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Dual effects of entomopathogenic fungi on control of the pest *Lobesia botrana* and the pathogenic fungus *Eutypella microtheca* on grapevine

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Abstract

Background: Entomopathogenic fungi (EPF) are the natural enemies of insect pests. Nevertheless, research on the use of EPF for simultaneous prevention of pest and disease agents on the same crop is limited. In this study, we explored the potential dual effects of three strains of the EPF *Metarhizium anisopliae* on the control of detrimental agents of *Vitis vinifera* L., including different developmental stages (larvae, pupae, and adult) of the insect pest *Lobesia botrana* and the phytopathogenic fungus *Eutypella microtheca*.

Methods: Laboratory pathogenicity trials were performed to examine the effects of the three *M. anisopliae* strains on the mortality rate of *L. botrana*. In addition, field trials were conducted to assess the biocontrol potential of one selected *M. anisopliae* strain on the larval stage of *L. botrana*. Moreover, inhibitory effects of the three EPF strains on *E. microtheca* growth were examined in vitro.

Results: All the *M. anisopliae* strains were highly effective, killing all stages of *L. botrana* as well as inhibiting the growth of *E. microtheca*. The in vitro mortality of larvae treated with the strains was over 75%, whereas that of treated pupae and adults was over 85%. The three EPF strains showed similar efficacy against larvae and adult stages; nevertheless, pupal mortality was observed to be strain dependent. Mortality of *L. botrana* larvae ranged from 64 to 91% at field conditions. Inhibition of *E. microtheca* growth reached 50% in comparison to the control.

Conclusions: Our study showed that *M. anisopliae* strains were highly effective in ensuring control of two different detrimental agents of *V. vinifera* L., providing new evidence to support the dual effects of entomopathogenic fungi.

Keywords: Entomopathogenic fungi, European grapevine moth, *Eutypella microtheca*, Grapevine, Ligninolytic fungus, *Lobesia botrana*

Background

The grapevine (*Vitis vinifera* L.) is one of the most cultivated fruit crops worldwide, with a cultivated area of 7.4 million ha. However, grapevine production is affected by several pests and diseases, resulting in high use of pesticides and chemicals [1–4]. Among the numerous pests and diseases of grapevine, the European grapevine moth (*Lobesia botrana* Den. and Schiff.) and the ligninolytic

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fungus (*Eutypella microtheca* Trouillas, W.M. Pitt and Gubler) are of considerable economic importance to vine production worldwide [5].

Lobesia botrana is one of the most relevant pests of vineyards [6]. Although this pest is endemic to the Palearctic Region [7], is economically more important in southern Europe (France, Spain, Italy and the Mediterranean islands) and South America (Argentina and Chile) [8–11], where can cause substantial crop losses from larvae feeding on young flower buds and fruit [12, 13]. Additionally, ligninolytic fungi, including *E. microtheca*, *Phaeoconiella chlamydospora*, and the genus *Phaeoacremonium* [5, 14, 15], are responsible for pathogenic symptoms, such as wood necrosis and/or discoloration, vascular infections, and decay, which are commonly known as grapevine trunk diseases [16]. These two biological agents are highly detrimental to vine production worldwide. While *L. botrana* is one of the most injurious insect pests of table and wine grape production [17], the economic costs caused by the effects of ligninolytic fungi on grape production is estimated to be more than 1.5 billion dollars per year [18].

Conventional methods of preventing and controlling *L. botrana* and ligninolytic fungi involve the use of chemical compounds [15, 19, 20]. Additionally, the management and prevention of *L. botrana* in vineyards involve mating disruption [21–24]. However, these methods have been ineffective, as this pest continues to proliferate in vineyards. Moreover, the widespread use of chemical products in controlling crop pests and diseases have been widely criticised because of their negative impacts on biodiversity and human health [25, 26]. To the best of our knowledge, researchers are yet to develop an effective, economic, and eco-friendly method for preventing and managing *L. botrana* infestation and *E. microtheca* infection. Owing to the negative impacts of synthetic pesticides on the environment, biopesticides such as entomopathogenic fungi (EPF), have been identified as a promising alternative for environmentally friendly pest management programmes [27].

EPF are a group of environmentally safe fungi used for biological control of insects and other arthropod pests of agricultural crops. They comprise a wide range of morphologically, phylogenetically, and ecologically diverse fungal species [28]. A wide range of EPF species have been established as important biocontrol agents in many natural and artificial ecosystems [29], to defend against a large number of insect species [30]. Additionally, recent studies suggest that EPF possess inhibitory actions against some phytopathogenic fungal species [31–34]. Thus, there is growing interest in the use of these organisms in integrated pest management (IPM) programmes [35]. However, research on the use of EPF in

the simultaneous prevention and management of several pest and disease agents of the same crop is limited.

Here, we examined the effectiveness of three different strains of the EPF *Metarhizium anisopliae* in controlling *L. botrana* at both laboratory and field conditions, as well as growth of *E. microtheca* in vitro. Therefore, the findings of this study serve as a basis for the use of EPF in pest and disease control providing new evidence for the inclusion of EPF as dual biocontrol agents in vineyards.

Methodology

Biological material

Entomopathogenic fungal strains

EPF were obtained from soils under vine crops in San Juan, Argentina (31° 65' 67" S; 68° 58' 51" W). Specimens were isolated from soil samples using the *Tenebrio molitor* larval baiting technique [36]. Three *M. anisopliae* strains (Metsc.) Sorok. (CEP413, CEP589 and CEP591) were selected for trials based on preliminary inhibitory tests against *L. botrana* [32]. The strains were first identified morphologically [37] and then genetically [38]. The *M. anisopliae* strains showed cylindrical conidia (between 5 and 9 µm of length), with an olive-green colouring, which is characteristic of the species. The isolated EPF were preserved in the Fungal Entomopathogens Collection of the 'Centro de Estudios Parasitológicos y de Vectores' (CEPAVE-CONICET, La Plata, Buenos Aires, Argentina).

Lobesia botrana specimens

The effects of *M. anisopliae* strains against the fifth instar larvae (L₅), pupae (P_p), and adults (A_d) stages of *L. botrana* were examined. Newly emerged larvae were obtained from a breeding colony in Mendoza, Argentina (33° 01' 52" S, 68° 46' 34" W). Fifth instar larvae of *L. botrana* were selected for trials based on previous studies reporting that damage by *L. botrana* increases with larval age [39]. The larvae were reared on an artificial diet [40], which was provided ad libitum during the complete growth period. Individuals from all stages were maintained in a growth chamber under a 16-h photoperiod, at a temperature of 25 ± 5 °C, and relative humidity ranging from 30 to 50%. This procedure has been reported to be effective in producing large numbers of larvae [41].

Phytopathogenic fungal strain

The phytopathogenic fungal strain was obtained from a vine culture (31° 76' 10" S, 68° 57' 94" W) located in San Juan Province, Argentina. Fourteen plants displaying clear symptoms of eutypiosis, including chlorotic and small leaves as well as short, rough internodes, stunted shoots, and dark brown wood with hard wedge-shaped consistency [42], were selected from the field.

The infected plants were uprooted, and infected tissues were excised and taken to the laboratory. We collected 2–5 subsamples from each symptomatic wood fragment. To remove external non-parasitic microorganisms, the surface of each subsample was sterilised as follows: 30 s in 70% ethanol, 2 min in 3.5% NaOCl, and 30 s in 70% ethanol [43]. The subsamples were then cultured in Petri dishes containing malt extract agar medium (MEA) and on plates containing potato glucose agar (PGA) (Britania®). Both MEA and PGA culture media were supplemented with 100 mg/mL of streptomycin sulphate, 50 mg/mL of chlortetracycline HCl, and 5 mg/mL of dichloran to prevent bacterial and yeast contamination [44]. Plates were immediately cultured in the dark in a growth chamber at 25 °C and inspected daily over a period of 4 weeks until fresh mycelia were observed. Subsequently, successive isolations were performed to derive pure cultures of the fungal strain.

The identification of the phytopathogenic fungus was based first on morphological observations, followed by molecular identification [45]. Genomic DNA was extracted from the mycelium growing on liquid Peptone Malt (MP) medium at 21 days using a DNeasy UltraClean Microbial Kit (Qiagen®, Germany), following the manufacturer's protocol. Then, the extracted DNA was purified, and the internal transcribed spacer (ITS) region of the complete rDNA (ITS1, ITS2 and 5.8S) was amplified by PCR. The PCR products were sent to Macrogen Inc® (Seoul, Korea) for purification and sequencing. The strain CC58 was identified as *E. microtheca* (SINAVIMO #9287) and preserved in the Centro de Investigación y Extensión Forestal Andino Patagónico (CIEFPAP) mycological collection, Chubut, Argentina.

EPF control on *L. botrana* in vitro

The evaluation of EPF efficacy in vitro and in vivo can be achieved using different application techniques, such as dipping insects in a spore suspension [46, 47], using topical micro-applications [48–51], or spraying EPF formulations directly to the insects or plants [52, 53]. In this study, EPF pathogenicity across different stages of *L. botrana* was tested using a micro-application technique, which ensures a higher level of precision, accuracy, and reliability of the pest susceptibility [54], and is useful to compare insect susceptibility to EPF among different developmental stages. A laboratory experiment was performed to examine the effects of three EPF strains on the mortality rate of *L. botrana* using 240 fifth instar larvae (L_5), 240 pupae (P_p), and 240 adults (A_d) of *L. botrana* using a topical micro-application technique [50]. EPF were applied to un-cocooned pupae [6, 55, 56]. The experiment comprised four groups: (1) insects treated with EPF strain CEP413, (2) insects treated with

EPF strain CEP589, (3) insects treated with EPF strain CEP591, and (4) control group (insects treated with an EPF-free solution). Treatment groups were topically treated [50, 54] with a 1 μ L drop (1×10^6 c/mL) of the respective strain using a Hamilton® micro dispenser (7000 Series Syringes®). The control group was treated with 1 μ L of distilled water. Mortality rates were measured using a subset of 20 individuals per replicate. After fungal application, the L_5 , P_p , and A_d of *L. botrana* were carefully transferred to sterile 90-mm Petri dishes lined with sterile filter paper (Whatman®). Immediately, dishes were incubated in growth chambers at 27 °C. Considering the high competition for food among larvae [57], an artificial diet [40] was offered ad libitum during the study period. Adults were carefully inoculated immediately after pupal eclosion and transferred to plastic trays (20 cm \times 10 cm \times 15 cm). The adults were nourished during the experimental period with a 5% ascorbic acid solution provided using a plastic syringe [41]. Since mortality over long time periods is not representative of field conditions, the mortality rates of L_5 , P_p , and A_d of *L. botrana* were measured up to the 7th day of the experiment. Dead European grapevine moths were carefully removed from the Petri dish to avoid horizontal transmission. To prevent adults from escaping from the trays, dead individuals were not removed until the end of the experiment. Abbott's equation [58] was used to obtain the corrected mortality (CM).

EPF control on *L. botrana* in the field

Efficacy of EPF on the control of the larval stage of *L. botrana* was tested in the field. Based on the corrected mortality (CM) observed in the laboratory experiments, we selected one *M. anisopliae* strain (CEP591) for field trials. The trial was repeated across different seasons over 1 year (September 2018–March 2019): (S_1) during spring, when plants displayed well developed inflorescences, (S_2) during early summer, when plants had undeveloped bunches, and (S_3) during late summer, which comprised the vine veraison stage. Trials were carried out in an experimental field ("Las Melizias") located in San Juan, Argentina (31° 58' 58" S; 68° 26' 28" W), which is planted with *V. vinifera* L. cv. Merlot since 2011. The field trial was performed within an approximate area of 4.63 ha. No chemical or biological insecticides were added to vines 45 days before trials or during trials. To avoid pupation during trials, we performed the field experiments with newly emerged L_3 – L_4 larvae of *L. botrana*. Trials consisted of two treatments (EPF+ and EPF–), with six replicates each treatment. Micro-application technique was used. For the EPF+ treatment, 20 larvae were topically treated with 1 μ L drop (1×10^6 c/mL) of CEP591. The control group

(EPF–) were treated with 1 μ L of sterile distilled water. After larvae were topically treated, they were immediately brought to the experimental field and carefully placed on selected grapevine bunches in groups of 20 treated individuals per bunch. The grapevine bunches were then immediately covered with a fabric mesh of $500 \times 500 \mu\text{m}$ of pore diameter. Fabric bands were then attached from the top of the mesh to the rachis of each bunch to enclose the experimental unit. This helped to maintain a closed system, preventing the entry or exit of insects into the experimental unit. Treatments were spatially separated by at least 20 m. After 7 days, bunches were carefully removed from plants and transferred to the laboratory in closed plastic containers to determine larvae mortality. Environmental temperature (T°) and relative humidity (RH) during the three seasons were obtained from an automatic weather station (Davis Instruments[®] – Mod. Vantage pro-2).

***Eutypella microtheca* growth assessment**

We examined the inhibitory rate of the three strains of EPF (CEP413, CEP589, and CEP591) on the growth of *E. microtheca* strain (CC58). CC58 was used in this study because it was the most abundant phytopathogenic strain affecting grapevine plants at the study site (see above for detailed geolocation information). CEP413, CEP589, and CEP591 were co-cultured with CC58 in separate Petri dishes containing potato glucose agar (PGA) media (Britania[®]). To achieve this, a 5-mm mycelial disc, obtained from the edges of a 10-days-old culture of CC58, was placed at the centre of a dish containing 20 mL of PGA. Immediately, four discs of one EPF strain (from 10-days-old cultures) of the same diameter as those of the CC58 strain were placed carefully at four sites at approximately 3 cm from the centre of the Petri dish [59]. The Petri dishes were inverted to prevent conidia from falling onto the agar medium and then incubated in the dark at $28 \pm 2^\circ\text{C}$. The growth of the *E. microtheca* colony was measured every 96 h on two perpendicular axes over the following 20 days under a stereomicroscope (Lancet Instruments[®], Model ZTX-30Y-C2, China) using a digital calliper. The control was determined by measuring the radial growth of *E. microtheca* strain growing in isolation (agar discs without EPF) on separate Petri dishes (indicative of the potential growth of the *E. microtheca* strain). The percentage of growth inhibition (GI) was calculated with the following formula: $\text{GI} (\%) = ((A - B)/A) \times 100$, where A represents the radial growth (mm) of the control treatment, and B represents the radial growth (mm) of the pathogen with the EPF setup [60]. Three replicate plates were prepared for each phytopathogenic/EPF strain combination and for the control treatment.

Statistical analyses

Effects of CEP413, CEP589, and CEP591 on mortality rate of larvae (L_5), pupae (P_p), and adults (A_d) of *L. botrana* in vitro were analysed using a one-way ANOVA (independent factor: EPF strains, response variable: corrected mortality (CM)). Efficacy exhibited by CEP591 over *L. botrana* in the field was analysed using a one-way ANOVA (independent factor: seasons, response variable: CM). Antagonistic effects of EPF on *E. microtheca* growth were also analysed using a one-way ANOVA (independent factor: EPF strains, response variable: pathogen inhibition growth (%)). A post hoc Fisher's LSD test was performed to analyse differences among treatments. All statistical analyses were performed using Infostat[®] statistical software [61].

Results

Biological control of EPF on *L. botrana* in vitro

All *M. anisopliae* strains tested in this study were able to infect *L. botrana* at any developmental stage, including larvae (L_5), pupae (P_p), and adults (A_d) (Fig. 1a–c, respectively). No significant differences were observed among EPF strain efficacy for L_5 ($F=0.22$, $p=0.8097$) and A_d ($F=0.12$, $p=0.8905$). Nevertheless, for P_p stage significant differences in EPF strain efficacy were observed ($F=33.25$, $p=0.0006$). CEP591 was observed to be more effective (99.9%) than CEP589 (81.6%) and CEP413 (79.98%) in controlling the pupal stage of *L. botrana* (Fig. 2). Overall, the mortality of the control group was lower than 15% in all cases.

Biological control of EPF on *L. botrana* in the field

At field conditions, larvae of *L. botrana* were susceptible to EPF infection across all seasons. Nevertheless, *L. botrana* mortality was significantly affected by the season ($F=6.92$, $p=0.0074$). Overall, larvae of *L. botrana* were more susceptible to CEP591 at early spring (91%) and early summer (81.5%) than in late summer (Table 1). In late summer, CM drastically decreased to 64.9%. Mortality of control larvae was relatively constant across seasons ($F=1.11$, $p=0.3551$), which rounded 5%.

Inhibitory effects of EPF on *E. microtheca*

The growth of *E. microtheca* was significantly affected by the EPF strains ($F=151.49$, $p<0.0001$). There was a significant decrease in the growth of *E. microtheca* treated with CEP413, CEP589, and CEP591 compared with that of the control group (Fig. 3). The inhibitory percentage of the strain CEP413 ($65.2\% \pm 12.24$) was significantly higher than those of CEP591 ($55.07\% \pm 10.11$) and

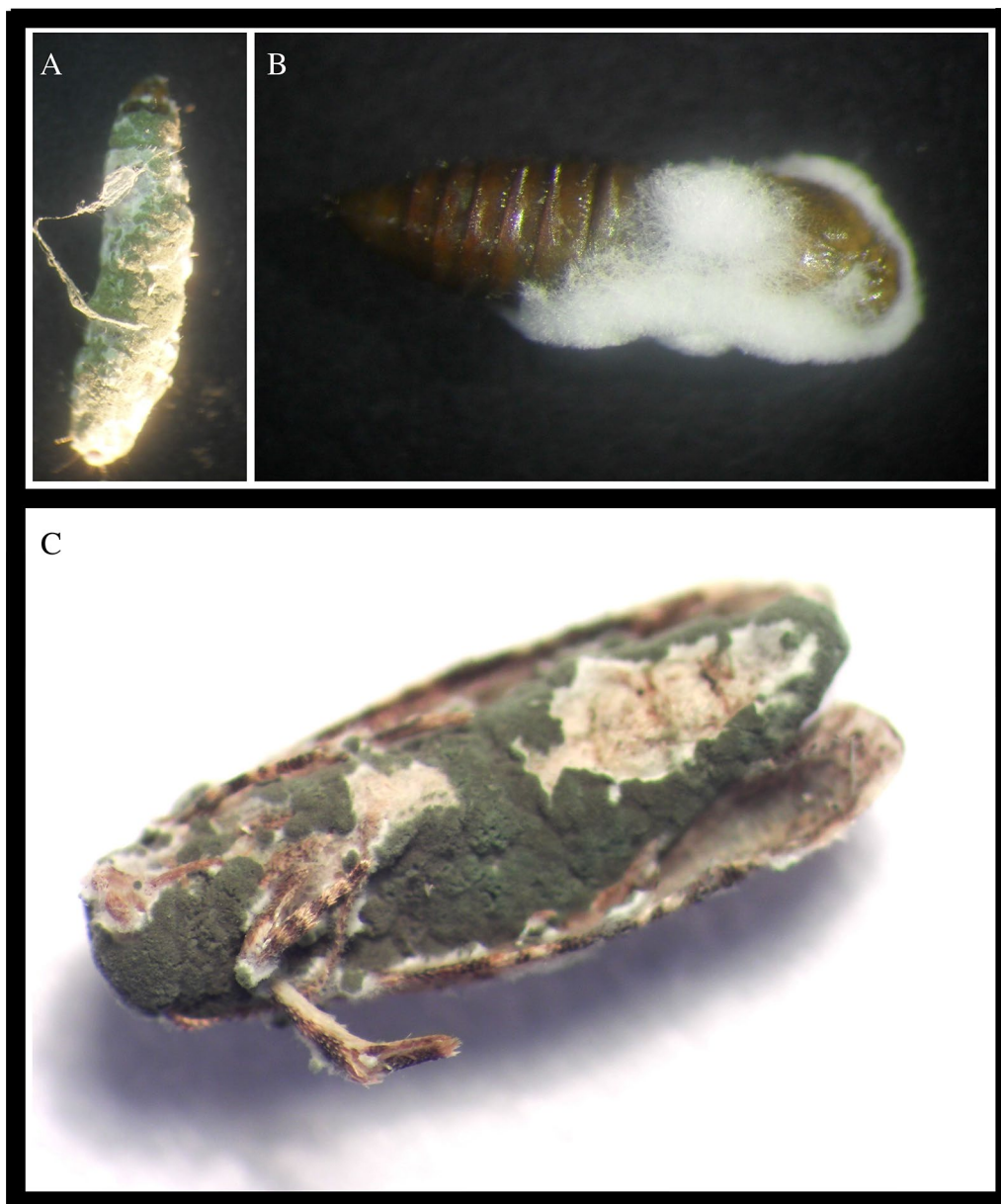


Fig. 1 Effects of the EPF *Metarhizium anisopliae* on *Lobesia botrana*. Effective infection of *Metarhizium anisopliae* strains can be observed for larvae (a), pupae (b), and adults (c)

CEP589 ($50.27\% \pm 9.12$) on *E. microtheca* by the end of the trial (after 480 h) ($F=7.8, p=0.0013$).

Discussion

Strains of *M. anisopliae* were highly effective to controlling two detrimental agents of *V. vinifera* L. in vitro and in vivo. EPF strains showed high mortality rates in all developmental stages of *L. botrana* and reduced the growth of the phytopathogenic fungus *E. microtheca*.

Overall, the three *M. anisopliae* strains were highly effective to controlling *L. botrana* at the L₅, P_p, and A_d stages. The average corrected mortality (CM) of larvae treated with the EPF strains was over 75%, while that of treated pupae and adults was over 85%. However, the CM of pupae treated with CEP591 was significantly higher than that with the other two strains, causing approximately 100% of mortality. This result is particularly important given that the pupal stage is highly resistant to fungal infection, at least in some

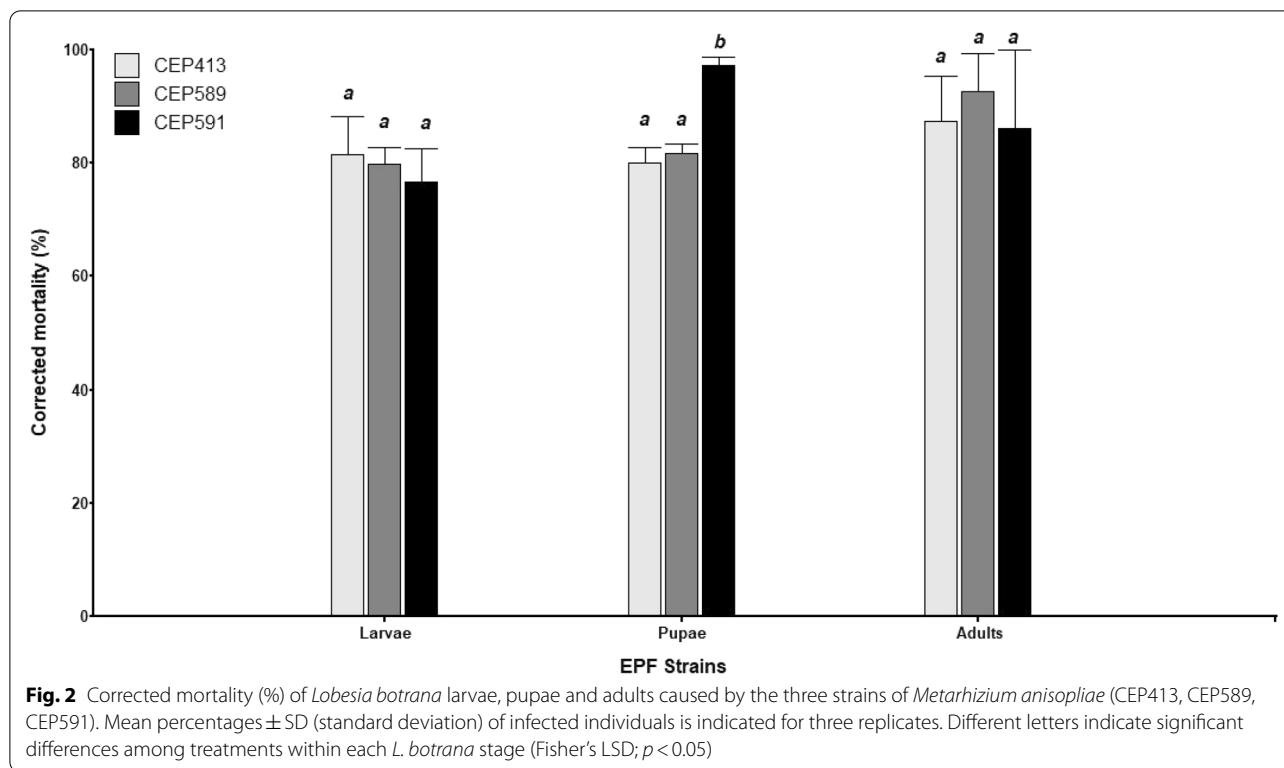


Table 1 Evaluation of EPF at field conditions

EPF strain	CM (%)—S ₁	CM (%)—S ₂	CM (%)—S ₃
CEP591	91 \pm 9.8 ^a	81.58 \pm 17.53 ^a	64.97 \pm 6.88 ^b
Temperature	22.5 \pm 1.2 °C	25.4 \pm 2.5 °C	21.7 \pm 2.1 °C
RH	52 \pm 12.2%	64 \pm 8.4%	61 \pm 3.9%

Corrected mortality (CM) \pm SD (standard deviation) for L₃–L₄ larvae of *Lobesia botrana* caused by *Metarhizium anisopliae* strain CEP591 at field conditions. All trials were performed in vivo (*Vitis vinifera* L. cv. Merlot) on an experimental field located in San Juan (Argentina) (31° 58' 58" S; 68° 26' 28" W). The trial was initiated at early spring (S₁) and repeated at early summer (S₂) and late summer (S₃), comprising a complete vine productive season. Letter's a, b indicates significant differences between seasons (Fisher's LSD; $p < 0.05$)

Lepidoptera [62], Coleoptera [63], and Blattodea [64] species. Consistent with our results, previous studies have also shown the effectiveness of EPF strains in controlling immature stages of *L. botrana*, including larvae and pupae [32]. Similarly, recent findings [65] showed that *Metarhizium* and *Beauveria*, which were isolated from soils under vineyards in Argentina, were effective against the fifth-instar larvae of *L. botrana* in vitro. Additionally, several studies have shown that *Metarhizium* is a successful EPF genus in the biological control of some lepidopteran pests in vitro, such as *Spodoptera litura*, *S. frugiperda*, *Tuta absoluta*, and *Plutella xylostella* [66–69], suggesting that strains of this genus have

the potential to be used as biopesticides against several lepidopteran species.

Previous studies on the effectiveness of EPF strains against *L. botrana* have been particularly focused on a single developmental stage of the insect's life cycle [55, 65, 70, 71]. In this study, we have shown the strong effectiveness of *M. anisopliae* strains against different developmental stages of *L. botrana*, including larvae, pupae, and adults in vitro. These results agree with other studies, which have demonstrated the role of EPF strains as an efficient method controlling all developmental stages of different insect pests such as *Megalurothrips sjostedti* [72], *S. litura* [68], *Dermanyssus gallinae* [73], and *Tetranychus urticae* [74]. Differences in EPF effectiveness between the larval and pupal stage of *L. botrana* may be associated with differences in the ecdysis time between both stages. Larvae, in contrast to pupae, may have shorter time intervals between successive ecdysis, which has been reported to be an important factor in insect resistance to fungal infection [75, 76].

In the field, the strain CEP591 was shown to be effective in killing *L. botrana* larvae across seasons. Nevertheless, efficacy of CEP591 in controlling *L. botrana* in the field was dependent on the season. In late summer, it was observed that CEP591 was less pathogenic to *L. botrana*, causing a mortality of around 64% compared to the other two seasons, in which larval mortality ranged

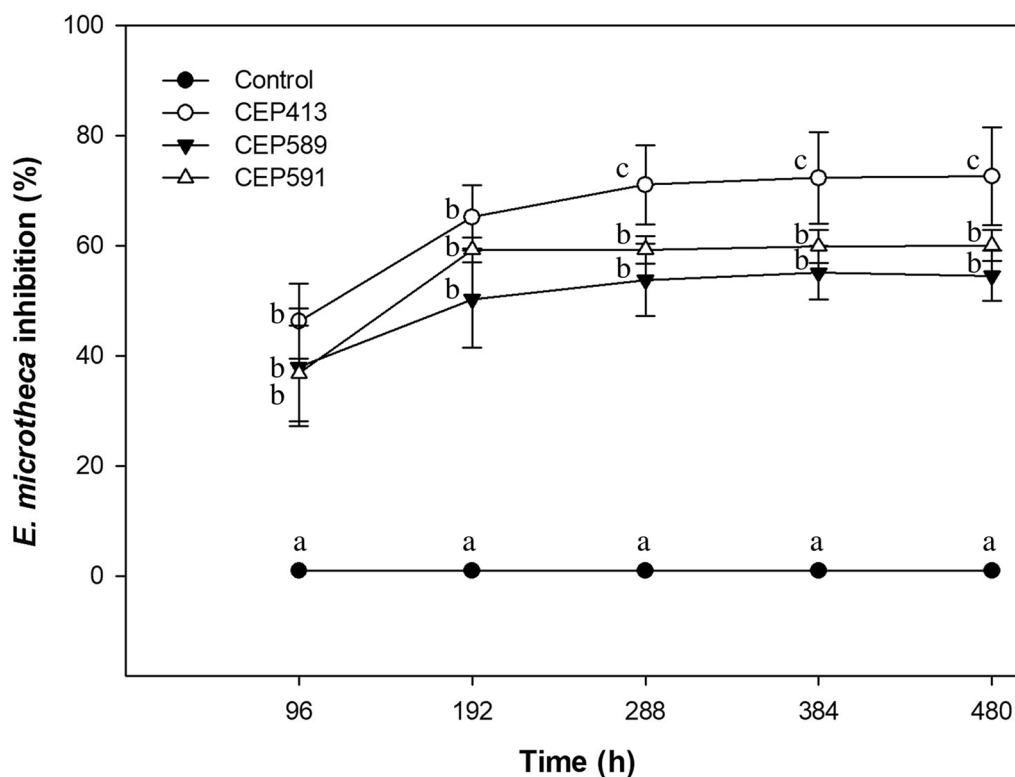


Fig. 3 Growth inhibition (%) of the phytopathogenic fungus *Eutypella microtheca* caused by the three strains of *Metarhizium anisopliae* (CEP413, CEP589, CEP591) over 20 days. Control consisted of agar plugs without fungal spores. Mean percentages \pm SD (standard deviation) is indicated for three replicates. Different letters indicate significant differences among treatments for each measured time (Fisher's LSD; $p < 0.05$)

from 81 to 91%. Our results agree with previous results, which have reported a marked decline in the biocontrol efficacy when insect's pests are challenged by EPF in the field compared to laboratory trials [77–79]. For example, Rodríguez et al. [77] observed that the *M. anisopliae* dose required to kill 50% of *Varroa destructor* (Acari) in vitro was considerably lower (3.8×10^5 c/mL) to that required under natural conditions (5×10^{10} c/mL). Similar observations were found by Altimira et al. [55] using the EPF *Beauveria pseudobassiana* to control *L. botrana* in vitro and in vivo. This efficacy variation of EPF in vitro versus in vivo may be associated with abiotic factors, including temperature, relative humidity, and UV radiation, which are known to influence some physiological insect processes such as conidia germination [55, 80, 81].

The results here showed that the three *M. anisopliae* strains were also effective inhibiting the *E. microtheca* growth when co-cultured during 20 days at 28 °C in the dark. The three tested strains significantly inhibited the growth of *E. microtheca* by over 50%. The strain CEP413 was, however, more effective than CEP589 and CEP591 in inhibiting the proliferation and growth of *E. microtheca* over time. Different EPF species, such as *Beauveria*

bassiana, *Metarhizium brunneum*, *M. anisopliae*, *Lecanicillium lecanii*, and *Isaria javanica* [33, 34, 82, 83] have been identified as suitable candidates for controlling several phytopathogenic fungi, including *Rhizoctonia solani*, *Pythium myriotylum* [84], *Sphaerotheca fuliginea* [85], and *Botrytis cinerea* [86]. However, to the best of our records this is the first study reporting an antagonistic effect of *M. anisopliae* over *E. microtheca*, showing that *M. anisopliae* strains are also appropriate candidates for use in biological control of one of the most important pathogens of *V. vinifera* L.

Conclusions

The present study revealed that three strains of *M. anisopliae* were highly effective infecting and killing different developmental stages of *L. botrana* in vitro and in vivo. Additionally, the same EPF fungal strains were able to inhibit the growth of the phytopathogen *E. microtheca* in vitro. Our results showed that *M. anisopliae* strains have the potential to be used in a program of integrated pest management aimed to control different detrimental agents of vines. Additional research, however, is

required to improve and sustain the efficacy of EPF under natural conditions, particularly under different environmental conditions.

Abbreviations

EPF: Entomopathogenic fungi; IPM: Integrated pest management; MEA: Malt extract agar; PGA: Potato glucose agar; MP: Liquid peptone malt; CM: Corrected mortality; L₅: Fifth instar larvae; P_p: Pupae; A_d: Adults.

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Authors' contributions

Conceptualisation: JAS, JC, FV; methodology and trials: JAS, JC, MR; Statistical analysis: JAS, BL, MD; writing and revision: JAS, MGT; resources: FV, BL, DP. All authors read and approved the final manuscript.

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Availability of data and materials

Figures, and data sheets are available by request to the corresponding author.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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References

- Čepo DV, Pelajić M, Vrček IV, Krivohlavek A, Žuntar I, Karoglan M. Differences in the levels of pesticides, metals, sulphites and ochratoxin A between organically and conventionally produced wines. *Food Chem.* 2018;246:394–403.
- Herrero-Hernández E, Andrades MS, Álvarez-Martín A, Pose-Juan E, Rodríguez-Cruz MS, Sánchez-Martín MJ. Occurrence of pesticides and some of their degradation products in waters in a Spanish wine region. *J Hydrol.* 2013;486:234–45.
- Hildebrandt A, Guillamón M, Lacorte S, Tauler R, Barceló D. Impact of pesticides used in agriculture and vineyards to surface and groundwater quality (North Spain). *Water Res.* 2008;42(13):3315–26.
- Marsala RZ, Capri E, Russo E, Bisagni M, Colla R, Lucini L, et al. First evaluation of pesticides occurrence in groundwater of Tidone Valley, an area with intensive viticulture. *Sci Total Environ.* 2020;736:139730.
- Paolinelli-Alfonso M, Serrano-Gomez C, Hernandez-Martinez R. Occurrence of *Eutypella microtheca* in grapevine cankers in Mexico. *Phytopathol Mediterr.* 2015;54(1):86–93.
- Vicente-Díez I, Blanco-Pérez R, Chelkha M, Puelles M, Pou A, Campos-Herrera R. Exploring the use of entomopathogenic nematodes and the natural products derived from their symbiotic bacteria to control the grapevine moth, *Lobesia botrana* (Lepidoptera: Tortricidae). *Insects.* 2021;12(11):1033.
- Rank A, Ramos RS, da Silva RS, Soares JRS, Picanço MC, Fidelis EG. Risk of the introduction of *Lobesia botrana* in suitable areas for *Vitis vinifera*. *J Pest Sci.* 2020;93:1167–79.
- Varela LG, Lucchi A, Bagnoli B, Nicolini G, Ioriatti C. Impacts of standard wine-making process on the survival of *Lobesia botrana* larvae (Lepidoptera: Tortricidae) in infested grape clusters. *J Econ Entomol.* 2013;106:2349–53.
- Gutiérrez AP, Ponti L, Gilioli G, Baumgärtner J. Climate warming effects on grape and grapevine moth (*Lobesia botrana*) in the Palearctic region. *Agric For Entomol.* 2018;20:255–71. <https://doi.org/10.1111/afe.12256>.
- Altimira F, Vitta N, Tapia E. Integrated pest management of *Lobesia botrana* with microorganism in vineyards: an alternative for clean grapes production. *IntechOpen.* 2021. <https://doi.org/10.5772/intechopen.99153>.
- Dagatti CV, Becerra VC. Ajuste de modelo fenológico para predecir el comportamiento de *Lobesia botrana* (Lepidoptera: Tortricidae) en un viñedo de Mendoza, Argentina. *Rev Soc Entomol Argent.* 2015;74(3–4):117–22.
- Satar G, Aslan MM, Ücük C. Two haplotypes of *Lobesia botrana* (Denis & Schiffermüller, 1775) predominate in two adjacent regions of southern Turkey. *Phytoparasitica.* 2020;48:149–58.
- Tasin M. Sex, wine and chemical communication in grapevine moth *Lobesia botrana*. PhD Dissertation, Swedish University of Agricultural Sciences, Acta Universitatis Agriculturae Sueciae, Alnarp, Sweden; 2005.
- Yacoub A, Gerbore J, Magnin N, Chambon P, Dufour MC, Corio-Costet MF, et al. Ability of *Pythium oligandrum* strains to protect *Vitis vinifera* L. by inducing plant resistance against *Phaeoemoniella chlamydospora*, a pathogen involved in Esca, a grapevine trunk disease. *Biol Control.* 2016;92:7–16.
- Mondello V, Songy A, Battiston E, Pinto C, Coppin C, Trotel-Aziz P, et al. Grapevine trunk diseases: a review of fifteen years of trials for their control with chemicals and biocontrol agents. *Plant Dis.* 2018;102(7):1189–217.
- Bertsch C, Ramírez-Suero M, Magnin-Robert M, Larignon P, Chong J, Abou-Mansour E, et al. Grapevine trunk diseases: complex and still poorly understood. *Plant Pathol.* 2013;62(2):243–65.
- Vassiliou VA. Control of *Lobesia botrana* (Lepidoptera: Tortricidae) in vineyards in Cyprus using the mating disruption technique. *Crop Prot.* 2009;28:145–50.
- Hofstetter V, Buyck B, Croll D, Viret O, Couloux A, Gindro K. What if esca disease of grapevine were not a fungal disease? *Fungal Divers.* 2012;54(1):51–67.
- Fontaine F, Gramaje D, Armengol J, Smart R, Nagy ZA, Borgo M, et al. Grapevine trunk diseases. A review. Paris: OIV Publications; 2016;24. ISBN: 979-10-91799-60-7. fhal-01604038f.
- Ioriatti C, Anfora G, Angeli G, Mazzoni V, Trona F. Effects of chlorantraniliprole on eggs and larvae of *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae). *Pest Manag Sci.* 2009;65(6):717–22.
- Lance DR, Leonard DS, Mastro VC, Walters ML. Mating disruption as a suppression tactic in programs targeting regulated lepidopteran pests in US. *J Chem Ecol.* 2016;42(7):590–605.
- Ioriatti C, Anfora G, Tasin M, De Cristofaro A, Witzgall P, Lucchi A. Chemical ecology and management of *Lobesia botrana* (Lepidoptera: Tortricidae). *J Econom Entomol.* 2011;104(4):1125–37.
- Louis F, Schmidt-Tiedemann A, Schirra KJ. Control of *Sparganothis piliferiana* Schiff. and *Lobesia botrana* Den. & Schiff. in German vineyards using sex pheromone-mediated mating disruption. *IOBC/WPRS Bull.* 2002;25:1–9.
- Lucchi A, Sambado P, Royo ABJ, Bagnoli B, Conte G, Benelli G. Disrupting mating of *Lobesia botrana* using sex pheromone aerosol devices. *Environ Sci Pollut Res.* 2018;25(22):22196–204.

25. Geiger F, Bengtsson J, Berendse F, Weisser WW, Emmerson M, Morales MB, et al. Persistent negative effects of pesticides on biodiversity and biological control potential on European farmland. *Basic Appl Ecol*. 2010;11(2):97–105.
26. Hallenbeck WH, Cunningham-Burns KM, editors. *Pesticides and human health*. Berlin: Springer-Verlag New York; 2012. p. 159.
27. Glare T, Caradus J, Gelernter W, Jackson T, Keyhani N, Köhl J, et al. Have biopesticides come of age? *Trends Biotechnol*. 2012;30(5):250–8.
28. Araújo JP, Hughes DP. Diversity of entomopathogenic fungi: which groups conquered the insect body? *Adv Genet*. 2016;94:1–39.
29. Cuthbertson AG, Audsley N. Further screening of entomopathogenic fungi and nematodes as control agents for *Drosophila suzukii*. *Insects*. 2016;7(2):24.
30. Khater HF. Ecosmart biorational insecticides: alternative insect control strategies. In: *Advances in integrated pest management*. Croatia: InTech; 2011. p. 17–60.
31. Yun HG, Kim DJ, Gwak WS, Shin TY, Woo SD. Entomopathogenic fungi as dual control agents against both the pest *Myzus persicae* and phytopathogen *Botrytis cinerea*. *Mycobiology*. 2017;45(3):192–8.
32. Aguilera Sammaritano J, Deymié M, Herrera M, Vazquez F, Cuthbertson A, López-Lastra C, et al. The entomopathogenic fungus, *Metarhizium anisopliae* for the European grapevine moth, *Lobesia botrana* Den. & Schiff. (Lepidoptera: Tortricidae) and its effect to the phytopathogenic fungus, *Botrytis cinerea*. *Egypt J Biol Pest Control*. 2018. <https://doi.org/10.1186/s41938-018-0086-4>.
33. Kang BR, Han JH, Kim JJ, Kim YC. Dual biocontrol potential of the entomopathogenic fungus, *Isaria javanica*, for both aphids and plant fungal pathogens. *Mycobiology*. 2018;46(4):440–7.
34. Barra-Bucarei L, France Iglesias A, Gerding González M, Silva Aguayo G, Carrasco-Fernández J, Castro JF, et al. Antifungal activity of *Beauveria bassiana* endophyte against *Botrytis cinerea* in two solanaceae crops. *Microorganisms*. 2020;8(1):65.
35. Ali S, Zhang C, Wang Z, Wang XM, Wu JH, Cuthbertson AG, et al. Toxicological and biochemical basis of synergism between the entomopathogenic fungus *Lecanicillium muscarium* and the insecticide matrine against *Bemisia tabaci* (Gennadius). *Sci Rep*. 2017;7(1):1–14.
36. Meyling NV. Methods for isolation of entomopathogenic fungi from the soil environment. Department of Ecology (Frederiksberg Denmark University of Copenhagen). 2007;1–18. <http://www.orgprints.org/11200>.
37. Bridge PD, Williams MAJ, Prior C, Paterson RRM. Morphological, biochemical and molecular characteristics of *Metarhizium anisopliae* and *M. flavoviride*. *Microbiology*. 1993;139(6):1163–9.
38. Aguilera Sammaritano JA, López Lastra CC, Leclercque A, Vazquez F, Toro ME, D'Alessandro C, et al. Control of *Bemisia tabaci* by entomopathogenic fungi isolated from arid soils in Argentina. *Biocontrol Sci Technol*. 2016;26(12):1668–82.
39. Delbac L, Thiery D. Damage to grape flowers and berries by *Lobesia botrana* larvae (Denis & Schiffenmüller) (Lepidoptera: Tortricidae), and relation to larval age. *Aust J Grape Wine Res*. 2016;22(2):256–61.
40. Ferreira A, Bastos M, Aguiar A. Criação de traça da uva *Lobesia botrana* Den. y Schiff. In: *Actas do VI Encontro Nacional de Protecção Integrada*. Castelo Branco; 2003. p. 83–88.
41. Herrera ME, Dagatti CV, Becerra VC. Método práctico de cría masiva de *Lobesia botrana* Den. & Schiff. (Lepidoptera: Tortricidae) en condiciones de laboratorio. *Rev Soc Entomol Argent*. 2016;75(3–4):160–4.
42. García-Jiménez J, Vicent A, Armengol J. Las enfermedades de madera en vid, un problema creciente. *Vida Rural*. 2002;146:32–6.
43. White PJ, Brown PH. Plant nutrition for sustainable development and global health. *Ann Bot*. 2010;105(7):1073–80.
44. Munkvold GP. *Eutypa* dieback of grapevine and apricot. *Plant Health Prog*. 2001;2(1):9.
45. Rosa-Manzano MB, Pildain MB, Pappano DB, Rajchenberg M. Aislamiento e identificación de *Eutypella microtheca* (9287) en vides de la provincia de San Juan con síntomas de hoja de malvón (#A2-024). In: *4º Congreso Argentino de Fitopatología*. Mendoza, Argentina; 2017. <http://aafitopatologos.com.ar/wp/wp-content/uploads/2017/06/Libro-de-res%C3%BAmenes-4%C2%B0-CAF.pdf?05a317>.
46. Lecuona RE, Turica M, Tarocco F, Crespo DC. Microbial control of *Musca domestica* (Diptera: Muscidae) with selected strains of *Beauveria bassiana*. *J Med Entomol*. 2005;42(3):332–6.
47. Gołębiowski M, Bojke A, Tkaczuk C. Effects of the entomopathogenic fungi *Metarhizium robertsii*, *Metarhizium flavoviride*, and *Isaria fumosorosea* on the lipid composition of *Galleria mellonella* larvae. *Mycologia*. 2021;113(3):525–35. <https://doi.org/10.1080/00275514.2021.1877520>.
48. Reinert JA, Knauf TA, Maranz SJ, Bishr M. Effect of *Beauveria bassiana* fungus on the boxelder and red shouldered bugs (Hemiptera: Rhopalidae). *Fla Entomol*. 1999;82(3):469–74. <https://doi.org/10.2307/3496873>.
49. Trissi AN, El Bouhssini M, Al Salti MN, Abdulhai M, Skinner M, Parker BL. Virulence of *Beauveria bassiana* against Sunn pest, *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae) at different time periods of application. *J Entomol Nematol*. 2012;4(5):49–53.
50. Klingen I, Westrum K, Meyling NV. Effect of Norwegian entomopathogenic fungal isolates against *Otiorynchus sulcatus* larvae at low temperatures and persistence in strawberry rhizospheres. *Biol Control*. 2015;81:1–7. <https://doi.org/10.1016/j.biocontrol.2014.10.006>.
51. Yu D, Xie R, Wang Y, Xie T, Xu L, Huang B. The G-protein coupled receptor GPRK contributes to fungal development and full virulence in *Metarhizium robertsii*. *J Invertebr Pathol*. 2021;83: 107627. <https://doi.org/10.1016/j.jip.2021.107627>.
52. Kim JC, Lee MR, Kim S, Park SE, Lee SJ, Shin TY, Kim WJ, Kim J. Transcriptome analysis of the Japanese pine sawyer beetle, *Monochamus alternatus*, infected with the entomopathogenic fungus *Metarhizium anisopliae* JEF-197. *J Fungi*. 2021;7:373. <https://doi.org/10.3390/jof7050373>.
53. Li J, Xie J, Zeng D, Xia Y, Peng G. Effective control of *Frankliniella occidentalis* by *Metarhizium anisopliae* CQMa421 under field conditions. *J Pest Sci*. 2020;94(1):111–7. <https://doi.org/10.1007/s10340-020-01223-9>.
54. Hicks B. Optimization of *Beauveria bassiana* in a spray formulation against *Choristoneura fumiferana*. *Can J For Res*. 2016;46(4):543–7. <https://doi.org/10.1139/cjfr-2015-0435>.
55. Altamira F, De La Barra N, Rebufel P, Soto S, Soto R, Estay P, et al. Potential biological control of the pupal stage of the European grapevine moth *Lobesia botrana* by the entomopathogenic fungus *Beauveria pseudobassiana* in the winter season in Chile. *BMC Res Notes*. 2019;12(1):1–6.
56. Güven Ö, Aydın T, Karaca I, Butt T. Biopesticides offer an environmentally friendly solution for control of pine processionary moth (*Thaumetopoea wilkinsoni* Tams) larvae and pupae in urban areas. *Biocontrol Sci Technol*. 2020;31(1):35–52. <https://doi.org/10.1080/09583157.2020.1826905>.
57. Thiéry D, Monceau K, Moreau J. Larval intraspecific competition for food in the European grapevine moth *Lobesia botrana*. *Bull Entomol Res*. 2014;104(4):517–24.
58. Abbott WS. A method of computing the effectiveness of an insecticide. *J Econ Entomol*. 1925;18(2):265–7.
59. Nally MC, Pesce VM, Maturano YP, Muñoz CJ, Combina M, Toro ME, et al. Biocontrol of *Botrytis cinerea* in table grapes by non-pathogenic indigenous *Saccharomyces cerevisiae* yeasts isolated from viticultural environments in Argentina. *Postharvest Biol Technol*. 2012;64(1):40–8.
60. Jiang C, Shi J, Liu Y, Zhu C. Inhibition of *Aspergillus carbonarius* and fungal contamination in table grapes using *Bacillus subtilis*. *Food Control*. 2014;35(1):41–8.
61. Di Rienzo JA, Casanoves F, Balzarini MG, Gonzalez L, Tablada M, Robledo CW. InfoStat versión 2020. Centro de Transferencia InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. 2020. <http://www.infostat.com.ar>.
62. Ullah MI, Altaf N, Afzal M, Arshad M, Mehmood N, Riaz M, et al. Effects of entomopathogenic fungi on the biology of *Spodoptera litura* (Lepidoptera: Noctuidae) and its reduviid predator, *Rhynocoris marginatus* (Heteroptera: Reduviidae). *Int J Insect Sci*. 2019;11:1179543319867116.
63. Schapovaloff ME, Alves LFA, Fantí AL, Alzogaray RA, López Lastra CC, Ode P. Susceptibility of adults of the cerambycid beetle *Hedypathes betulinus* to the entomopathogenic fungi *Beauveria bassiana*, *Metarhizium anisopliae*, and *Purpureocillium lilacinum*. *J Insect Sci*. 2014. <https://doi.org/10.1093/jis/14.1.127>.
64. Gutierrez AC, Gołębiowski M, Pennisi M, Peterson G, García JJ, Manfrino RG, et al. Cuticle fatty acid composition and differential susceptibility of three species of cockroaches to the entomopathogenic fungi *Metarhizium anisopliae* (Ascomycota, Hypocreales). *J Econ Entomol*. 2015;108(2):752–60.
65. López-Plantey R, Papura D, Couture C, Thiéry D, Pizzuolo PH, Bertoldi MV, et al. Characterization of entomopathogenic fungi from vineyards in Argentina with potential as biological control agents against the European grapevine moth *Lobesia botrana*. *BioControl*. 2019;64(5):501–11.

66. Contreras J, Mendoza JE, Martínez-Aguirre MR, García-Vidal L, Izquierdo J, Bielza P. Efficacy of entomopathogenic fungus *Metarhizium anisopliae* against *Tuta absoluta* (Lepidoptera: Gelechiidae). *J Econ Entomol.* 2014;107(1):121–4.
67. García GC, González MMB, Bautista MN. Patogenicidad de aislamientos de hongos entomopatógenos contra *Spodoptera frugiperda* (Lepidoptera: Noctuidae) y *Epilachna varivestis* (Coleoptera: Coccinellidae). *Rev Colomb Entomol.* 2011;37(2):217.
68. Sowjanya-Sree K, Padmaja V, Murthy YL. Insecticidal activity of destruxin, a mycotoxin from *Metarhizium anisopliae* (Hypocreales), against *Spodoptera litura* (Lepidoptera: Noctuidae) larval stages. *Pest Manag Sci.* 2008;64(2):119–25.
69. Zafar J, Shoukat RF, Zhang Y, Freed S, Xu X, Jin F. *Metarhizium anisopliae* challenges immunity and demography of *Plutella xylostella*. *Insects.* 2020;11(10):694.
70. Cozzi G, Somma S, Haidukowski M, Logrieco AF. Ochratoxin A management in vineyards by *Lobesia botrana* biocontrol. *Toxins.* 2013;5(1):49–59.
71. Gholizad LM, Inan C, Nalçacıoğlu R, Hamzezhadeh A, Demirbağ Z. Bacterial flora of *Lobesia botrana* ([Denis & Schiffermüller]) (Lepidoptera: Tortricidae) as a possible microbial control agent. *Acta Zool Bulg.* 2017;69(4):583–91.
72. Ekési S, Maniania NK. 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.* 2000;94(3):229–36.
73. Tavassoli M, Ownag A, Pourseyed SH, Mardani K. Laboratory evaluation of three strains of the entomopathogenic fungus *Metarhizium anisopliae* for controlling *Dermanyssus gallinae*. *Avian Pathol.* 2008;37(3):259–63.
74. Bugeme DM, Knapp M, Boga HI, Ekési S, Maniania NK. Susceptibility of developmental stages of *Tetranychus urticae* (Acari: Tetranychidae) to infection by *Beauveria bassiana* and *Metarhizium anisopliae* (Hypocreales: Clavicipitaceae). *Int J Trop Insect Sci.* 2014;34(3):190–6.
75. Vey A, Fargues J. Histological and ultrastructural studies of *Beauveria bassiana* infection in *Leptinotarsa decemlineata* larvae during ecdysis. *J Invertebr Pathol.* 1977;30(2):207–15. [https://doi.org/10.1016/0022-2011\(77\)90221-x](https://doi.org/10.1016/0022-2011(77)90221-x).
76. Vestergaard S, Gillespie AT, Butt TM, Schreiter G, Eilenberg J. Pathogenicity of the hyphomycete fungi *Verticillium lecanii* and *Metarhizium anisopliae* to the western flower thrips, *Frankliniella occidentalis*. *Biocontrol Sci Technol.* 1995;5(2):185–92.
77. Rodríguez M, Gerding M, France A, Ceballos R. *Metarhizium anisopliae* var. *anisopliae* Qu-M845 isolate to control *Varroa destructor* (Acari: Varroidae) in laboratory and field trials. *Chil J Agric Res.* 2009;69(4):541–7. <https://doi.org/10.4067/s0718-58392009000400009>.
78. Lobo LS, Rodrigues J, Luz C. Effectiveness of *Metarhizium anisopliae* formulations against dengue vectors under laboratory and field conditions. *Biocontrol Sci Technol.* 2016;26(3):386–401. <https://doi.org/10.1080/09583157.2015.1123220>.
79. Keyser CA, Fernandes ÉKK, Rangel DEN, Foster RN, Jech LE, Reuter KC, et al. Laboratory bioassays and field-cage trials of *Metarhizium* spp. isolates with field-collected Mormon crickets (*Anabrus simplex*). *BioControl.* 2017;62(2):257–68. <https://doi.org/10.1007/s10526-016-9782-8>.
80. Singh H, Joshi N. Management of the aphid, *Myzus persicae* (Sulzer) and the whitefly, *Bemisia tabaci* (Gennadius), using biorational on capsicum under protected cultivation in India. *Egypt J Biol Pest Control.* 2020;30(67):1–9. <https://doi.org/10.1186/s41938-020-00266-5>.
81. Brancini GT, Bachmann L, Braga GÜL. Timing and duration of light exposure during conidia development determine tolerance to ultraviolet radiation. *FEMS Microbiol Lett.* 2021. <https://doi.org/10.1093/femsle/fnab133>.
82. Nguyen HQ, Quyen DT, Nguyen SLT, Van Hanh VU. An extracellular antifungal chitinase from *Lecanicillium lecanii*: purification, properties, and application in biocontrol against plant pathogenic fungi. *Turk J Biol.* 2015;39(1):6–14.
83. Lozano-Tovar MD, Garrido-Jurado I, Quesada-Moraga E, Raya-Ortega MC, Trapero-Casas A. *Metarhizium brunneum* and *Beauveria bassiana* release secondary metabolites with antagonistic activity against *Verticillium dahliae* and *Phytophthora megasperma* olive pathogens. *Crop Prot.* 2017;100:186–95.
84. Ownley BH, Griffin MR, Klingeman WE, Gwinn KD, Moulton JK, Pereira RM. *Beauveria bassiana*: endophytic colonization and plant disease control. *J Invertebr Pathol.* 2008;98(3):267–70.
85. Goettel MS, Koike M, Kim JJ, Aiuchi D, Shinya R, Brodeur J. Potential of *Lecanicillium* spp. for management of insects, nematodes and plant diseases. *J Invertebr Pathol.* 2008;98(3):256–61.
86. Shin TY, Bae SM, Woo SD. Screening and characterization of antimicrobial substances originated from entomopathogenic fungi. *J Asia-Pac Entomol.* 2016;19(4):1053–9.

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