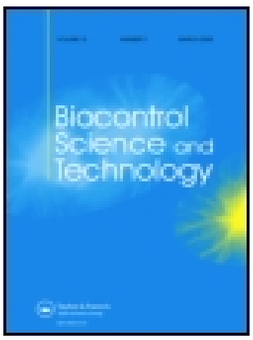

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RESEARCH ARTICLE

Applying local entomopathogenic fungi strains to the soil can control *Ceratitis capitata* (Diptera: Tephritidae) Wiedemann adults

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ABSTRACT

Ceratitis capitata causes direct and indirect damage to fruit production worldwide, reducing productivity and increasing costs. Currently, there is no effective control strategy for fruit fly pupae in the soil. We selected virulent local entomopathogenic fungi (EF) strains for application against pupae of *C. capitata* and evaluated their effects on the post-emergence survival of adults in laboratory conditions. A cage was designed to study mortality in apparently healthy *C. capitata* adults that emerged from soils previously treated with EF conidia. This approach allowed the selection of four strains of *Beauveria bassiana (*Balsamo) Vuillemin (Hypocreales: Cordycipitaceae) and two strains of *Metarhizium anisopliae* (Metschnikoff) Sorokin (Hypocreales: Clavicipitaceae) that were virulent to *C. capitata* pupae. The application of *B. bassiana* strains LCB53 and LCB289 caused 44.4 and 60.1% corrected pupae mortality and 83.5 and 88.7% corrected mortality of pupae plus adults, respectively. The median lethal time after adult emergence was 4.0 and 3.5 days for LCB53 and LCB289, respectively. The application of conidial preparations to natural soil reduced insect emergence and the adult life span and represents a promising strategy for fruit fly integrated management.

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Biological control; *Metarhizium*; *Beauveria*; pupae; adult mortality

Introduction

Fruit flies are a key pest of fruit production worldwide. In addition to direct damage, quarantine regulations may require postharvest treatment that increases production costs (Dias, Zotti, Montoya, Carvalho, & Nava, 2018). Integrated pest management of fruit flies usually employs orchard sanitation, cover sprays, soil drenches with insecticide, mass trapping, toxic bait applications, and, in recent decades, sterile male releases (Gordello, Sarwar, Vargas, Leblanc, & Inouye, 2015; Paranhos, Nava, & Malavasi, 2019). Postharvest control of fruit flies include cold quarantine, hydrothermal treatment, and gamma radiation (Grout, 2016). A general concern about the development of resistant populations, food and environment contamination with pesticide residues, and other side effects of insecticides

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led to development of microbial biopesticides (Arthurs & Dara, 2019; Nollet & Rathore, 2015). In addition, fruit flies possess cryptic behaviour in three stadia in their life cycle: inside fruits during egg and larval development and the pupal stadia in the soil. In these developmental phases, fruit flies are sheltered from insecticide applications.

Efforts have been made to select new strains of entomopathogenic fungi (EF) for different pests, but most of them are applied in the aerial part of plants, whereas fewer studies have examined efficient EF for soil-dwelling pests (Jaronski, 2010). In general, soil is the natural repository of entomopathogens where bacteria and fungi can dwell as saprophytes, colonising fragments of insect cadavers' and organic matter (Solter, Hajek, & Lacey, 2017). *Metarhizium* and *Beauveria* species can establish in the soil and colonise the plant rhizosphere (Castro et al., 2016; Nishi & Sato, 2018; Ramos, Portal, Lysøec, Meyling, & Klingen, 2017). Their adaptation to living in the soil makes them candidates for the control of insect pests that spend part of their life cycle in this environment (Lovett & St. Leger, 2015; St. Leger, 2008).

The soil application of virulent strains of EF can potentially be incorporated into the integrated management of pests possessing cryptic behaviour or a life stage in the soil (Jackson, Alves, & Pereira, 2000). For example, soil application of *M. anisopliae* (s.l.) and *B. bassiana* caused mortality of the white grub *Polyphylla fullo* (Erler & Ates, 2015). The application of *M. brunneum* ARSEF4556 and V275 resulted in mortality greater than 70% in the chestnut weevil *Curculio elephas* and the chestnut tortrix *Cydia splendana*, which pupate in the soil after damaging nut kernels (Asan et al., 2017). Although melanization and sclerotisation of puparia represent great challenges to infection of entomopathogens, larvae and pupae of *C. capitata* are exposed to infection by EF and nematodes in the soil (Dias et al., 2018). In studies in Kenya, Ekesi, Maniania, and Lux (2002) found strains of *M. anisopliae* that were highly virulent to three African Tephritidae species when applied in sterilised soil, showing their potential to kill fruit flies inside the puparia. Later studies confirmed the potential for controlling fruit flies by applying EF in the soil, also revealing significant effects of soil characteristics and fungal formulations on the efficiency of *Ceratitis* species control (Garrido-Jurado, Torrent, Barrón, Corpas, & Quesada-Moraga, 2011; Maniania & Ekesi, 2016; Quesada-Moraga, Valverde-García, & Garrido-Jurado, 2012).

Although the application of a virulent strain of EF in the soil could target the pupae of fruit flies, it has not been widely applied. Probably, the large variation of pupae mortality reported in the literature (40–80%) resulted in a lack of confidence in its efficacy. However, most of these studies do not compute the postemergence effect of EF on adult survival, thus may underestimate the efficiency of control achieved. Adding this information to the current knowledge about the integrated management of *C. capitata* could influence the usage decision of fruit growers. The objective of this work was to select virulent EF among local strains to control *C. capitata* during the cryptic life cycle stadia in soil and evaluate their effects on the postemergence survival of adults under controlled conditions.

Materials and methods

Biological materials

Insect rearing

Insects were reared following methods described by Silva Neto et al. (2012). Briefly, the insects were initially obtained from pupae recovered from naturally infested fruits

collected from farms located in Petrolina (Pernambuco, Brazil) in 2015 and disposed into plastic trays containing vermiculite. Adults were fed an artificial diet containing sugar and hydrolysed yeast (Biones®, Quatá, São Paulo, Brazil) and distilled water *ad libitum* (Silva Neto et al., 2012). Egg-laying occurred in a voile fabric positioned in a lateral overture of the cages. The eggs were collected, seeded onto the artificial diet, and incubated in a growth chamber at 25.0 ± 2°C with 60 ± 10% relative humidity and a 14/10 h photoperiod. Larvae were reared on an artificial diet prepared with distilled water (60.0%), sugarcane bagasse (13.4%), sugar (8.4%), soy flour (8.4%), beer yeast (8.4%), sodium benzoate (0.3%), and citric acid (2.0%). Late 3rd instar larvae (prepupae) were transferred to flasks containing vermiculite inside methacrylate cages (30 × 30 × 30 cm) covered with voile fabric and maintained in a growth chamber under the same environmental conditions described above.

Fungal strains and propagules production

Initially, forty-seven strains of *Beauveria* spp. and *Metarhizium* spp. were assessed for their virulence to pupae of *C. capitata*. The strains were obtained from different hosts and soil samples collected from experimental farms of Embrapa Semi-Arid and stored in the Microbial Collection of Agronomic Interest (CMISA). Of these, 17 strains were pathogenic to *C. capitata* pupae (Table 1). The fungal strains were cultured in Sabouraud dextrose agar (SDA; 40.0 g glucose, 20.0 g yeast extract, 10.0 g peptone, 15.0 g agar, 1000 ml of water) and incubated at 28.0 ± 0.5°C in the dark for 15 days. Disks from the outer limits of the colonies were collected and stored in sterile distilled water at 5°C. Propagules for the experiments were produced by collecting conidial suspensions extracted from densely colonised plates using a 0.05% v/v sterile Triton X-100 solution as a dispersant.

Conidia for technical grade preparations were produced as described by Jaronski (2014). After 15 days, when fungi achieved high conidiation in plastic bags, the mixture of substrate and propagules was transferred to paper trays and dried in a forced-air drying oven at 35°C until 10% w/w moisture content. Fungal conidia were then extracted using a Mycoharvester (MH5, VBS Agriculture Ltd, Cornwall, UK).

Table 1. Identification, original host, and origin of fungal strains pathogenic to *C. capitata* selected in the sterile substrate assay.

Strain	Original host	Locality
B. bassiana LCB52	*D. sacharalis* (Lepidopetera)	Juazeiro, BA
B. bassiana LCB53	Soil	Juazeiro, BA
B. bassiana LCB56	*C. sordidus* (Coleoptera)	Petrolina, PE
B. bassiana LCB62	*Deois* sp. (Hemiptera)	Juazeiro, BA
B. bassiana LCB66	*C. sordidus* (Coleoptera)	Juazeiro, BA
B. bassiana LCB81	*Spodoptera* sp. (Lepidoptera)	Petrolina, PE
M. anisopliae LCB244	Soil	Juazeiro, BA
M. anisopliae LCB 245	*Spodoptera* sp. (Lepidoptera)	Petrolina, PE
B. bassiana LCB252	Soil	Petrolina, PE
M. anisopliae LCB 255	Soil	Petrolina, PE
B. bassiana LCB259	Soil	Petrolina, PE
M. anisopliae LCB 275	Soil	Petrolina, PE
B. bassiana LCB284	Soil	Petrolina, PE
B. bassiana LCB286	Soil	Petrolina, PE
B. bassiana LCB289	Soil	Petrolina, PE
M. anisopliae LCB 312	Soil	Petrolina, PE
M. anisopliae LCB 313	Soil	Petrolina, PE

Selection of pathogenic fungal strains

Modified cage design

A cage was designed to estimate adult mortality post-emergence. The cage had a transparent body and an inverted funnel, both built with poly(ethylene terephthalate) (PET) and 100 mm in diameter. The cage also had a lower compartment built in opaque polyvinyl chloride (PVC), that could be used in different lengths according to the experiment. The lower compartment could be filled with a substrate, a soil sample, or an undisturbed soil column (Figure 1). Access for gas exchange and water and diet supply were built in the upper part of the cage. After emergence, the adults migrated to the upper part of the cage attracted by light, water, and food, allowing the removal and analysis of the lower compartment.

Pupae colonisation and adult mortality

Conidial suspensions of the fungal strains in a 0.05% v/v sterile Triton X-100 solution were added to the PVC column (100 mm depth) containing autoclaved substrate (sand + vermiculite 1:1 added with 10% v/v of water) to achieve 10^7 conidia g^{-1} and mixed. The control treatment was mixed only with Triton solution. Groups of twenty late 3rd instar larvae (prepupae) were transferred to the columns. Larvae that did not bury into the substrate within 6 h were removed from the experimental analysis.

The number of adult flies that emerged in the experiments was recorded after 8 days, and the pupae were recovered to confirm EF infection. Dead pupae that were recovered from the substrate without apparent fungal colonisation were surface sterilised and transferred to Petri dishes containing moistened sterile filter paper. The adults that migrate for the upper

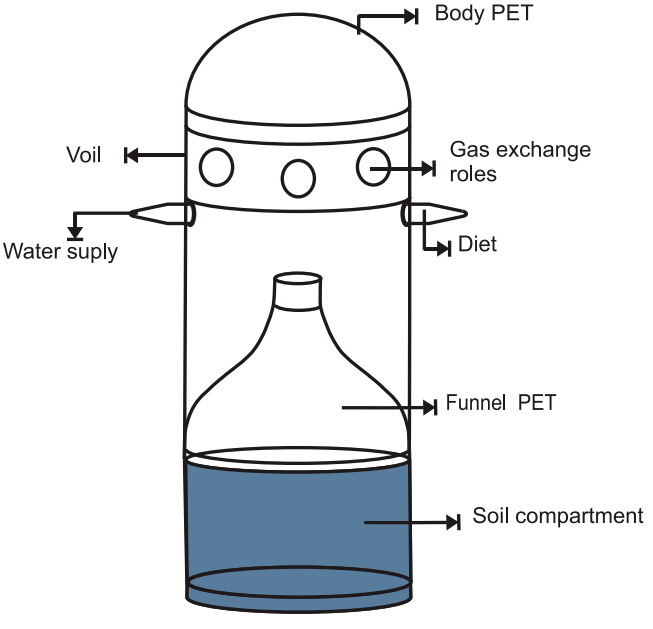


Figure 1. Cage developed for experiments evaluating the virulence of entomopathogens to fruit fly pupae. The lower compartment is built using PVC and can assume different lengths, receiving soil or substrate where larvae can be deposited. The aerial compartment, which was built using transparent PET, contains water and an artificial diet to receive emerging adults.

compartment of the cage received water and artificial diet *ad libitum* and were monitored for survival for further 10 days. Surface sterilisation of adults and pupae was performed by immersing the cadavers in 70% v/v ethanol and 1.0% v/v sodium hypochlorite (30 seconds each) followed by 3 washes in autoclaved distilled water.

The experiment was a completely randomised design with six repetitions using larvae from 3 independent insect cohorts (two repetitions for each group). Insect mortality was corrected by the control treatment (natural mortality) using the Schneider-Orelli's formula (Püntener, 1992). Only data from strains with average corrected mortality different from zero were included in statistical analyses. Corrected mortality data (pupae and adults) were transformed into the proportion of dead insects, subject to a homoscedasticity test and analysed using analysis of variance. Averages were compared using the Scott-Knot test ($p < 0.05$).

Pathogenic strain virulence to pupae in sterile soil columns

Ten milliliters of conidial suspensions containing 10^7 conidia ml^{-1} of the previously selected pathogenic strains were mixed with 500 g of autoclaved sandy soil (Red-Yellow Ultisol, clay 80.0 g kg^{-1}; silt 140.0 g kg^{-1}; sand 780.0 g kg^{-1}; organic matter 7.8 g kg^{-1}, and 5.4% humidity w/w), obtaining a final concentration of 2×10^6 conidia g^{-1}. Autoclaved distilled water was added to achieve 70% water retention capacity (described below). After mixing, soil samples were transferred to the PVC compartment (150 mm length) of the cages. The soil columns were maintained at 70% of field capacity during the experiments by controlling the weight of the columns. Moistening was achieved using a handheld sprayer with autoclaved distilled water.

Twenty prepupae of *C. capitata* were transferred to each column, and the upper compartments were attached. After all flies in the control treatment had emerged, the aerial compartments were detached and closed using voile fabric. Soil columns were examined and pupal and adult fly mortality were assessed over further 10 days.

The experiment was conducted using three replications with 20 insects and repeated twice using different cohorts of insects. After natural death correction using the Schneider-Orelli's formula (Püntener, 1992), mortality data were transformed to the proportion of dead pupae or adults. The data set did not exhibit a normal distribution and was analysed using the Kruskal–Wallis nonparametric *H* test followed by a post hoc Dunn's Multiple Comparison test ($p < 0.05$).

Fungal virulence to pupae in columns containing natural soil

Preparing soil columns

Soil samples were collected from the subsurface layer (20–40 cm) of a sandy Yellow Ultisol (clay 60.0 g kg^{-1}; silt 150.0 g kg^{-1}; sand 790.0 g kg^{-1}; organic matter 10.8 g kg^{-1}) and a clayey Vertisol (clay 520.0 g kg^{-1}; silt 207.5 g kg^{-1}; sand 272.5 g kg^{-1}; organic matter 28.8 g kg^{-1}) and sieved in 2.0-mm mesh. Bulk soil densities of the original sites were obtained by collecting undisturbed soil cores between 5 and 10 cm using stainless steel rings (Blake & Hartge, 1986). After shade drying, the soil samples were sieved in 2.0-mm mesh again to eliminate soil clods and root fragments. Three samples were collected to evaluate the moisture content. The columns (200 mm length × 100 mm diameter) were closed on their bases with a water-permeable nylon tissue, and the columns were packed with soil to obtain a bulk density similar

to the surface layer of the Ultisol (1.10–1.20 kg.l) and the Vertisol (1.45–1.50 kg.l). Average water retention capacity (WR) was 9.7% w/w for the Ultisol and 17.4% w/w for the Vertisol. Water volume to be applied to the columns in the simulated irrigation was standardised as the soil WR plus 10%.

Mortality of C. capitata *upon application of conidial suspensions to the soil*

Suspensions containing 5×10^7 conidia ml^{-1} were prepared by dispersing the technical grade preparation in 1.0 L of 0.05% v/v Triton X-100. The suspensions were applied to the soil columns using drip irrigation and adjusted to the infiltration rate observed in the column. Similar to procedures applied for fertigation, 40% of the water volume was initially applied. Then, the conidial suspension was dispersed in 30% of the water volume and applied. Finally, pure water was applied to complete the volume and helping the conidial distribution along the soil column length. In addition to the selected local strains, we added the commercial strains *B. bassiana* IBCB 66 and *M. anisopliae* IBCB 425 to this experiment. Conidia of all strains were produced in the laboratory as previously described. The control treatment received only distilled water. The columns were stored overnight on a plastic tray to allow free draining of water excess.

Twenty-four hours later, twenty prepupae of *C. capitata* were transferred to each column, and the upper compartment was attached. After the complete emergence of the control treatment, fly mortality was estimated as previously described. After emergence, adult mortality was registered daily to evaluate the median lethal time of emerged adults. The experiment was conducted in a factorial design (a sandy Ultisol and a clayey Vertisol, seven fungi strains plus one control treatment) in a randomised block design with three columns per treatment and repeated twice. After correction using Schneider-Orelli's formula, mortality data were transformed to the proportion of dead pupae or adults divided by the number of pupae that effectively buried into the soil.

Data were evaluated using Lilliefors and Levene tests for homoscedasticity and homogeneity of variance, respectively. Percentage data were transformed by applying the equation: $X' = arcsen\sqrt{X/100}$, where X and X' are observed and transformed data, respectively. Data were analysed using analysis of variance followed by a post hoc Duncan Multiple Comparison test ($p < 0.05$). The results were presented as the original percentage and are the average of two experiments.

ANOVA did not show a significant effect of soil characteristics in postemergence adult mortality, and data were pooled to investigate the effect of EF strains on the mortality curve. Daily data of mortality for each strain were analysed using the Kaplan-Meier procedure to compare mortality curves to the control using the Mantel–Cox test and to determine median mortality time.

Results and discussion

Selection of pathogenic strains in sterilised substrate

Only seventeen strains from the initial 47 tested (36%) caused corrected mortality in *C. capitata* that was higher than zero (Tables 1 and 2). However, the use of the modified cage showed that some adults that emerged apparently healthy died hours or a few days after the emergence. There was a significant effect of the fungal strains

on the mortality of pupae ($F_{16;\ 85} = 4.352$; $p < 0.01$) and adults ($F_{16;\ 85} = 5.837$; $p < 0.01$). The strains of *B. bassiana* LCB53, LCB66, LCB81, LCB259, LCB259, and LCB289, and the strains of *M. anisopliae* LCB245 and LCB312, caused significantly higher pupae mortality, while *B. bassiana* LCB5, LCB66, LCB289, and LCB252 and *Metarhizium* spp. LCB245, LCB257, and LCB312 caused significantly higher mortality of adults after their emergence from the substrate. *B. bassiana* LCB53 and LCB289, and *M. anisopliae* LCB245 produced the highest total mortality (pupae plus adults) according to the Scott-Knot test ($p < 0.05$). *B. bassiana* LCB66, LCB81, and *M. anisopliae* LCB312 produced total mortality greater than 50%, and they were also selected for subsequent studies.

Quesada-Moraga, Ruiz-García, and Santiago-Alvarez (2006) and Beris, Papachristos, Fytrou, Antonatos, and Kontodimas (2013) also reported pupae mortality ranging from 18 to 50% of *C. capitata* treated with EF. However, in both studies, the authors dipped the pupae into conidial suspensions. The method applied by both studies was efficient to select pathogenic strains, but a large number of conidia was applied with a higher contact with the puparia. The approach adopted in this work closely imitated field conditions. This study allowed flies to contact fungal propagules while burying themselves inside the substrate as larvae, later with puparia, and also as newly emerged adults during their return to the surface.

Fungal virulence to pupae in soil columns

Insect mortality in sterilised soil columns

The experiment performed using autoclaved soil columns confirmed that the selected fungal strains were able to infect *C. capitata* pupae in the test conditions. There was a

Table 2. Corrected mortality (%) of *C. capitata* when larvae in the third instar (prepupae) were transferred to a column containing substrate previously treated with a conidial suspension of different strains of *M. anisopliae* and *B. bassiana*. The results are the average of two experiments with 3 repetitions and 20 insects for each repetition.

	Inferior[1]			
Strains	Pupae	Dead/deformed	Adults[2]	Total[3]
Bb LCB52	1.67 c[4]	3.33 a	20.00 b	25.0 c
Bb LCB53	31.67 a	0.00 a	33.33 a	65.0 a
Bb LCB56	1.67 c	6.67 a	13.33 c	21.7 c
Bb LCB62	1.67 c	1.67 c	16.67 b	20.0 c
Bb LCB66	18.33 a	3.33 a	36.67 a	58.3 b
Bb LCB81	28.33 a	0.00 a	23.33 b	51.7 b
Ma LCB244	6.67 b	3.33 a	16.67 c	26.7 c
Ma LCB245	21.66 a	3.33 a	57.50 a	82.5 a
Bb LCB252	6.67 b	1.67 a	36.67 a	45.0 b
Ma LCB255	1.66 c	0.00 a	18.33 b	20.0 c
Bb LCB259	16.66 a	5.00 a	13.33 c	35.0 c
Ma LCB275	10.00 b	1.67 a	26.67 a	38.3 b
Bb LCB284	19.17 a	1.67 a	21.67 b	42.5 b
Bb LCB286	8.32 b	0.00 a	26.67 b	35.0 c
Bb LCB289	20.00 a	6.67 a	61.67 a	88.3 a
Ma LCB312	18.33 a	3.33 a	36.67 a	58.3 b
Ma LCB313	5.00 b	3.33 a	16.67 c	25.0 c

[1]Insect mortality in the lower compartment of the cage; [2]Mortality of adults; [3]Sumation of insect mortality in all compartments of the cage. [4]Average followed by the same letters in the column did not differ from each other by the Scott-Knot test ($P < 0.05$). Data from the control treatment were applied as natural mortality for corrected mortality calculations using Schneider-Orelli's formulae.

significant effect of EF on the death of *C. capitata* pupae ($H = 22.335$; $p < 0.01$) and adults ($H = 37.544$; $p < 0.01$) according to a Kruskal–Wallis H test. Strains LCB289 and LCB63 showed the highest mortality after adult emergence and total mortality among the strains tested (Table 3). *B. bassiana* strains were the most effective EF in the two experiments, causing the highest total mortality. Similar to the initial experiment, pupae mortality varied from 6.3 to 23.7%, while total mortality varied from 40.0 to 83.3%, confirming a strong effect of EF on adult survival. Similar results of postemergence mortality of fruit flies were reported by Ekesi et al. (2002). Our results indicate that some infection occurred on the larva penetrating in the soil and in the puparia likely by contact with fungi propagules. However, insects were also infected via contact of adults with infective propagules while displacing through bulk soil to the surface during emergence, as demonstrated by a large number of infected cadavers recovered from the upper segment of the cage.

Effect of physical–chemical characteristics of non-sterile soils on fruit fly mortality

There was a significant interaction of soil characteristics and EF strains on the fruit fly mortality ($F_{7;30} = 3.287$; $p < 0.05$). In general, pupae mortality was significantly ($F_{1:30} = 4.843$; $p < 0.05$) higher in the sandy Ultisol column (Figure 2). The strains LCB53, LCB81, and LCB289 showed the highest mortality in sandy Ultisol based on the Duncan test ($p < 0.05$), with results statistically similar to the commercial strains included in the experiment (Figure 2A). Strains LCB53 and LCB63 showed insect mortality of 88.0 and 76.0%, respectively, in the clayey Vertisol and were statistically similar to LCB81 (82.0%) and the commercial strain *M. anisopliae* IBCB425 (74.0%) (Figure 2B). There was no significant difference in the number of sick or deformed fruit flies emerging from the Ultisol columns, but there was a significant difference among strains in the Vertisol. *B. bassiana* LCB53 and LCB81 caused high total mortality of *C. capitata* in both soils closely followed by LCB289 and the commercial strains. Similar to the initial experiments, there was a significant amount of insect mortality after the flies emerged from the soil columns.

Statistical analysis did not show a significant difference in adult mortality after emergence between the soils, so data from different soils were pooled together for survival

Table 3. Corrected mortality (%) of *C. capitata* after the introduction of third instar larvae (prepupae) in autoclaved sandy soil previously infested with conidial suspensions (10^6 conidia g^{-1}) of selected virulent strains of *B. bassiana* and *M. anisopliae*.

	Inferior[1]			
Strains	Pupae	Dead/deformed	Adults[2]	Total[3]
Bb LCB53	21.67 a	3.33 a	52.50 a	77.50 a
Bb LCB66	18.33 a	3.33 a	31.67 b	53.33 b
Bb LCB81	18.42 a	0.00 a	28.33 b	46.67 b
Ma LCB245	6.67 b	1.67 a	31.67 b	40.00 b
Bb LCB289	20.00 a	6.67 a	56.67 a	83.33 a
Ma LCB312	8.33 b	0.00 a	21.67 b	30.00 b

[1] Insect mortality in the lower compartment of the cage; [2] Mortality of adults (%) in relation to the total of larvae used in the experiment found in the aerial compartment of the cage; [3]Sumation of insect mortality in all compartments of the cage; [4]Small letters indicate significant differences among trains by Kruskal-Wallis test followed by a post hoc Dunn's Multiple Comparison test ($p < 0.05$). Data from the control treatment were applied as natural mortality for corrected mortality calculation using Schneider-Orelli's formulae.

analysis (Figure 3). Although most strains did not achieve 50% median mortality after emergence, death curves were significantly different from the control treatment based on the Mantel–Cox test ($p < 0.05$) except for LCB312 (Figure 3).

Strains LCB53 and LCB289 showed the highest adult mortality with median mortality times of 4.13 and 3.65 days, respectively (Figure 3), representing a substantial reduction in the life span of adult flies. Considering that the infected insects modify their flight activity, feeding, and sexual behaviour hours or even days before dying from EF infection (Bernardo & Singer, 2017; Dimbi, Maniania, & Ekesi, 2009), it is possible that some infected

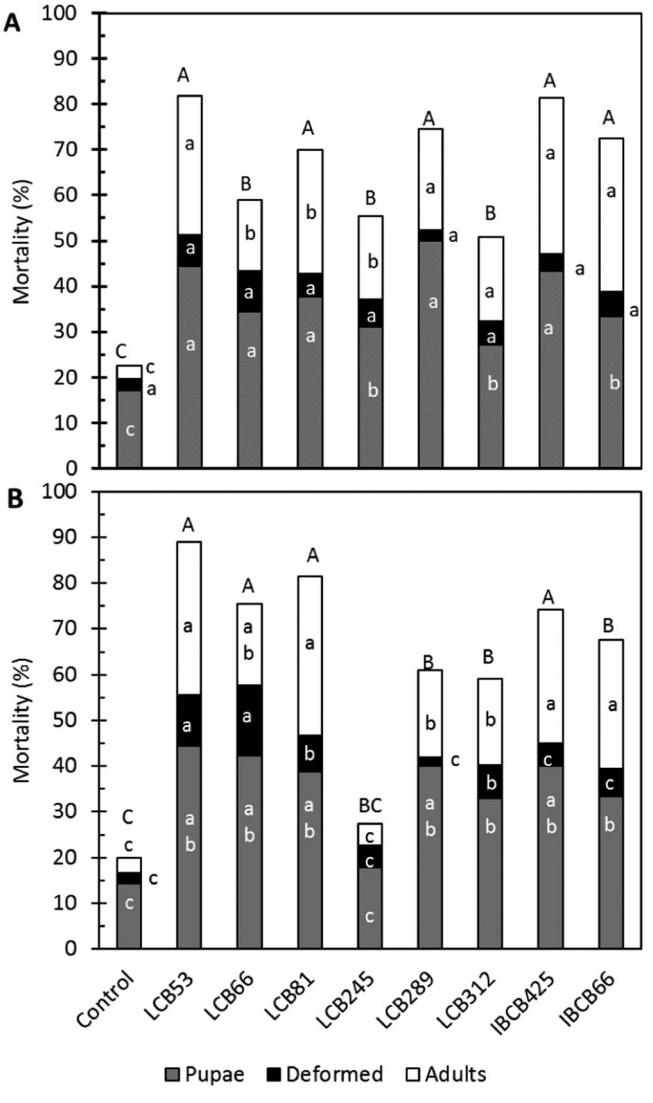
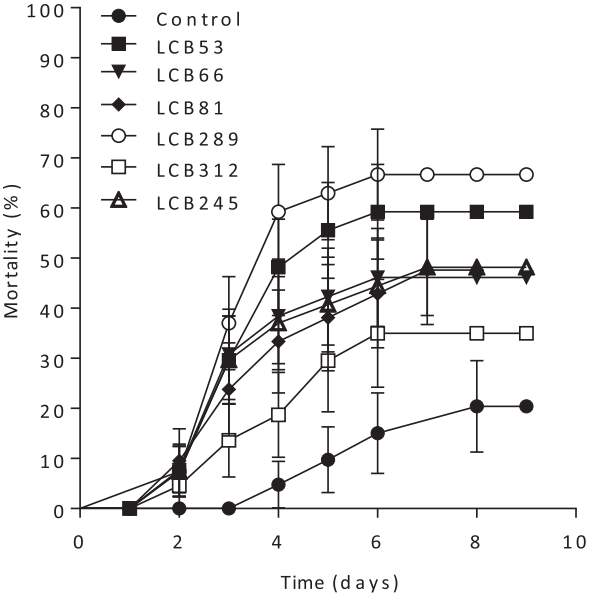


Figure 2. Mortality of pupae, adults, and total mortality of *Ceratitis capitata* caused by the application of fungal strains in soil columns containing sandy Ultisol (A) and clayey Vertisol (B). Small letters inside columns compare mortality among fungi in different development stages, while capital letters in the top of the columns compare total mortality among treatments. Different letters show significant differences among treatments by the Tukey test ($p < 0.05$).

flies would not disperse in the orchard and engage in reproductive activity. Alternatively, biocontrol efficiency could even be enhanced if infected insects emerging from soil could spread infective propagules amid the local and immigrant fruit fly populations.

To our knowledge, the infection of emerging adults has been underestimated in most previous studies. Failure of accounting for this late effect of the EF on mortality could undervalue the effects of EF biocontrol agents applied to the soil to the control of fruit flies. The commercial strains IBCB66 and ICBC245, i.e. were highly virulent against *C. capitata* pupae and adults in a previous study using sterile and nonsterile soil conditions (Almeida, Filho,



Treatments	Median lethal time (days)	Mantel-Cox χ^2 test[a]
Control	Und.[b]	-
LCB53	4.13 (2.26 – 6.55)	11.004*
LCB66	Und.	6.496*
LCB81	Und.	4.921*
LCB245	Und.	5.484*
LCB289	3.65 (1.66 – 8.18)	13.540**
LCB312	Und.	2,429[ns]

[a] Comparing death curves of treatments with the control treatment. Numbers followed by asterisks differ significantly from control by the Mantel-Cox test (**significant at $p< 0.01$; *significant at $p< 0.05$); [b]Undefined.

Figure 3. Mortality curve and Kaplan-Meier statistics for *C. capitata* adults that emerged from soil columns previously treated with conidial suspensions of *B. bassiana* and *M. anisopliae* strains.

Oliveira, & Raga, 2007). However, the experiments were separately conducted for each insect development phase, and a possible postemergence effect on insect survival was not evaluated.

Soil application of formulations containing virulent strains of EF is an attractive method to control fruit flies, especially in irrigated areas using water as a vehicle for propagules application. However, the soil environment can also represent a hurdle for EF propagules. Although it is a physically more stable environment than a leaf surface, temperatures in the superficial layers can reach over 30°C, becoming detrimental to the survival of fungi propagules mainly because these conditions are usually accompanied by low water availability (Wilson et al., 2017). In addition, the soil environment possesses a diverse microbiota containing antagonistic fungi and bacteria that can inhibit EF survival, reducing biocontrol activity (Jaronski, 2007).

Some studies showed the existence of highly competitive EF strains able to colonise the soil environment and plant rhizosphere, establishing dense populations that have been proposed as biocontrol agents of plant pathogens (Lozano-Tovar, Garrido-Jurado, Quesada-Moraga, Raya-Ortega, & Trapero-Casas, 2017; Parsa, Ortiz, Gómez-Jiménez, Kramer, & Vega, 2018). The lower temperature and UV-radiation exposition under the fruit-tree canopy significantly increase EF survival than in leaf surface exposed to sunlight (Ekesi et al., 2002). Furthermore, the usage of intensive irrigation, such as that used by fruit growers in the Brazilian semiarid region, reduces the risk caused by soil desiccation, and continuous water application could move EF propagules to lower layers of the soil. Further studies should address the conidial permeation in soil layers and the residual effect of the strain to recommend the best EF management strategy for soil application against fruit flies.

The application of EF preparations to the soil has emerged as a potential strategy for control of soil-dwelling pests with consistent results against fruit flies. In summary, the results obtained in this work allowed the selection of local strains *B. bassiana* highly virulent to *C. capitata* when applied in soils with different physical–chemical properties Moreover, the evidence collected in this work suggests that a large amount of infection also occurs while the adults are moving through the soil as they die 3–5 days after emergence.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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