Variation in the reproduction of the biological control nematode *Deladenus siricidicola* on *Amylostereum areolatum* strains

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Introduction

- A single lineage of the nematode *Deladenus siricidicola* has been used as the primary biocontrol agent of the invasive woodwasp *Sirex noctilio* throughout the Southern Hemisphere[1, 3].
- The fungus *Amylostereum areolatum* has a symbiotic association with female *S. noctilio* and is used to mass-produce nematodes for the Sirex biocontrol program.
- Three other nematode lineages (A, C, D) have been identified in Australia and New Zealand with lineage B (the Kamona commercial strain) and D (a novel strain) being the most dominant[2].
- This unexpected diversity creates an opportunity to select additional strains for use in Sirex biological control programs.

Aim

To compare reproduction rates of different *D. siricidicola* strains from lineages B and D on four different strains of *A. areolatum*.

Methods

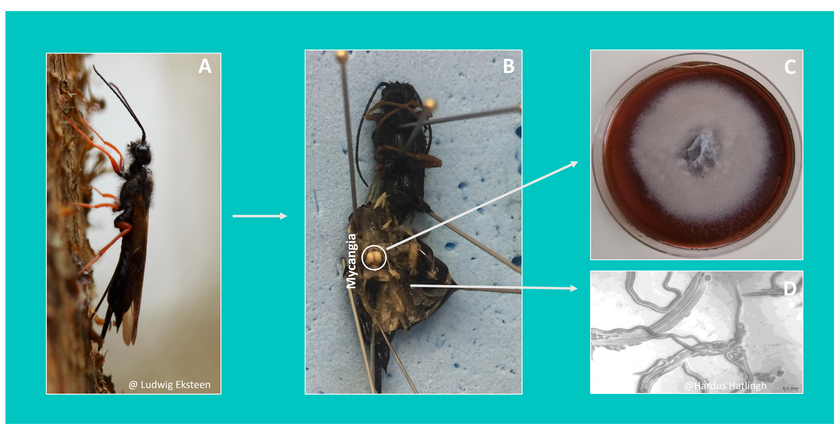


Fig. 1. *Sirex noctilio* woodwasps were dissected to collect the symbiotic fungus *A. areolatum* and the parasitic nematode *D. siricidicola*. A: female *S. noctilio*; B: dissected female *S. noctilio*; C: *Amylostereum areolatum* recovered from mycangia; D: *Deladenus siricidicola* recovered from infected woodwasp.

Experimental design

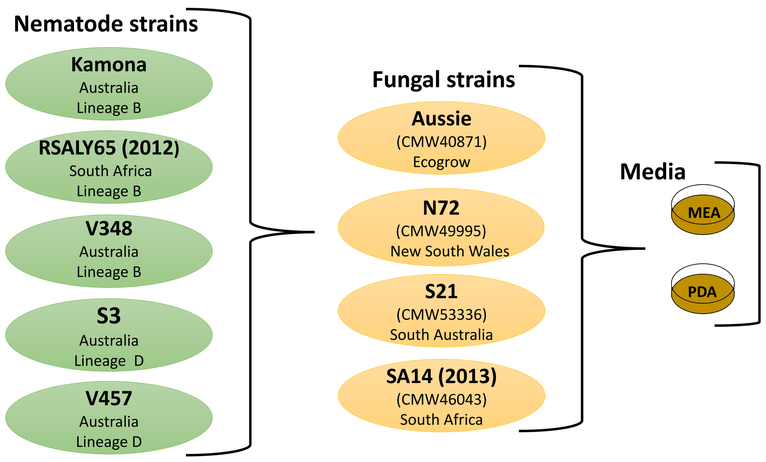
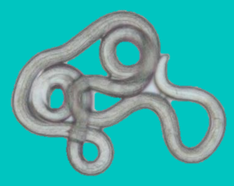


Fig. 2. Each of five strains of *D. siricidicola* were grown on four strains of *A. areolatum* to identify nematode-fungus compatibility. Lineage D nematodes are genetically different from the widely used biocontrol strain, lineage B. Strains were collected from different localities and woodwasps. CMW number refers to fungal culture collection at FABI and Ecogrow to *A. areolatum* strain used for commercial nematode mass-production in Australia.



Biocontrol nematode reproduction varies among fungal strains.

Nematode strains in lineage D are potential biocontrol agents to use in different environments and the woodwasp *S. noctilio* populations.

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Experimental procedures

Table 1. Experimental assays used to determine *D. siricidicola* reproduction rates on *A. areolatum* fungal strains. There were three replicates of each nematode-fungus-media-combination, and the experiment was duplicated.

Day 1 (Fungus inoculation assay)	Day 5 (Egg inoculation assay)	Day 30 (Harvesting and counting assays)
❖ ~ 5 mm Ø fungal plug inoculated onto MEA or 1/3 PDA media in 9 cm Petri dish ❖ Incubated at 23°C for 5 days	❖ Eggs of each nematode strain harvested from parent cultures ❖ ~ 530 eggs inoculated on each plate ❖ Incubated at 23°C for 25 days	❖ Nematodes were harvested and counted from each plate as a measure of the reproduction rate

Results

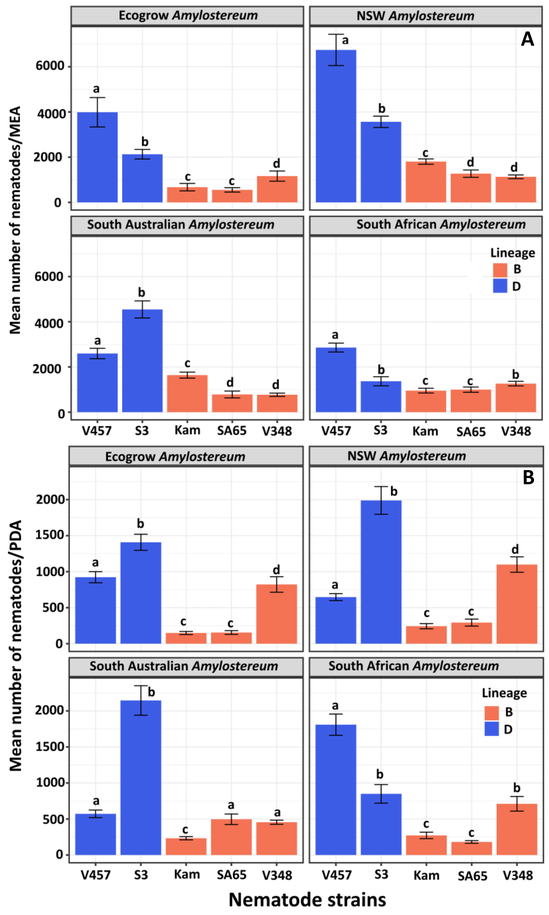

Fig. 3. Mean number of nematodes (±SE) of *D. siricidicola* on four different *A. areolatum strains* grown on MEA and PDA, 25 days after nematode egg inoculation. A: reproduction rate on MEA media; B: reproduction rate on PDA media. Different letters indicate significant differences between nematode strains with each fungal strain.

Conclusions

- Reproduction rates differed significantly between different *D. siricidicola* - *A. areolatum* strain on both media types (**Fig. 3A & B**), reflecting different nematode-fungus compatibilities.
- Lineage D had higher reproduction rates than most lineage B strains across all fungal-media combinations (**Fig. 3A & B**), suggesting differential adaptation potential and fitness.
- Kamona performed best on MEA media and "wild" *Amylostereum* strains (New South Wales and South Australian *Amylostereum*) (**Fig. 3A**), suggesting that the switch to a new rearing strain may improve the current biocontrol program.
- Results highlight why nematode mass production should consider the effect of diversity and nematode-fungus interaction patterns in biocontrol programs.

Key references

1. Bedding RA (2009). Controlling the pine-killing woodwasp, *Sirex noctilio*, with nematodes. In Hajek AE, Glare TR, O'Callahan M (*eds*). *Use of Microbes for Control and Eradication of Invasive Arthropods*. *Springer* pp 213-235.
2. Eshetu *et al.* (2022). Unexpected diversity in historical biological control programs: Population genetics of the nematode *Deladenus siricidicola* in Australia and New Zealand. *Biological Control, submitted.*
3. Fitza *et al.* (2019). The global diversity of *Deladenus siricidicola* in native and non-native populations. *Biological control* 132, 57–65.

Acknowledgments

FABI
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