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Performance of *Sirex noctilio*'s biocontrol agent *Deladenus siricidicola*, in known and predicted hosts

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HIGHLIGHTS

- Growth of Sirex fungus was lower on hybrid pine substrate than on *Pinus taeda*.
- Survival of Sirex biocontrol nematodes was lower in hybrid pine than *P. taeda*.
- Plant chemistry may contribute to differences in fungus and nematode performance.

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ABSTRACT

Survival of the free-living mycetophagous form of *Deladenus siricidicola*, the major biological control agent of Sirex woodwasp, *Sirex noctilio*, was tested in known (*Pinus taeda*) and predicted novel (*P. elliottii* subsp. *elliottii* × *P. caribaea* var. *hondurensis*) hybrid host taxa. Trials were established in the field to simulate nematode dispersal both naturally by infected wasps and following commercial inoculation, as well as in the laboratory under controlled conditions. Nematodes showed reduced survival in hybrid pine compared with *P. taeda* for all tree-associated treatments, but performed equivalently in petri-dish bioassays containing substrate of each taxon. Growth of *Amylostereum areolatum*, the food source of *D. siricidicola* was lower on plates containing ground hybrid substrate than on plates containing ground *P. taeda*. Some physical differences were found between taxa, including differences in bordered pit diameters, tracheid widths, and basic density, but these did not consistently explain reduced performance. More plant secondary compounds (predominantly oleoresins) were present in hybrid taxa than in *P. taeda*, and in standing trees compared with felled trees. Our results suggested that *D. siricidicola* may not be as effective in hybrid pine taxa for the biological control of *S. noctilio* as it is in its current known host taxa, possibly because of reduced growth of its food source, *A. areolatum* in hybrid pine.

1. Introduction

Sirex woodwasp, *Sirex noctilio* F. (Hymenoptera: Siricidae) and its obligate symbiotic fungus, *Amylostereum areolatum* (Chaillet ex Fr.) Boidin (Russulales: Amylostereaceae) are major invasive pests of softwoods worldwide. In concert, the duo kills trees through the combined action of the wasp's phytotoxic mucus and growth of the fungus, both deposited into trees during oviposition (Ryan and Hurley, 2012). The two species are established as exotic invaders in Australia, New Zealand, South Africa, China, South America and North America (Adams et al., 2011; Li et al., 2015), and have gradually expanded their Australian geographic range, reaching southern (temperate) Queensland in 2009 (Carnegie and Bashford, 2012). Climate modelling predicts subtropical regions of Queensland will support *S. noctilio* establishment (Carnegie et al., 2006), suggesting the wasp is likely to continue to move north into valuable subtropical pine estates, where previously unencountered hosts, particularly southern pines (*Pinus elliottii* (Engelm) var. *elliottii* and *P. caribaea* var. *hondurensis* (Sénécl.) W.H. Barrett and Golfari) (Pinales: Pinaceae) and their hybrids, are grown in large-scale plantations (Gavran, 2014). No *Pinus* species are known to be resistant to Sirex, although the level of susceptibility varies between species (Ryan and Hurley, 2012): it is likely that these hybrids will be suitable hosts but uncertain how host characteristics may interact with biological control (Bedding, 2009).

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The parasitic nematode, *Deladenus* (=*Beddingia*) *siricidicola* Bedding (Tylenchida: Neotylenchidae), is the most effective biological control agent used in *S. noctilio* management worldwide, but shows very variable and inconsistent parasitism rates within and between regions (Hurley et al., 2007). Mechanisms behind the variation in biological control success using nematodes have not been clearly explained (Hurley et al., 2008; Slippers et al., 2012). Extremely low genetic diversity of *D. siricidicola* may affect their ability to adapt to novel environments and hosts (Mlonyeni et al., 2011), and aspects of the host trees themselves may also affect nematode survival (Bedding, 2009). The efficacy of the standard biological control program developed in winter rainfall regions appears to be reduced in different climatic conditions, as was the case in South America and South Africa (Slippers et al., 2012). Thus, there are concerns that if the woodwasp establishes in the subtropical pine estate in Australia, the current biological control practice may not be effective.

Deladenus siricidicola has a free-living fungal-feeding form that is mass-produced for commercial inoculation in Sirex biological control programs (Slippers et al., 2012). In proximity to Sirex larvae, female nematodes shift phase to a parasitic form that ultimately sterilises female Sirex by entering the ovaries and eggs, and is then transmitted (as the free-living, fungal-feeding form) during oviposition by the infected wasp (Slippers et al., 2012). The free-living form is thus spread naturally when infected *S. noctilio* females lay packets of nematodes in place of viable eggs into trees, and operationally by inoculating laboratory-cultured nematodes into felled trees. The success of the biological control program is influenced by interactions between wasp, nematodes and fungal strains (Morris et al., 2012; Slippers et al., 2015), other insects and fungi (Yousuf et al., 2014), climatic conditions (Hurley et al., 2007) and host tree characteristics (Bedding, 2009).

Loblolly pine, *Pinus taeda* L., native to SE USA, is grown extensively in South America and, along with *P. elliottii* is the most commonly planted commercial pine species in Brazil and Argentina (Klasmer and Botto, 2012) and the most susceptible to *S. noctilio* (Fenili et al., 2000; Iede et al., 2012). In this host, *S. noctilio* parasitism by *D. siricidicola* varies between 0 and 90% (Iede et al., 2012; Nahrung et al., 2015). *Pinus taeda* is grown commercially in southern Queensland and northern New South Wales, where it is readily attacked by *S. noctilio* (Carnegie et al., 2005), albeit less successfully than *P. radiata* (Nahrung et al., 2015). A commercial hybrid *Pinus* taxon, *Pinus elliottii* subsp. *elliottii* x *P. caribbea* var. *hondurensis*) (PEE × PCH) was developed in 1958 (Nikles, 1996) and is now widely grown in coastal plantation regions of south-eastern Queensland. The performance of Sirex wasp, fungus and nematode in this host is unknown, and hence the overall impact of the Sirex wasp in this host is impossible to predict. Here, we use field and laboratory studies, in the absence of *S. noctilio*, to compare performance and survival of both the *Amylostereum* fungus and the free-living mycetophagous form of *D. siricidicola* on these different host taxa. We also compare physical and chemical properties of the two hosts with potential to impact on the survival and development of nematodes and/or fungus, including moisture content, wood density, tracheid and bordered pit diameter.

2. Materials and methods

2.1. Nematode survival and development

2.1.1. Field trials

Field trials were established in a subtropical plantation (Beerburrum, Site 1, PEE × PCH F2 hybrids) and a higher altitude, temperate plantation (Passchendaele, Site 2, *P. taeda*) (Fig. 1) in Queensland, and intended to mimic (a) natural nematode spread by ovipositing females (i.e. into standing trees, n = 5/site) and (b) commercial inoculation into felled trees (n = 5/site). Because *S. noctilio* is not yet present in the subtropics (Site 1), surrogate techniques were used to introduce *A. amylostereum* and *D. siricidicola* into all trees. All trees were poisoned using standard trap tree plot techniques designed to stress trees and make them susceptible to *S. noctilio* oviposition (Haugen et al., 1990; Gitau et al., 2013) to provide conditions similar to those that nematodes would encounter either deposited by Sirex females (standing trees), or during artificial inoculation (felled trees). Ten trees at each site were poisoned with Dicamba (3,6-dichloro-2-methoxybenzoic acid) at a rate of 1 mL/10 cm circumference in March 2012. In May 2012, five trees at each site were felled, and five were left standing. Each tree was inoculated at four points (approximately 0, 90, 180 and 270 degrees around the stem) at each 1 m interval along the trunk for 4 m (i.e. 16 inoculation points per tree) with a slurry of nematode rearing media containing both nematodes and the *Amylostereum* fungus supplied by Ecogrow Environment Ltd. Nematodes were mixed to a concentration of approximately 1 million nematodes/500 mL of Amylostereum rearing slurry, and inoculated into the tree using the standard hammer punch method (Carnegie and Bashford, 2012), such that each inoculation point received approximately 995 nematodes. Samples were taken by removing a 4 cm diameter core around the inoculation point to a depth of 1 cm with a hole saw at each inoculation height (1–4 m) one and three months after inoculation. The outer bark was removed to minimise inclusion of any bark feeding nematodes, and then cores were soaked in 30 mL of tap water for 12 h and the supernatant examined under a dissecting microscope (40×) for the presence of nematodes. The proportion of cores per tree that scored positive for nematodes was calculated, and results for each sample time and treatment were compared using chi-square tests.

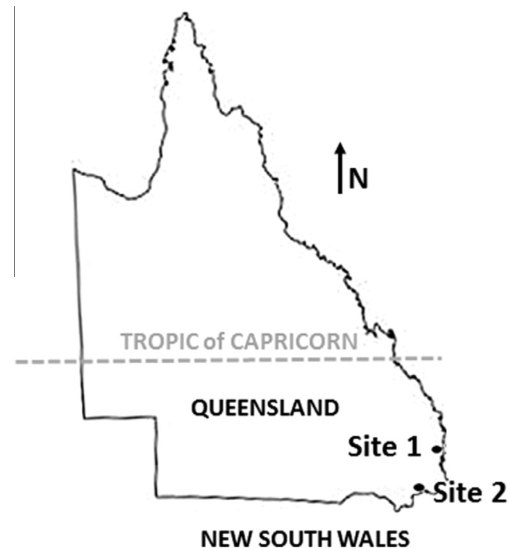

Fig. 1. Map of Queensland, Australia, showing the location of the two field sites. Site 1 (Beerburrum) comprises hybrid pine grown in the subtropics; Site 2 (Passchendaele) comprises *Pinus taeda* grown in higher altitude, temperate conditions.

2.1.2. Laboratory trials

To enable comparison between taxa under identical conditions, three inoculated billets from the top of each felled tree from the field trial above were taken to the laboratory immediately following inoculation, and stored in a controlled environment room (20 °C, 60% RH). One billet from each tree was destructively