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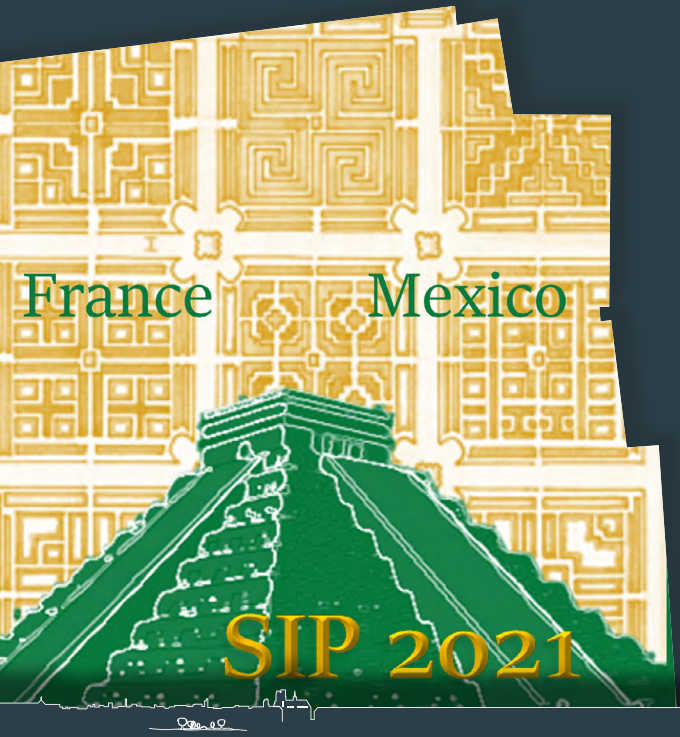
VIRTUAL MEETING | 2021

28th June - 2nd July 2021

2021 International Congress on Invertebrate Pathology and Microbial Control & 53rd Annual Meeting of the Society for Invertebrate Pathology



LOCATION

VIRTUAL MEETING

CONVENORS

Dr Cristina Del Rincon Castro

LE STUDIUM / MARIE SKŁODOWSKA-CURIE RESEARCH FELLOW

FROM University of Guanajuato - Mexico

IN RESIDENCE AT Insect Biology Research Institute (IRBI), University of Tours / CNRS - FR

Dr Elisabeth Herniou

Insect Biology Research Institute (IRBI), University of Tours / CNRS - FR

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ABSTRACTS

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Maurine Villiers, Communication & Events Manager

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MICROBIAL CONTROL WITH FUNGI
POSTERS

The ingestion of Metarhizium-colonized plants produces direct and indirect effects on the cotton leafworm Spodoptera littoralis

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The present work reveals the occurrence of intermediate *S. littoralis* larval mortality levels related to the foliar application of the entomopathogenic endophytic fungus *Metarhizium brunneum*. The mortality rates of larvae fed on *Metarhizium*-colonized melon leaves were 45.0% and 87.5%, and the average survival times were 6.6 and 3.1 days in experiments performed with melon discs and with the whole plant, respectively. Notably, these mortality levels were not associated with fungal outgrowth and were not caspase 1, 3-7 and 8 mediated. This work also shows the existence of significant sublethal effects of food consumption by *Spodoptera littoralis* larvae challenged by the fungal endophyte when feeding on colonized leaves. In this regard, in experiments performed in planta, plant damage increased larval mortality in both fungally challenged and control larvae. There was also a meaningful effect of exposure to *Metarhizium*-colonized plants in female fecundity and egg fertility of the adults emerging from pupae developing from surviving larvae. Experiments performed with foliar discs showed a non-realistic situation due to the prior damage caused to the plant. Hence, the present work presents new findings revealing the high potential of endophytic entomopathogenic fungi to improve the outcome of foliar applications against chewing insects in the short, mid- and long term, by the reduction of the reproductive potential of surviving adults.

Tick cuticle lipids may limit infection by entomopathogenic fungi

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Chemical composition of tick cuticle acts as a barrier to pathogens. Here, we tested the toxicity of total cuticle extracts from four ixodid tick species predominant in Brazil to conidia of *Metarhizium robertsii* IP 146 and *Beauveria bassiana* s.l. IP 361. Cuticular lipids were extracted using hexane, and fungal conidia were scraped from culture plates and suspended in tick cuticle extracts and incubated overnight at room temperature. In the control groups, conidia of IP 146 or IP 361 were also suspended in hexane for the same period. After exposure to the cuticle extracts or hexane (control), the conidia were re-suspended in PBS solution and propidium iodide. The viability of conidia was examined in a FACSCanto II flow cytometer by the acquisition of 10,000 events, and the data were analysed using the FACSDiva software. The extracts from Amblyomma sculptum caused apoptosis to 81% of *M. robertsii* and 36% of *B. bassiana* treated conidia, whereas extracts from Dermacentor nitens caused apoptosis of 64% and 66% to M. robertsii and B. bassiana, respectively. Cuticular extracts from *Rhipicephalus microplus* or *Rhipicephalus sanguineus* caused low (<8%) conidial apoptosis on both isolates, IP146 or IP361, which did not differ from the control group. This study indicates a natural tolerance of *A. sculptum* and *D. nitens* to fungal infection in comparison to *R. microplus* and *R. sanguineus*, although the mechanisms in which they are used for host defence remains uncertain.

Conidial production from granules of Metarhizium humberi microsclerotia on soil samples

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Metarhizium humberi produces structures named microsclerotia which are promising to control soil-dwelling arthropod pests. This study compared the efficacy of microsclerotia of *M. humberi* IP46, formulated as granules or pellets, to produce conidia on soil samples. Microsclerotia were produced by liquid fermentation in a medium with C:N 30:1 ratio, at 250 rpm, for 4 days. The granules were prepared using a 1:1 biomass:excipient ratio and water as liquid binder. Granulation was performed using a 0.5 mm sieve. Pellets were prepared by extrusion-spheronization at the same biomass:excipient ratio. Granules and pellets size distribution was determined by sieving. Soil samples were collected from open grounds or vegetated regions, autoclaved, and distributed in Petri dishes. Granules or pellets were distributed over the soil samples and incubated for 10 days. Then, the soil in each dish was suspended in 0.01% Tween 80 and an aliquot inoculated onto CTC selective medium for entomopathogenic fungi; the colonies and the conidia produced were counted on the fourth day of cultivation. Granules size distribution ranged from 0.1 to 0.7 mm, whereas the pellets size ranged between 0.4 and 0.8 mm. The conidial production from pellets was significantly lower compared to the production from granules, which was approximately $3x10^8$ conidia g^{-1}, with viability higher than 90%. Conidial yield from granules or pellets applied to the soil collected from vegetated regions was higher than the production from those applied to the open ground soils. Formulated microsclerotia have biotechnological potential for controlling arthropod pests that inhabit the soil.

Influence of abiotic factors on the persistence and viability of microsclerotia produced by the entomopathogenic fungus Metarhizium spp. (Hypocreales: Clavicipitaceae)

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Some species of the entomopathogenic fungus *Metarhizium* may produce microsclerotia (MS) as resistance spores to counteract adverse nutritional or environmental conditions. These propagules can be used as active material of a commercial biopesticide for the control of soil-dwelling stages of some important geophilic insect pests. In this study, the MS production and quality of *Metarhizium brunneum* EAMb 09/01-Su and EAMa 01/58-Su and *M. robertsii* EAMa 01/158-Su strain have been evaluated. The three strains were able to produce MS in liquid fermentations with values of produced conidia per MS ranging between 2×10^{10} and 4.73×10^{10} conidia per gram of MS. Soil texture had a significant effect on EAMa 01/58-Su MS germination and subsequent production of conidia being higher in sandy soils than in clayey soils. The best combination of temperature and soil humidity for the germination of the EAMa 01/58-Su MS was 22.7°C and 7.28%, respectively. Furthermore, the exposure of these MS to UV-B during 4, 8, 24 and 48 hours did not significantly affect their capacity to germinate and produce conidia, demonstrating their photo-resistance. Finally, storage temperature was evaluated over a year, the MS showed longer shelf life when stored at lower temperature, being the -80 oC the one that provided the highest viability of the MS with 2×10^9 conidia per gram, after 8 months of storage, while at 25°C its viability decreased to 1×10^7 conidia per gram in first four-months of storage.

Digging into the past: *Metarhizium brunneum* as control agent against the sugar beet weevil *(Asproparthenis punctiventris)*

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The sugar-beet weevil (*Asproparthenis punctiventris*, Germ.) is one of the main pests in sugar beet cultivation and causes major damage in Austria. Although biological control of the pest with the entomopathogenic fungus *Metarhizium* spp. was already mentioned and tested in the late 19th century, no further studies have been conducted since then. Therefore, a three-year study in Lower Austria has been initiated to test the effectiveness of *M. brunneum* in preventing larval development and the control of the beetles by using two types of *Metarhizium* formulations (i.e. GranMet GR and GranMet WP). GranMet-GR (active ingredient *M. brunneum* BIPESCO 5) was applied in eleven arable fields with a concentration of 100 kg ha^{-1}. Fields were sampled in spring and autumn to assess Metarhizium abundance and control efficacy. The desired fungal density of 5,000 *M. brunneum* CFU g^{-1} soil (dry weight) and more was reached and even exceeded after the first application of the GranMet-GR in the soil, on its own or in combination with the liquid formulation (1×10^{12} spores ha^{-1}). At all the sites, a strikingly high abundance of indigenous *Metarhizium* was found. Genotyping confirmed successful establishment of the applied strain BIPESCO 5 in the treated fields. The fungus was able to persist on treated sugar beet leaves for more than three weeks. In addition, more than 50% of the collected beetles were infected with the applied strain after spray application. Additional trials will be performed in 2021 to confirm the applicability of the *Metarhizium* based sugar beet weevil control.

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