**Growth of the *Sirex*-parasitic nematode *Deladenus siricidicola* on the white rot fungus
*Amylostereum***

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Abstract

The Kamona strain of the nematode *Deladenus siricidicola* has been extensively used as a biological control agent against invasive *Sirex noctilio* woodwasps in the Southern Hemisphere, where it sterilizes female hosts. In North America, a non-sterilizing (NS) strain of *D. siricidicola*, thought to have been introduced with *S. noctilio*, is commonly found parasitizing this invasive woodwasp. Species of *Deladenus* that parasitize *Sirex* have a parasitic form, as well as a mycophagous form. The mycophagous form feeds on *Sirex* fungal symbionts in the genus *Amylostereum*. The goal of this study was to compare reproduction of NS and Kamona *D. siricidicola* when feeding on four isolates of *Amylostereum areolatum* (three introduced and one native in North America) and one native strain of *A. chailletii* isolated from *Sirex nigricornis*. Mycophagous forms of the two *D. siricidicola* strains displayed relatively similar production of offspring when feeding on most of the *A. areolatum* found associated with *S. noctilio* in this continent, except for strain BD on which NS produced more offspring than the biological control strain Kamona. Growth of both nematodes was greater on the introduced versus the native *A. areolatum* isolates.

Key Words: Biological control; invasive arthropod; *Sirex noctilio*; white rot fungus; fungal-feeding nematode

1. Introduction

The nematode *Deladenus siricidicola*, Kamona strain has been used for decades as a biological control agent against invasive *Sirex noctilio* woodwasps that attack pines in the Southern Hemisphere. *Sirex noctilio* and its symbiotic white rot fungus *Amylostereum areolatum* were first collected from an established population in North America in 2004 (Hoebeke et al., 2005). While the Kamona strain of *D. siricidicola* acts to sterilize adult female *S. noctilio*, in North America, a non-sterilizing (NS) strain of this nematode species, thought to have been introduced with *S. noctilio*, is also now commonly found (Kroll et al., 2013; Yu et al., 2009). Native *Sirex* and *Deladenus* species also occur in North America. *Deladenus* are mainly fungal feeding and can live for many generations feeding on *Amylostereum* spp., but change to the infective form when near *Sirex* larvae (Bedding, 1972).

Based on sequencing of the intergenic spacer region (IGS), previous studies have suggested that *A. areolatum* IGS BD and D were introduced with *S. noctilio* to North America, and *A. areolatum* IGS BE is native to this continent (Nielsen et al., 2009). *Sirex noctilio* has often been found carrying *A. areolatum* IGS BD and D, and rarely carrying *A. chailletii* (Wooding et al., 2013), which is usually found associated with native species of *Sirex*.

To develop a successful and environmentally safe biological control program, it is important to study how associated organisms will interact within a given system. Since *Sirex* larvae are surrounded by *Amylostereum* in the xylem, and *Deladenus* nematodes feed on this fungus, species and strains of fungi could influence both presence and size of *D. siricidicola* colonies, thus impacting whether *S. noctilio* become parasitized. It is now known

that *Sirex* species are less tightly associated with fungal strains than previously thought (Hajek et al., 2013; Wooding et al., 2013) and recent work has shown that *D. siricidicola* Kamona reproduction is affected by fungal strain (Morris et al., 2012, 2014). However, no similar studies have been conducted on the NS strain. This study compares the fitness of the recently discovered NS strain of *D. siricidicola* with *D. siricidicola* Kamona by quantifying the ability of the nematode strains to develop and reproduce when feeding on different isolates of *Amylostereum* associated with *Sirex* in North America.

2. Materials and methods

2.1. Nematode and fungal strains

To obtain *D. siricidicola* NS, *S. noctilio* collected from infested trees were dissected and nematodes were reared in petri dishes containing ½PDAh (Morris et al., 2012) inoculated with *A. areolatum*. Nematode strain was determined via molecular characterization of the mtCO1 gene, which was compared to *D. siricidicola* mtCO1 sequences in GenBank (JQ241275, Leal et al., 2012). *Deladenus siricidicola* Kamona was imported in 2014 from Ecogrow Environment (Westgate, NSW), the Australian commercial producer of this nematode for biological control of *S. noctilio*. A colony of the Kamona strain was maintained in a quarantine facility and all experiments with this strain were conducted under quarantine.

Isolates of *Amylostereum* spp. used had been obtained from siricids collected in North America (Table 1). Four isolates are *A. areolatum*, commonly found associated with invasive *S. noctilio*, and one isolate is *A. chailletii*, a fungal species which is usually associated with native *Sirex*. One of the *A. areolatum* strains included is thought to be native

to North America (BE) while the other three strains of *A. areolatum* are thought to have been introduced with *S. noctilio* (Nielsen et al., 2009). *S. noctilio* and *S. nigricornis* can co-infest the same tree and thus *A. chailletii* used in this study was isolated from *S. nigricornis*.

2.2. Bioassays

Petri dishes containing ½PDAh were inoculated with one of the five fungal isolates and incubated for five days. All bioassays were conducted at 23°C without light. Nematodes and eggs were removed from colony dishes of *D. siricidicola* Kamona or NS by flooding the dishes with autoclaved water + gentamicin (6.25 mg/L). The resulting suspension was filtered through a 60 µm filter (Swinnex, Millipore) to eliminate juvenile and adult nematodes. The resulting egg suspension was adjusted to 167 eggs/20µl. Three 20µl drops of the final suspension were transferred to prepared fungal dishes, placing drops close to the edge of the dish and equidistant from each other. Therefore, each dish received approximately 500 eggs.

After 27d, three dishes of each treatment (Table 1) were thoroughly rinsed with tap water to remove all nematodes and eggs, creating a separate suspension from each dish. Nematodes and eggs within five 20µl drops of the suspension from each plate were counted and the number of nematodes plus eggs/plate was calculated.

Each treatment was replicated 2-4 times. To compare the production of nematodes and eggs on different fungal isolates after 27d, we used analysis of variance with main effects of nematode strain, fungal isolate and the interaction between fungal isolate and nematode strain. Means were separated using Tukey HSD posthoc tests.

3. Results and discussion

The interaction between nematode and fungal treatment was highly significant ($F_{4,89} = 7.182$; $P < 0.001$). *Deladenus siricidicola* Kamona grew best on *A. areolatum* strains D_{no}, D_{ni} and BD, which are assumed to have been introduced to North America with *S. noctilio* (Nielsen et al., 2009) (Fig. 1). Growth was intermediate on the native strain *A. areolatum* BE. The NS strain of *D. siricidicola* displayed greatest reproduction when grown on *A. areolatum* BD, followed by both *A. areolatum* D strains. Reproduction of *D. siricidicola* NS on *A. areolatum* BE was significantly less than BD and D_{no}. Neither nematode strain increased in numbers when offered *A. chailletii* (Ch). Comparing nematode strains, the only significant difference was seen for *A. areolatum* BD on which *D. siricidicola* NS increased to higher densities than Kamona ($P < 0.001$). Both nematode strains grew faster on introduced isolates of *A. amylostereum* (BD, D_{no} and D_{ni}) than on the native isolate (BE) (NS: $F_{1,86} = 27.0868$, $P < 0.0001$; Kamona: $F_{1,86} = 35,1503$, $P < 0.0001$).

When developing biological control methods, understanding interactions between the organisms involved within a system is important. Knowing the reproductive performance of these nematode strains when feeding on different isolates of *A. areolatum* may help with decisions about which fungus to use to mass-produce the nematode for biological control. Morris et al. (2012, 2014) and Hurley et al. (2012) tested growth of *D. siricidicola* on different strains and species of *Amylostereum*. Results from our study differ from those of bioassays with Kamona by Morris et al. (2014) in which highest reproduction was seen on *A. areolatum* BE and reproduction was very poor on *A. areolatum* D. While our study tested these same fungal strains, surprisingly Kamona reproduced best on *A. areolatum* D_{no} and reproduction on *A. areolatum* BE was intermediate.

This study provides a better understanding of how these two nematode strains could potentially interact in nature in North America. The mycophagous forms of both *D. siricidicola* strains displayed relatively similar production of offspring when feeding on most of the *A. areolatum* found associated with *S. noctilio* in this continent, except for strain BD on which NS nematodes reproduced more than the biological control strain Kamona.

If both strains of nematode occurred in the same tree and were located within the same fungal colonies, potential interactions between the two nematode strains could include food resource competition as well as potential strain hybridization, as Akhurst (1975) has shown hybridization among *D. siricidicola* strains to be possible.

Based on our results, if a *Sirex* larva was surrounded by *A. chailletii*, *D. siricidicola* NS and Kamona might not infect that larva because both nematode strains do not readily feed on *A. chailletii*. If a *S. noctilio* larva was surrounded by the introduced *A. areolatum* strains that are currently present in North America, then either of the nematode strains might occur within that fungal culture and would possibly infect the larva. We included *A. areolatum* BE because it is native and has historically been associated with native *Sirex*. We wanted to evaluate whether NS would grow well on BE in order to determine whether NS would be in the vicinity of native *Sirex* surrounded by symbiotic BE. Both nematode strains grew better on introduced *A. areolatum* rather than the native *A. areolatum*, although there was still growth on the native fungus. We found that growth of the two *D. siricidicola* strains was equal on *A. areolatum* BE, suggesting that either strain of nematode has an equal chance of being near native *Sirex* larvae having BE as a symbiont.

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Table 1. Strains of *Amylostereum* included in nematode reproduction bioassays.

Isolate ID	Species	Isolation from	Original host	Date isolated	IGS strain
BE	<i>A. areolatum</i>	Warrensburg, NY	<i>S. nigricornis</i>	19 Sep 2009	BE
BD	<i>A. areolatum</i>	Fulton County, NY	<i>S. noctilio</i>	19 Feb 2008	BD
Dni	<i>A. areolatum</i>	Oswego County, NY	<i>S. nigricornis</i>	19 Sep 2007	D
Dno	<i>A. areolatum</i>	Oswego County, NY	<i>S. noctilio</i>	Jan 2006	D
Ch	<i>A. chailletii</i>	New Haven, NY	<i>S. nigricornis</i>	21 Sep 2007	G

Figure 1 Legend

Fig. 1. The number of nematodes and eggs of *D. siricidicola* produced per plate by 27 days (white = Kamona; grey = NS). Lower case letters compare the growth of *D. siricidicola* Kamona when feeding on different fungal isolates. Uppercase letters compare the growth of *D. siricidicola* NS when feeding on different fungal isolates. Asterisks indicate differences between the two nematode strains feeding on the same fungal isolate.

BD, BE, D_{ni}, D_{no} = Strains of *A. areolatum*; Ch = Strain of *A. chailletii*.

Figure