

Association of the native parasitic nematode *Deladenus proximus* with individuals and populations of the native woodwasp *Sirex nigricornis*

Saskya van Nouhuys^{1,3}  | David C. Harris¹ | Fred M. Stephen² |
Larry D. Galligan² | Ann E. Hajek¹ 

¹Department of Entomology, Cornell University, Ithaca, New York, USA

²Department of Entomology, University of Arkansas, Fayetteville, Arkansas, USA

³Centre for Ecological Sciences, Indian Institute of Science, Bangalore, India

Correspondence

Ann E. Hajek, Department of Entomology, Cornell University, 6126 Comstock Hall, 129 Garden Ave., Ithaca, New York 14853-2601, USA
Email: aeh4@cornell.edu

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Abstract

1. Parasitic stages of the North American native dimorphic nematode *Deladenus proximus* develop within larvae of the native North American siricid *Sirex nigricornis* and sterilize many of the eggs in adult females. *Sirex nigricornis* were reared from felled trees or trapped in Arkansas and Louisiana in 2017–2019 and parasitism by *D. proximus* was evaluated.
2. *Deladenus proximus* parasitized 0.16–0.43 of *S. nigricornis* individuals, among sites and years. Parasitized males were smaller than healthy males, and more males were parasitized than females emerging from the same trees.
3. Rate of parasitism increased with increasing *S. nigricornis* density per tree, and, as per tree density increased, so did the proportion that was male.
4. Egg sterilization (woodwasp eggs killed and inhabited by nematodes) was partial, with an average proportion of 0.66–0.88 woodwasp eggs sterilized by *D. proximus* per female *S. nigricornis*, differing significantly by site. As the numbers of eggs in a female increased, the proportion of eggs sterilized declined.
5. Comparing data from this study with others, a trend is reported for variable levels of egg sterilization in *Sirex* females, with 0 or 100% sterilization often occurring in novel host/parasite associations and partial (averages between 50% and 100%) sterilization occurring when host/parasite associations have co-evolved.

KEYWORDS

biological control, density dependence, forest entomology, invasive species, nematode parasitism, rate of parasitism, Siricidae, woodwasp

INTRODUCTION

Woodwasps (Family Siricidae) are sawflies whose larvae develop within trees while eating fungal-digested wood. Most woodwasps develop in dead and dying trees, but the European woodwasp *Sirex noctilio* F. is an exception in that it can develop in weakened trees. This Eurasian pine specialist invaded and significantly damaged exotic pine plantations in the Southern Hemisphere, beginning in approximately 1900 in New Zealand and then continued to cause economic damage as it spread to Australia, South America and

South Africa (Hurley et al., 2007). *Sirex noctilio* was first found in northeastern North America in 2004 and has been spreading in North America since then (Liebhold & Hajek, 2021). Because of its importance to forestry, much more is known of the biology and ecology of *S. noctilio* than other woodwasps, and we often rely on this when initiating and interpreting studies of other woodwasp species. *Sirex* females are accompanied by obligate fungal mutualistic symbionts: white rot fungi in the genus *Amylostereum*. For *S. noctilio*, *Amylostereum areolatum* (Chaillat ex Fr.) Boidin is vectored by females and then grows within pines where it is required to assist

S. noctilio in overcoming tree defences as well as facilitating larval nutrition (Krivak-Tetley & Hajek, 2021).

Biological control programs have been developed for managing the invasive *S. noctilio* in the Southern Hemisphere, including introductions of the parasitic nematode, *Deladenus siricidicola* Bedding (Hajek & Morris, 2021a). *Deladenus siricidicola* is dimorphic, with a mycophagous stage that feeds on the *S. noctilio* fungal symbiont, and a parasitic stage that lives within siricid larvae and adults. The mycophagous stage allows the nematodes to increase and disperse within trees to find *S. noctilio* larvae to parasitize. The parasitic nematode stage increases within woodwasp larvae and then can invade and kill eggs when female *S. noctilio* have pupated (referred to as egg sterilization). Nematodes are then vectored during oviposition, when adult female *S. noctilio* deposit nematode-filled eggs and accessory fluid filled with nematodes into trees. *Deladenus siricidicola* parasitism of adult female *S. noctilio* also reduces the size and number of woodwasp eggs produced (Bedding, 1972).

Other species within the woodwasp genus *Sirex* are also parasitized by species of *Deladenus* nematodes (Bedding & Akhurst, 1978; Hajek & Morris, 2021b). Because *D. siricidicola* has been found parasitizing other *Sirex* species and an associated beetle (Bedding & Akhurst, 1978), non-target impacts are of concern; there is interest in North America in potentially using a native North American species of *Deladenus* for biological control of *S. noctilio*, should it become an economically important pest. The goal for biological control would be high prevalence of parasitism in the host woodwasp population and high levels of egg sterilization within woodwasp individuals, as occur in Australia with *D. siricidicola* and *S. noctilio*. However, we need to know more about the biology of native *Deladenus* in North America. Therefore, researchers have been investigating *Deladenus proximus* Bedding associated with the native pine specialist in eastern North America, *Sirex nigricornis* F., that can co-infest pines with *S. noctilio* (Hajek et al., 2017). Extensive sampling (Hartshorn, Fisher, et al., 2016; Morris et al., 2020; Zieman et al., 2015) across the southeast and east as well as specifically in Arkansas and Louisiana has determined that *D. proximus* is the only *Deladenus* species parasitizing *S. nigricornis* in that region; *S. noctilio* does not occur in Arkansas and Louisiana at this time.

Deladenus proximus was described in 1974 from a male *S. nigricornis* collected in South Carolina, USA (Bedding, 1974). This dimorphic nematode species is also known to parasitize *S. noctilio* (Morris et al., 2013) and has a mycophagous stage that feeds either on *A. areolatum* or *A. chailletii* (Pers.) Boidin (Morris et al., 2014). Both of these fungi are vectored by *S. nigricornis* (Hajek et al., 2013). Studies of *D. proximus* parasitizing *S. nigricornis* have documented between 0% and 44% of the individuals in the population parasitized in five southeastern and mid-western states, with highly variable egg sterilization (Fisher et al., 2017; Hartshorn, Chase, et al., 2016; Zieman et al., 2015) (Table 1).

We conducted this study to investigate parasitism of female and male *S. nigricornis* by *D. proximus* in native host/parasite associations within southeastern US pine forests. Using trap-caught females, and females and males reared in the lab from naturally infested wood, we investigated the prevalence (proportion of *S. nigricornis* individuals

parasitized) of the nematode in *S. nigricornis* populations at different sites, among trees that varied in *S. nigricornis* density within a site, and between male and female *S. nigricornis*. Using just the females, we measured the proportion of eggs sterilized and its variation related to site and number of eggs in the ovaries. We discuss our findings with respect to what is known about population prevalence and egg sterilization by different *Deladenus* species in relation to co-evolved versus novel woodwasp/nematode associations.

MATERIALS AND METHODS

Sites and trapping methods

For this study, *S. nigricornis* were sampled from pine forests in Arkansas and Louisiana. The two sites in Arkansas, here called Wedington and Hard Mile, were located in recently thinned stands of shortleaf pine, *Pinus echinata* Mill., in Ozark National Forest (Table 2). The site in Louisiana, Hunt Plywood, was on the edges of stands of loblolly pine, *Pinus taeda* L., adjacent to a wood processing facility. Traps used to catch females in Arkansas (2017–2018) and Louisiana (2019) were 125 litre plastic trash cans containing freshly cut pine bolts and foliage, with the base of an intercept panel trap sealed to a matching opening cut in the lid of the trash can (Hartshorn, Galligan, et al., 2016). Traps were visited every 3–4 days to collect living female *S. nigricornis*, which were then maintained in individual vials at 4°C until dissection. Traps were checked from early October to early December. All trapped *S. nigricornis* were females, totalling 550 individuals.

Laboratory rearing from naturally infested wood

During October and November 2016 and 2017, seven and four smaller, suppressed *P. echinata* were felled, respectively, at the Wedington Management area of Ozark National Forest. Felled trees remained in the forest where they were naturally attacked by *S. nigricornis* during that same fall. In February or March of the following year, these felled trap trees were cut into 75 or 100 cm bolts, yielding 10.5 ± 1.6 (mean \pm SE) bolts/tree (range: 5–17), with the number of bolts/tree depending on tree height. The bolts were placed individually in ventilated 113.6 litre plastic trash cans, large plastic boxes, or wooden cabinets located indoors. They were checked three times each week for emergence, which began in early July and ended in late October. Emerging *S. nigricornis* were placed in individual vials and stored at 4°C until dissection. A total of 1351 reared *S. nigricornis* males and females were included in the study.

Evaluation of parasitization and egg sterilization by *D. proximus*

The size of *S. nigricornis* was quantified using the pronotum width (Kroll et al., 2013). To record the presence of nematodes, *S. nigricornis*

TABLE 1 Sterilization of siricid eggs for different host/*Deladenus* combinations, including co-evolved associations and associations that are presumed to be novel or for which the history of association is unknown

<i>Deladenus</i> species	Source of <i>Deladenus</i>	Siricid species	Novel or co-evolved association ^a	Location	% <i>Sirex</i> parasitized	% of eggs sterilized/ female	Reference
<i>D. siricidicola</i>	Sopron, Hungary from <i>S. juvencus</i> (or re-isolate made in Tasmania = Kamona)	<i>S. noctilio</i>	Novel?	Tasmania	Usually >70%	≈100%	Bedding, 1972; Bedding & Akhurst, 1974
<i>D. siricidicola</i>	Commercially available Kamona strain	<i>S. noctilio</i>	Isolated from <i>S. noctilio</i>	Queensland, Australia	24–54%	Partial	Nahrung et al., 2015
<i>D. siricidicola</i>	Native	<i>S. noctilio</i>	Co-evolved	Galicia, Spain	36.7%	≈90%	Lombardero et al., 2016
<i>D. siricidicola</i>	?	<i>S. cyaneus</i>	Co-evolved	?	?	≈100% ^b	Bedding, 1974
<i>D. siricidicola</i>	?	<i>S. juvencus</i>	Co-evolved	?	?	≈100% ^b	Bedding, 1974
<i>D. siricidicola</i>	Native	<i>S. nitobei</i>	Co-evolved	Japan	?	0% ^b	Bedding, 1972
<i>D. siricidicola</i> ^a	Accidental introduction	<i>S. noctilio</i> ^a	Unknown	North Island, New Zealand	?	0%	Zondag, 1974
<i>D. siricidicola</i> ^a	Accidental introduction	<i>S. noctilio</i> ^a	Unknown	New York, USA	27.9%	0%	Kroll et al., 2013
<i>D. siricidicola</i> ^a	Accidental introduction	<i>S. noctilio</i> ^a	Unknown	Southern Ontario, Canada	avg. 50%; 1 site 95%	0%	Yu et al., 2009
<i>D. siricidicola</i> ^a	Accidental introduction	<i>S. nigricornis</i>	Unknown	Southern Ontario, Canada	24%	0%	Haavik, Yu, et al., 2016
<i>D. proximus</i>	Native	<i>S. nigricornis</i>	Co-evolved	Louisiana, USA	7.5% (2009); 1.1% (2010); 4.6% (2011)	100%	Zieman et al., 2015
<i>D. proximus</i>	Native	<i>S. nigricornis</i>	Co-evolved	Illinois, USA	23.5% (2011); 33.3% (2012)	100%	Zieman et al., 2015
<i>D. proximus</i>	Native	<i>S. nigricornis</i>	Co-evolved	South Carolina, USA	31.3%	100%	Zieman et al., 2015
<i>D. proximus</i>	Native	<i>S. nigricornis</i>	Co-evolved	Arkansas & Louisiana	16–43%	79%	This paper
<i>D. proximus</i>	Native	<i>S. nigricornis</i>	Co-evolved	Arkansas	16.3% (2009); 39.3% (2010)	61.5% (2009); 66.3% (2010) ^c	Fisher et al., 2017
<i>D. proximus</i>	Native	<i>S. nigricornis</i>	Co-evolved	Arkansas & Mississippi	12–31%	78–90%	Hartshorn, Chase, et al., 2016
<i>D. nevexii</i>	Native	<i>Xeris</i> spp.	Co-evolved	?	?	100% ^b	Bedding, 1974
<i>D. nevexii</i>	Native	<i>Sirex cyaneus</i>	Co-evolved	?	?	0% ^b	Bedding, 1974
<i>D. nevexii</i>	Native	<i>Urocerus californicus</i>	Co-evolved	?	?	25–75% ^b	Bedding, 1974
<i>D. imperialis</i>	Native	<i>S. imperialis</i>	Co-evolved	Pakistan	?	0% ^b	Bedding, 1974

^aExact invasive sources of *S. noctilio* and *D. siricidicola* have not been identified despite international surveys (Bittner et al., 2017; Boissin et al., 2012; Fitza et al., 2019; Morris et al., 2020).

^bDetailed data not available.

^cValues estimated from Fisher et al., 2017.

were then dissected in Syracuse watch glasses following the methods described by Thomsen and Harding (2011). Nematodes were found within or surrounding the reproductive tissues and in the hemocoels of both males and females, and, in females, nematodes could also be within the eggs. Ovaries were removed from the females and placed in a separate dish containing 2–3 mL of insect Ringer's solution (7.5 g NaCl, 0.35 g KCl, 0.21 g CaCl₂, 1 L distilled water), and all eggs were counted for subsamples of females from each collection date at each site (Table S1). For a subsample of the parasitized females in which total eggs had been counted, eggs were examined using 63x magnification with bottom illumination to quantify the proportion of eggs containing nematodes. Under these conditions, live nematodes were visible moving inside of *S. nigricornis* eggs. Any egg that did not appear to contain living nematodes was torn open with fine-tipped forceps for confirmation. If the female contained fewer than 100 eggs, all eggs were examined for nematodes but if she contained more than 100 eggs, only 100 eggs were randomly chosen and evaluated. Using this evaluation method, an average of $77.4 \pm 2.4\%$ of eggs per female ($n = 131$) were evaluated for sterilization (Table S1).

Data analysis

The rate of nematode parasitism in *S. nigricornis* populations was analysed using the trapped and reared woodwasps from Arkansas (Hard Mile site and Wedington sites 2017, 2018) and Louisiana (Hunts Plywood site, 2019). All wasps were used to assess parasitism, except the majority of reared males from Wedington in 2018, which were destroyed before they could be evaluated for parasitism. We used a logistic regression model, with presence of nematode parasitism (yes or no) as a response variable. The predictor variables were site, and year within site for the sites sampled two years.

The association of parasitism with number of eggs in the ovaries was analysed using the subset of female wasps from which the number of eggs was counted ($n = 716$ females, Table S1). A standard least squares model was used, with egg number as the response variable, and nematode parasitism (yes/no), pronotum size, pronotum size \times nematode parasitism, site and year within site for the sites sampled two years as predictor variables.

The proportion of sterilized eggs in female *S. nigricornis* was compared among sites and years using a standard least squares model, using the subset of data for which we had counted and evaluated the eggs in the ovaries for nematode parasitism ($n = 131$ females, Tables 3 and S1). The predictor variables were site, year nested within site where the site was sampled over two years, numbers of eggs in the ovaries and Julian date. The interactions, including the interaction of site and egg number, did not significantly contribute to the model and so were removed.

Analyses on the basis of woodwasp sex were conducted using the lab-reared male ($n = 776$) and female ($n = 360$) *S. nigricornis* that emerged in 2017 from the seven trees from the Wedington site. The wasps collected in the same way the following year (emerging 2018) were not included because most of the males were destroyed before being assessed for parasitism. We also could not use the trapped individuals to compare nematodes by sex because only female woodwasps fly to the traps. A standard least squares model was used to analyse the association of wasp size with the explanatory variables sex, density of wasps in the tree of emergence and Julian date. A logistic regression model was used to analyse parasitism status (presence of nematodes as yes or no) with respect to the explanatory variables sex, pronotum size, pronotum size \times sex, tree and Julian date. Using this same subset of data, we analysed the relationship between number of *S. nigricornis* emerging from a tree and the sex ratio (proportion of *S. nigricornis* male), using linear regression.

TABLE 2 Sample site locations

Years	State	Site	GPS
2017–2018	Arkansas	Wedington Management Area, Ozark National Forest	36°08'22.03" N 94°26'31.41" W
2017–2018	Arkansas	Hard Mile, Ozark National Forest	35°36'25.28" N 94°18'14.69" W
2019	Louisiana	Hunt Plywood, nr Kisatchie National Forest	31°35'44.38" N 92°24'58.62" W

TABLE 3 The numbers of parasitized and unparasitized female *S. nigricornis* and the number of females dissected to assess rate of egg sterilization in each sample

Site-year	Number of <i>S. nigricornis</i> parasitized by <i>D. proximus</i>		Number of parasitized female <i>S. nigricornis</i> for which egg sterility was quantified
	No	Yes	
Hard Mile (AR) 2017	136	53	15
Hard Mile (AR) 2018	54	40	40
Hunt Plywood (LA) 2019	201	37	36
Wedington (AR) 2017	290	71	16
Wedington (AR) 2018	133	59	24

All of the statistical analyses were done using the statistical software package JMP version 15 (2021), and the residuals in the models were checked and found to be distributed normally.

RESULTS

Prevalence of nematodes in wasp populations and egg sterilization

On average, the proportion of wasps parasitized by *D. proximus* was $0.30 \pm \text{SE } 0.03$. This varied between sites ($\chi^2 = 30.42$, $p < 0.0001$), and years within sites that were sampled over two years ($\chi^2 = 9.67$, $p < 0.046$), from 0.16 at Hunt Plywood in Louisiana in 2019 to 0.43 at Hard Mile in Arkansas in 2018 (Figure 1).

Parasitized females contained fewer eggs than unparasitized females (mean number of eggs: 144.04 ± 6.09 vs. 183.58 ± 5.72 , respectively; $F_{1,716} = 54.95$; $P < 0.0001$). This difference increased for especially small wasps (interaction of parasitism with pronotum size $F_{1,716} = 15.59$; $P < 0.0001$). The proportion of eggs sterilized within an individual female parasitized by *D. proximus* on average was 0.79 ± 0.02 and ranged from 0.37 to 1.0 among individuals (two outliers had only 0.1 egg sterilization). This differed between sites ($F_{2,123} = 4.53$; $p = 0.013$), with individuals from Hard Mile being sterilized at a higher rate (0.87 ± 0.02) than those from Hunt Plywood (0.76 ± 0.04) or Wedington (0.70 ± 0.04) (planned least squares

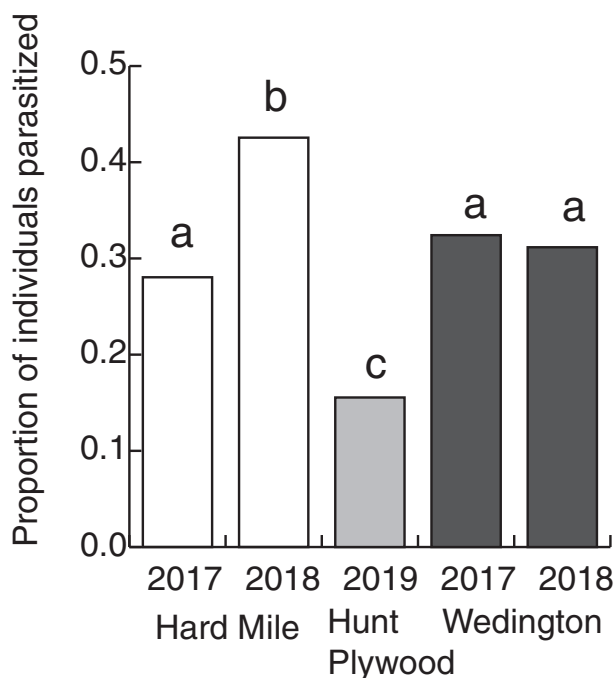


FIGURE 1 The proportion of *S. nigricornis* parasitized by *D. proximus* at each site. Those marked with different letters differ significantly at $P < 0.01$ in a logistic regression model of the relationship of parasitism with collection site and year within collection site (see Data Analysis section)

contrast, $F_{1,123} = 6.98$; $p = 0.009$) (Figure 2). Within the sites evaluated over two years, there was no difference in sterilization between years ($F_{2,123} = 1.89$; $p = 0.155$). As the number of eggs in the ovaries

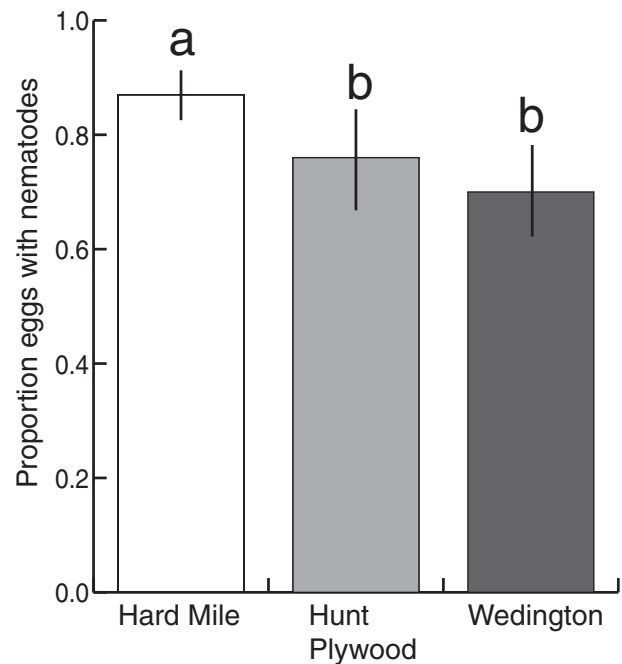


FIGURE 2 (a) The mean (SE) proportion of eggs sterilized in *S. nigricornis* at each site. Those marked with different letters differ significantly in the full least squares regression model at $p = 0.009$ (see Data Analysis section)

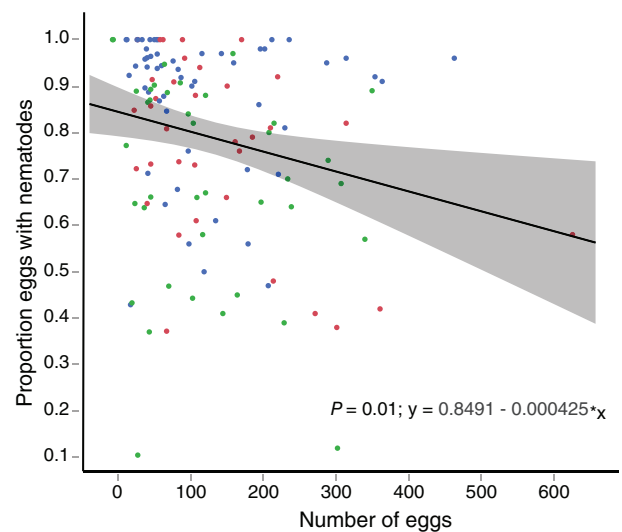


FIGURE 3 The relationship between proportion of eggs sterilized and number of eggs in a female *S. nigricornis*. Each point is one female from Hunt Plywood (red), Hard Mile (blue) and Wedington (green). The shaded band is the 95% confidence interval for the regression line. The negative association is significant at $p = 0.009$ in the full least squares regression model and does not differ between sites

increased, the proportion of them parasitized decreased ($F_{1,123} = 7.02$; $p = 0.009$) (Figure 3), which did not differ significantly between sites (no interaction of site with egg number). Overall 100% of eggs were sterilized in just 15% of the females sampled, while 42% had >90% sterilization.

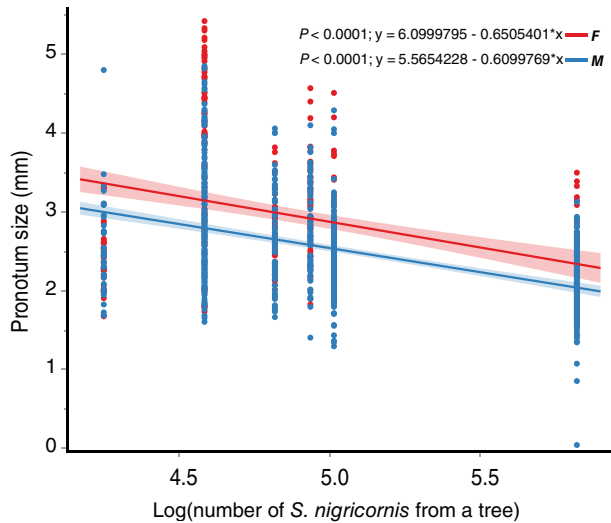


FIGURE 4 Linear regression of female (red) and male (blue) *S. nigricornis* pronotum size and the log of the number of *S. nigricornis* emerging from each tree. The shaded bands are the 95% confidence intervals for the regression lines. The negative association of size with density is significant at $P < 0.0001$ in the full least squares regression for both males and females (see Data Analysis section)

Sirex nigricornis sex, density and nematode parasitism by tree

Among *S. nigricornis* reared from naturally infested pines in 2017, a higher fraction of the population was male (0.69) than female. Males were smaller than females (mean female pronotum width = 2.90 ± 0.05 mm; male = 2.46 ± 0.02 mm) ($t = 10.37$; $P < 0.0001$). Both male and female wasps were larger when developing in trees at a lower density of *S. nigricornis* ($t = 20.45$; $P < 0.0001$) (Figure 4), and smaller wasps emerged later in the season than earlier emerging larger woodwasps ($t = 13.00$; $P < 0.0001$).

Deladenus proximus parasitism of males was much higher than females ($\chi^2 = 31.19$; $P < 0.0001$) (males: 38.3%; females: 19.2%). Although parasitized males were slightly smaller than healthy males (2.32 ± 0.03 mm vs. 2.56 ± 0.03 mm), this trend did not hold for parasitized females (which leads to an interaction between sex and woodwasp size; $\chi^2 = 5.05$; $p = 0.024$, with respect to parasitism). Both parasitized and unparasitized woodwasps emerged throughout the season, with parasitized woodwasps emerging on average two days later than unparasitized woodwasps ($\chi^2 = 22.65$; $P < 0.0001$).

Among the trees from which *S. nigricornis* were reared, parasitism varied ($\chi^2 = 57.50$; $P < 0.0001$), with a range of 0 to 0.42 of the emerging woodwasp parasitized per tree (mean = 0.27 ± 0.04). In addition, there was a strong positive association of parasitism with host density in a tree ($\chi^2 = 7.40$; $p = 0.006$) (Figure 5a), such that none of the *S. nigricornis* emerging from the tree with the lowest host density ($n = 68$) were parasitized, and the tree with the greatest *S. nigricornis* emergence ($n = 327$) had the greatest rate of parasitism.

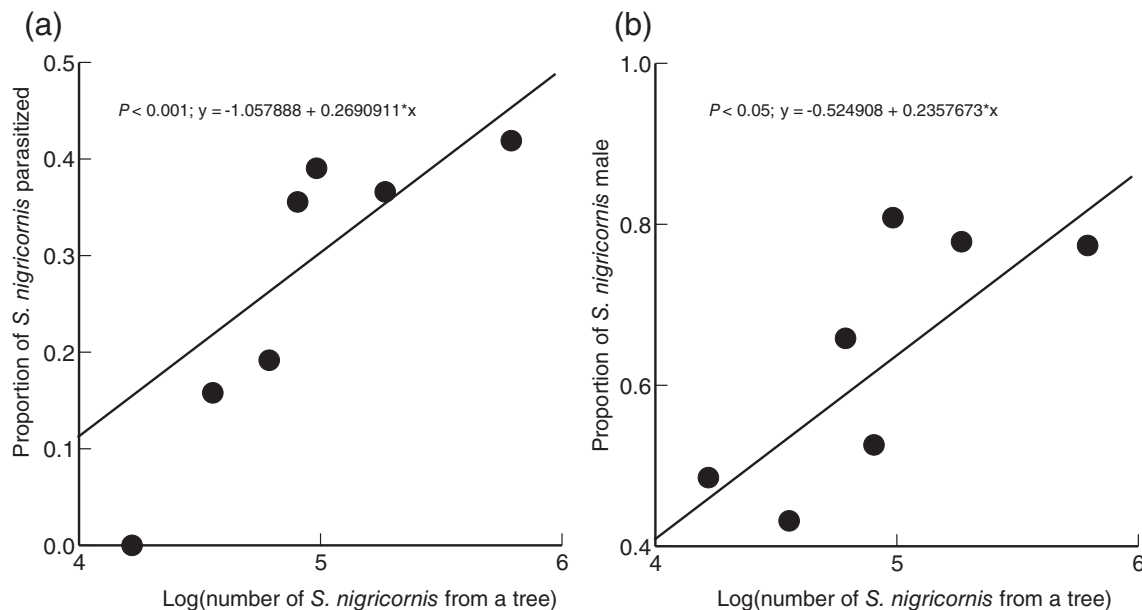


FIGURE 5 Linear regression of the a) proportion of *S. nigricornis* parasitized by *D. proximus* and b) proportion of the sample that is male, for the individuals emerging from trees at Wedington in 2017. Each point represents one tree. Equations refer to regression lines drawn in the figures but overall results for panel a) in this study are based on the full logistic regression models (see Data Analysis section) ($\chi^2 = 7.40$; $p = 0.006$)

Similarly, the proportion of the woodwasps emerging that were male was positively associated with the total number of *S. nigricornis* emerging from a tree (Figure 5b) ($r^2 = 0.58$; $p = 0.05$).

DISCUSSION

Fraction of the population parasitized, fecundity and proportion of eggs sterilized

In this study, approximately a third of naturally occurring *S. nigricornis* were parasitized by *D. proximus*, ranging from 0.16 to 0.43 of individuals between sites and years (Figure 1). Previous studies conducted in five southeastern and midwestern states reported parasitism of *S. nigricornis* by *D. proximus* ranging from 0% to 44% (Fisher et al., 2017; Hartshorn, Chase, et al., 2016; Zieman et al., 2015). Thus, it appears that less than 50% of *S. nigricornis* are parasitized by *D. proximus* under natural conditions. The only other report quantifying the level of parasitism of a native siricid by a native nematode is from low-density populations of *S. noctilio* in Galicia, Spain (Lombardero et al., 2016), where a total of 36.8% parasitism by *D. siricidicola* occurred across 248 *S. noctilio*. It is notable that in both the present study and the study by Hartshorn, Chase, et al. (2016), parasitism by *D. proximus* from Arkansas was greater than *D. proximus* from Louisiana. How conditions at sites in Arkansas versus Louisiana differentially impacted *D. proximus* could be explored further. For example, some sites included in the present study differed in tree species. The impact of tree species for this host/parasite system is not known. However, Nahrung et al. (2016) found that tree species can impact performance of *D. siricidicola* on *S. noctilio* in Australia. In the present study, the Arkansas sites were in highlands or mountains, and >450 km from the Louisiana site, which was in the south-central plains. Therefore, weather would have differed to some extent between sites. Differential weather could have had an impact on the nematodes as Yousuf et al. (2014) found that temperature could influence *S. noctilio*/*D. siricidicola* relations.

We found that parasitized *S. nigricornis* females had on average 21% fewer eggs than unparasitized females, a general trend also reported by Hartshorn, Chase, et al. (2016). Among the parasitized females, an average of 79% of their eggs contained nematodes. For the purposes of biological control, high levels of parasitism and egg sterilization in the host population would be advantageous, leading quickly to a steep decline of the host population. For instance, when developing *D. siricidicola* for biological control of *S. noctilio* in Australia, parasite prevalence was close to 100% of the host population, and parasitized female *S. noctilio* were 100% sterilized, that is, all eggs in a parasitized female woodwasp had been killed and contained juvenile nematodes to be vectored (Bedding & Akhurst, 1974) (Table 1). Bedding and Akhurst (1978) stated that *D. siricidicola* parasitized both *S. noctilio* and *S. juvencus* in Europe and the strain of *D. siricidicola* introduced to Australia originated from *S. juvencus*, from Hungary. This strain was released in Australia against a strain of *S. noctilio* of uncertain origin, so there is a possibility the high parasitism

and sterilization recorded there was a newly associated host/pathogen combination (i.e., the *S. noctilio* that had been introduced had not come from Hungary, where *D. siricidicola* infected both *S. noctilio* and *S. juvencus*).

However, for a natural parasite population to persist, there must be some unparasitized hosts surviving each generation (Montovan et al., 2015). This would necessarily occur if not all eggs within individual females are sterilized, so that each mother contributes both healthy offspring and nematodes to a new tree. Alternatively, if females are entirely sterilized, but the prevalence of parasitism in the host population is low enough and multiple females oviposit in the same tree, nematodes from infected females can disperse within the tree to find healthy larvae from different mothers (Zondag, 1974). The average sterilization of *S. nigricornis* eggs by *D. proximus* has been over 50% in all studies to date (Table 1). When 20–30 eggs were examined per female, some parasitized females had 70–100% sterilized eggs (Fisher et al., 2017; Hartshorn, Chase, et al., 2016). In our study, where 100 eggs were examined per female (or all of the eggs, if there were fewer than 100), two females had 10% sterilization, while all others had $\geq 37\%$. Thus, results from these studies found that *D. proximus* usually only partially sterilizes a female *S. nigricornis* ensuring that juvenile nematodes emerging from woodwasp eggs have the chance to encounter healthy sibling larvae to parasitize and thus disperse to other trees. In stark contrast, Zieman et al. (2015) found 100% sterilization in all eggs examined from 112 females from six US state x year combinations (with low sample sizes from half). Curiously, in Galicia, Spain, sterilization of *S. noctilio* by *D. siricidicola* was reported as $\approx 90\%$ (Lombardero et al., 2016) and perhaps that still provides enough healthy eggs for survival of nematode lineages. Quantifying levels of sterilization across studies of native systems, sterilization seems to average above 60% (Table 1).

Researchers have reported that dimorphic *Deladenus* show three different patterns of egg sterilization, 0%, partial and 100%. Bedding (1972) reported that *D. siricidicola* juveniles were present in virtually all eggs within each parasitized female of *S. noctilio* in Australia, but when the same strain of *D. siricidicola* parasitized *Sirex nitobei* F. from Japan, no eggs were sterilized. This lack of any sterilization is also the situation with the introduced strain of *D. siricidicola* of unknown origin parasitizing invasive *S. noctilio* in North America (Kroll et al., 2013; Yu et al., 2009). When there is no egg sterilization, nematodes are still vectored to new trees by infected ovipositing females via accessory gland fluids introduced when female woodwasps oviposit. Based on a worldwide survey, Bedding (1972) reported that the specific combinations of *D. siricidicola* strain and *Sirex* species could result in either total sterilization or no sterilization, with the ability for nematodes to enter eggs being attributed to whether the siricid eggs were mature at the time that nematodes migrated within pupating female woodwasps to the reproductive system.

Less than 100% parasitism with total sterilization can be sustainable for hosts that are often aggregated, so that nematodes vectored to a new tree will be able to find healthy hosts to parasitize (Zondag, 1974). Invasive *S. noctilio* in North America can be highly

aggregated, for example, almost 500 *S. noctilio* emerged from one pine in central New York State (Kroll et al., 2013). Partial to no sterilization is suitable for siricids at low densities (for example, Zondag, 1974) or for hosts not often in aggregated distributions. In these instances, nematodes can parasitize larvae from surviving woodwasp eggs so that the nematode offspring can be vectored to another tree. Numbers of *S. nigricornis* emerging from trees are reported here for the first time as being variable but able to reach high densities (Figure 4a). However, in this study, there were too few parasitized females per tree to compare level of sterilization among trees with different woodwasp densities.

Association of parasitism of *S. nigricornis* in individual trees with density and sex

Using *S. nigricornis* reared from individual trees, we found a strong positive association of parasitism by *D. proximus* and density of *S. nigricornis* within trees. At one extreme, none of the 68 wasps developing in the tree with the lowest density were parasitized, and at the other extreme, 42% of the 327 wasps developing in the highest density tree were parasitized (Figure 4a). There are several possible mechanisms leading to this positive density dependence. It could be that the nematodes encounter hosts more at higher woodwasp densities within trees, so more of them get parasitized (Rohani et al., 1994). It could also be that competition among woodwasp larvae leads to low defence against nematodes at higher densities (Morrill & Forbes, 2012). Finally, it could be that nematodes fare better in specific trees for the same reason that wasps do. This could potentially be due to the success of growth of the woodwasp fungal symbiont which is also the food for the mycophagous stages of the nematode (Morris et al., 2014). Whatever the mechanism, a consequence of positive host-density dependence might be facilitation of the spread of the nematode in the woodwasp population, since trees infected with the most woodwasps were parasitized at higher frequencies and therefore would contribute most to the population dispersing to new trees. Woodwasp dispersal generally decreases with decreasing size in *S. noctilio* (for example, Gaudon et al., 2016). While Zieman et al. (2015) and this article found that *D. proximus* parasitism of *S. nigricornis* did not decrease female size, Hartshorn, Chase, et al. (2016) found the opposite. Therefore, the extent that *D. proximus* parasitism of vectors can potentially impact its transmission via impacting dispersal ability of hosts will require further study.

We also found that parasitism of males was much higher than females. This may be because trees with a high density of *S. noctilio* are both strongly male-biased and parasitized at a high rate. Curiously, male *S. noctilio* parasitized with *D. siricidicola* are not sterile, and Bedding (1972) found that they do not vector nematodes to females when mating, although nematodes are abundant within the testes of parasitized adult males. Whether *D. proximus* are also not transmitted from males to females during *S. nigricornis* mating is not known, so the consequences of the high *D. proximus* parasitism of male *S. nigricornis* remains to be determined. However, if males play no part in

transmitting the nematode, the association of the nematodes with high density host trees would not be particularly advantageous to the nematodes' spread.

On average, male *S. nigricornis* were smaller than females in this study although the typical great variability in size occurred, as reported for both *S. nigricornis* and *S. noctilio* (Haavik, Allison, et al., 2016). *Deladenus proximus*-parasitized male *S. nigricornis* were smaller than healthy males in our study, as was also found by Zieman et al. (2015). In contrast, Haavik, Allison, et al. (2016) reported that *Deladenus* infection did not impact size of *S. nigricornis* males, although the nematode species in this study were not identified and could have included *D. siricidicola* as well as *D. proximus*. If males do not transmit the nematode, then this size variation is of little consequence to *D. proximus* being vectored. If males do transmit the nematode through mating then parasitized males may be at a disadvantage as smaller individuals may disperse shorter distances, live shorter lives and potentially have lower mating success. Although we found that parasitized females had fewer eggs, parasitized and healthy females did not differ in size. Zieman et al. (2015) also found no difference in female size between parasitized and healthy females. However, Hartshorn, Chase, et al. (2016) found that *D. proximus*-infected *S. nigricornis* females were smaller than healthy females, and Bedding (2009) reported that the sizes of *S. noctilio* females decreased to different extents when parasitized by *D. siricidicola* from different source populations. Clearly, the impact of nematode infection on *Sirex* size is not consistent. Maybe this is not surprising given the generally high variability of adult woodwasp size.

In summary, associations between the native woodwasp *S. nigricornis* and its native parasitic nematode *D. proximus* were investigated in the southeastern US. Levels of egg sterilization were variable, averaging 79%. This rate of sterilization may assist in retention of the nematodes in a host population; none of the locations we studied averaged 0 or 100% egg sterilization, which has been found in other associations (Table 1). Prevalence of nematode parasitism in *S. nigricornis* populations differed by site, agreeing with Hartshorn, Chase, et al. (2016) that Arkansas had high prevalence; however, this level was nowhere near the high prevalences reported for *S. noctilio* in Australia (Bedding & Akhurst, 1974). *Deladenus proximus* had a positively density dependent relation to the host within trees and parasitized more males than females. Therefore, some attributes of this nematode appear very positive towards assisting in regulation of *S. nigricornis*, although the impact of *D. proximus* on *S. noctilio* now needs to be investigated.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Saskya van Nouhuys  <https://orcid.org/0000-0003-2206-1368>

Ann E. Hajek  <https://orcid.org/0000-0001-5740-4717>

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

Table S1. Parasitism of *Sirex nigricornis* by *Deladenus proximus*: Numbers of eggs/female for healthy and parasitized females, parasitism by site and year and egg sterilization.

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