹	ns	2.12; 0.034 ^a	ns	ns
Araneae AIC=573.0	283	ns C=T	10.51; <0.001 LA>BS	–2.50; 0.012 ¹	ns	ns	ns	ns

¹See text and Figs. 2, 3, 4 and 5 for differences along time and the interaction treatment * time for Dermaptera

respectively). Due to the short time elapsed between the fungal application and the evaluation of larval burial (2 days), as expected, no effect of *M. brunneum* on burial success was detected. However, adult emergence was reduced by *M. brunneum* for up to three months after fungal application, with the combination of *L. arundinaceum* and *M. brunneum* being the most effective at reducing *C. capitata* emergence relative to bare soil (92.5%; Fig. 2). Although *M. brunneum* also reduced the activity density of ground-dwelling predatory beetles (up to 93 days after the application), it showed no clear negative effects on earwigs, no effects on spiders, and a positive effect on ants (up to 65 days after the application), as already observed in olive groves for the same strain of *M. brunneum* (Garrido-Jurado et al. 2011b). Therefore, our initial hypothesis that the enhanced activity of predators in the seeded cover of *L. arundinaceum* could be impaired by *M. brunneum*, proved only partially correct. Yet, the combined use of a ground cover and *M. brunneum* against the

soil-dwelling stages of *C. capitata* is compatible and they seem to work synergistically.

The lack of heavy rain from the start of the assay until mid-autumn (Fig. 2) could explain the long-term effect of *M. brunneum* on *C. capitata* observed in both bare soil and the *L. arundinaceum* cover. Indeed, the inoculum could be still detected in the soil 150 days after treatment at a concentration of 5×10^4 CFU g⁻¹. Surprisingly, almost 40 and 200 mm of rain, which could have washed the fungus from the upper layers of the soil where *C. capitata* pupates, were registered around mid-October and mid-November, respectively. This higher-than-expected persistence of *M. brunneum* (keep in mind that a second fungal application toward the end of the summer had been initially planned) could be an important asset of *M. brunneum*. Indeed, field experiments have shown that this fungus can persist over 250 days in the soil (Hernández et al. 2023). This result, though, could also be related to *M. brunneum* multiplying in the soil when

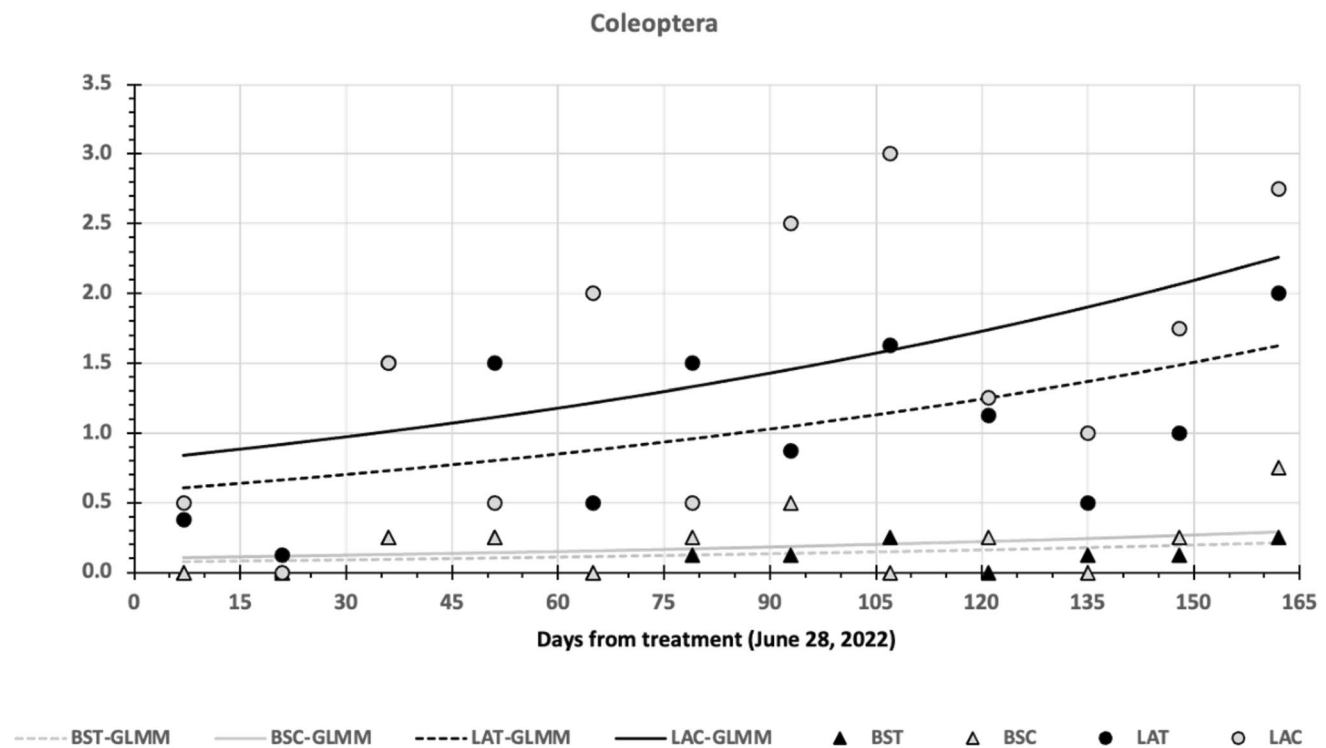


Fig. 3 Coleoptera activity density in $4 \times 4 \times 4$ m³ cages with either bare soil (BS) or a seeded cover of *L. arundinaceum* (LA) combined with a treatment of *M. brunneum* (T; applied on June 28, 2022) or control (C). Pitfall traps were sampled every 15 days from treatment

to 11 December, 2022; Dots and triangles represent the measured values as lines, the GLMMs fitted. Lines are significantly different ($P < 0.050$, see Table 4)

insect hosts are present, as shown in studies where fungal concentration increased in the presence of susceptible hosts (Vestergaard et al. 2003; Meyling and Eilenberg 2007; EFSA 2020). The infected teneral adults of *C. capitata* recovered in our study can be taken as evidence of the reproduction of *M. brunneum* EAMa 01/58-Su strain in the soil. Interestingly, our methodology, which included the washing of the adults captured in the traps before their processing, likely underestimated the impact of *M. brunneum* on emerged flies, as the percentage of infested insects should most probably be higher than that of infected ones. Infection of non-target insects with *M. brunneum* following its soil application has been documented for Coleoptera in Danish alfalfa fields (Vestergaard et al. 2003) and Coleoptera and Hemiptera in a Danish fir plantation (Nielsen et al. 2006). Therefore, fungal infection of non-target insects could explain the lower activity density of predatory beetles recorded in fungus-treated compared to control plots (Table 4; Fig. 3). A completely different situation occurred to the other three groups of predators examined in this study (ants, earwigs, and spiders). As soil-dwelling arthropods have co-evolved with pathogenic microorganisms, which are widespread in the soil, like EPF, these arthropods have developed strategies to cope with infection (Vanninen et al. 1999). In turn, these strategies have been overcome by entomopathogenic microorganisms

in an ongoing arms race (Ortiz-Urquiza and Keyhani 2013). Tephritids, for instance, present a puparium, which makes the pupal stage less susceptible to EPF (Ekesi et al. 2002). This has been related to the nature of the puparium, which originates from the cuticle of LIII, which remains in tephritids as an extra protecting layer for the pupa conferring a barrier to penetration by EPF (Ekesi et al. 2002). Indeed, the cuticle is the first and major protective barrier against pathogen infections in arthropods. Puparia, though, do not occur in ants, which did even better in *M. brunneum*-treated than in control plots, in spiders, for which this fungus was neutral, and earwigs. Ants, produce antimicrobial secretions inhibiting EPF (Bot et al. 2001; Rodrigues et al. 2009; Garrido-Jurado et al. 2011b), and can avoid and differentially manage prey depending on their infectious potential (Pereira and Detrain 2020), a phenomenon observed in larvae of *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) as well (Ríos-Moreno et al. 2018). Additionally, ants, as other eusocial insects, have evolved many effective defense mechanisms against pathogens (Cremer et al. 2007; Meunier 2015), like self- and allo-grooming (Reber et al. 2011) or nest cleaning (Bot et al. 2001; Jackson and Hart 2009; Diez et al. 2012). These behaviors, which complement individual immune defense are called social immunity (Diehl and Meunier 2018). Because Cruz-Miralles et al.

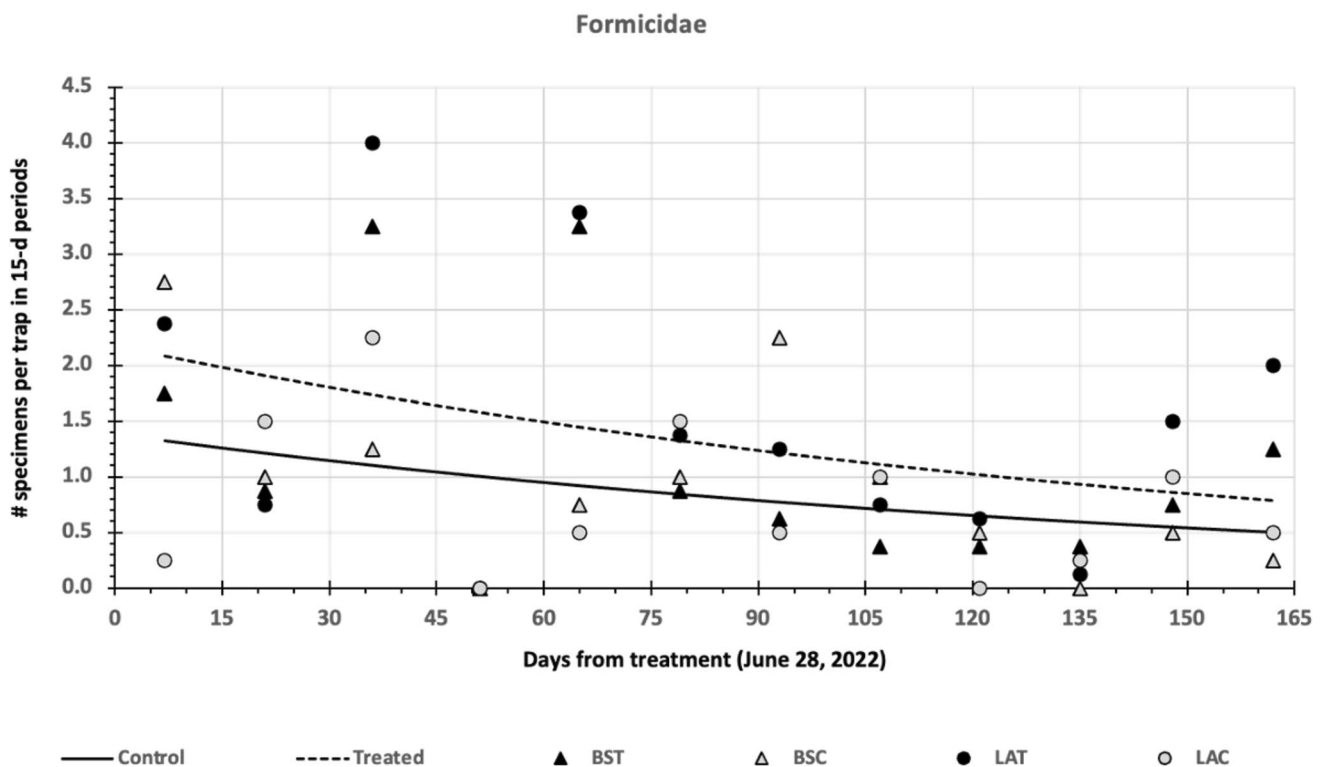


Fig. 4 Formicidae activity density in $4 \times 4 \times 4$ m³ cages with either bare soil (BS) or a seeded cover of *L. arundinaceum* (LA) combined with a treatment of *M. brunneum* (T; applied on June 28, 2022) or control (C). Pitfall traps were sampled every 15 days from treatment

to 11 December, 2022; Dots and triangles represent the measured values as lines, the GLMMs fitted. Lines are significantly different ($P < 0.050$, see Table 4)

(2022) identified the occurrence of intra-guild interference (which could include competition, predation, or risk avoidance behavior) between ants and beetles in a cover of *L. arundinaceum*, the effective defense mechanisms identified in ants may have allowed this group to have an additional advantage over ground-dwelling beetles resulting in an ant-mediated negative effect of *M. brunneum* on beetles. Hence, the negative effects observed on beetles in our study (Fig. 3) could be attributed to both the direct and indirect effects of fungal application.

Social immunity is not exclusive to eusocial insect species, like ants, bees, or termites, and can be found in less complex forms of group living, such as the simple family units of earwigs. In these units, parents may cover the nest with feces with antimicrobial activity (Diehl et al. 2015) and females, which do not avoid EPF-contaminated environments, can mitigate the associated costs of pathogen exposure by adjusting their level of egg care (Diehl and Meunier 2018). In particular, the presence of pathogens shortened the duration of egg abandonment, which favors the removal of pathogens from the egg surface (Boos et al. 2014) and favored the construction of a nest, a form of hygienic

behavior that may help cleaning the nest from pathogen spores by shifting the sands around (Arathi et al. 1999). Same as with ants, social immunity in earwigs could explain the lack of clear negative impacts of the application of *M. brunneum* on the activity density of this group of omnivorous predators (Fig. 5). Although Fischhoff et al. (2018) reported reduced survival of the wolf spider *Schizocosa ocreata* (Hentz) (Araneae: Lycosidae) after soil application of *M. brunneum* in a field microcosm study, these authors suggested that potential interference between fungal application and *S. ocreata* should be examined at larger scales. Our study provides evidence of absence of impact of *M. brunneum* EAMa 01/58-Su strain on ground-dwelling spiders (Fig. 6). To sum up, these results point at this strain as mostly safe for the predatory guild of *C. capitata* occurring in the soil. The harmful effects detected on beetles disappeared three months after the fungal application. Therefore, the simultaneous use of a ground cover of *L. arundinaceum* as a conservation biological control method to increase the populations of the predators of *C. capitata*, and that of *M. brunneum* as an augmentative biological control method, can enhance the control efficacy against the soil-dwelling

Dermaptera

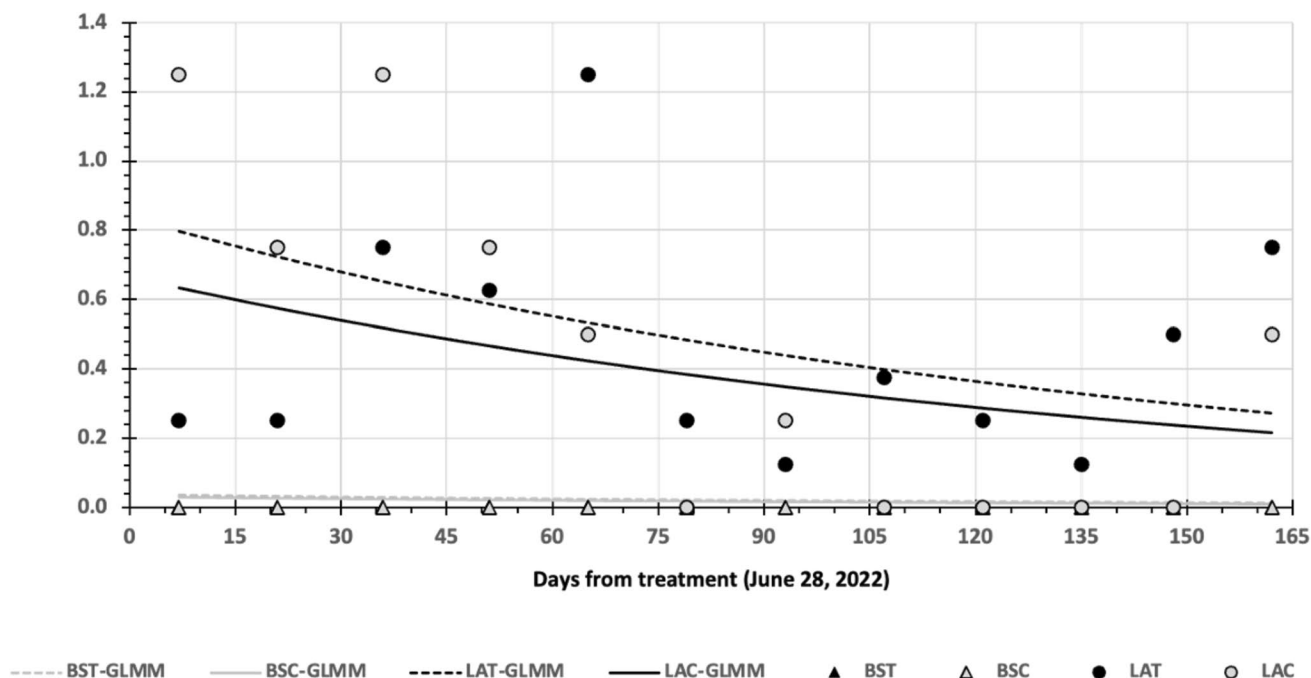


Fig. 5 Dermaptera activity density in $4 \times 4 \times 4$ m³ cages with either bare soil (BS) or a seeded cover of *L. arundinaceum* (LA) combined with a treatment of *M. brunneum* (T; applied on June 28, 2022) or control (C). Pitfall traps were sampled every 15 days from treat-

ment to 11 December, 2022; Dots and triangles represent the measured values as lines, the GLMMs fitted. Lines are significantly different ($P < 0.050$, see Table 4)

stages of this fruit fly. Moreover, as the use of *L. arundinaceum* also favors the conservation along the year of some key above-ground natural enemies of other important fruit pests, like mites, thrips, and aphids (Aguilar-Fenollosa et al. 2011a, b, c; Aguilar-Fenollosa and Jacas 2013; Gómez-Marco et al. 2015; Gómez-Martínez et al. 2018), such a ground cover appears as a strong and sustainable conservation biological control method against fruit orchard pests.

Commercial fruit orchards, including citrus, usually contain one single cultivar or two, when they are self-incompatible, but usually reach harvest maturity at around the same time. As fruit flies prefer fully ripe fruit to oviposit (Aluja and Mangan 2008), most fruit within an orchard become susceptible to *C. capitata* around the same time. The window period for *C. capitata* oviposition could extend for about two months, from the moment when fruit become receptive until unharvested fruit either mummify or decompose and can no longer support *C. capitata* immature development (this window would shrink in case that sanitation is applied to unharvested fruit). Therefore, one single treatment of *M. brunneum* applied before harvest, with an efficiency extending up to three months, could be enough to reduce the emergence of flies developing on those fruits. Only in case of heavy rain, a second treatment could be advisable. Because such a treatment would not reduce damage in the

orchard where the application takes place during the ongoing season but reduce the density of further developing adult populations, it could ideally be part of an area-wide IPM program, where this ground application could target hotspots on a collective basis. To calculate the cost for the farmer of the different combinations considered in this study, we have followed (1) Aguilar-Fenollosa et al. (2011c) for the establishment and maintenance of bare soil and the seeded cover of *L. arundinaceum* and (2) Sosa-Gómez (2017) and Lee et al. (2023) for the soil application of *M. brunneum*. Based on these calculations, the most cost-effective tactic would be the use of a ground cover of *L. arundinaceum* in combination with a single soil application of *M. brunneum* (Table 5).

Author contributions

JAJ and EQM conceived and designed research. JCM, YM, and IGJ conducted the experiments. OD contributed *C. capitata* larvae. MVI and JAJ analyzed data. JAJ prepared the first draft of the manuscript, which was read, modified, and eventually approved by all authors.

Araneae

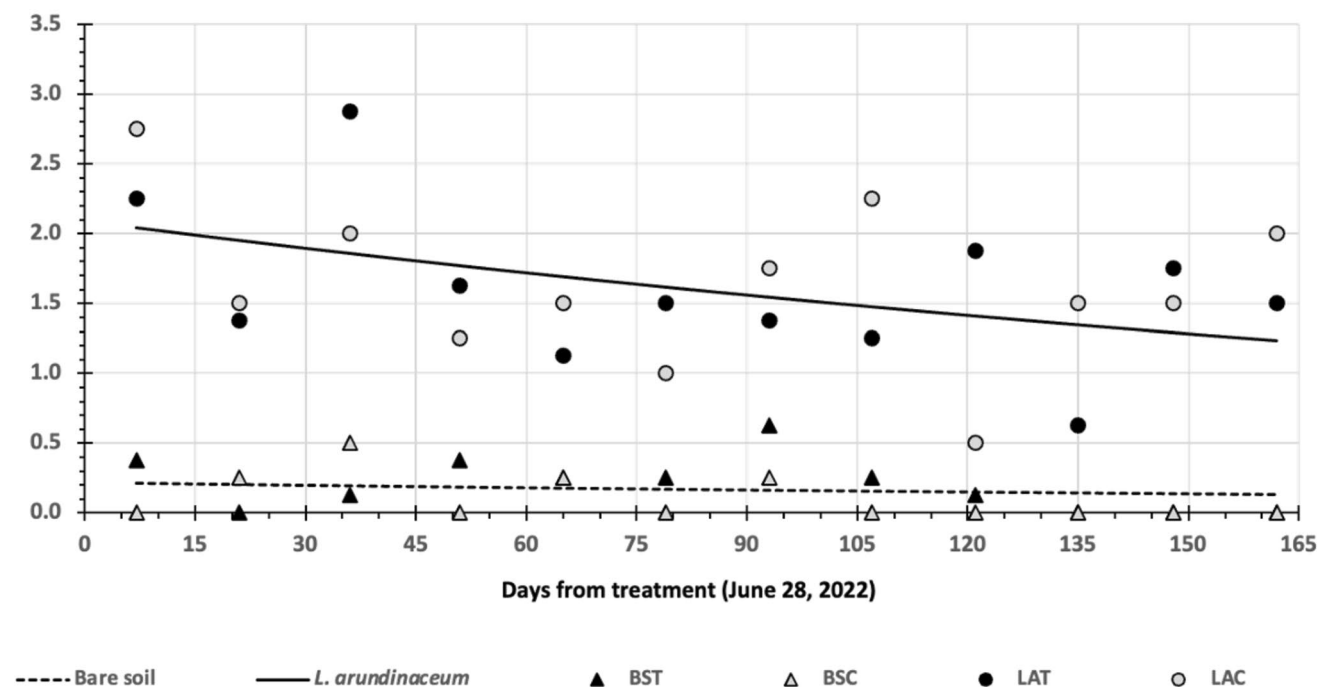


Fig. 6 Araneae activity density in $4 \times 4 \times 4$ m³ cages with either bare soil (BS) or a seeded cover of *L. arundinaceum* (LA) combined with a treatment of *M. brunneum* (T; applied on June 28, 2022) or control (C). Pitfall traps were sampled every 15 days from treatment to 11

December, 2022; Dots and triangles represent the measured values as lines, the GLMMs fitted. Lines are significantly different ($P < 0.050$, see Table 4)

Table 5 Cost-effectiveness of the different management options for the ground cover against *C. capitata*: bare soil either by use of herbicides (BS-herb) or mechanical means (BS-mech), a seeded cover of *L. arundinaceum*, and an application of *M. brunneum*

Ground cover/treatment	Costs (€ ha ⁻¹)				Total cost € ha ⁻¹ (A)	Adult emergence reduction % (B)	A/B
	BS-herb	BS-mech	<i>L. arundi- naceum</i>	<i>M. brunneum</i>			
	199.2	51.0	38.0	35.0			
BS-herb	✓				199.2	–	–
BS-mech		✓			51.0	–	–
BS-herb. + <i>M. brunneum</i>	✓			✓	234.2–269.2 ¹	59.4	3.9–4.5
BS-mech. + <i>M. brunneum</i>		✓		✓	86.0–121.0 ¹	59.4	1.4–2.0
<i>L. arundinaceum</i>			✓		38.0	48.9	0.8
<i>L. arundinaceum</i> + <i>M. brunneum</i>			✓	✓	73.0–108.0 ¹	92.5	0.8–1.2

Adult emergence values were obtained as means from efficacies reported in Table 2

¹Cost in case that a second treatment with *M. brunneum* is required (i.e., heavy rain)

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10340-023-01705-6>.

Acknowledgements We thank M. Piquer (UJI) and A. Benages (COCALNI, Cooperativa Citrícola de Les Alqueries) for technical support and provision of the experimental orchard, respectively, and A. Nieto from the University of Córdoba for technical support in fungal detection. Financial support has been provided by the European Union's Horizon 2020 Program for Research and Innovation grant

number 818184 (FF-IPM) and PRIMA grant number 1812 (PLANT-B), and Excellence Project PROYEXCEL00808 from the Andalusian Regional Government.

Funding Open Access funding provided thanks to the CRUE-CSIC agreement with Springer Nature. Open Access funding provided thanks to the CRUE-CSIC agreement with Springer Nature. Financial support has been provided by the European Union's Horizon 2020 Program for Research and Innovation Grant Number 818184 (FF-IPM)

and PRIMA Grant Number 1812 (PLANT-B), the Spanish Ministry of Science and Innovation through the Project PID2019-103844RB-I00, and the Andalusian Regional Government through the Excellence Project PROYEXCEL00808.

Data availability Raw data will be available at UJI public repository (<http://repositori.uji.es/xmlui/>).

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Abbott WS (1925) A Method of computing the effectiveness of an insecticide. *J Econ Entomol* 18:265–267. <https://doi.org/10.1093/jee/18.2.265a>
- Aguilar-Fenollosa A, Jacas JA (2013) Effect of ground cover management on Thysanoptera (thrips) in clementine mandarin orchards. *J Pest Sci* 86:469–481. <https://doi.org/10.1007/s10340-013-0494-x>
- Aguilar-Fenollosa E, Ibáñez-Gual MV, Pascual-Ruiz S, Hurtado-Ruiz M, Jacas JA (2011a) Effect of ground-cover management on spider mites and their phytoseiid natural enemies in clementine mandarin orchards (I): tetranychid mite dynamics. *Biol Control*. <https://doi.org/10.1016/j.biocontrol.2011.06.012>
- Aguilar-Fenollosa E, Ibáñez-Gual MV, Pascual-Ruiz S, Hurtado M, Jacas JA (2011b) Effect of ground-cover management on spider mites and their phytoseiid natural enemies in clementine mandarin orchards (II): top-down regulation mechanisms. *Biol Control* 59:171–179. <https://doi.org/10.1016/j.biocontrol.2011.06.012>
- Aguilar-Fenollosa E, Pascual-Ruiz S, Hurtado MA, Jacas JA (2011c) Efficacy and economics of ground cover management as a conservation biological control strategy against *Tetranychus urticae* in clementine mandarin orchards. *Crop Prot* 30(10):1328–1333. <https://doi.org/10.1016/j.cropro.2011.05.011>
- Akaike H (1974) A new look at the statistical model identification. *IEEE Trans Autom Control* 19:716–723
- Aluja M, Mangan RL (2008) Fruit fly (Diptera: Tephritidae) host status determination: critical conceptual, methodological, and regulatory considerations. *Annu Rev Entomol* 53:473–502. <https://doi.org/10.1146/annurev.ento.53.103106.093350>
- Arathi HS, Burns I, Spivak M (1999) Ethology of hygienic behavior in the honey bee *Apis mellifera* L. (Hymenoptera: Apidae): behavioral repertoire of hygienic bees. *Ethology* 106:365–379. <https://doi.org/10.1046/j.1439-0310.2000.00556.x>
- Bates D, Maechler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. *J Stat Softw* 67(1):1–48
- Beris EI, Papachristos DP, Fytros A, Antonatos SA, Kontodimas DC (2013) Pathogenicity of three entomopathogenic fungi on pupae and adults of the Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae). *J Pest Sci* 86:275–284. <https://doi.org/10.1007/s10340-012-0468-4>
- Boos S, Meunier J, Pichon S, Kölliker M (2014) Maternal care provides antifungal protection to eggs in the European earwig. *Behav Ecol* 25:754–761. <https://doi.org/10.1093/beheco/aru046>
- Bot ANM, Currie CR, Hart AG, Boomsma JJ (2001) Waste management in leaf-cutting ants. *Ethol Ecol Evol* 13:225–237. <https://doi.org/10.1080/08927014.2001.9522772>
- CABI (Centre for Agriculture and Bioscience International) (2023) Invasive species compendium. <https://doi.org/10.1079/cabicompendium.12367>. Accessed April 1, 2023
- Cremer S, Armitage SAO, Schmid-Hempel P (2007) Social immunity. *Curr Biol* 17:R693–702. <https://doi.org/10.1016/j.cub.2007.06.008>
- Cruz-Miralles J, Guzzo M, Ibáñez-Gual MV et al (2022) Ground-coverers affect the activity density of ground-dwelling predators and their impact on the Mediterranean fruit fly, *Ceratitis capitata*. *Biocontrol* 67:583–592. <https://doi.org/10.1007/s10526-022-10168-0>
- De Meyer M, Ekesi S (2016) Exotic invasive fruit flies (Diptera: Tephritidae): in and out of Africa. In: Ekesi S, Mohamed SA, De Meyer M (eds) Fruit fly research and development in Africa: towards a sustainable management strategy to improve horticulture. Springer, Switzerland, pp 127–150
- Dias NP, Montoya P, Nava DE (2022) A 30-year systematic review reveals success in tephritid fruit fly biological control research. *Entomol Exp Appl* 170:370–384. <https://doi.org/10.1111/eea.13157>
- Diehl JMC, Meunier J (2018) Surrounding pathogens shape maternal egg care but not egg production in the European earwig. *Behav Ecol* 29(1):128–136. <https://doi.org/10.1093/beheco/arx140>
- Diehl JM, Körner M, Pietsch M, Meunier J (2015) Feces production as a form of social immunity in an insect with facultative maternal care. *BMC Evol Biol* 15:40. <https://doi.org/10.1186/s12862-015-0330-4>
- Diez L, Deneubourg JL, Detrain C (2012) Social prophylaxis through distant corpse removal in ants. *Naturwissenschaften* 99:833–842. <https://doi.org/10.1007/s00114-012-0965-6>
- EFSA (European Food Safety Authority), Anastassiadou M, Arena M, Auteri D, Brancato A, Bura L, Carrasco Cabrera L, Chaidiftou E, Chiusolo A, Crivellente F, De Lentdecker C, Egsmose M, Fait G, Greco L, Ippolito A, Istace F, Jarrah S, Kardassi D, Leuschner R, Lostia A, Lythgo C, Magrans O, Mangas I, Miron I, Molnar T, Padovani L, Parra Morte JM, Pedersen R, Reich H, Santos M, Sharp R, Szentes C, Terron A, Tiramani M, Vagenende B, Villamar-Bouza L (2020) Conclusions on the peer review of the pesticide risk assessment of the active substance *Metarhizium brunneum* BIPESCO 5/F52. *EFSA J* 18(10):e06274. <https://doi.org/10.2903/j.efsa.2020.6274>
- EFSA PLH Panel (EFSA Panel on Plant Health), Bragard C, Dehnen-Schmutz K, Di Serio F, Gonthier P, Jacques M-A, Jaques Miret JA, Justesen AF, Magnusson CS, Milonas P, NavasCortes JA, Parnell S, Potting R, Reignault PL, Thulke H-H, Van der Werf W, Vicent Civera A, Yuen J, Zappala L, Bali EM, Papadopoulos N, Papanastassiou S, Czwienczek E, MacLeod A (2020) Pest categorisation of non-EU Tephritidae. *EFSA J* 18(1):e05931. <https://doi.org/10.2903/j.efsa.2020.5931>
- Ekesi S, Maniania NK, Lux SA (2002) Mortality in three African tephritid fruit fly puparia and adults caused by the entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana*. *Biocontrol Sci Technol* 12(1):7–17. <https://doi.org/10.1080/09583150120093077>
- Ekesi S, Maniania NK, Lux SA (2003) Effect of soil temperature and moisture on survival and infectivity of *Metarhizium anisopliae* to four tephritid fruit fly puparia. *J Invert Pathol* 83(2):157–167. [https://doi.org/10.1016/S0022-2011\(03\)00069-7](https://doi.org/10.1016/S0022-2011(03)00069-7)

- Ekesi S, Maniania NK, Mohamed SA, Lux SA (2005) Effect of soil application of different formulations of *Metarhizium anisopliae* on African tephritid fruit flies and their associated endoparasitoids. *Biol Control* 35(1):83–91. <https://doi.org/10.1016/j.biocontrol.2005.06.010>
- Enkerlin W, Gutiérrez Ruelas J, Pantaleón R, Soto Litera C, Villaseñor Cortés A, Zavala López JL, Orozco Dávila D, Montoya Gerardo P, Silva Villarreal L, Cotoc Roldán E, Hernández López F, Arenas Castillo A, Castellanos Domínguez D, Valle Mora A, Rendón Arana P, Cáceres Barrios C, Midgarden D, Villatoro Villatoro C, Lira Prera E, Zelaya Estradé O, Castañeda Aldana R, López Culajay J, Ramírez y Ramírez F, Liedo Fernández P, Ortíz Moreno G, Reyes Flores J, Hendrichs J (2017) The moscamed regional programme: review of a success story of area-wide sterile insect technique application. *Entomol Exp Appl* 164:188–203. <https://doi.org/10.1111/eea.12611>
- Escudero-Colomar LA, Vilajeliu M, Batllori L (2008) Seasonality in the occurrence of the Mediterranean fruit fly [*Ceratitis capitata* (Wied.)] in the north-east of Spain. *J Appl Entomol* 132:714–721. <https://doi.org/10.1111/j.1439-0418.2008.01372.x>
- Fischhoff IR, Burtis JC, Keesing F, Ostfeld RS (2018) Tritrophic interactions between a fungal pathogen, a spider predator, and the blacklegged tick. *Ecol Evol* 8:7824–7834. <https://doi.org/10.1002/ece3.4271>
- Garrido-Jurado I, Torrent J, Barrón V, Corpas A, Quesada-Moraga E (2011a) Soil properties affect the availability, movement, and virulence of entomopathogenic fungi conidia against puparia of *Ceratitis capitata* (Diptera: Tephritidae). *Biol Control* 58(3):277–285. <https://doi.org/10.1016/j.biocontrol.2011.05.017>
- Garrido-Jurado I, Ruano F, Campos M, Quesada-Moraga E (2011b) Effects of soil treatments with entomopathogenic fungi on soil dwelling non-target arthropods at a commercial olive orchard. *Biol Control* 59:239–244. <https://doi.org/10.1016/j.biocontrol.2011.07.001>
- Garrido-Jurado I, Valverde-García P, Quesada-Moraga E (2011c) Use of a multiple logistic regression model to determine the effects of soil moisture and temperature on the virulence of entomopathogenic fungi against pre-imaginal Mediterranean fruit fly *Ceratitis capitata*. *Biol Control* 59(3):366–437. <https://doi.org/10.1016/j.biocontrol.2011.09.011>
- Gava CAT, Paranhos BAJ (2023) Combining the virulent *Beauveria bassiana* (Balsam) Vuillemin LCB289 and nematode strains to control pupae of *Ceratitis capitata* Wiedemann. *Biocontrol Sci Technol* 33(4):383–396. <https://doi.org/10.1080/09583157.2023.2191300>
- Gava CAT, da Silva JC, Simões WL, Paranhos BAJ (2021) Impact of soil texture on conidia movement and residual effect of entomopathogenic fungi applied through irrigation to control fruit-fly pupae in mango orchards. *Biol Control* 163:104559. <https://doi.org/10.1016/j.biocontrol.2021.104559>
- Gómez-Marco F, Tena A, Jacas JA, Urbaneja A (2015) Early arrival of predators control *Aphis spiraecola* (Hemiptera: Aphididae) colonies in citrus clementine. *J Pest Sci* 89:69–79. <https://doi.org/10.1007/s10340-015-0668-9>
- Gómez-Martínez MA, Jaques JA, Ibáñez-Gual MV, Pina T (2018) When the ground cover brings guests: is *Anaphothrips obscurus* a friend or a foe for the biological control of *Tetranychus urticae* in clementines? *J Pest Sci* 91:613–623. <https://doi.org/10.1007/s10340-017-0926-0>
- Hernández I, Sant C, Martínez R, Almazán M, Caminal M, Quero V, El-Adak M, Casanova A, Garrido-Jurado I, Yousef-Yousef M, Quesada-Moraga E, Lara JM, Fernández C (2023) Persistence of *Metarhizium brunneum* (Ascomycota: Hypocreales) in the soil is affected by formulation type as shown by strain-specific DNA markers. *J Fungi* 9(2):229. <https://doi.org/10.3390/jof9020229>
- Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. *Biom J* 50:346–363. <https://doi.org/10.1002/bimj.200810425>
- Jacas JA, Karamaouna F, Vercher R, Zappalà L (2010) Citrus pest management in the northern Mediterranean basin (Spain, Italy and Greece). In: Ciancio A, Mukerji K (eds) Integrated management of arthropod pests and insect borne diseases. Integrated management of plant pests and diseases. Springer, Dordrecht, pp 3–27
- Jackson DE, Hart AG (2009) Does sanitation facilitate sociality? *Anim Behav* 77:e1–e5. <https://doi.org/10.1016/j.anbehav.2008.09.013>
- Kapranas A, Chronopoulou A, Peters A, Antonatos S, Lytra I, Milonas P, Papachristos D (2023) Early and off-season biological control of medfly with entomopathogenic nematodes: from laboratory experiments to successful field trials. *Biol Control* 179:105173. <https://doi.org/10.1016/j.biocontrol.2023.105173>
- Köhl J, Booij K, Kolnaar R, Ravensberg WJ (2019) Ecological arguments to reconsider data requirements regarding the environmental fate of microbial biocontrol agents in the registration procedure in the European Union. *Biocontrol* 64:469–487. <https://doi.org/10.1007/s10526-019-09964-y>
- Lee D, Johnson MA, Aristizábal LF, Shriner S, Chan C, Miyasaka S, Wall M (2023) Economic benefits from managing coffee berry borer (*Hypothenemus hampei*) in Hawaii. *InSects* 14(4):350. <https://doi.org/10.3390/insects14040350>
- Mathis M, Blom JF, Nemecek T, Bravin E, Jeanneret P, Daniel O, de Baan L (2022) Comparison of exemplary crop protection strategies in Swiss apple production: multicriteria assessment of pesticide use, ecotoxicological risks, environmental and economic impacts. *Sustain Prod Consum* 31:512–528. <https://doi.org/10.1016/j.spc.2022.03.008>
- Meunier J (2015) Social immunity and the evolution of group living in insects. *Philos Trans R Soc B Biol Sci* 370:20140102. <https://doi.org/10.1098/rstb.2014.0102>
- Meyling N, Eilenberg J (2007) Ecology of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in temperate agroecosystems: potential for conservation biological control. *Biol Control* 43:145–155. <https://doi.org/10.1016/j.biocontrol.2007.07.007>
- Mochi DA, Monteiro AC, De Bortoli SA, Dória HO, Barbosa JC (2006) Pathogenicity of *Metarhizium anisopliae* for *Ceratitis capitata* (Wied.) (Diptera: Tephritidae) in soil with different pesticides. *Neo Entomol* 35:382–389. <https://doi.org/10.1590/S1519-566X200600300014>
- Monzó C, Mollá Ó, Castañera P, Urbaneja A (2008) Activity-density of *Pardosa cribata* in Spanish citrus orchards and its predatory capacity on *Ceratitis capitata* and *Myzus persicae*. *Biol Control* 54:393–402. <https://doi.org/10.1007/s10526-008-9199-0>
- Monzó C, Sabater-Muñoz B, Urbaneja A, Castañera P (2011) The ground beetle *Pseudophonus rufipes* revealed as predator of *Ceratitis capitata* in citrus orchards. *Biol Control* 56:17–21. <https://doi.org/10.1016/j.biocontrol.2010.09.004>
- Nielsen C, Vestergaard S, Harding S, Wolsted C, Eilenberg J (2006) Biological control of *Strophosoma* spp. (Coleoptera: Curculionidae) in greenery (*Abies procera*) plantations using Hyphomycetes. *Biocontrol Sci Technol* 16(6):583–598. <https://doi.org/10.1080/09583150500532824>
- Ortiz-Urquiza A, Keyhani NO (2013) Action on the surface: entomopathogenic fungi versus the insect cuticle. *InSects* 4(3):357–374. <https://doi.org/10.3390/insects4030357>
- Pereira H, Detrain C (2020) Prophylactic avoidance of hazardous prey by the ant host *Myrmica rubra*. *InSects* 11(7):444. <https://doi.org/10.3390/insects11070444>
- Pla I, García de Oteya J, Tur C, Martínez MA, Laurín MC, Alonso E, Martínez M, Martín A, Sanchis R, Navarro MC, Navarro MT, Argilés R, Briasco M, Dembilio O, Dalmau V (2021) Sterile insect technique programme against Mediterranean fruit fly in

- the Valencian community (Spain). *InsEcts* 12:415. <https://doi.org/10.3390/insects12050415>
- Quesada-Moraga E, Ruiz-García A, Santiago-Alvarez C (2006) Laboratory evaluation of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* against puparia and adults of *Ceratitis capitata* (Diptera: Tephritidae). *J Econ Entomol* 99(6):1955–1966. <https://doi.org/10.1093/jee/99.6.1955>
- R Core Team (2023) R: a language and environment for statistical computing. R foundation for statistical computing, Vienna. www.r-project.org/
- Reber A, Purcell J, Buechel SD, Buri P, Chapuisat M (2011) The expression and impact of antifungal grooming in ants. *J Evol Biol* 24:954–964. <https://doi.org/10.1111/j.1420-9101.2011.02230.x>
- Ríos-Moreno A, Quesada-Moraga E, Garrido-Jurado I (2018) Treatments with *Metarhizium brunneum* BIPESCO5 and EAMa 01/58-Su strains (Ascomycota: Hypocreales) are low risk for the generalist predator *Chrysoperla carnea*. *J Pest Sci* 91:385–394. <https://doi.org/10.1007/s10340-017-0905-5>
- Riviere C, Bethinger A, Bergez JE (2022) The effects of cover crops on multiple environmental sustainability indicators: a review. *Agronomy* 12(9):1–17. <https://doi.org/10.3390/agron-omy12092011>
- Rodrigues A, Cable RN, Mueller UG, Bacci M, Pagnocca FC (2009) Antagonistic interactions between garden yeasts and micro-fungal garden pathogens of leafcutting ants. *Antonie Leeuwenhoek* 96:331–342. <https://doi.org/10.1007/s10482-009-9350-7>
- Scheepmaker JWA, Butt TM (2010) Natural and released inoculum levels of entomopathogenic fungal biocontrol agents in soil in relation to risk assessment and in accordance with EU regulations. *Biol Sci Technol* 20(5):503–552. <https://doi.org/10.1080/09583150903545035>
- Sosa-Gómez DR (2017) Chapter 13 - microbial control of soybean pest insects and mites. In: Lacey LA (ed) *Microbial control of insect and mite pests: from theory to practice*. Academic Press, Cambridge, Massachusetts, pp 199–208. <https://doi.org/10.1016/B978-0-12-803527-6.00013-5>
- Vanninen I, Hokkanen H, Tyni-Juslin J (1999) Screening of field performance of entomopathogenic fungi and nematodes against cabbage root flies (*Delia radicum* L. and *D. floralis* (Fall.); Diptera, Anthomyiidae). *Acta Agric Scand* 49:167–183. <https://doi.org/10.1080/09064719909362513>
- Vestergaard S, Cherry A, Keller S, Goettel M (2003) Safety of hyphomycete fungi as microbial control agents. In: Hokkanen HMT, Hajek AE (ed) *Environmental impacts of microbial insecticides: need and methods for risk assessment*. Springer, New York, pp 35–62. https://doi.org/10.1007/978-94-017-1441-9_3
- Weldon C (2020) *Ceratitis capitata* (Mediterranean fruit fly). *CABI Compendium*. <https://doi.org/10.1079/cabicompendium.12367>
- Yousef M, Quesada-Moraga E, Garrido-Jurado I (2015) Compatibility of herbicides used in olive orchards with a *Metarhizium brunneum* strain used for the control of preimaginal stages of tephritids in the soil. *J Pest Sci* 88:605–612. <https://doi.org/10.1007/s10340-014-0632-0>
- Yousef M, Garrido-Jurado I, Ruíz-Torres M, Quesada-Moraga E (2017) Reduction of adult olive fruit fly populations by targeting preimaginals in the soil with the entomopathogenic fungus *Metarhizium brunneum*. *J Pest Sci* 90:345–354. <https://doi.org/10.1007/s10340-016-0779-y>
- Yousef M, Alba-Ramírez C, Garrido Jurado I, Mateu J, Raya Díaz S, Valverde-García P, Quesada-Moraga E (2018) *Metarhizium brunneum* (Ascomycota; Hypocreales) treatments targeting olive fly in the soil for sustainable crop production. *Front Plant Sci*. <https://doi.org/10.3389/fpls.2018.00001>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.