Peer Community In Zoology

Optimizing fungal pathogen strategies for oriental fruit fly control

Kévin Tougeron based on peer reviews by *François Verheggen* and *Papa Djibril Faye*

Anais Chailleux, Oumou N. Coulibaly, Babacar Diouf, Samba Diop, Ahmad Sohel, Thierry Brevault (2024) Dose, temperature and formulation shape Metarhizium anisopliae virulence against the oriental fruit fly: lessons for improving on-target control strategies. bioRxiv, ver. 2, peer-reviewed and recommended by Peer Community in Zoology.
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Using entomopathogenic fungi for biological control is an effective method for controlling certain crop pests, with the perspective of reducing the use of chemical pesticides. Yet, the efficiency of pathogenic fungi is dependent upon many factors that need to be evaluated to improve biological control potential in the fields (Lacey, 2001). The article by Chailleux et al. (2024) presents an exciting contribution to the field of biological pest control, specifically focusing on using entomopathogenic fungi to manage the oriental fruit fly, *Bactrocera dorsalis*. This fly, a member of the Tephritidae family, is a major threat to orchards in Asia, the Pacific and Africa, as it attacks fruit and causes considerable damage, in addition to having a relatively rapid biological invasion dynamic (Clarke et al. 2005).

The objective of the Chailleux et al. (2024) study was to evaluate the virulence of *Metarhizium anisopliae* spores (strain Met69) on *B. dorsalis* adult flies according to various conditions: the inoculation dose and spore load, the formulation (adjuvant) and temperature conditions. The focus on host specificity and on-target applications was conducted to ensure minimal impact on non-target organisms, which is crucial for sustainable agriculture. The main challenge in this system was to achieve high strain virulence to kill wild individuals with a low number of spores—therefore limiting impact on non-target species such as natural enemies—but with a sufficient incubation period to allow transmission from mass-reared insects to wild conspecifics (Leite et al. 2022). A comparison of different inoculation methods is also provided and is interesting from a methodological point of view for future studies or even large-scale applications.

Using a well-designed experimental setup, the authors show that high pathogenicity (measured by LD50) is achievable even at low spore doses and independently of the fly's sex. Lethal action speed was, however,

dependent on the dose. Regarding temperature, the authors demonstrated that mycelium growth was affected by the mean temperature but, most importantly, by daily fluctuation regimes; night and day temperature alternation allowed faster growth than constant temperature. These notions of thermal fluctuations are still under-researched in terms of their modulating role in biological control yet seem central to understanding them, as the authors demonstrate here. The correlation between increased virulence and specific abiotic factors, such as temperature, offers valuable additional insights into the bioecology of the insect host and the fungal pathogen. Chailleux et al. finally point out the need for careful selection of adjuvants in formulations and pay attention to interactions with the abiotic environment to avoid compromising the effectiveness of biological control agents. Indeed, the survival rate of inoculated flies increased in the presence of the corn starch adjuvant, but this effect decreased with temperature. As corn starch unexpectedly delayed mortality, the authors suggest a potential for enhancing conspecific transmission

From a broader perspective, the study emphasizes the importance of standardizing virulence evaluation to optimize biological control strategies like auto-dissemination or vectoring with sterile males, particularly in field conditions. The study contributions are timely and essential for advancing sustainable pest management strategies and improving inoculation methods. The findings underscore the need for field trials to refine these strategies, particularly in Africa, where climatic factors may affect pathogen efficacy and fly behavior. I recommend publishing this article in a referenced journal like the Peer Community Journal.

References:

Chailleux, A. Coulibaly, ON, Diouf B, Diop S, Sohel A, Brevault T (2023) Dose, temperature and formulation shape Metarhizium anisopliae virulence against the oriental fruit fly: lessons for improving on-target control strategies. bioRxiv, ver.2 peer-reviewed and recommended by PCI Zoology https://doi.org/10.1101/2023.12.14.571642

Clarke, A. R. et al. (2005). Invasive phytophagous pests arising through a recent tropical evolutionary radiation: the Bactrocera dorsalis complex of fruit flies. Annu. Rev. Entomol., **50**, 293-319. https://doi.org/10.1146/annurev.ento.50.071803.130428

Lacey, L. A. (2001). Formulation of microbial biopesticides: beneficial microorganisms, nematodes and seed treatments. J Invertebr Pathol, **77**, 147. https://doi.org/10.1006/jipa.2000.5005

Leite, M. O. et al. (2022). Laboratory risk assessment of three entomopathogenic fungi used for pest control toward social bee pollinators. Microorganisms, **10**, 1800. https://doi.org/10.3390/microorganisms10091800

Reviews

Evaluation round #1

DOI or URL of the preprint: https://doi.org/10.1101/2023.12.14.571642
Version of the preprint: 1

Authors' reply, 09 August 2024

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Decision by Kévin Tougeron, posted 06 May 2024, validated 06 May 2024

Revisions needed

As the authors will see from the reviews by two experts in the field, the manuscript has many merits and could be recommended by PCI Zoology, provided several clarifications are made.
I would draw the authors' attention to the need to place the results of this study in a more general context, to see what could be extrapolated from the analyses, for example on other systems.

Reviewed by François Verheggen, 21 February 2024

Metarhizium anisopliae is known for its potential as a biological control agent of the oriental fruit fly, Bactrocera dorsalis. Chailleux and colleagues have performed an interresting study demonstrating the importance of the inoculation dose, the presence of adjuvant in the formulation and the air temperature on Metarhizium virulence. Given the importance of the fruit fly species and the need for identifying efficient control strategies, I believe this manusript is of prime importance.
Moreover, the study has been properly designed and executed. Below a just minor comments, that should be taken into account to increase clarity. Authors should pay special attention to th eincomplete methodology section regarding the role of the adjuvant.

Material and Method:

"As this design provided high mortality even for the smallest dose, we modified our inoculation method to be able to reduce the spore load on flies. To this end, we used a paint brush with a small number of hairs (8, 4, 2, and 1 hair)"

=> It is not clear to me what has been modified in the inoculation method, and what is the role of the paint brush.

"Among the 30 flies of each type"

=> Do you mean of each sex?

" The same inoculation procedure of flies as described above was adopted but only with the dose of 40 084 926 spores.cm^{-2}. "

=> I believe you should justify this choice.

"The quantity of spores in the inoculation tube was kept constant. Tests were led"

=> The methodology section on the evaluation of the role of the adjuvant on fly mortality if incomplete.

Results:

"The dose threshold to obtain fast and high mortality was at the slope change, between 40 084 926 and 8 016 985 spores.cm−2, where the LT 50 jumped from 3.07 to 3.63 days"

=> Is that really a significant jump ?

" The LT 50 kept small, 5.64 days, with 2 004 246 spores.cm−2 un but reach 22.9 days with …"

=> what does "un" means? "reach" should either read "reaches" or "reached"

"1,69 e+3"

=> 1.69 e+3

"92.2% ± 0.9 and 99.5% ± 0.2 after 48 h."

=> 92.2 ± 0.9 **%** and 99.5 ± 0.2 % after 48 h.

"Surprisingly, the lowest growth was observed at the intermediate May temperature (20.7-24.3°C)."

=> "Surprisingly" calls for a discussion. Delete it from here and make sure to discuss it in the discussion section.

"Survival of flies was negatively affected by spore inoculation"

=> Do you mean method of inoculation or inoculation load?

"Sex was the only factor having only main effect "

=> Clarify what you mean

”but this effect might be interpreted cautiously because it was unbalanced between fertile and sterile population.”

=> That sounds like a discussion element

”but significant interaction between adjuvant and month temperature (table 3) reveled”

=> revealed

Reviewed by **Papa Djibril Faye**, 02 May 2024

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