Metarhizium anisopliae pathogenesis of mosquito larvae: a verdict of accidental death.

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Introduction

Mosquitoes vector a wide range of diseases (e.g. dengue, yellow fever and malaria) which have devastating impacts on human health. Over half the world's population is at risk of mosquito-transmitted diseases[1].

Recent studies show that *Metarhizium anisopliae,* a soil borne fungal pathogen of terrestrial insects, offers an environmentally friendly alternative to chemicals for the control of mosquitoes yet the mechanism of how this terrestrial pathogen kills the aquatic larval stage is unclear.

We demonstrate for the first time that *M. anisopliae* kills mosquito larvae *via* a mechanism that does not follow the traditional host-pathogen response, as the species have not evolved to interact.

Methods

A number of methodologies were utilised to determine the mode of pathogenesis. Including:

1. Spectrophotometry to assess Caspase activity,
2. Fluorescent microscopy to asses conidia viability and the damage cause by the fungus to the mosquito,
3. qPCR to determine transcript levels of *Ae. aegypti* response genes and *M. anisopliae* pathogenicity genes.

Results

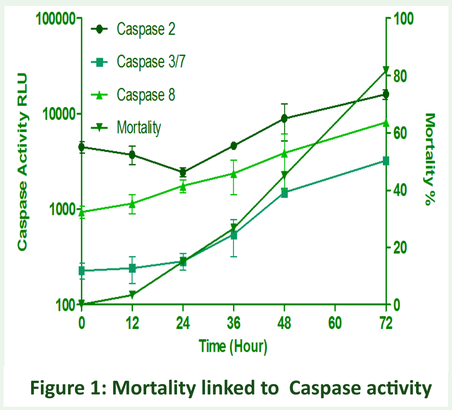

Figure 1: Mortality linked to Caspase activity

Caspase activity of *Metarhizium* treated larvae was concomitant with larval mortality between 36—72 hr. post infection (pi).

Results

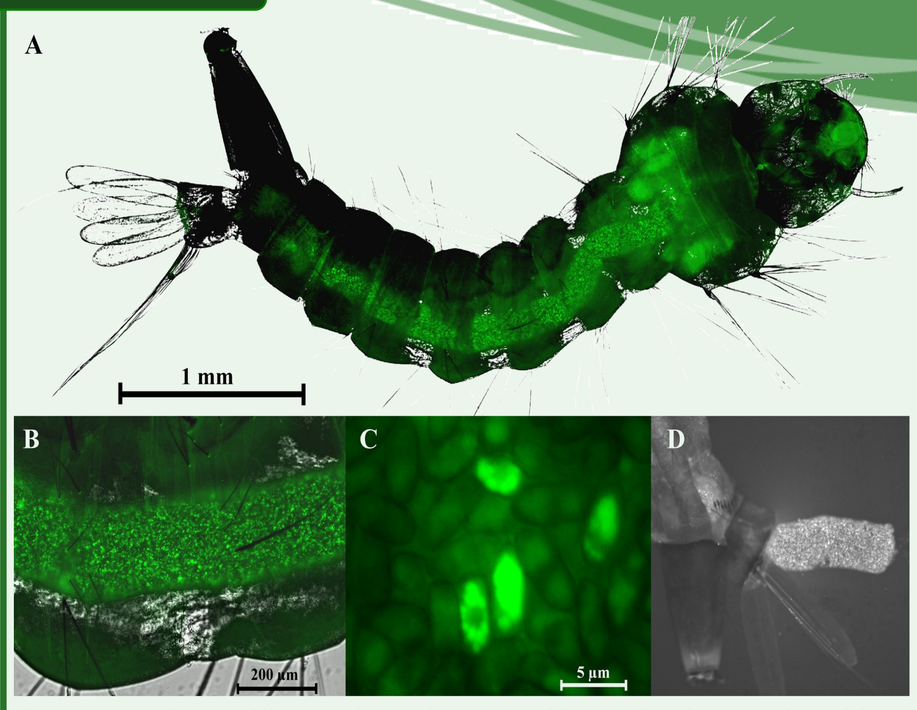

Figure 2. *Metarhizium* conidia expressing GFP in the gut and faecal pellets demonstrating activity

Conidia appear contained within the gut lumen (Fig. 2A—B) with no evidence of conidia invading the haemocoel.

Conidia actively expressing GFP inside the gut of the insect (x40 mag, Fig. 2C).

Mosquito maintains bodily functions, producing compact faecal pellets, containing conidia still actively expressing GFP (Fig. 2D).

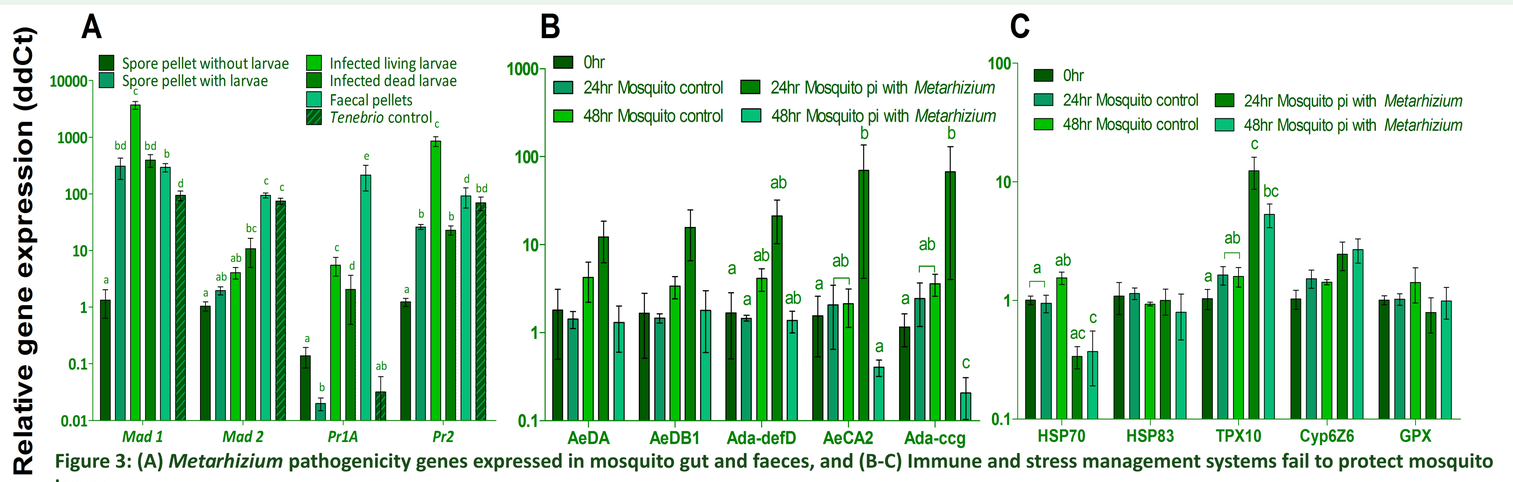

Figure 3: (A) *Metarhizium* pathogenicity genes expressed in mosquito gut and faeces, and (B-C) Immune and stress management systems fail to protect mosquito larvae

Fungal Pathogenicity

(Fig. 3A)

- ***Pr1, Pr2, Mad1*** and ***Mad2*** play a key role in fungal pathogenicity, expression of the genes analysed was shown to be generally much higher in the gut lumen and remained high in the faecal pellets (p <0.001).

Larval defence response

(Fig. 3B)

- Mosquitoes did not mount a strong Antimicrobial peptide (AMP) mediated defence response
- Significant down regulation of AeCA2 and Ada-ccg (*cecropins*) 48hr pi — coinciding with larval mortality.

Larval stress response

(Fig. 3C)

- *Hsp70* down regulated after 48hr—predispose the mosquito to apoptosis
- *TPX10* increased expression—Important role in detoxification of reactive oxygen species. May be an attempt to contain apoptosis.

Conclusions

- **Mortality of mosquito larvae exposed to *M. anisopliae* is multifactorial**. It is not due to invasion and colonisation of the host but entails *M. anisopliae* proteases, triggering stress induced apoptosis which ultimately leads to host death, hence the verdict of accidental death.
- Key pathogenic determinants are expressed in the mosquito larvae but the **established infection process of the terrestrial host is not observed**.
- **The mosquito larvae did not mount a strong defence response to *M. anisopliae*** - Larvae have either not evolved appropriate receptors identifying *M. anisopliae* as a pathogen, as is the case for terrestrial hosts[2] or the lack of interaction between the fungus and insect limits the mosquitoes ability to recognise an attempted infection.
- *Cecropins* and *Hsp70* genes were down regulated as larval death occurs **linking mortality to autolysis though *Hsp70* mediated Caspase activity.**
- ***M. anisopliae* retains pre-formed pathogenic determinants which mediate host mortality, but unlike true aquatic fungal pathogens, does not recognise and colonise the larval host.**

References

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