*Published in : Journal of Invertebrate Pathology (2026), vol. 216, 108559*
*DOI: 10.1016/j.jip.2026.108559*
*Status : Postprint (Author's version)*

# BIOCONTROL POTENTIAL OF *METARHIZIUM* SPP. AGAINST *MYZUS PERSICAE* AND BEET MILD YELLOWING VIRUS IN THE POST-NEONICOTINOID ERA

Ibtissem Ben Fekih [a,*], Kenza Dessauvages [a], Mathilde Scheifler [b], Grégoire Noël [a], Tariq M. Butt [c], Joachim Carpentier [a], Frédéric Francis [a]

[a] *Functional and Evolutionary Entomology, Terra, Gembloux Agro-Bio Tech, University of Liege, Passage des Déportés 2, 5030 Gembloux, Belgium*
[b] *Evolution and Ecophysiology Group, Functional and Evolutionary Entomology, Terra, Gembloux Agro-Bio Tech, University of Liege, 5030 Gembloux, Belgium*
[c] *Department of Biosciences, Faculty of Science and Engineering, Swansea University, Singleton Park, Swansea SA2 8PP, UK*
** Corresponding author : E-mail address: ibtissem.benfekih@uliege.be (I. Ben Fekih).*

**ABSTRACT**

Entomopathogenic fungi (EPF) are promising biocontrol agents that can also function as plant endophytes, mediating interactions within plant–insect–pathogen systems. Here, we evaluated four EPF isolates, two endemic (*Metarhizium brunneum* strain GxABT-2 and *Metarhizium majus* strain GxABT-3) and two reference strains (*M. brunneum* ARSEF4556 and V275), for their efficacy against the green peach aphid (*Myzus persicae*) and their ability to limit beet mild yellowing virus (BMYV) transmission. Concentration-dependent bioassays assessed aphid mortality, fecundity, and post-mortem fungal development, while seed treatments evaluated the endophytic colonization and impact on BMYV establishment. All isolates caused > 80% aphid mortality at the highest concentration ($1 \times 10^8$ conidia $ml^{-1}$), whether applied directly (spray or immersion of aphids) or indirectly (spray or immersion of leaves), with GxABT-2 producing the greatest reduction in fecundity. Three post-mortem developmental stages (melanized, non-sporulating, and sporulating) were primarily observed at lower concentrations ($1 \times 10^5$ and $1 \times 10^6$ conidia $ml^{-1}$). Overall, melanized aphid cadavers were more frequent with endemic isolates, whereas sporulating cadavers predominated in the V275 and ARSEF4556 treatments. Endophytically colonized plants exhibited significantly lower BMYV loads, with GxABT-2 being the most effective. These results highlight the potential of endemic *Metarhizium* spp. as sustainable biocontrol agents in a post- neonicotinoid era and underscore the importance of understanding isolate-specific variability in plant–fungus interactions for optimizing viral suppression.

**KEYWORDS:** APHID ; BETA VULGARIS BIOCONTROL ; DUAL ACTION ; METARHIZIUM SPP. ; YELLOWING VIRUS

*Published in : Journal of Invertebrate Pathology (2026), vol. 216, 108559*
*DOI: 10.1016/j.jip.2026.108559*
*Status : Postprint (Author's version)*

# 1. Introduction

Sugar beet (*Beta vulgaris* ssp. *vulgaris* L.) is an economically important crop, accounting for 20% of the world's sugar (Biancardi et al., 2010). Sugar beet is attacked by a wide range of insect pests of which aphids, namely the green peach aphid (*Myzus persicae* Sulzer) and the black bean aphid (*Aphis fabae* Scopoli), cause considerable losses directly through feeding and indirectly mainly through the transmission of virus diseases (Hossain et al., 2021; Zhu et al., 2024). Aphid-transmitted beet yellows virus (BYV) and beet mild yellowing virus (BMYV) cause yield losses of up to 50% and 30%, respectively (Hossain et al., 2021; Borgolte et al., 2024). Until recently, aphids' control was dependent on the use of neonicotinoid insecticides (Hauer et al., 2017). However, these insecticides have been withdrawn or restricted in their use due to the risks they pose to human health and pollution of the environment (Wood and Goulson, 2017; Laurent et al., 2024; Wyckhuys et al., 2024). This has prompted the search for benign alternatives with a particular focus on biocontrol agents or biopesticides (van Lenteren et al., 2018; Francis et al., 2022a; Wyckhuys et al., 2024).

Entomopathogenic fungi (EPF)-based biopesticides are among the most promising alternatives and have been shown to be efficacious against a wide range of arthropod pests, including aphid species like *M. persicae* (Ekesi and Maniania, 2000; Shan and Feng, 2010; Jandricic et al., 2014; Biryol et al., 2022). The infection and developmental processes of EPF are similar among the different species, involving spore adhesion to the host surface, germination and penetration of the host cuticle, colonization of the haemocoel, and ultimately emergence and sporulation at the surface of the insect cuticle (Amnuaykanjanasin et al., 2013; Butt et al., 2016). Besides their entomopathogenic potential, many EPF species can also develop endophytically inside a wide range of host plants and concomitantly stimulate growth and increase plant resilience to biotic and abiotic stresses (Shaalan et al., 2021; Fite et al., 2023). The physiological changes in the plant elicited by endophytic EPF also impact pest fitness and survival (Francis et al., 2022b; Panwar and Szczepaniec, 2024). Most often EPF reduce aphid fitness, survival and fecundity (Jaber and Araj, 2018; Mahmood et al., 2019; Allegrucci et al., 2020). However, there are a few reports where endophytic EPF increase aphid populations compared to untreated controls (Clifton et al., 2018, Rasool et al., 2021). These negative effects can range from mild to severe and are dependent on the fungal strain, host plant and target pest. For example, Rasool et al. (2021) reported that inoculation of wheat and bean with the EPF *Metarhizium robertsii* (Metchnikoff) Sorokin and *Beauveria bassiana* (Balsamo) Vuillemin reduced populations of Bird cherry-oat aphid (*Rhopalosiphum padi* L.) and *A. fabae* compared with control treatments, whereas *Metarhizium brunneum* (Petch) strain Met52 increased the populations of both aphids. Interestingly, the Met52 strain (=BIPESCO 5, V275) of *M. brunneum* also increased populations of soybean aphid (*A. glycines* Matsumura) on soya (Clifton et al., 2018) but negatively impacted *M. persicae* on sweet pepper, *Capsicum annum* L. (Jaber and Araj, 2018).

Many strains of endophytic EPF have been reported to suppress a wide range of plant pathogenic fungi through modulation of the plant's defenses (Hao et al., 2021). For example, *M. brunneum* Met52 inoculated sweet pepper significantly reduced incidence and severity of different *Fusarium* species in controlled greenhouse trials (Jaber and Alananbeh, 2018). Comparatively little is known about

*Published in : Journal of Invertebrate Pathology (2026), vol. 216, 108559*
*DOI: 10.1016/j.jip.2026.108559*
*Status : Postprint (Author's version)*

the antagonistic effects of fungal endophytes against plant virus diseases. Jaber and Salem (2014) reported lower virus titer levels of zucchini yellow mosaic virus in squash (*Cucurbita pepo* L.) colonized endophytically by *B. bassiana* compared to the endophyte-free plants. Maize plants inoculated by *M. anisopliae* (Metsch.) Sorokin resulted in reduced severity and titer levels of sugarcane mosaic virus compared with controls but had no significant effect on maize chlorotic mottle virus (Kiarie et al., 2020).

The current study focuses on the assessment of four strains of *Metarhizium* spp. (three of *M. brunneum* and one of *M. majus* (J.R. Johnst.)) in tripartite interactions involving *M. persicae*, sugar beet, and BMYV. Specifically, we aim to: (i) evaluate the virulence of two Belgian endemic fungal isolates of *M. brunneum* and one of *M. majus*, on *M. persicae* survival and fecundity through both direct and indirect exposure to fungal inoculum; (ii) investigate their ability to colonize sugar beet plants following seed treatment; and (iii) determine their potential to "protect" the host plant against BMYV infection.

# 2. Materials and methods

## 2.1. PLANT MATERIAL

Uncoated, unprimed, and some coated with hymexazol (Tachigaren 70 WS) sugar beet seeds were provided by the Royal Belgian Institute for Beet Improvement (IRBAB, Belgium). Seed planting and plant growth were conducted in a plant growth chamber at 23 ± 2°C under a 16:8 LD photoperiod. Seeds were sown in seed trays using a universal potting soil (TERS50, La Plaine Chassart, Fleurus, Belgium). At two-true leaf stage vegetative growth, the plants were transplanted into 10 cm diameter pots and kept in a growth chamber under the controlled conditions mentioned above. Sugar beet plants from coated seedlings were kept for aphid rearing while plants from the uncoated and unprimed seeds were kept for experiments dealing with fungal treatment.

## 2.2. BEET MILD YELLOWING VIRUS – INFECTED MYZUS PERSICAE: APHIDS REARING AND VIRUS MAINTENANCE

A clone of BMYV-viruliferous *M. persicae*, originally provided by SESVanderHave (Belgium), was maintained on healthy sugar beet plants. The aphids were manually transferred onto fresh sugar beet plants using a fine brush. The infested plants were kept in net insect cages (BugDorm 4 M4545, MegaView Science Co. Ltd., Taichung, Taiwan) at 23 ± 2°C under 16:8 LD photoperiod. Sugar beet plants at the four-leaf stage were supplied biweekly to sustain the viruliferous colony.

## 2.3. FUNGAL IDENTIFICATION AND MAINTENANCE

The reference isolates used were *M. brunneum* strains ARSEF 4556 and V275. Both have been extensively studied and characterized (Kortsinoglou et al. 2024; Saud et al. 2021). The V275 strain is

*Published in : Journal of Invertebrate Pathology (2026), vol. 216, 108559*
*DOI: 10.1016/j.jip.2026.108559*
*Status : Postprint (Author's version)*

the same as the Ma43, F52 and BIPESCO 5 strains which are the active ingredients in Lallemand's M52™, Novozymes Met52™ and Agrifutur's Granmet GR™, respectively.

Two local EPF, *M. brunneum* and *M. majus*, were isolated from sugar beet rhizospheres collected at two field sites in Gembloux, Belgium (site 1: 50°29′54.3″N 4°46′51.4″E, and site 2: 50°33′48.9″N 4°40′15.2″E) in Gembloux (Belgium), as described by Dessauvages et al. (2024). Soil samples collected from plant rhizospheres were sieved through a 2 mm mesh, and 20 ml of soil portions were placed into 30 mL plastic cups. One milliliter of tap water was added to standardize moisture. Each soil sample was subsequently baited with larvae of *Tenebrio molitor* L. (Coleoptera: Tenebrionidae), following the method of Zimmermann (1986), and incubated in closed boxes at 21°C in the dark. A moistened paper towel was included in each box to maintain high humidity. The cups were inverted every two days for 4–5 weeks and inspected daily to record larval mortality. Cadavers were surface-sterilized in 70% ethanol for 10 s, rinsed three times in sterile distilled water, and dried on sterile filter paper. The sterilized specimens were then incubated on Sabouraud Dextrose Agar (SDA) supplemented with streptomycin (0.5 mL of 0.6 g $mL^{-1}$), tetracycline (0.5 mL of 0.05 g $mL^{-1}$), and cycloheximide (1 mL of 0.05 g $mL^{-1}$) at 23°C in the dark. Fungal outgrowth was monitored up to 5–8 days. Purified fungal colonies were subcultured and maintained on SDA media at 23 ± 1°C in the dark. Conidia were harvested from 14-day old sporulating cultures unless indicated otherwise.

The identity of the fungal isolates was verified based on morphological features using the key developed by Humber (2012). The isolates were also characterized using molecular techniques as described by Dessauvages et al. (2024). Fourteen-day-old fungal cultures were frozen using liquid nitrogen and ground twice for 1 min each at 30 Hz using a Retsch-MM 400 (Verder scientific, Germany) with 3 mm sterile steel beads (Macherey-Nagel: MN 740814.50 NucleoSpin Bead Tubes Type D). DNA was extracted using a DNeasy Plant Mini kit (Qiagen, Germany), following the manufacturer's instructions.

PCR amplification targeting the 5′- intron region of Elongation Factor 1-alpha (EF1- α intron region) was performed using the primer pairs EF2F (5′-GGAGGACAAGACTCACATCAACG-3′)/ EFjR (5′-TGYTCNCGRGTYTGNCCRTCYTT-3′) as described by Meyling et al. (2012). The PCR mixture contained 25 μl of Q5 High-Fidelity 2x Master Mix (New Englands Biolabs, United-Kingdom), 2.5 μl of each primer (10 μM), and 5 μl genomic DNA (10 ng/μl), resulting in a final volume of 50 μl. After 30 s of initial denaturation at 98°C, a touchdown protocol was applied with 8 cycles of 10 s at 98°C, 30 s from 70 to 63°C (reducing annealing temperature by 1°C per cycle), and 30 s at 72°C. Thirty-five cycles were then performed with the same conditions, with a fixed annealing temperature of 63°C followed by a final extension of 10 min at 72°C. The PCR products were analyzed via electrophoresis on 1% agarose gels in 1 × TAE buffer with SYBR Safe (Invitrogen, USA), then purified with a NucleoSpin gel and PCR clean-up (Macherey-Nagel, Germany). Sanger sequencing was conducted by Eurofins Genomics (Ebersberg, Germany). Sequence identity for fungal strains was confirmed by BLAST search against reference sequences in GenBank (NCBI).

*Published in : Journal of Invertebrate Pathology (2026), vol. 216, 108559*
*DOI: 10.1016/j.jip.2026.108559*
*Status : Postprint (Author's version)*

## 2.4. FUNGAL SUSPENSIONS

Fungal inoculum of each of the strains was prepared by harvesting conidia from 20-day-old cultures and suspending them in 10 ml sterilized 0.03% (v/v) Tween 80. The suspension was filtered through multiple layers of sterile cheesecloth to remove any hyphae. Conidial concentration was determined using a Burker hemocytometer (Marienfeld, Germany) and diluted to final concentrations of $1 \times 10^5$, $1 \times 10^6$, $1 \times 10^7$, $1 \times 10^8$ conidia $ml^{-1}$. Conidial viability was determined by plating 100 µl of $1 \times 10^5$ conidia $ml^{-1}$ suspension of each strain on SDA plates. After 24 h of incubation at 23°C, germinated and non-germinated conidia were counted. Suspensions from all strains with viability > 90% were used for aphid bioassays and seed treatments. During the incubation period prior to use, conidial suspensions were stored at 4°C under refrigeration.

## 2.5. VIRULENCE BIOASSAYS THROUGH DIRECT AND INDIRECT EXPOSURE

Spray and immersion-based bioassays were conducted to investigate the direct and indirect effects of tested *Metarhizium* strains against *M. persicae*. A total of fifty apterous young adults (fourth instar) of *M. persicae* were used for each treatment. Detached sugar beet leaves used as food source were the youngest leaves, which were also the smallest, taken from plants at the four-leaf stage. The selected leaves were gently inserted into the surface of 1.5% water-agar within bottom of the 30 ml plastic cups, using sterile forceps to keep the petiole base in contact with the agar and maintain leaf turgor following the protocol by Ben Fekih et al. (2019). Experimental cups were capped with perforated lids to allow ventilation. Five replications were performed for each fungal concentration and treatment. Cups were incubated at 23°C, 70% RH and LD 18:6h.

### 2.5.1. DIRECT EXPOSURE TO FUNGAL INOCULUM

*Aphid treatment by spray.* Ten apterous fourth instar *M. persicae* per experimental cup were collected from infested sugar beet plants using a dedicated fine brush and placed into Petri dishes (60 mm diameter). Each aphid group was sprayed using the spray top (trigger with 0.5 mm fine mist nozzle) of a 40 ml polypropylene bottle sprayer (WHITE SPRAY, Vanhalst. Belgium). The spray top was inserted into a 2 ml Eppendorf tube that served as the reservoir for the conidia suspension. Prior to the treatments, the spray volume was calibrated by spraying multiple times into a graduated container. Based on the calibrations, 10 fine sprays delivered approximately 1 ml of suspension. During application, the full 1 ml volume was sprayed from a distance of approximately 10 cm to ensure uniform coverage of the aphids. Control (Ct) aphids were sprayed with 0.03% aqueous Tween 80. After treatment, aphids were transferred onto filter paper to absorb excess droplets, then to experimental cups containing fresh sugar beet leaves secured into water agar as described above.

*Aphid treatment by immersion.* Aphid treatment by immersion involved placing 1 ml of conidial suspension of different concentrations into 60 mm diameter Petri dishes. Ten apterous young adults were gently collected from sugar beet infested plants using a fine brush and dipped simultaneously into the 1 ml conidial suspensions for 15 s to ensure adequate conidial contact. After immersion, aphids were carefully collected using a fine brush and transferred onto filter paper to absorb excess liquid and allow separation of any clustered individuals, reducing potential shielding effects.

*Published in : Journal of Invertebrate Pathology (2026), vol. 216, 108559*
*DOI: 10.1016/j.jip.2026.108559*
*Status : Postprint (Author's version)*

Subsequently, aphids were carefully picked up one by one and placed onto non-treated sugar beet leaves secured in water agar as described above. Control aphids underwent the same procedure but were immersed in 0.03% aqueous Tween 80. A new clean brush was used for each treatment to avoid cross contamination.

### 2.5.2. Indirect exposure to fungal inoculum

*Leaf treatment by spray.* For each treatment, five youngest and smallest sugar beet leaves from plants at the 4–5 leaf stage were individually placed into Petri dishes (60 mm diameter). Leaf spraying was performed using the same spray equipment and method as described for aphid treatment by spray. A total of 1 ml of conidia suspension was evenly sprayed over both leaf surfaces from a distance of 10 cm to ensure uniform coverage. Control leaves were sprayed similarly with 0.03% aqueous Tween 80. Treated leaves were placed on sterile filter paper and left to dry for around 15 min under a laminar flow hood. Each treated leaf was subsequently secured in 1.5% water–agar in plastic cups (30 mL) as described above, prior to the introduction of ten apterous young adults of *M. persicae*.

*Leaf treatment by immersion.* As described above, five youngest and smallest sugar beet leaves were placed individually in Petri dishes (60 mm diameter) containing 1 ml of conidia suspension. Using sterile forceps, leaves were gently manipulated in the suspension to ensure full surface contact. Leaves were held in the suspension for 15 s, with repositioning as needed to maintain conidial exposure. Control leaves were dipped similarly in 0.03% aqueous Tween 80. Following treatments, leaves were placed on sterile filter paper and left to dry under a laminar flow for 15 min before being placed in water-agar as highlighted above. Ten healthy aphids were placed on each leaf and incubated under the same conditions as outlined earlier.

### 2.5.3. Aphid survival and nymph production monitoring

For each bioassay, aphid mortality was monitored daily up to 8 days post inoculation (DPI). The number of first-instar nymphs produced was recorded and removed from the cups daily for the 8-day monitoring period. Aphid cadavers were surface-sterilized by immersion in 70% ethanol for 1 min followed by three rinses with sterile distilled water with excess liquid being removed using filter paper. The sterilized cadavers were placed on a sterile moist filter paper and incubated in darkness at 23°C. Cadavers were examined daily for up to 5 days to assess post-mortem fungal development under a binocular stereo microscope. Based solely on external morphological features, cadavers were categorized as follows: (1) Melanized cadavers showing uniform darkening of the cuticle (melanization) without visible hyphal outgrowth; (2) Non-sporulating cadavers with no visible external fungal structures or sporulation; and (3) Sporulating cadavers showing conspicuous external fungal outgrowth with initially white mycelia turning dark green, characteristic of *Metarhizium* infection.

*Published in : Journal of Invertebrate Pathology (2026), vol. 216, 108559*
*DOI: 10.1016/j.jip.2026.108559*
*Status : Postprint (Author's version)*

## 2.6. ENDOPHYTIC POTENTIAL OF FUNGAL STRAINS AND THEIR IMPACT ON BEET MILD YELLOWING VIRUS TRANSMISSION

### 2.6.1. SEED STERILIZATION AND INOCULATIONS

Uncoated sugar beet seeds were surface-sterilized following the protocol by Rasool et al. (2021). Briefly, the seeds were dipped in 70% ethanol (EtOH) for 3 min, followed by 2% sodium hypochlorite (NaClO) for 10 min, and then rinsed seven times with sterile distilled water. The seeds were air-dried on sterile filter paper for 30 min. To verify the efficacy of surface sterilization,100 µl of the final rinse water was plated onto SDA and incubated in darkness at 23°C for 10 days. Verification tests were conducted simultaneously. Immediately after sterilization, the seeds were inoculated and subsequently sown.

For inoculations, 20 ml of $1 \times 10^8$ conidia $ml^{-1}$ suspension was prepared for each treatment (fungal isolates and 0.03% Tween 80 for Ct) in a 250 ml glass conical flask. A total of 30 surface sterilized sugar beet seeds were immersed in each treatment for 24 h and agitated at 100 rpm at 25 ± 2°C. Treated seeds were sown in nursery trays and transferred at the two-true leaf stage to 10 cm diameter plastic pots containing non- sterilized universal potting soil (TERS50, La Plaine Chassart, Belgium). The treated plants were placed on separate plastic plates to prevent cross-contamination during irrigation. Plants from each treatment were kept in separate cages in a growth chamber maintained at 25 ± 2°C, 60–70% RH, and LD 18:6h.

### 2.6.2. PLANT INOCULATION WITH BEET MILD YELLOWING VIRUS

Adults of *M. persicae* reared on BMYV- infested sugar beet plants were used to vector BMYV to twenty fungal treated and non-treated sugar beet plants (four-leaf stage). Each plant was introduced with five BMYV- viruliferous *M. persicae* and subsequently covered with microperforated plastic bags to prevent aphid escape. Five days after introduction, the different plants (fungal treated and control) were sprayed with an acetamiprid-based systemic insecticide (KB® Multisect) at the manufacturer's recommended dose to eliminate *M. persicae*. The aphid-free plants were then maintained in a growth chamber at 23°C under LD 16:8h for a period of 4 weeks. Following the virus incubation phase, four leaf discs (1.5 cm diameter) were collected from each sugar beet plant (eight-leaf stage) using a 5 ml Eppendorf tube as described in Dessauvages et al. (2024). A double antibody sandwich ELISA (DAS-ELISA) was performed on the different samples, following the manufacturer's protocol (Loewe Biochemica GmbH, Sauerlach, Germany). First, a 96- well ELISA microplate was coated with a 1:250 dilution of IgG in coating buffer (pH 9.6) and incubated for 4 h at 37°C. The different leaf samples were ground in sample mesh bags (AGDIA EMEA SARL, ACC 00930/050; bag dimensions 12 × 12.5 cm, mesh area 9 × 11.5 cm) using a hand roller. The homogenates were subsequently diluted in sample buffer (pH 7.4) at a ratio of 1:20. After washing the coated plates three times with wash buffer (pH 7.2–7.4), duplicates of each diluted sample (100 µl) were prepared and added to the ELISA microplates.

After overnight incubation at 4°C, another round of washing was performed. Afterwards, both plates were treated with an AP-conjugate diluted to 1:250 in conjugate buffer (pH 7.4) and incubated at

*Published in : Journal of Invertebrate Pathology (2026), vol. 216, 108559*
*DOI: 10.1016/j.jip.2026.108559*
*Status : Postprint (Author's version)*

37°C for 4 h. Then, a final wash was performed and a substrate solution of 4- nitrophenylphosphate-disodium-salt in substrate buffer (pH 9.8) was added. The plates were kept at room temperature in the dark for 1–2 h. Color development was measured at 415 nm using an iMark Microplate Reader (Bio-Rad). Samples were considered virus-infected if their absorbance (at OD405) values were at least twice as high as those of the negative control samples.

#### 2.6.3. FUNGAL COLONIZATION PATTERNS IN TREATED SUGAR BEET PLANTS

Plants used for fungal colonization assessment were inoculated at the seed stage as previously described in section 2.6.1(Seed sterilization and inoculations). Preliminary data (unpublished) showed that sugar beet plants endophytically colonized by *M. brunneum* ARSEF 4556 exhibited significantly lower BMYV loads compared to those colonized by *M. brunneum* V275 (Fig. SM1). Based on this assessment, *M. brunneum* ARSEF 4556 was selected, along with local isolates to assess the endophytic growth and BMYV transmission. Four-leaf stage plants, based on fully expanded leaves, were randomly selected before aphid infestation to assess the ability of the different fungal isolates to colonize plant tissue. The remaining sugar beet plants were infested with BMYV- viruliferous *M. persicae* for BMYV transmission experiment (section 2.6.2.). Five infested plants from each of the fungal treatments were randomly selected, and aphids removed using a fine paint brush (mechanical treatment). The remaining infested plants were treated with an acetamiprid-based systemic insecticide to remove the aphids (chemical treatment). Plants from chemical and mechanical treatments were kept in a controlled temperature room and DAS-ELISA performed when the plants reached the eight-leaf stage. This experiment helped determine (1) the colonization pattern of each fungal isolate at the four- and eight- leaf stage and (2) the impact of the chemical insecticides on the fungal endophytic colonization in comparison to mechanical treatment. Different sections of the plants such as petioles (2.0 cm), leaves (3.0 × 3.0 cm), and roots (1.5 cm), were taken and surface-sterilized as described in Parsa et al. (2013). The plant pieces were immersed for 2 min each in 70% and 2% NaClO, followed by three rinses in ddH2O sterile water and left for 30 min to dry under a Laminar flow hood. Each sterilized section was trimmed using sterile scalpels and cut into six pieces of different sizes (around 1 cm for roots and 0.5 cm for each of the hypocotyls, petioles, and leaves) and partially inserted into SDA media supplemented with the three antibiotics mentioned above and incubated in the dark at 23°C. The plates were checked daily for fungal outgrowth over a 14-day period. The reisolated fungi were identified based on colony color changes and conidial morphology using the key of Humber (2012).

## 2.7. STATISTICAL ANALYSIS

Data from survival times and death events were combined using the 'surv' function from the 'survival' (Therneau et al., 2024) package. To model the survival data, the Kaplan–Meier survival estimate was fitted using the 'survfit' function within the same package, allowing for an estimation of aphid survival over time. Median lethal concentrations ($LC_{50}$) were estimated through concentration–response modelling using the log-logistic model (LL.2) in the R drc package, and confidence intervals were calculated via the delta method.

*Published in : Journal of Invertebrate Pathology (2026), vol. 216, 108559*
*DOI: 10.1016/j.jip.2026.108559*
*Status : Postprint (Author's version)*

Mycosis development on aphid cadavers and nymph production were examined throughout GLMMs with replicate (1|replicate) as a random effect to account for experimental blocking. We implemented a tiered modeling approach where primary models used Poisson distributions (log link), with negative binomial distributions substituted when overdispersion was detected (deviance/df > 1.5), automatically selecting the appropriate model family for each combination of used application method (immersion or spray) × target group (aphids or leaves).

In the case of mycosis development, models specified 'Group' (Melanized/NonSporulating/Sporulating) as a fixed factor. In the case of nymph production, it was the fungal isolate that was compared. Then, *post-hoc* pairwise comparisons were conducted via estimated marginal means (*emmeans* package) (Lenth, 2023) with Tukey adjustment for multiple comparisons (family-wise $\alpha = 0.05$), with all estimates reported as mean ± SEM. Analyses were implemented using lme4::glmer ()/glmer.nb() (Bates et al., 2015) for model fitting and lmerTest (Kuznetsova et al., 2017) for significance testing, with the entire pipeline automated across all Method × Target combinations (n = 4). For the nymph production, two nested models were compared for each fixed effect: a full model incorporating all the fixed effects and a reduced model excluding each time one both fixed effect (ex. Model_All vs. Mod_no_Target) using nlmeR package (Pinheiro, 2025). The model selection was performed via likelihood ratio tests (LRTs) to assess whether the concentration, target, the fungal strains, and application methods significantly improved model fit. Additional analyses were performed using generalized linear mixed models (GLMMs) with Poisson or Negative Binomial distributions to further examined the relationship between aphid mortality (count of dead aphids) and nymph production (count of nymphs) at the highest applied concentration using glmmTMB package (Brooks et al., 2017). Model selection was guided by assessment of overdispersion, where ratios exceeding 1.5 indicated the need for a Negative Binomial model to account for extra-Poisson variation. Random intercepts for replicates were included to control for experimental blocking effects, and simplified Poisson GLMs were used when mixed models did not converge.

Plant colonization data were examined through a binomial generalized linear mixed model (GLMM), with fungal strains and plant sections (roots, petiole, and leaf) considered as fixed effects. The random effects accounted for variation among individual plants and the different pieces sampled from each plant section. To assess the effect of fungal treatments on BMYV transmission by *M. persicae*, a linear mixed-effects model (LMM) was applied to compare absorbance values, which served as a proxy for viral load (Lange et al., 2021). Treatment effect was further examined through analyses of variance and Tukey's pairwise comparisons were performed in case of significant differences. Analyses and data visualizations were run in R (version 4.1.3; RStudio Team, 2022), with significance levels set at p-value < 0.05.

*Published in : Journal of Invertebrate Pathology (2026), vol. 216, 108559*
*DOI: 10.1016/j.jip.2026.108559*
*Status : Postprint (Author's version)*

# 3. Results

## 3.1. FUNGAL IDENTIFICATION

From a total of 54 *T. molitor* larvae used to bait 18 soil samples (9 from each site), only two cadavers exhibited typical hypocrealean infection. Based on mycelial color and conidial shape, these fungal isolates were preliminarily assigned to the genus *Metarhizium*. The isolates displayed cottony colonies with a white mycelial margin, and colony color ranged from olive green to yellow-green or dark green during growth.

DNA sequencing followed by NCBI BLAST search revealed that the isolates recovered from site 1, *M. majus* strain GxABT-3, shared 99.3% sequence homology with *M. majus* KVL 12–29 (GenBank accession number KM391931). Similarly, the fungal isolate recovered from site 2, *M. brunneum* strain GxABT-2, showed 100% sequence homology with *M. brunneum* strain KVL 12–30 (GenBank accession number KM391920) and was named. Both sequences have been deposited in NCBI under GenBank accession number OQ368831 and OQ368832 for *M. brunneum* strain GxABT-2 and *M. majus* strain GxABT-3, respectively. Conidia dimensions measured were 9.5 ± 0.7 × 4.2 ± 0.6 μm for *M. majus* and 6.7 ± 0.7 × 2.9 ± 0.3 μm for *M. brunneum*. Together, the morphological and molecular data confirmed the accurate taxonomic identification of these isolates.

## 3.2. BIOASSAY

### 3.2.1. VIRULENCE PATTERNS OF FUNGAL ISOLATES IN RELATION TO DOSAGE AND APPLICATION METHOD

Overall, aphid cumulative mortality 8 days post treatment was concentration-dependent independent of fungal isolate and application method. However, this mortality was marginally higher for aphids after direct exposure (sprayed or immersed) than those exposed to leaves pre-treated (sprayed or immersed) with conidia (Table 1; Table SM1). At the concentration of $1 \times 10^8$ conidia $ml^{-1}$ (aphid spray), all fungal treatments significantly reduced the median survival time (MST) of *M. persicae* compared with the control (Log-rank test: $\chi^2 = 614$, df = 19, $p < 0.001$), and no significant differences were detected among fungal treatments. Under the same condition of treatment and applied concentration and 8 days post-treatment, cumulative mortality reached 100% for *M. brunneum* ARSEF4556, V275, and GxABT-2, to be 98% for *M. majus* GxABT-3. The MST were not significantly different among isolates: 3 days for *M. brunneum* strains ARSEF4556 and GxABT-2, 4 days for V275, and 3 days for *M. majus* strain GxABT-3 (Table 1). With aphid immersion at the same concentration, significant differences in MST were observed only between *M. brunneum* strain V275 and *M. majus* GxABT-3, at 4 and 3 days, respectively. No significant differences were detected among the other isolates.

Similarly, to aphid spray pattern, at $1 \times 10^8$ conidia $ml^{-1}$, aphids feeding on fungal-immersed leaves showed non-significant MST of 3, 5, and 4 days for *M. brunneum* strain ARSEF4556, GxABT-2, and *M. majus* strain GxABT-3, respectively. Notably, a non-significant MST of 6 and 5 days was observed for *M. brunneum* strain V275 and GxABT-2, respectively. This pattern differed when aphids fed on fungal-

*Published in : Journal of Invertebrate Pathology (2026), vol. 216, 108559*
*DOI: 10.1016/j.jip.2026.108559*
*Status : Postprint (Author's version)*

sprayed leaves. Under this latter application, a significant difference in MST was observed only between *M. brunneum* strain V275 and *M. majus* strain GxABT-3, at 4 and 8 days, respectively. Under indirect exposure to fungal inoculum at 8 days, cumulative aphid mortality was slightly higher for aphids fed on leaves immersed in a conidial suspension than on sprayed leaves (Table 1).

At the lowest concentration ($1 \times 10^5$ conidia $ml^{-1}$), only *M. brunneum* isolate GxABT-2 exceeded 50% mortality under all application methods (Table SM2). With aphid spray, at the lowest concentration, *M. brunneum* strain ARSEF4556, V275, GxABT-2, and GxABT-3 still caused mortality rates of 50, 62, 62, and 38%, respectively. The MST did not differ significantly among isolates, recorded at 7 days for V275 and GxABT-2, and 8 days for ARSEF4556. Likewise, no significant differences were found in MST of *M. persicae*. For *M. majus* GxABT-3, mortality did not reach 50%, and MST could not be determined.

The estimation of the median lethal concentration ($LC_{50}$) for the different tested strains of *M. brunneum* and *M. majus* GxABT-3 showed a different pattern. For *M. brunneum* GxABT-2, regardless of the application method, a concentration lower than $1 \times 10^5$ conidia $ml^{-1}$ was sufficient to reach the $LC_{50}$ (Table SM2). In contrast, *M. brunneum* strain ARSEF4556 required a concentration below $1 \times 10^5$ conidia $ml^{-1}$ only when applied via aphid immersion, whereas strain V275 achieved this level only when applied by aphid spray. Similarly, under aphid spray, *M. majus* GxABT-3 also required a concentration below $1 \times 10^5$ conidia $ml^{-1}$ to reach the $LC_{50}$.

### 3.2.2. Assessment of mycosis development on aphid cadavers

Across 4,000 observations, melanized cadavers were relatively rare (2.7%; N = 109) compared with sporulating (28.5%; N = 1,138) and non-sporulating (30.7%; N = 1,228) cadavers (Table 2). When pooled across application methods and targets, melanized cadavers (N = 109) occurred primarily at lower concentrations, with 39.4, 32.1, and 25.7% observed at $1 \times 10^5$, $1 \times 10^6$, and $1 \times 10^7$ conidia $ml^{-1}$, respectively, whereas only 2.8% occurred at $1 \times 10^8$ conidia $ml^{-1}$. At the highest concentration, V275 generated significantly more sporulating than non- sporulating cadavers (z-ratio = -5.80; $p < 0.001$), whereas GxABT-2 (z- ratio = 4.61; $p < 0.001$) and GxABT-3 (z-ratio = 2.76; $p < 0.01$) exhibited the opposite pattern. No significant difference between sporulating and non-sporulating cadavers was detected for ARSEF4556 at $1 \times 10^8$ conidia $ml^{-1}$ (Table 2). By fungal treatment, melanized cadavers following GxABT-2, GxABT-3, and ARSEF4556 occurred at all three lower concentrations ($1 \times 10^5$–$1 \times 10^7$ conidia $ml^{-1}$). Notably, melanized cadavers at $1 \times 10^8$ conidia $ml^{-1}$ were observed only for GxABT-3, whereas for V275 they occurred exclusively at $1 \times 10^5$ conidia $ml^{-1}$.

Detailed analysis of post-mortem signs (Table SM3) revealed substantial variability in cadaver type among isolates, with no consistent pattern in melanization across application methods or targets (aphids vs. leaves). Except for the leaf-spray application of GxABT-3 at $1 \times 10^5$ conidia $ml^{-1}$, where no sporulating cadavers were observed, both sporulating and non-sporulating cadavers occurred across isolates and concentrations, without a consistent pattern among methods or targets. Following aphid spray, GxABT-2 and GxABT-3 produced more non- sporulating than sporulating cadavers at $1 \times 10^8$ conidia $ml^{-1}$, whereas ARSEF4556 and V275 showed no significant difference. In contrast, following aphid immersion, GxABT-3, V275, and ARSEF4556 produced significantly more

*Published in : Journal of Invertebrate Pathology (2026), vol. 216, 108559*
*DOI: 10.1016/j.jip.2026.108559*
*Status : Postprint (Author's version)*

sporulating than non-sporulating cadavers at $1 \times 10^8$ conidia $ml^{-1}$, while GxABT-2 showed no difference.

*Published in : Journal of Invertebrate Pathology (2026), vol. 216, 108559*
*DOI: 10.1016/j.jip.2026.108559*
*Status : Postprint (Author's version)*

***Table 1.*** *Virulence patterns of fungal isolates at the lowest (1 × $10^5$) and highest (1 × $10^8$) conidia $ml^{-1}$ concentrations using direct (A) application to aphids by spraying or immersion, and indirect (B) application to aphid-infested leaves by spraying or immersion. Cumulative mortality (± SE, %) and median survival time (d) of Myzus persicae treated with Metarhizium brunneum (ARSEF4556, V275, GxABT-2) and M. majus (GxABT-3). Letters indicate statistically homogeneous groups within each concentration, based on pairwise survival analysis with Bonferroni correction (p < 0.05).*

(A) **Direct application**

| | | Spray aphid | | | Immersion aphid | | |
|---|---|---|---|---|---|---|---|
| Concentration (conidia $ml^{-1}$) | Fungal strains | Cumulative mortality ± SE [%] | Median survival time [d] (95% CI) | Significance group (log-rank) | Cumulative mortality ± SE [%] | Median survival time [d] (95% CI) | Significance group (log-rank) |
| $10^8$ | ARSEF4556 | 100 ± 0 | 3 (3 – 3) | e | 94 ± 3.4 | 3 (3 – 4) | def |
| | V275 | 100 ± 0 | 4 (3 – 4) | ef | 98 ± 2 | 4 (4 – 4) | adf |
| | GxABT-2 | 100 ± 0 | 3 (3 – 4) | e | 82 ± 5.4 | 6 (4 – 7) | abcdef |
| | GxABT-3 | 98 ± 2 | 3 (3 – 4) | ef | 100 ± 0 | 3 (3 – 4) | e |
| $10^5$ | ARSEF4556 | 50 ± 7 | 8 (8 – NA) | abc | 70 ± 6.5 | 5.5 (4 – 8) | ac |
| | V275 | 62 ± 6.9 | 7 (6 – NA) | abc | 36 ± 6.8 | NA | abcd |
| | GxABT-2 | 62 ± 6.9 | 7 (6 – NA) | abc | 56 ± 7 | 6.5 (5 – NA) | abcd |
| | GxABT-3 | 38 ± 6.9 | NA | bd | 46 ± 7 | NA | abcd |

*Published in : Journal of Invertebrate Pathology (2026), vol. 216, 108559*
*DOI: 10.1016/j.jip.2026.108559*
*Status : Postprint (Author's version)*

(B) **Indirect application**

| Concentration (conidia $ml^{-1}$) | Fungal strains | Spray leaf | | | Immersion leaf | | |
|---|---|---|---|---|---|---|---|
| | | Cumulative mortality ± SE [%] | Median survival time [d] (95% CI) | Significance group (log-rank) | Cumulative mortality ± SE [%] | Median survival time [d] (95% CI) | Significance group (log-rank) |
| $10^8$ | ARSEF4556 | 78 ± 5.9 | 5 (3 – 6) | abcd | 92 ± 3.8 | 3 (3 – 3) | def |
| | V275 | 96 ± 2.8 | 4 (3 – 6) | acdf | 90 ± 4.2 | 6 (6 – 7) | bc |
| | GxABT-2 | 78 ± 5.9 | 6 (5 – 7) | abc | 80 ± 5.7 | 5 (4 – 7) | abcd |
| | GxABT-3 | 68 ± 6.6 | 8 (6 – 8) | b | 94 ± 3.4 | 4 (4 – 5) | adf |
| $10^5$ | ARSEF4556 | 16 ± 5.2 | NA | d | 58 ± 7 | 7 (6 – NA) | abc |
| | V275 | 16 ± 5.2 | NA | d | 38 ± 6.9 | NA | bcd |
| | GxABT-2 | 73 ± 6.3 | 6 (5 – 7) | a | 56 ± 7 | 7.5 (7 – NA) | abc |
| | GxABT-3 | 30 ± 6.5 | NA | bd | 42 ± 7 | NA | abcd |

Within each concentration (in panels A and B), recorded parameters were compared among fungal treatments, application methods and targets (aphid or leaf). NA: Not applicable (cumulative mortality did not reach 50% within 8 days, preventing calculation of median survival time).

*Published in : Journal of Invertebrate Pathology (2026), vol. 216, 108559*
*DOI: 10.1016/j.jip.2026.108559*
*Status : Postprint (Author's version)*

***Table 2.*** *Mean number (mean ± SEM) of Myzus persicae cadavers by post-mortem status (melanized, non-sporulating, sporulating) for each treatment x concentrations combination, pooled across application methods and targets. Letters indicate significant differences between cadaver types within each boxed group (Tukey- adjusted pairwise comparisons via emmeans, α = 0.05). Absence of letters denotes no comparison possible. Data were analyzed using generalized linear mixed models (Poisson family; replicate as random effect; model selection based on overdispersion diagnostics).*

| | | Applied concentration (conidia ml$^{-1}$) | | | |
|---|---|---|---|---|---|
| **Treatment** | **Cadaver status** | **$10^5$** | **$10^6$** | **$10^7$** | **$10^8$** |
| Control | Melanized | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| | Non-sporulating | 1.4 ± 0.3 | 2.7 ± 0.2 | 2.7 ± 0.2 | 2.1 ± 0.1 |
| | Sporulating | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| GxABT-2 | Melanized | 0.7 ± 0.2a | 0.2 ± 0.1a | 1.2 ± 0.3a | 0.0 ± 0.0 |
| | Non-sporulating | 3.8 ± 0.3c | 3.7 ± 0.4b | 3.9 ± 0.7b | 5.7 ± 0.5b |
| | Sporulating | 1.7 ± 0.3b | 2.8 ± 0.2b | 2.4 ± 0.3b | 2.7 ± 0.4a |
| GxABT-3 | Melanized | 1.1 ± 0.2a | 0.7 ± 0.2a | 0.1 ± 0.1a | 0.2 ± 0.1a |
| | Non-sporulating | 2.3 ± 0.3b | 2.4 ± 0.3b | 5.0 ± 0.6b | 5.8 ± 0.6c |
| | Sporulating | 0.6 ± 0.2a | 4.0 ± 0.5c | 3.3 ± 0.6b | 3.5 ± 0.6b |
| ARSEF4556 | Melanized | 0.2 ± 0.1a | 0.9 ± 0.3a | 0.1 ± 0.1a | 0.0 ± 0.0 |
| | Non-sporulating | 2.7 ± 0.5b | 1.3 ± 0.3a | 2.7 ± 0.4b | 4.3 ± 0.4a |
| | Sporulating | 2.2 ± 0.2b | 4.3 ± 0.4b | 6.0 ± 0.4c | 4.9 ± 0.5a |
| V275 | Melanized | 0.3 ± 0.1a | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| | Non-sporulating | 1.4 ± 0.3b | 1.5 ± 0.2a | 3.6 ± 0.4a | 2.8 ± 0.3a |
| | Sporulating | 2.2 ± 0.4b | 4.2 ± 0.3b | 5.5 ± 0.3b | 7.0 ± 0.3b |

Each boxed group represents a comparison between cadaver status for a given treatment and concentration.

*Published in : Journal of Invertebrate Pathology (2026), vol. 216, 108559*
*DOI: 10.1016/j.jip.2026.108559*
*Status : Postprint (Author's version)*

### 3.2.3. APHID NYMPH PRODUCTION FOLLOWING FUNGAL TREATMENT

The total number of *M. persicae* nymphs recorded 8 days post- treatment was significantly influenced by fungal isolates (LRT: $\chi^2(4) = 1420.8$, $p < 0.001$), applied concentration (LRT: $\chi^2(3) = 285.01$, $p < 0.001$), target (LRT: $\chi^2(1) = 560.42$, $p < 0.001$) and application method (LRT: $\chi^2(1) = 40.43$, $p < 0.001$). The lowest numbers recorded was when aphids had been exposed to conidia through immersion or spraying, independent of isolate and was particularly pronounced at the higher concentrations (Table 3). To further explore these patterns, GLMM analysis at $10^8$ conidia $ml^{-1}$ revealed significant negative relationships between aphid mortality and nymph production, along with distinct patterns of treatment efficacy across application methods and target organisms (Fig. SM2). For aphid-targeted treatments, both immersion and spray methods showed highly significant negative relationships (d.f. = 25; $p < 0.001$), with immersion producing the strongest effect ($\beta = -0.46$) followed by spray ($\beta = -0.41$). In contrast, leaf-targeted treatments displayed weaker but still significant negative effects, with coefficients of − 0.08 for immersion and − 0.14 for spray ($p < 0.05$). These results show that the relationship between aphid mortality and nymph production at $1 \times 10^8$ conidia $ml^{-1}$ is strongly influenced by both application method and the target organism, with direct aphid treatments leading to significantly higher mortality.

There were a few anomalies. For example, aphids placed on leaves sprayed with the highest concentration of GxABT-2 produced virtually no nymphs. Aphids placed on leaves immersed in a $10^7$ conidia $ml^{-1}$ suspension of ARSEF4556 produced a similar nymph number as in the control (Table 3).

## 3.3. ENDOPHYTIC POTENTIAL OF DIFFERENT FUNGAL SPECIES

Surface sterilization efficacy was confirmed by plating the final rinse water, which showed no microbial contamination throughout the incubation period. Furthermore, untreated control plants remained free of microbial contamination during the endophytic colonization assays. Following seed treatments, the different fungal species readily colonized various sugar beet plant tissues (roots, petioles, and leaves), with colonization rate increasing from the four-leaf stage to the eight-leaf stage of plant growth (Fig. 1). At the four-leaf stage, both GxABT-2 and GxABT-3 showed a slightly greater ability to colonize the host plants compared to ARSEF4556, although the difference was not statistically significant. However, significantly more fungus was recovered from below-ground plant parts than from petiole or leaf tissue (Table 4). Although each isolate exhibited slightly different colonization patterns, there were no overall statistical differences among the four isolates.

Before assessing viral load, aphids were removed using a chemical insecticide. To determine whether this method of aphid removal affected the detection of fungal colonization, an additional factor, "mechanical application," was introduced. Overall, the chemical treatment did not impair the ability of the tested fungal species to colonize the different plant parts. None of the treatments significantly affected the colonization capacity of *M. brunneum* GxABT-2 and V275 or *M. majus* GxABT-3. Interestingly, however, the ability of *M. brunneum* ARSEF4556 to colonize the various plant tissues was reduced following chemical application (Fig. 2).

*Published in : Journal of Invertebrate Pathology (2026), vol. 216, 108559*
*DOI: 10.1016/j.jip.2026.108559*
*Status : Postprint (Author's version)*

***Table 3*** *Mean number (mean ± SEM) of Myzus persicae nymphs produced across treatments and by applied method. Letters indicate significant differences (Tukey- adjusted pairwise comparisons via emmeans, $\alpha = 0.05$) between treatment groups within each concentration.*

| | | | Applied concentration (conidia ml$^{-1}$) | | | |
|---|---|---|---|---|---|---|
| **Target** | **Method** | **Treatment** | **$10^5$** | **$10^6$** | **$10^7$** | **$10^8$** |
| Aphids | Immersion | Control | 41.0 ± 3.6d | 32.0 ± 2.8b | 30.6 ± 2.5d | 32.4 ± 2.1b |
| | | GxABT-2 | 8.6 ± 0.8a | 5.8 ± 1.0a | 2.2 ± 0.9b | 0.4 ± 0.2a |
| | | GxABT-3 | 16.4 ± 2.1bc | 9.4 ± 0.9a | 6.0 ± 1.1c | 2.0 ± 0.5a |
| | | ARSEF4556 | 22.0 ± 1.4b | 8.4 ± 0.4a | 1.8 ± 0.6ab | 0.8 ± 0.4a |
| | | V275 | 19.8 ± 1.7bc | 5.4 ± 1.0a | 1.4 ± 0.5ab | 1.0 ± 0.5a |
| | Spray | Control | 27.2 ± 3.8c | 32.8 ± 2.9b | 29.2 ± 3.3c | 25.6 ± 1.7b |
| | | GxABT-2 | 10.2 ± 1.3a | 7.0 ± 1.1a | 2.6 ± 0.8ab | 0.4 ± 0.2a |
| | | GxABT-3 | 16.8 ± 1.9b | 8.8 ± 0.9a | 3.4 ± 0.5b | 2.0 ± 0.3a |
| | | ARSEF4556 | 14.2 ± 1.0ab | 10.6 ± 0.8a | 0.6 ± 0.4a | 0.6 ± 0.4a |
| | | V275 | 14.4 ± 0.7ab | 7.0 ± 0.7a | 0.8 ± 0.4ab | 0.8 ± 0.5a |
| Leaf | Immersion | Control | 28.4 ± 3.9ab | 31.2 ± 2.1c | 35.6 ± 1.8c | 34.2 ± 2.3c |
| | | GxABT-2 | 33.4 ± 1.6b | 28.0 ± 1.8c | 10.2 ± 1.9a | 3.4 ± 0.9a |
| | | GxABT-3 | 30.4 ± 2.5ab | 18.0 ± 5.0b | 15.2 ± 2.6ab | 18.4 ± 1.4b |
| | | ARSEF4556 | 21.8 ± 2.8a | 12.4 ± 3.9a | 37.2 ± 6.6c | 19.6 ± 1.7b |
| | | V275 | 21.8 ± 2.2a | 20.2 ± 0.7b | 19.6 ± 2.9b | 20.2 ± 2.5b |
| | Spray | Control | 28.4 ± 3.9b | 35.2 ± 3.7c | 38.6 ± 7.2c | 34.4 ± 2.4c |
| | | GxABT-2 | 12.8 ± 1.2a | 10.6 ± 1.2b | 11.0 ± 1.7a | 0.0 ± 0.0 |
| | | GxABT-3 | 27.8 ± 1.7b | 22.2 ± 4.6a | 12.6 ± 1.0a | 18.8 ± 2.7b |
| | | ARSEF4556 | 12.2 ± 1.0a | 24.6 ± 3.2a | 18.6 ± 2.0b | 11.4 ± 2.3a |
| | | V275 | 13.6 ± 0.8a | 19.2 ± 3.0a | 21.4 ± 3.5b | 9.4 ± 1.6a |

Each boxed group represents a comparison between treatments for a given combination of application method, target, and concentration.

*Published in : Journal of Invertebrate Pathology (2026), vol. 216, 108559*
*DOI: 10.1016/j.jip.2026.108559*
*Status : Postprint (Author's version)*

### 3.4. IMPACT OF FUNGAL TREATMENTS ON BEET MILD YELLOWING VIRUS TRANSMISSION

Regardless of the method of applied treatment, all sugar beet plants were infected with BMYV. However, differences in viral load were observed across treatments (LMM: $\chi^2 = 35.7$, df = 3, $p < 0.001$) with significantly reduced levels in EPF treated plants than untreated sugar beet plants. Plants colonized by *M. brunneum* GxABT-2, *M. majus* GxABT- 3 and *M. brunneum* ARSEF4556 had 40.6, 24.8, and 18.6% lower viral loads than controls, respectively. There were no significant differences between *M. majus* GxABT-3 and *M. brunneum* strains ARSEF4556 and GxABT-2 (Table 5).

Overall, sugar beet colonization by EPF was not impeded following viral infection even after removal of the aphids mechanically or chemically. However, the viral load was lower in EPF-treated plants than in the control, with the lowest load recorded in plants endophytically colonized by *M. brunneum* GxABT-2 (Fig. 3).

## 4. Discussion

Our study demonstrates that *M. persicae* is highly susceptible to *Metarhizium* infection, highlighting the potential of EPF to simultaneously suppress aphid populations and limit aphid-vectored BMYV in sugar beet. Distinct post-mortem fungal development patterns (melanized, non-sporulating, and sporulating cadavers) were observed, varying with fungal strains, concentration and treatment method. These patterns indicate strain-specific infection dynamics with practical implications for biocontrol, as sporulation enhances fungal persistence in the field. Seed treatment enabled effective endophytic colonization of sugar beet plants, further reducing BMYV transmission. Among the tested strains, the endemic *M. brunneum* GxABT-2 showed the most consistent performance, combining strong direct aphid suppression with effective indirect viral mitigation. These findings underscore the dual potential of endemic *Metarhizium* species as sustainable tools against aphid-borne viruses in sugar beet cultivation.

*Natural occurrence and virulence of Metarhizium isolates.* Hypocrealean EPF like *Metarhizium* spp. are widely distributed in soils of various terrestrial ecosystems, including forests, hedgerows, and agricultural fields (Scheepmaker and Butt, 2010). To our knowledge, this study is the first to document the natural occurrence of *M. brunneum* and *M. majus* in Belgian soils and demonstrate their virulence against BMYV-*M. persicae* pathosystem. Both species were isolated from soil sampled before sugar beet harvest. It is not uncommon to recover *Metarhizium* species from tilled (i.e. disturbed) soils (Meyling and Eilenberg, 2007) but most often the number and diversity are higher in undisturbed (forest, grassland) habitats (Scheepmaker and Butt, 2010).

Our bioassays confirmed a clear concentration-dependent impact of all tested *Metarhizium i*solates on *M. persicae* survival, with significantly higher mortality and shorter median survival times at the highest concentration ($1 \times 10^8$ conidia $ml^{-1}$). Direct exposure (spraying or immersion) produced consistently higher mortality than indirect exposure through pre-treated leaves, highlighting the

*Published in : Journal of Invertebrate Pathology (2026), vol. 216, 108559*
*DOI: 10.1016/j.jip.2026.108559*
*Status : Postprint (Author's version)*

importance of maximizing conidial contact with aphids (Wraight and Ramos, 2002; Shah and Pell, 2003). Mortality rates of 98–100% following aphid contamination by all three *M. brunneum* strains and *M. majus* by spraying align with previous findings on *Metarhizium* species' potential for aphid control (Saranya et al., 2010; Lee et al., 2015; Mweke et al., 2019). However, our study did not detect significant interspecific or intraspecific variation in mortality rates at higher concentrations following spray application. This contrasts with Reingold et al. (2021), who reported significant intraspecies variation in the virulence of *M. brunneum* isolates against *M. persicae.* The variation could be due to intrinsic differences in virulence or environmental conditions, especially temperature and humidity. Notably, *M. brunneum* strain GxABT-2 demonstrated remarkable virulence even at the lowest tested concentration, independent of application method. The efficacy of GxABT-2 may reflect local adaptation to the host plant and environment, as has been observed for other endemic *Metarhizium* isolates (Bidochka et al., 2001; Meyling and Eilenberg, 2007). *M. brunneum* strains ARSEF4556 and V275, and *M. majus* GxABT-3 achieved low $LC_{50}$ values under specific application methods, reflecting their high virulence. However, the variability across application methods suggests that infection success is sensitive to the method of application, likely due to differences in conidial adhesion and infection efficiency, as reported for other EPF (Inglis et al., 2001). Where insects acquire conidia by walking over a treated surface, fewer conidia will adhere. Some studies suggest that the long stilt-like legs of aphids minimize body contact with the leaf surface which reduces the likelihood of aphids acquiring sufficient conidia from treated leaf surfaces (Hall, 1979). The number of conidia adhering will depend on the concentration and aphid behavior. When aphids alight on a leaf, they typically spend some time wandering, waving their antennae, and making explorative short probes, to locate suitable feeding sites (Pettersson et al., 2007). The whole process can take a few minutes during which time they can acquire conidia. The fact that significant mortality was observed for *M. persicae* even at the low concentrations on pre-treated leaves, suggests that the aphids must be spending a significant amount of time wandering before settling to feed and in the process acquiring sufficient inoculum to cause infection.

The extensively studied reference strains *M. brunneum* V275 and ARSEF4556 have proven efficacy against a wide range of insect plant pests (Nielsen et al., 2006; Ansari et al., 2009, Klingen et al., 2015, Asan et al., 2017, Sonmez et al., 2017¨ ). Commercial products based on *M. brunneum* V275/F52/BIPESCO5 have also proven to be highly efficacious against a range of soil and foliar pests (Gill et al., 2024; Zottele et al., 2023). Our findings demonstrate that these reference strains are also highly virulent against *M. persicae*. The comparable or superior performance of endemic strains, particularly GxABT-2, underscores the potential value of locally sourced *Metarhizium* isolates for biological control, which may be better suited to local environmental conditions and host plants. Previous studies have evaluated the virulence of native isolates (Lopes et al., 2013; Korosi et al., 2019), but few have included reference strains for direct comparison (Yubak Dhoj et al., 2008). While Yubak Dhoj et al. (2008) found that indigenous isolates of *M. anisopliae* and *B. bassiana* were more virulent than exotic strains against white grubs, Lopes et al. (2013) reported low virulence of indigenous EPF from a banana plantation against the banana weevil, *Cosmopolites sordidus* (Germar).

*Published in : Journal of Invertebrate Pathology (2026), vol. 216, 108559*
*DOI: 10.1016/j.jip.2026.108559*
*Status : Postprint (Author's version)*



(Manoussopoulos et al., 2019; Francis et al., 2022b; Maluta et al., 2022). The higher mortality observed after direct application to aphids (immersion and spraying) compared to indirect exposure via treated leaves highlights the importance of thorough coverage of host surfaces, consistent with previous findings that immersion enhances infection rates (Wraight and Ramos, 2002). For all tested fungal isolates, the cumulative mortality and MST clearly depended on the conidial concentration, which is also consistent with the well-known concentration–response relationship for hypocrealean EPF (Inglis et al., 2001). Interestingly, contrasting outcomes have been reported for *M. brunneum* in plant-mediated interactions with aphids. Rasool et al. (2021) found that seed inoculation of wheat and bean plants with *M. brunneum* KVL 04–57 (V275) unexpectedly increased aphid densities, whereas *M. robertsii* and *B. bassiana* reduced them. This variation was attributed to EPF-induced shifts in plant secondary metabolism rather than differences in colonization success. Although the present study focused on direct and indirect pathogenic effects rather than plant- mediated responses, the consistently high aphid mortality observed suggests that strain virulence and application method were the dominant factors under the experimental conditions. Overall, these findings highlight the complexity of EPF–plant–insect interactions and underscore the importance of distinguishing physiological from pathogenic effects when evaluating fungal biocontrol potential.

Regarding post-mortem infection dynamics, our study revealed predominance of sporulating and non-sporulating cadavers, with few melanized individuals. The presence of melanized cadavers, none of which showed mycosis, suggests that melanization, a known insect immune response (Gillespie et al., 1997), successfully inhibited fungal development in a small subset of aphids (Dubovskiy et al., 2013; Butt et al., 2016). Melanized cadavers in our study most likely reflect a strong aphid immune response rather than successful fungal colonization. Extensive melanization creates a highly toxic internal environment that prevents fungal development and external sporulation (Butt, unpublished; St Leger et al., 1996; Butt et al., 2016). For example, constitutive

expression of the subtilisin protease Pr1 in *Metarhizium* accelerates infection but also triggers massive melanization, which is lethal to both host and pathogen, resulting in rapid death without sporulation (St Leger et al., 1996). The melanization cascade, driven by phenoloxidase, produces reactive intermediates and oxidative stress capable of overwhelming fungal cells (Dubovskiy et al., 2013; Smith et al., 2022), and melanin can encapsulate fungal propagules, inhibiting enzymes required for infection. While occasional fungal cells may be present, further development is unlikely in this environment, as also observed for endophytic EPF causing internal melanization without external growth (Garrido-Jurado et al., 2017). In contrast, external sporulation occurs only when the fungus successfully colonizes the haemocoel prior to producing conidia at the host surface (Butt et al., 2016). Collectively, these observations highlight the importance of aphid humoral defenses in limiting mycosis and explain the distinction between melanized and non-melanized cadavers observed in this study.

Variations in the proportions of sporulating versus non-sporulating cadavers across isolates, concentrations, and application methods further suggest complex interactions between host

*Published in : Journal of Invertebrate Pathology (2026), vol. 216, 108559*
*DOI: 10.1016/j.jip.2026.108559*
*Status : Postprint (Author's version)*

immune defenses and fungal virulence traits, influencing infection outcomes beyond host death. For instance, *M. brunneum* V275 produced significantly more sporulating cadavers at the highest concentration, suggesting a superior ability to complete external sporulation, a trait essential for fungal transmission (Hajek and Leger, 1994). Variability in the proportions of sporulating and non-sporulating cadavers across treatments indicates that post-mortem outcomes depend on isolate-specific and context- dependent interactions between host immune responses and the fungal capacity to complete sporulation, without a consistent directional relationship between these outcomes. Non-sporulating cadavers may result from infections where the fungus killed the host but was unable to overcome residual immune defenses, environmental conditions, or antimicrobial compounds in the host cuticle that prevent external sporulation (Butt and Goettel, 2000). Together, these findings highlight that both virulence (killing the host) and fitness-related traits such as sporulation are critical determinants of the biocontrol potential of fungal isolates.

*Impact of fungal treatment on aphid fecundity*. Overall, fungal treatments at the highest concentration significantly reduced *M. persicae* survival and reproduction, with a strong negative correlation between mortality and fecundity. This combined lethal and sublethal effect is consistent with previous reports on the virulence of the *M. brunneum* complex, which include rapid disease progression and suppression of aphid reproduction (Reingold et al., 2021). Beyond mortality, EPF exposure influenced aphid fitness, particularly nymph production, with application method further influence overall nymph production at 8 days post-treatment. Direct contact with EPF isolates either by immersion or spraying at higher concentrations markedly reduced fecundity, likely due to rapid systemic colonization of the host haemocoel that impaired aphid reproductive capacity. This pattern mirrors findings in other insect-EPF systems where EPF reduce reproductive output (Butt and Goettel, 2000). Also, aphids that were indirectly exposed to conidia or low concentrations of inoculum could continue to exhibit vivipary, albeit lower than control insects. EPF have been shown to negatively affect mating fitness and fecundity in a wide range of insect species, including khapra beetle, mosquitoes, fruit flies (Scholte et al., 2006; Cossentine et al., 2016; Mohammed et al., 2019; Liao et al., 2024). However, a previous study found no effect of EPF on pre-mortem reproduction of aphids exposed to EPF conidia (Jandricic et al., 2014).

*Endophytic colonization and EPF-mediated reduction of BMYV load.* All tested *Metarhizium* isolates successfully colonized sugar beet tissues following seed treatment, with a consistent pattern of higher fungal recovery from roots compared to leaves and petioles. This pattern of endophytic colonization is similar to that previously reported. For example, seed inoculation of broad bean (*Vicia faba*) with *M. brunneum* BIPESCO 5 (=V275) resulted in more inoculum being detected in the roots, the stem, and least in the leaf tissue (Jaber and Enkerli, 2016). A similar pattern was observed for *M. robertsii* seed inoculated Austrian winter pea (*Pisum sativum*), rye (*Sevale cereale*), and winter canola (*Brassica napus*), although more fungus was recovered from pea than rye or canola (Ahmad et al., 2020). Seed inoculation with *M. anisopliae* and *M. flavoviride* of cucumber and tomato, respectively, also resulted in more fungus being recovered from the roots than the stem or leaves (Shaalan et al., 2021; Zheng et al., 2023; Ghaffari et al., 2025). Seed sterilization has been shown to improve endophytic establishment of wheat by *M. anisopliae* (Gonzalez-Guzm´ an et al., 2020´ ).

*Published in : Journal of Invertebrate Pathology (2026), vol. 216, 108559*
*DOI: 10.1016/j.jip.2026.108559*
*Status : Postprint (Author's version)*

Altogether, these observations demonstrate that EPF can enter plants via radicles or roots and grow rapidly within plant tissues without eliciting negative plant responses (Donga et al., 2018). Foliar applications typically result in more inoculum being recovered from leaves then stems and little or no fungus in roots, as reported for *M. anisopliae* in sugarcane (Donga et al., 2018) and tomato (Ghaffari et al., 2025).

Applying EPF during seed sowing offers a convenient way of inoculating sugar beet with beneficial strains. This approach may also confer protection against subterranean pests such as the sugar beet weevil (*Asproparthenis punctiventris* (Germar, 1824)) and the sugar beet root maggot (*Tetanops myopaeformis* (Roder, 1881)) (Jaronski, 2007; Zottele et al., 2023). Using non-sterilized universal potting soil with its inherent microbial communities did not appear to interfere with fungal inoculum establishment or endophytic colonization, as none of the control plants grown in the same soil yielded EPF. This indicates that naturally occurring *Metarhizium* did not contribute to the infections detected in treated plants. Fungi recovered from treated plants exhibited phenotypic traits consistent with the applied isolates. However, because molecular confirmation was not performed, individual colonies cannot be unequivocally assigned to the inoculated strains. Nevertheless, the absence of growth in controls and the consistency of colony morphology suggest that colonization likely originated from the applied EPF. The use of non-sterile soil is consistent with previous work designed to maintain realistic growing conditions for endophytic EPF (Lopez and Sword, 2015; Clifton et al., 2018; Russo et al., 2019), and our results indicate that background soil microbes did not confound the observed colonization patterns.

The successful endophytic colonization of sugar beet plants by all tested EPF isolates implies their potential to significantly reduce BMYV load, with *M. brunneum* GxABT-2 strain showing the strongest and statistically significant reduction compared to ARSEF4556. Meanwhile, *M. majus* GxABT-3 exhibited a numerically intermediate effect that was not significantly different from either *M. brunneum* strain. This underscores the potential benefits of exploring native hypocrealean EPF for biological control, as they may outperform established commercial strains and provide better protection against virus transmission in specific agricultural settings.

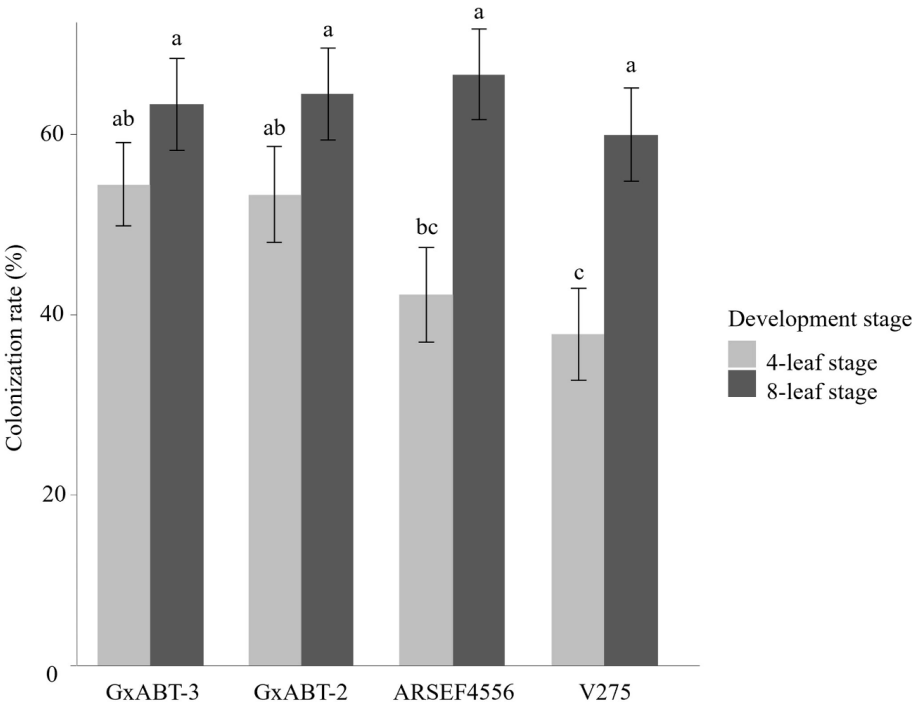
Although all plants became infected, viral titers were markedly lower in EPF-treated plants, indicating that fungal endophytes can suppress viral replication or spread within the host. This finding aligns with previous reports showing that fungal endophytes have antagonistic effects against plant viruses. For example, squash (*C. pepo*) endophytically colonized with *B. bassiana* isolates was shown to have lower virus titer levels of zucchini yellow mosaic virus than endophyte-free plants (Jaber and Salem, 2014). Gonzalez-Mas et al. (2019)´ reported that endophytic *B. bassiana* colonization of melon plants, *Cucumis melo* L., confers protection against persistent (cucurbit aphid-borne yellows virus, Polerovirus) and non-persistent (cucumber mosaic virus, Cucumovirus) plant viruses transmitted by *A. gossypii*. Kiarie et al. (2020) suggested endophytic *M. anisopliae* as a potential candidate for inducing resistance against sugarcane mosaic virus and its use for the management of maize lethal necrosis. Non-EPF endophytes such as *Hypocrea lixii* Pat., *Trichoderma harzianum* Rifai and *T. asperellum* Samuels, Lieckf. & Nirenberg also increase plant resilience to

*Published in : Journal of Invertebrate Pathology (2026), vol. 216, 108559*
*DOI: 10.1016/j.jip.2026.108559*
*Status : Postprint (Author's version)*

insect-transmitted plant viruses (Elsharkawy et al., 2013; Vitti et al., 2016; Gonzalez-Mas et al., 2019´ ).

Altogether, these observations suggest that endophytic EPF are activating plant defenses which interfere with virus replication. However, this may be augmented by poor aphid development on EPF colonized plants (Jaber and Araj, 2018). Reducing viral load is crucial for managing plant health and minimizing yield losses caused by virus infections. Lower viral titers could decrease symptom severity and slow virus spread through vector populations such as the highly polyphagous vector, *M. persicae*. Utilizing EPF as endophytes offers a promising sustainable alternative to chemical controls, potentially reducing pesticide reliance while enhancing crop resilience.

***Fig. 1.*** *Overall colonization rate of different Metarhizium brunneum strains and M. majus throughout sugar beet plants at two growth stages following fungal treatments. Significance letters (a, b, c) indicate statistically different treatments based on post-hoc Tukey tests ($p < 0.05$).*

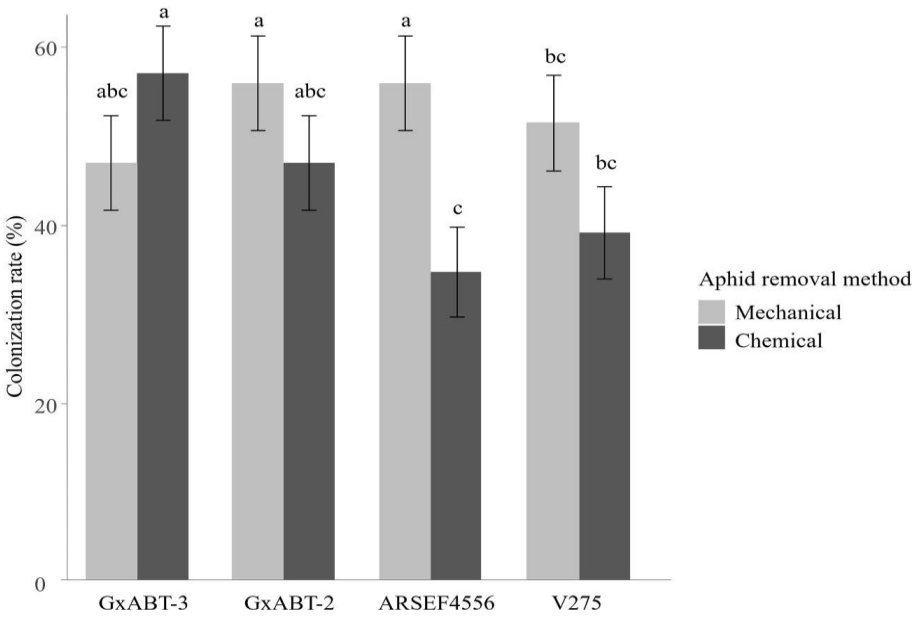


*Published in : Journal of Invertebrate Pathology (2026), vol. 216, 108559*
*DOI: 10.1016/j.jip.2026.108559*
*Status : Postprint (Author's version)*

***Table 4.*** *Percentage colonization (± SE) of sugar beet tissues (root, petiole, and leaf) by the four Metarhizium strains at the four- and eight-leaf stages. Significance letters indicate statistically distinct groups based on post-hoc pairwise comparisons using the estimated marginal means (emmeans) method.*

| Treatment | Root | Petiole | Leaf |
|---|---|---|---|
| 4-leaf stage | | | |
| *M. brunneum* GxABT-2 | 80.0 ± 7.4 ab | 43.3 ± 9 cde | 33.3 ± 8.8 cdef |
| *M. majus* GxABT-3 | 85.7 ± 5.5 a | 50.0 ± 7.8 bcd | 19.4 ± 6.7 ef |
| *M. brunneum* ARSEF4556 | 63.3 ± 9.0 abc | 33.3 ± 8.8 cdef | 26.7 ± 8.2 def |
| *M. brunneum* V275 | 76.7 ± 7.9 ab | 26.7 ± 8.2 def | 6.7 ± 4.6f |
| 8-leaf stage | | | |
| *M. brunneum* GxABT-2 | 96.7 ± 3.3 a | 43.3 ± 9.2 d | 46.7 ± 9.3 d |
| *M. majus* GxABT-3 | 96.7 ± 3.3 a | 53.3 ± 9.2 cd | 33.3 ± 8.8 d |
| *M. brunneum* ARSEF4556 | 83.3 ± 7.0 a | 63.3 ± 9.0 bcd | 50.0 ± 9.3 d |
| *M. brunneum* V275 | 90.0 ± 5.6 a | 43.3 ± 9.2 d | 43.3 ± 9.2 d |

***Fig. 2.*** *Impact of mechanical and chemical aphid removal treatments on the overall colonization rate of different Metarhizium brunneum strains and M. majus throughout plants at the eight-leaf stage. Significance letters (a, b, c) indicate statistically different treatments based on post-hoc Tukey tests ($p < 0.05$).*

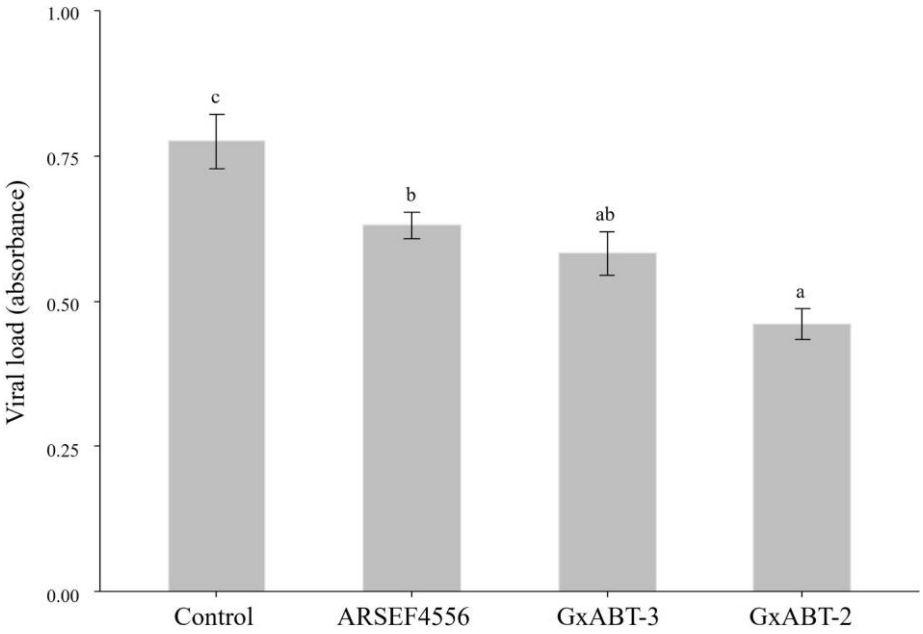


*Published in : Journal of Invertebrate Pathology (2026), vol. 216, 108559*
*DOI: 10.1016/j.jip.2026.108559*
*Status : Postprint (Author's version)*

***Table 5.*** *Mean absorbance (± SE) of viral load in plants and percentage viral load reduction (%) following fungal treatment. Different letters indicate significant differences between treatments (Tukey's test, $p < 0.05$).*

| Treatment | Mean absorbance | Viral load reduction (%) | Significance |
|---|---|---|---|
| Control | 0.78 ± 0.04 | 0.0 | c |
| *M. brunneum* ARSEF4556 | 0.63 ± 0.04 | 18.6 | b |
| *M. majus* GxABT-3 | 0.58 ± 0.04 | 24.8 | ab |
| *M. brunneum* GxABT-2 | 0.46 ± 0.04 | 40.6 | a |

***Fig. 3.*** *Impact of fungal treatments (Metarhizium brunneum and M. majus) and a non-treated control on beet mild yellowing virus viral load in sugar beet plants. Significance letters (a, b, c) indicate statistically different treatments based on post-hoc Tukey tests ($p < 0.05$).*




# 5. Conclusions

We demonstrate the significant potential of Metarhizium species, particularly the locally adapted M. brunneum GxABT-2 strain, as effective biocontrol agents against the green peach aphid (M. persicae) and BMYV in sugar beet. Our results demonstrate that these Metarhizium strains not only cause substantial aphid mortality in a concentration- and application method-dependent manner but also successfully endophytically colonize sugar beet plants, with a preference for root tissues. Importantly, Metarhizium colonization significantly reduced viral loads in infected plants, suggesting a promising role for fungal endophytes in suppressing virus transmission and enhancing plant resilience. These findings support the integration of native Metarhizium isolates into sustainable pest and disease management strategies, offering an environmentally friendly

*Published in : Journal of Invertebrate Pathology (2026), vol. 216, 108559*
*DOI: 10.1016/j.jip.2026.108559*
*Status : Postprint (Author's version)*

alternative to chemical pesticides that could improve crop health and yield. **CREDIT AUTHORSHIP CONTRIBUTION STATEMENT**

**Ibtissem Ben Fekih:** Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Kenza Dessauvages:** Writing – review & editing, Visualization, Software, Formal analysis, Data curation. **Mathilde Scheifler:** Writing – review & editing, Software, Formal analysis, Data curation. **Gregoire Noël:** ¨ Writing – review & editing, Software, Formal analysis. **Tariq M. Butt:** Writing – review & editing, Writing – original draft, Conceptualization. **Joachim Carpentier:** Writing – review & editing, Data curation. **Frédéric Francis:** Writing – review & editing, Validation, Supervision, Conceptualization.

## DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## ACKNOWLEDGEMENTS

The authors thank IRBAB, and in particular Andre Wauters and Chlo´ ·e Dufrane, for providing sugar beet seeds used in the biological assays. We are also grateful to Mark Goettel for valuable comments on the manuscript, to Martyn J. Wood (Swansea University) for fruitful discussions during fungal isolation experiments, and to Emilie Berra (Gembloux Agro-Bio Tech) for technical support. I.B.F. was funded by the National Fund for Scientific Research F.R.S.-FNRS (PDR T.0088.21), and T.M.B. was supported by UK Research and Innovation Building a Green Future strategic theme (grant number UKRI239).

## APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jip.2026.108559.

*Published in : Journal of Invertebrate Pathology (2026), vol. 216, 108559*
*DOI: 10.1016/j.jip.2026.108559*
*Status : Postprint (Author's version)*

# References

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

Allegrucci, N., Velazquez, M.S., Russo, M.L., Vianna, M.F., Abarca, C., Scorsetti, A.C., 2020. Establishment of the entomopathogenic fungus *Beauveria bassiana* as an endophyte in *Capsicum annuum* and its effects on the aphid pest *Myzus persicae* (Homoptera: Aphididae). Rev. Biol. Trop. 68, 1084–1094. https://doi.org/ 10.15517/rbt.v68i4.41218.

Amnuaykanjanasin, A., Jirakkakul, J., Panyasiri, C., Panyarakkit, P., Nounurai, P., Chantasingh, D., Eurwilaichitr, L., Cheevadhanarak, S., Tanticharoen, M., 2013. Infection and colonization of tissues of the aphid *Myzus persicae* and cassava mealybug *Phenacoccus manihoti* by the fungus *Beauveria bassiana*. BioControl 58, 379–391. https://doi.org/10.1007/s10526-012-9499-2.

Ansari, M.A., Evans, M., Butt, T.M., 2009. Identification of pathogenic strains of entomopathogenic nematodes and fungi for wireworm control. Crop Prot. 28, 269–272. https://doi.org/10.1016/j.cropro.2008.11.003.

Asan, C., Hazir, S., Cimen, H., Ulug, D., Taylor, J., Butt, T., Karagoz, M., 2017. An innovative strategy for control of the chestnut weevil Curculio elephas (Coleoptera: Curculionidae) using Metarhizium brunneum. Crop Prot. 102, 147–153. https://doi. org/10.1016/j.cropro.2017.08.021.

Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67, 1–48. https://doi.org/10.18637/jss.v067.i01.

Ben Fekih, I., Jensen, A.B., Boukhris-Bouhachem, S., Pozsgai, G., Rezgui, S., Rensing, C., Eilenberg, J., 2019. Virulence of two entomophthoralean fungi, *Pandora neoaphidis* and *Entomophthora planchoniana*, to their conspecific (*Sitobion avenae*) and Heterospecific (*Rhopalosiphum padi*) aphid hosts. Insects 10, 54. https://doi.org/ 10.3390/insects10020054.

Biancardi, E., McGrath, J.M., Panella, L.W., Lewellen, R.T., Stevanato, P., 2010. Sugar beet, in: Bradshaw, J.E. (Ed.), Root and Tuber Crops. Springer, New York, pp. 173–219. 10.1007/978-0-387-92765-7_6.

Bidochka, M.J., Kamp, A.M., Lavender, T.M., Dekoning, J., De Croos, J.N., 2001. Habitat association in two genetic groups of the insect-pathogenic fungus *Metarhizium anisopliae*: uncovering cryptic species? Appl. Environ. Microbiol. 67, 1335–1342. https://doi.org/10.1128/AEM.67.3.1335-1342.2001.

Biryol, S., Demirbag, Z., Erdo˘ gan, P., Demir, I., 2022. Development of ˘ *Beauveria bassiana* (Ascomycota: Hypocreales) as a mycoinsecticide to control green peach aphid, *Myzus persicae* (Homoptera: Aphididae) and investigation of its biocontrol potential. J. Asia Pac. Entomol. 25, 101878. https://doi.org/10.1016/j.aspen.2022.101878.

Borgolte, S., Varrelmann, M., Hossain, R., 2024. Time point of virus yellows infection is crucial for yield losses in sugar beet, and co-infection with beet mosaic virus is negligible under field conditions. Plant Pathol. 73, 2056–2070. https://doi.org/ 10.1111/ppa.13954.

Brooks, M.E., Kristensen, K., van Benthem, K.J., Magnusson, A., Berg, C.W., Nielsen, A., Skaug, H.J., Maechler, M., Bolker, B.M., 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. R Journal 9, 378–400. https://doi.org/10.32614/RJ-2017-066.

*Published in : Journal of Invertebrate Pathology (2026), vol. 216, 108559*
*DOI: 10.1016/j.jip.2026.108559*
*Status : Postprint (Author's version)*

Butt, T.M., Coates, C.J., Dubovskiy, I.M., Ratcliffe, N.A., 2016. Entomopathogenic Fungi: New insights into host-pathogen interactions. Adv. Genet. 94, 307–364. https://doi. org/10.1016/bs.adgen.2016.01.006.

Butt, T.M., Goettel, M.S., 2000. Bioassays of entomogenous fungi, in: Lacey, L.A. (Ed.), Bioassays of entomopathogenic microbes and nematodes. CABI Books. pp. 141–195. 10.1079/9780851994222.0141.

Clifton, E.H., Jaronski, S.T., Coates, B.S., Hodgson, E.W., Gassmann, A.J., 2018. Effects of endophytic entomopathogenic fungi on soybean aphid and identification of *Metarhizium* isolates from agricultural fields. PLoS One 13, e0194815. https://doi. org/10.1371/journal.pone.0194815.

Cossentine, J., Robertson, M., Buitenhuis, R., 2016. Impact of acquired entomopathogenic fungi on adult *Drosophila suzukii* survival and fecundity. Biol. Control 103, 129–137. https://doi.org/10.1016/j.biocontrol.2016.09.002.

Dessauvages, K., Scheifler, M., Francis, F., Ben Fekih, I., 2024. A new isolate *Beauveria bassiana* GxABT-1: efficacy against *Myzus persicae* and promising impact on the beet mild yellow virus-aphid associatio. Insects 15, 697. https://doi.org/10.3390/ insects15090697.

Donga, T.K., Vega, F.E., Klingen, I., 2018. Establishment of the fungal entomopathogen *Beauveria bassiana* as an endophyte in sugarcane, *Saccharum Officinarum*. Fungal Ecol. 35, 70–77. https://doi.org/10.1016/j.funeco.2018.06.008.

Dubovskiy, I.M., Whitten, M.M.A., Yaroslavtseva, O.N., Greig, C., Kryukov, V.Y., Grizanova, E.V., Mukherjee, K., Vilcinskas, A., Glupov, V.V., Butt, T.M., 2013. Can insects develop resistance to insect pathogenic fungi? PLoS One 8, e60248. https:// doi.org/10.1371/journal.pone.0060248.

Ekesi, S., Maniania, N.K., 2000. Susceptibility of *Megalurothrips sjostedti* developmental stages to *Metarhizium anisopliae* and the effects of infection on feeding, adult fecundity, egg fertility and longevity. Entomol. Exp. Appl. 94, 229–236. https://doi.org/10.1046/j.1570-7458.2000.00624.x.

Elsharkawy, M.M., Shimizu, M., Takahashi, H., Ozaki, K., Hyakumachi, M., 2013. Induction of systemic resistance against cucumber mosaic virus in *Arabidopsis thaliana* by *Trichoderma asperellum* SKT-1. Plant Pathol. J. 29, 193–200. https://doi. org/10.5423/PPJ.SI.07.2012.01.

Fite, T., Kebede, E., Tefera, T., Bekeko, Z., 2023. Endophytic fungi: Versatile partners for pest biocontrol, growth promotion, and climate change resilience in plants. Front. Sustain. Food Syst. 7, 1322861. https://doi.org/10.3389/fsufs.2023.1322861.

Francis, F., Fingu-Mabola, J.C., Ben Fekih, I., 2022a. Direct and endophytic effects of fungal entomopathogens for sustainable aphid control: a review. Agriculture 12, 2081. https://doi.org/10.3390/agriculture12122081.

Francis, F., Then, C., Francis, A., Gbangbo, Y.A.C., Iannello, L., Ben Fekih, I., 2022b. Complementary strategies for biological control of aphids and related virus transmission in sugar beet to replace neonicotinoids. Agriculture 12, 1663. https:// doi.org/10.3390/agriculture12101663.

Garrido-Jurado, I., Resquín-Romero, G., Amarilla, S.P., Ríos-Moreno, A., Carrasco, L., Quesada-Moraga, E., 2017. Transient endophytic colonization of melon plants by entomopathogenic fungi after foliar application for the control of *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae). J. Pest. Sci. 90, 319–330. https://doi.org/ 10.1007/s10340-016-0767-2.

Ghaffari, S., Karimi, J., Cheniany, M., Seifi, A., Loverodge, J., Butt, T.M., 2025. Endophytic entomopathogenic fungi enhance plant immune responses against tomato leafminer. J. Invertebr. Pathol. 209, 108270. https://doi.org/10.1016/j.jip.2025.108270.

Gillespie, J.P., Kanost, M.R., Trenczek, T., 1997. Biological mediators of insect immunity. Annu. Rev. Entomol. 42, 611–643. https://doi.org/10.1146/annurev.ento.42.1.611.

Gill, S., Kunkel, B.A., Klick, S., O'Donnell, S., Leslie, A., 2024. Insecticides used to manage spotted lanternflies in nurseries, 2023. Arthropod Manag. Tests 49, tsae047. 10.1093/amt/tsae047.

Gonzalez-Guzm´ an, A., Sacrist´ an, D., S´ anchez-Rodríguez, A.R., Barr´ on, V., Torrent, J., del ´ Campillo, M.C., 2020. Soil nutrients effects on the performance of durum wheat inoculated with entomopathogenic fungi. Agronomy 10, 589. https://doi.org/ 10.3390/agronomy10040589.

Gonzalez-Mas, N., Quesada-Moraga, E., Plaza, M., Fereres, A., Moreno, A., 2019. Changes ´ in feeding behaviour are not related to the reduction in the transmission rate of plant viruses by *Aphis gossypii* (Homoptera: Aphididae) to melon plants colonized by *Beauveria bassiana* (Ascomycota: Hypocreales). Biol. Control 130, 95–103. https:// doi.org/10.1016/j.biocontrol.2018.11.001.

Hajek, A.E., Leger, R.J.S., 1994. Interactions between fungal pathogens and insect hosts. Annu. Rev. Entomol. 39, 293–322. https://doi.org/10.1146/annurev.en.39.010194.001453.

Hall, R.A., 1979. Pathogenicity of *Verticillium lecani* conidia and blastospores against the aphid, *Macrosiphoniella sanborni*. Entomophaga 24, 191–198. https://doi.org/ 10.1007/BF02375133.

Hao, Q., Albaghdady, D.M.D., Xiao, Y., Xiao, X., Mo, C., Tian, T., Wang, G., 2021. Endophytic *Metarhizium anisopliae* is a potential biocontrol agent against wheat *Fusarium* head blight caused by *Fusarium graminearum*. J. Plant Pathol. 103, 875–885. https://doi.org/10.1007/s42161-021-00866-6.

Hauer, M., Hansen, A.L., Manderyck, B., Olsson, Å., Raaijmakers, E., Hanse, B., Stockfisch, N., Marl¨ ander, B., 2017. Neonicotinoids in sugar beet cultivation in ¨ Central and Northern Europe: efficacy and environmental impact of neonicotinoid seed treatments and alternative measures. Crop Prot. 93, 132–142. https://doi.org/ 10.1016/j.cropro.2016.11.034.

Hossain, R., Menzel, W., Lachmann, C., Varrelmann, M., 2021. New insights into virus yellows distribution in Europe and effects of beet yellows virus, beet mild yellowing virus, and beet chlorosis virus on sugar beet yield following field inoculation. Plant Pathol. 70, 584–593. https://doi.org/10.1111/ppa.13306.

Humber, R.A., 2012. Identification of entomopathogenic fungi. In: Lacey, L.A. (Ed.), Manual of Techniques in Invertebrate Pathology, 2nd ed.,. Academic Press, San Diego, pp. 151–187. https://doi.org/10.1016/B978-0-12-386899-2.00006-3.

Inglis, G.D., Goettel, M.S., Butt, T.M., Strasser, H., 2001. Use of hyphomycetous fungi for managing insect pests. In: Butt, T.M., Jackson, C., Magan, N. (Eds.), Fungi as Biocontrol Agents: Progress, Problems and Potential. CABI publishing, Wallingford, UK, pp. 23–69. https://doi.org/10.1079/9780851993560.0023.

Jaber, L.R., Alananbeh, K.M., 2018. Fungal entomopathogens as endophytes reduce several species of *Fusarium* causing crown and root rot in sweet pepper (*Capsicum annuum* L.). Biol. Control 126, 117–126. https://doi.org/10.1016/j.biocontrol.2018.08.007.

Jaber, L.R., Araj, S.E., 2018. 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 116, 53–61. https://doi.org/10.1016/j. biocontrol.2017.04.005.

Jaber, L.R., Enkerli, J., 2016. Effect of seed treatment duration on growth and colonization of *Vicia faba* by endophytic *Beauveria bassiana* and *Metarhizium brunneum*. Biol. Control 103, 187–195. https://doi.org/10.1016/j.biocontrol.2016.09.008.

Jaber, L.R., Salem, N.M., 2014. Endophytic colonisation of squash by the fungal entomopathogen *Beauveria bassiana* (Ascomycota: Hypocreales) for managing Zucchini yellow mosaic virus in cucurbits. Biocontrol Sci. Tech. 24, 1096–1109. https://doi.org/10.1080/09583157.2014.923379.

Jandricic, S.E., Filotas, M., Sanderson, J.P., Wraight, S.P., 2014. Pathogenicity of conidia- based preparations of entomopathogenic fungi against the greenhouse pest aphids *Myzus persicae, Aphis gossypii,* and *Aulacorthum solani* (Hemiptera: Aphididae). J. Invertebr. Pathol. 118, 34–46. https://doi.org/10.1016/j.jip.2014.02.003.

Jaronski, S.T., 2007. Soil ecology of the entomopathogenic Ascomycetes: a critical examination of what we (think) we know. In: Ekesi, S., Maniania, N.K. (Eds.), Use of Entomopathogenic Fungi in Biological Pest Management. CABI publishing, Wallingford, UK, pp. 91–143.

Kiarie, S., Nyasani, J.O., Gohole, L.S., Maniania, N.K., Subramanian, S., 2020. Impact of fungal endophyte colonization of maize (*Zea mays* L.) on induced resistance to thrips- and aphid-transmitted viruses. Plants 9, 416. https://doi.org/10.3390/ plants9040416.

Kortsinoglou, A.M., Wood, M.J., Myridakis, A.I., Andrikopoulos, M., Roussis, A., Eastwood, D., Butt, T., Kouvelis, V.N., 2024. Comparative genomics of *Metarhizium brunneum* strains V275 and ARSEF 4556: Unraveling intraspecies diversity. G3 (Bethesda) 14, jkae190. 10.1093/g3journal/jkae190.

Klingen, I., Westrum, K., Meyling, N.V., 2015. Effect of norwegian entomopathogenic fungal isolates against *Otiorhynchus sulcatus* larvae at low temperatures and persistence in strawberry rhizospheres. Biol. Control 81, 1–7. https://doi.org/ 10.1016/j.biocontrol.2014.10.006.

Korosi, G.A., Wilson, B.A., Powell, K.S., Ash, G.J., Reineke, A., Savocchia, S., 2019. Occurrence and diversity of entomopathogenic fungi (*Beauveria* spp. and *Metarhizium* spp.) in Australian vineyard soils. J. Invertebr. Pathol. 164, 69–77. https://doi.org/10.1016/j.jip.2019.05.002.

Kuznetsova, A., Brockhoff, P.B., Christensen, R.H.B., 2017. lmerTest package: tests in linear mixed effects models. J. Stat. Softw. 82, 1–26. https://doi.org/10.18637/jss. v082.i13.

Lange, T.M., Wutke, M., Bertram, L., Keunecke, H., Kopisch-Obuch, F., Schmitt, A.O., 2021. Decision strategies for absorbance readings from an enzyme-linked immunosorbent assay – a case study about testing genotypes of sugar beet (*Beta vulgaris* L.) for resistance against beet necrotic yellow vein virus (BNYVV). Agriculture 11, 956. https://doi.org/10.3390/agriculture11100956.

Laurent, M., Bougeard, S., Caradec, L., Ghestem, F., Albrecht, M., Brown, M.J.F., De Miranda, J., Karise, R., Knapp, J., Serrano, J., Potts, S.G., Rundlöf, M., Schwarz, J., Attridge, E., Babin, A., Bottero, I., Cini, E., de la Rúa, P., Di Prisco, G., Dominik, C., Dzul, D., García Reina, A., Hodge, S., Klein, A.M., Knauer, A., Mand, M., Martínez López, V., Serra, G., Pereira-Peixoto, H., Raimets, R., Schweiger, O., Senapathi, D.,Stout, J.C., Tamburini, G., Costa, C., Kiljanek, T., Martel, A.-C., LE, S., Chauzat, M.P., 2024. Novel indices reveal that pollinator exposure to pesticides varies across biological compartments and crop surroundings. Sci. Total Environ. 927, 172118. https://doi.org/10.1016/j.scitotenv.2024.172118.

*Published in : Journal of Invertebrate Pathology (2026), vol. 216, 108559*
*DOI: 10.1016/j.jip.2026.108559*
*Status : Postprint (Author's version)*

Lee, W.W., Shin, T.Y., Bae, S.M., Woo, S.D., 2015. Screening and evaluation of entomopathogenic fungi against the green peach aphid, *Myzus persicae*, using multiple tools. J. Asia Pac. Entomol. 18, 607–615. https://doi.org/10.1016/j. aspen.2015.07.012.

Lenth, R., 2023. emmeans: estimated marginal means (least-squares means). R package version 1.8.7. https://CRAN.R-project.org/package=emmeans.

Liao, A., Cavigliasso, F., Savary, L., Kawecki, T.J., 2024. Effects of an entomopathogenic fungus on the reproductive potential of *Drosophila* males. Ecol. Evol. 14, e11242. https://doi.org/10.1002/ece3.11242.

Lopes, R.B., Mesquita, A.L.M., Tigano, M.S., Souza, D.A., Martins, I., Faria, M., 2013. Diversity of indigenous *Beauveria* and *Metarhizium* spp. in a commercial banana field and their virulence toward *Cosmopolites sordidus* (Coleoptera: Curculionidae). Fungal Ecol. 65, 356–364. https://doi.org/10.1016/j.funeco.2013.06.007.

Lopez, D.C., Sword, G.A., 2015. The endophytic fungal entomopathogens *Beauveria bassiana* and *Purpureocillium lilacinum* enhance the growth of cultivated cotton (*Gossypium hirsutum*) and negatively affect survival of the cotton bollworm (*Helicoverpa zea*). Biol. Control 89, 53–60. https://doi.org/10.1016/j.biocontrol.2015.03.010.

Mahmood, Z., Steenberg, T., Mahmood, K., Labouriau, R., Kristensen, M., 2019. Endophytic *Beauveria bassiana* in maize affects survival and fecundity of the aphid *Sitobion avenae*. Biol. Control 137, 104017. https://doi.org/10.1016/j. biocontrol.2019.104017.

Maluta, N., Castro, T., Lopes, J.R.S., 2022. Entomopathogenic fungus disrupts the phloem-probing behavior of *Diaphorina citri* and may be an important biological control tool in citrus. SC Rep. 12, 7959. https://doi.org/10.1038/s41598-022- 11789-2.

Manoussopoulos, Y., Mantzoukas, S., Lagogiannis, I., Goudoudaki, S., Kambouris, M., 2019. Effects of three strawberry entomopathogenic fungi on the prefeeding behavior of the aphid *Myzus persicae*. J. Insect Behav. 32, 99–108. https://doi.org/ 10.1007/s10905-019-09709-w.

Meyling, N.V., Pilz, C., Keller, S., Widmer, F., Enkerli, J., 2012. Diversity of Beauveria spp. isolates from pollen beetles Meligethes aeneus in Switzerland. J. Invertebr. Pathol. 109, 76–82. https://doi.org/10.1016/j.jip.2011.10.001.

Meyling, N.V., 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.

Mohammed, A.A., Kadhim, J.H., Hasan, A.M.H., 2019. Laboratory evaluation of entomopathogenic fungi for the control of Khapra beetle (Coleoptera : Dermestidae) and their effects on the beetles' fecundity and longevity. J. Agric. Urban Entomol. 35, 1–11. https://doi.org/10.3954/1523-5475-35.1.1.

Mweke, A., Akutse, K.S., Ulrichs, C., Fiaboe, K.K.M., Maniania, N.K., Ekesi, S., 2019. Efficacy of aqueous and oil formulations of a specific *Metarhizium anisopliae* isolate against *Aphis craccivora* Koch, 1854 (Hemiptera: Aphididae) under field conditions. J. Appl. Entomol. 143, 1182–1192. https://doi.org/10.1111/jen.12705.

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, 583–598. https://doi. org/10.1080/09583150500532824.

Panwar, N., Szczepaniec, A., 2024. Endophytic entomopathogenic fungi as biological control agents of insect pests. Pest Manag. Sci. 80, 6033–6040. https://doi.org/ 10.1002/ps.8322.

Pettersson, J., Tjallingii, W.F., Hardie, J., 2007. Host-plant selection and feeding. in: van Emden, H.F., Harrington, R. (Eds.), Aphids as crop pests. CABI, Wallingford, UK, pp. 87–113. 10.1079/9780851998190.0087.

Pinheiro, J., Bates, D., R Core Team, 2025. nlme: linear and nonlinear mixed effects models. R package version 3.1-168. https://CRAN.R-project.org/package=nlme.

Rasool, S., Vidkjaer, N.H., Hooshmand, K., Jensen, B., Fomsgaard, I.S., Meyling, N.V., 2021. Seed inoculations with entomopathogenic fungi affect aphid populations coinciding with modulation of plant secondary metabolite profiles across plant families. New Phytol. 229, 1715–1727. https://doi.org/10.1111/nph.16979.

Reingold, V., Kottakota, C., Birnbaum, N., Goldenberg, M., Lebedev, G., Ghanim, M., Ment, D., 2021. Intraspecies variation of *Metarhizium brunneum* against the green peach aphid, *Myzus persicae*, provides insight into the complexity of disease progression. Pest Manag. Sci. 77, 2557–2567. https://doi.org/10.1002/ps.6294.

Russo, M.L., Pelizza, S.A., Vianna, M.F., Allegrucci, N., Cabello, M.N., Toledo, A.V., Mourelos, C., Scorsetti, A.C., 2019. Effect of endophytic entomopathogenic fungi on soybean *Glycine max* (L.) Merr. growth and yield. J. King Saud Univ. Sci. 31, 728–736. https://doi.org/10.1016/j.jksus.2018.04.008.

Saranya, S., Ushakumari, R., Jacob, S., Philip, B.M., 2010. Efficacy of different entomopathogenic fungi against cowpea aphid, *Aphis craccivora* (Koch). J. Biopestic. 3, 138–142. https://api.semanticscholar.org/CorpusID:53969189.

Saud, Z., Kortsinoglou, A.M., Kouvelis, V.N., Butt, T.M., 2021. Telomere length de novo assembly of all 7 chromosomes and mitogenome sequencing of the model entomopathogenic fungus, *Metarhizium brunneum*, by means of a novel assembly pipeline. BMC Genomics 22, 87. https://doi.org/10.1186/s12864-021-07390-y.

Scheepmaker, J.W.A., Butt, T.M., 2010. Natural and released inoculum levels of entomopathogenic fungal biocontrol agents in soil in relation to risk assessment and in accordance with EU regulations. Biocontrol Sci. Tech. 20, 503–552. https://doi. org/10.1080/09583150903545035.

Scholte, E.J., Knols, B.G.J., Takken, W., 2006. Infection of the malaria mosquito *Anopheles gambiae* with the entomopathogenic fungus *Metarhizium anisopliae* reduces blood feeding and fecundity. J. Invertebr. Pathol. 91, 43–49. https://doi.org/ 10.1016/j.jip.2005.10.006.

Shaalan, R. S., Gerges, E., Habib, W., Ibrahim, L., 2021. Endophytic colonization by *Beauveria bassiana* and *Metarhizium anisopliae* induces growth promotion effect and increases the resistance of cucumber plants against *Aphis gossypii*. J. Plant Prot. Res. 61, 358–370. 10.24425/jppr.2021.139244.

Shah, P.A., Pell, J.K., 2003. Entomopathogenic fungi as biological control agents. Appl. Microbiol. Biotechnol. 61, 413–423. https://doi.org/10.1007/s00253-003-1240-8.

Shan, L.T., Feng, M.G., 2010. Evaluation of the biocontrol potential of various *Metarhizium* isolates against green peach aphid *Myzus persicae* (Homoptera: Aphididae). Pest Manag. Sci. 66, 669–675. https://doi.org/10.1002/ps.1928.

*Published in : Journal of Invertebrate Pathology (2026), vol. 216, 108559*
*DOI: 10.1016/j.jip.2026.108559*
*Status : Postprint (Author's version)*

Sonmez, E., Demir, ¨ I., Bull, J.C., Butt, T.M., Demirba ̆ g, Z., 2017. Pine processionary moth ˇ (*Thaumetopoea pityocampa*, Lepidoptera: Thaumetopoeidae) larvae are highly susceptible to the entomopathogenic fungi *Metarhizium brunneum* and *Beauveria bassiana*. Biocontrol Sci. Tech. 27, 1168–1179. https://doi.org/10.1080/ 09583157.2017.1387643.

St Leger, R.J., Joshi, L., Bidochka, M.J., Roberts, D.W., 1996. Construction of an improved mycoinsecticide overexpressing a toxic protease. PNAS 93, 6349–6354. https://doi.org/10.1073/pnas.93.13.6349.

Smith, D.F.Q., Dragotakes, Q., Kulkarni, M., Marie Hardwick, J., Casadevall, A., 2022. *Galleria mellonella* immune melanization is fungicidal during infection. Commun. Biol. 5, 1364. https://doi.org/10.1038/s42003-022-04340-6.

Therneau, T.M., Lumley, T., Elizabeth, A., Cynthia, C., 2024. A package for survival analysis in R. package (version 3.8-3). https://cran.r-project.org/web/packages/ survival/index.html. van Lenteren, J.C., Bolckmans, K., Kohl, J., Ravensberg, W.J., Urbaneja, A., 2018. ¨ Biological control using invertebrates and microorganisms: plenty of new opportunities. BioControl 63, 39–59. https://doi.org/10.1007/s10526-017-9801-4.

Vitti, A., Pellegrini, E., Nali, C., Lovelli, S., Sofo, A., Valerio, M., Scopa, A., Nuzzaci, M., 2016. *Trichoderma harzianum* T-22 induces systemic resistance in tomato infected by cucumber mosaic virus. Front. Plant Sci. 7, 1520. https://doi.org/10.3389/ fpls.2016.01520.

Wood, T.J., Goulson, D., 2017. The environmental risks of neonicotinoid pesticides: a review of the evidence post 2013. Environ. Sci. Pollut. Res. Int. 24, 17285–17325. https://doi.org/10.1007/s11356-017-9240-x.

Wraight, S.P., Ramos, M.E., 2002. Application parameters affecting field efficacy of *Beauveria bassiana* foliar treatments against Colorado potato beetle *Leptinotarsa decemlineata*. Biol. Control 23, 164–178. https://doi.org/10.1006/bcon.2001.1004.

Wyckhuys, K.A.G., Gu, B., Ben Fekih, I., Finger, R., Kenis, M., Lu, Y., Subramanian, S., Tang, F.H.M., Weber, D.C., Zhang, W., Hadi, B.A.R., 2024. Restoring functional integrity of the global production ecosystem through biological control. J. Environ. Manag. 370, 122446. https://doi.org/10.1016/j.jenvman.2024.122446.

Yubak Dhoj, G.C., Keller, S., Nagel, P., Kafle, L., 2008. Virulence of *Metarhizium anisopliae* and *Beauveria bassiana* against common white grubs in Nepal. Formosan Entomol. 28, 11–20. https://doi.org/10.6661/TESFE.2008002.

Zheng, Y., Liu, Y., Zhang, J., Liu, X., Ju, Z., Shi, H., Mendoza-Mendoza, A., Zhou, W., 2023. Dual role of endophytic entomopathogenic fungi: Induce plant growth and control tomato leafminer *Phthorimaea absoluta*. Pest Manag. Sci. 79, 4557–4568. https://doi.org/10.1002/ps.7657.

Zhu, Y., Stahl, A., Rostas, M., Will, T., 2024. Temporal and species-specific resistance of ´ sugar beet to green peach aphid and black bean aphid: mechanisms and implications for breeding. Pest Manag. Sci. 80, 404–413. https://doi.org/10.1002/ps.7770.

Zottele, M., Mayrhofer, M., Embleton, H., Enkerli, J., Eigner, H., Tarasco, E., Strasser, H., 2023. Integrated biological control of the sugar beet weevil *Asproparthenis punctiventris* with the fungus *Metarhizium brunneum* : New application approaches. Pathogens 12, 99. https://doi.org/10.3390/pathogens12010099.

Zimmermann, G., 1986. The 'Galleria bait method' for detection of entomopathogenic fungi in soil. J. Appl. Entomol. 102, 213–215.