Within-Tree Distributions of the *Sirex noctilio* Fabricius (Hymenoptera: Siricidae) – Parasitoid Complex and Development of an Optimal Sampling Scheme

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ABSTRACT Little is known about the introduced European woodwasp, *Sirex noctilio* F. (Hymenoptera: Siricidae), and its hymenopteran parasitoid complex in North America. To assess within-tree and landscape-level densities and distributions of these insects, and develop a more cost effective approach for sampling and monitoring *S. noctilio*, 18 infested pines (12 *P. resinosa* Aiton and six *P. sylvestris* L.) were felled from six stands within three regions in central New York and cut into 0.5-m bolts. Bolts were carefully split to recover all siricids (*S. noctilio* and native siricids) and parasitoids. In total, 2,558 siricids were recovered; 1,972 siricid larvae and 586 teneral adult *S. noctilio*. Parasitism of siricids, a majority of which were *S. noctilio* based on larval rearing results, was 16.4% with *Ibalia leucospoides ensiger* Norton causing 10.7% of the documented mortality. Numbers of siricids and parasitoids declined 33–86% from the northern to the southern sampling regions, peak insect densities occurred in sections of the bole 15–19 cm in diameter and numbers of insects were generally higher in *P. sylvestris* than *P. resinosa* according to the highest ranked zero-inflated poisson and probit regression models. Bark thickness was not correlated with siricid or parasitoid species densities. We also describe sampling plans where as few as two 0.5-m samples from infested trees provided reliable within-tree insect densities.

KEY WORDS invasive insect, conifer pest, pine mortality, native parasitoids

Sirex noctilio F. (Hymenoptera: Siricidae), a Palearctic woodwasp, was first detected in North America near Fulton, NY in 2004 (Hoebeke et al. 2005). In its native habitat, *S. noctilio* is considered a minor pest (Gilmour 1965), but in areas of accidental introduction it has caused extensive damage to pines, especially in plantation settings in the Southern Hemisphere (Madden 1988, Tribe 1995). In North America, concern is greatest for economically important southern pine plantations and expansive western pine forests, where softwood commodities are valued at 8.2 billion and 10.7 billion dollars, respectively (Borchert et al. 2007). Uncontrolled spread of *S. noctilio* also may have ecological impacts as many threatened species use pine stands as habitat (Evans-Goldner 2008).

Successful tree colonization by *S. noctilio* involves a series of events. Early in tree attack, females make single drills with their ovipositor and inject a toxic mucus that physiologically changes host trees and facilitates establishment of a fungal symbiont, *Amylostereum areolatum* (Fr.) Boiden, spores of which are also inoculated during oviposition (Coutts 1965). As trees become more susceptible to colonization, females will make multiple drills and eggs will also be deposited along with the mucus and spores (Coutts and Dolezal 1969). *Sirex noctilio* larvae feed on fungal hyphae (Talbot 1977) and typically go through six or seven instars within infested trees (Morgan and Stewart 1966, Neumann and Minko 1981).

A suite of Nearctic and Palearctic hymenopteran parasitoids have been used as biocontrol agents in areas where *S. noctilio* has been accidentally introduced, and parasitism as high as 70% has been documented (Taylor 1976). Two subspecies of *Ibalia leucospoides* (Ibaliidae), *I. l. leucospoides* (Hockenwarth) from Europe (Miller and Clark 1935); *I. l. ensiger* Norton from North America (Zondag 1969, Nuttall 1970); as well as several ichneumonids (collectively referred to as rhyssines), including *Megarhyssa nortoni* (Cresson) from North America (Zondag 1969) and *Rhyssa persuasoria* (L.) from Europe (Miller and Clark 1935) were introduced to control *S. noctilio* infestations in New Zealand and Australia and subsequently naturalized. In addition, *Rhyssa lineolata* (Kirby), a North American species, also was successfully established after accidental introduction into New Zealand (Zondag and Nuttall 1961).

Stem diameter and bark thickness may be important determinants for successful *S. noctilio* infestation and subsequent parasitism. Larger diameter areas of the tree bole provide abundant resources, and developing *S. noctilio* larvae may be out of reach of parasitoid

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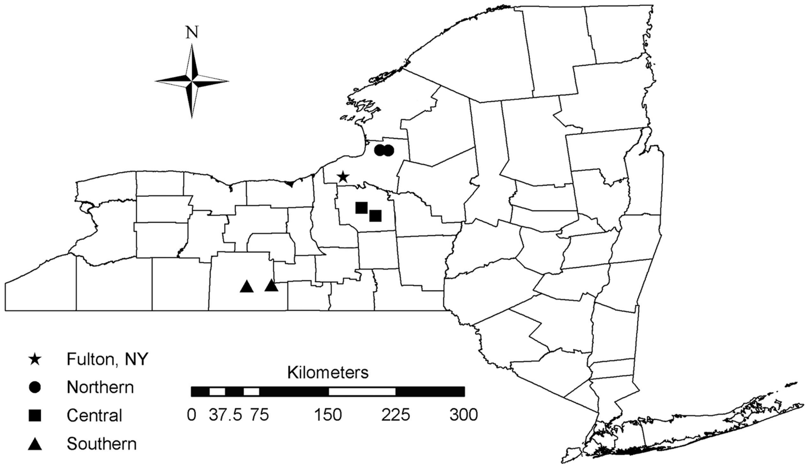

Fig. 1. Twelve red and six Scots pines were harvested from six monoculture pine stands in three regions in central New York. Fulton, NY is also indicated as *S. noctilio* was discovered there in 2004.

ovipositors. These refugia from parasitism would not be available higher on the bole where there is less wood to "hide" in. Access to different host larval stages and host larvae at differing depths can be related to differences in parasitoid ovipositor length (Price 1972, Sivinski et al. 2001). Because *I. leucospoides* are endoparasitoids of eggs and early instars, they use *S. noctilio* oviposition drills to access host larvae (Hanson 1939, Taylor 1978) so is unlikely to be directly affected by stem diameter or bark thickness. In contrast, rhyssines are ectoparasitoids that actively drill through the wood to reach host larvae (Taylor 1976), and though they have longer ovipositors than *I. leucospoides,* stem diameter and bark thickness may influence parasitism in this group.

Current sampling methodology for *S. noctilio* and its parasitoids rely on affixing cages to trees (Taylor 1978) or felling, transporting, and rearing sections that appear to be infested (Long et al. 2009). These methods do not indicate densities for the entire tree, which can provide information on within-tree distributions of infestation and insect mortality, nor do they allow estimation of stand and landscape-level densities that can provide insights into population dynamics and are critical for development of infestation thresholds for management. Quantifying within-tree densities of wood-boring insects (and their parasitoids) require felling, transporting, and dissecting entire trees (Fierke et al. 2005) or monitoring entire trees for adult emergence. Development of an optimal sampling method that uses the smallest number of samples per tree needed to achieve statistically reliable estimates (Stephen and Taha 1976, Crook et al. 2007) reduces the time and effort required for more intensive techniques (Morris 1960, Fierke et al. 2005). These methods facilitate increased sampling effort across the larger geographic areas necessary to elucidate spatial patterns of insect populations (Southwood and Henderson 2000).

The first objective of this study was to determine spatial patterns of *S. noctilio* and parasitoid attack. Specifically, we wanted to elucidate infestation patterns of *S. noctilio* and its parasitoids as well as percent parasitism among infested stands in central New York State as well as within infested tree boles. Second, we wanted to investigate if bark thickness was correlated to within-tree distributions of *S. noctilio* or parasitoids. Third, we wanted to develop an optimal subsampling method, based on whole-tree sampling, to estimate within-tree densities of *S. noctilio* and its parasitoids.

Materials and Methods

Tree Sampling. Infested trees were identified in six unmanaged monoculture red pine, *Pinus resinosa* Aiton, and Scots pine, *Pinus sylvestris* L., stands along a longitudinal gradient in New York State (Fig. 1). Sampled stands were grouped into northern, central, and southern regions (Table 1). Extensive searches failed to identify Scots pine stands in the south central region of the state, thus trees were felled from two red pine stands. In June of 2008, 18 pines (12 red pine and six Scots pine) were felled with at least two trees taken from each stand and higher numbers from larger stands. Trees sampled were the first trees encountered at each site exhibiting signs of *S. noctilio* infestation, e.g., resin beads and reddening and wilting crowns. Sampled trees ranged in diameter at breast height (dbh) from 8.3 to 25.3 cm and heights ranged from 3.5 to 18 m.

Trees were cut ≈15 cm from the ground. Total infested height up the bole was determined and entire trees were sectioned into 0.5-m bolts. Infested length was determined by measuring the distance between the lowest and highest signs of infestation on the bole (resin beads associated with oviposition drills or larval galleries on cut ends of bolts). Bolts >8 cm in diameter were labeled to indicate sample site, tree number, and height from the ground. Generally, siricids do not inhabit smaller diameter wood, but smaller bolts also were examined and if signs of infestation were found, bolts were also labeled and transported to the State

Table 1. Location and tree descriptions for infested pine trees harvested from six monoculture pine stands in New York State

Region	Location	Latitude	Longitude	Tree species	No. felled	Ave. dbh (cm)	Ave. infested length (m)
North	Orwell, NY	43.570422° N	−75.992861° W	Red pine	2	11.8	12.3
North	Richland, NY	43.573608° N	−76.043983° W	Scots pine	3	10.3	5.5
Central	Syracuse, NY	42.972136° N	−76.187381° W	Red pine	3	16.4	11.2
Central	Tully, NY	42.769183° N	−76.074408° W	Scots pine	3	19.8	11.7
South	South Bradford State Forest	42.299119° N	−77.112772° W	Red pine	3	15	13
South	Cameron State Forest	42.249286° N	−77.416347° W	Red pine	4	13.5	9.8

Number of trees felled depended upon stand size.

University of New York, College of Environmental Science and Forestry for dissection.

Insect Sampling. To suspend larval development, bolts were stored at 4°C and later split using a 4-ton electric log splitter to recover siricid and parasitoid larvae. Bolts were split along galleries identified on the ends of bolts and then subsequently down to 2- to 5-cm slivers to facilitate recovery of all larvae. Recovered larvae were placed in glass vials and subsequently monitored for eclosion. Digital images were taken of a number of parasitoid larvae before and after eclosion, which allowed identification of Ibaliid and Rhyssine larvae to the species and genus level, respectively (Fig. 2). In general, Ibaliid larvae were much smaller than Rhyssine larvae, but when a larva was encountered whose size was questionable, mouthparts were examined as these are markedly different between the two genera. Voucher specimens of adults were deposited in the SUNY-ESF insect museum, Syracuse, NY.

Siricid larvae are distinctive with a dark schlerotized posterior spine on their abdomen. However, *S. noctilio* larvae cannot be distinguished visually from larvae of our native siricids, *S. nigricornis* F. and *S. edwardsii* Brullé, and so are referred to collectively as siricids. A relatively small number of siricid larvae were injured or killed during the splitting process (42) and were included in data analyses. There was high mortality of siricids placed in rearing vials with only 216 of 1,211 of the siricid pupae and larvae eclosing to adults. Although all of the 216 siricids that eclosed were *S. noctilio,* it is likely that a few of the larvae that perished were natives. This number was likely very low, however, as subsequent rearing of adults from infested host material the next year yielded <1% native siricids (P. T. Eager, unpublished data). Parasitoids emerged from 49 of the siricid larvae in rearing vials and all were *I. l. ensiger.*

Within-Tree Distributions. Diameter of each 0.5-m bolt was measured at the midpoint by using a diameter tape before splitting. Larval counts were recorded for each bolt. Total number of siricids included living and dead siricid larvae, pupae and teneral adults, plus all parasitoid larvae (as they each fed on a siricid larva).

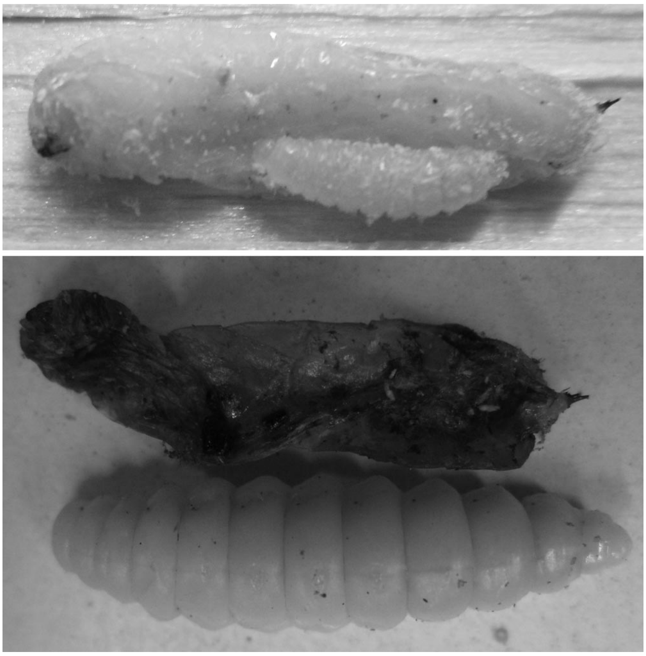

Fig. 2. Hymenopteran parasitoids emerging from siricid larvae recovered from infested pines in central New York. A) *I. l. ensiger* larva and B) Rhyssine larva (*Rhyssa persuasoria* or *R. lineolata*).

Table 2. Summary statistics for *S. noctilio* and siricid parasitoid larvae found in *P. resinosa* and *P. sylvestris* harvested in 2008 from various sites in central New York

Tree species	Insect	Mean no./tree ± SE	Mean density/m^3 ± SE	Mean % parasitism/ tree ± SE
Red pine ($n = 12$)	Total siricids	101.2 ± 34.1 (range = 4–337)	822.4 ± 207.9	
	Dead siricids	29.1 ± 11.6	303.5 ± 105.5	
	Living siricids	48.1 ± 18.5	331.4 ± 102.8	
	I. l. ensiger	18.5 ± 10.9	127.2 ± 54.9	10.8 ± 3.6
	Rhyssines	5.5 ± 2.7	52.0 ± 30.7	4.4 ± 1.8
Scots pine ($n = 6$)	Total siricids	219.3 ± 103.0 (range = 5–704)	2057.5 ± 714.8	
	Dead siricids	61.7 ± 33.4	413.9 ± 116.6	
	Living siricids	112.7 ± 51.5	1202.7 ± 552.6	
	I. l. ensiger	26.3 ± 11.3	228.5 ± 83.5	10.6 ± 2.8
	Rhyssines	21.7 ± 9.4	178.2 ± 99.8	8.3 ± 3.5

Although *R. persuasoria* and *R. lineolata* are documented parasitoids of *Monochamus* spp (Coleoptera: Cerambycidae) (Naves et al. 2005), and 92 longhorned beetle larvae were found during bolt dissections, all parasitoids found were associated with siricid larval remains and so all were assumed to have emerged from siricids.

Zero-inflated poisson (ZIP) regression models were fitted to the count data for each species group (siricids, *I. l. ensiger*, and rhyssines) within a given bolt by using the ZIP function in Stata 11.2 (StataCorp 2011). Zero-inflated rather than standard poisson models were necessary to account for overdispersion because of an excess of zeros (Vuong test $z \geq 4.23$, $P < 0.001$). Candidate models included combinations of covariates representing region (north, central, south); individual stand; species (Scots or red pine); and bolt diameter (cm). Akaike's Information Criterion (AIC_c; Burnham and Anderson 2002) were used to identify the most parsimonious models (Appendix A). Bolts were nested within tree as they were nonindependent subsamples and robust standard errors were calculated using the Huber-White sandwich estimator available in Stata.

Bark Thickness. A Swedish bark depth gauge was used to assess bark thickness at three evenly spaced intervals around the midpoint of each bolt and these values were averaged for each bolt. Assumptions of normality for bark thickness were tested using the Shapiro–Wilk test and data transformed using the Box–Cox method (Box and Cox 1964). Regression analysis was used to evaluate correlation of bark thickness with siricid and parasitoid densities. Insect densities were the dependent variables and bark thickness was the independent variable. Bolts varied in bark thickness as well as siricid densities and so in construction of the model, bolts were considered the sample unit and were nested within individual trees and trees were considered a random effect. Statistical analyses were performed in JMP 8 (SAS Institute 2009) and considered significant at $\alpha = 0.05$.

Optimal Sampling Method. Best subsets regression in Minitab 15 (Minitab Inc. 2007) was used to determine which combinations of 0.5-m bolts best estimated within-tree siricid and parasitoid densities. Best subsets regression allows evaluation of nine values (or subsamples) to be tested at once. Because of this, bolts labeled with whole numbers (0, 1, 2, etc.) for height-off-the-ground were regressed against within-tree counts of siricids, *I. l ensiger,* and rhyssines, followed by bolts taken at half meter intervals (0.5, 1.5, 2.5, etc.). Bolts consistently used in successive best-subsets regression runs were identified and those consistently present were included in a final best-subsets regression to determine the most efficient number and the most effective combination of bolts, in terms of standard error and adjusted R^2 values, at estimating within-tree densities for each insect species.

Linear regression analyses between whole-tree siricid and parasitoid totals, and totals in the best 2-, 3-, 4-, and -5-bolt combinations for predicting species densities were conducted using JMP 8. Five trees <8.5 m in height were not tall enough to contain the required number of bolt combinations and so were excluded from some of these analyses. Regression equations were used to estimate within-tree siricid and parasitoid densities for all trees that had corresponding bolts. Equations created using only red pine resulted in accurate estimates for within-tree species densities of Scots pine. Similarly, equations constructed using all trees resulted in accurate within-tree species densities for both red and Scots pine. Matched pair analysis using paired *t*-tests was completed in JMP 8 to determine if there were statistically significant differences between observed and estimated species densities. Percent bias between observed and estimated species densities was calculated for all trees and presented as mean bias, which indicates the extent to which estimated values over- or underestimate species densities as compared with observed densities.

Results

Insect Population Estimates and Percent Parasitism. In total, 2,558 siricids were recovered from sampled trees (Table 2). Of these, 1,972 were immature siricids and 586 were teneral *S. noctilio* adults. There were 719 dead siricid larvae in sampled trees, with cause of mortality unknown. In total, 384 *I. l. ensiger* were recovered, 218 in red pine and 166 in Scots pine (range, 0–133 for individual trees). There were 202 rhyssines recovered, 65 from red pine and 137 from Scots pine (range, 0–54 for individual trees). Densities based on wood volume (m^3) are provided to facilitate comparisons with future studies (Table 2).

Table 3. Best supported models predicting the number of siricids

Model	Variable	Siricids			*I. l. ensiger*			Rhyssines		
		β	SE	Factor change	β	SE	Factor change	β	SE	Factor change
Count	Region (Central)	−0.5446	0.2754*	0.76	−0.6508		0.73	−2.2659	0.4303**	0.33
	Region (South)	−0.8439	0.3838*	0.66	−1.8162	0.1972**	0.41	−3.9752	0.8605**	0.14
	Stand (Onondaga)	—			1.5603	0.3873**	1.84	3.7082	1.0013**	4.26
	Stand (SBSF)	—			0.7945	0.5379**	1.40	1.4762	0.7557	1.86
	Species (Scots Pine)	0.4041	0.2921	1.20	−0.8723	0.2987**	0.67	—		
	Diameter	0.7695	0.2581**	30.82	1.1565	0.3285**	172.78	1.3012	0.2680**	329.19
	Diameter^2	−0.0220	0.0081**	0.05	−0.0348	0.2660**	0.01	−0.0371	0.0079**	0.01
	Constant	−3.2757	1.7332		−6.5827	0.0087**		−7.8648	1.9126**	
						1.6916**				
	Region (Central)	—			3.5807	0.9818**	35.90	—		31.31
Inflation	Stand (Onondaga)	1.5192	0.5612**	4.57	—			3.4438	0.9508**	0.25
	Stand (Heiberg)	—			—			−1.3823	0.6487	
	Species (Scots Pine)	—			−4.4447	0.9050**	0.01	—		
	Diameter	0.0989	0.0425*	1.10	—			—		
	Constant	−2.6931	0.6669**		0.3321	0.4547		0.6588	0.3854	

(Wald χ^2 = 68.28, df = 5, $P < 0.001$), *I. l. ensiger* (Wald χ^2 = 922.55, df = 7, $P < 0.001$), and rhyssines (Wald χ^2 = 1756.26, df = 6, $P < 0.001$) from 342 bolt samples from 18 trees in New York State.

For each variable, in each portion of the model (predicted count vs inflation factor to predict zero counts) is the estimated coefficient (β) with standard error (SE). Standard errors were adjusted to account for non-independence of samples taken from 18 individual trees.

"—" indicates a variable not included in the given model. Significance of the z-score testing for non-zero coefficients is indicated by "*" where $P < 0.05$ and "**" where $P < 0.01$. Also shown is the factor change (akin to odds ratios) for the expected count or probability of having a zero count in the inflation portion of the model.

The total count of each species group varied with region, species, specific stand, or both, and bolt diameter (Table 3). The most parsimonious predictive model indicated that all three insect species groups declined in abundance from the northern, to the central, and then to the southern region. There was some model selection uncertainty with respect to whether tree species affected the "zero" counts for siricids or the total number of rhyssines above and beyond stand differences. However, the final model indicated that Scots pine hosted larger numbers of siricids than red pines in the central and northern stands (Fig. 3A), and also higher numbers of *I. l. ensiger* in the central stands (Fig. 3B). All three groups responded similarly to bolt diameter, indicating a peak in abundance around diameters of 15.4–18.8 cm (Fig. 3A–C).

Overall percent parasitism was 16.4 ± 3.8%. The highest ranked predictive model indicated that the probability of parasitism varied with bolt diameter, region, and stand (AIC$_c$ w_i = 0.74), with minor model selection uncertainty regarding the effect of tree species (w_i = 0.22). The most parsimonious model (Wald χ^2 = 41.28, df = 5, $P < 0.001$) indicated that total parasitism was 1.7 and 3.6 times greater in the central and northern region, respectively, compared with the southern stands, and this varied nonlinearly with bolt diameter indicating a peak in parasitism around 15 cm in diameter (Fig. 4). The model explained a small fraction of the total variation in parasitism (Pseudo-R^2 = 0.18), but had a moderate predictive accuracy (area under the receiver-operating characteristic curve = 0.78).

Bark Thickness. There was no significant difference in bark thickness between red pine (2.7 ± 0.2 mm) and Scots pine bolts (3.1 ± 0.3 mm) (N = 337; F = 1.4; df = 1, 336; P = 0.23); thus, the two species were pooled for analysis. There was no effect of bark thickness on densities of siricids (N = 337; F = 0.65; df = 1, 335; P = 0.43); *I. l. ensiger* (F = 0.64; df = 1, 335; P = 0.45); or Rhyssines (F = 4.4; df = 1, 335; P = 0.06).

Optimal Sampling Method. The best 2-, 3-, 4-, and -5-bolt predictors of within-tree species densities were determined by best subsets regression analyses (Table 4). Linear regression of species density estimates derived using the equations and actual within-tree species densities indicated significant correlations. Matched pair analysis indicated no significant difference between observed and estimated densities for any insect species (Table 5). Mean bias estimates indicated that estimates and observed values differed by <1%.

Discussion

Our data indicate there were significant differences in insect densities by region with more insects predicted to be present in infested pines in the north. Although *S. noctilio* was discovered initially in Fulton, NY in 2004, subsequent surveys revealed it was relatively widespread in central and western New York (Dodds et al. 2007) and so the initial introduction point is unknown. The northern stands were approximately equidistant (≈43 km) from Fulton, NY as the central sites, whereas the southern sites were further away (≈127 km). There may be landscape factors, e.g., large water bodies, urban areas, affecting dispersal, and colonization that may account for density differences between the northern and central stands.

There were higher predicted insect densities by tree species with higher numbers in Scots pine than in red pine for the Northern and Central regions. Scots pine is a non-native tree species planted in the northeastern United States, but it is a species that *S. noctilio* uses in its native habitat. It may be that *S. noctilio* is

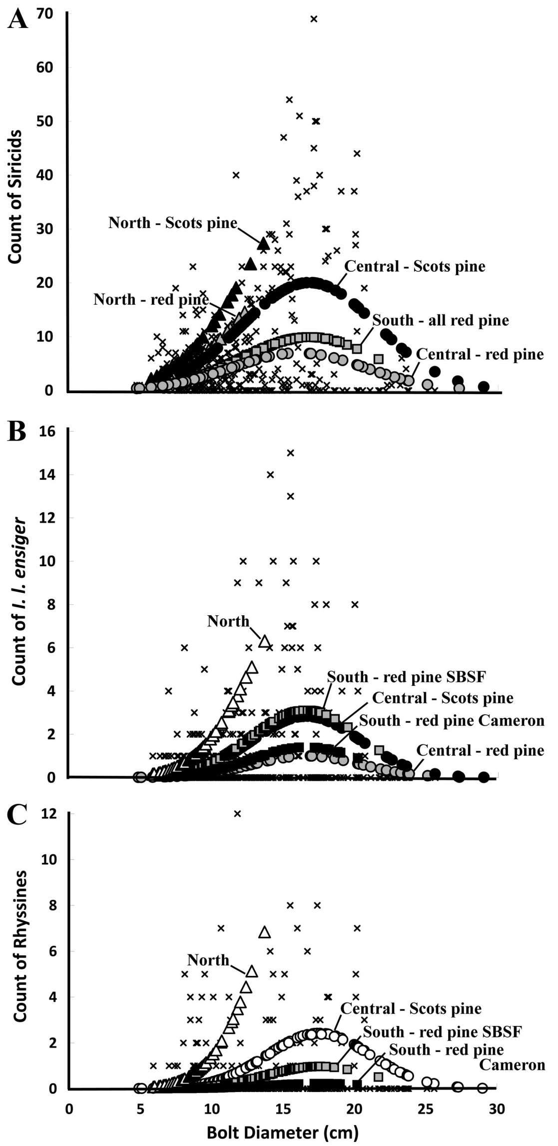

Fig. 3. Numbers of insects observed in 0.5-m tree bolts sampled in New York State in June 2008. Observed counts are shown as "×" for siricids (A), *I. l. ensiger* (B), and rhyssines (C). Model predicted counts are indicated by symbols representing different stands within the Northern (triangles), Central (circles), and Southern regions (squares). Note that only red pine was measured in the southern region.

exhibiting a preference for this tree as a known host over red pine, a novel host, or it may be that red pine is more successful at defending itself against attack. More research is necessary to provide insights into this particular aspect of *S. noctilio* host utilization.

We found significantly higher numbers of insects in bolts with diameters ranging from ≈14 to 20 cm in diameter. This suggests there may be some preference, or constraint, for oviposition, and understanding why may be important in elucidating patterns of siricid attack and subsequent parasitism. This portion of the tree may be more attractive for *S. noctilio* oviposition because larvae can escape avian predation (Spradbery 1990) and perhaps parasitism if they are deeper within the tree. Using this logic, it would seem that more siricids would be found in the lower section of the tree, however, this was not the case and so there must be a trade-off relative to host quality or another aspect of the insect's life history requirements in the larger diameter bolts.

Numbers of parasitoids varied spatially, among regions and stands, as well as between tree species and within the tree bole, but were similar to those of their siricid hosts (Fig. 3A and B and C). Parasitism was higher in infested trees taken from the northern stands followed by the central Scots pine stand and the southern red pine stands (Fig. 4). It is unknown why parasitism was so low in the central red pine stand but it may be associated with low numbers of siricids in the stand overall (Fig. 3A). Maximum parasitism occurred in bolts ranging from ≈13 to 17 cm in diameter (Fig. 4) and so siricids occupying smaller bolts were not parasitized more successfully, indicating that diameter is not a constraint to parasitism. Continued monitoring of parasitism is needed to provide more insights into spatial as well as temporal variation in parasitism and importance of the various parasitoids.

Ibalia leucospoides is the most successful parasitoid in many areas where it has been introduced against *S. noctilio* (Eldridge and Taylor 1989, Eskiviski et al. 2004, Tribe and Cillié 2004). Results of this study indicate *I. l. ensiger* is contributing to *S. noctilio* mortality in North America with parasitism at close to 11%. Long et al. (2009) documented *I. l. ensiger* parasitism at ≈20.5%, however, their study quantified percent parasitism based only on emerged insects. Methodologies employed in this study should provide a more complete assessment of parasitism as a percentage of each of these groups of insects would not have emerged using standard rearing methods because of mortality from other agents. Using only live siricids and assuming these would have emerged as adults, percent parasitism by *I. l. ensiger* in this study would have been calculated as ≈17%, which is closer to that documented by Long et al. (2009).

Relative importance of introduced parasitoid species in the southern hemisphere varies with locality. *Megarhyssa nortoni* and *R. persuasoria* are effective at reducing *S. noctilio* populations in Tasmania (Taylor 1978), whereas *I. leucospoides* was more effective on mainland Australia (Eldridge and Taylor 1989, Haugen 1990, Neumann et al. 1993). In South Africa and South America, *I. leucospoides* has been the most successful parasitoid (Eskiviski et al. 2004, Tribe and Cillié 2004, Hurley et al. 2007). It has been suggested that *I. l. ensiger* may be the most successful parasitoid in the United States (Long et al. 2009) and although our data appear to support this (rhyssine parasitism was <7%), a caveat is that trees were taken in June, after the April and May emergence and oviposition period of rhyssine parasitoids. We did find rhyssine larvae in sampled trees, however, small larvae (feeding externally on the larger siricid larvae) may not have survived the

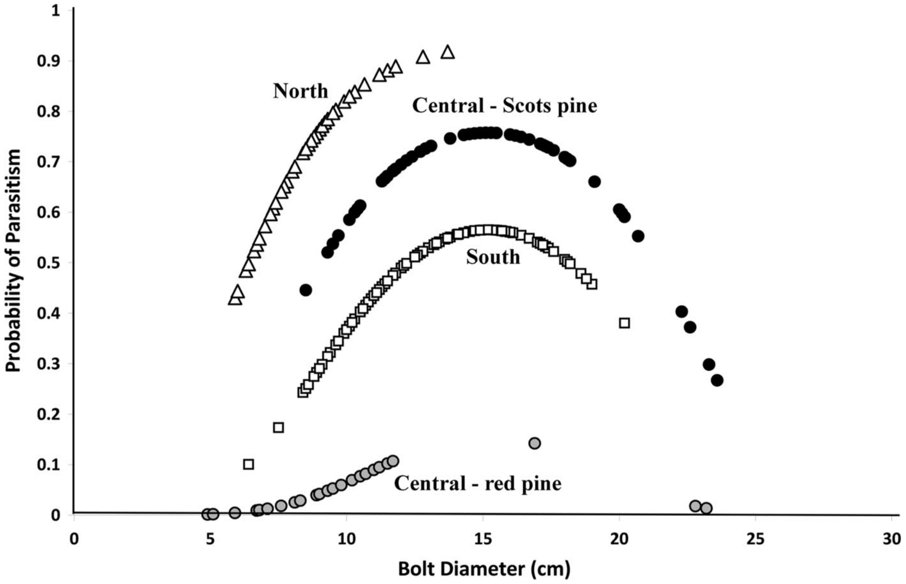

Fig. 4. Predicted probability of parasitism across the range of bolt diameters observed in the Northern, Central, and Southern regions of New York State. Note that total parasitism levels varied between Scots Pine and Red Pine stands in the central region.

splitting process. We also would add that rearing from our trees would not have yielded rhyssine adults until the next year. This also was likely the case with the previous New York rearing study (Long et al. 2009) as those sampled trees were felled in late June and adults reared from July through October and so rhyssines parasitizing siricids in those trees in April and May would not have emerged until the next year.

By carefully splitting sample bolts, we found 719 dead siricid larvae (28% of the total number of siricid larvae). This significant mortality could not be readily attributed to a specific agent. Although a thorough investigation of potential causes was beyond the scope of this research, a majority of dead larvae had mycelia protruding from their exoskeleton (similar to that of *Bauveria* spp.) and many of the other dead larvae were blackened and liquefied. It is unknown if these were primary or secondary agents and this aspect merits further research.

Penteado et al. (2000) and Long et al. (2009) hypothesized that bark thickness may constrain *S. noctilio* oviposition and subsequent parasitoid attack. Our results indicate this variable explains little biologically relevant variation in presence or densities of insect species or in parasitism. In fact, we found 26 *S. noctilio* larvae as well as three *I. l. ensiger* in the lowest 0.5-m portion of a *P. sylvestris* with a bark thickness of 16.3 mm, the highest measurement of bark thickness in our study. Also, 12 rhyssines were found in a bolt with a bark thickness of 9.7 mm, more than three times thicker than the mean thickness across all bolts. All sampled trees (and bolts) exhibited a range of bark thickness and even those with thick bark still had crevices that could be exploited by ovipositing insects.

Table 4. The best 2-, 3-, 4-, and 5-bolt predictors of within-tree *S. noctilio* and parasitoid densities using best-subsets regression analysis

Species	No. of bolts	Optimal ½ m bolts	No. trees	Equation R^2 (adj.)	Equation
Siricids	2	4.5, 8.5	13	97.2%	Est. density = 19.91 + 8.67*SUM 2 bolts
	3	1.5, 4.5, 8.5	13	97.1%	Est. density = −15.69 + 8.47*SUM 3 bolts
	4	2, 4.5, 6, 8.5	13	99.0%	Est. density = −5.21 + 5.61*SUM 4 bolts
	5	1.5, 2, 4.5, 6, 8.5	13	94.3%	Est. density = −19.50 + 5.29*SUM 5 bolts
I. l. ensiger	2	6, 8.5	13	97.8%	Est. density = 3.73 + 8.57*SUM 2 bolts
	3	1.5, 5.5, 6	16	94.2%	Est. density = 1.85 + 6.70*SUM 3 bolts
	4	1.5, 4.5, 5.5, 6	16	97.3%	Est. density = 1.61 + 5.10*SUM 4 bolts
	5	1.5, 4.5, 5.5, 6, 8	13	98.0%	Est. density = 1.96 + 3.98*SUM 5 bolts
Rhyssines	2	2.5, 5	17	80.8%	Est. density = 2.93 + 6.05*SUM 2 bolts
	3	5, 8,8.5	13	91.3%	Est. density = 2.96 + 5.37*SUM 3 bolts
	4	3, 4.5, 7, 8.5	13	97.3%	Est. density = 0.38 + 3.65*SUM 4 bolts
	5	3, 4.5, 7, 8, 8.5	13	99.7%	Est. density = 0.57 + 3.15*SUM 5 bolts

All equations were significant ($P < 0.0001$). Numbers of trees used to develop specific equations differed based on tree height (some trees were not tall enough to contain the bolts consistently designated).

Table 5. Comparison of observed and estimated within-tree species densities in trees that had all requisite bolts (i.e. were >8.5 m tall)

Tree no.	*S. noctilio*					*I. l. ensiger*					Rhyssines				
	Obs. density	Estimated density				Obs. density	Estimated density				Obs. density	Estimated density			
		2 bolt	3 bolt	4 bolt	5 bolt		2 bolt	3 bolt	4 bolt	5 bolt		2 bolt	3 bolt	4 bolt	5 bolt
1	334	340.7	297.8	364.8	329.5	133	132.3	129.1	129.2	137.3	25	15	19.1	22.3	22.7
3	84	106.6	94.4	67.7	65.1	5	3.7	15.3	11.8	9.9	4	2.9	3	4	3.7
7	4	19.9	0	0	0	0	3.7	1.9	1.6	2	0	2.9	3	0.4	0.6
8	111	89.3	196.1	112.5	181.4	13	3.7	15.3	11.8	13.9	0	2.9	3	0.4	0.6
10	337	254	374	359.2	424.7	30	20.9	22	27.1	25.8	5	9	8.3	4	3.7
11	704	730.9	687.5	667.5	620.4	75	72.3	82.2	83.3	65.7	54	33.2	62	47.9	54.2
12	291	271.4	255.4	303.1	287.2	41	46.6	35.3	42.4	33.8	41	51.3	29.8	47.9	41.6
13	88	63.3	102.9	95.7	123.3	11	3.7	8.6	6.7	5.9	4	2.9	3	4	3.7
14	4	19.9	0	0.4	0	0	3.7	1.9	1.6	2	0	2.9	3	0.4	0.6
15	38	54.6	35.1	17.2	12.2	0	3.7	1.9	1.6	2	0	2.9	3	0.4	0.6
16	108	132.6	94.4	95.7	75.7	1	3.7	1.9	1.6	2	1	2.9	3	0.4	0.6
17	32	63.3	26.7	34	17.5	7	12.3	8.6	6.7	5.9	3	2.9	3	4	3.7
18	118	106.6	111.4	140.5	144.4	24	29.5	28.6	22	33.8	25	15	19.1	25.9	25.8
Mean	173.3	173.3	175.1	173.7	175.5	26.2	26.1	27.1	26.7	26.2	11.8	11.3	12.5	12.5	12.5
Bias		-0.69	0.12	0.17	0.18		0.73	0.80	0.82	0.80		0.83	0.83	0.93	0.92
t		0.00	0.20	0.08	0.18		0.00	0.69	0.55	0.00		0.00	0.02	0.00	0.30
df		12	12	12	12		12	12	12	12		12	12	12	12
P		1.00	0.85	0.94	0.86		0.99	0.50	0.59	1.00		0.61	0.99	1.00	0.98

Mean percent bias indicates how much calculated density estimates over (negative values) or under-estimate densities relative to actual densities.

Whole-tree sampling of wood-boring insects requires transportation, storage, and monitoring of large amounts of host material, thus incurring considerable expenditures of time, space, and funds (Fierke et al. 2005). An optimal sampling approach reduces host material needed (Stephen and Taha 1976, Crook et al. 2007). For example, Penteado et al. (2000) recommended sampling the middle 50% of infested *Pinus taeda* L. for surveys of *S. noctilio* and its parasitoids. However, even sampling the middle 50% would still result in a significant amount of tree material to be transported and monitored for insect emergence and would not yield whole-tree densities. Despite the small sample size in this study, our results indicated that even a 2-bolt sampling method was statistically reliable and could be used to accurately extrapolate to whole-tree densities.

The decision to use a particular subsampling scheme should be based on the insect group being investigated (siricids or parasitoids, see Table 4), keeping in mind that there is within-tree variability in densities and that increasing the number of trees will increase confidence in values obtained. If equations presented here are adopted to answer particular research questions, they should be validated by whole-tree sampling or alternate equations developed (and validated) based on techniques described above. These caveats are recommended as these equations were developed using data from red and Scots pine in central New York in 2008 and there may be variation of within-tree densities of the insects of interest based on pine species, study location, or year that were not addressed in the current study.

To better monitor species introductions and subsequent infestation, it is important to establish a baseline understanding of how introduced species are adapting to their new environments. This study provides important information on within-tree and landscape level siricid densities and how the native parasitoid complex is responding to the *S. noctilio* infestation. These findings can be compared with future research as the infestation spreads to ecologically and economically important southern and western *Pinus* plantations and forests. Subsampling techniques developed here can also be repeated in other areas to reduce sampling expenses and facilitate stand and landscape level comparisons of woodwasp densities as well as development of management protocols. Further, we recommend that future studies should be conducted to evaluate siricid mortality because of pathogens and the role pathogens play in natural settings.

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Appendix A. Weight of evidence behind candidate zero-inflated Poisson regression models predicting the count of siricids (N = 248 nonzero and 94 zero observations); *Ibalia l. ensiger* (N = 113 nonzero and 229 zero observations); and rhyssines (N = 71 nonzero, 271 zero observations) in 342 bolts sampled from 18 trees in New York State

Count variables	Inflation variables	LL	K	ΔAIC_c	w_i	Σw_i
Siricids						
Region, species, diam(poly)	Stand(1), diam	−1396.44	9	0.00	0.64	0.64
Region, species, diam(poly)	Stand(1), species, diam	−1395.59	10	1.30	0.33	0.97
Region, species, diam(poly)	Stand(6), diam	−1393.95	13	7.02	0.02	0.99
Region, species, diam(poly)	Stand(1)	−1401.96	8	8.04	0.01	1.00
I. l. ensiger						
Region, stand(2), species, diam(poly)	Region(1), species	−403.90	11	0.00	0.86	0.86
Region(1), stand(2), species, diam(poly)	Region(1), species	−407.55	10	4.30	0.10	0.96
Region, stand(2), diam(poly)	Region(1), species	−408.51	10	6.22	0.04	1.00
Rhyssines						
Region, stand(2), diam(poly)	Stand(2)	−255.56	10	0.00	0.64	0.64
Region, stand(2), species, diam(poly)	Stand(2)	−255.51	11	2.90	0.15	0.79
Region, stand(1), species, diam(poly)	Stand(2)	−257.55	10	3.98	0.09	0.88
Region, stand(2), species, diam(poly)	Region	−256.58	11	5.04	0.05	0.93
Region, stand(2), species, diam(poly)	Species	−258.88	10	6.64	0.02	0.95

For each model the variables used to predict the count versus the inflation factor to predict zero responses are indicated along with the model log-likelihood (LL), number of estimated parameters (K), difference in AIC_c (given $N = 342$) and the AIC_c model wt (w_i). The set of models shown for each species grouping comprise at least 95% of the total model wt (Σw_i).