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Eat or be eaten: fungus and nematode switch off as predator and prey

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ABSTRACT

Use of the parasitic nematode *Deladenus siricidicola* to control invasive pine-killing *Sirex noctilio* woodwasps in the Southern Hemisphere is one of the most successful examples of classical biological control. Both nematode and woodwasp rely on the white rot fungus, *Amylostereum areolatum*, for continued survival, and the nematode is commercially mass produced in its mycophagous phase on this fungus. We tested the hypothesis that a role reversal can occur wherein fungal hyphae invade and kill nematode eggs. *Deladenus siricidicola* eggs were exposed to *A. areolatum* to quantify the number of eggs lost to fungal invasion. Additionally, *A. areolatum* and *A. chailletii* were observed via cryogenic scanning electron microscopy and fluorescence microscopy to document their ability to parasitize eggs and adults of *D. siricidicola*, *D. proximus*, and an undescribed *Deladenus* species. This study reports evidence of a basidiomycete destroying nematode eggs, as well as a novel trapping mechanism used to capture and parasitize three species of adult female *Deladenus* nematodes.

Introduction

Many species of fungi, spanning Basidiomycota, Ascomycota, Zygomycota, and Chytridiomycota, have been found to have antagonistic relationships toward nematodes and their eggs, often using them for nutrition (Chen and Dickson, 2004). Barron (1977, 1992) and Lopez-Llorca et al. (2007) suggested that wood rot fungi in particular, which are often in a nitrogen-limited environment, might be expected to use nematodes as a nitrogen supplement. Additionally, Barron (1977) suggested that because many fungi are parasitized by stylet-bearing fungal-feeding nematodes, these fungi might have evolved an antagonistic relationship towards such nematodes as a protective strategy.

The basidiomycete *Amylostereum areolatum* (Russulales: Amylostereaceae) is a wood rot fungus integral to an intriguing ecological system. The fungus is necessary for the development of the invasive pine-killing woodwasp, *Sirex noctilio* (Thompson et al., 2014), as well as being the fungal food source for the mycophagous phase of the most successful biological control agent used to target *S. noctilio*, the parasitic nematode *Deladenus siricidicola* (Tylenchida: Neotylenchidae) (Bedding, 2009). Due to the invasive pest status of *S. noctilio* (Borchert et al., 2007), the role that *A. areolatum* plays for both the woodwasp and the nematode necessitates an understanding of the many individual interactions occurring between woodwasp, fungus, and nematode.

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In adult female *S. noctilio*, fragments of *A. areolatum* called 'oidia' or 'arthrospores' are carried within paired internal organs at the base of the ovipositor called 'mycangia' (Coutts and Dolezal, 1969). Oidia are injected into the pine tree during *S. noctilio* oviposition. The fungus is critical to *S. noctilio* in two main ways. First, phytotoxic venom is simultaneously injected into the tree along with the fungus, and in concert, the fungus and venom cause lethal pine wilt of the tree (Coutts, 1969; Bordeaux and Dean, 2012; Bordeaux et al., 2012). This allows *S. noctilio* larvae to develop in the xylem unhindered by tree defenses. The second critical role played by *A. areolatum* relative to *S. noctilio* is that early instar larvae require the fungus to develop to adults (Madden and Coutts, 1979) and are unable to survive to adulthood in the absence of the fungus (Ryan and Hurley, 2012). The symbiotic fungus liberates organic compounds from the wood, which are, remarkably, externally digested by the larvae (Thompson et al., 2014).

The nematode *D. siricidicola* has a dual life strategy, in which fungal feeding (mycophagous) nematodes in the tree feed exclusively on the growing hyphal tips of *A. areolatum*. In the presence of woodwasp larvae, however, the nematode switches its life strategy and develops into a parasitic form. Females of the parasitic form of *D. siricidicola* invade woodwasp larvae, eventually leading to sterilization of parasitized *S. noctilio* hosts, although parasitization of a woodwasp by a *Deladenus* nematode does not always lead to sterilization (Kroll et al., 2013; Yu et al., 2009; Zondag, 1975). A single generation of the parasitic nematodes is followed by numerous mycophagous generations (Bedding, 2009; Bedding and Iede, 2005). Mycophagous generations can continue indefinitely, a trait which is exploited for the mass production of this nematode for biological control.

Morris et al. (2014) described how reproduction of two species of *Deladenus* nematodes, *D. siricidicola* and *D. proximus*, varied significantly when feeding on different species and strains of *Amylostereum* fungus; in some cases, nematodes failed to lay many eggs when feeding on a given fungus, but in other cases, nematodes laid many eggs, but the eggs were subsequently grown over by the fungus and failed to hatch. Additionally, an earlier study showed that *D. siricidicola* numbers increased most successfully when feeding on the slowest growing *A. areolatum* strains (Morris et al., 2012). Given nematophagy in Basidiomycota may be more widespread than previously thought (Lopez-Llorca et al., 2007), the *Deladenus–Amylostereum* relationship warranted closer scrutiny.

Studies were carried out to investigate the interactions between four associated pairs of *Amylostereum–Deladenus* species and strains. The pairings were chosen either based on natural associations found in the field or, in the case of *D. siricidicola* strain Kamona, because the associated fungal strain (*A. areolatum* BDF) is used to mass produce the nematode for biological control. Given that most *D. siricidicola* eggs are laid at the growing edge of a fungal colony and hatch between 3 and 5 d later (E.E. Morris, unpublished data), it was hypothesized that if the fungus was provided with more nutrients, hyphae would overgrow the stationary nematode eggs before the eggs could hatch, ultimately producing fewer hatched juveniles. Cultures of two strains of *A. areolatum* were inoculated with eggs of two strains of *D. siricidicola*. The ability of these fungal strains to grow over and kill *Deladenus* eggs, with and without rich growing medium, was quantified, in order to estimate how much impact a given fungal strain can have on nematode population growth. The ability of these fungal species and strains to invade and kill their associated nematodes' eggs, juveniles and adults was further studied via fluorescence microscopy. Cryogenic scanning electron microscopy was used to examine a strain of *A. areolatum* invading *D. siricidicola*.

Materials and methods

Fungal cultures

To establish a fungal culture, a 3 mm diameter plug from the edge of a growing culture was transferred to the center of a 90-mm diameter Petri plate containing half-strength potato dextrose agar (Difco, Sparks, MD) with a total of 25 g l^{-1} agar to make this medium harder (1/2PDAh) (R.A. Bedding, personal communication). The fungal cultures were incubated for 5 d at 23 °C in darkness prior to use in experiments.

Nematode strains

Four combinations of nematode and fungus were included in this study (Table 1). The Kamona strain of *D. siricidicola* was obtained in 2006 from Ecogrow Environment (Queanbeyan,

Table 1 – Nematodes and fungi included in this study

Nematode species	Nematode strain	Fungal species and IGS[a] strains tested	Insect fungal host	Fungus collection location
Deladenus siricidicola	Kamona	*A. areolatum* BDF	*Sirex juvencus*[b]	Sopron, Hungary[b]
	Non-sterilizing	*A. areolatum* BD	*Sirex noctilio*	Fulton Co., NY
Deladenus proximus		*A. areolatum* BE	*Sirex nigricornis*	Warrensburg, NY
Deladenus sp. (undescribed)		*A. chailletii* G	*Sirex californicus*	Seattle, WA

a Strains identified by intergenic spacer regions (IGS), as described in Nielsen et al. (2009) and Hajek et al. (2013).

b This fungal strain is used to mass produce *D. siricidicola* Kamona. While detailed records have been kept on the isolation of this nematode, originally isolated from *S. juvencus* in Sopron, Hungary and later reisolated from *S. noctilio* in the Kamona forest of Tasmania, there is no official record for where this fungal strain was initially isolated, the information is given for *D. siricidicola* Kamona, instead.

N.S.W., Australia) where it is mass-produced for biological control of *S. noctilio*. *Deladenus siricidicola* strain Kamona was kept under USDA, APHIS permit in a quarantine facility (the Sarkaria Arthropod Research Laboratory, Ithaca, New York) for the duration of our experiments. Nematodes were initially grown on *A. areolatum* BDF (from Ecogrow Environment) and were maintained inside brown paper bags in a 23 °C incubator with no light. Cultures were subcultured every 2 weeks.

A colony of *D. siricidicola* (isolate 718-12) used for studies was established by transferring juvenile nematodes dissected from a single parasitized *S. noctilio* male and placing them on 1/3PDA plates previously inoculated with *A. areolatum* BD strain. The *S. noctilio* host had emerged from a Scots pine (*Pinus sylvestris*) collected from Tioga County, Pennsylvania in 2012. Nematodes were identified via molecular characterization as *D. siricidicola*, as described in Kroll et al. (2013), and were found to be the same as the *D. siricidicola* investigated in that study, which does not sterilize the North American population of *S. noctilio*.

A colony of *D. proximus* used for these studies was established by transferring juvenile nematodes dissected from a single parasitized *S. nigricornis* male and placing them on 1/3PDA plates previously inoculated with *A. areolatum* BE strain. The *S. nigricornis* host had emerged from a Scots pine collected from Warren County, New York in 2012. Nematodes were identified via molecular and morphological characterization as described in Morris et al. (2014).

A colony of an undescribed *Deladenus* species was established by transferring juvenile nematodes dissected from a single parasitized *S. californicus* male and placing them on 1/3PDA plates previously inoculated with *A. chailletii*. The host *S. californicus* emerged from a *P. moniticola* × *P. strobus* hybrid tree from King County, Washington State in 2009.

Egg survival assays

Two experiments were conducted: the first to test the impact of *A. areolatum* BD strain on egg hatching of *D. siricidicola* 718-12, and the second to test the impact of *A. areolatum* BDF strain on hatching of *D. siricidicola* Kamona eggs. For nematode growth assays, there were three treatments: two treatments included *A. areolatum*, growing either on 1/2PDAh medium or on water agar (i.e., faster or slower, respectively) and the control treatment only included 1/2PDAh medium and no fungus. Assays were conducted at 23 °C in darkness. To inoculate the treatment plates with eggs, nematode colonies were flooded with distilled water and the liquid containing nematodes and eggs was filtered once through a Swinnex filter holder (Millipore) equipped with a 60 μm filter, which allowed eggs to pass through into the filtrate. The concentration of eggs in the stock suspension was estimated by counting all eggs from each of ten 20 μl samples under a dissecting microscope at 20× magnification. The suspension was then concentrated so it would contain 500 eggs per 20 μl drop. For each treatment, 20 μl was added to each of 15 5-d old cultures of *A. areolatum* at the growing margin of the fungal colony or, in the case of the control treatment, 1 cm from the center of the plate. Three replicate plates per treatment were destructively sampled every 24 hr for 5 d to quantify the number of hatched juveniles per plate. For each sampling period, plates were flooded with water and ten 20 μl samples of each of the washings were examined under a dissecting microscope to volumetrically estimate the total number of hatched juveniles per plate. Each of the two experiments was repeated for a total of three experimental replicates per nematode/fungus combination.

Microscopy

Four nematode–fungal combinations were evaluated (Table 1). For each combination, fungal cultures were established and inoculated with nematodes as above. Nematode colonies were grown at 23 °C for up to 25 d, colonies were examined with a dissecting microscope, and eggs and nematodes at different infection stages were removed with a minuten pin. Such samples were either mounted in a drop of water on a microscope slide with a cover slip for direct viewing at 100–400× magnification under light microscopy, or were stained for fluorescence microscopy.

To stain fungal hyphae for fluorescence, sample nematodes were placed on a microscope slide in a drop of Calcofluor/Evans Blue (1.0 %, 0.5 %). A drop of 1 M potassium hydroxide was added, and a coverslip was placed on top. Samples were then examined under fluorescence using an excitation wavelength of 440 nm.

Both types of slides were examined to observe fungal infection. Additionally, a sterile scalpel was used to excise agar medium containing fungus and nematodes of both treatments. These excised pieces were placed on a slide and viewed via light microscopy at 100–400× magnification.

Selective staining of fungal cystidia

To confirm the role of cystidia, which do not stain with Calcofluor, some nematodes exhibiting possible fungal parasitism were removed with a minuten pin. These samples were placed on a slide in a drop of aqueous safranin solution (1 % w/v), which stains cystidia deeply (Talbot, 1964). A cover slip was added, and samples were examined under a compound microscope at 100–400× magnification.

Cryogenic scanning electron microscopy

Deladenus siricidicola Kamona colonies were established as above on 90 mm Petri plates, with one modification: prior to fungal inoculation, autoclaved cellophane was placed on the 1/2PDAh medium. Colonies were kept at 23 °C for 2 weeks, at which time they were observed under a dissecting microscope (20× magnification) for signs of fungal parasitism. A scalpel was used to excise 1 cm × 0.5 cm rectangles of cellophane containing fungus and nematodes. Samples were quickly attached to brass stubs using TissueTek® and frozen at −80 °C in liquid nitrogen for up to 2 weeks until they were transferred to the cryostage of a scanning electron microscope (Hitachi 4500) for viewing.

Statistical analysis

In the egg survival assays, the total numbers of juveniles observed per day were $\log_{10}$ transformed $(x + 1)$ and analyzed

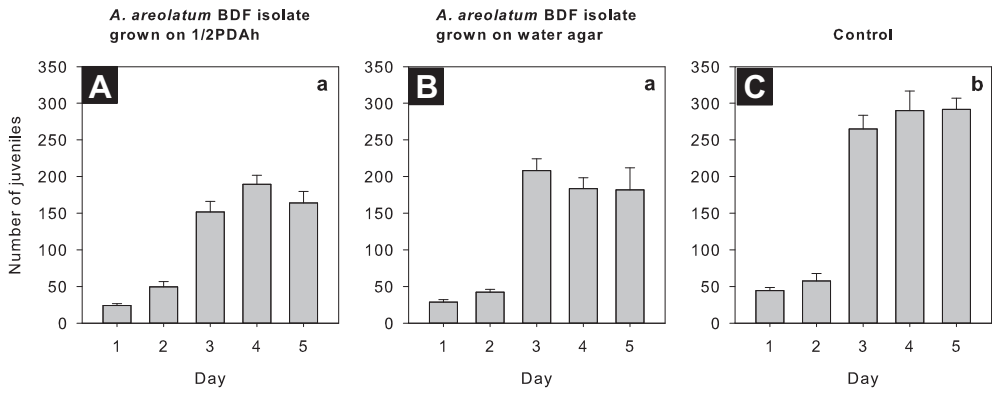

Fig 1 – Mean numbers (±SE) of *D. siricidicola* Kamona juveniles after 5 d when: (A) grown on *A. areolatum* BDF on 1/2PDAh medium; (B) grown on *A. areolatum* BDF on water agar; or (C) with no fungus. Lower case letters in the top right corners of graphs denote significant differences in the total juveniles over all days combined.

using a two-way ANOVA with treatment, day, and the interaction between treatment and day as main effects. Experimental replicate was coded as a random effect. Means were separated with Tukey's LSM test (SAS, 2013).

Results

Egg survival assays

Both day and treatment had significant effects on the average total *D. siricidicola* Kamona juveniles produced (day: $F_{1, 127} = 241.31$; $p < 0.0001$; treatment: $F_{2, 127} = 9.68$; $p = 0.0001$). *Amylostereum areolatum* BDF strain grown on 1/2PDAh medium and grown on water agar produced significantly fewer juveniles (115.7 ± 11.2; 128.9 ± 13.7, respectively) (mean ± SE) than the control treatment of 1/2PDAh only (189.8 ± 18.6) (Fig 1). There was no interaction between treatment and day ($F_{2, 127} = 0.39$; $p = 0.68$).

Both day and treatment had significant effects on the total *D. siricidicola* 718-12 juveniles produced (day: $F_{1, 127} = 165.53$; $p < 0.0001$; treatment: $F_{2, 127} = 10.20$; $p < 0.0001$). *Amylostereum areolatum* BD strain grown on 1/2PDAh medium produced significantly fewer juveniles, with an average of 89.4 ± 8.9 juveniles (mean ± SE), when compared with either *A. areolatum* BD grown on water agar (133.4 ± 13.4) or the control treatment of 1/2PDAh only (132.0 ± 15.0) (Fig 2). There was no interaction between treatment and day ($F_{2, 127} = 1.02$; $p = 0.36$).

For both nematode strains in the 1/2PDAh medium plus fungus treatment, eggs were observed initially, but some were then grown over by the fungus and appeared darkened. For the water agar treatment, this was observed only for *D. siricidicola* Kamona grown on *A. areolatum* BDF strain.

Microscopy

Cryogenic scanning electron microscopy showed *D. siricidicola* Kamona eggs overgrown by *A. areolatum* BDF strain (Fig 3A), as well as the apparent penetration of an egg by a hyphal tip (Fig 3B). Light microscopy revealed piles of darkened nematode eggs that never hatched overgrown by fungus (Fig 3C), while fluorescence microscopy showed fungus growing inside of eggs (Fig 3D). Fungus was observed growing over masses of eggs that did not hatch for all *Deladenus–Amylostereum* combinations tested.

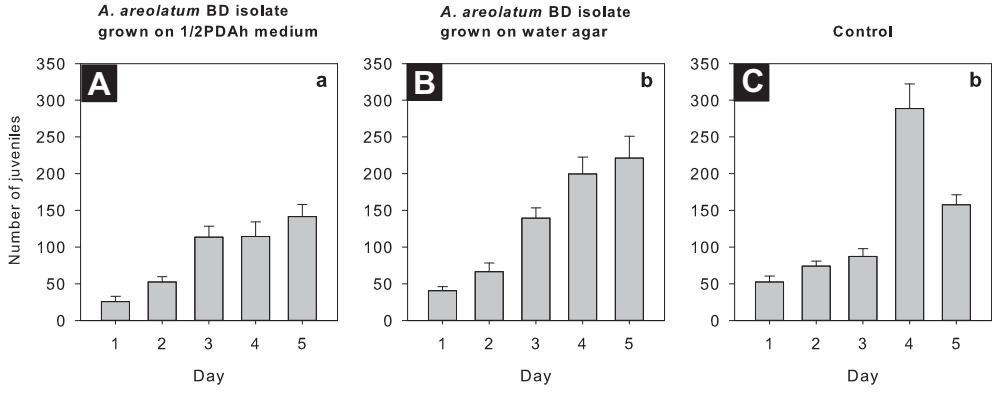

Fig 2 – Mean numbers (±SE) of non-sterilizing *D. siricidicola* juveniles after 5 d when: (A) grown on *A. areolatum* BD on 1/2PDAh medium; (B) grown on *A. areolatum* BD on water agar; or (C) with no fungus. Lower case letters in the top right corners of graphs denote significant differences in the total juveniles over all days combined.

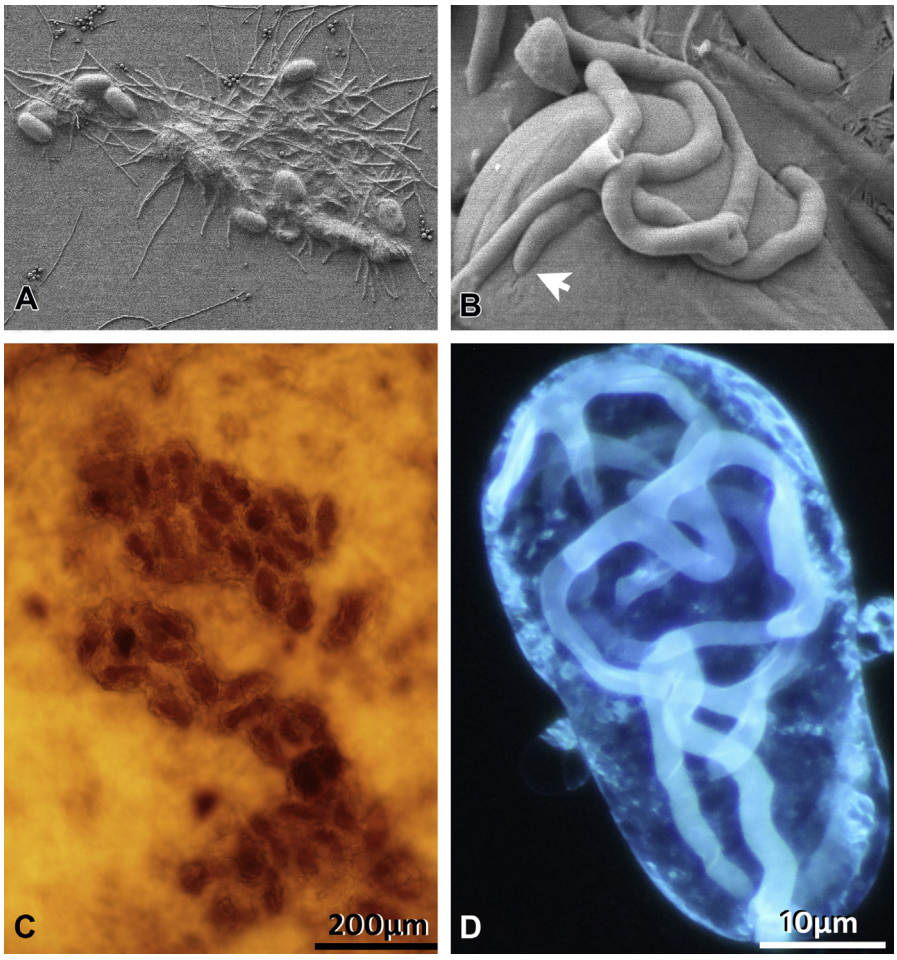

Fig 3 – *Deladenus* eggs attacked by *Amylostereum areolatum*. (A) *D. siricidicola* Kamona eggs attacked by *A. areolatum* BDF strain, cryogenic scanning electron microscopy. (B) Cryogenic scanning electron microscopy of a *D. siricidicola* Kamona egg, apparently penetrated by *A. areolatum* BDF strain hyphal tip (arrow). (C) Darkened *D. proximus* eggs attacked by *A. areolatum* BE strain. (D) Assimilative hyphae of *A. areolatum* BE strain inside of a *D. proximus* egg, under fluorescence staining. Images in the same column share the same size bar.

Additionally, adult female nematodes were found parasitized by fungal hyphae for all *Deladenus*–*Amylostereum* combinations tested (Fig 4A–D). Hyphae always invaded adult females via the vulva, and these nematodes were frequently found thrashing vigorously despite invasion of the fungus through the vulva. Fungal-parasitized nematodes were tethered to the substratum via fungal cystidia, which attached to the nematode vulva at one end, and grew into the substratum at the other end. Fluorescence microscopy revealed extensive hyphal growth within nematode bodies (Fig 4B, D), which eventually killed the nematode and grew out from the cuticle along the length of the body. Cystidia in fungal cultures stained deeply red with safranin, as did the cystidia tethering parasitized nematodes to the substratum (Fig 4C). Living juvenile nematodes were never found parasitized by hyphae, although occasionally a juvenile was observed hatching from an egg which was surrounded by fungus-killed eggs, and such juveniles were essentially blocked from moving away from the egg mass. Adult males were never observed to be parasitized. Likely, this is because the vulva of the female is the only natural opening large enough for hyphae to enter. Additionally, gravid females appeared to move less than juveniles and males, which may have made them easier targets for fungal entry.

Discussion

This study provides the first evidence that three species of *Deladenus* nematodes can be parasitized by their *Amylostereum* food source. Previously, it has been noted that to culture *D. siricidicola* on *A. areolatum*, a "balance must be maintained between nematodes and fungus since the nematodes can breed successfully only on the growing edge of the fungus" (Bedding, 1972). The results of this study provide insight into what drives the balance between *Amylostereum* fungi and *Deladenus* nematodes and help to at least partially explain prior findings of suppression of reproductive output of *Deladenus* when grown on different species and strains of *Amylostereum* (Morris et al., 2012, 2014).

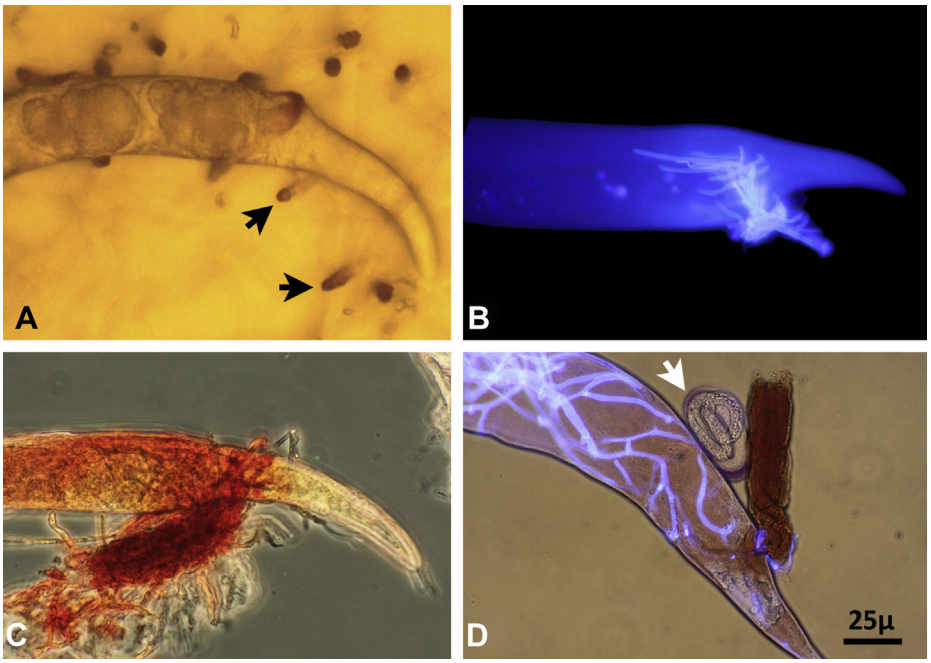

Fig 4 – Parasitized adult female *Deladenus* vulvas. (A) *D. siricidicola* NS strain nematode surrounded by cystidia (black arrows), early stage of infection. (B) Undescribed *Deladenus* species parasitized by *A. chailletii* hyphae. (C) Safranin staining of *A. areolatum* BD strain cystidium penetrating adult female *D. siricidicola* NS strain vulva. (D) Fluorescence staining showing internal hyphae of *A. areolatum* BD strain within adult female nematode body. White arrow indicates healthy nematode egg.

Fungal antagonists of nematodes include those fungi that have an adverse effect on nematodes, and these can either be nematophagous or non-nematophagous (Chen and Dickson, 2004). Nematophagy occurs across all major groups of fungi and is believed to have evolved multiple times (Barron, 1992). Established categories of these fungi include: (1) predatory, (2) endoparasitic, (3) toxin-producing, and (4) egg-parasitic fungi, and it is worth noting that individual fungal species can belong to more than one category (Chen and Dickson, 2004). Results of the present study suggest that *A. areolatum* and *A. chailletii* are predatory and also may be toxin-producing and egg-parasitic, although this is less clear.

In the present study, *A. areolatum* hyphae growing on 1/2PDAh medium frequently grew over *D. siricidicola* eggs which then darkened and did not hatch, ultimately leading to a significant reduction in number of juveniles. Cryogenic scanning electron microscopy did not reveal any apparent formation of appressoria. However, penetration of a nematode egg can also occur via simple hyphae (Chen and Dickson, 2004), and in this study fluorescence microscopy revealed extensive assimilative hyphae inside nematode eggs despite the absence of appressoria.

It is difficult to draw parallels between this study and other literature on fungi attacking nematode eggs, as the majority of the literature focuses on root-knot and cyst nematodes, due to difficulties with extracting nematode eggs from soil (Stirling and Mankau, 1979). Egg-parasitic fungi attacking root-knot and cyst nematodes have been described from Ascomycota, Zygomycota, and Oomycota (Lopez-Llorca et al., 2007). However, Basidiomycota are noticeably absent from this literature, despite being represented in other categories of nematophagous fungi (Barron, 1977; Lopez-Llorca et al., 2007; Dackman et al., 1992).

Classification of those fungi which kill nematode eggs can be challenging. The literature can refer to them as either "egg-destroying" or "egg-parasitic," or both, and it is not always easy to distinguish the difference. For example, Lopez-Llorca et al. (2007) refer to *Pochonia rubescens* and *P. chlamydosporia* as egg-destroying fungi, but specify the fungi both act "as true parasites" by directly penetrating and infecting eggs, as well as indirectly acting on eggs via the distortion of embryos. Gintis et al. (1983) discusses the latter mode of action, stating that fungi may initially grow saprotrophicly on mucilage associated with eggs but eventually inhibit egg hatching via diffusible metabolites. Once the egg is physiologically disordered, it becomes vulnerable to fungal invasion (Gintis et al., 1982, 1983). "Egg-destroying" seems to be the term applied when the relationship between fungus and nematode is in a gray area, where a fungus weakens or kills a nematode egg and then enters it, instead of the other way around. While it has not been unusual for a few eggs produced by a normal mycophagous *D. siricidicola* female to be non-viable, this is always a small percentage (E.E. Morris, unpublished data). This observation and the results from this study demonstrate that *A. areolatum* is killing *D. siricidicola* eggs. However, whether the eggs are killed before or after the fungus penetrates will need to be addressed in future studies.

Due to the low nitrogen availability in wood, it has been hypothesized that basidiomycete wood rot fungi would be particularly suited to facultative nematophagy, especially when the nematode prey is a fungal grazer (Barron, 1977). Levels of nitrogen have been shown to influence the

production of trapping devices in some species of nematophagous fungi, such as *Arthrobotrys oligospora* (Scholler and Rubner, 1994), and frequently observations of nematophagy are made when a given fungus is grown on low nutrient medium such as water agar medium (Barron, 1992). In the present study, however, richer medium resulted in increased predation of nematodes and eggs over water agar medium, presumably due to the increased growth rate of the fungi. A previous study found that *A. areolatum* BDF grew faster than *A. areolatum* BD on all media tested, although water agar was not included as a treatment (Morris et al., 2012). In the present study, *A. areolatum* BDF growing on water agar medium had a significant effect on the ability of eggs to hatch; however, *A. areolatum* BD growing on water agar medium did not. The ability of *A. areolatum* BDF to grow faster than *A. areolatum* BD may account for the difference in egg predation on water agar. These results point more toward the latter motivation for nematophagy suggested by Barron (1977), which is the possibility that nematophagy could arise as a protective device to incapacitate mycophagous nematodes.

All combinations of *Amylostereum* and *Deladenus* tested showed evidence of parasitism of adult female mycophagous nematodes. The fungal entry route consistently occurred via the vulva, and frequently fungal invasion was observed in a live, vigorous host. This method of capture of a nematode, as well as the ability to kill and invade nematode eggs, is a novel observation among Basidiomycota.

Nematophagous basidiomycetes employ numerous methods of prey capture, including traps, adhesive nets, and special spore structures (Lopez-Llorca et al., 2007). Although *A. areolatum* forms asexual arthrospores in culture (Talbot, 1964), these were never observed playing a role in nematode infection. However, another structure formed by both *A. areolatum* and *A. chailletii*, the encrusted cystidium (Talbot, 1964), does appear to play a role in nematode infection. Cystidia tethered parasitized adult female nematodes by the vulva to the substratum. While it is not uncommon for a female nematode to be colonized via natural openings such as the vulva or oral opening (Chen and Dickson, 2004), the role of cystidia in the present study suggests a novel trapping method. The function of cystidia in Basidiomycota is largely unknown, although some studies suggest they may provide protection from fungal grazers, which would be consistent with our observations. Nakamori and Suzuki (2007) found that cystidia of the fruit bodies of *Russula bella* and *Strobilurus ohshimae* increased Collembola mortality in lab studies, as dead Collembola were found held in place by the cystidia. Barron and Dierkes (1977) found cystidia of *Hohenbuehelia* bearing hourglass or spherical secretory cells at their tips, although they could not demonstrate a nematode-capturing ability for these cells. For some nematophagous fungi, trap adhesives are activated upon contact with nematode cuticle, a response which is thought to be mediated by lectin binding (Nordbring-Hertz and Mattiasson, 1979). In the present study, the mechanism used by the fungus to attach to a nematode is unknown.

A limitation to this study is that it took place in a laboratory setting in Petri plates and, therefore, it is hard to extrapolate what might be occurring between these organisms within larval woodwasp galleries in pine trees. If the parasitic relationship does exist within pine trees, this would be notable in that the vast majority of nematophagous fungi have been described from soil habitats (Barron, 1977; Lopez-Llorca et al., 2007). One exception is *Esteya vermicola* (Ascomycota), which preys on the pinewood nematode, *Bursaphelenchus xylophilus*, within pine trees (Wang et al., 2008, 2011; Liou et al., 1999).

In addition to perhaps providing the fungus with supplementary nitrogen or protecting the fungus from mycophagy, a possible benefit to *Amylostereum* from consuming *Deladenus* nematodes emerges when one considers the relationship between *Sirex* woodwasps and their *Amylostereum* fungal symbionts. For example, an important way that *A. areolatum* arrives at a new *Pinus* tree is via ovipositing *S. noctilio* (Thomsen and Koch, 1999). Given that *D. siricidicola* parasitism of *S. noctilio* can have a negative impact on the woodwasp, either by sterilizing females or by reducing the total number of eggs produced by females (Bedding, 2009; Kroll et al., 2013), it might benefit the fungus to reduce the number of nematodes able to parasitize *S. noctilio*.

This study investigated a hypothesized role reversal wherein hyphae of two species of *Amylostereum* fungi that are the food source of mycophagous nematodes can, in turn, invade and kill these same nematodes. Two strains of *D. siricidicola* eggs were shown to be killed and filled with assimilative hyphae when exposed to multiple isolates of *A. areolatum*. Cryogenic scanning electron microscopy and fluorescence microscopy further revealed the ability of both *A. areolatum* and *A. chailletii* to parasitize living adult females of *D. siricidicola*, *D. proximus*, and an undescribed *Deladenus* species. This study reports the first evidence of a basidiomycete destroying nematode eggs, as well as a novel trapping mechanism used to capture and parasitize adult nematodes.

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