agriculture

MDPI

*Article*

# *Metarhizium* Associated with Coffee Seedling Roots: Positive Effects on Plant Growth and Protection against *Leucoptera coffeella*

**Mayara L. Franzin [1], Camila C. Moreira [2], Leila N. P. da Silva [1], Elem F. Martins [1], Marcos A. M. Fadini [3], Angelo Pallini [1], Simon L. Elliot [1] and Madelaine Venzon [4,*]**

[1] Department of Entomology, Federal University of Viçosa, Viçosa 36570-000, Minas Gerais, Brazil
[2] Department of Entomology and Acarology, "Luiz de Queiroz" College of Agriculture, University of São Paulo, Piracicaba 13418-900, São Paulo, Brazil
[3] Department of Agriculture Science, Federal University of São João Del-Rei, Sete Lagoas 35701-970, Minas Gerais, Brazil
[4] Agriculture and Livestock Research Enterprise of Minas Gerais (EPAMIG), Viçosa 36570-000, Minas Gerais, Brazil
* Correspondence: madelaine@epamig.br

**Abstract:** *Metarhizium* species can be mutualistic symbionts of plants. They are able to colonize roots, promote plant growth and provide protection against pests. We previously found *Metarhizium robertsii* and *M. brunneum* associated with coffee roots in a diversified coffee system. Here, we investigated whether these fungi, when inoculated in coffee seedlings, can associate with roots, improve seedling growth and indirectly protect against the coffee leaf miner (CLM) *Leucoptera coffeella* (Lepidoptera: Lyonetiidae). We performed a greenhouse experiment with coffee seedlings using suspensions of each *Metarhizium* species applied as soil drenches to potted seedlings. We also challenged these plants with CLM infestation (two adult couples per plant). We recovered *Metarhizium* spp. from most of the seedling roots 43 days after fungal inoculation. Plants inoculated with *M. robertsii* showed a 30% leaf area increase compared to the control. Both isolates promoted protection against CLM in coffee seedlings, reducing the percentual of leaf area mined and prolonging CLM development time by two days versus controls. Besides this protection provided by *Metarhizium*, *M. robertsii* also improves seedling growth. Therefore, these *Metarhizium* species could be considered for the development of inoculants for coffee seedlings.

**Keywords:** endophytes; coffee leaf miner; *Metarhizium robertsii*; *Metarhizium brunneum*; plant growth promotion; plant protection

check for updates

**Citation:** Franzin, M.L.; Moreira, C.C.; da Silva, L.N.P.; Martins, E.F.; Fadini, M.A.M.; Pallini, A.; Elliot, S.L.; Venzon, M. *Metarhizium* Associated with Coffee Seedling Roots: Positive Effects on Plant Growth and Protection against *Leucoptera coffeella*. *Agriculture* **2022**, *12*, 2030. https://doi.org/10.3390/agriculture12122030

Academic Editor: Zhengqun Zhang

Received: 19 October 2022
Accepted: 22 November 2022
Published: 28 November 2022



## 1. Introduction

The fungal genus *Metarhizium* (Hypocreales: Clavicipitaceae) has a multifunctional lifestyle. It can acquire nutrients by infecting insects, being considered an insect pathogen [1], and by colonizing plants to form a mutualistic symbiosis [2–7]. As insect-pathogenic fungi, *Metarhizium* species can infect and kill all life stages of economically important arthropod pests, such as termites, locusts, grasshoppers, cockroaches, whiteflies, thrips, mosquitoes and ticks [8,9], which have made them important tools for biological control [10,11]. The use of *Metarhizium* as a biological control agent is mainly in inundative control [8] and there are a number of commercial products based on *Metarhizium* registered around the world [9,12,13].

*Metarhizium* is also a symbiont of agriculturally and economically important plants, such as tomato, bean, corn, wheat and soybean [7]. When *Metarhizium* colonizes plants, it can confer benefits such as growth promotion [14–16], nutrient transfer [3] and protection against insect pests [15,16] and diseases [17,18]. In turn, the host plant provides the fungus with photosynthetic compounds [19]. This association of *Metarhizium* plus plant can be endophytic [3] and through rhizosphere competence [20]. Endophytic fungi develop within plant tissues without causing any noticeable symptoms of disease in the plant [21,22]. Meanwhile, rhizosphere-competent fungi grow in the rhizosphere without colonizing plant

tissues [23]. *Metarhizium* species can associate with roots [16,24], stems and leaves [16,25]. However, they are most commonly found inhabiting roots [16,26–28].

The ability of *Metarhizium* to associate with plants can be considered in the context of it being phylogenetically closer to the grass endophytes *Claviceps* and *Epichloë* than to animal-pathogenic Hypocreales [29]. These authors also showed that *Metarhizium* harbors genes that codify plant-degrading enzymes in its genomes, indicating that *Metarhizium* may have evolved from a plant symbiont lifestyle, as was initially proposed by Spatafora et al. [30]. Our current understanding is that some species in the genus have maintained their role as plant symbionts but have more recently subsequently acquired the ability to infect and kill insects [31,32].

Colonization of roots by fungi increases the surface area from which plants can scavenge nutrients, facilitating the absorption of soil nutrients, which results in increased photosynthetic ability and enhanced plant growth [33]. The protection against herbivores can arise from the production of fungal secondary metabolites in plants. *Metarhizium* can produce destruxins [34], a secondary metabolite toxic to insects that was detected in cowpea plants [35], potato [36], and tomato leaves [37]. These responses in planta also can be activated by abscisic acid (ABA): during the early stages of fungal infection in bean plants, ABA reduces immune responses in plants during endophytic colonization by *Metarhizium robertsii* and increases immune responses to pathogenic colonization by *Fusarium solani* [38]. These results suggest that ABA plays a central role in differential responses to endophytic colonization in plants. Studies indicate that protection in plants can be caused by the induction of plant resistance since fungi colonizing plants can at first be recognized as potential invaders, triggering immune responses such as transcription factors involved in resistance against herbivores [16,39].

Several studies have reported enhanced plant growth and indirect negative effects of plant-associated *Metarhizium* on herbivores feeding on above-ground plant parts. Canassa et al. [40] found that root colonization of *Phaseolus vulgaris* by *M. robertsii* can suppress spider mites feeding on leaves and improve plant growth. *Metarhizium brunneum* inoculated on the roots of sweet pepper *Capsicum annum* increased plant growth while negatively affecting life history parameters of the aphid *Myzus persicae* (prolonged development time, delayed onset of reproduction and reduced birth rate [15]). Maize seeds treated with *M. brunneum*, *M. anisopliae,* and *M. robertsii* increase leaf collar formation, stalk length, average ear biomass and average stalk, and foliage biomass [14].

The coffee leaf miner (CLM) *Leucoptera coffeella* (Lepidoptera: Lyonetiidae) is a pest that damages coffee plants. It reduces seedling quality through reductions in photosynthetic leaf area and defoliation [41,42]. This pest often attains high population levels in many coffee-producing regions in Brazil, causing defoliation by up to 70% and reducing coffee yields by 50% [43]. Thus, it is important to produce healthy coffee seedlings in order to avoid planting CLM-infested seedlings. The use of pesticides is the most common measure for controlling CLM [44]. However, the rampant use of pesticides is damaging to the health and integrity of ecosystems [45]. Given the ability of *Metarhizium* species to affect herbivores negatively when associated with roots, their use as inoculants could protect coffee seedlings against CLM.

During field studies (MLF unpubl. data), *M. robertsii* and *M. brunneum* were isolated from coffee roots collected in a diversified coffee system in the municipality of Patrocínio–Cerrado (savannah like) biome of Minas Gerais, using the bait insect method [46,47]. Here, the potential of those two *Metarhizium* species, when in association with coffee roots to protect coffee seedlings from CLM damage, was evaluated. We hypothesized that *Metarhizium* species inoculated in coffee seedlings can (a) associate with roots and, as a consequence of this, (b) improve seedling growth and (c) indirectly promote protection against CLM.

## 2. Materials and Methods

### *2.1. Fungal Isolates, Plants and Insects*

The isolates were RD-20.114 of *M. robertsii* and RD-20.120 of *M. brunneum*. These were obtained from coffee roots in 2020, using larvae of *Tenebrio molitor* (Coleoptera: Tenebri-

onidae) as a bait insect [46,47]. They were collected in a diversified coffee system at an Experimental Research Station of the Agriculture and Livestock Research Enterprise of Minas Gerais (EPAMIG) in Patrocínio, Savannah-like biome of Minas Gerais (18°9′48″ S and 46°59′00″ W). These isolates are maintained in tubes on slanted PDA at 5 ºC.

Coffee seedlings—*Coffea arabica* variety "IAC 44"—were obtained from the coffee nursery of the Plant Pathology Department at Federal University of Viçosa (UFV), Viçosa, MG, Brazil. They were three months old ("orelha de onça" stage, meaning "jaguar ear": with the two cotyledons open), planted in soil. These seedlings were cultivated without use of pesticides and were visually inspected to ensure the absence of pests and diseases before setting up the experiment. The coffee seedlings were transplanted into 3 l pots (one seedling per pot) containing substrate MecPlant® (commercial substratum based on Pinus bark) and kept in a greenhouse until fungal inoculation, totalizing 60 pots for all experiments. Each seedling was watered (30 mL) every two days. Monthly fertilization consisted of 30 mL of 4 g $L^{-1}$ ammonium sulfate per seedling. The experiment was set up three months after transplantation when plants had at least six pairs of true leaves.

CLM-infested leaves were collected from plants at Diogo Alves de Mello Experimental Station at UFV-Viçosa, MG, Brazil (20º45′14″ S; 42º52′55″ W). The rearing was kept in the Laboratory of Agroecology of EPAMIG-Viçosa, MG, Brazil, at 23 ± 1 ºC and 12:12 (L:D). Mined leaves were maintained in transparent acrylic cages (40 × 40 × 40 cm) with their petioles inserted into plastic boxes (20 × 10 cm) with flexible polyurethane foam sections soaked in tap water. When adults emerged from infested leaves, they were transferred to new cages with clean coffee leaves to ensure continuity of the CLM life cycle [48].

### 2.2. Fungal Suspensions

Conidia of *M. robertsii* and *M. brunneum* from stock cultures were plated on Petri dishes (9 cm diameter) containing potato dextrose agar (PDA) supplemented with 0.05 g $L^{-1}$ chloramphenicol and incubated in darkness at 26 °C for 15 days. Subsequently, *Metarhizium* conidia were harvested from the Petri dishes with a sterilized metal spatula and inoculated into plastic bags containing rice. For this, the bags with 100 g of rice (polished parboiled type 1) and 30 mL of sterile distilled water were autoclaved for 15 min at 1.0 atm pressure to 120 ºC. The bags were then kept in a laminar flow cabinet until completely cooled. Rice bags with *Metarhizium* conidia were kept in darkness at 26 °C for 7 days [49]. After that, 50 g of rice grains with conidia were suspended in 1 L of sterile Tween solution 0.05%. These suspensions were filtrated twice using bilayer sterile cheesecloth so as to remove rice grains and hyphal fragments and were then vortexed for 30 s.

The concentrations of suspensions were adjusted to 1 × $10^8$ conidia $mL^{-1}$ in sterile Tween solution 0.05%, with the aid of a Neubauer haemocytometer. We checked conidial germination by transferring 150 µL of the suspension onto Petri dishes (9 cm diameter) with PDA + chloramphenicol (0.05 g $L^{-1}$), incubating these at 26 °C for 24 h and counting germinated conidia. Suspension of both isolates presented germination rates higher than 98%.

### 2.3. Treatments

Seedling inoculation was carried out by soil drench placing 30 mL of suspension (1 × $10^8$ conidia $mL^{-1}$ in sterile Tween solution 0.05%) on the soil close to the stem. Controls received 30 mL of blank Tween 0.05%. The three treatments were *M. robertsii*, *M. brunneum*, and the control, with 20 coffee seedlings per treatment. The experiment was conducted in a completely randomized design. Each potted coffee seedling was kept in a cylindrical cage (30 cm diameter × 60 cm height) made with wire rods (3 mm diameter) and covered with gauze (Figure 1). The experiment was maintained in a greenhouse at the Laboratory of Insect–Microorganism Interactions, UFV.

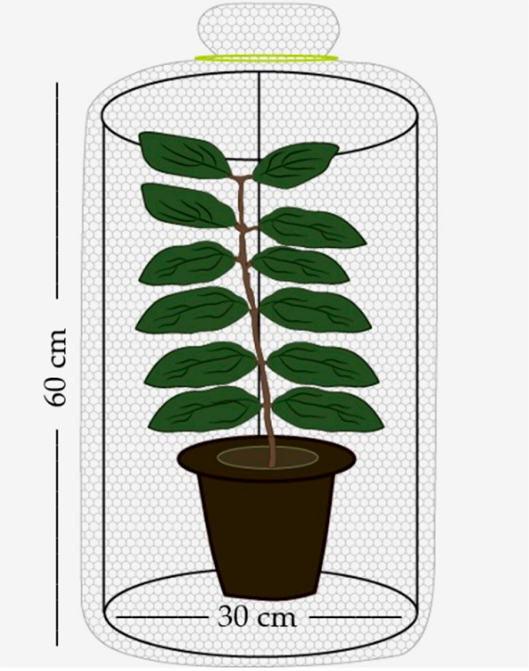


**Figure 1.** Drawing of cylindrical cage with a coffee seedling.

### *2.4. Colonization of M. robertsii and M. brunneum in Roots of Coffee Seedlings*

Roots from coffee seedlings were collected at the end of the experiment in order to evaluate the presence or absence of *Metarhizium*. Each root was shaken in a standardized manner to remove non-rhizosphere soil [50]. Subsequently, we ground 2 g of roots of each plant individually with a mortar and pestle and then suspended them in 5 mL of sterile 0.01% Tween, in a 15 mL Falcon® tube. Tubes were rotated for one hour in a rotary shaker at 150 rpm [51]. The suspensions were then vortexed for 15 s and aliquots of 100 µL from each suspension were plated in three Petri dishes (9 cm diameter) with semi-selective medium and spread with a sterile Drigalski spatula. The semi-selective medium for hypocrealean fungi consisted of 10 g peptone, 20 g dextrose, 15 g agar and 1 L distilled water. After sterilizing the medium, we added 0.05 g $L^{-1}$ of cycloheximide and tetracycline, 0.6 g $L^{-1}$ streptomycin and 0.175 g $L^{-1}$ CTAB (cetyltrimethylammonium bromide) [10]. Petri dishes were incubated in darkness at 26 °C for 14 days. We then evaluated the frequency of fungal colonization in roots. Only plants that presented at least one *Metarhizium* colony were considered positive for fungal association.

### *2.5. Effects of M. robertsii and M. brunneum on Coffee Seedling Growth*

To evaluate whether *Metarhizium* species have the potential to promote the growth of coffee seedlings, we evaluated the numbers of leaves, leaf areas, lengths of aerial parts and roots, stem diameters, and fresh and dry weights of aerial parts and roots. Since the seedlings had different initial sizes, the length of aerial parts and the numbers of leaves of each coffee seedling prior to treatments were measured. At the end of the experiment, 43 days after fungal inoculation, we repeated measurements of the aerial parts and leaf counts. We calculated increases in these values as proportional to the initial values for each plant. We used ImageJ software to measure leaf area [52], a tape measure to evaluate the length, digital calipers to measure the diameter and a precision balance to evaluate the weight.

### *2.6. Effects of M. robertsii and M. brunneum on L. coffeella*

Eight days post-inoculation, each plant was infested with two males and two females of CLM. After 48 h, we removed the adults and counted the eggs laid by the females on each seedling with a pocket magnifier. From this time, CLM development time (from egg to adult) and the number of mines, pupae and adults were daily evaluated. The seedlings

were evaluated until there was no more adult emergence, at ca. 40 days. On the last day of evaluation, all leaves from seedlings were removed and had pictures taken of them. We evaluated the percentage of leaf area that had been mined by measuring the total leaf area and mined area of the images with ImageJ software [52].

The survival and reproductive performance of CLM adults that emerged from seedlings were evaluated. For this, we used 26 CLM couples formed from adults that emerged from *M. robertsii*-inoculated plants and 30 from each of *M. brunneum*-inoculated plants and control plants. Each couple was placed inside a plastic pot (500 mL) covered with PVC, containing one clean untreated coffee leaf from coffee seedlings maintained in a greenhouse. The leaf petiole was inserted in a plastic container (3 mL) with water to maintain turgidity [48]. We evaluated the survival of males and females and numbers of eggs per female under a stereomicroscope daily until their death.

### *2.7. Statistical Analyses*

Inoculation with *M. robertsii*, *M. brunneum* and controls were used as explanatory variables to investigate if they can associate with coffee roots and the effect on seedling growth and CLM parameters. To examine if *Metarhizium* species can associate with roots, the frequencies with which the fungi were recovered from coffee seedling roots were analyzed. The samples were scored as positive or negative for *M. robertsii* or *M. brunneum*. Based on the confirmation of the colonization by *Metarhizium* species, we excluded the inoculated coffee seedlings that were negative for fungus from all the analyses (effect on coffee seedling growth and CLM parameters). To examine coffee seedling growth variables (number of leaves, leaf area, stem diameter, length of roots and aerial part, fresh and dry mass of roots and aerial part) we used Analysis of Deviance (F-tests) assuming normal distribution. Pairwise comparisons were performed with the emmeans R-package (adjustment method: Tukey) [53]. Survival analyses with censored Weibull distributions were carried out to test CLM development times to adults and the survival of emerged males and females. We used generalized linear models (GLM) adjusted to a Poisson distribution to analyze CLM count data of number of eggs, mines, pupae and emerged adults per plant and the numbers of eggs per emerged CLM female. We used Analysis of Deviance with $\chi^2$ tests and with pairwise comparisons as above. For percentage of leaf area mined, we used GLM adjusted to a Binomial distribution. We realized Analysis of Deviance with $\chi^2$ tests and pairwise comparisons as above. R (R Core Team, 2018) was used to analyze all data.

## 3. Results

### *3.1. Colonization of M. robertsii and M. brunneum in Roots of Coffee Seedlings*

Coffee seedlings of the control had no *Metarhizium* isolate recovered from roots (up to day 43). In contrast, both isolates were recovered from the roots of fungus-treated coffee seedlings. At 43 days after inoculation, *M. robertsii* was recovered from 18 of 20 coffee seedlings (90%) and *M. brunneum* from 15 of 20 seedlings (75%).

### *3.2. Effects of M. robertsii and M. brunneum on Growth of Coffee Seedlings*

Only the inoculation of *M. robertsii* enhanced seedling growth. There were no differences among treatments in the numbers of leaves ($F = 1.56$, $p = 0.22$; Figure 2a), lengths of aerial parts ($F = 0.37$, $p = 0.68$; Figure 2b) and roots ($F = 0.29$, $p = 0.743$; Figure 2c), stem diameters ($F = 1.23$, $p = 0.30$; Figure 2d), fresh masses of roots ($F = 0.19$, $p = 0.82$; Figure 2e) and of aerial parts ($F = 0.29$, $p = 0.74$; Figure 2f) and dry masses of roots ($F = 0.78$, $p = 0.46$; Figure 2g) and of aerial parts ($F = 0.37$, $p = 0.69$; Figure 2h). However, the inoculation of *M. robertsii* in coffee seedlings increased the leaf area by 30% compared to the uninoculated control ($t = 6.253$, $p < 0.001$, $df = 1$; Figure 2i).

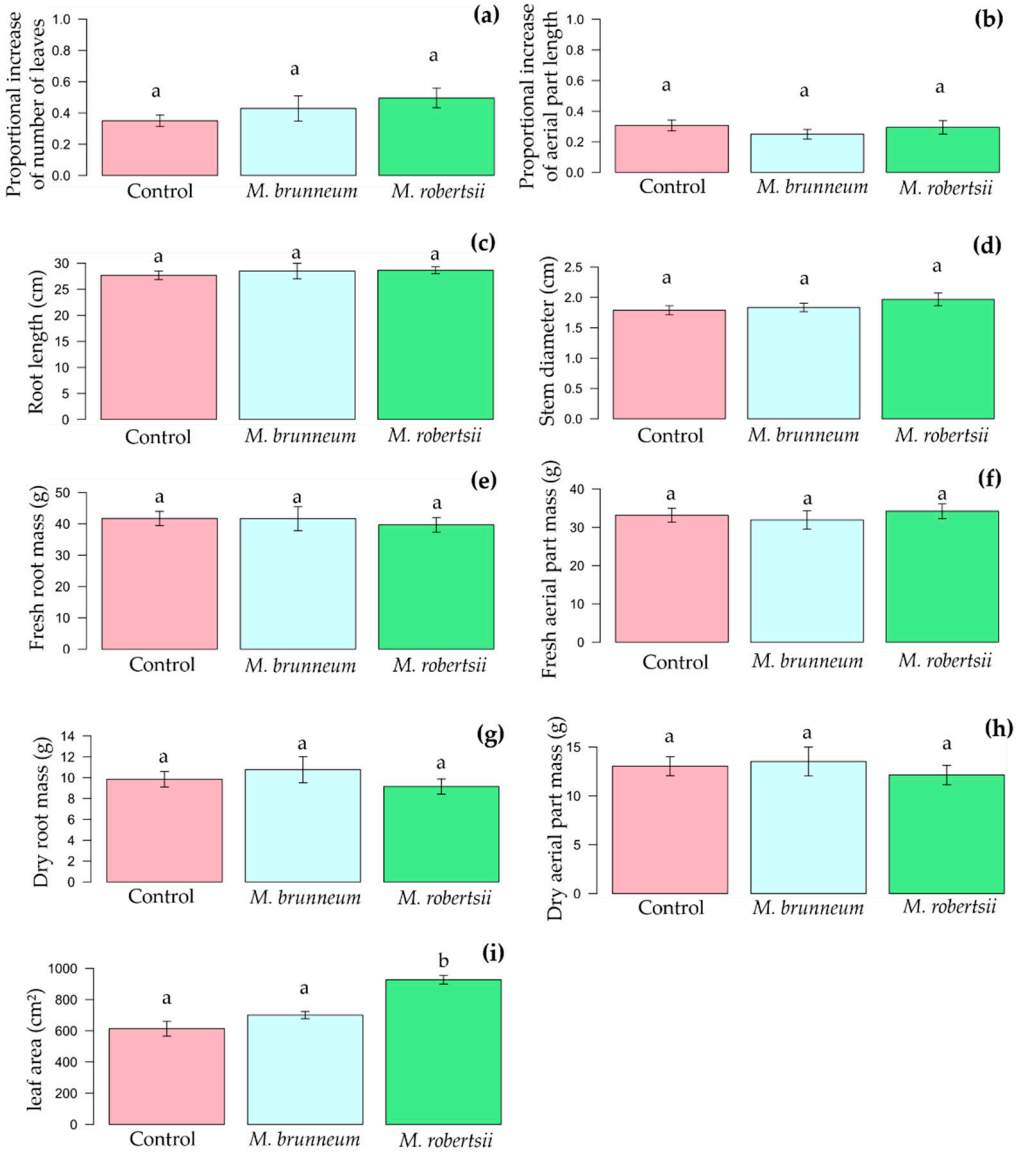


**Figure 2.** Growth variables of coffee seedlings inoculated with *Metarhizium robertsii* or *Metarhizium brunneum* by soil drench. (**a**) number of leaves; (**b**) length of aerial part; (**c**) length of roots; (**d**) stem diameter; (**e**) fresh mass of roots; (**f**) fresh mass of aerial part; (**g**) dry mass of roots; (**h**) dry mass of aerial part; (**i**) leaf area. Inoculation of 30 mL of each isolate suspension was to the surface of the soil in each coffee seedling while the control received a sterile solution of Tween 0.05%. Bars with the same letters are not statistically different.

### *3.3. Effects of M. robertsii and M. brunneum on L. coffeella*

Both isolates promoted protection against CLM. In coffee seedlings inoculated with *M. robertsii* ($z = 2.39$, $p = 0.04$) and *M. brunneum* ($z = 5.68$, $p < 0.001$), CLM presented delays of two days in the developmental times compared to uninoculated controls (Figure 3). There was no difference in the effects of inoculation with the two fungi on development times ($z = 1.95$, $p = 0.12$; Figure 3).

Fungal inoculation did not decrease the numbers of eggs ($\chi^2 = 1.95$, $p = 0.88$; Figure 4a), mines ($\chi^2 = 24.43$, $p = 0.11$; Figure 4b) or pupae ($\chi^2 = 26.59$, $p = 0.11$; Figure 4c) of CLM. Nevertheless, inoculation of *M. robertsii* reduced the numbers of emerged CLM adults by a third compared to the control ($z = 2.86$, $p = 0.01$; Figure 4d).

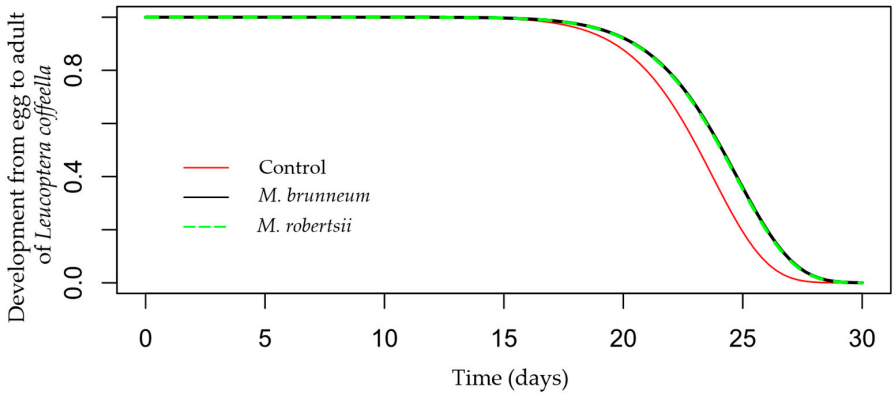


**Figure 3.** Developmental time from egg to adult of *Leucoptera coffeella* in coffee seedlings inoculated with *Metarhizium robertsii* and *Metarhizium brunneum* by soil drench. Inoculation of 30 mL of each isolate suspension was to the surface of the soil in each coffee seedling while the control received a sterile solution of Tween 0.05%.

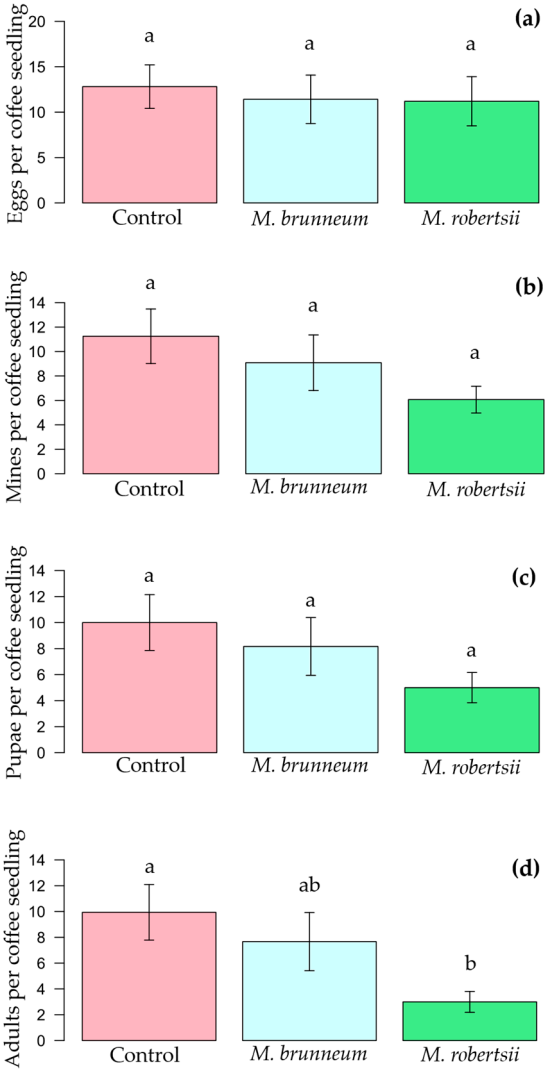


**Figure 4.** Development of *Leucoptera coffeella* in coffee seedlings with *Metarhizium robertsii* and *Metarhizium brunneum* inoculation by soil drench. (**a**) Number of eggs; (**b**) number of mines; (**c**) number of pupae; (**d**) number of adults. Inoculation of 30 mL of each isolate suspension was to the surface of the soil in each coffee seedling while the control received a sterile solution of Tween 0.05%. Bars with the same letters are not statistically different.

Considering the damage caused by the pest insect, the percentage of mined leaf area was lower in plants inoculated with *M. robertsii* (0.08 ± 0.02%; t = 9.23, $p < 0.001$) and *M. brunneum* (0.28 ± 0.10%; z = 7.16, $p < 0.001$) when compared to the control (1.38 ± 0.17%) (Figure 5). The observed differences in mined leaf area in the two inoculated treatments were statistically significant (z = 3.58, $p = 0.001$; Figure 5).

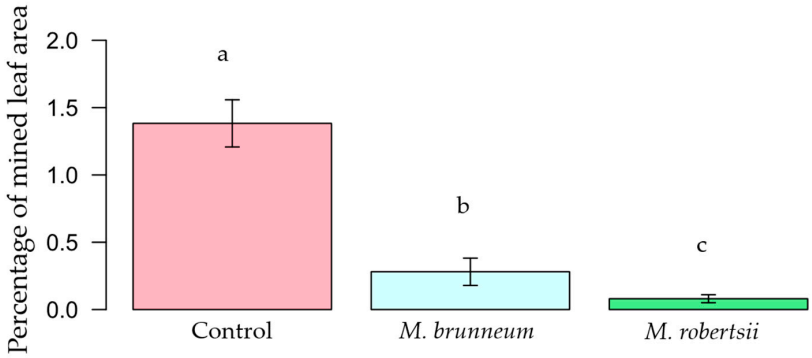


**Figure 5.** Percentage of leaf area mined by *Leucoptera coffeella* in coffee seedlings inoculated with *Metarhizium robertsii* and *Metarhizium brunneum* by soil drench. Inoculation of 30 mL of each isolate suspension was to the surface of the soil in each coffee seedling while the control received a sterile solution of Tween 0.05%. Bars with the same letters are not statistically different.

The survival of emerged females ($\chi^2 = 3.37$, $p = 0.18$) and males ($\chi^2 = 2.82$, $p = 0.24$) were similar in all treatments (Figure 6). Females who emerged from plants inoculated with *M. robertsii* produced half the eggs that females who emerged from the control produced (z = 2.46, $p = 0.03$; Figure 7). The eggs of females that emerged from the *M. brunneum* treatment were similar to the control (z = 1.89, $p = 0.13$; Figure 7) and *M. robertsii* treatment (z = 0.77, $p = 0.71$; Figure 7).

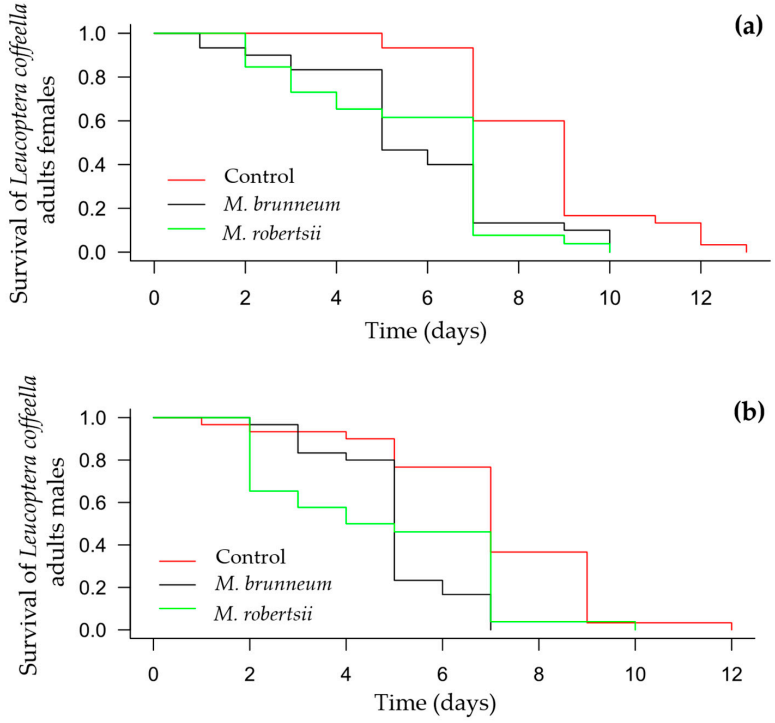


**Figure 6.** Survival of *Leucoptera coffeella* adults emerged from coffee seedlings with *Metarhizium robertsii* and *Metarhizium brunneum* inoculation by soil drench. (**a**) Survival of *L. coffeella* females; (**b**) Survival of *L. coffeella* males. Inoculation of 30 mL of each isolate suspension was to the surface of the soil in each coffee seedling while the control received a sterile solution of Tween 0.05%.

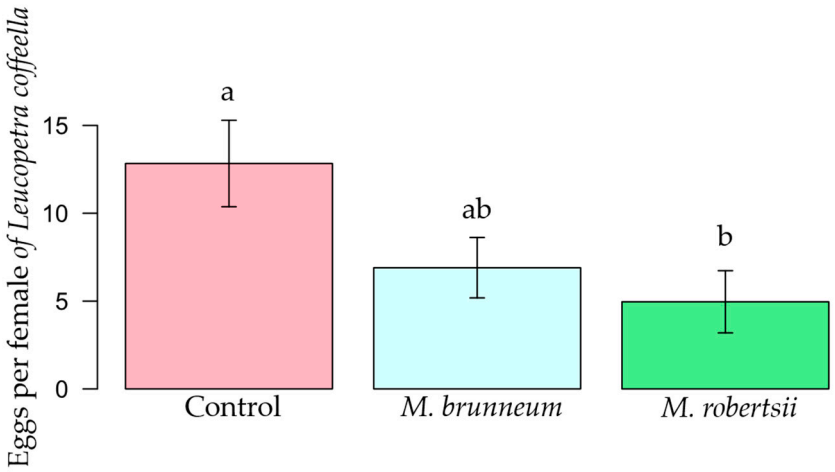


**Figure 7.** Number of eggs per female of *Leucoptera coffeella* emerged in coffee seedlings with *Metarhizium robertsii* and *Metarhizium brunneum* inoculation by soil drench Inoculation of 30 mL of each isolate suspension was to the surface of the soil in each coffee seedling while the control received a sterile solution of Tween 0.05%. Bars with the same letters are not statistically different.

## 4. Discussion

We showed that both *Metarhizium* isolates can successfully be applied as soil drenches to coffee seedlings where they establish associations with the roots. *Metarhizium robertsii* increases leaf area by a considerable 30%, an effect not seen from inoculation with *M. brunneum*. Furthermore, both *Metarhizium* isolates promoted some degree of protection against CLM in coffee seedlings as hypothesized, both reducing the percentage of mined leaf area and prolonging the insect developmental time. This effect was greater with *M. robertsii* with an additional reduction in the numbers of adults and eggs that emerged from inoculated plants.

Considering the colonization of coffee roots, *Metarhizium robertsii* appeared to be more efficient than *M. brunneum*, although a firm conclusion would require a study more specifically focused on this question and with appropriate sample sizes. However, studies with others plants show both species with similar root colonization capacity [14,54]. The ability of *Metarhizium* to colonize plant tissues varies by fungal species and strain, environmental conditions and host species [55]. Here, both *Metarhizium* isolates were in the same environmental conditions, and on the same host species. However, we used only one isolate for each *Metarhizium* species. Therefore, it is entirely possible that the difference found is due to the isolates rather than species. A study of these effects that considered a range of isolates of different species would be of great value, although this would present logistical difficulties.

Because we did not sterilize the surface of coffee seedling roots, it was not possible to distinguish between endophytic colonization by the fungi and growth only on the root surface. While such a point is important to understand how the fungi affect the plants, this was not the aim of the present study and so remains for future investigation. It is worth noting, however, that the initial stages of *Metarhizium* endophytic colonization involve rhizosphere colonization [56]. Likewise, in both associations, fungi can promote plant growth and protection against pests [3,4,15].

Inoculation with *M. robertsii* increased the total leaf area of coffee seedlings, indicating that this fungus acts as a growth promoter in coffee seedlings. EPF colonization may enhance plant growth by facilitating nutrient uptake through the plant root system or by translocating nitrogen from insect cadavers in the soil to the plant in exchange for carbon. [3,27]. We are aware of no previous study of *Metarhizium* inoculation in coffee plants. However, studies with other crops, such as sweet pepper (*Capsicum annum*) and sweet corn (*Zea mays*), have shown that *M. brunneum* inoculation can improve plant growth [14,15], a result we did not observe here.

We showed that plants inoculated with *M. robertsii* and *M. brunneum* reduced the total leaf area mined by 70% and prolonged CLM development time by two days compared

to uninoculated controls. Furthermore, *M. robertsii* reduced the number of emerged CLM adults by a third and the females who emerged produced half the eggs produced by the control females. Numerically, the data do not show a great discrepancy between *Metarhizium*-inoculated and uninoculated plants (see Figures 3–5 and 7). However, this degree of protection against coffee pests in our study corresponds to the infestation couples of CLM for 48 h. Under natural conditions, coffee seedlings are subject to more than two CLM females, which live for about 13 days. Therefore, the effects that we found here will become more expressive in coffee nurseries.

The main aim of this study was to determine if inoculation with *Metarhizium* would provide a protective effect against an important herbivorous pest, in this case, CLM. Endophytic EPF can promote indirect protection against insects. Mutualist associations between plants and *Metarhizium* species may prolong the developmental time of herbivores, delay the onset of reproduction and also reduce birth rates [15,16,40]. It was suggested that the mechanisms of these systemic responses are fungal metabolites that could be produced and transported through the plant's vascular system, either directly affecting herbivores or mediating indirect effects through the upregulation of plant defenses [35,36,57]. The fungal metabolites could be alkaloids, saponins, tannins, phenolic acids, steroids, quinones, and terpenoids, which are insect antagonists [58]. A further aspect to bear in mind is that endophytic fungi can induce changes in the emission of volatile compounds, which can, in turn, influence how insects choose plants for oviposition [59].

The insect's feeding mode is an important feature of its interaction with the plant, which will affect any indirect protection of the plant by endophytic EPF. This is because chewing, sucking, mining and galling insects often respond differently to plant defenses [60]. Sucking insects are more strongly affected by endophytic EPF inoculation than the others [60] because fungal metabolites can be transported through plant vasculature [61]. It was argued that intracellular EPF growth is limited because of nutrient availability in the plant intercellular space and the absence of cell walls and cell membrane-degrading fungal enzymes [62]. Therefore, it was suggested that fewer effects of endophytic EPF occur in mining insects, as they do not feed directly on the plant vascular bundles, where EPF growth may be more extensive. In spite of this, we show here the negative effects of *Metarhizium* spp. against a mining insect. It would be interesting to examine the degree of intercellular growth in this case; meanwhile, we could expect even greater effects on sucking insects [60].

Brazil is the world's largest producer and exporter of coffee [63]. However, climate-changing conditions and rising global temperatures are major threats to coffee production across the world [64]. There is strong evidence that rising temperatures and altered rainfall patterns are already affecting coffee yields, quality, pests and diseases [64]. Rising temperatures may promote pest insects by allowing them to produce more generations in a year [65,66]. CLM specifically is influenced by climatic conditions, with dry and hot areas being more favorable for this pest to reach high infestation levels [41]. In order to control heavy infestations of CLM, current management involves intensive insecticide use, such as chlorantraniliprol and organophosphate. As a consequence, problems with CLM resistance to conventional insecticides are frequent [44,67]. Therefore, the rising temperatures associated with indiscriminate insecticide use have increased the costs of CLM control, besides the negative impacts on the environment and human health.

The development of microbial inoculants that improve coffee seedling growth and have negative effects on CLM is a promising strategy to reduce costs (whether economical or in terms of environmental and human health) with pesticides. Our results are consistent with studies that report the indirect effect of EPF on reducing insect pest damage. The most common effects reported are delays in insect developmental time, feeding deterrence, retardation of insect growth, reduced survival and oviposition (reviewed in Bamisile et al. [27]). Studies of inoculation of entomopathogenic fungi in coffee are restricted to *Beauveria bassiana* [68,69], a biological control agent of coffee berry borer *Hypothenemus hampei* Ferrari (Coleoptera: Curculionidae), another key coffee pest worldwide [70]. Our study is therefore novel with respect

to *Metarhizium* species and their colonization of coffee roots (here via soil drench), which improves plant growth and protection against CLM. Hence, we showed that *M. brunneum* and *M. robertsii* could be considered for the development of inoculants for coffee seedlings. Further studies are necessary to test the viability of using such a strategy in adult coffee plants in the field. Additionally, microscopical and molecular studies are also needed to elucidate the mechanisms behind the association of *Metarhizium* species and coffee roots as was performed with other plants (papers by Bidochka and St leger). Thereby, it will be possible to explain how the physiological processes occur inside the plant and may encourage new tests with other organisms.

**Author Contributions:** Conceptualization, M.L.F. and C.C.M.; methodology, M.L.F., L.N.P.d.S. and E.F.M.; formal analysis, M.A.A.M.F.; investigation, M.L.F.; resources, A.P., S.L.E. and M.V.; data curation, M.L.F., C.C.M. and M.A.A.M.F.; writing—original draft preparation, M.L.F.; writing—review and editing, C.C.M., A.P., S.L.E. and M.V.; visualization, M.L.F.; supervision, C.C.M., M.A.A.M.F., S.L.E. and M.V.; project administration, A.P. and M.V.; funding acquisition, A.P. and M.V. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by 'Conselho Nacional de Desenvolvimento Científico e Tecnológico' (CNPq, 312784/2021-0), 'Fundação de Amparo à Pesquisa de Minas Gerais' (FAPEMIG, CAG-PPM-00270/18), 'Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-Brasil' (CAPES)-Finance Code 001 and Consórcio Brasileiro de Pesquisa e Desenvolvimento do Café (CBP&D-Café, 10.18.20.049.00.04).

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

**Acknowledgments:** We are grateful to members of the laboratory of Insect–Microorganism Interactions, UFV, for their help with laboratory protocols. We thank Jéssica Letícia Abreu Martins for her support in preparing the drawing of the cylindrical cage.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Hajek, A.E.; St. Leger, R.J. Interactions between fungal pathogens and insect hosts. *Annu. Rev. Entomol.* **1994**, *39*, 293–322. [CrossRef]
2. Fisher, J.J.; Rehner, S.A.; Bruck, D.J. Diversity of rhizosphere associated entomopathogenic fungi of perennial herbs, shrubs and coniferous trees. *J. Invertebr. Pathol.* **2011**, *106*, 289–295. [CrossRef] [PubMed]
3. Behie, S.W.; Zelisko, P.M.; Bidochka, M.J. Endophytic insect-parasitic fungi translocate nitrogen directly from insects to plants. *Science* **2012**, *336*, 1576–1577. [CrossRef] [PubMed]
4. Behie, S.W.; Bidochka, M.J. Nutrient transfer in plant–fungal symbioses. *Trends Plant Sci.* **2014**, *19*, 734–740. [CrossRef] [PubMed]
5. Keyser, C.A.; De Fine Licht, H.H.; Steinwender, B.M.; Meyling, N.V. Diversity within the entomopathogenic fungal species *Metarhizium flavoviride* associated with agricultural crops in Denmark. *BMC Microbiol.* **2015**, *15*, 249. [CrossRef]
6. Krell, V.; Jakobs-Schoenwandt, D.; Vidal, S.; Patel, A.V. Cellulase enhances endophytism of encapsulated *Metarhizium brunneum* in potato plants. *Fungal Biol.* **2018**, *122*, 373–378. [CrossRef]
7. Stone, L.B.L.; Bidochka, M.J. The multifunctional lifestyles of *Metarhizium*: Evolution and applications. *Appl. Microbiol. Biotechnol.* **2020**, *104*, 9935–9945. [CrossRef]
8. Roberts, D.W.; Hajek, A.E. Entomopathogenic fungi as bioinsecticides. In *Frontiers in Industrial Mycology*; Leatham, F.G., Ed.; Springer: New York, NY, USA, 1992; pp. 144–159.
9. Zimmermann, G. Review on safety of the entomopathogenic fungus *Metarhizium anisopliae*. *Biocontrol Sci. Technol.* **2007**, *17*, 879–920. [CrossRef]
10. Kepler, R.M.; Ugine, T.A.; Maul, J.E.; Cavigelli, M.A.; Rehner, S.A. Community composition and population genetics of insect pathogenic fungi in the genus *Metarhizium* from soils of a long-term agricultural research system. *Environ. Microbiol.* **2015**, *17*, 2791–2804. [CrossRef] [PubMed]
11. Brunner-Mendoza, C.; del Rocío Reyes-Montes, M.; Moonjely, S.; Bidochka, M.J.; Toriello, C. A review on the genus *Metarhizium* as an entomopathogenic microbial biocontrol agent with emphasis on its use and utility in Mexico. *Biocontrol Sci. Technol.* **2019**, *29*, 83–102. [CrossRef]
12. Bioinsumos do Ministério da Agricultura, Pecuária e Abastecimento (Mapa) Program. Bioinsumos App. (Version 1.0.0). 2020. Available online: https://play.google.com/store/apps/details?id=br.embrapa.bioinsumos (accessed on 2 October 2022).

13. MAPA Ministério da Agricultura Pecuária e Abastecimento. Available online: http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons (accessed on 20 August 2022).
14. Liao, X.; O'Brien, T.R.; Fang, W.; St. Leger, R.J. The plant beneficial effects of *Metarhizium* species correlate with their association with roots. *Appl. Microbiol. Biotechnol.* **2014**, *98*, 7089–7096. [CrossRef] [PubMed]
15. Jaber, L.R.; Araj, S.E. Interactions among endophytic fungal entomopathogens (Ascomycota: Hypocreales), the green peach aphid *Myzus persicae* Sulzer (Homoptera: Aphididae), and the aphid endoparasitoid *Aphidius colemani* Viereck (Hymenoptera: Braconidae). *Biol. Control* **2018**, *116*, 53–61. [CrossRef]
16. Ahmad, I.; Jiménez-Gasco, M.M.; Luthe, D.S.; Shakeel, S.N.; Barbercheck, M.E. Endophytic *Metarhizium robertsii* promotes maize growth, suppresses insect growth, and alters plant defense gene expression. *Biol. Control* **2020**, *144*, 104167. [CrossRef]
17. Sasan, R.K.; Bidochka, M.J. Antagonism of the endophytic insect pathogenic fungus *Metarhizium robertsii* against the bean plant pathogen *Fusarium solani* f. sp. *Phaseoli*. *Can. J. Plant Pathol.* **2013**, *35*, 288–293. [CrossRef]
18. Chairin, T.; Petcharat, V. Induction of defense responses in longkong fruit (*Aglaia Dookkoo* Griff.) against fruit rot fungi by *Metarhizium guizhouense*. *Biol. Control* **2017**, *111*, 40–44. [CrossRef]
19. Behie, S.W.; Moreira, C.C.; Sementchoukova, I.; Barelli, L.; Zelisko, P.M.; Bidochka, M.J. Carbon translocation from a plant to an insect-pathogenic endophytic fungus. *Nat. Commun.* **2017**, *8*, 14245. [CrossRef]
20. Hu, G.; Leger, R.J.S. Field studies using a recombinant mycoinsecticide (*Metarhizium anisopliae*) reveal that it is rhizosphere competent. *Appl. Environ. Microbiol.* **2002**, *68*, 6383–6387. [CrossRef]
21. Wilson, D. Endophyte—The evolution of a term, and clarification of its use and definition. *Oikos* **1995**, *73*, 274–276. [CrossRef]
22. Mantzoukas, S.; Eliopoulos, P.A. Endophytic entomopathogenic fungi: A valuable biological control tool against plant pests. *Appl. Sci.* **2020**, *10*, 360. [CrossRef]
23. Morgan, J.A.W.; Bending, G.D.; White, P.J. Biological costs and benefits to plant-microbe interactions in the rhizosphere. *J. Exp. Bot.* **2005**, *56*, 1729–1739. [CrossRef]
24. Behie, S.W.; Jones, S.J.; Bidochka, M.J. Plant tissue localization of the endophytic insect pathogenic fungi *Metarhizium* and *Beauveria*. *Fungal Ecol.* **2015**, *13*, 112–119. [CrossRef]
25. Batta, Y.A. Efficacy of endophytic and applied *Metarhizium anisopliae* (Metch.) Sorokin (Ascomycota: Hypocreales) against larvae of *Plutella xylostella* L. (Yponomeutidae: Lepidoptera) infesting *Brassica napus* plants. *Crop Prot.* **2013**, *44*, 128–134. [CrossRef]
26. Wyrebek, M.; Huber, C.; Sasan, R.K.; Bidochka, M.J. Three sympatrically occurring species of *Metarhizium* show plant rhizosphere specificity. *Microbiology* **2011**, *157*, 2904–2911. [CrossRef]
27. Bamisile, B.S.; Dash, C.K.; Akutse, K.S.; Keppanan, R.; Wang, L. Fungal endophytes: Beyond herbivore management. *Front. Microbiol.* **2018**, *9*, 544. [CrossRef]
28. Ramos, Y.; Taibo, A.D.; Jiménez, J.A.; Portal, O. Endophytic establishment of *Beauveria bassiana* and *Metarhizium anisopliae* in maize plants and its effect against *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) larvae. *Egypt. J. Biol. Pest Control* **2020**, *30*, 212410370. [CrossRef]
29. Gao, Q.; Jin, K.; Ying, S.H.; Zhang, Y.; Xiao, G.; Shang, Y.; Duan, Z.; Hu, X.; Xie, X.Q.; Zhou, G.; et al. Genome sequencing and comparative transcriptomics of the model entomopathogenic fungi *Metarhizium anisopliae* and *M. acridum*. *PLoS Genet.* **2011**, *7*, e1001264. [CrossRef]
30. Spatafora, J.W.; Sung, G.H.; Sung, J.M.; Hywel-Jones, N.L.; White, J.F. Phylogenetic evidence for an animal pathogen origin of ergot and the grass endophytes. *Mol. Ecol.* **2007**, *16*, 1701–1711. [CrossRef]
31. Hu, X.; Xiao, G.; Zheng, P.; Shang, Y.; Su, Y.; Zhang, X.; Liu, X.; Zhan, S.; St. Leger, R.J.; Wang, C. Trajectory and genomic determinants of fungal-pathogen speciation and host adaptation. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 16796–16801. [CrossRef]
32. Barelli, L.; Moonjely, S.; Behie, S.W.; Bidochka, M.J. Fungi with multifunctional lifestyles: Endophytic insect pathogenic fungi. *Plant Mol. Biol.* **2016**, *90*, 657–664. [CrossRef]
33. Clark, R.B.; Zeto, S.K. Mineral acquisition by arbuscular mycorrhizal plants. *J. Plant Nutr.* **2000**, *23*, 867–902. [CrossRef]
34. Roberts, D. Toxins of entomopathogenic fungi. In *Microbial Control of Pests and Plant Disease*; Burges, H.D., Ed.; Academic Press: London, UK, 1981; pp. 1970–1980.
35. Golo, S.; Gardner, D.R.; Grilley, M.M.; Takemoto, J.Y.; Krasnoff, S.B.; Pires, M.S.; Fernandes, É.K.K.; Bittencourt, V.R.E.P.; Roberts, D.W. Production of destruxins from *Metarhizium* Spp. fungi in artificial medium and in endophytically colonized cowpea plants. *PLoS ONE* **2014**, *9*, e104946. [CrossRef] [PubMed]
36. Ríos-Moreno, A.; Garrido-Jurado, I.; Resquín-Romero, G.; Arroyo-Manzanares, N.; Arce, L.; Quesada-Moraga, E. Destruxin A production by *Metarhizium brunneum* strains during transient endophytic colonisation of *Solanum tuberosum*. *Biocontrol Sci. Technol.* **2016**, *26*, 1574–1585. [CrossRef]
37. Resquín-Romero, G.; Garrido-Jurado, I.; Delso, C.; Ríos-Moreno, A.; Quesada-Moraga, E. Transient endophytic colonizations of plants improve the outcome of foliar applications of mycoinsecticides against chewing insects. *J. Invertebr. Pathol.* **2016**, *136*, 23–31. [CrossRef] [PubMed]
38. Hu, S.; Bidochka, M.J. Abscisic acid implicated in differential plant responses of *Phaseolus vulgaris* during endophytic colonization by *Metarhizium* and pathogenic colonization by *Fusarium*. *Sci. Rep.* **2021**, *11*, 11327. [CrossRef] [PubMed]
39. Liao, X.; Lovett, B.; Fang, W.; St Leger, R.J. *Metarhizium robertsii* produces indole-3-acetic acid, which promotes root growth in arabidopsis and enhances virulence to insects. *Microbiology* **2017**, *163*, 980–991. [CrossRef]

40. Canassa, F.; Tall, S.; Moral, R.A.; de Lara, I.A.R.; Delalibera, I.; Meyling, N. V Effects of bean seed treatment by the entomopathogenic fungi *Metarhizium robertsii* and *Beauveria bassiana* on plant growth, spider mite populations and behavior of predatory mites. *Biol. Control* **2019**, *132*, 199–208. [CrossRef]
41. Reis, P.R.; Souza, J.C.; Venzon, M. Manejo ecológico das principais pragas do cafeeiro. *Inf. Agropecu.* **2002**, *23*, 83–99.
42. Tomaz, M.A.; Martins, L.D.; Rodrigues, W.N. Produção de mudas e plantio. In *Café Arábica: Do Plantio à Colheita*; Sakiyama, N.S., Martinez, H.E.P., Tomaz, M.A., Borém, A., Eds.; UFV: Viçosa, Brazil, 2015; pp. 46–63.
43. Reis, P.R.; Souza, J.C. Manejo integrado do bicho-mineiro, *Perileucoptera cofeella* (Guérin-Mèneville) (Lepidoptera: Lyonetiidae) e seus reflexos na produção de café. *An. Soc. Entomol. Bras.* **1996**, *25*, 77–82. [CrossRef]
44. Leite, S.A.; Dos Santos, M.P.; Resende-Silva, G.A.; Da Costa, D.R.; Moreira, A.A.; Lemos, O.L.; Guedes, R.N.C.; Castellani, M.A. Area-wide survey of chlorantraniliprole resistance and control failure likelihood of the Neotropical coffee leaf miner *Leucoptera coffeella* (Lepidoptera: Lyonetiidae). *J. Econ. Entomol.* **2020**, *113*, 1399–1410. [CrossRef]
45. Nicolopoulou-Stamati, P.; Maipas, S.; Kotampasi, C.; Stamatis, P.; Hens, L. Chemical pesticides and human health: The urgent need for a new concept in agriculture. *Front. Public Health* **2016**, *4*, 148. [CrossRef]
46. Zimmermann, G. The '*Galleria* bait method' for detection of entomopathogenic fungi in soil. *J. Appl. Entomol.* **1986**, *102*, 213–215. [CrossRef]
47. Sharma, L.; Oliveira, I.; Torres, L.; Marques, G. Entomopathogenic fungi in portuguese vineyards soils: Suggesting a '*Galleria-Tenebrio*-bait method' as bait- insects *Galleria* and *Tenebrio* significantly underestimate the respective recoveries of *Metarhizium* (*robertsii*) and *Beauveria* (*bassiana*). *MycoKeys* **2018**, *38*, 1–23. [CrossRef]
48. Martins, E.F.; Franzin, M.L.; Perez, A.L.; Schmidt, J.M.; Venzon, M. Is *Ceraeochrysa cubana* a coffee leaf miner predator? *Biol. Control* **2021**, *160*, 104691. [CrossRef]
49. Jaronski, S.T.; Jackson, M.A. Mass production of entomopathogenic hypocreales. In *Manual of Techniques in Invertebrate Pathology*; Lacey, L.A., Ed.; Academic Press: London, UK, 2012; pp. 255–284.
50. Moreira, C.C. *The Double Life of an Insect Pathogen: Metarhizium as a Plant Symbiont and Its Genetic Diversity in Coffee Agroecosystems*; Universidade Federal de Viçosa: Viçosa, Brazil, 2016.
51. Lacey, L.A. *Manual of Techniques in Invertebrate Pathology*, 2nd ed.; Lacey, L.A., Ed.; Elsevier Ltd.: Yakima, WA, United States, 2012; ISBN 9780123868992.
52. Abràmoff, M.D.; Hospitals, I.; Magalhães, P.J.; Abràmoff, M. Image processing with ImageJ. *Biophotonics Int.* **2004**, *11*, 36–42.
53. Lenth, R.V.; Buerkner, P.; Herve, M.; Love, J.; Riebl, H.; Singmann, H. Packpage "Emmeans". 2021. Available online: https://cran.r-project.org/web/packages/emmeans/index.html (accessed on 2 October 2022).
54. Lahey, S.; Angelone, S.; DeBartolo, M.O.; Coutinho-Rodrigues, C.; Bidochka, M.J. Localization of the insect pathogenic fungal plant symbionts *Metarhizium robertsii* and *Metarhizium brunneum* in bean and corn roots. *Fungal Biol.* **2020**, *124*, 877–883. [CrossRef]
55. Lovett, B.; St. Leger, R.J. Stress is the rule rather than the exception for *Metarhizium*. *Curr. Genet.* **2015**, *61*, 253–261. [CrossRef]
56. Barelli, L.; Moreira, C.C.; Bidochka, M.J. Initial stages of endophytic colonization by *Metarhizium* involves rhizoplane colonization. *Microbiology* **2018**, *164*, 1531–1540. [CrossRef] [PubMed]
57. Mantzoukas, S.; Chondrogiannis, C.; Grammatikopoulos, G. Effects of three endophytic entomopathogens on sweet sorghum and on the larvae of the stalk borer *Sesamia nonagrioides*. *Entomol. Exp. Appl.* **2015**, *154*, 78–87. [CrossRef]
58. Gouda, S.; Das, G.; Sen, S.K.; Shin, H.S.; Patra, J.K. Endophytes: A treasure house of bioactive compounds of medicinal importance. *Front. Microbiol.* **2016**, *7*, 1538. [CrossRef] [PubMed]
59. Jallow, M.F.A.; Dugassa-Gobena, D.; Vidal, S. Influence of an endophytic fungus on host plant selection by a polyphagous moth via volatile spectrum changes. *Arthropod-Plant Interact.* **2008**, *2*, 53–62. [CrossRef]
60. Gange, A.C.; Koricheva, J.; Currie, A.F.; Jaber, L.R.; Vidal, S. Meta-analysis of the role of entomopathogenic and unspecialized fungal endophytes as plant bodyguards. *New Phytol.* **2019**, *223*, 2002–2010. [CrossRef]
61. Jaber, L.R.; Ownley, B.H. Can we use entomopathogenic fungi as endophytes for dual biological control of insect pests and plant pathogens? *Biol. Control* **2018**, *116*, 36–45. [CrossRef]
62. Ullrich, C.I.; Koch, E.; Matecki, C.; Schafer, J.; Burkl, T.; Rabenstein, F.; Kleespies, R.G. Detection and growth of endophytic entomopathogenic fungi in dicot crop plants. *J. Kult.* **2017**, *69*, 291–302. [CrossRef]
63. FAO Food and Agriculture Organization of the United Nations. Available online: http://faostat.fao.org/faostat (accessed on 20 July 2022).
64. Watts, C. *A Brewing Storm: The Climate Change Risks to Coffee*; The Climate Institute: Australia, New Zealand, 2016.
65. Gregory, P.J.; Johnson, S.N.; Newton, A.C.; Ingram, J.S.I. Integrating pests and pathogens into the climate change/food security debate. *J. Exp. Bot.* **2009**, *60*, 2827–2838. [CrossRef]
66. IPPC Secretariat. *Scientific Review of the Impact of Climate Change on Plant Pests—A Global Challenge to Prevent and Mitigate Plant Pest Risks in Agriculture, Forestry and Ecosystems*; FAO: Rome, Italy, 2021; ISBN 9789251344354.
67. Fragoso, D.B.; Guedes, R.N.C.; Picanço, M.C.; Zambolim, L. Insecticide use and organophosphate resistance in the coffee leaf miner *Leucoptera coffeella* (Lepidoptera: Lyonetidae). *Bull. Entomol. Res.* **2002**, *92*, 203–212. [CrossRef]
68. Posada, F.; Vega, F.E. Inoculation and colonization of coffee seedlings (*Coffea arabica* L.) with the fungal entomopathogen *Beauveria bassiana* (Ascomycota: Hypocreales). *Mycoscience* **2006**, *47*, 284–289. [CrossRef]
69. Posada, F.; Aime, M.C.; Peterson, S.W.; Rehner, S.A.; Vega, F.E. Inoculation of coffee plants with the fungal entomopathogen *Beauveria bassiana* (Ascomycota: Hypocreales). *Mycol. Res.* **2007**, *111*, 748–757. [CrossRef]
70. Oliveira, C.M.; Auad, A.M.; Mendes, S.M.; Frizzas, M.R. economic impact of exotic insect pests in brazilian agriculture. *J. Appl. Entomol.* **2013**, *137*, 1–15. [CrossRef]