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Parasitism of *Sirex noctilio* by non-sterilizing *Deladenus siricidicola* in northeastern North America

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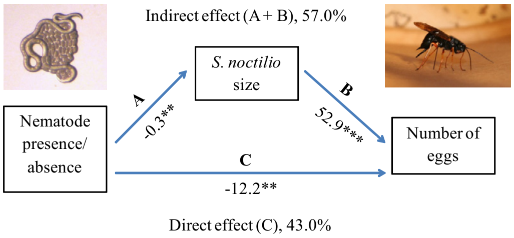
HIGHLIGHTS

- *S. noctilio* with non-sterilizing (NS) *D. siricidicola* produced fewer eggs.
- Fecundity was also mediated by the effect of NS *D. siricidicola* on host size.
- *S. noctilio* with NS *D. siricidicola* emerged earlier than unparasitized woodwasps.
- There was an inverse relationship between NS *D. siricidicola* and parasitoids.
- NS *D. siricidicola* was present in all sites and reached 57.7% parasitism.

GRAPHICAL ABSTRACT

Direct and indirect effects of parasitism by non-sterilizing *Deladenus siricidicola* nematodes on egg production in *Sirex noctilio*. Left photo: Mycophagous *D. siricidicola* with eggs Right photo: *S. noctilio female* drilling with ovipositor (Photos: E. Erin Morris).



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ABSTRACT

The parasitic nematode *Deladenus siricidicola* Bedding (Tylenchida: Neotylenchidae) has been extensively used for biological control of the wood wasp *Sirex noctilio* F. (Hymenoptera: Siricidae), an invasive pest of pine trees (*Pinus* spp.) in the Southern Hemisphere. The virulent strain of *D. siricidicola* used for biological control sterilizes *S. noctilio* females, although non-sterilizing strains of this nematode also occur. A non-sterilizing (NS) strain is established in the most recent invasion of *S. noctilio*, in northeastern North America. This study analyzed the effects of the NS strain of *D. siricidicola* on invasive *S. noctilio* collected from New York State and Pennsylvania. Nematode parasitism had a direct negative relationship on the number of eggs produced by the pro-ovigenic adult female *S. noctilio*. Nematode presence also negatively influenced the number of eggs indirectly, due to smaller size in nematode-parasitized females. Nematode-parasitized females produced an average of 29 fewer eggs than healthy females. On average, male and female nematode-parasitized *S. noctilio* emerged earlier from trees than healthy *S. noctilio*, and the size of female *S. noctilio* was not related to emergence date. *S. noctilio* with NS nematodes were found in 44.0% of trees and 26.9% of all individuals diagnosed, reaching 27.9 ± 26.0% parasitism (mean ± S.D.) when averaged across sites. There was greater parasitism of female *S. noctilio* than males. We also examined parasitism by hymenopteran parasitoids in association with parasitism by NS *D. siricidicola*. Parasitism by nematodes averaged 31.9 ± 35.4% per tree, while parasitism by hymenopteran parasitoids averaged 41.8 ± 19.6%. In terms of management, NS *D. siricidicola* may be less effective in providing biological control compared with sterilizing *D. siricidicola* or parasitic hymenopterans; the latter two natural enemies prevent all or most reproduction of *S. noctilio*, while the NS strain is primarily associated with a reduction in *S. noctilio* size and fecundity.

1. Introduction

The woodwasp *Sirex noctilio* F. (Hymenoptera: Siricidae) has been the focus of extensive biological control programs using the

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parasitic nematode *Deladenus siricidicola* Bedding (Tylenchida: Neotylenchidae) in the Southern Hemisphere, where this Eurasian woodwasp species was first introduced around 1900 (Slippers et al., 2012a). In northeastern North America there are three native species of *Sirex* (*Sirex nigricornis* F., *S. nitidus* (T.W. Harris) and *S. cyaneus* F.) (Schiff et al., 2012), and in 2004 the first individual of an established population of the pine-specialist (*Pinus* spp.) *S. noctilio* was collected in northeastern North America (Hoebeke et al., 2005). Within native ranges, siricid woodwasps perform an ecological role of decomposers (Middlekauff, 1960; Spradbery and Kirk, 1978). However, *S. noctilio* is the only species of Siricidae that is able to weaken and kill healthy trees where it has been introduced. Most female siricids carry a phytotoxic mucous and a symbiotic white rot fungus from the genus *Amylostereum* (Russulales: Amylostereaceae), which they inject into trees during oviposition. The fungus and mucous inhibit the tree vascular system as the wood dries to moisture levels suitable for larval and pupal development (Talbot, 1977). Larvae acquire digestive enzymes from the fungus as well as nutrition from wood decomposed by the fungus (Parkin, 1942; Kukor and Martin, 1983; Martin, 1987; Thompson et al., 2012), and early instars also eat fungal hyphae (Madden and Coutts, 1979). Adult *S. noctilio* do not feed; females are pro-ovigenic, and the size of adult females can be related to potential egg number (Madden, 1974; Fukuda et al., 1993).

The life cycle of the nematode *D. siricidicola* is closely linked with its hosts as well as the symbiotic fungus associated with *S. noctilio*, *Amylostereum areolatum* (Chaillet ex Fr.) Boidin. Both *S. noctilio* larvae and mycophagous *D. siricidicola* require *A. areolatum* within the host tree. *D. siricidicola* has a unique life history involving two different forms: mycophagous forms of *D. siricidicola* can survive in a tree while feeding on *A. areolatum* and then give rise to a parasitic form when in the vicinity of *S. noctilio* larvae. The parasitic form invades larval *S. noctilio* and then reproduces during host pupation. At high levels of parasitism, *D. siricidicola* has been documented killing the larval host, but more commonly the nematode remains quiescent until host pupation, when females reproduce and juveniles move to the reproductive organs (Zondag, 1969; Bedding, 1972).

The physiological effects of *D. siricidicola* on *S. noctilio* depend on the strains of both the nematode and the host (Bedding, 1972, 2009). Most research has been conducted on the sterilizing strains of *D. siricidicola* (e.g. Kamona and Sopron strains) which move within pupating female hosts to their ovaries and invade eggs, sterilizing the females. The other effects of the sterilizing nematode strains on *S. noctilio* include reductions in egg number and varying effects on *S. noctilio* size, including smaller and larger parasitized woodwasps as well as no size difference, comparing parasitized with unparasitized individuals (Bedding, 1972, 1979; Eskiviski et al., 2004; Hurley et al., 2008; Villacide and Corley, 2008). In males, *D. siricidicola* invade the testes, but only after sperm have moved to the seminal vesicles, so parasitism does not sterilize males. In addition, males do not transmit the nematode to females (Bedding, 1972). In Europe and Japan, where *D. siricidicola* is native (Bedding and Akhurst, 1978), there are non-sterilizing strains of the nematode, which do not enter siricid eggs but remain associated with female ovaries (Williams et al., 2009; Yu et al., 2009). Non-sterilizing (NS) *D. siricidicola* were also found in *S. noctilio* introduced to New Zealand (Zondag, 1975). While both types of nematode parasitism result in transportation of nematodes to new trees when*S. noctilio* oviposit, we hypothesized that whether this nematode is sterilizing or not would have vastly different impacts on *S. noctilio* population densities.

For classical biological control, a virulent strain of *D. siricidicola* was selected and release protocols were developed in Australia. Forest managers then introduced the nematode to areas where populations of *S. noctilio* were present (Slippers et al., 2012a). *S. noctilio* populations are reduced to low densities after this management approach; however, after 10 years populations are able to increase again, and *D. siricidicola* is often reintroduced augmentatively (Bedding, 2009; Carnegie and Bashford, 2012). Injecting *D. siricidicola* into trees containing healthy *S. noctilio* has been a very successful biological control approach in Australia, South America and New Zealand; parasitism levels of up to 100% have been reported in Australia (Zondag, 1969; Carnegie and Bashford, 2012). However, more recent introductions of *D. siricidicola* in the summer rainfall areas of South Africa have not been as successful overall, potentially due to low moisture levels within pine trees (Hurley et al., 2008).

The invasion by *S. noctilio* detected in northeastern New York State represents the first establishment of this invasive woodwasp in an area with native pine trees, *Sirex* species and associated communities. Although this invasion is not considered problematic for the small-scale pine industry in the north, there is concern that it will severely impair pine tree plantations in the southern United States when it expands its range southward as expected (Carnegie et al., 2006; Borchert et al., 2007; Yemshanov et al., 2009). In 2007–2008, a NS strain of *D. siricidicola* was found parasitizing *S. noctilio* in Ontario pine forests[1], and this strain is considered to have arrived with *S. noctilio* introduced to North America (Yu et al., 2009). The effects of NS *D. siricidicola* on *S. noctilio* in North America have not been examined.

Hymenopteran parasitoids are another group of natural enemies that have been used in biological control of *S. noctilio* in the Southern Hemisphere. Studies on controlling *S. noctilio* by introducing parasitoid wasps in the Southern Hemisphere have resulted in parasitism levels of 20–70% (Hurley et al., 2007; Cameron, 2012). These parasitoids are not host specific and parasitize an individual by laying eggs on or within the developing *Sirex* larva (Taylor, 1976). Some of the hymenopteran parasitoids introduced against *S. noctilio* in the Southern Hemisphere were collected from North America, where they are associated with native Siricidae (Taylor, 1976; Spradbery and Kirk, 1978; Murphy, 1998; Hajek and Morris, in press). In northeastern North America the most common hymenopteran parasitoids associated with *S. noctilio* are *Rhyssa lineolata* (Kirby), *R. persuasoria* (L.),*Megaryhssa nortoni* (Cresson) and *Ibalia leucospoides ensiger* Norton (Long et al., 2009; Ryan et al., 2012).

This study aimed to evaluate the amount of parasitism by the NS strain of *D. siricidicola* and the characteristics associated with nematode-parasitized *S. noctilio* in northeastern North America. Based on research on the sterilizing strains of *D. siricidicola*, we expected that nematode parasitism would be related to smaller females and fewer eggs than unparasitized females. Emergence timing of nematode-parasitized individuals was expected to be similar to the emergence timing of unparasitized *S. noctilio*. We also examined the association between nematode presence and parasitism by hymenopteran parasitoids on *S. noctilio*: we anticipated low levels of parasitism by hymenopterans where parasitism levels by nematodes were high and vice versa.

2. Materials and methods

2.1. Sampling

Twenty-five mature red pine (*Pinus resinosa* Sol. ex Aiton) and Scotch pine (*Pinus sylvestris* L.) trees with signs of siricid oviposition, as described by Hoebeke et al. (2005), were felled in June and July, 2011, from seven sites in New York State and northern Pennsylvania (Table 1). Sites in Pennsylvania were located toward

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[1] Williams et al. (2009) refer to this strain as the "North American strain".

Table 1
Site locations for mature red and Scotch pine trees from which *S. noctilio* and associated insects were reared in 2011. Not all *S. noctilio* were dissected for use in this study.

Site	Location	County	State	# Trees	Harvest date	*Pinus* species	*S. noctilio*	*S. nigricornis*
A	Arnot forest	Tompkins	NY	2	6 July	*P. sylvestris*	15	4
H	Triangle	Broome	NY	1	Collected by APHIS[a]	*P. resinosa*	473	0
HB	Heiberg forest	Onondaga	NY	2	20 June	*P. resinosa*	24	0
MC	Collected by APHIS[a]	Oswego or Onondaga	NY	1	Collected by APHIS[a]	*P. sylvestris*	34	0
MR	Tioga state forest	Tioga	PA	6	29 June	*P. resinosa*	60	1
P	Collected by APHIS[a]	Oswego or Onondaga	NY	3	Collected by APHIS[a]	Unknown[a]	115	0
V	Tioga state forest	Tioga	PA	10	29 June	*P. resinosa*	644	13
Total				25			1365	18

[a] These trees were obtained from USDA APHIS PPQ (Mattydale, NY, USA) and came from areas in Onondaga and Oswego Counties, but the exact locations or harvest dates are not known. In the ‘P’ site it is unknown whether trees were *P. resinosa* or *P. sylvestris*.

the southern edge of the *S. noctilio* range (NAPIS, 2013). Portions of trees with resin beads were cut into 70 cm long bolts and put into fiber barrels (77.5 cm tall × 51.4 cm diam.) with window screening covering the top. Numerous bolts were put in the same barrel, but bolts from different trees were never mixed. All emerging adult *Sirex* spp. and hymenopteran parasitoid were collected daily from 28 June to 26 October, 2011. Insects were kept individually in clear, plastic 29 mL cups with lids at 4 °C until processing. *Sirex* were identified according to Schiff et al. (2006) with revisions made by H. Goulet (personal communication); *Sirex edwardsii* Brullé records were subsequently changed to *S. nigricornis* according to Schiff et al. (2012). A total of 1365 *S. noctilio* (516 females, 849 males), 18 *S. nigricornis* and 800 hymenopteran parasitoids from the genera *Rhyssa, Megarhyssa* and *Ibalia* were collected. Emerging hymenopteran parasitoids were identified to genus by E.R. Hoebeke (Cornell University). Percent parasitism by hymenopterans was calculated using the total number of *Sirex* spp. collected per tree (not only *S. noctilio* because it is unknown which *Sirex* species has been parasitized) with the equation: % parasitism by Hymenoptera = Number of parasitoids/(*S. noctilio* + *S. nigricornis* + parasitoids)*100. The average number of hymenopteran parasitoids per tree was then calculated.

2.2. Sirex diagnosis

To examine parasitism by *D. siricidicola*, 253 female and 824 male *S. noctilio* were dissected. Female *S. noctilio* used for this study were not used in other experiments and had not mated or oviposited before they were dissected. As a metric of woodwasp size, the pronotum width of each *S. noctilio* female was measured (in mm) with a digital caliper. Pronotum width was used because this structure is sclerotized and does not change after the woodwasp dies (Madden, 1974). Pronotum widths for males were not measured. Males and females were then dissected at a magnification of 45X and the presence or absence of nematodes within the abdomens was documented. Ovaries and eggs were observed to detect nematode presence and locations in ovaries of females (within or outside of eggs) and eggs were counted. Nematode parasitism levels for male and female *S. noctilio* dissected were calculated per tree as: % nematode parasitism = number of nematode-parasitized individuals/total number of *S. noctilio* dissected*100. Nematode parasitism was then averaged for trees containing nematodes. *S. nigricornis* were not included in nematode analyses because of low numbers of specimens.

2.3. Molecular analysis of D. siricidicola

To confirm the strain of *D. siricidicola* present within *S. noctilio*, nematodes from 13 *S. noctilio* were randomly selected for molecular analysis using *in silico* RFLP, including at least one sample from each study site (Table 1). An additional sample of *D. siricidicola* was included for molecular analysis from the only *S. noctilio* female we found with nematodes present within the eggs. RFLP methods were based on use of AciI to cut a mtCO1 sequence, thus using the polymorphism described by Yu et al. (2009) to differentiate between Kamona and the non-sterilizing *D. siricidicola*. This method is described in detail in Morris et al. (2013) and more briefly below. *D. siricidicola* were sampled from the ovaries of parasitized female *S. noctilio*. Nematodes were preserved in 70% ethanol and kept at −20 °C until DNA isolation and extraction were performed. Vials containing nematodes were maintained in a water bath at 56 °C overnight for tissue lysis, then DNA was extracted using a QIAamp DNA Micro Kit (Qiagen, Valencia, CA). Primers and reaction conditions for PCR amplification of mtCO1 were as described in Ye et al. (2007). The samples were purified for sequencing using a QIAQuick PCR Purification Kit (Qiagen) according to manufacturer's instructions and eluted in double distilled water. PCR products were sequenced in both directions by the Core Laboratory Center (CLC) at Cornell University. The “noc173” strain of *D. siricidicola*, which is confirmed to be the NS strain (Morris et al., 2013), was included to compare the samples to a known strain. DNA sequences from the mtCO1 gene were subjected to *in silico* enzyme digestion with CodonCode Aligner (version 3.7.1) to search for the diagnostic restriction site patterns.

2.4. Statistical analyses

S. noctilio size (represented by pronotum width) and egg number were related for nematode-parasitized or unparasitized adult females through regression analysis using a general linear model (GLM; SAS 9.3, SAS Institute Inc., 2012). Nematode parasitism (presence or absence) was set as the predictor variable with egg number and pronotum width as the response variables. The interaction term size*eggs was analyzed to determine whether this relationship changed as a result of nematode infection, as other studies have found relationships between woodwasp size and egg number (Madden, 1974; Fukuda et al., 1993; Fukuda and Hijii, 1997). A second GLM analysis was performed to compare sizes of female *S. noctilio* within the same tree that were parasitized versus non-parasitized by NS *D. siricidicola*.

To further test the hypothesis that *S. noctilio* size influenced the relationship between nematode presence/absence and egg number, we performed the Sobel Test, a test of statistical mediation based on linear regression (Sobel, 1982). The Sobel Test yields a Wald test statistic and a *P* value, which describe whether a second predictor variable (woodwasp size) contributes to the relationship between the first predictor variable (nematode presence/absence) and the response variable (egg number). After using the Sobel test to confirm that mediation occurred, path analysis was used to determine the mediation coefficients and the variance accounted for by the direct effect of nematode parasitism on egg number and the indirect effect with size as an intermediate variable (see Wooton, 1994; Clough, 2012). Tests of statistical mediation were conducted using Mplus version 5.1 (Muthén and Muthén, 2011)

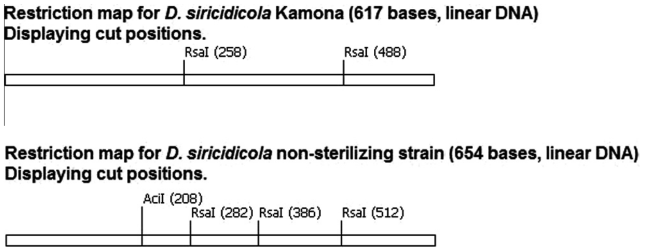

Fig. 1. Restriction enzyme map distinguishing the sterilizing *D. siricidicola* Kamona strain and the non-sterilizing *D. siricidicola* strain.

with a maximum likelihood estimation procedure. Indirect effects were tested using the product of coefficients technique with bias-corrected bootstrapping (based on 2000 draws) and 95% confidence intervals.

We used analysis of variance (ANOVA) to test the hypothesis that nematode parasitism was not related to earlier emergence date (in Julian days) in male and female *S. noctilio*. We performed linear regression to analyze the relationship between emergence date and female size. ANOVA was also used to compare parasitism percentages in males versus females. The relationship between percent nematode parasitism and percent parasitism by hymenopterans per tree was examined using linear regression. These tests were performed with the *car* package (Fox and Weisberg, 2011) of the software RStudio, R version 2.15.0 (R Development Core Team, 2012; RStudio, 2012).

3. Results

3.1. Identification of D. siricidicola

All *D. siricidicola* samples tested were classified as the NS strain using *in silico* restriction enzyme analysis of sequences of the mtCO1 gene. The combined presence of one AciI restriction site and three RsaI sites indicated all specimens were NS *D. siricidicola* (Fig. 1). All samples had identical sequences, and a reference sequence was submitted to GenBank (KC894733).

3.2. Parasitism by NS D. siricidicola

In males, all nematodes were found in the abdomen, within the hypertrophied testes. Nematodes were located in the ovaries of 113 females (outside of eggs) and were found within the eggs of one female, collected from the V site. However, molecular analysis of these nematodes identified the strain as NS. *D. siricidicola*. Nematodes were not found within the eggs of any other *S. noctilio* females collected from these sites.

NS *D. siricidicola* occurred in all sites and in *S. noctilio* from 11 of the 25 trees sampled (44.0%): six of nine trees in New York and five of 16 trees in Pennsylvania (Table 2). Parasitism of *S. noctilio* by NS *D. siricidicola* ranged from 0.8% to 57.7% of individuals per site. Average parasitism across all individuals diagnosed was 26.9%. NS *D. siricidicola* parasitism was consistently higher in female *S. noctilio* (45.9 ± 20.3%) than in males (23.4 ± 26.7%) ($F_{1,1076} = 58.14$, $P < 0.001$). Parasitism by NS nematodes averaged 27.9 ± 26.0% across all sites and 31.9 ± 35.4% per tree; 26.2 ± 26.0% in New York trees and 0.6 ± 1.6% in Pennsylvania trees.

3.3. NS D. siricidicola parasitism in S. noctilio females

On average, *S. noctilio* females parasitized by NS *D. siricidicola* had 79.6 ± 47.3 eggs, 29 fewer than the mean for unparasitized females (108.3 ± 55.5 eggs), and pronota were 0.3 mm smaller (3.2 ± 0.8 mm) on average than unparasitized females (3.5 ± 0.8 mm) (Fig. 2A and B). The GLM results were significant for the relationships between nematode presence/absence, pronotum width and egg number for *S. noctilio* females in all trees ($R^2 = 0.71$, $F_{2,251} = 312.6$, $P < 0.001$). NS *D. siricidicola* presence/absence was significantly related to pronotum width ($F_{1,252} = 563.6$, $P < 0.001$) and the number of eggs ($F_{1,252} = 10.8$, $P = 0.001$). The GLM interaction term between pronotum width and egg number was not significant ($F_{3,250} = 1.4$, $P = 0.24$), indicating that the effect of female size on egg number is not dependent on whether or not nematodes are present. Pronotum width was smaller in parasitized females than unparasitized females in trees containing both ($F_{1,202} = 4.8$, $P = 0.026$).

The Sobel test for statistical mediation results showed that both the direct effect of nematode parasitism and the indirect effect, with pronotum width as a mediator, are significant (Fig. 3). Path analysis showed that 57.0% of the variance in egg number can be explained indirectly, considering the relationship between nematode parasitism and *S. noctilio* size, and 43.0% of the variance can be explained by the path directly between nematode parasitism and egg number. Thus, one aspect of nematode parasitism is a reduction in the size of the insect, but there is also a direct relationship between nematode parasitism and reduced egg number which is not accounted for by body size.

3.4. NS D. siricidicola parasitism and emergence

Male and female *S. noctilio* parasitized by nematodes emerged earlier on average than unparasitized *S. noctilio*. The results of the ANOVAs were significant for all individuals (Root standard error (RSE) = 14.7, $F_{1,1076} = 320.8$, $P < 0.001$), males (RSE = 14.8, $F_{1,823} = 271.9$, $P < 0.001$) and females (RSE = 13.5, $F_{1,252} = 35.3$, $P < 0.001$) (Fig. 3A and B). However, the relationship between emergence time and female woodwasp size was not significant for all females (RSE = 0.8, $R^2 = 0.01$, $F_{1,252} = 2.82$, $P = 0.09$), nematode-parasitized females (RSE = 0.79, $R^2 = 0.03$, $F_{1,252} = 3.19$, $P = 0.07$) or for unparasitized females (RSE = 0.8, $R^2 = 0.01$, $F_{1,252} = 2.0$, $P = 0.16$) (Fig. 4).

3.5. Parasitism by hymenopterans and nematodes

Parasitic Hymenoptera associated with *Sirex* emerged from all 25 trees. *Ibalia* was the most abundant by far, accounting for 89.0% of parasitoids. Per tree, hymenopteran parasitoids ranged from 9.5% parasitism at the ‘P’ site to 68.0% at the ‘A’ site, with a mean of 41.2 ± 23.7% across all sites (Table 3). The linear equation fitting the relationship between percent parasitism by nematodes and hymenopteran parasitoids was: % nematodes = 35.56–0.63 * (% parasitoids) ($P = 0.01$, $F_{1,21} = 7.7$, adjusted $R^2 = 0.27$). Thus, the percentages of the two groups of natural enemies of *S. noctilio* appear to be inversely related see (Fig. 5).

4. Discussion

We found NS *D. siricidicola* to be widespread, in *S. noctilio* from all sites. NS *D. siricidicola* were found within the eggs of only one specimen in this study, and they have not been found within the eggs of any other *S. noctilio* females collected from these areas in New York or Pennsylvania. Parasitism by the NS strain was associated with smaller *S. noctilio* pronotum width and lower egg number compared with unparasitized females. The effects of *D. siricidicola* on *S. noctilio* depend on the strain of the nematode and host; the Kamona and Sopron strains of *D. siricidicola* selected for biological control can sterilize female *S. noctilio* without reducing *S. noctilio* size (Bedding, 1979), although other authors found a reduction (Eskiviski et al., 2004; Villacide and Corley, 2008) or an

Table 2
Average percent parasitism by *D. siricidicola* nematodes per tree in study sites.

Trees containing nematodes			Number of *S. noctilio*		Percent nematode parasitism		
Site	Number	Percent	Total diagnosed	With nematodes	Total	Females	Males
A	1	50.0	7	1	14.3	33.3	0.0
H	1	100.0	409	235	57.5	82.4	55.7
HB	1	50.0	16	2	12.5	33.3	11.0
MC	1	100.0	26	15	57.7	50.0	58.3
MR	1	16.7	43	1	2.3	50.0	0.0
P	2	66.7[a]	68	34	50.0[a]	53.6[a]	38.4[a]
V	4	40.0[a]	508	4	0.8[a]	18.8[a]	0.4[a]
Total	11		1077	292			
Mean		48.0			27.9 ± 26.0	45.9 ± 20.3	23.4 ± 26.7

[a] The means are calculated across all trees with *D. siricidicola* in the P and V sites.

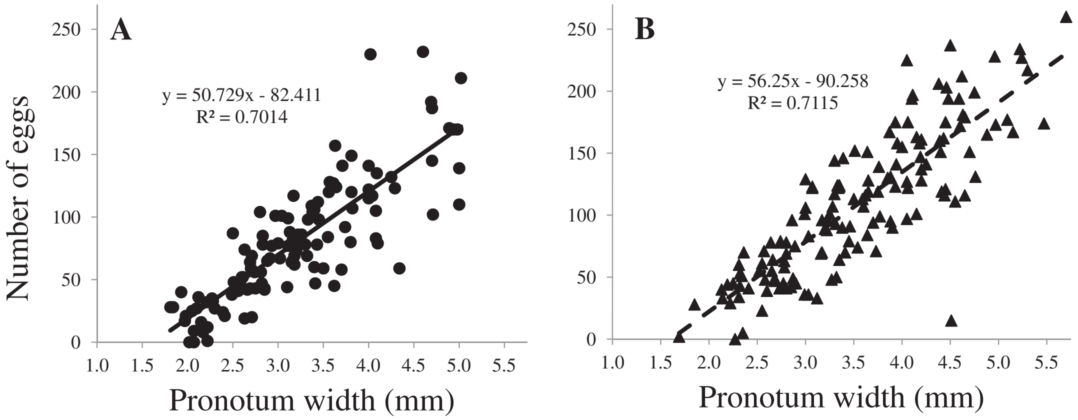

Fig. 2. Number of eggs as a function of size (pronotum width) in *S. noctilio* (A) with and (B) without parasitism by *D. siricidicola*.

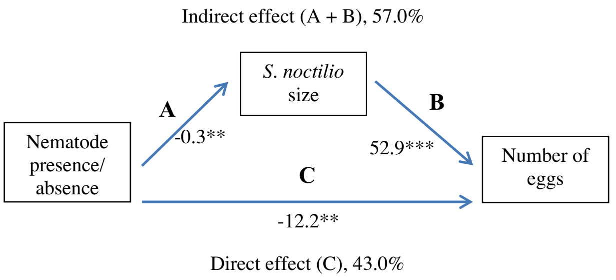

Fig. 3. Schematic diagram of *S. noctilio* size (measured by pronotum width) as a mediator for the effect of nematode parasitism on egg number. In the mediation analysis, paths A and B are analyzed for the indirect effect, while C shows the direct effect of nematode parasitism on egg number. Effect labels include the path and percentage of variance explained. Numbers below arrows indicate the coefficient for the path with asterisks for significance: $^{**}P < 0.01$, $^{***}P < 0.001$.

increase (Hurley et al., 2008) in the size of *D. siricidicola*-parasitized females, which may be a result of nematode dosage in trees (Bedding and Akhurst, 1978) or the strain of *S. noctilio*. The effectiveness of using sterilizing *D. siricidicola* as a biological control agent also relies on the dispersal of female *S. noctilio* and oviposition by unparasitized and sterile, parasitized *S. noctilio* females within the same tree. In contrast, the NS strain is associated with normal eggs and is thought to be injected into trees during oviposition by NS-parasitized females; thus, NS nematode dispersal can occur from females to their own offspring, and does not rely on oviposition of unparasitized individuals in the same trees as parasitized individuals. Bedding (1972) posited that NS *D. siricidicola* may have originated in solitary woodwasps or those present at low densities, when multiple females would not inject healthy eggs and nematodes into the same tree; in this case if the nematodes sterilized female *Sirex*, they would not be transported to another tree. Host sterilization by nematodes may be related to wood wasp density as well as the trade-off between virulence and dispersal. Since the continuation of the nematode population relies on dispersal and reproduction of *S. noctilio*, the NS strains may be more prevalent where sterilization of the *Sirex* host could lead to very low densities or local extinction of both the *Sirex* host and this parasite.

For *S. noctilio* parasitized by nematodes and without nematodes, we found linear relationships between egg number and size, as measured by pronotum width. Working in Tasmania, Madden (1974) also derived an equation for egg number and pronotum width in *S. noctilio*, but found different equations fit the relationship: $\log(\text{egg }\#) = 1.46 + 1.72 * (\text{pronotum width})$ and $\text{egg }\# = 28.84 * 52.5^{(\text{pronotum width})}$. The pronotum width vs. log (egg number) graph in Madden (1974) shows a pattern similar to the results of the present study, although the best regression equation we derived is with egg number as the response variable rather than the log of egg number. We assume that the *S. noctilio* described by Madden (1974) were not parasitized by nematodes based on the timing of nematode introduction in Tasmania (Hajek et al., 2005). Tasmanian *S. noctilio* described by Madden (1974) had a greater maximum size (7.3 mm pronotum width) and egg number (approx. 400) than the individuals in the present study (maxima of 5.7 mm and 260 eggs, respectively).

Parasitism by *D. siricidicola* is related to egg number directly and indirectly, as a function of female size in these populations of *S. noctilio*; this indicates an effect of the nematode on *S. noctilio* fecundity in northeastern North America. Females containing nematodes were smaller and had fewer eggs on average than unparasitized females. Eskiviski et al. (2004) and Villacide and Corley (2008) found smaller size and fewer eggs in *S. noctilio* parasitized by the Kamona strain of *D. siricidicola*. However, Hurley et al.

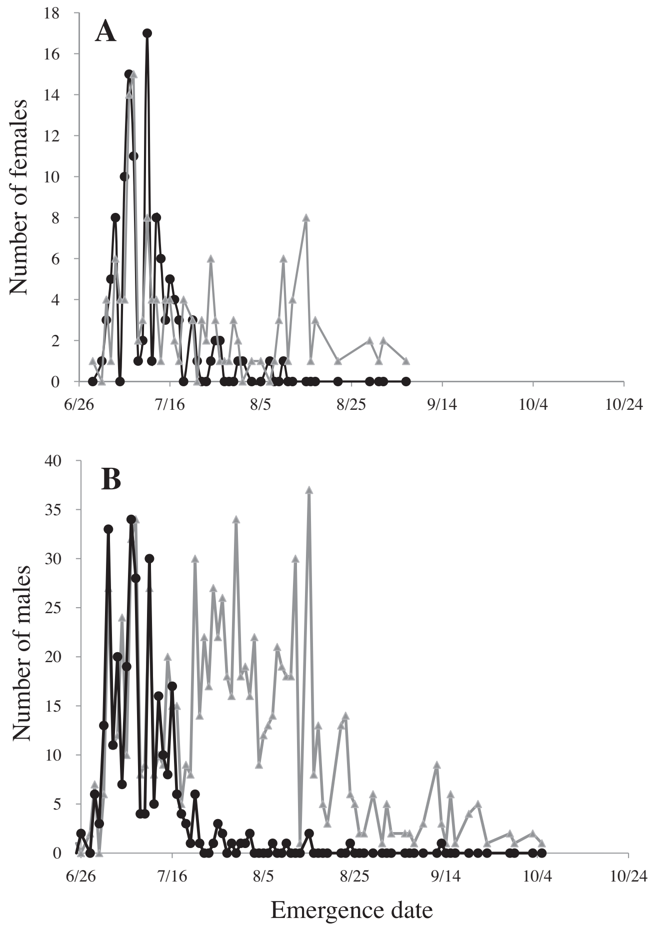

Fig. 4. Emergence dates of *S. noctilio* with and without nematode parasitism: (A) females and (B) males. Circles represent *S. noctilio* with nematode parasitism, triangles are without nematodes.

Table 3
Average parasitism by hymenopteran parasitoids per tree at each site.

Number					Mean parasitoid parasitism/tree (%)	SD parasitism/tree
Site	Trees	Ibalia	Rhyssa	Megarhyssa		
A	2	12	0	0	68.0	9.9
H	1	220	32	0	34.8	NA
HB	2	15	13	3	62.0	1.8
MC	1	5	0	0	12.8	NA
MR	6	63	9	0	60.0	21.2
P	3	14	3	0	9.5	5.4
V	10	385	24	2	41.0	8.6
Total	25	714	81	5		
Mean					41.2	23.7

(2008) found Kamona strain-parasitized female *S. noctilio* to be larger than unparasitized females. The Kamona strain was chosen for biological control because it sterilized eggs with little reduction in female size (Bedding, 1979), although the studies cited above indicate that this can vary by location. Fukuda and Hijii (1997) found that nematode-parasitized individuals of the siricid *Xeris spectrum* (L.) contained fewer eggs than unparasitized females. However, data from the latter study do not implicitly indicate an effect of nematodes on fecundity because female size was not considered as a mediating variable.

Several mechanisms could contribute to the direct effect of nematode parasitism on fecundity. Parasitism by NS *D. siricidicola* may affect *S. noctilio* egg number through consumption of fat stores, thereby depleting the energy available for egg production, as adult female *S. noctilio* do not feed. *S. noctilio* are pro-ovigenic, so the number of eggs should be related to the amount of fat stores the larvae possess. Little to no fat stores in nematode-parasitized adult male and female *S. noctilio* have been observed in these and other populations (Bedding, 1972; E.E.M., unpublished results). Depletion of fat stores in nematode-parasitized individuals could also affect woodwasp immune response, as hemocytes are produced from the fat body in many insect species (e.g. Strand, 2008; Castillo et al., 2011).

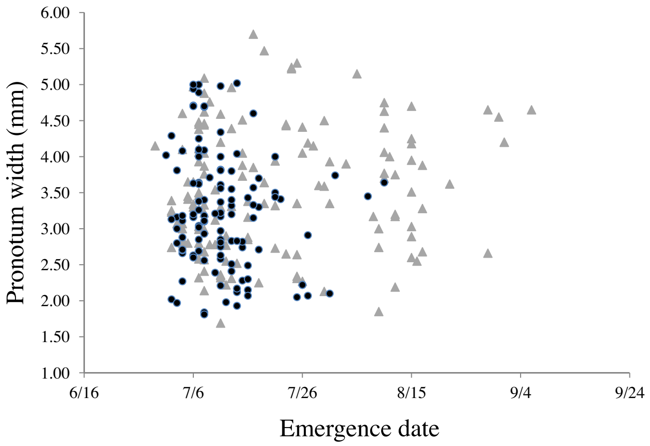

Fig. 5. Emergence dates of female *S. noctilio* versus pronotum size. Symbols are as in Fig. 4.

NS nematodes have been found in the present study as well as in *S. noctilio* in New Zealand (Zondag, 1975), in *X. spectrum* (Fukuda and Hijii, 1997) and *Sirex nitobei* (Bedding, 1972) in Japan, and in some Belgian *S. noctilio* that were not sterilized by the typically sterilizing Sopron strain of *D. siricidicola*, likely due to the strain of *S. noctilio* (Bedding, 1972). To date, nearly all *D. siricidicola* found in *S. noctilio* in New York and Pennsylvania have been within female ovaries but outside of the eggs, with the exception of one *S. noctilio* female from our 'V' site, in which *D. siricidicola* nematodes were found within eggs. This is the only occurrence of sterilization found in over 1000 dissections of *S. noctilio* females from this region of North America since 2008 (S.J.L. and A.E.H., unpublished data). However, molecular analysis revealed these nematodes to be the NS strain; this suggests that it is possible for the so-called "non-sterilizing" strain to rarely occur within *S. noctilio* eggs. This aspect of the relationship of *D. siricidicola* strains with *S. noctilio* in North America can be explored further using RFLP methods to differentiate between the NS and Kamona *D. siricidicola* strains. In this paper, we employed an RFLP method based on a polymorphism in CO1 (Morris et al., 2013) and another method based on a polymorphism associated with a microsatellite sequence has been described by Leal et al. (2012).

Nematode-infected females and males emerged earlier on average than unparasitized individuals. This finding is contrary to the observations of Fukuda and Hijii (1997) on *X. spectrum*, indicating that different species of Siricidae may exhibit varied responses to nematode infection. Early emergence of nematode-parasitized individuals in North American *S. noctilio* may indicate that these individuals hatched from the first eggs laid during the oviposition period of females or that they emerged sooner due to physiological stress. Stressed insects would also be smaller (e.g. Gullan and Cranston, 2005; Stoks et al., 2006; Diamond and Kingsolver, 2011). However, in our study early emergence did not appear to be associated with the size of females and therefore is not likely to be related to physiological stress in this population. *S. noctilio* that emerge earlier would have first access to new host trees, followed by later-emerging individuals. If emerging *S. noctilio* females are parasitized by nematodes, they would inoculate trees with nematodes, which could later parasitize other *S. noctilio* in the same tree.

Nematode-parasitized female *S. noctilio* in our study were smaller than unparasitized individuals. Smaller size could be considered an undesirable effect of nematode parasitism in terms of the impact of these nematodes as a biological control agent; the nematode will not be spread as far by smaller woodwasps as it would by larger individuals (Bedding, 1979). However, smaller size may contribute to controlling North American populations of *S. noctilio*, through smaller dispersal distances and lower reproductive potential than larger females on average (Madden, 1974; Villacide and Corley, 2008). Thus, parasitism by NS *D. siricidicola* could decrease the rate of range expansion of *S. noctilio.* In general, the threat of this invasive woodwasp in North America is high because healthy *S. noctilio* can fly as far as 100 km and have high reproductive potential (Bedding, 1979). *S. noctilio* are well suited to warm climatic regions, such as the southern United States, but can also survive the harsh winters of the northern U.S. (Haugen, 1991; Borchert et al., 2007; Bedding, 2009; Ryan et al., 2012). However, the spread of *S. noctilio* populations in the U.S. may be slowed by the activity of *D. siricidicola* and hymenopteran parasitoids as well as the relatively low density of host trees in the Northeast where *S. noctilio* was introduced (APHIS, 2008).

In contrast to other areas where *S. noctilio* has become invasive, in North America the woodwasp competes with native siricids and faces native natural enemies that have coevolved with these hosts and with pine trees. Hymenopteran parasitoids appear to have an important effect on the populations, with parasitism reaching 68.0% of *Sirex* spp. in this study. When released as biological control agents in the Southern Hemisphere, parasitism levels of hymenopteran parasitoids have ranged from 70% to 90% (Bedding, 1993; Nuttall, 1989), although the average in northeastern North America has been estimated around 20% in other studies (Long et al., 2009; Ryan et al., 2012; Zylstra and Mastro, 2012).

The average parasitism by NS *D. siricidicola* found in this study (26.9% of all individuals diagnosed) differs from levels reported in other studies performed in this region. Ryan et al. (2012) found parasitism by NS *D. siricidicola* in southern Ontario to be approximately 38% across all individuals dissected, and the percentage of nematode parasitism varied between 0% and 100% per tree. Zylstra and Mastro (2012) observed maximum parasitism by *D. siricidicola* of only 3.3% in northeastern New York. We found that nematode parasitism levels varied by tree and by site, which agrees with these studies. In particular, we found lower levels of NS *D. siricidicola* per tree for sites closer to the edge of the *S. noctilio* spread, in Pennsylvania.

In most sites in the present study, the percentage of NS *D. siricidicola*-parasitized *S. noctilio* tended to be lower at sites with a greater percent of parasitoids and vice versa. *Ibalia*, *Rhyssa* and *Megarhyssa* have been found to carry the nematode *Deladenus wilsoni* Bedding (Bedding and Akhurst, 1978), but dissected adult parasitoids collected in the present study were never found to contain *Deladenus* nematodes (E.E.M., unpublished results). However, in some parasitoid species, nematodes attacking hosts can kill parasitoid larvae (Kaya and Hotchkin, 1981; Battisti, 1994; Zaki et al., 1997), which has not been examined for these populations of hymenopteran parasitoids and *S. noctilio*. Rhyssines and other parasitoids detect the presence of the symbiotic fungus in the oviposition sites of *Sirex* to locate prey in order to deposit their eggs (Madden, 1968; Spradbery, 1970). It is possible that parasitoids are also able to detect nematodes within oviposition sites and avoid laying their eggs on or in nematode-parasitized *Sirex* larvae. Avoidance of nematode-parasitized prey is known for other species of Hymenoptera (Shannag and Capinera, 2000; Everard et al., 2009) and specifically for ichneumonids (Lacey et al., 2003; Harvey and Griffin, 2012), although not all hymenopteran parasitoids avoid prey containing nematodes (Sher et al., 2000). For biological control of*S. noctilio,* parasitoids have a greater impact than NS *D. siricidicola* because they kill the individual, while NS nematodes are only associated with a reduction in fecundity and adult size, the latter of which would decrease dispersal capabilities (Villacide and Corley, 2008). However, nematodes may also kill larval parasitoid wasps or deter adults from ovipositing, thereby reducing the impact of parasitoids on *S. noctilio* populations; further study of relationships among natural enemies attacking *S. noctilio* is necessary to better understand interactions.

Introduction of the sterilizing strain of *D. siricidicola* has been considered for biological control of *S. noctilio* populations in North America (Williams et al., 2012). *Deladenus* nematodes are oligoxenic, specific to a group of hosts rather than a single species, although they tend to be associated with one species of fungal symbiont (Bedding and Akhurst, 1978), and there is concern about non-target effects of the sterilizing nematode. Some degree of hybridization has been observed between different species of *Deladenus* (Akhurst, 1975), so there is certainly potential for the sterilizing and NS strains of *D. siricidicola* to hybridize (Williams et al., 2012). If hybridization occurs, the resulting strain may be less virulent, which would render biological control using *D. siricidicola* less effective (Slippers et al., 2012b). Other considerations about introduction of the sterilizing strain for biological control include the effects of low temperature on nematode population growth and high temperature thresholds, compatibility of the sterilizing strain with the *A. areolatum* strain native in northeastern North America, and whether or not the sterilizing strain of *D. siricidicola* will effectively sterilize the *S. noctilio* that was introduced to North America (Williams et al., 2012), which at present appears to consist of an unknown number of introductions from Europe and the Southern Hemisphere (Boissin et al., 2012). The interactions between parasitoid hymenopterans and *D. siricidicola* will also be important to understand if the sterilizing strain of the nematode affects the influence of parasitoid wasps through interference or competition.

5. Conclusions

NS *D. siricidicola* has been found to be widespread in New York and Pennsylvania. NS *D. siricidicola* may not have the capacity to make eggs inviable, but the nematode is associated with a reduction in the size and fecundity of females. In areas where NS *D. siricidicola* is well-established, it can reach high levels of parasitism in *S. noctilio* populations. The activity of this nematode and hymenopteran parasitoids may be contributing to reductions in *S. noctilio* population growth and could potentially affect range expansion. Knowledge about the relationships between hymenopteran parasitoids, *S. noctilio,* native siricids, and NS *D. siricidicola* as well as *D. siricidicola* strains used in biological control in northeastern North America will be important to designing plans for management and control of this invasive woodwasp.

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References

Akhurst, R.J., 1975. Cross-breeding to facilitate the identification of *Deladenus* spp., nematode parasites of wood wasps. Nematologica 21, 267–272.

APHIS, 2008. Proposed program for the control of the wood wasp *Sirex noctilio* F. (Hymenoptera: Siricidae) in the northeastern United States. <http://www.aphis.usda.gov/plant_health/ea/downloads/SirexEA-final-northeast.pdf> (Date accessed: 10.02.12).

Battisti, A., 1994. Effects of entomopathogenic nematodes on the spruce web-spinning sawfly *Cephalcia arvensis panzer* and its parasitoids in the field. Biocontrol Sci. Technol. 4, 95–102.

Bedding, R.A., 1972. Biology of *Deladenus siricidicola* (Neotylenchidae) an entomophagous-mycetophagous nematode parasitic in siricid wood wasps. Nematologica 18, 482–493.

Bedding, R.A., 1979. Manipulating the entomophagous-mycetophagous nematode, *Deladenus siricidicola*, for biological control of the wood wasp *Sirex noctilio* in Australia. In: Current Topics in Forest Entomology. USDA-Forest Service Gen. Tech. Rep. WO-8, pp. 144–147.

Bedding, R.A., 1993. Biological control of *Sirex noctilio* using the nematode *Deladenus siricidicola*. In: Bedding, R.A., Akhurst, R.J., Kaya, H.K. (Eds.), Nematodes and the Biological Control of Insect Pests. CSIRO Publishing, Hobart, pp. 11–20.

Bedding, R.A., 2009. Controlling the pine-killing wood wasp, *Sirex noctilio* with nematodes. In: Hajek, A.E., Glare, T.R., Callaghan, M. (Eds.), Use of Microbes for Control and Eradication of Invasive Arthropods. Springer, Dordrecht, pp. 213–235.

Bedding, R.A., Akhurst, R.J., 1978. Geographical distribution and host preferences of *Deladenus* species (Nematoda: Neotylenchidae) parasitic in siricid wood wasps and associated hymenopterous parasitoids. Nematologica 24, 286–294.

Boissin, E., Hurley, B., Wingfield, M.J., Vasaitis, R., Stenlid, J., Davis, C., DeGroot, P., Ahumada, R., Carnegie, A., Goldarazena, A., Klasmer, P., Wermelinger, B., Slippers, B., 2012. Retracing the routes of introduction of invasive species: the case of the *Sirex noctilio* wood wasp. Mol. Ecol. 21, 5728–5744.

Borchert, D., Fowler, G., Jackson, L., 2007. Organism pest risk analysis: risk to the conterminous United States associated with the wood wasp, *Sirex noctilio* Fabricius, and the symbiotic fungus, *Amylostereum areolatum* (Fries: Fries) Boidin. USDA-APHIS-PPQ-CPHST-PERAL (Rev. 1).

Cameron, E.A., 2012. Parasitoids in the management of *Sirex noctilio*: looking back and looking ahead. In: Slippers, B., de Groot, P., Wingfield, M.J. (Eds.), The Sirex Wood Wasp and Its Fungal Symbiont. Springer, Dordrecht, pp. 103–117.

Carnegie, A.J., Bashford, R., 2012. Sirex wood wasp in Australia: current management strategies, research and emerging issues. In: Slippers, B., de Groot, P., Wingfield, M.J. (Eds.), The Sirex Wood Wasp and Its Fungal Symbiont. Springer, Dordrecht, pp. 175–202.

Carnegie, A.J., Matsuki, M., Haugen, D.A., Hurley, B.P., Ahumada, R., Klasmer, P., Sun, J., Iede, E.T., 2006. Predicting the potential distribution of *Sirex noctilio* (Hymenoptera: Siricidae), a significant exotic pest of *Pinus* plantations. Ann. For. Sci. 63, 119–128.

Castillo, J.C., Reynolds, S.E., Eleftherianos, I., 2011. Insect immune responses to nematode parasites. Trends Parasitol. 27, 537–547.

Clough, Y., 2012. A generalized approach to modeling and estimating indirect effects in ecology. Ecology 93, 1809–1815.

Diamond, S.E., Kingsolver, J.G., 2011. Host plant quality, selection history and trade-offs shape the immune responses of *Manduca sexta*. Proc. R. Soc. B 278, 289–297.

Eskiviski, E.R., Faraldo, G., De Coll, O.R., 2004. Effects of the nematode Beddingia (=Deladenus) siricidicola parasitism in adult stage of wood wasp Sirex noctilio, In: Proceedings of the Conference the 11th Jornadas Técnicas Forestales y Ambientales, Monte Carlo.

Everard, A., Griffin, C.T., Dillo, A.B., 2009. Competition and intraguild predation between the braconid parasitoid *Bracon hylobii* and the entomopathogenic nematode *Heterorhabditis downesi*, natural enemies of the large pine weevil, *Hylobius abietis*. Bull. Entomol. Res. 99, 151–161.

Fox, J., Weisberg, S., 2011. AR Companion to Applied Regression, Second ed. Sage, Thousand Oaks.

Fukuda, H., Hijii, N., 1997. Delayed emergence and reduced body-weight of *Xeris spectrum* (Hymenoptera: Siricidae) due to nematode parasitism. J. For. Res. 2, 183–186.

Fukuda, H., Kajimura, H., Hijii, N., 1993. Fecundity of the wood wasp, *Sirex nitobei* Matsumura, in relation to its body size. J. Jpn. Forest. Soc. 75, 405–408.

Gullan, P.J., Cranston, P.S., 2005. The Insects – An Outline of Entomology, third ed. Blackwell Publishing, Malden.

Hajek, A.E., Morris, E.E. *Sirex noctilio*, in: Van Driesche, R.G. (Ed.), Saving Trees, Saving Forests: Use of Classical Biological Control to Preserve Native U.S. and Canadian Forests, USDA, Forest Service, FHTET, (in press).

Hajek, A.E., McManus, M.L., Delalibera Jr., I., 2005. Catalogue of Introductions of Pathogens and Nematodes for Classical Biological Control of Insects and Mites. USDA, Forest Service, FHTET-2005-05.

Harvey, C.D., Griffin, C.T., 2012. Host activity and wasp experience affect parasitoid wasp foraging behaviour and oviposition on nematode-parasitized larvae of the forestry pest *Hylobius abietis*. Ecol. Entomol. 37, 269–282.

Haugen, D.A., 1991. Woodchip sampling for the nematode *Deladenus siricidicola* and the relationship with the percentage of *Sirex noctilio* parasitized *Pinus radiata* plantations. Aust. Forest. 54, 3–8.

Hoebeke, E.R., Haugen, D.A., Haack, R.A., 2005. *Sirex noctilio*: discovery of a palearctic siricid wood wasp in New York. Newslett. Michigan Entomol. Soc. 50, 24–25.

Hurley, B.P., Slippers, B., Wingfield, M.J., 2007. A comparison of control results for the alien invasive wood wasp, *Sirex noctilio*, in the southern hemisphere. Agric. For. Entomol. 9, 159–171.

Hurley, B.P., Slippers, B., Croft, P.K., Hatting, H.J., van der Linde, M., Morris, A.R., Dyer, C., Wingfield, M.J., 2008. Factors influencing parasitism of *Sirex noctilio* (Hymenoptera: Siricidae) by the nematode *Deladenus siricidicola* (Nematoda: Neotylenchidae) in summer rainfall areas of South Africa. Biol. Control 45, 450–459.

Kaya, H.K., Hotchkin, P.G., 1981. The nematode *Neoaplectana carpocapsae* Weiser and its effect on selected ichneumonid and braconid parasites. Environ. Entomol. 10, 474–478.

Kukor, J.J., Martin, M.M., 1983. Acquisition of digestive enzymes by siricid wood wasps from their fungal symbiont. Science 220, 1161–1163.

Lacey, L.A., Unruh, T.R., Headrick, H.L., 2003. Interactions of two idiobont parasitoids (Hymenoptera: Ichneumonidae) of codling moth (Lepidoptera: Torticidae) with the entomopathogenic nematode *Steinernema carpocapsae* (Rhabditida: Steinermatidae). J. Invertebr. Pathol. 83, 230–239.

Leal, I., Foord, B., Davis, C., de Groot, P., Mlonyeni, X.O., Slippers, B., 2012. Distinguishing isolates of *Deladenus siricidicola*, a biological control agent of *Sirex noctilio*, from North America and the Southern Hemisphere using PCR-RFLP. Can. J. For. Res. 42, 1173–1177.

Long, S.J., Williams, D.W., Hajek, A.E., 2009. *Sirex* species (Hymenoptera: Siricidae) and their parasitoids in *Pinus sylvestris* in eastern North America. Can. Entomol. 141, 153–157.

Madden, J.L., 1968. Behavioural responses of parasites to the symbiotic fungus associated with *Sirex noctilio* F. Nature 218, 189–190.

Madden, J.L., 1974. Oviposition behaviour of the wood wasp, *Sirex noctilio* F. Aust. J. Zool. 22, 341–351.

Madden, J.L., Coutts, M.P., 1979. The role of fungi in the biology and ecology of wood wasps (Hymenoptera: Siricidae). In: Batra, L.R. (Ed.), Insect-Fungus Symbiosis, Allanheld. Osmun & Co., Montclair, pp. 165–174.

Martin, M.M., 1987. Acquired enzymes in the siricid wood wasp *Sirex cyaneus*. In: Martin, M.M. (Ed.), Invertebrate–Microbial Interactions. Cornell University Press, Ithaca, pp. 37–48.

Middlekauff, W.W., 1960. The siricid wood wasps of California. Bull. California Insect Surv. 6, 59–77.

Morris, E.E., Kepler, R.M., Long, S.J., Williams, D.W., Hajek, A.E., 2013. Phylogenetic analysis of *Deladenus* nematodes parasitizing northeastern North American *Sirex* species. J. Invertebr. Pathol. 113, 177–183.

Murphy, S.T., 1998. Indigenous siricid spp. parasitoid communities and principal biological control agents of *Sirex noctilio* in Australasia: a review. USDA, Forest Service FHTET 98–13, 31–35.

Muthén, L.K., Muthén, B.O., 2011. Mplus User's Guide, sixth ed. Muthén & Muthén, Los Angeles.

NAPIS (National Agricultural Pest Information System), 2013. "Survey Status of Sirex Wood wasp – *Sirex noctilio* (2010 to present)". Purdue University Published: 01/08/2013. <http://pest.ceris.purdue.edu/map.php?code=ISBBADA&year=3year> (Date accessed: 01.14.13).

Nuttall, M.J., 1989. *Sirex noctilio* F., sirex wood wasp (Hymenoptera: Siricidae). In: Cameron, P.J., Hill, R.L., Bain, J., Thomas, W.P. (Eds.), A review of biological control of pests and weeds in New Zealand 1974–1987, Technical Communication No. 10. C.A.B. International & DSIR, Wallingford, pp. 299–306.

Parkin, E.A., 1942. Symbiosis and siricid wood wasps. Ann. Appl. Biol. 29, 268–274.

R Development Core Team, 2012. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org> (Date accessed: 11.01.12).

RStudio, 2012. RStudio: Integrated development environment for R (Version 0.96.122) [Computer software]. Boston, MA. <http://www.rstudio.org/> (Date accessed: 11.1.12).

Ryan, K., De Groot, P., Nott, R.W., Drabble, S., Ochoa, I., Davis, C., Smith, S.M., Turgeon, J.J., 2012. Natural enemies associated with *Sirex noctilio* (Hymenoptera: Siricidae) and *S. nigricornis* in Ontario, Canada. Environ. Entomol. 41, 289–297.

SAS Institute Inc., 2012. What's New in SAS® 93 and SAS® Analytical Products 12.1. SAS Institute Inc., Cary (p. 354).

Schiff, N.M., Valley, S.A., LaBonte, J.R., Smith, D.R., 2006. Guide to the siricid wood wasps of North America. USDA Forest Service FHTET-2006-15.

Schiff, N.M., Goulet, H., Smith, D.R., Boudreault, C., Wilson, A.D., Scheffler, B.E., 2012. Siricidae (Hymenoptera: Symphyta: Siricoidea) of the Western Hemisphere. Canadian Journal of Arthropod Identification 21. <http://www.biology.ualberta.ca/bsc/ejournal/sgsbws_21/sgsbws_21.html> (Date accessed: 12.06.12).

Shannag, H.K., Capinera, J.L., 2000. Interference of *Steinernema carpocapsae* (Nematoda: Steinernematidae) with *Cardiochiles diaphaniae* (Hymenoptera:

Braconidae), a parasitoid of melonworm and pickleworm (Lepidoptera: Pyralidae). Environ. Entomol. 29, 612–617.

Sher, R.B., Parrella, M.P., Kaya, H.K., 2000. Biological control of the leafminer *Liriomyza trifolii* (Burgess): Implications for intraguild predation between *Diglyphus begini* Ashmead and *Steinernema carpocapsae* (Weiser). Biol. Control 17, 155–163.

Slippers, B., de Groot, P., Wingfield, M.J., 2012a. The Sirex Wood wasp and its Fungal Symbiont: Research and Management of a Worldwide Invasive Pest. Springer, Dordrecht.

Slippers, B., Hurley, B.P., Mlonyeni, X.O., Groot, P., Wingfield, M.J., 2012b. Factors affecting the efficacy of *Deladenus siricidicola* in biological control systems. In: Slippers, B., de Groot, P., Wingfield, M.J. (Eds.), The Sirex Wood wasp and its Fungal Symbiont. Springer, Dordrecht, pp. 119–133.

Sobel, M.E., 1982. Asymptotic confidence intervals for indirect effects in structural equation models. In: Leinhardt, S. (Ed.), Sociological Methodology. American Sociological Association, Washington, D.C., pp. 290–312.

Spradbery, J.P., 1970. Host finding by *Rhyssa persuasoria* (L.), an ichneumonid parasite of siricid wood wasps. Anim. Behav. 18, 103–114.

Spradbery, J.P., Kirk, A.A., 1978. Aspects of the ecology of siricid wood wasps (Hymenoptera: Siricidae) in Europe, North Africa and Turkey with special reference to the biological control of *Sirex noctilio* F. in Australia. Bull. Entomol. Res. 68, 341–359.

Stoks, R., De Block, M., McPeak, M.A., 2006. Physiological costs of compensatory growth in a damselfly. Ecology 87, 1566–1574.

Strand, M.R., 2008. The insect cellular immune response. Insect Sci. 15, 1–14.

Talbot, P.H.B., 1977. The *Sirex-Amylostereum-Pinus* association. Annu. Rev. Phytopathol. 15, 41–54.

Taylor, K.L., 1976. The introduction and establishment of insect parasitoids to control *Sirex noctilio* in Australia. Biol. Control 21, 429–440.

Thompson, B.M., Grebenok, R.J., Behmer, S.T., Gruner, D.S., 2012. Microbial symbionts shape the sterol profile of the xylem-feeding wood wasp, *Sirex noctilio*. J. Chem. Ecol. (8 Dec. DOI: 10.017/s10886-012-0222-7).

Villacide, J.M., Corley, J.C., 2008. Parasitism and dispersal potential of *Sirex noctilio*: implications for biological control. Agric. For. Entomol. 10, 341–345.

Williams, D.W., Zylstra, K.E., Mastro, V.C., 2009. Biological control of *Sirex noctilio* with *Beddingia siricidicola*: 2008 update. In: Proceedings 20th US Department of Agriculture Interagency Research Forum on Invasive Species. Gen Tech Rep NRS-P-51 USDA Forest Service, Newtown Square.

Williams, D.W., Zylstra, K.E., Mastro, V.C., 2012. Ecological considerations in using *Deladenus* (=*Beddingia*) *siricidicola* for the biological control of *Sirex noctilio* in North America. In: Slippers, B., de Groot, P., Wingfield, M.J. (Eds.), The *Sirex* Wood wasp and Its Fungal Symbiont. Springer, Dordrecht, pp. 135–148.

Wooton, T.J., 1994. Predicting direct and indirect effects: an integrated approach using experiments and path analysis. Ecology 75, 151–165.

Ye, W., Giblin-Davis, R.M., Davies, K.A., Purcell, M.F., Scheffer, S.J., Taylor, G.S., Center, T.D., Morris, K., Thomas, W.K., 2007. Molecular phylogenetics and the evolution of host plant associations in the nematode genus *Fergusobia* (Tylenchida: Fergusobiinae). Mol. Phylogenet. Evol. 45, 123–141.

Yemshanov, D., McKenney, D.W., de Groot, P., Haugen, D., Sidders, D., Joss, B., 2009. A bioeconomic approach to assess the impact of an alien invasive insect on timber supply and harvesting: a case study with *Sirex noctilio* in eastern Canada. Can. J. For. Res. 39, 154–168.

Yu, Q., de Groot, P., Leal, I., Davis, C., Ye, W., Foord, B., 2009. Characterization of *Deladenus siricidicola* (Tylenchida: Neotylenchidae) associated with *Sirex noctilio* (Hymenoptera: Siricidae) in Canada. Int. J. Nematol. 19, 23–32.

Zaki, F.N., Awadallah, K.T., Gesraha, M.A., 1997. Competitive interaction between the braconid parasitoid *Meteorus rubens* Nees and the entomogenous nematode *Steinernema carpocapsae* (Weiser) on larvae of *Agrotis ipsilon* Hufn. (Lep., Noctuidae). J. Appl. Entomol. 121, 151–153.

Zondag, R., 1969. A nematode parasitism of *Sirex noctilio* (F.) in New Zealand. NZ J. Sci. 12, 732–747.

Zondag, R., 1975. A non-sterilising strain of *Deladenus siricidicola*, In: Forestry Research Institute Report 1974, Rotorua, pp. 51–52.

Zylstra, K.E., Mastro, V.C., 2012 12, 83, insectscience.org/12.83.