

Sirex and associate community

Influence of the community of associates on *Sirex noctilio* brood production is contextual

Laurel J. Haavik^{1,2*}, Bernard Slippers³, Brett P. Hurley⁴, Kevin J. Dodds⁵, Taylor Scarr^{6,7}, Jean J. Turgeon¹
and Jeremy D. Allison^{1,4}

¹Natural Resources Canada-Canadian Forest Service, Great Lakes Forestry Centre, 1219 Queen Street E, Sault Ste. Marie, ON, P6A 2E5, Canada

²Current address of corresponding author: USDA Forest Service, Forest Health Protection, 1992 Folwell Avenue, St Paul, MN, 55108, USA, laurel.haavik@usda.gov

³Department of Biochemistry, Genetics and Microbiology, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria 0002 Gauteng, South Africa

⁴Department of Zoology and Entomology, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria 0002 Gauteng, South Africa

⁵USDA Forest Service, Forest Health Protection, 271 Mast Road, Durham, NH, 03824, USA

⁶Ontario Ministry of Natural Resources, Forest Health and Silviculture Section, 70 Foster Drive, Sault Ste. Marie, ON, P6A 6V5

⁷Current address: Natural Resources Canada-Canadian Forest Service, Great Lakes Forestry Centre, 1219 Queen Street E, Sault Ste. Marie, ON, P6A 2E5, Canada

Abstract

1. Competition and predation are important components of biotic resistance, which helps define the invasibility of an ecosystem.
2. To search for evidence of biotic resistance to the European woodwasp, *Sirex noctilio* Fabricius, in North America, we used cages to experimentally exclude the community of associates (natural enemies and competitors) from infested logs. Specifically, we assessed *S. noctilio* brood production in pine forests in Ontario and New York, where there was a rich existing community of associates (other wood borers, bark beetles and associated fungi, and parasitoids), and in South Africa, where siricid wasps and pines are not native and a similar associate community is not present. In addition, in Ontario, we excluded associates by size, and for different periods of time to identify important associates and their temporal dynamics.
3. We found evidence that biotic factors limit *S. noctilio* in North America, whereby exclusion of natural enemies and competitors had a positive influence on the abundance or presence of *S. noctilio* brood in Ontario and New York. This influence was absent in South Africa.
4. It is unclear which member(s) of the associated insect community in North America were most important in limiting *S. noctilio* brood production, though they likely acted quickly (<two weeks) following *S. noctilio* oviposition.
5. Further study is needed to determine whether associates have limited *S. noctilio* populations in pine forests throughout northeastern North America, and which specific natural enemies and/or competitors are important.

Keywords

Biotic resistance; enemy release; invasive species; natural enemy exclusion; wood borer

Introduction

Competition and predation are dominant biotic factors that shape the abundance and distribution of invertebrates (Halaj and Wise 2001; Kaplan and Denno 2007). As such, these biotic factors have potential to limit the impact of invasive herbivorous pests (Brockerhoff and Liebhold 2017; Nunez-Mir et al. 2017). Determining how competition and predation naturally operate in different ecosystems where invaders have established and spread is useful for understanding the dynamics of invasive pests, predicting their potential impact, and for developing effective management strategies for them. Such studies of invasive bark- and wood-boring insects in North America are important, given that these insects comprise a large portion of invertebrate invaders (Aukema et al. 2010; Lovett et al. 2016).

The cryptic nature of woodborers, which reside beneath tree bark throughout much of their development, creates an empirical challenge. Studies have examined the consequences of subcortical interactions among bark- and wood-boring beetle larvae on the survival of bark beetle larvae by rearing larvae with and without members of the associate community (Lawson et al. 1997; Linit and Stephen 1983; Schroeder and Weslien 1994b). These studies all observed a reduction in bark beetle larval survival and imply that predatory and competitive subcortical interactions with associates limit bark beetle populations, (but see (Flamm et al. 1989)). The impact of these subcortical associations on woodborers has received less empirical attention in field conditions, although laboratory assays suggest that the effects are similar (Dodds et al. 2001; Schoeller et al. 2012; Ware and Stephen 2006).

The woodwasp, *Sirex noctilio* F., native to Europe and introduced to several other continents, is an interesting case study in which to explore the importance of competition and predation in population dynamics of invasive woodboring insects. *Sirex noctilio* development is typically complete within one year, although development can take two or more years in cold climates (Ryan et al. 2012a; Spradbery and Kirk 1978). In North America, in the mid-summer, females drill through the bark of pines and into

Sirex and associate community

the wood to lay eggs along with a phytotoxic mucus [including noctilisin (Bordeaux et al. 2014) and other potential toxins (Wang et al. 2016)] and a symbiotic fungus [*Amylostereum* spp., (Gaut 1969; Wooding et al. 2013)], which act synergistically to overcome mechanisms of tree resistance and render pines suitable hosts for developing larvae (Coutts 1969; Coutts and Dolezal 1969). In North America, *S. noctilio* carries its traditional mutualist, *A. areolatum*, in the majority of cases, although in a few cases it carries *A. chailletii*, native to North America and traditionally associated with *S. nigricornis* (Hajek et al. 2013; Olatinwo et al. 2013; Wooding et al. 2013). Following oviposition into the sapwood, larvae feed in and create meandering tunnels through the wood, using a combination of *Amylostereum* and bacterial symbionts to digest it (Madden 1981; Thompson et al. 2014; Thompson et al. 2013).

The presence of biotic factors that have potential to limit *S. noctilio* varies among different geographic areas within its invaded range. *Sirex noctilio* was first detected in North America in 2005 (de Groot et al. 2006; Hoebeke et al. 2005) and currently occupies pine forests in the northeastern US and eastern Canada (Ontario, Quebec). These forests are patchy on the landscape and composed of native pines (*Pinus resinosa* Aiton, *P. banksiana* Lamb., *P. strobus* L.), the naturalized *P. sylvestris* L. (native to Europe, Skilling 1990) is also common. Pines in natural stands are often mixed with hardwoods; and plantations, especially of *P. resinosa*, are common. *Sirex noctilio*-caused tree mortality in North America has mostly been limited to unmanaged and stressed pine, especially *P. sylvestris* (Ayres et al. 2014; Dodds et al. 2010), and availability of these suitable pines may limit *S. noctilio* populations (Haavik et al. 2016). In contrast, in South Africa, where *S. noctilio* was detected in 1994 (Tribe 1995), *S. noctilio* infests non-native pine trees, planted in monoculture stands in commercial plantations. *Sirex noctilio* has been reported to infest all the pine species planted in South Africa, where the most dominant species planted are *P. patula*, *P. radiata*, *P. elliottii*, *P. taeda*, *P. greggii* and *P. elliottii x caribea* hybrid (Richardson and Higgins 1998). *Sirex noctilio*-induced tree mortality in South Africa has been considerable (Hurley et al. 2007, 2012).

Sirex and associate community

Besides differences in availability of susceptible hosts, North America and South Africa also differ in the community of natural enemies and competitors associated with *S. noctilio*. Because of the presence of native pine trees and siricid wasps in North America, there is a rich community of insects that colonize favored hosts of *S. noctilio* (Coyle and Gandhi 2012; Dodds et al. 2012; Ryan et al. 2012a; Ryan et al. 2012b). Two widely distributed parasitoid genera of the Siricinae in North America, *Ibalia* (Hymenoptera: Ibalidae) and *Rhyssa* (Hymenoptera: Ichneumonidae), both parasitize *S. noctilio* (Coyle and Gandhi 2012; Ryan et al. 2012a). *Ibalia* spp. are endoparasitic koinobionts that attack eggs and early-stage siricid larvae, have a similar seasonal phenology as *S. noctilio*, and complete one generation per year in North America (Coyle and Gandhi 2012). *Rhyssa* spp. are ectoparasitic idiobionts that attack late-stage siricid larvae (Coyle and Gandhi 2012; Spradbery and Kirk 1978). In addition, native woodborers (primarily Cerambycidae and native Siricidae) and bark beetles (Curculionidae: Scolytinae), and the fungi that they vector, frequently co-occur with *S. noctilio* and *Amylostereum*, and likely compete with both for weakened and dying pines (Foelker 2016; Haavik et al. 2015; Ryan et al. 2012b). South Africa, however, does not have a diverse assemblage of insects associated with pine. Biological control programs have resulted in the introduction and establishment of *Ibalia leucospoides* and the parasitic nematode, *Deladenus siricidicola* (Hurley et al. 2007). Besides these introduced natural enemies, the most dominant species co-occurring with *S. noctilio* is an invasive *Pissodes* spp. of North American origin (Wondafrash et al. 2019). Other introduced insects occurring on pine that are less commonly reported in association with *S. noctilio* are *Orthotomicus erosus*, *Hylastes angustatus* and *Hylurgus angustatus* (Roux et al. 2012).

No studies have experimentally quantified the impact of the community of associates on *S. noctilio* larval survival in different geographic areas of its invaded range. Reduced *S. noctilio* larval survival could occur as a consequence of (1) predation from other wood-boring insect larvae or associated predaceous species; (2) parasitism by *Rhyssa* and/or *Ibalia*; (3) interactions between the *S.*

Sirex and associate community

noctilio fungal symbiont *Amylostereum* and fungi introduced by other bark- and wood-boring insect larvae or (4) predation by woodpeckers. Our specific objectives were to (1) assess whether there was an impact of the community of associates (natural enemies and competitors) on *S. noctilio* brood production in multiple pine forest types that vary in the diversity of pine-associated insects; (2) examine if this impact varied with exposure to different components of the community filtered by size; and (3) examine whether temporal dynamics of associates affected *S. noctilio* brood production (i.e., the effect of asynchrony in colonization of host material by *S. noctilio* and associates). We evaluated the null hypothesis that excluding the community of associates would not influence *S. noctilio* brood production in any of the three locations, regardless of the diversity of the community of pine insects.

Materials and Methods

Experiment #1: Exclusion of all associates

This experiment was established at three sites where *S. noctilio* was present; one in the USA, one in Canada and one in South Africa. Methods used at the three sites were similar with slight variations. For all experiments, we used mesh screen cages secured to logs exposed to *S. noctilio* to exclude some or all of the associate community (Table 1), following the method of Linit and Stephen (1983). For Experiment 1, we excluded the entire community of associate insects and predatory vertebrates (i.e., woodpeckers) with fine-grain cages (8 mesh/inch; TWP Berkeley, California). The mesh cages were equipped with Velcro® closures, sewn directly onto the wire mesh. To prevent cages from collapsing down on the bole, we screwed three wooden struts (10-15 cm long, cut from 5x5 cm lumber) into logs, equidistant apart (e.g. at 0°, 120°, and 240°), near both ends of cages, and encircled the struts in a hoop of plastic tubing (1.75 cm in diameter), screwed to the struts (Fig. 1). Cages were then placed overtop the hoops and the ends tightened against the bole with zip-ties so that ~125 cm of the bole was

Sirex and associate community

enclosed in the caged area. To prevent insects from infesting the bole outside the caged area and their progeny entering the treatment area by mining through the bark and phloem tissue, the experimental sections were separated from the rest of the bole by scoring through the bark and phloem down to the xylem 3-5 cm from the point where the zip-ties were fastened and inside the caged area. Although ~125 cm were caged, only 1 m sections were collected and placed in rearing drums the following year (the 1 m section represented ca. 50 cm on each side of the exact center of the caged portion of the bole).

Table 1. Experimental set-up of treatments via location and experiment, and statistical models used.

Exp. description	Exp. no.	Response	Location	Year	Sample size (n)	Model
Exclusion of associates	1	<i>S. noctilio</i> F1	New York	2010 – 2011	10	Hurdle
	1	<i>S. noctilio</i> F1	Ontario	2013 – 2014	6	Hurdle
	1	<i>S. noctilio</i> F1	South Africa	2014 – 2015	15	GLM
Exclusion of associates by size	2	<i>S. noctilio</i> F1	Ontario	2013 – 2014	10	Hurdle
	2	<i>I. leucospoides</i> F1	Ontario	2013 – 2014	10	GLM
Exclusion of associates in time	3	<i>S. noctilio</i> F1	Ontario	2013 – 2014	6	Hurdle
	3	<i>I. leucospoides</i> F1	Ontario	2013 – 2014	6	GLM

Hurdle models used negative binomial distribution of errors; GLM = generalized linear model, Poisson distribution of errors; F1 = F1 generation (brood)



Fig. 1. Images of Experiment 1 (a) and 2 (b) set-up in Ontario after cages were removed for the exposed treatment (Experiments 1 and 3 only). The set-up for Experiment 3 was identical to that of 1.

New York, USA. On 29-June, 2011 ten *P. resinosa* on the Ft. Drum, NY army base were girdled to the xylem with a timber scribe, all branches on the lower 4 m were cut at the bole, and left standing. Two cages were installed per tree, one with its top ~3.5-4 m above ground and a second with its top at ~1.5-2 m above ground. All cages were seeded with two male and two female wasps that emerged from

Sirex and associate community

logs infested in 2010. All cages were installed and infested 1-July, 2011. By 8-July, 2011 all female wasps had died and one cage, haphazardly selected, was removed from each tree (i.e., the exposed treatment). Trees were left standing in the field until April 2012 when they were felled and the two sections on each tree that had been caged were cut and removed from the field. These logs were then placed individually in rearing drums at the USDA APHIS Sirex lab in North Syracuse, New York. Wasp emergence was monitored daily from Monday to Friday from May to August until emergence ceased. Only *S. noctilio* were counted and collected.

Ontario, Canada. On Simcoe County Forest land (mixed pine) near Angus, Ontario, we felled 22 healthy, but small *P. sylvestris* (7–11 cm in diameter at breast height, dbh, and 15–20 m tall) over a three-week period in July 2013. We cut a 3–4 m section from the mid-bole of each pine and placed each end of these logs onto a cinder block (25x10x10 cm). On the same day, we then secured mesh cages (1 m long) to each horizontal log with heavy duty, plastic zip-ties at each end. Two cages were placed on each log. Each caged bole section received two male and two female *S. noctilio*, 1.5–3 weeks after pines were cut and caged, in an attempt to create a physically suitable (i.e. stressed) host for optimal survival of *S. noctilio* brood [see (Madden 1971)]. We obtained adult *S. noctilio* from infested pines in Innisfil, Ontario, which were cut in late June 2013, and stored at the Ontario Ministry of Natural Resources and Forests Tree Seed Plant Facility (OMNRF-TSPF) in large outdoor tents. Resin beading in response to oviposition of the parent generation was visible on most logs. In late June 2014, logs were collected from the field site and transported to the OMNRF-TSPF, where we placed them into cardboard rearing tubes in a covered shed with open sides (Ryan et al. 2012a). We collected, identified, and counted adult wasps (*S. noctilio*, *S. nigricornis*, *Ibalia*, and *Rhyssa*), wood borers and bark beetles from rearing tubes 5x per week from early July through December.

Mpumalanga, South Africa. The trial in South Africa was established in a compartment of *Pinus patula* in the Rooihogte plantation in Mpumalanga near Carolina. On 5-6 November 2014, 15 *P. patula*

Sirex and associate community

were felled and a 6 m section from the mid-bole was limbed and placed on cinder blocks to raise the logs above ground high enough to allow the attachment of two cages per log (cages were identical to those described above). On 12-November, 2014, and based on wasp availability at the time, both cages on five logs received two female *S. noctilio* and the cages on ten remaining logs received one female *S. noctilio*. On 19-November, 2014 both cages on all 15 logs received two female and three male *S. noctilio*. On 12-December, 2014 one cage, haphazardly selected, was removed from each bole. The boles were left in the field on the cinder blocks until ca. 1 month before *S. noctilio* emergence when both caged portions from each bole were collected and placed individually in rearing cages at the FABI Biocontrol Centre, University of Pretoria. Wasp emergence was monitored daily Monday to Friday from early October until late November. Only *S. noctilio* were collected and counted.

Experiment #2: Exclusion of associates by size

For Experiment 2, we excluded increasingly larger portions of the associate community by using different sized mesh cages. Cages with small mesh (same size as for Experiment 1) excluded the entire community (bark beetles, wood borers, parasitoids, woodpeckers), those with medium mesh (5 mesh/inch; TWP Berkeley, California) excluded woodpeckers and larger insects (presumably wood borers and some parasitoids), and those with large mesh (1 mesh/inch; TWP Berkeley, California) excluded woodpeckers only. The mesh cages were set up the same as those used in Experiment 1, except that the cages with large mesh had small mesh skirting with Velcro® at each end and zip-ties were used to join the long edge of the cages to encircle the tree bole. This experiment was only established at the Ontario site, using the same methods as for Experiment 1, except that three cages (one of each of the mesh sizes described above) were placed on each log.

Experiment #3: Exclusion of associates in time

For Experiment 3, we excluded the associate community (with small mesh cages) from the experimental log sections for different periods of time with four treatments: (1) continuously protected from all associates; (2) exposed to associates for two weeks immediately after *S. noctilio* oviposition and then protected throughout the remainder of *S. noctilio* brood development; (3) exposed to associates for four weeks immediately after *S. noctilio* oviposition and then protected throughout the remainder of *S. noctilio* brood development; (4) exposed to associates continuously. This experiment was only established at the Ontario site, using the same methods as for Experiment 1.

We were not able to replicate Experiments 2 and 3 in South Africa and New York due to limited abundance of parent generation *S. noctilio*, finances and availability of technical support in those locations.

Data analysis

In the R statistical environment (R Core Team 2018), we examined histograms of response variables to determine appropriate models and error terms. Because experimental set-up varied slightly in each location, separate models were constructed for each location and experiment. The effect of treatment on number of *S. noctilio* or *I. leucospoides* brood in the F1 generation was tested with a generalized linear model with a negative binomial distribution of errors (package = MASS), and differences among treatments were determined by least squares. In order to account for a high frequency of zeros in the data, we used a hurdle model with a negative binomial distribution of errors (package = pscl, Zeileis et al. 2008) to test for the effect of treatment on number of *S. noctilio* or *I. leucospoides* brood. Relative to generalized linear models, hurdle models are advantageous because zero and non-zero responses are tested separately (Zeileis et al. 2008). Hurdle models determine if the frequency of non-zero, as well as zero responses differs more than by chance among the treatments

(Zeileis et al. 2008). We compared the log likelihood of generalized linear models and hurdle models for each treatment-location combination to determine which model fit the data best (larger values of log likelihood indicate better model fit; see Table 1 for final model selections). A subset of the data from Ontario collected for Experiment 3 was analyzed again for Experiment 1, so the results could be examined along with similar data from New York and South Africa. Data are archived at the Great Lakes Forestry Centre, Sault Ste. Marie, Ontario, Canada.

Results

Experiment #1: Exclusion of all associates

In New York, the effect of exclusion from associates on brood production was significant ($z = 2.4$; $df = 5$; $P = 0.0157$). More tree sections exposed to associates than protected from associates had no *S. noctilio* brood (7 vs. 1 section). However, among tree sections that did produce *S. noctilio* brood (11 sections), there was no difference between treatments in the number of *S. noctilio* that emerged ($z = 1.2$; $df = 5$; $P = 0.238$; Fig. 2a). In Ontario, the effect of exclusion from associates was also significant. However, in contrast to New York, there was not a significant difference between treatments in the number of tree sections that had no *S. noctilio* brood ($z = 1.6$; $df = 5$; $P = 0.0992$), but among tree sections that had some *S. noctilio* brood (7 sections), greater numbers of *S. noctilio* emerged from sections protected from associates ($z = 2.2$; $df = 5$; $P = 0.026$; Fig. 2b). In South Africa, the effect of exclusion from associates on *S. noctilio* brood was not significant ($z = 1.1$; $df = 1,28$; $P = 0.297$; Fig. 2c).

Sirex and associate community

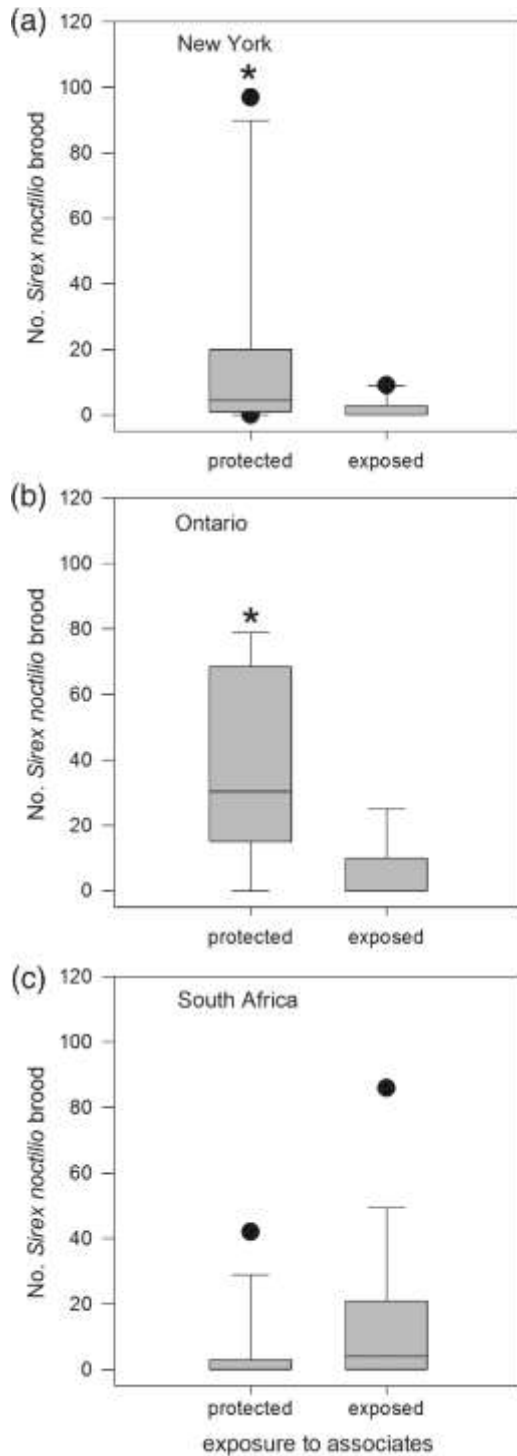


Fig. 2. Boxplots of *S. noctilio* brood (zero and non-zero combined) following exclusion of associates (Exp. 1) in all three geographic locations (a–c). Boxes are bounded by the first and third quartiles; the internal solid line represents the median. Asterisk indicates statistical significance between treatments with zero (a) and non-zero (b) *S. noctilio* brood according to Chi square estimation in hurdle models.

Sirex and associate community

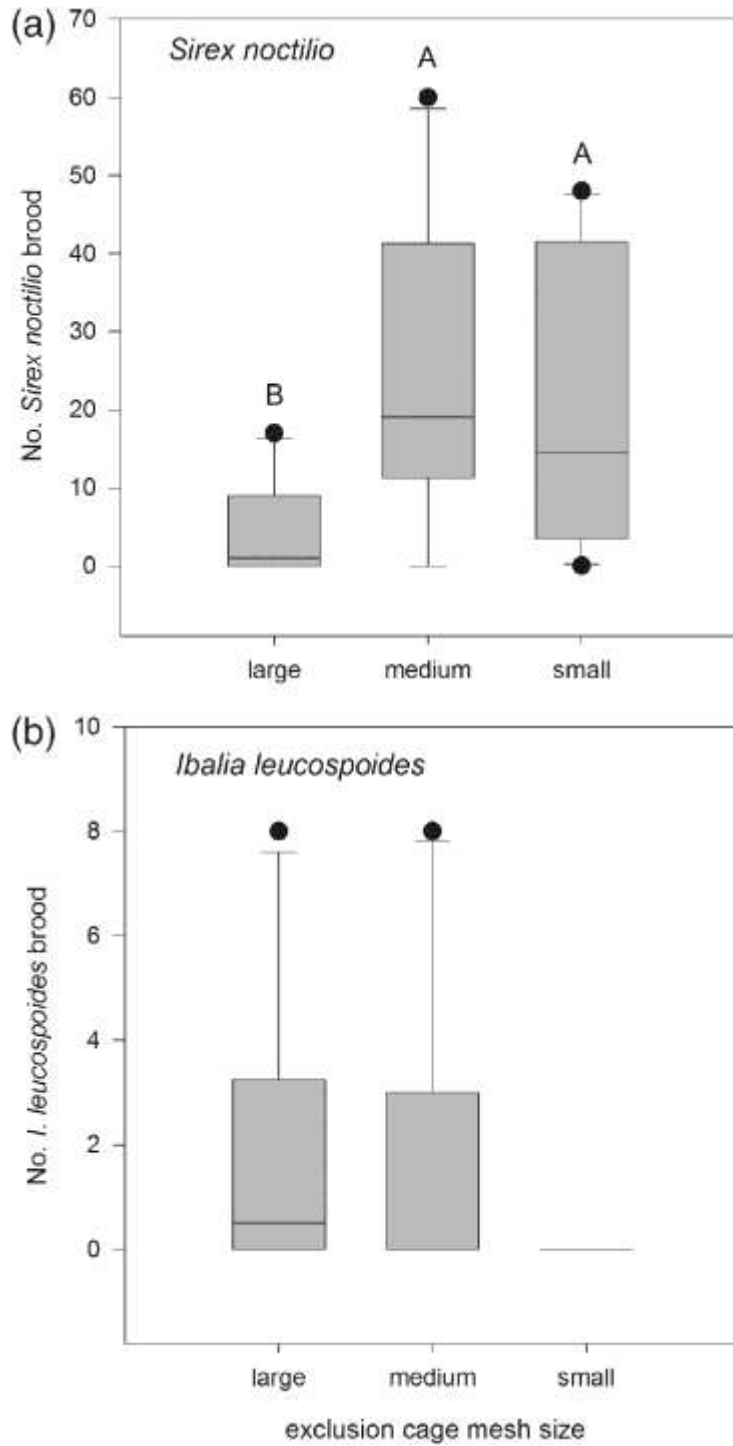


Fig. 3. Boxplots of (a) *S. noctilio* and (b) *I. leucospoides* brood (zero and non-zero brood combined) following exclusion of associates by size (Exp. 2) in Ontario. Boxes are bounded by the first and third quartiles; the internal solid line represents the median. Different letters indicate statistical significance between treatments with non-zero brood according to Chi square estimation in the hurdle model.

Experiment #2: Exclusion of associates by size

The effect of exclusion cage mesh size on *S. noctilio* brood was significant ($z = 3.19$; $df = 7$; $P = 0.001$). Among tree sections with some *S. noctilio* brood (22 sections), more wasps emerged from sections with small and medium mesh cages than sections with large mesh cages (Fig. 3a). However, there was not a significant difference among treatments in the number of sections that had no *S. noctilio* brood ($z = 2.2$; $df = 7$; $P = 0.074$). The effect of mesh size of exclusion cage on *I. leucospoides* brood production was not significant ($z = 0$; $df = 2,27$; $P = 0.995$; Fig. 3b). No *I. leucospoides* emerged from small mesh cages, although the variance in *I. leucospoides* brood among sections with medium and large mesh cages was high. No Cerambycidae, *S. nigricornis*, or *Rhyssa* spp. were recovered from the log sections; only a few bark beetles were recovered from logs and those were not in high enough numbers for analysis.

Experiment #3: Exclusion of associates in time

The effect of timing of exclusion cage removal on *S. noctilio* brood was significant ($z = 2.21$; $df = 3,20$; $P = 0.027$). Among tree sections with some *S. noctilio* brood (16 sections), greater numbers of *S. noctilio* emerged from sections protected from associates for the duration of the study than all other treatments (Fig. 4a). There were no significant differences in the number of *S. noctilio* that emerged among cages exposed for two weeks, four weeks, or the full duration of the experiment post *S. noctilio* oviposition (Fig. 4a). There was not a significant difference among treatments in the number of tree sections that had no *S. noctilio* brood ($z = 1.6$; $df = 5$; $P = 0.099$). The effect of timing of exclusion cage on *I. leucospoides* brood was significant ($z = 34.5$; $df = 3,20$; $P < 0.001$). Greater numbers of *I. leucospoides* emerged from sections in cages exposed to associates for only two weeks immediately following *S. noctilio* oviposition than those exposed for four weeks or for the entire experiment (Fig. 4b). Since no *I. leucospoides* emerged from any sections protected from associates for the entire experiment, that

Sirex and associate community

treatment was not included in the comparison. No wood borers or *S. nigricornis* emerged from log sections; only a few *Rhyssa* spp. and bark beetles emerged from logs and those were not in high enough numbers for analysis.

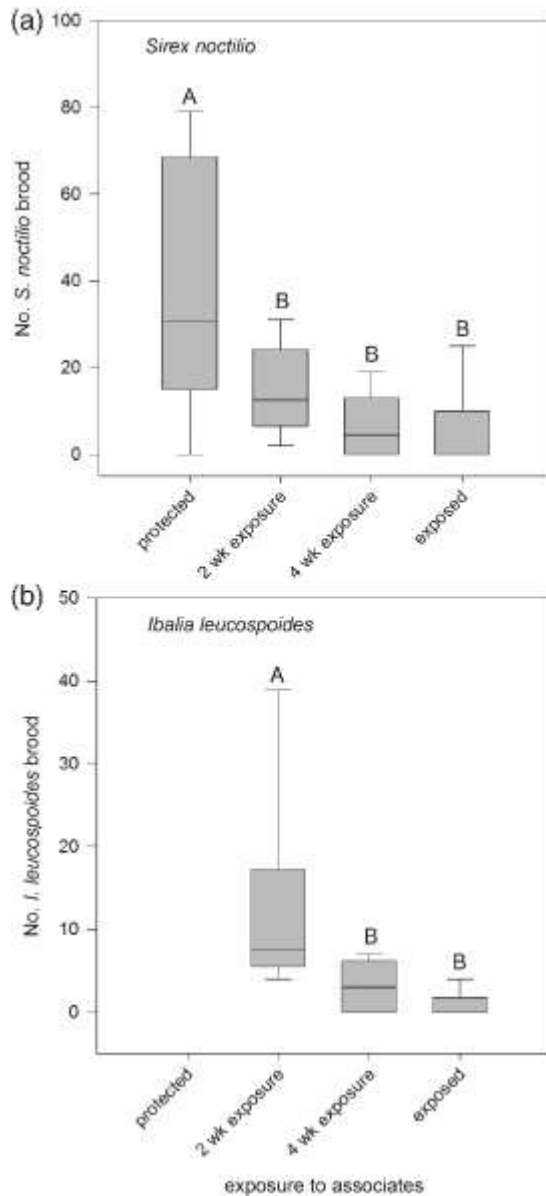


Fig. 4. Boxplots of (a) *S. noctilio* and (b) *I. leucospoides* brood (zero and non-zero brood combined) following exclusion of associates in time (Exp. 3) in Ontario. Boxes are bounded by the first and third quartiles; the internal solid line represents the median. Different letters indicate statistical significance between treatments with non-zero (a) and zero + non-zero (b) brood according to Chi square estimation in the hurdle (a) and generalized linear (b) models.

Discussion

Our study revealed that in North America the community of insects associated with pines has the potential to limit the success of the exotic invader *S. noctilio*. This adds to evidence that biotic resistance is important in limiting invasion success in forest ecosystems (Nunez-Mir et al. 2017). Specifically, under experimental *in situ* conditions, protection from competitors and predators had a positive influence on the abundance or presence of *S. noctilio* brood in Ontario and New York, yet this influence was apparently absent in South Africa, where the community of pine insects is extremely limited (Hurley et al. 2007; Roux et al. 2012; Wondafrash et al. 2019). It is unclear which member(s) of the associated insect community in North America were most important in limiting *S. noctilio* brood production, though they likely acted quickly (<two weeks) following *S. noctilio* oviposition. Various bark beetles, including *Ips* spp., have been documented arriving at artificially stressed trap trees in New York (Dodds et al. 2012; Zylstra et al. 2010) and their associated fungi can have negative effects on *Sirex* and *Amylostereum* spp. (Ryan et al. 2011; Yousef et al. 2014).

For *S. noctilio*, protection from associates throughout the duration of development was more effective than excluding associates for only two or four weeks following oviposition. Longer exclusion times result in protection from early arriving phloem feeding species such as *Ips* spp. and their associated fungi, phloem-sapwood species such as *Monochamus* spp., and later arriving ambrosia beetles that inhabit the sapwood. The egg and early-instar parasitoid, *Ibalia leucospoides*, was significantly less abundant in logs exposed for longer than two weeks. The reduction in *I. leucospoides* emerging from logs exposed for four weeks and continuously post-oviposition is most likely a consequence of mortality agents acting indirectly (i.e., the agent(s) kill the host *S. noctilio*). The absence of a reduction in *S. noctilio* coincident with the effect observed in *I. leucospoides* could be evidence that this unknown mortality factor(s) acted preferentially on *S. noctilio* parasitized by *I. leucospoides*.

Sirex and associate community

Any member or combination of members of the community of subcortical insects or their associated fungi could have been responsible for limiting *S. noctilio* survival. It does not appear that woodpeckers were important predators of *S. noctilio*, as we did not observe any evidence of woodpecker foraging on the exposed logs (Experiment 1 or 3). However, woodpeckers often forage in particular habitats (Bull et al. 1986) and placement of cages lower on tree boles or logs placed in the understory may have been outside the normal habitat for most species.

Potential competitors were not analyzed as part of our study because their flight phenologies and generation times differ enough from that of *S. noctilio* (Foelker et al. 2016; Ryan et al. 2012b) that many of them could have easily been missed if counted, i.e., emergence before logs were removed from the field, or not allowed access to *S. noctilio* brood if logs were removed from the experiment too early. It was also not possible to identify species or feeding guild of insects using adult emergence holes in the bark, because the size and shape of those holes overlaps considerably within the community (Ayres et al. 2009). This was a trade-off; we chose to measure the effect of the entire community of associates on *S. noctilio* brood and pinpoint the timing of this effect rather than attempt to identify exactly which members of the community were most important. *Sirex nigricornis*, the pine-colonizing woodwasp native to North America, probably did not compete (at least successfully) with *S. noctilio*, because we would have collected it if it had colonized logs, given that its flight period is considerably later in the season than that of *S. noctilio* (Foelker et al. 2016; Haavik et al. 2013; Ryan et al. 2012a). Whatever their identity, important associates easily and willingly entered large mesh, but not medium or small mesh cages, which suggests that they may have been large insects or had difficulty/did not invest the time or energy required to pass through the mesh.

The parasitoids *Rhyssa* spp. and *Megarhyssa* spp., and the cleptoparasitoid *Pseudorhyssa* spp. are all large insects (Coyle and Gandhi 2012) that could have entered large mesh, but probably not medium or small mesh cages. These natural enemies all seem to be important players in the parasitoid

Sirex and associate community

complex that attacks *S. noctilio* in North America, yet their flight phenologies do not align with that of *S. noctilio* (Foelker et al. 2016; Ryan et al. 2012a). *Rhyssa* spp. may have a density-dependent response to *S. noctilio* in North America, as has been reported elsewhere (Taylor 1978), but this has proved difficult to determine, because of the asynchrony in phenology and the possibility of a second, short generation of *Rhyssa* in the spring before *S. noctilio* completes development (Foelker 2016; Haavik et al. 2016). Our study was not replicated over several pine forests, and our results may have been influenced by patchiness in the distribution of competitors and natural enemies across the landscape. Aggregation of the bark- and wood-inhabiting pine insect community is dictated by spatial and temporal availability of dead and dying pines. A meta-analysis of herbivorous insects found that competition may be highly variable both in space and time (Kaplan and Denno 2007), which further complicates determining whether competition limits *S. noctilio*. Our experiments suggest that the community present within pine trees in North America can influence *S. noctilio* survival; however, it is clear that the degree of influence will be based on many interacting localized factors. Additionally, because the experiment was not replicated in pine forests within geographic locations, it is possible that variation in unknown or unmeasured site-level factors contributed to the observed patterns of caging effects among locations. We assumed that such site-level variation was minimal, because the same pattern was observed in both New York and Ontario, the pine forest used in South Africa was representative of pine plantation forests in South Africa, and the unknown variables would have to differentially affect caged and uncaged bole portions.

Other studies that excluded associates in pine insect communities found that timing of exclusion was important for determining the importance of natural enemies in limiting bark beetle brood production (Lawson et al. 1997; Linit and Stephen 1983; Riley and Goyer 1986). Studies that examined the influence of predation and competition found that both were important (Schroeder and Weslien 1994a; Schroeder and Weslien 1994b). Some exclusion studies reported a large amount of variability

Sirex and associate community

within treatments (Riley and Goyer 1986; Schroeder and Weslien 1994a), as did we, which highlights the inherent difficulty in such studies with subcortical insects in natural habitats. This implies that there is considerable variability in local associate abundance and/or successful establishment of brood (i.e., frequency of non-ovipositing females). Other studies were able to report the percentage reduction in bark beetle brood attributable to natural enemies or competitors or both, which was either substantial ($\geq 78\%$) (Miller 1984; Schroeder and Weslien 1994a; Schroeder and Weslien 1994b) or not (15 – 30%) (Lawson et al. 1997; Linit and Stephen 1983; Riley and Goyer 1986).

Some members of the associate community probably limit *S. noctilio* survival and population growth in North America, though which members and to what degree remains unknown. Also of concern is that a difference exists between what was observed in the experimental conditions created in our study and what occurs on the landscape. We found this to be the case in a previous study in Ontario, where associates were important in limiting *S. noctilio* brood when experimentally manipulated, but the same measures in the natural population revealed that associates were considerably less important (Haavik et al. 2015). In that study, tree resistance was found to be of paramount importance in limiting *S. noctilio* brood, a factor we did not measure in the current study. Future studies should strive to isolate the importance of specific predators and competitors on *S. noctilio* populations in North America. This would require scrutinizing members of the subcortical pine community in their arrival to and departure from colonized trees, especially during the two weeks after the *S. noctilio* flight period, followed by examination of brood size and survivorship.

Sirex and associate community

Authors' contributions

JDA, KJD, and JJT conceived the study. JDA, KJD, BS, BPH, LJH, and JJT conducted the study. LJH analyzed the data. LJH and JDA led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Acknowledgements

Authors thank Sarah Drabble, Reg Nott, Nick Boyonoski, Sarah Crispell, Katelyn Kotska, Chanel Fall, Cydney Mihell, Brett Marshall, Garrett Dubois, Rodger Voss, Jason Wagner, Travis Ganter, Hardus Hatting and Elder Batista for field and lab assistance, Al Foley for access to the Ontario Tree Seed Facility, Peter Smallidge for access to the Cornell University Arnot Forest, and Rodger Voss, Jason Wagner and Travis Ganter for access to the Ft. Drum Military Base. This collaborative work was funded in part by Natural Resources Canada, The Ontario Ministry of Natural Resources, and the USDA Forest Service-Forest Health Protection. Authors have no conflict of interest to declare.

References

- Aukema, J.E., McCullough, D.G., Von Holle, B., Liebhold, A.M., Britton, K., & Frankel, S.J. (2010). Historical accumulation of nonindigenous forest pests in the continental United States. *Bioscience*, 60, 886-897.
- Ayres, M.P., Sullivan, J.M., Harrison, T., & Lombardero, M.J. (2009). Diagnosing the presence of *Sirex noctilio* from examination of dead and dying pine trees. Report for USDA APHIS, 20 p.
- Ayres, M.P., Pena, R., Lombardo, J.A., & Lombardero, M.J. (2014). Host use patterns by the European woodwasp, *Sirex noctilio*, in its native and invaded range. *PLoS ONE*, 9, e90321.

Sirex and associate community

Bordeaux, J.M., Lorenz, W.W., Johnson, D., Badgett, M.J., Glushka, J., Orlando, R., & Dean, J.F.D. (2014).

Noctilisin, a venom glycopeptide of *Sirex noctilio* (Hymenoptera: Siricidae), causes needle wilt and defense gene responses in pines. *Journal of Economic Entomology*, 107, 1931-1945.

Brockerhoff, E.G., & Liebhold, A.M. (2017). Ecology of forest insect invasions. *Biological Invasions*, 19, 3141-3159.

Bull, E.L., Peterson, S.R., & Thomas, J.W. (1986). Resource partitioning among woodpeckers in northeastern Oregon. USDA Forest Service Res. Note, PNW-RN-444, 20 p.

Coutts, M.P. (1969). The mechanism of pathogenicity of *Sirex noctilio* on *Pinus radiata* II. Effects of *S. noctilio* mucus. *Australian Journal of Biological Science*, 22, 1153-1161.

Coutts, M.P., & Dolezal, J.E. (1969). Emplacement of fungal spores by the woodwasp, *Sirex noctilio*, during oviposition. *Forest Science*, 15, 412-416.

Coyle, D.R., & Gandhi, K.J.K. (2012). The ecology, behavior, and biological control potential of hymenopteran parasitoids of woodwasps (Hymenoptera: Siricidae) in North America. *Environmental Entomology*, 41, 731-749.

de Groot, P., Nystrom, K., & Scarr, T. (2006). Discovery of *Sirex noctilio* (Hymenoptera: Siricidae) in Ontario, Canada. *The Great Lakes Entomologist*, 39, 49-53.

Dodds, K.J., Graber, C., & Stephen, F.M. (2001). Facultative intraguild predation by larval Cerambycidae (Coleoptera) on bark beetle larvae (Coleoptera: Scolytidae). *Environmental Entomology*, 30, 17-22.

Dodds, K.J., de Groot, P., & Orwig, D. (2010). The impact of *Sirex noctilio* in *Pinus resinosa* and *Pinus sylvestris* stands in New York and Ontario. *Canadian Journal of Forest Research*, 40, 212-223.

Dodds, K.J., Zylstra, K.E., Dubois, G.D., & Hoebeke, E.R. (2012). Arboreal insects associated with herbicide-stressed *Pinus resinosa* and *Pinus sylvestris* used as *Sirex noctilio* trap trees in New York. *Environmental Entomology*, 41, 1350-1363.

Sirex and associate community

- Flamm, R.O., Coulson, R.N., Beckley, P., Pulley, P.E., & Wagner, T.L. (1989). Maintenance of a phloem-inhabiting guild. *Environmental Entomology*, 18, 381-387.
- Foelker, C.J. (2016). Beneath the bark: associations among *Sirex noctilio* development, bluestain fungi, and pine host species in North America. *Ecological Entomology*, 41,676-684.
- Foelker, C.J., Standley, C.R., Parry, D., & Fierke, M.K. (2016). Complex ecological relationships among an assemblage of indigenous hymenopteran parasitoids, the exotic European woodwasp (*Sirex noctilio*; Hymenoptera: Siricidae), and a native congener. *The Canadian Entomologist*, 148, 532-542.
- Gaut, I.P.C. (1969). Identity of the fungal symbiont of *Sirex noctilio*. *Australian Journal of Biological Sciences*, 22, 905-914.
- Haavik,L.J., Meeker, J.R., Johnson, W., Ryan, K., Turgeon, J.J., & Allison, J.D. (2013). Predicting *Sirex noctilio* and *S. nigricornis* emergence using degree days. *Entomologia Experimentalis et Applicata*, 149, 177-184.
- Haavik, L.J., Dodds, K.J., & Allison, J.D. (2015). Do native insects and associated fungi limit non-native woodwasp, *Sirex noctilio*, survival in a newly invaded environment? *PLoS ONE*, 10, e0138516.
- Haavik, L.J., Dodds, K.J., Ryan, K., & Allison, J.D. (2016). Evidence that the availability of suitable pine limits non-native *Sirex noctilio* in Ontario. *Agricultural and Forest Entomology*, 18, 357-366.
- Hajek, A.E., Nielsen, C., Kepler, R.M., Long, S.J., & Castrillo, L. (2013). Fidelity among *Sirex* woodwasps and their fungal symbionts. *Microbial Ecology*, 65, 753-762.
- Halaj, J., & Wise, D.H. (2001). Terrestrial trophic cascades: how much do they trickle? *The American Naturalist*, 157, 262-281.
- Hoebeke, E.R., Haugen, D.A., & Haack, R.A. (2005). *Sirex noctilio*: discovery of a palearctic siricid woodwasp in New York. *Newsletter of the Michigan Entomological Society*, 50, 24-25.

Sirex and associate community

- Hurley, B.P., Slippers, B., & Wingfield, M.J. (2007). A comparison of control results for the alien invasive woodwasp, *Sirex noctilio*, in the southern hemisphere. *Agricultural and Forest Entomology*, 9, 159-171.
- Hurley, B., Croft, P., Verleur, M., Wingfield, M.J., & Slippers, B. (2012). The control of the *Sirex* woodwasp in diverse environments: the South African experience. In: Slippers, B., de Groot, P., Wingfield, M.J. (eds), *The Sirex woodwasp and its fungal symbiont: research and management of a worldwide invasive pest*. Springer, New York, pp. 247-264.
- Kaplan, I., & Denno, R.F. (2007). Interspecific interactions in phytophagous insects revisited: a quantitative assessment of competition theory. *Ecology Letters*, 10, 977-994.
- Lawson, S.A., Furuta, K., & Katagiri, K. (1997). Effect of natural enemy exclusion on mortality of *Ips typographus japonicus* Nijima (Col.: Scolytidae) in Hokkaido, Japan. *Journal of Applied Entomology*, 121, 89-98.
- Linit, M.J., & Stephen, F.M. (1983). Parasite and predator component of within-tree southern pine beetle (Coleoptera: Scolytidae) mortality. *The Canadian Entomologist*, 115, 679-688.
- Lovett, G.M. et al. (2016). Nonnative forest insects and pathogens in the United States: impacts and policy options. *Ecological Applications*, 26, 1437-1455.
- Madden, J.L. (1971). Some treatments which render Monterey pine (*Pinus radiata*) attractive to the wood wasp *Sirex noctilio* F. *Bulletin of Entomological Research*, 60, 467-472.
- Madden, J.L. (1981). Egg and larval development in the woodwasp, *Sirex noctilio* F. *Australian Journal of Zoology*, 29, 493-506.
- Miller, M.C. (1984). Effect of exclusion of insect associates on *Ips calligraphus* (Germ.) (Coleoptera, Scolytinae) brood emergence. *Journal of Applied Entomology*, 97, 298-304.

Sirex and associate community

- Nunez-Mir, G.C., Liebhold, A.M., Guo, Q., Brockerhoff, E.G., Jo, I., Ordonez, K., & Fei, S. (2017). Biotic resistance to exotic invasions: its role in forest ecosystems, confounding artifacts, and future directions. *Biological Invasions*, 19, 3287-3299.
- Olatinwo, R., Allison, J.D., Meeker, J., Johnson, W., Streett, D., Aime, M.C., & Carlton, C. (2013). Detection and identification of *Amylostereum areolatum* (Russulales: Amylostereaceae) in the mycangia of *Sirex nigricornis* (Hymenoptera: Siricidae) in central Louisiana. *Environmental Entomology*, 42, 1246-1256.
- R Development Core Team. (2018). R: A language and environment for statistical computing. ISBN 3-900051-07-0, URL <http://www.R-project.org> edn. R Foundation for Statistical Computing, Vienna, Austria.
- Richardson, D.M., & Higgins, S.I. (1998) Pines as invaders in the southern hemisphere. In: Richardson, D.M. (ed) Ecology and biogeography of *Pinus*. Cambridge University Press, Cambridge, UK.
- Riley, M.A., & Goyer, R.A. (1986). Impact of beneficial insects on *Ips* spp. (Coleoptera: Scolytidae) bark beetles in felled loblolly and slash pines in Louisiana. *Environmental Entomology*, 15, 1220-1224.
- Roux, J., Hurley, B.P., & Wingfield, M.J. (2012). Diseases and pests of eucalypts, pines and wattle. pp. 303-336 In Bredkamp, B.V., & Upfold, S.J. (Eds) *South African Forestry Handbook*. 5th edn. South African Institute of Forestry.
- Ryan, K., Moncalvo, J.M., de Groot, P., & Smith, S.M. (2011). Interactions between the fungal symbiont of *Sirex noctilio* (Hymenoptera: Siricidae) and two bark beetle-vectored fungi. *The Canadian Entomologist*, 143, 224-235.
- Ryan, K. et al. (2012a). Natural enemies associated with *Sirex noctilio* (Hymenoptera: Siricidae) and *S. nigricornis* in Ontario, Canada. *Environmental Entomology*, 41, 289-297.
- Ryan, K., de Groot, P., & Smith, S.M. (2012b). Evidence of interaction between *Sirex noctilio* and other species inhabiting the bole of *Pinus*. *Agricultural and Forest Entomology*, 14, 187-195.

Sirex and associate community

- Schoeller, E.N., Husseneder, C., & Allison, J.D. (2012). Molecular evidence of facultative intraguild predation by *Monochamus titillator* larvae (Coleoptera: Cerambycidae) on members of the southern pine beetle guild. *Naturwissenschaften*, 99, 913-924.
- Schroeder, L.M., & Weslien, J. (1994a). Interactions between the phloem-feeding species *Tomicus piniperda* (Col.: Scolytidae) and *Acanthocinus aedilis* (Col.: Cerambycidae), and the predator *Thanasimus formicarius* (Col.: Cleridae) with special reference to brood production. *Entomophaga*, 39, 149-157.
- Schroeder, L.M., & Weslien, J. (1994b). Reduced offspring production in bark beetle *Tomicus piniperda* in pine bolts baited with ethanol and α - pinene, which attract antagonistic insects. *Journal of Chemical Ecology*, 20, 1429-1444.
- Skilling, D.D. (1990). *Pinus sylvestris* L., Scotch pine. Silvics Manual: Conifers. USDA Forest Service. http://na.fs.fed.us/spfo/pubs/silvics_manual/Volume_1/vol1_Table_of_contents.htm. pp. 489-496.
- Spradbery, J.P., & Kirk, A.A. (1978). Aspects of the ecology of siricid woodwasps (Hymenoptera: Siricidae) in Europe, North Africa and Turkey with special reference to the biological control of *Sirex noctilio* F. in Australia. *Bulletin of Entomological Research*, 68, 341-359.
- Taylor, K.L. (1978). Evaluation of the insect parasitoids of *Sirex noctilio* (Hymenoptera: Siricidae) in Tasmania. *Oecologia*, 32, 1-10.
- Thompson, B.M., Bodart, J., McEwen, C., & Gruner, D.S. (2014). Adaptations for symbiont-mediated external digestion in *Sirex noctilio* (Hymenoptera: Siricidae). *Annals of the Entomological Society of America*, 107, 453-460.
- Thompson, B.M., Grebenok, R.J., Behmer, S.T., & Gruner, D.S. (2013). Microbial symbionts shape the sterol profile of the xylem-feeding woodwasp, *Sirex noctilio*. *Journal of Chemical Ecology*, 39, 129-139.

Sirex and associate community

- Tribe, G.D. (1995). The woodwasp *Sirex noctilio* Fabricius (Hymenoptera: Siricidae), a pest of *Pinus* species, now established in South Africa. *African Entomology*, 3, 215-217.
- Wang, T., Zhao, M., Rotgans, B.A., Ni, G., Dean, J.F.D., Nahrung, H.F., & Cummins, S.F. (2016). Proteomic analysis of the venom and venom sac of the woodwasp, *Sirex noctilio* - towards understanding its biological impact. *Journal of Proteomics*, 146, 195-206.
- Ware, V.L., & Stephen, F.M. (2006). Facultative intraguild predation of red oak borer larvae (Coleoptera: Cerambycidae). *Environmental Entomology*, 35, 443-447.
- Wondafrash, M., Slippers, B., Hurley, B.P., & Garnas, J. (2019). Local antagonism and resource partitioning between two invasive pine plantation pests. *Agricultural and Forest Entomology*, 21, 276-285.
- Wooding, A.L., Wingfield, M.J., Hurley, B.P., Garnas, J.R., de Groot, P., & Slippers, B. (2013). Lack of fidelity revealed in an insect-fungal mutualism after invasion. *Biology Letters*, 9, 20130342.
- Yousef, F., Gurr, G.M., Carnegie, A.J., Bedding, R.A., Bashford, R., Gitau, C.W., & Nicol, H.I. (2014). The bark beetle, *Ips grandicollis*, disrupts biological control of the woodwasp, *Sirex noctilio*, via fungal symbiont interactions. *FEMS Microbiology Ecology*, 88, 38-47.
- Zeileis, A., Kleiber, C., & Jackman, S. (2008). Regression models for count data in R. *Journal of Statistical Software*, 27, 1-25.
- Zylstra, K.E., Dodds, K.J., Francese, J.A., & Mastro, V.C. (2010). *Sirex noctilio* in North America: the effect of stem-injection timing on the attractiveness and suitability of trap trees. *Agricultural and Forest Entomology*, 12, 243-250.