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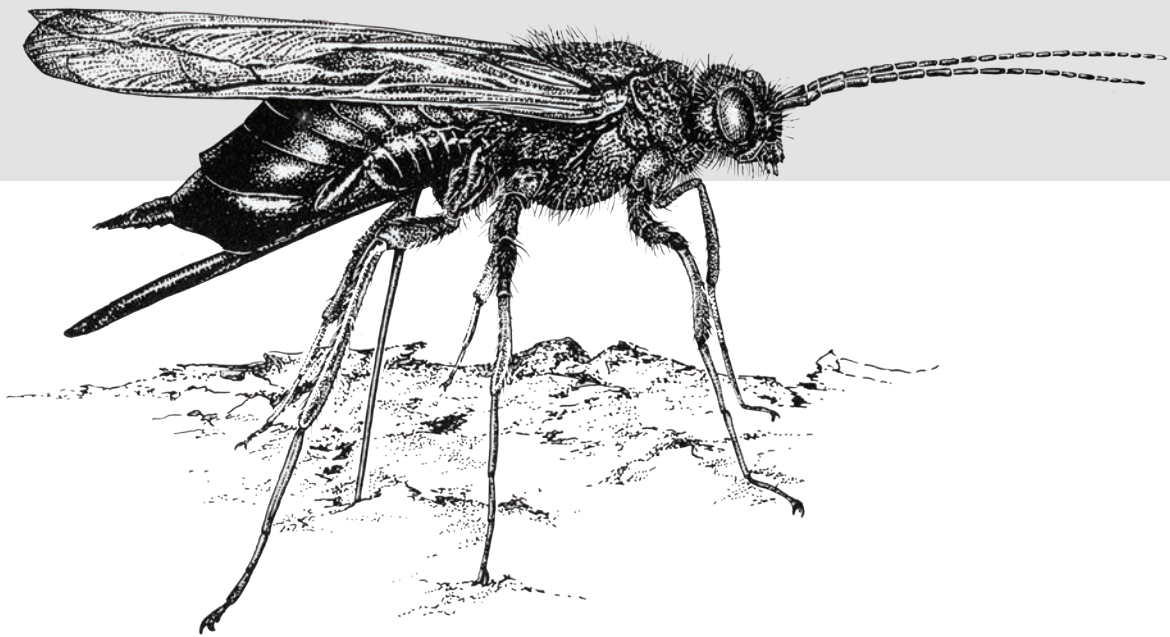
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BIOLOGY AND ECOLOGY OF *SIREX NOCTILIO* IN NORTH AMERICA

Edited by Ann E. Hajek, Laurel J. Haavik, and Fred M. Stephen



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BIOLOGY AND ECOLOGY OF *SIREX NOCTILIO* IN NORTH AMERICA

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Cover Images: (top) red crown of dead Scots pine (center) in September 2014, attacked and colonized by *Sirex noctilio* in summer 2014 (image by L.J. Haavik); (bottom) line drawing of *Sirex noctilio* (illustration from Taylor, K.L. 1981. The Sirex woodwasp: Ecology and control of an introduced forest insect, pp. 230–248. In: R.L. Kitching and Jones, R.E. [eds.] The Ecology of Pests: Some Australian Case Histories. CSIRO, Melbourne, Australia; reprinted with permission)

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Dedication

We dedicate this review to Dr. Richard (Dick) Reardon in recognition of his outstanding contributions to promoting and supporting our understanding of forest entomology, biological control, invasive species, and insect conservation. Dick received his BS from Utah State University in wildlife management in 1966 after which he moved to the University of Michigan where he studied forest entomology, receiving an MS in 1968 and a PhD in 1975. Dick worked for the USDA for 53 years in the Northeast Forest Experiment Station in Hamden, Connecticut, in Forest Health Protection in Hamden, Connecticut, at the Pacific Southwest Forest and Range Experiment Station in Davis, California and in Forest Health Protection, Morgantown, West Virginia. Throughout this time, Dick's work involved biological control of invasive plants and arthropods and development of the biological insecticides Btk and Gypchek for gypsy moth management. A long-standing contribution has also been Dick's leadership and involvement in producing outreach publications to improve information transfer between scientists, land managers, and the public. Throughout his career Dick promoted over 100 publications, including FHTET and FHAASST publications like this one.



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**CHAPTER
1****Global biogeography of *Sirex noctilio*
with emphasis on North America****Andrew M. Liebhold^{1,2} and Ann E. Hajek³**¹USDA Forest Service Northern Research Station, Morgantown, West Virginia 26505 USA²Faculty of Forestry and Wood Sciences, Czech University of Life Sciences Prague, Forestry and Wood Sciences, 165 21, Prague, Czech Republic³Department of Entomology, Cornell University, Ithaca, New York 14853-2601 USA**Abstract**

Sirex noctilio is a Eurasian species in the woodwasp (Siricidae) insect family, a small and poorly understood group of insects. Several siricid species have invaded various world regions due to their frequent presence in wood moving in international trade. Like most other woodwasps, feeding by *S. noctilio* occurs in weakened or dying pines in its native range, and populations exist there at relatively low levels. But in non-native regions of the Southern Hemisphere, *S. noctilio* populations have thrived, colonizing hosts in plantations of non-native pines planted at high densities. Under such conditions, *S. noctilio* reproduction can drive populations to epidemic densities, causing considerable tree mortality. Invasions in the Southern Hemisphere started with colonization of New Zealand around 1900, then Australia in 1952, South America in 1980, and South Africa in 1994. Given the reputation of *S. noctilio* as a successful invader and its repeated interception from imported cargo at ports, invasion of North America by this species was greatly anticipated with caution. Those fears came true when it was detected in a trap placed in New York near Lake Ontario in 2004. Subsequent delimitation surveys, and discovery of larvae within trees, confirmed the presence of the species over a large 19-county area, indicating that populations had been present for many years and eradication was considered infeasible. Subsequently, *S. noctilio* has been found in nine northeastern US states and two eastern Canadian provinces and appears to be expanding its range. To date, however, populations in North America remain low and have caused little damage, similar to what is seen throughout most of its native range. Large regions of pine-dominated forests are still likely to be invaded, but it is not clear whether these regions are climatically suitable and whether populations will continue to remain at low densities as it spreads into regions with abundant pine forests.

Introduction

The species *Sirex noctilio* is a member of the Siricidae (woodwasp or horntail) family in the order Hymenoptera. The Hymenoptera exhibit considerable variation in life histories, including groups with species playing diverse ecosystem roles such as nectar/pollen-feeders, predators, parasitoids, and herbivores. The hymenopteran order is also characterized by major differences in the morphology and ecological niches of immature and mature forms; in some groups, adults provision immatures, and this is sometimes associated with varying levels of social behavior.

The Siricidae belong to the suborder Symphyta, which is considered the more evolutionarily primitive branch of the Hymenoptera and contains only herbivorous insects, including sawflies and woodwasps. The Siricidae is a relatively small family, with only about 100 known species that are almost entirely native to the Northern Hemisphere. All of these species exist most of their lives as larvae that bore in woody tissue of trees. Within the Siricidae, there are two subfamilies: the Tremicinae that mostly feed on hardwoods and the Siricinae that feed on conifers. A total of 23 species in the Siricidae are known to be native to North America (Schiff et al. 2012) (Table 1).

Table 1. Siricidae native to North America, north of Mexico (from Schiff et al. 2012).

SUBFAMILY	SPECIES	HOSTS ¹	NATIVE RANGE
Tremicinae	<i>Tremex columba</i>	Wide variety of hardwoods; main hosts are hickories, maples, and elms	Transcontinental N. America
	<i>Xeris caudatus</i>	Fir , spruce, pine, Douglas-fir	Western N. America
	<i>Xeris chiricahua</i>	Host tree not known	Western N. America
	<i>Xeris indecisis</i>	Wide host range in Pinaceae	Western N. America
	<i>Xeris melancholicus</i>	Wide host range in Pinaceae	Eastern and north-central N. America
	<i>Xeris morrisoni</i>	Fir , Douglas-fir, spruce	Western N. America
	<i>Xeris tarsalis</i>	Wide host range in Cupressaceae	Western N. America
Siricinae	<i>Sirex abietinus</i>	Fir , spruce, hemlock	Western N. America
	<i>Sirex areolatus</i>	Very wide host range with major hosts in Cupressaceae	Western N. America
	<i>Sirex behrensii</i>	Pine , Douglas-fir	Western N. America
	<i>Sirex californicus</i>	Pine , with a few other spp.	Western N. America
	<i>Sirex cyaneus</i>	Fir , larch, spruce, pine	Eastern and north-central N. America
	<i>Sirex longicauda</i>	Fir , pine, Douglas-fir	Western N. America
	<i>Sirex nigricornis</i>	Pine , spruce	Eastern and north-central N. America
	<i>Sirex nitidus</i>	Spruce , fir, larch, Douglas-fir, hemlock, pine, cypress	Transcontinental N. America
	<i>Sirex obesus</i>	Pine	Western N. America
	<i>Sirex varipes</i>	Very wide host range in Pinaceae	Western N. America
	<i>Sirex xerophilus</i>	Pine	Western N. America
	<i>Urocerus albicornis</i>	Very wide host range in Pinaceae, hemlock	Transcontinental N. America
	<i>Urocerus californicus</i>	Very wide host range in Pinaceae	Western N. America
	<i>Urocerus cressoni</i>	Wide host range in Pinaceae	Eastern and north-central N. America
	<i>Urocerus flavicornis</i>	Wide host range in Pinaceae, cedar	Transcontinental N. America
	<i>Urocerus taxodii</i>	Bald cypress	Eastern N. America

¹ Tree genera are in bold, when siricid species predominantly use that genus as a host.

All Siricidae share a common life history and exploit similar ecological niches. Adult females oviposit directly into tree xylem, and larvae spend their entire lives boring through woody tissue. This tissue is composed almost entirely of cellulose and lignin, neither of which can be directly digested by any insect species. Like other xylem-chewing insects (e.g., ambrosia beetles), siricids have co-evolved with mutualistic fungi that break down this material into compounds that can be readily assimilated. As described in chapters 4 and 5, the association of *S. noctilio* with its microbial symbionts is only partially understood, and even less is known about fungal symbionts of other species of Siricidae.

Most siricids colonize weakened and/or dying trees that lack physiological resistance to defend themselves. The degree to which hosts must be weakened prior to colonization varies considerably among siricid species. For example, in North America the native siricid *S. nigricornis* is only found colonizing severely weakened trees (see Chapter 6). These weakened trees may be dying as a result of wind damage, attack by other insects or diseases or suppression by larger trees. Invasive populations of *S. noctilio* in North America have behaved similarly, though in rare instances more vigorous trees have been colonized in addition to highly suppressed individuals in the same stand. *Sirex noctilio* is native across Eurasia (Fig. 1) but little is known about this species through most of this region. It is known that within the native range of *S. noctilio* in Europe, tree colonization is almost entirely limited to weakened and dying trees (Wermelinger and Thomsen 2012; Lombardero et al. 2016). However, within non-native *S. noctilio* populations in the Southern Hemisphere, colonization of seemingly vigorous trees is fairly common, particularly during outbreaks. It is not fully understood why outbreaks of *S. noctilio* occur in the Southern Hemisphere and why they sometimes colonize healthy trees there, while this generally does not happen in Europe and North America (see Chapter 10).

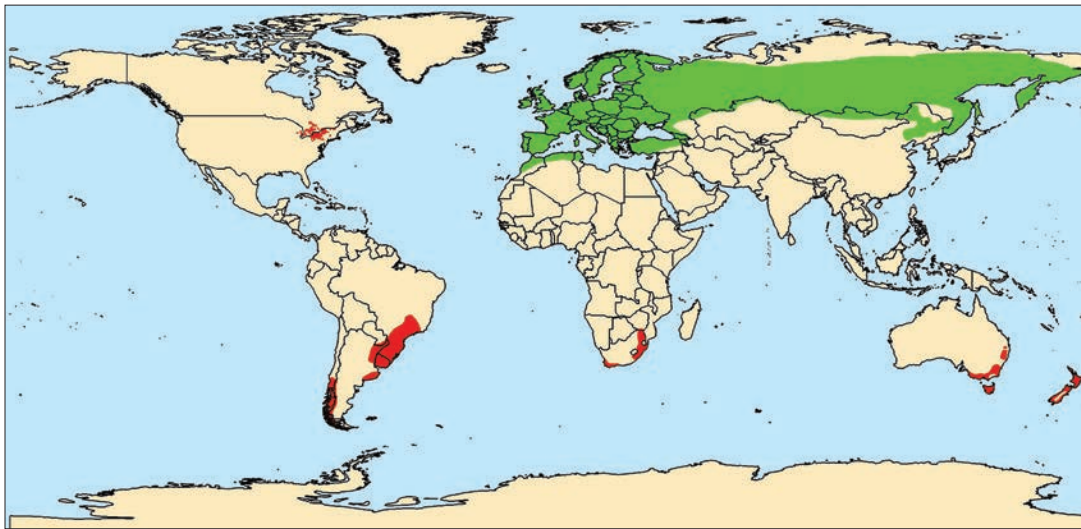


Figure 1. Global distribution of native and non-native *Sirex noctilio* populations. Native range is shown in green and invaded range in red.

History of *S. noctilio* global spread

Even though the Siricidae is a relatively small insect family, there are at least seven species that have successfully invaded new regions; three of these species have invaded North America (Table 2). Invasion success in this group can be attributed in part to their tendency to be accidentally transported with wood and wood packaging material, such as wooden pallets, crates, and dunnage. Burnip et al. (2010) reported

Table 2. Siricidae known to have invaded world regions outside their native ranges.

SUBFAMILY	SPECIES	HOSTS ¹	INVADED RANGE	NATIVE RANGE	CITATIONS
Siricinae ²	<i>Sirex areolatus</i>	Very wide host range with major hosts in Cupressaceae	Europe	North America	Schiff et al. 2012
	<i>Sirex noctilio</i>	Pine , Douglas-fir	N. America, S. America, Australia, New Zealand, S. Africa	Europe, North Africa, Northeast Asia	Schiff et al. 2012
	<i>Urocerus albicornis</i>	Very wide host range in Pinaceae, hemlock	Europe	Transcontinental North America	CABI 2020a
	<i>Urocerus gigas</i>	Wide host range in Pinaceae, cypress	S. America	Europe, North Asia, North Africa	Schiff et al. 2012
	<i>Urocerus sah</i>	Pinaceae	N. America	Central Asia, Turkey, N. Africa	Schiff et al. 2012
Tremicinae	<i>Eriotremex formosanus</i>	Oak, hickory , sweetgum, pine	N. America	East Asia	Schiff et al. 2012
	<i>Tremex fuscicornis</i>	Poplar, black locust, maple	Australia, Chile	Europe, Asia	CABI 2020b; Schiff et al. 2012

¹ Tree genera are in bold, when siricid species predominantly use that genus as a host.

² There has been confusion for many years regarding *Sirex cyaneus* and species with similar appearances from North America and Europe. Schiff et al. (2012) state that misidentifications have occurred and there is confusion about names, but *S. cyaneus* is native to North America and the newly named *S. torvus* is native to Europe but is morphologically similar and has previously been called *S. cyaneus*.

that between 2001 and 2008, there were live siricid life stages discovered during inspections of 34 shipments arriving at New Zealand ports. Among these, the most common species intercepted were *S. noctilio*, *S. juvencus*, and *Urocerus gigas*. Two of these species have invaded the Americas (Table 2), illustrating their ubiquitous presence in the wood packaging pathway and its potential to drive invasions. In reaction to the growing realization of the risk associated with woodboring insects present in trade, from 2003 to 2005 the International Plant Protection Convention implemented the International Standard for Phytosanitary Measures-15 (ISPM-15). This phytosanitary standard requires all member countries of the World Trade Organization to enforce a system of mandatory heat treatment or fumigation of wood packaging used in international trade. Despite the partial success of ISPM-15 in reducing accidental pest introductions, there are several incidences of live *S. noctilio* emerging from ISPM-15-treated wood (Burnip et al. 2010). Thus, the risk of new introductions of *S. noctilio* and other species of Siricidae around the world has not been completely eliminated.

Sirex noctilio predominantly attacks pines (*Pinus* spp.). While pines are not native to the Southern Hemisphere, they have been planted there extensively for timber and pulp production. In several portions of the Southern Hemisphere, pines are planted at high densities in monocultures, a setting that is apparently ideal for *S. noctilio* population growth. Worldwide, the first invasion of *S. noctilio* occurred in the North Island of New Zealand around 1900 (Bain et al. 2012). But it wasn't until the late 1920's that it was identified as a pest, when it was found killing trees in overstocked plantations of radiata pine (*Pinus radiata*, also called Monterey pine) native to North America. During a period of drought between 1946 and 1951, intense outbreaks developed in this region, and tree mortality became very high in densely stocked stands (> 1,000 stems/ha or > 2,400 stems/ac). These outbreaks subsequently subsided during the 1950's as forestry practices were modified to include intermediate cuttings (thinning) to reduce stocking to 200–300 stems/ha (500–750 stems/ac). *Sirex noctilio* is now rarely observed in New Zealand and is not currently considered a significant

forest pest. The reasons for this change in population behavior are not entirely known but may have resulted both from changes in forest management practices (thinning to lower stand densities) and biological control using imported natural enemies. Several hymenopteran parasitoid species were introduced, and the species *Rhyssa persuasoria persuasoria*, *Megarhyssa nortoni nortoni*, and *Ibalia leucospoides leucospoides* became well established, at times causing high levels of mortality. The parasitic nematode *Deladenus siricidicola* was apparently introduced along with founding *S. noctilio* populations and significantly contributes to control in New Zealand.

Following the massive outbreaks in New Zealand, *S. noctilio* was next discovered in Australia in 1952 (Carnegie et al. 2012). The initial invasion occurred in Tasmania, but by 1961 it was discovered in Victoria, near Melbourne. The insect continued to expand its range, arriving in South Australia and New South Wales in 1980 and in Queensland by 2009. The species has invaded most of the country's pine plantations, except those in Western Australia and coastal subtropical portions of New South Wales and Queensland.

Like New Zealand, large portions of Australia are planted with radiata pine for timber production. But typical site conditions in Australian pine stands are drier; consequently, these stands are more susceptible to *S. noctilio* outbreaks. The worst such outbreak occurred from 1987 to 1990 when over 5 million trees were killed during the "Green Triangle outbreak" in southeastern South Australia. Following this outbreak, the Australian Forestry Council developed a national strategy for managing *S. noctilio* populations (Haugen and Underwood 1990). Parasitoid populations had previously been introduced in the 1960's and 1970's but had not been effective at maintaining populations at low levels. The new national strategy outlined a system, still in place, that focuses on minimizing *S. noctilio* spread via quarantine and detection monitoring as well as population control using silviculture, population monitoring, and inoculative releases of the Kamona strain of the nematode *Deladenus siricidicola* (see Chapter 6). While these practices appear to have prevented the recurrence of another massive outbreak, *S. noctilio* remains a significant problem in Australian forestry, and research there continues to address this pest.

South America was the next continent to be invaded, with *S. noctilio* being discovered in Uruguay in 1980 (Maderni 1998). It subsequently spread and was discovered in Chile in 1981, in Argentina in 1985, and finally in Brazil in 1988. In New Zealand and Australia, the primary host of *S. noctilio* is radiata pine. This is also the principal host in certain parts of South America (e.g., northern Argentina and all of Chile), but elsewhere in South America the species has colonized other pine species, including loblolly (*P. taeda*) and slash (*P. elliotii*) pines in Brazil as well as lodgepole (*P. contorta*) and ponderosa (*P. ponderosa*) pines in Argentine Patagonia. As is the case elsewhere in the Southern Hemisphere, *S. noctilio* outbreaks develop in densely stocked stands. The onset of outbreaks is often related to drought conditions, and outbreaks result in extensive tree mortality (Lantschner et al. 2019). South American countries have largely adopted the same *S. noctilio* management tools used elsewhere in the Southern Hemisphere, namely silviculture, classical biological control using parasitoids, and inoculative biological control using the nematode *D. siricidicola*.

Africa is the last continent in the Southern Hemisphere to be invaded by *S. noctilio*. To date, the only country where the species is established is South Africa. *Sirex noctilio* was first discovered near Cape Town in 1994, and it subsequently spread through the majority of South African regions where pines are grown in plantations. The most common hosts for which *S. noctilio* is a problem in the country are radiata and patula (*P. patula*) pines, although loblolly, slash and slash-Caribbean (*P. caribaea*) hybrid pines are also colonized. Despite *S. noctilio* having been established the longest in the Western Cape region, outbreaks there are limited in extent. In contrast, extensive outbreaks and tree mortality have occurred in the Eastern Cape and KwaZulu-Natal regions, most likely due to the management of these stands at higher stocking levels for pulpwood production (Hurley et al. 2007). Similar to other areas in the Southern Hemisphere, biological control has been the principal approach to managing the pest. While damage still is extensive, it has subsided from levels experienced following the initial invasion (Hurley et al. 2007).

Following invasion of each continent, populations have expanded into climatically suitable regions with available hosts. Unfortunately, *S. noctilio* may frequently be present in harvested logs; when these logs

are transported long distances, this facilitates spread. Lantschner et al. (2014) found that within a continent, the radial rate of invasive *S. noctilio* range expansion in various world regions varied from 12 to 82 km (7.5–51 mi) per year, with the highest spread rates occurring in the warmest climates, perhaps due to higher insect reproductive rates.

Invasion of North America by *S. noctilio*

Given its reputation as a destructive forest pest in other invaded areas, there has been considerable concern about potential damage that *S. noctilio* could cause should it invade North America. Ciesla (2003) argued that the large expanse of unmanaged pine forests in western and eastern North America would create ample resources for outbreaks of *S. noctilio*, and that these outbreaks could move into more intensively managed stands. Further evidence of risk included repeated historical interceptions of *S. noctilio* at North American ports; between 1993 and 2001, USDA inspectors found 103 shipments containing wood packaging contaminated with *S. noctilio* (Ciesla 2003).

This perception of invasion risk was proven true in September 2004 when a single *S. noctilio* adult was collected in a funnel trap deployed near Fulton, Oswego County, New York, as part of the USDA Cooperative Agricultural Pest Survey (CAPS) for exotic bark beetles (Hoebeke et al. 2005). The trap was baited with cis-verbenol, ipsdienol, and methyl butenol and deployed as part of CAPS detection monitoring at high-risk sites, such as near ports. The Fulton trap was deployed approximately 16 km (10 mi) from the port of Oswego (on Lake Ontario) and near the Oswego River, which is used for inland commercial barge passage.

In 2005, the presence of a reproducing population was confirmed by molecular analysis of *S. noctilio* larvae discovered in symptomatic trees in the vicinity of the initial trap capture. An attempt was made to delimit the population by deploying additional traps in an area in New York and adjoining Ontario within 100 km (62 mi) or more from the initial detection location. Several of these traps captured *S. noctilio* adults, indicating the presence of populations over a large area. By the end of 2005, detections were made in four Canadian and four US counties, and sites with trap captures were separated by hundreds of kilometers. Given this evidence of the large geographic extent of established populations, it was concluded that the population had most likely been established for several years and that eradication would not be feasible.

Additional delimitation surveys in 2006 confirmed *S. noctilio* presence in 19 Canadian counties across Ontario and in 24 US counties in New York and Pennsylvania. By 2020, *S. noctilio* was known from nine northeastern US states (New York, Pennsylvania, Vermont, Connecticut, Ohio, Michigan, New Jersey, Massachusetts, and New Hampshire) and from two Canadian provinces (Ontario and Quebec) (Fig. 2). Detection of spreading populations is difficult due to lack of highly active attractant lures (see Chapter 3) and the labor intensive challenge of searching for and finding infested host trees. While it is assumed that *S. noctilio* populations are continuing to spread slowly, there currently is no nationally coordinated survey program underway in the US or Canada, so there remains some uncertainty about the current geographical extent of established populations.

Across most of the area of northeastern North America currently invaded by *S. noctilio*, pine hosts comprise a relatively small proportion of forest composition (Fig. 3). While there are some areas of native pitch (*P. rigida*), red (*P. resinosa*), white (*P. strobus*), and jack (*P. banksiana*) pine, most forests in this region are dominated by broadleaf trees, and a large fraction of pines occur in widely scattered stands that were mostly planted 70 or more years ago. These plantations are often comprised of several different pine species, but *S. noctilio* is particularly common in non-native Scots pine (*P. sylvestris*) plantations. Many of these plantations are largely abandoned and not managed, resulting in high stocking levels with many suppressed trees that are ideal *S. noctilio* hosts. However, given the scattered distribution of these plantations, it appears that there are insufficient densities of host trees across the landscape to permit regional outbreaks to develop. Consequently, *S. noctilio* is presently an uncommon insect across most of its invaded range. This, along with

Figure 2. Historical records of first detections of *Sirex noctilio* in eastern North America (updated from Dodds and de Groot 2012). Site of initial detection in 2004 near Fulton, New York is shown with a “★”.

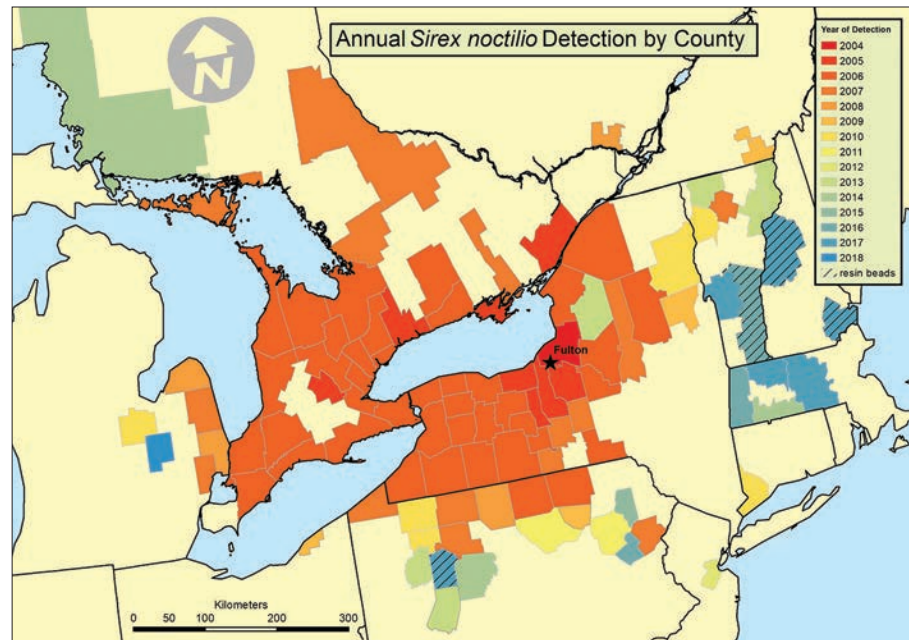
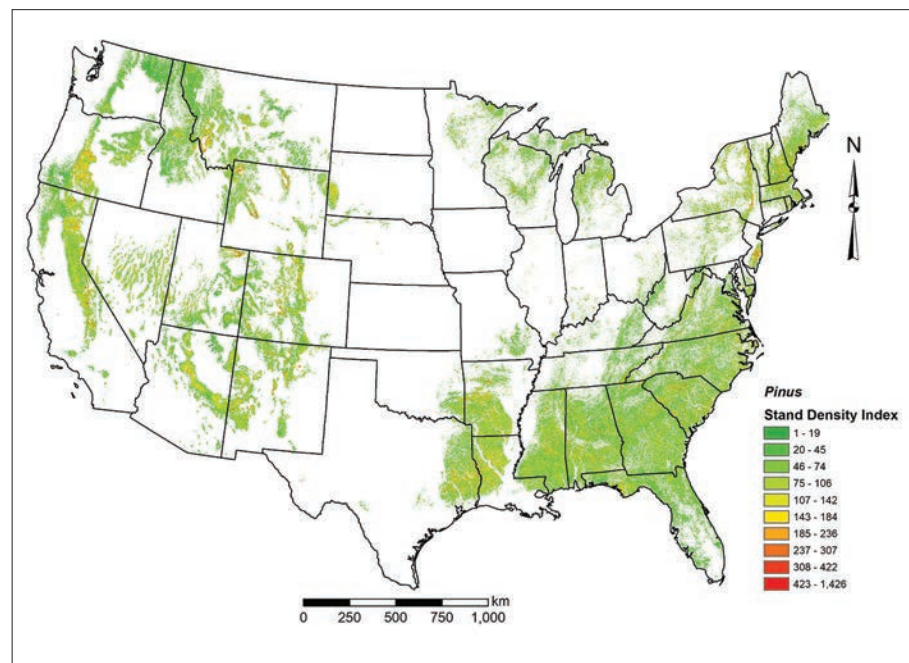


Figure 3. Density of pines (all species) across the conterminous US (data used to create map are from the USDA Forest Service Individual Tree Species Parameter Map database, <https://www.fs.fed.us/foresthealth/applied-sciences/mapping-reporting/individual-tree-parameter-maps.shtml>).



the low level of surveillance currently conducted in the region, means that the current range of *S. noctilio* in North America is poorly defined. Most likely, the species has expanded well beyond areas shown in Figure 2, but additional survey data are lacking.

Researchers have used molecular methods to investigate the origin of *S. noctilio* that is now present in North America. *Sirex noctilio* has a large native distribution (Fig. 1), but data on the genomes are only available from a few native areas. Genetic samples are more easily available from invaded areas. Analyses from northeastern North America suggest that *S. noctilio* has been introduced at least twice to North America (Bittner et al. 2017). An evaluation conducted to hypothesize worldwide invasion patterns suggested that

S. noctilio populations in North America originated from an unknown location in Europe and from South America (Boissin et al. 2012).

Prospects for future invasions by *S. noctilio*

Sirex noctilio has already invaded every continent where pines exist (Fig. 1). Thus, there is limited potential for future long-distance invasion of new continents by this species. Nevertheless, there remain many areas within these continents that are still uninvaded. For example, the only portion of the African continent where *S. noctilio* is known to be established is South Africa, but there are several other countries (e.g., Mozambique, Tanzania, Kenya, Angola) with extensive pine plantations where *S. noctilio* invasion can be anticipated. Interestingly, there are also portions of Asia where *S. noctilio* is not native or currently present and may invade in the future, including large areas of native and exotic pine stands in southeastern Asia and the Indian subcontinent.

North America contains more suitable habitat that currently has not been invaded by *S. noctilio* than any other continent. Native and planted pine stands are extensive through western and southeastern North America (Fig. 3). In the Southern Hemisphere, many pine species native to North America are widely planted and have proven to be excellent hosts for *S. noctilio*, so there is reason for concern about the potential for damage when *S. noctilio* expands its North American range. However, climate-matching studies indicate that most of the pine region in the North American West may be only marginally suitable for *S. noctilio* (Ireland et al. 2018). This conclusion should be questioned and further investigated, however, since their maps also classify regions such as Argentine Patagonia as being climatically marginal for *S. noctilio*, even though the species has proven to be a serious pest in these regions.

There may be additional factors besides climate and host availability that may limit the future range of *S. noctilio*, or at least limit its outbreak range (see Chapter 10). While several of the most abundant pine species native to western North America are frequently attacked by *S. noctilio* in the insect's alien range, there is no guarantee this insect will increase to outbreak densities once it becomes established in the native range of these tree species. Thus, there is no certainty about whether the extensive damage caused by *S. noctilio* in the Southern Hemisphere will be replicated anywhere in North America once this species expands its range.

Acknowledgments

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CHAPTER
2Host range and life history of *Sirex noctilio*
in North AmericaFlora Krivak-Tetley¹ and Ann E. Hajek²¹Department of Biological Sciences, Dartmouth College, Hanover, New Hampshire 03755 USA²Department of Entomology, Cornell University, Ithaca, New York 14853-2601 USA**Abstract**

Sirex noctilio colonizes a wide number of *Pinus* species in its native Eurasian range. Since its introduction to North America and spread through the northeastern United States, Ontario, and Quebec, *S. noctilio* has primarily attacked three hard pine species in the region: the native red pine (*P. resinosa*) and jack pine (*P. banksiana*), and the introduced Scots pine (*P. sylvestris*). Many native North American pine species growing outside of the current introduced range of *S. noctilio* have been found to be susceptible in Southern Hemisphere countries where they are utilized as plantation trees, suggesting that these species will be potential hosts when *S. noctilio* disperses into areas where they grow. Female *S. noctilio* oviposit in stressed or weakened pines, where they deposit a nutritional mutualist fungus, *Amylostereum areolatum*, and venom gland secretions along with eggs. This venom reduces vascular function of the tree and, in combination with *A. areolatum*, initiates the process of tree death, which occurs over time as larvae hatch and begin to develop within the tree. Most larvae mature in one year, with a minority (up to ~25%) requiring more than one year. During this time, larvae tunnel into the xylem, feeding and passing through multiple instars before overwintering and eventually emerging during midsummer. Important natural enemies include several native hymenopteran parasitoids that parasitize variable percentages (typically <25%) of *S. noctilio* larvae in North America. A strain of the parasitic nematode, *Deladenus siricidicola*, that was introduced to North America with invading *S. noctilio* is present but has little impact on *S. noctilio* populations. *Sirex noctilio* vary greatly in body size, a characteristic that impacts dispersal, and populations are usually male biased. A small percentage of attacked pines can produce large numbers of adult *S. noctilio*, which can result in rapid increases in population density, often observed over several years in newly invaded areas.

Introduction

Sirex noctilio is a member of the woodwasp family, Siricidae, and completes its life cycle in pine trees (Fig. 1). This species is known as a scavenger or secondary pest in its native range, where it exclusively attacks

suppressed and weakened pines (Lombardero et al. 2016). In the Southern Hemisphere, however, non-native populations are well known for causing substantial tree mortality and economic losses in plantations of exotic pines. Factors such as high stocking levels, delayed thinning, and drought have been found to contribute to outbreaks in the Southern Hemisphere.



Figure 1. (a) Adult female (left) and male (right) *Sirex noctilio* resting on end of cut pine log. (b) *Sirex noctilio* larva inside a gallery. (c) Two male *S. noctilio*, demonstrating the large body size variation in this species (images by [a] Vicky Klasmer, Instituto Nacional de Tecnología Agropecuaria, Bugwood.org, [b] M.P. Ayres, and [c] L.J. Haavik).

An established *S. noctilio* population was first detected in North America in 2004, and the species now occurs through much of the northeastern United States and eastern Canada (see Chapter 1). The basic biology and life history of this invasive woodwasp as well as its interactions with host trees, fungal symbionts, and natural enemies will be described in this chapter. We have emphasized information gained from research in North America when possible. For a more detailed account of the biology and life history, largely based on many decades of experience with high *S. noctilio* populations in the Southern Hemisphere, see Ryan and Hurley (2012).

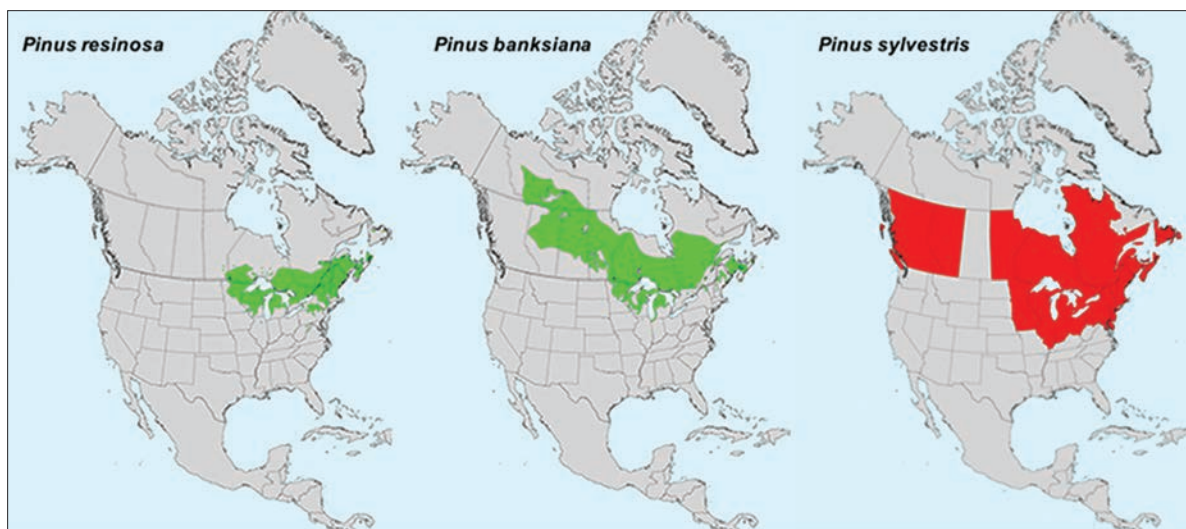


Figure 2. *Sirex noctilio* has three primary hosts in its current range in North America. Maps show ranges of the native *Pinus resinosa* (red pine; left) and *P. banksiana* (jack pine; center), and USDA hardiness zones suitable for the introduced *P. sylvestris* (Scots pine). Green indicates native range, red indicates introduced (maps by Virginia Tech Department of Forest Resources and Environmental Conservation with data from Little [1971]).

North American pine hosts of *S. noctilio*

Sirex noctilio is native to Eurasia where its preferred tree hosts include a wide range of pine (*Pinus*) species, although successful reproduction has also been reported in other conifer genera (Spradbery and Kirk 1978). Since its introduction to North America, *S. noctilio* has primarily colonized three hard pine species with overlapping ranges in the northeastern United States and eastern Canada: the native red pine (*P. resinosa*) and jack pine (*P. banksiana*), and the introduced Scots pine (*P. sylvestris*) (Fig. 2). The heaviest early infestations of *S. noctilio* were recorded in Scots pine stands in New York and southern Ontario. This pine species, like red pine, tends to occur frequently in small, isolated stands across the region; many of these are windbreaks or abandoned plantations with abundant smaller, suppressed trees. While jack pine is common in regions of the northern Midwest and Ontario that have been invaded by *S. noctilio*, it is only colonized by *S. noctilio* under certain conditions, particularly in mixed pine stands (Haavik et al. 2016a), and may be a less suitable larval host than the two commonly colonized species (Haavik et al. 2017). Early reports suggested

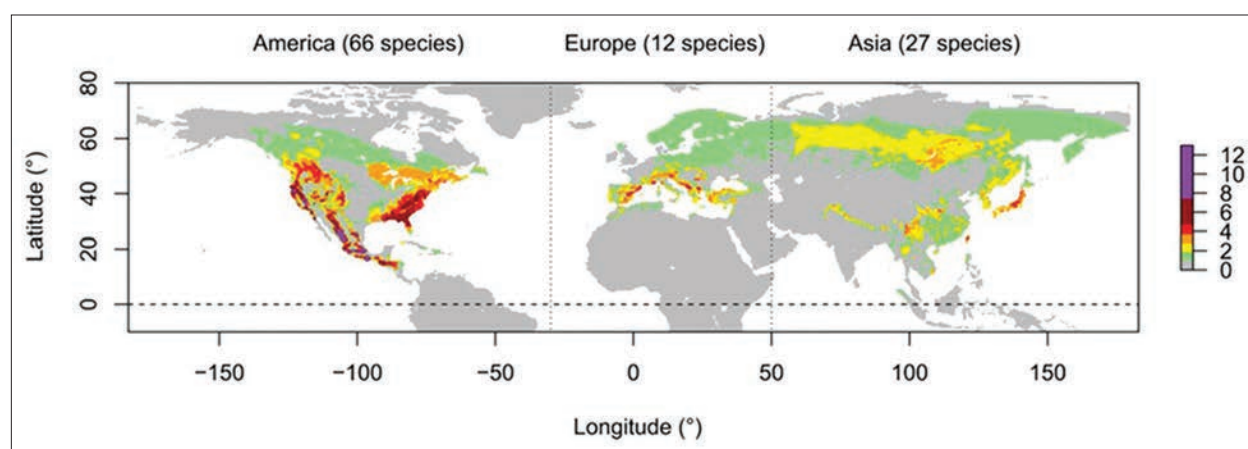


Figure 3. North America has 66 native *Pinus* species, more than any other continent. This is due to a combination of high local species richness (regional number of species shown here using a color scale from 0 to 12) and high beta diversity, or variation in species composition across different habitats on the continent (figure from Nobis et al. [2012]; reprinted with permission).

that eastern white pine (*P. strobus*) also could be utilized as a host (Dodds et al. 2007), but instances of colonization have been rare and widespread attacks in white pine have not occurred. At the southern and eastern edges of its range, *S. noctilio* has likely already come into contact with Virginia pine (*P. virginiana*) and pitch pine (*P. rigida*), but no attacks in these species have been reported as of 2020.

North America has the highest level of pine diversity in the world with over 66 native *Pinus* species on the continent (Nobis et al. 2012) (Fig. 3). As the range of *S. noctilio* expands in North America, this woodwasp will come into contact with native pines that have suffered high levels of mortality in plantations in the Southern Hemisphere as well as novel native pines that are not widely utilized elsewhere as plantation trees. While it is unknown how *S. noctilio* will interact with these species when the two meet in North America, the highly successful host utilization observed in the Southern Hemisphere suggests the same outcome is likely in North America (Table 1). The California native, radiata pine or Monterey pine (*P. radiata*), is a preferred host in New Zealand, Australia, Chile, and winter rainfall regions of South Africa. The southern pines, including loblolly pine (*P. taeda*) and slash pine (*P. elliottii*), are preferred hosts in Brazil, but in other regions are reported to be less susceptible. In Argentina, lodgepole pine (*P. contorta*) is susceptible, particularly in overstocked stands experiencing drought stress. *Sirex noctilio* activity has also been reported in Argentina in ponderosa pine (*P. ponderosa*), but with lower tree mortality (Lantschner et

Table 1. North America contains 16 pine (*Pinus*) species that are known to be suitable hosts for *Sirex noctilio* or grow within the woodwasp's current continental range. Pine species are shown grouped by subsection within the genus *Pinus*. Columns indicate whether the pine species is native to North America, whether *S. noctilio* is currently present in the North American range of the pine, and whether each species has been confirmed as a suitable host.

SUBSECTION	SCIENTIFIC NAME	COMMON NAME	NATIVE TO NORTH AMERICA	RANGE OVERLAP WITH <i>S. NOCTILIO</i> ¹	CONFIRMED HOST OF <i>S. NOCTILIO</i>
Australes	<i>Pinus caribaea</i>	Caribbean pine	yes	no	yes
	<i>Pinus echinata</i>	shortleaf pine	yes	no	yes
	<i>Pinus elliotii</i>	slash pine	yes	no	yes
	<i>Pinus palustris</i>	longleaf pine	yes	no	yes
	<i>Pinus patula</i>	Mexican weeping pine	yes	no	yes
	<i>Pinus radiata</i>	Monterey pine	yes	no	yes
	<i>Pinus rigida</i>	pitch pine	yes	likely	no
	<i>Pinus taeda</i>	loblolly pine	yes	no	yes
Contortae	<i>Pinus banksiana</i>	jack pine	yes	yes	yes
	<i>Pinus contorta</i>	lodgepole pine	yes	no	yes
	<i>Pinus virginiana</i>	Virginia pine	yes	likely	yes
Pinus	<i>Pinus resinosa</i>	red pine	yes	yes	yes
	<i>Pinus sylvestris</i>	Scots pine	no	yes	yes
Ponderosa	<i>Pinus jeffreyi</i>	Jeffrey pine	yes	no	yes
	<i>Pinus ponderosa</i>	ponderosa pine	yes	no	yes
Strobus	<i>Pinus strobus</i>	eastern white pine	yes	yes	rarely

¹Based on range overlap of pine species and *S. noctilio* in North America as of 2020 (see also Chapter 1, Fig. 2).

al. 2019). Ultimately, as *S. noctilio* spreads in North America, its impacts to North American pine species will likely depend on the complex interplay of tree species susceptibility, management practices, *S. noctilio* life history traits, natural enemy and competitor communities that influence *S. noctilio* population growth, and landscape-level or environmental factors that influence *S. noctilio* dispersal and the availability of suitable hosts (Krivak-Tetley et al. 2021; see Chapters 10 and 11).

General biology and behavior of *S. noctilio*

Life cycle of *S. noctilio*

Like all Hymenoptera (the insect order for wasps and bees), *S. noctilio* is holometabolous and undergoes complete metamorphosis. Adult female *S. noctilio* deposit eggs, the symbiotic fungus *Amylostereum areolatum*, and phytotoxic venom in the xylem of weakened pine trees during a summer flight season (Fig. 4a; see next section). At the oviposition site, trees can respond by expressing resin, which may be the only indication that *S. noctilio* are developing within a pine (Fig. 5 a,b; see Chapter 11). After oviposition, eggs usually hatch in 16–28 days. Larvae require at least one year to develop (see life history), and adults subsequently live for only 1–2 weeks (Ryan and Hurley 2012). Thus, the majority of the *S. noctilio* life cycle is spent in the larval stage (Figs. 4b, 5c), tunneling inside the sapwood of the xylem and avoiding the heartwood in the middle of the trunk. As larvae tunnel and feed, they progress through a variable number of instars (6–12) and pack the galleries behind them with wood shavings created during feeding. Galleries are primarily oriented with the wood grain, but gallery shapes and sizes vary by tree moisture level (Madden 1981). Larvae pupate near the surface under the bark (Fig. 4c). After pupation, new *S. noctilio* adults chew their way out through the bark, creating round emergence holes of variable sizes, depending on the size of the emerging adult wasp (Fig. 4c, 5d,e). *Sirex noctilio* has extremely high levels of body-size variation (Fig. 1c; see life history). This has been hypothesized to be due to a combination of factors, including variation in larval nutrition linked to the growth of *A. areolatum* within the xylem, environmental factors, and genetic differences.

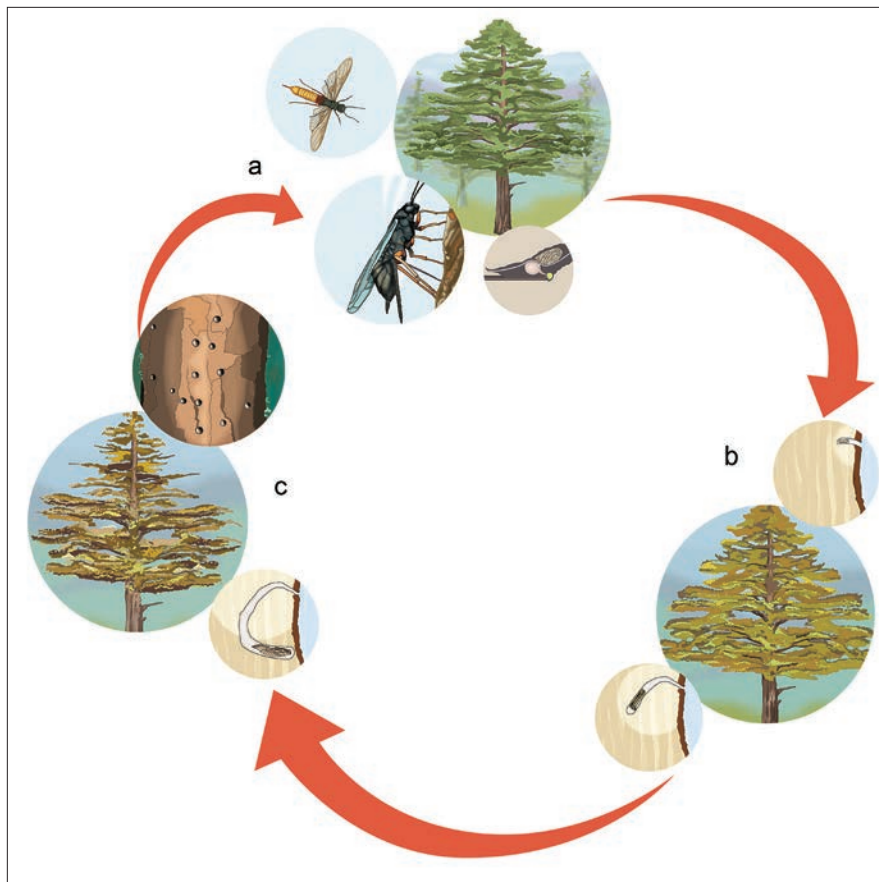
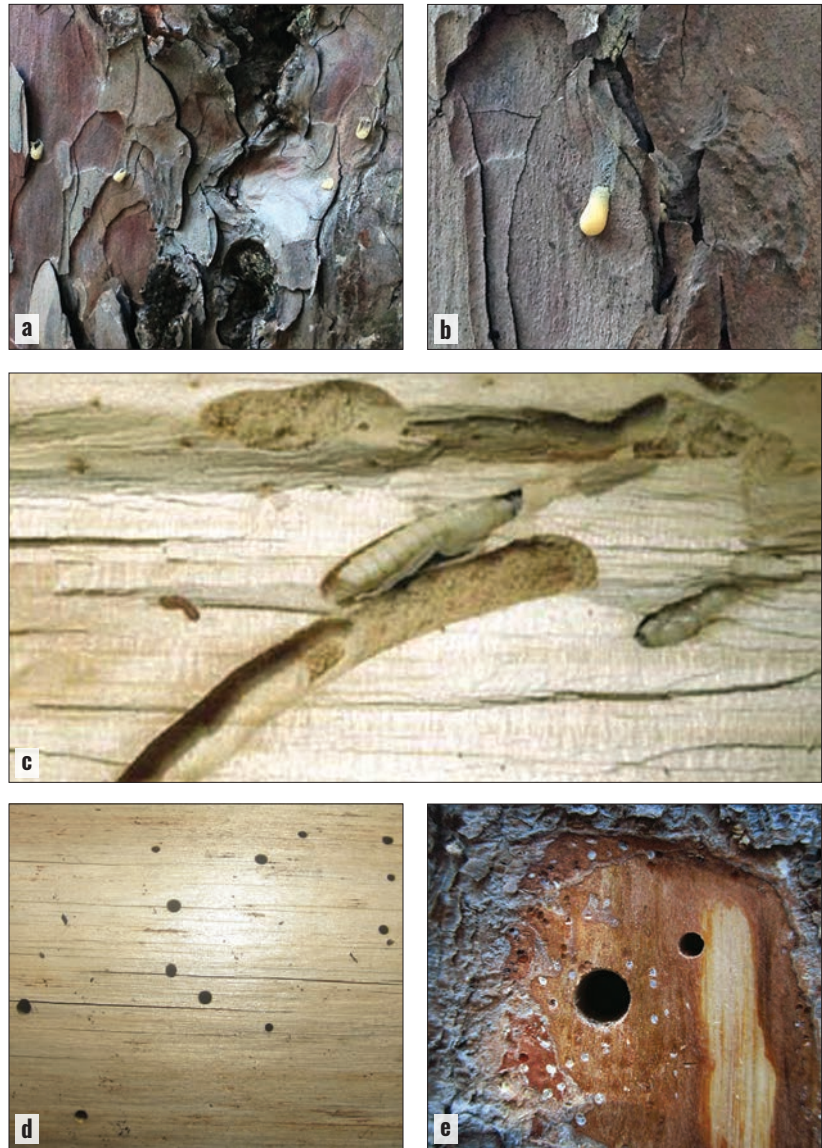


Figure 4. Life cycle of *Sirex noctilio*. (a) After seeking a mate, female *S. noctilio* find a suitable pine tree for oviposition during the summer flight season, depositing a combination of eggs, venom gland secretions, and the mutualist fungus *Amylostereum areolatum* via drills into the sapwood. (b) If oviposition is successful, eggs hatch and larvae tunnel in the xylem, passing through multiple instars as they grow. Tree vigor declines, with systemic loss of vascular function, resulting in visible needle wilt and chlorosis. The majority of *S. noctilio* larvae take one year to develop (with up to ~25% taking longer), (c) pupating near the surface and then emerging as adults during the summer flight season leaving round emergence holes visible on the outside of the trunk (image by Glenda Brits, Senior Graphic Designer, Department for Education Innovation, University of Pretoria, South Africa).

Figure 5. Photographs showing (a) a group of resin beads and (b) a close-up view of a single resin bead formed on *Pinus resinosa* after *Sirex noctilio* oviposition; (c) larvae and larval galleries inside the sapwood (outer xylem), (d) a range of sizes of *S. noctilio* emergence holes in an older dead *P. contorta* trunk with bark removed (photo from a plantation in Argentina), and (e) emergence holes on a recently killed tree with some bark removed (images by [a,b,d,e] F. Krivak-Tetley and [c] William M. Ciesla, Forest Health and Management International, Bugwood.org).



Emerging adults do not feed but instead rely on stored energy for mating, dispersal, host location, and oviposition during their adult life span. Immediately after emergence, adult wasps are attracted to light, which may play a role in mate searching and dispersal. When males are abundant, they have been observed in small swarms at the tops of trees, at times attracting females (Morgan and Stewart 1966; Madden 1988; F. Krivak-Tetley personal observation). Females visit these male aggregations (also called leks), likely attracted by male aggregation pheromones that have been found to attract both sexes (Cooperband et al. 2012; see Chapter 3). This behavior has not been documented in North America, possibly because population densities have been relatively low and adults have tended to be widely dispersed. During mating, females may be selective, sometimes rejecting potential mates but often mating with multiple males (Caetano and Hajek 2017). As is typical of all wasps and bees, unmated female *S. noctilio* can still lay viable eggs, but these unfertilized eggs will produce only male offspring.

After mating, female dispersal occurs, although females are thought to remain in the area of their natal tree (the tree they emerged from) if attractive hosts occur nearby. Alternatively, longer distance dispersal occurs in a smaller proportion of the population. Laboratory flight mill studies have shown that *S. noctilio* adults can be strong fliers and that larger *S. noctilio* adults are able to fly farther (Gaudon et al. 2016). As a result, in the Southern Hemisphere spread rates of 12–82 km (7.5–51 mi) per year have been documented, with faster spread in hotter regions that have more constant annual temperatures (Lantschner et al. 2014).

Interactions among *S. noctilio*, its fungal symbiont, and pine trees

The capacity of *S. noctilio* to weaken and kill living trees is facilitated by the wasp's obligate partnership with the fungus *A. areolatum* (see Chapter 4), combined with the phytotoxic effects of its venom gland secretions. Similar to other woodwasps that have nutritional fungal mutualists, *S. noctilio* females carry *A. areolatum* in paired mycangia that are located in the wasp's abdomen adjacent to the ovipositor and oviduct (Fig. 4a; see Chapter 4). The fungus is injected into a tree at the time of oviposition (Madden and Coutts 1979). Successful establishment of *A. areolatum* in a host tree depends on the simultaneous introduction of venom gland secretions that cause physiological distress to the attacked tree, including a reduction in vascular function and transport of photosynthates, resulting in needle wilt and chlorosis (Coutts 1969). Bordeaux et al. (2014) isolated a water-soluble bioactive peptide named noctilisin from these secretions and, using bioassays, confirmed its ability to travel through the xylem and cause needle wilt in pine seedlings. Venom glands in *S. noctilio* are larger than those studied in other siricids, which may contribute to the ability of this woodwasp to colonize healthier trees than other conifer-infesting siricids (Spradbery 1973; Hajek et al. 2017). The phytotoxic activity of *S. noctilio* venom stimulates *A. areolatum* growth, and it is the combination of physiological stress to the host tree and successful fungal establishment that eventually leads to tree mortality.

Female wasps primarily seek stressed or weakened pine trees for oviposition (see Chapter 3). After selecting a host tree, the female uses her ovipositor to drill through the bark and 1 cm (0.4 in) or deeper into the wood. The ovipositor is sensitive to osmotic (resin) pressure (Madden 1974), and this probing activity helps the female to assess the relative health and defense capability of the tree (Fig. 4a). In cases of high osmotic pressure (likely indicating robust defense capability), the female will reject the site for oviposition and will move on without depositing eggs. In this case, she leaves only *A. areolatum* arthrospores and venom in a single drill before withdrawing her ovipositor. A female may work her way down and around the tree bole, probing and rejecting the same tree many times before moving on to evaluate a new tree. These single drills are thought to condition the tree for future successful attacks, with the repeated introductions of venom plus *A. areolatum* weakening the tree and increasing its future suitability as a host (Madden and Irvine 1971).

In the Northeast, *S. noctilio* attacks can be diagnosed by the presence of resin beads or occasionally resin drippings on the outside of trees at oviposition sites. The appearance of these beads varies among pine species and with the vigor and resin content of a tree. Attacks on *P. resinosa* typically form a single droplet at each oviposition site, with sites sometimes found in small clusters (Fig. 5a,b; see Chapter 11). The presence of oviposition drills can be further confirmed by shaving away the bark to reveal the phloem and cambial layer where oviposition drill holes will be visible if they are the source of the wound (Fig. 6). The native siricid *S. nigricornis* oviposits in a similar manner and has been found to co-occur in the same trees with *S. noctilio*, but *S. nigricornis* typically colonizes trees that are closer to death and no longer have enough resin production for the formation of beads (see Chapter 9).

When a site is deemed suitable for oviposition, the female creates multiple branching drills, depositing a combination of eggs, *A. areolatum*, and venom gland secretions in the drills (Fig. 4a). Individual oviposition sites commonly contain 2–4 drills that branch from each other in the bark/

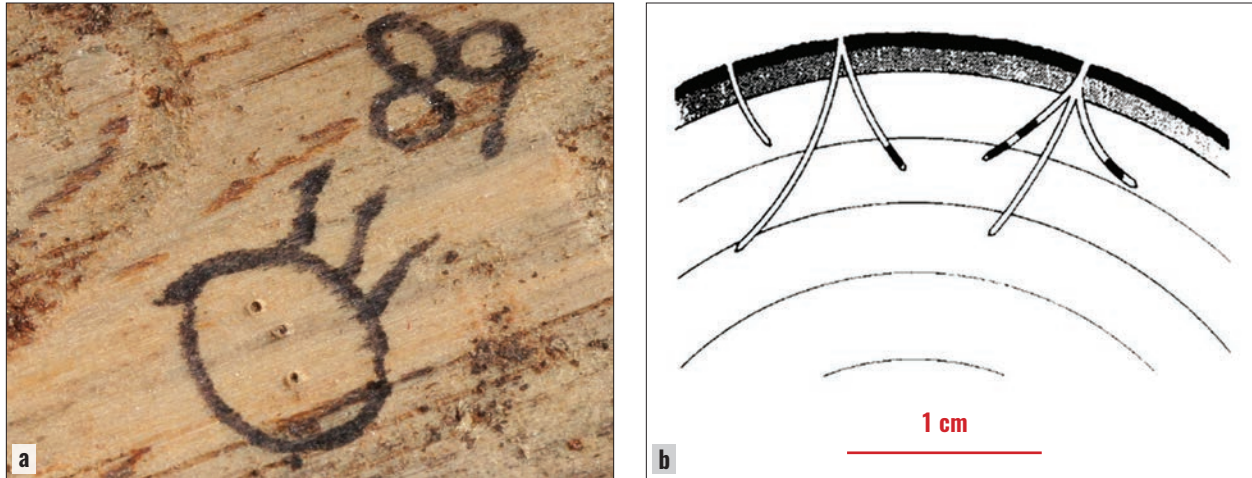


Figure 6. *Sirex noctilio* females oviposit in the xylem (sapwood) of the host tree. (a) One oviposition site with three branching drills that extend into the xylem (numbered and circled; bark and phloem removed). The female had inserted her ovipositor through the bark, drilled and laid an egg and then had withdrawn to the bark and subsequently angled to make another drill. This was repeated to create 3 drills. (b) A cross section of a tree, showing oviposition drills and the locations of eggs within them. One attack, with a single drill, was rejected for oviposition but would have contained fungus and venom (far left). The two oviposition sites with multiple drills, branching before entering the sapwood, contain eggs in some drills, but the last drill for each group contained only fungal arthrospores and venom (which are not visible) (images by [a] I.A. Caetano and [b] modified from Coutts and Dolezal [1969]).

phloem; eggs are placed in the initial drill(s) (Fig. 6). The final drill usually has no eggs and contains fungus plus venom, as with the ‘conditioning’ single drills (Coutts and Dolezal 1969). The number of eggs deposited at one oviposition site can be reliably estimated from the number of branching drills, with more drills indicating more eggs (Madden 1974). Thus, the number of drills per attack on a tree or across trees in a stand has important implications for potential reproductive output and population growth (Krivak-Tetley 2019). Trees with mostly single drills will likely yield few, if any, offspring, whereas trees with many branching drills have the possibility of producing large numbers of offspring. Nearby oviposition sites commonly contain sibling larvae (Bittner et al. 2017) as female *S. noctilio* typically oviposit multiple times once they have located a suitable host.

Sirex noctilio and *A. areolatum* have an obligate relationship, meaning successful larval development requires the growth of *A. areolatum* in host tree tissues. This begins with the establishment of the fungus adjacent to the oviposition site, and the fungus subsequently spreads into the surroundings areas, especially around woodwasp galleries where it is needed to decompose the wood for larval nutrition (see Chapter 5). Adult wasps subsequently provide dispersal services for *A. areolatum* after emergence from trees (see Chapter 4).

Natural enemies

The trajectory of larval development can be altered by the presence of two types of natural enemies: hymenopteran parasitoids and parasitic nematodes. A complex of native hymenopteran parasitoids attack *S. noctilio* larvae in North America. These include the ibaliid wasp *Ibalia leucospoides* and the rhyssines *Rhyssa persuasoria*, *R. lineolata*, and *Megarhyssa nortoni* (see Chapter 8). All of these species are solitary parasitoids, meaning that one *S. noctilio* larva yields one parasitoid. The parasitic nematode *Deladenus siricidicola* was introduced into North America with *S. noctilio*. Unlike the hymenopteran parasitoids, it reproduces within but does not kill *S. noctilio* larvae. While some strains of this species of nematode can sterilize females of some genotypes of *S. noctilio* (see Chapter 6), the *D. siricidicola* strain that co-invaded North America with *S. noctilio* does not sterilize females (see Chapter 7). However, the

nematode relies on the woodwasp as a dispersal agent and, at present in North America, has variable indirect demographic impacts (see Chapters 6 and 7). A native parasitic nematode known to infect native *S. nigricornis* in pines can infect *S. noctilio*, but notable population-level impacts of this species have not been observed to date in North America (see Chapter 7).

Life history of *S. noctilio* in North America

In North America, the primary adult flight period for *S. noctilio* stretches from July to September, with some reports of a longer season starting in June and lasting into October (Ryan et al. 2012; Hajek et al. 2017). Some studies reporting on phenology have included insects from Pennsylvania, hinting at the possibility of an extended emergence period as *S. noctilio* moves south, as hypothesized by Hartshorn et al. (2016). Adult males tend to emerge from trees slightly earlier than females, and models have projected emergence timing for areas of the eastern US that *S. noctilio* has not yet invaded (Myers et al. 2014). As in the native range, the majority of *S. noctilio* larvae require one year to develop, but all North American studies have reported some percentage of larvae requiring two or more years, and emergence after a second year has often ranged between 1 and 10% (Myers et al. 2014; Foelker et al. 2016; Hajek et al. 2017).

Sirex noctilio populations around the globe have been found to exhibit a greater than 3-fold within-sex variation in body size (Krivak-Tetley 2019). Adult body size varies from <6 mm to >37 mm in length. On average, females are larger than males. Females have an average body length of almost 20 mm compared to 14.3 mm for males across populations in New York, Vermont, and Pennsylvania (Krivak-Tetley 2019); in Ontario, females have average pronotum widths of 3.18 mm compared to 2.19 mm for males (Haavik et al. 2016b). Body size of *S. noctilio* has important demographic implications because larger females produce more eggs. Individual females have been found to carry as few as five eggs in very small woodwasps and >280 in the largest individuals.

Although extreme male bias in the sex ratio (more males than females) has been reported in some invasive *S. noctilio* populations in the Southern Hemisphere (Iede et al. 1998), studies in North America have found female:male sex ratios ranging from 1:3 to 1:5 (i.e., about 16–25% females) across a range of sampled populations (Long et al. 2009; Ryan et al. 2012; Myers et al. 2014; Haavik et al. 2016a), similar to sex ratios recorded in the native range (Lombardero et al. 2016).

Although the aggregation behavior of *S. noctilio* is not well understood, within one New York forest, *S. noctilio*-attacked pines were documented as aggregated within localized areas of 15–20 m (49–66 ft) (Foelker et al. 2018). Large numbers of adults (plus the parasitoids that each develop by consuming one *S. noctilio* larva) can emerge from individual pine stems. In both the native and invaded ranges, a small percentage of attacked pines frequently yields a majority of emerging *S. noctilio*. For example, at one infested red pine site in Pennsylvania in 2017, a range of 1–854 *S. noctilio* adults (and parasitoids) emerged from 10 individual infested pine stems in a localized area. From 2011 to 2019, of 189 *S. noctilio*-infested red and Scots pines in New York and Pennsylvania that were felled and where total emergence was followed, >100 *S. noctilio* emerged from 13% of stems while <100 adults emerged from 87% of stems (A.E. Hajek unpublished data). This is consistent with recent observations in the native range where over 90% of emerging insects were collected from <20% of attacked trees in 2013–2014 in Galicia, Spain (Lombardero et al. 2016).

Life history traits including sex ratio, fecundity, and parasitism rates influence population dynamics and the trajectory of invasions in new regions. Fecundity of North American *S. noctilio* is higher than that of their native counterparts in Europe, and the absence of sterilization by nematodes in North America boosts the potential for rapid population growth in these non-native populations (Krivak-Tetley 2019). However, *S. noctilio* sex ratios and hymenopteran parasitism rates in northeastern North America closely resemble those of native populations in Galicia, Spain where *S. noctilio* is considered a secondary mortality agent attacking weakened pines (Lombardero et al. 2016). As of 2020, impacts to pine forests have so far been

limited within the North American range of *S. noctilio*. In the early years of the invasion of North America, *S. noctilio* impacts were relatively high in localized areas where small, suppressed pines were abundant, with some stands losing 20% or more of standing stems over several years (Dodds et al. 2010; see Chapter 11). Such mortality of pines at individual sites occurred over a period of a few years, but high density populations were not sustained, likely due at least in part to the patchy distribution and variable susceptibility of host trees and the presence of the native parasitoid complex and competitors (see Chapters 10 and 11). Therefore, the dynamics of *S. noctilio* populations seem to change between when this woodwasp first invades a region versus when it has been present for several years.

The similarities in *S. noctilio* life history traits in North America and Galicia, Spain suggest that the future dynamics of North American populations may ultimately most closely resemble those in the woodwasp's native range. While initial invaders and a few subsequent generations build to higher densities as they utilize and kill the most susceptible trees, later generations will be at lower densities as they are confronted with healthier pine trees that are more resistant as well as intact communities of native natural enemies and competitors (Haavik et al. 2018; see Chapter 10).

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**CHAPTER
3****Chemical ecology and trapping techniques
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Abstract

Adult *Sirex noctilio* have chemoreceptors on their antennae and ovipositor, allowing them to perceive volatiles produced by stressed host trees and fungi. Woodwasp oviposition behavior is influenced by the presence/absence of both their fungal symbiont (*Amylostereum areolatum* for *S. noctilio*) and fungi associated with bark and woodboring beetles. Mate recognition is, at least in part, mediated by a contact sex pheromone on the female cuticle, and a putative volatile male-produced sex pheromone has been identified. Considerable research effort has been invested in developing sampling tools with high specificity and sensitivity for *S. noctilio*. Current *S. noctilio* survey and detection occurs primarily with intercept traps baited with host volatiles. The information generated by these traps is used to guide decisions about where and when to apply management treatments.

Introduction

Insects perceive their environment through olfaction (i.e., smell). This is true in terms of the number of species that rely on olfaction to interact with their biotic and abiotic environment, the number of behaviors mediated by volatile chemicals, and the complexity of structures and behaviors involved in the perception and transmission of chemical stimuli. In large part, the dominance of olfaction in insects is due to their small body size, which imposes limitations on their ability to transmit and receive stimuli along other modalities (e.g., sound and vision). In addition, chemical communication offers several advantages including: i) given the diversity of chemicals insects can synthesize and perceive, a large number of “channels” are available and, consequently, a high degree of specificity and information content is possible; ii) chemical signals and cues can be transmitted over large distances and around obstacles; and iii) insects can perceive and discriminate among chemical cues and signals with high levels of sensitivity and precision. The disadvantages of olfaction are primarily due to the passive nature of chemical cues and signals: they cannot be transmitted quickly

over long distances, normally the emitter cannot influence the direction of transmission, and localizing the source of chemical cues or signals over moderate to long distances requires sophisticated behavioral mechanisms (e.g., optomotor anemotaxis).

At low population densities, *S. noctilio* primarily attacks suppressed and subdominant pine trees. This resource can be rare at the landscape level and may also be colonized by a diverse community of bark and woodboring insects. These insects, and their fungal associates, can have negative consequences on both the availability and suitability of host trees for *S. noctilio*. Additionally, adult woodwasps do not feed and are short lived; male *S. noctilio* can live for approximately 12 days and females 5–7 days (Ryan and Hurley 2012). As a result, a delay of only 1–2 days in locating a mate or oviposition site can have significant consequences on brood production. A basic principle of insect population ecology is that the time until the first female oviposition event has significant consequences for population growth rates. In fact, delayed mating is one of the proposed mechanisms for how pheromone-based mating disruption can regulate population growth of pest species without preventing mate location. As a result, there should be strong selection for rapid host location and avoidance of suboptimal hosts in *S. noctilio*. As with other woodboring insects (e.g., Cerambycidae), chemical cues and signals likely mediate the process of host location and acceptance. In northeastern North America, *Sirex noctilio* has often been present at low population levels, making it difficult to obtain individuals for laboratory work or to conduct field experiments. Consequently, some researchers have worked with closely related, but more common native species of woodwasps. The primary focus of this chapter is *S. noctilio*, but in a few instances I include results of studies on other species of woodwasps.

Host location and acceptance

As mentioned above, adult *S. noctilio* do not feed; consequently, the process of locating host trees is primarily for oviposition and mating, which typically occurs in leks (aggregated groups) formed in the canopy of host trees. The process of oviposition by insect herbivores can generally be considered as two separate and sequential events: host location and host acceptance. Host location is generally thought to occur first, be initiated from a distance (i.e., before landing on the host plant), and be mediated by olfaction and vision. Olfactory stimuli usually have a higher degree of specificity; however, visual stimuli can play a key role in host location when perceived concomitantly with odor.

The principal hosts of *S. noctilio* are pines (*Pinus* spp; see Chapter 2). Bookwalter et al. (2019) caged female *S. noctilio* in the laboratory with bolts of seven pine species and observed that females spent significantly more time on *Pinus strobus*, *P. sylvestris*, and *P. elliotii* than *P. taeda*, *P. palustris*, *P. echinata*, and *P. virginiana*. Females were observed drilling in all of these pines but were more often seen drilling on *P. strobus*, *P. sylvestris*, and *P. virginiana*. Among these three hosts, significantly more exit holes were observed on *P. sylvestris* bolts. Haavik et al. (2017) caged pairs of *S. noctilio* on four host species and estimated oviposition and counted the number of woodwasps that subsequently emerged. Female oviposition behavior was affected by host tree, but in a manner that did not reflect larval performance (i.e., larval survivorship was not highest on the host most preferred for oviposition). Some field studies have reported that in North America *P. sylvestris*, a host in the native range, is more frequently attacked than other host pines (Dodds and de Groot 2012; Foelker et al. 2018; see Chapters 2 and 10). While this is consistent with a preference for a native host over novel host trees, *P. sylvestris* stands in North America are often unmanaged, overstocked, and on low quality sites (Dodds and de Groot 2012). The observed pattern of attack in North America may simply reflect a preference for stressed hosts.

Several studies have observed that *S. noctilio* preferentially attacks stressed and/or suppressed and subdominant trees, that attraction to host trees is increased by girdling or stem-injection of herbicide, and that artificially stressed trees have increased monoterpene loss through the bark (Zylstra et al. 2010; Ryan and Hurley 2012; Haavik et al. 2016, 2018; see Chapters 10 and 11). The volatiles released by felled *P. radiata*

change little over the first 3 weeks post felling, but some components (e.g., oxygenated compounds) are absent initially and increase with time post felling. Complementary electroantennogram studies reported electrophysiological responses for *S. noctilio* to several components of pine oil (Bashford and Madden 2012). Xu et al. (2019) reported that traps hung adjacent to girdled *P. sylvestris* var. *mongolica* captured significantly more female *S. noctilio* than those next to ungirdled trees. Qualitative and quantitative differences in the volatile organic compound (VOC) profiles of girdled and ungirdled trees were detected, and principal component analyses separated the VOCs of girdled and ungirdled *P. sylvestris* into two groups. Coincident with the observation that artificially stressed pines have increased release of monoterpene volatiles through the bark, is increased water loss through the bark and lower osmotic potential in the phloem tissue (Madden 1974). Female *S. noctilio* are hypothesized to assess host suitability while probing host tissues with their ovipositor and to prefer trees with low osmotic potential.

Larval *S. noctilio* rely on their fungal symbiont *Amylostereum areolatum* for nutrition, and mated females have been reported to be attracted to volatiles of their fungal symbiont (Fernández Ajó et al. 2015; Sarvary et al. 2016). The negative impact of bark and woodboring insects on *S. noctilio* brood survivorship may be indirect, mediated by the consequences of biotic interactions with *A. areolatum* in host pines. Female *S. noctilio* avoid ovipositing in host material already colonized by bark beetle fungal associates. Hajek et al. (2018) examined the oviposition behavior of *S. noctilio* towards uninfested pine and pine infested with different strains of the fungal symbiont, *A. areolatum*. They observed that females exhibited an oviposition preference for wood without the fungal symbiont present. Although reduced oviposition by females in host material infested with their fungal symbiont seems inconsistent with the report of attraction to fungal volatiles (Sarvary et al. 2016), it is possible that the effects observed in the two studies are mediated by chemicals operating at different spatial scales. Volatiles associated with host material infested with *A. areolatum* may mediate attraction to infested hosts. Once on the host tree, females may avoid areas already infested or attack hosts adjacent to the infested host. *Sirex noctilio* females have chemoreceptors (Fig. 1) on both their antennae and ovipositor, suggesting that this avoidance could be mediated by chemicals (volatile and non-volatile) associated with fungal activity detected by the antennae and/or ovipositor (see Chapter 10).

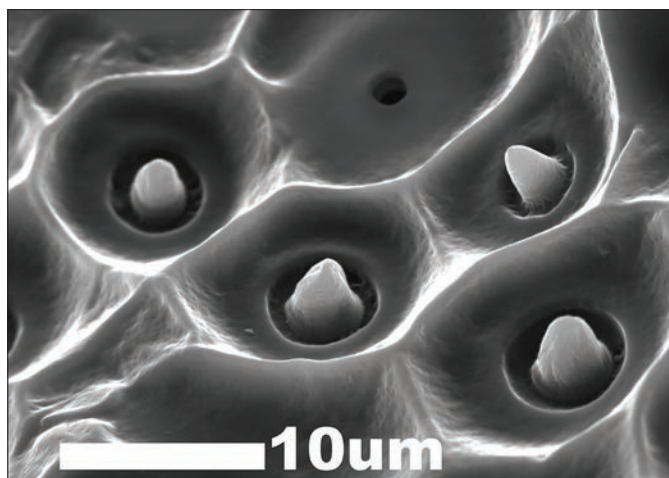


Figure 1. Scanning electron micrograph showing sensilla on the antenna of a male *Sirex noctilio* (image from Crook et al. [2008]; reprinted with permission).

The current model of *S. noctilio* oviposition proposes that females are able to detect moisture content and phloem resin osmotic pressure with sensilla on their ovipositor (Madden 1974; Bordeaux and Dean 2012). Depending on site quality (moisture content and resin osmotic potential) and female physiology, females lay up to 3 eggs per drill site, each in a separate tunnel (Madden 1974, 1988). In addition to the drills with eggs, females also create a drill without an egg that they inoculate with their fungal symbiont and a phytotoxic “venom”. Further, some drill sites contain only a single drill containing the fungus and venom. It has been hypothesized that these single-tunnel drill sites allow females to assess tree suitability and condition the tree and make it more likely to be attacked in the future (see Bordeaux and Dean 2012). While the significance of these single drills is not clear, it is clear that the fungus and venom are critical for tree death and are responsible for different symptoms. The venom has been characterized as a phytotoxic conditioning agent necessary for establishment of the fungal symbiont (Coutts 1969). The venom is produced in an

accessory gland in female *S. noctilio*. Preliminary studies identified a nonsulfated acid mucopolysaccharide as a major component of the accessory gland. This component is heat stable, has a molecular mass of 60–100 kD, dissociates from the venom in water, and further dissociates into 2–6 kD subunits that are as physiologically active on host tree tissues as the parent compound (Bordeaux and Dean 2012). Bordeaux et al. (2014) subsequently identified this heat stable factor as an 11-amino acid peptide, noctilisin.

Sirex noctilio has complete metamorphosis. Immature and adult life stages differ significantly in morphology, and feeding occurs exclusively in the larval stage. Larval development can take as little as 3 months to as long as three years depending on habitat. Even within a single host tree, development can range from one to two years. Despite this, emergence is generally concentrated over a period of a few weeks (Ryan and Hurley 2012; Haavik et al. 2013). Wasps are sexually mature at emergence, and males usually begin to emerge a few days before females. Shortly after emergence, males fly to the canopy and form aggregations that must be located by females for mating to occur. Because females are short-lived, this species is hypothesized to experience strong selection to rapidly locate mates (although *S. noctilio* can produce male progeny without mating, which reduces the strength of selection compared to diploid species with similar life spans). A male-produced sex pheromone could facilitate mate location and mitigate the costs associated with delayed mate location. Cooperband et al. (2012) reported that males were excited by and attracted to odors of males 2–5 days old, but not the odors of younger males. They identified (Z)-3-decen-1-ol and (Z)-4-decen-1-ol in male effluvia and reported that a 100:1:1 µg blend of (Z)-3-decen-1-ol, (Z)-4-decen-1-ol and (E,E)-2,4-decadienal was attractive in Y-tube bioassays. Field trials of this putative volatile sex pheromone did not observe an effect on trap capture. The fact that *S. noctilio* are aggregated spatially within stands and form leks in the canopy near the natal host may obviate the need for long-range pheromones. Additionally, adult *S. noctilio* are sexually dimorphic, with males having a bright orange band on the abdomen. Females may orient to the canopy near their natal host tree and subsequently use visual signals from males (e.g., the bright orange band on the abdomen) to locate leks and mates. Consistent with this model of mate location, male and female *S. noctilio* are strongly attracted to UV light in large wind-tunnel trials (Sarvary et al. 2015), and Allison et al. (2021) observed that traps baited with dead males captured more females than unbaited traps. Finally, in some cases, lek formation occurs at specific habitat features; in these cases, females can orient to these to search for mates.

Courtship is apparently initiated when females enter leks and approach males. Males then pursue females and repeatedly tap females with their legs and antennae (Crook et al. 2012; Caetano and Hajek 2017). Males will attempt copulation with fresh female cadavers, and this response is lost if female cuticular waxes are removed with an organic solvent. Böröczky et al. (2009) used behavior-guided fractionation to determine that the compounds (Z)-7-heptacosene, (Z)-7-nonacosene and (Z)-9-nonacosene were necessary and sufficient to recover the copulation response in males.

Applied chemical ecology: survey and detection

The detection of *S. noctilio* in North America was cause for concern because it has caused significant economic losses in plantations of non-native pines in the Southern Hemisphere. In fact, some of the pines affected in the Southern Hemisphere are native to North America. Management programs for *S. noctilio* in the Southern Hemisphere rely on silviculture to promote tree vigor and resistance to attack and on biological control to suppress populations. Effective survey and detection tools are important for determining when and where to implement these management tactics. For example, thinning operations can result in increased susceptibility to attack by *S. noctilio* if they occur during the flight period. Releases of the biological control agent *Deladenus siridicola* are most effective if they occur before wasp populations reach high levels. In the Southern Hemisphere, trap trees have been used to monitor *S. noctilio* populations; however, these are labor intensive, expensive to establish, and involve the use of herbicide, which is not generally accepted by the

public in forest settings. Detection of *S. noctilio* in the Northern Hemisphere motivated provincial, state, and federal agencies to invest substantial effort and resources to detect and delineate the distribution of *S. noctilio* in Canada and the US.

The modalities most commonly used by insects to obtain information about their environment are olfaction and vision. The development of survey and detection tools is predicated on understanding the identity and role of the visual and olfactory stimuli used by insects. Artificial copies of these stimuli can then be replicated and used to exploit the biology of the pest for management. Common targets are the olfactory and visual stimuli used by insects to locate hosts (e.g., trunk silhouette and host volatiles) and mates (e.g., sex pheromones). In *S. noctilio*, attempts have been made to exploit olfactory and visual stimuli to develop survey and detection tools.



Figure 2. An intercept panel trap deployed in a pine plantation in southern Ontario and baited with fresh host material (image by L.J. Haavik).

Survey and detection programs for *S. noctilio* primarily use intercept traps baited with synthetic blends of host volatiles (monoterpenes) (Bashford and Madden 2012) (Fig. 2). Their selection is based on observations that stressed host trees are more likely to be attacked than unstressed hosts and that artificial stress results in quantitative and qualitative changes in the volatile profile of host pines. The synthetic blend of monoterpenes attempts to exploit female *S. noctilio* host searching behavior by mimicking olfactory stimuli presented by attractive host trees. Numerous studies have demonstrated that traps baited with these volatiles capture more Siricidae (including *S. noctilio*) than unbaited traps and, as expected, these traps almost exclusively capture females. In the Southern Hemisphere, trap trees are used extensively for survey and detection of *S. noctilio*, and it has been suggested that in North America trap trees may perform better than synthetic lures. Consistent with this are observations that traps baited with host material (i.e., fresh branches with needles and split bole sections) capture more *S. noctilio* than blank traps and outperform traps baited with synthetic lures for *S. nigricornis* (Barnes et al. 2014). Although commercially available lures have been reported to work well in some cases (e.g., Hurley et al. 2015), these results suggest that they can be improved.

Intercept traps used for survey and detection programs of *S. noctilio* in North America are black and have a prominent silhouette designed to mimic the bole of host pines. Evidence that visual stimuli contribute to host location by female *S. noctilio* is limited. Unbaited black intercept traps captured more female *S. noctilio* than clear jar traps in walk-in wind-tunnel trials (Sarvary et al. 2015). Interpretation of this result is complicated by the fact that traps differed both in the silhouette presented and in shape/type. Further, trials in the same study that compared clear and black intercept traps observed no effect on female *S. noctilio* captures. Several studies have examined the effect of trap type on *S. noctilio* (Haavik et al. 2014; Hurley et al. 2015), and all report no effect of trap type. Allison and Redak (2017) performed a meta-analysis of the trapping literature for bark and woodboring insects and their associates and reported a significant effect of trap design for Siricidae, with panel traps outperforming multiple-funnel traps. The effect of trap placement along vertical understory-canopy gradients has received

limited attention. Martinez et al. (2014) observed that male *S. noctilio* fly higher than females and that when males were present on sticky mesh traps, females were observed to fly higher than when they were absent. The traps in that study were unbaited, so the observed effect on female flight behavior is likely mediated by a response of females to olfactory or visual stimuli associated with males. No effect of trap height on the capture of female *S. noctilio* was observed for traps baited with host volatiles (Battista et al. 2018) or host volatiles and a putative male aggregation pheromone (Hurley et al. 2015).

Conclusion

Although the woodwasp *S. noctilio* is a challenging research system, its chemical ecology is well studied. This research has supported the development of intercept traps baited with synthetic blends of host volatiles for survey and detection. These lures have had variable success, and experimental work in North America suggests that they are not competitive with natural sources of volatiles. Although IPM programs exist for *S. noctilio*, it continues to be a serious pest of plantation pines throughout the Southern Hemisphere. In addition, it continues to expand its range, and populations are the product of multiple introduction events. Available genomic evidence suggests that some of the invasions represent serial (i.e., bridgehead) introductions, while others are new invasions from native populations. This increases the potential for admixture creating unique gene pools, and this diversity is expected to result in variation in both life history traits and the pathogenicity of fungal symbionts, which could make pest management more difficult. More effective lures are needed for surveying and detecting introduction events and range expansion of established invasive populations, as well as to facilitate the timing and application of IPM tactics (e.g., silviculture and releases of biological control agents). The role of fungal volatiles and volatile pheromones in host and mate location are two promising areas that may provide improved tools for survey and detection in the future.

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**CHAPTER
4****Obligate mutualism of *Sirex noctilio*
with fungi****Ann E. Hajek¹ and Louela A. Castrillo²**¹Department of Entomology, Cornell University, Ithaca, New York 14853-2601 USA²USDA ARS, Emerging Pests and Pathogens Research Unit, Ithaca, New York 14853 USA**Abstract**

Sirex noctilio maintains an obligate mutualistic relationship with the symbiotic white rot fungus *Amylostereum areolatum*. This fungus is carried in the mycangia of adult females and is inoculated into wood with a phytotoxic venom before or during oviposition. The fungus grows in the outer sapwood around larvae as they develop and acts as an external rumen. The fungus and the phytotoxic venom together kill host pine trees. Other siricids can be more flexible than *S. noctilio* about their symbiotic associations with *Amylostereum* species, but it seems that *S. noctilio* is highly specific to its association with *A. areolatum*. *Amylostereum areolatum* is genetically diverse and is native to North America, Eurasia, and North Africa, but Eurasian strains were putatively introduced to North America along with *S. noctilio*. Cellulose-degrading bacteria also occur in the gut of *S. noctilio*, and while little is known about these associations, the activity of these bacteria is hypothesized as being complementary to *A. areolatum* in assisting *S. noctilio* in gaining nutrients from pine xylem.

Introduction

Insects do not have the enzymes needed to digest wood so associations with fungi and/or bacteria are required by wood-inhabiting insects. This includes woodwasps, as they generally complete their life cycle in dying or recently dead trees, using wood as the source for larval nutrients. This group includes the genus *Sirex*, as all known species are associated with fungi in the genus *Amylostereum* via symbiotic mutualism (Schiff et al. 2012). The woodwasp *Sirex noctilio* is obligately associated with *Amylostereum areolatum*, a basidiomycete fungus that females deposit within wood when laying their eggs. Larval survival and development depend on the presence and active growth of *A. areolatum* to provide nutrients that these insects cannot access on their own. In turn in this mutualism, *S. noctilio* vectors this fungus to new, susceptible trees, reducing tree resistance to enhance fungal survival via injection of phytotoxic venom. *Sirex noctilio* has also been

found to be associated with cellulose and hemicellulose-degrading bacteria whose actions are considered complementary to the fungus in relation to larval nutrition. In this chapter we will discuss the diversity and activity of *A. areolatum* and will add what is known about the bacterial symbionts.

General biology, taxonomy, and genetic diversity of the symbiont

The symbiont *A. areolatum* is one of four species in the basidiomycete genus *Amylostereum*. Of the three other species, *A. chailletii*, *A. laevigatum*, and *A. ferreum*, the first two are also associated with woodwasps (van der Nest et al. 2012). *Amylostereum areolatum* is a saprotrophic decay fungus found in logs or stumps of conifers (Vasiliauskas et al. 1998), causing the breakdown of lignin and resulting in changes in wood texture and color (hence the common name white rot fungus). Its host range includes *Pinus* (pines), *Abies* (firs), *Larix* (larches), and *Picea* (spruce). This fungus is native to Eurasia and North Africa (van der Nest et al. 2012), as well as North America (Hajek et al. 2013; Olatinwo et al. 2013), and it has been introduced to the Southern Hemisphere along with *S. noctilio*. In Europe, *A. areolatum* has been observed in two forms: i) the sexual stage, which forms the fruiting structures (basidiocarps) found on the bark of conifers; this stage produces sexual spores (basidiospores) on the surface of the basidiocarps, and ii) the asexual stage, forming arthrospores (fragments of fungal hyphae that function as spores; also called “oidia”; Fig. 1). Basidiospores are dispersed by wind, while arthrospores are vectored by *S. noctilio* females. In Europe, the fruiting structures are crust-like, corky, or leathery, flattened with bent and wavy edges, and are often colored deep brown to violet (Wermelinger and Thomsen 2012). Basidiocarps of *A. areolatum* are rarely found, while the asexual form is prevalent and is often found growing within wounded trees, indicating that the fungus reproduces primarily via the asexual mode due to its close association with the woodwasp (Thomsen and Koch 1999; Vasiliauskas and Stenlid 1999). *Amylostereum areolatum* does not need to produce airborne sexual spores for non-directed, aerial dispersal because *S. noctilio* will vector the asexual fungal spores and inject them into susceptible trees. Where asexual reproduction dominates (i.e., most locations), *A. areolatum* occurs as limited numbers of clonal lineages within areas. Sexual forms of *A. areolatum* have not been reported from North or South America.



Figure 1. Arthrospores (oidia) from mycangia of a female *Sirex noctilio* (image by A.E. Hajek).

Sirex noctilio vectors this fungus in both endemic and introduced sites. By its association with the woodwasp, the fungus is also considered invasive when introduced to new locations with *S. noctilio*. But while the woodwasp requires the fungus for survival, aside from being vectored by *S. noctilio*, the fungus exists without its insect host because it lives by decomposing wood. *Amylostereum areolatum* is not considered a strong pathogen (e.g., Vaartaja and King 1964), yet pine trees can be killed by a combination of this fungus along with *S. noctilio*'s venom gland secretion (noctilisin) that is injected into trees along with the fungus when females oviposit (Bordeaux et al. 2014). Thus, the association of *A. areolatum* with the woodwasp not only results in vectored dispersal but also greatly enhances the successful infection of trees by the fungus.

Multiple genetic variants or genotypes of *A. areolatum* have been observed in association with *S. noctilio*. Fungal isolates from endemic sites are generally more genetically diverse because of possible sexual recombination and also genetic exchange between compatible asexual forms. In areas of introduction (especially the Southern Hemisphere), where only the asexual form is present and the fungal genotypes present are determined by woodwasp introduction(s), the *A. areolatum* population is generally much less

diverse. Because of the close association between the woodwasp and its fungal symbiont, the diversity of fungal genotypes present in an area of introduction has been utilized in determining the number and possible routes of woodwasp introductions from endemic sites (e.g., Bergeron et al. 2011; Castrillo et al. 2015). Castrillo et al. (2015) found at least three distinct genotypes of *A. areolatum* associated with *S. noctilio* in the US. One of these was also widespread in Europe (present in Denmark, Hungary, and Spain), and the other two were from unrepresented source populations. This indicates multiple introductions to the US, possibly via different sources/routes.

Genetic diversity of *A. areolatum* has been examined by testing interactions between vegetatively growing isolates on culture media (e.g., Thomsen and Koch 1999; Vasiliauskas and Stenlid 1999) and/or by sequencing multiple genes (e.g., Nielsen et al. 2009; Bergeron et al., 2011; Castrillo et al. 2015). Vegetative growth tests are relatively easy to conduct because the fungus grows readily on simple media (Fig. 2), allowing analysis of interactions between two isolates growing near each other. Isolates of the same vegetative compatibility group (VCG) would continue to grow freely and intermingle along the line of contact, whereas those of different VCGs would have a clear zone with no growth between the two cultures, often marked by dark brown coloration (Fig. 2). Considerable genetic variation may be present among isolates of the same VCG however, requiring differentiation at a finer scale by other means such as sequencing multiple genes or by other molecular markers. For example, a study using a molecular approach to investigate introductions of *A. areolatum* to South America found multiple genotypes within the same VCG (Mlonyeni et al. 2018).

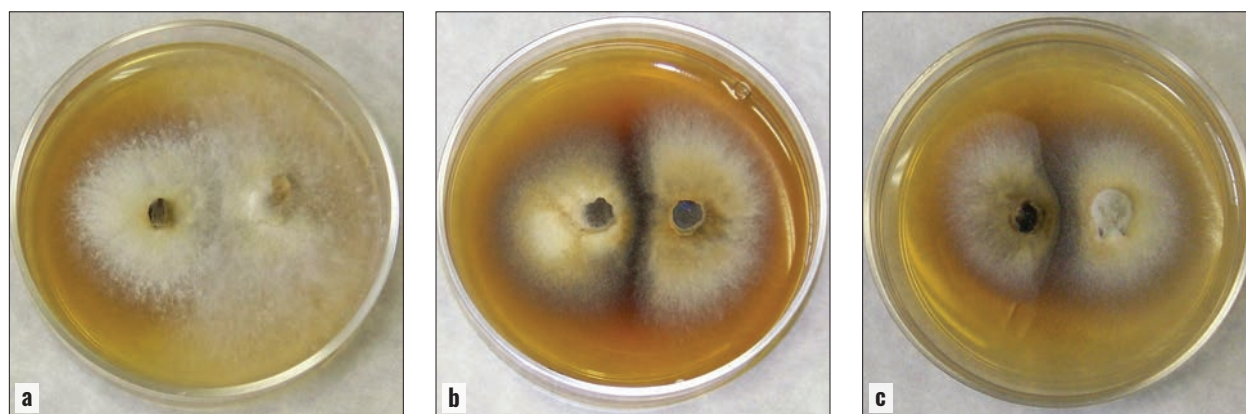


Figure 2. Vegetative compatibility group (VCG) testing of *Amylostereum* isolates. Strains of the same VCG have mycelia growing freely that intermingle along the line of contact (a: strains GR94 and SP12-E2), whereas those of different VCGs (incompatible) would have a clear zone between the two cultures, often marked by dark brown coloration (b: GR94 and AH1-17; c: B1352 and OtisAa). Strains GR94, AH1-17 and OtisAa were from *Sirex noctilio* collected in NY; SP12-E2 from *S. noctilio* from Spain; and B1352 from *Picea abies* in Germany (images from Castrillo et al. [2015]; reprinted with permission).

Life cycle of the fungal symbiont

Sirex noctilio is obligately associated with *A. areolatum* because the larvae need the fungus to act as an external rumen to digest wood (see Chapter 5). Wood degradation by *A. areolatum* also facilitates larval tunneling. Therefore, it is critical that the woodwasp carries this fungus along with it when laying eggs. Female *S. noctilio* have paired mycangia which are invaginations of the intersegmental membranes at the base of the ovipositor and which open into the oviduct (Fig. 3). Mycangia are filled with fungal arthrospores and lined with glands that are thought to provide nutrients to stimulate fungal growth. Arthrospores are injected into trees when a female woodwasp drills. She can produce a single drill without eggs or multiple drills that all have eggs except the last (Coutts and Dolezal 1969; Madden and Coutts 1979; see Chapter 2, Fig. 4). Fungus is not inserted in the same drills as eggs, except occasionally as a contaminant; fungal

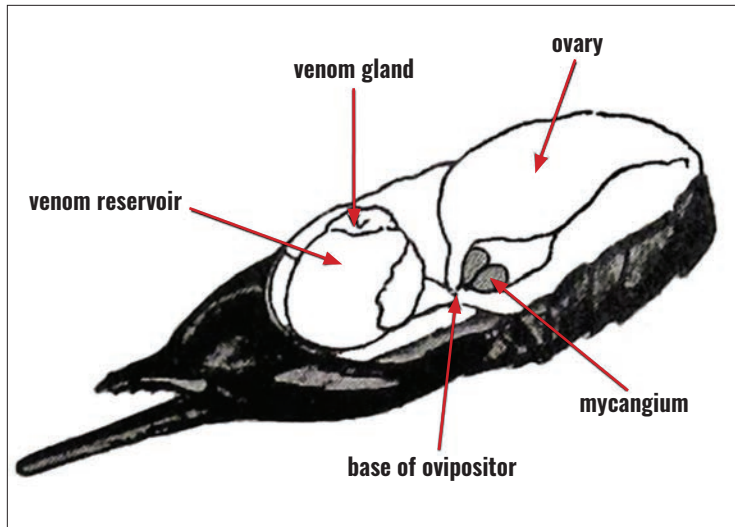


Figure 3. Interior of a *Sirex noctilio* adult female abdomen, showing the location of mycangia and venom gland (illustration based on a figure in “Controlling the Woodwasp *Sirex noctilio*,” an undated pamphlet from the Division of Entomology, Nematode Group, © Copyright CSIRO Australia).

arthrospores are deposited in the final egg-less drill and are accompanied by the phytotoxic venom. Once the arthrospores are deposited in the last drill, the fungus starts to grow in the wood, which results in localized drying of the wood. The number of drills is dependent in part on the moisture level of the wood, which is optimal at 60–70% moisture content (oven dry weight) (Madden and Coutts 1979). If the wood in a tree is quite wet, the female will initially create only single drills, and the resulting fungal growth dries the wood; this has been referred to as ‘conditioning’ the wood. After the osmotic pressure of the trees receiving only single drills is reduced, possibly a year or more after the initial attack (see Chapters 2 and 10), then a female would begin drilling to lay eggs as well as continuing to deposit fungus and venom in the last drills.

Once deposited in the drill, the arthrospores germinate and the fungus colonizes the wood, often growing more rapidly vertically than radially. Eggs hatch shortly after they have been laid (see Chapter 2). Larvae concentrate their feeding near the border of fungal growth in the sapwood (Thompson et al. 2014), and the resulting larval galleries are within the areas of fungal growth. From the early instars, female larvae carry arthrospores in hypopleural organs (Fig. 4), which are external pockets formed by folds of the skin on the dorsum of each side of the larval body, between the first and second abdominal segments. Each hypopleural organ contains fungal filaments surrounded by a wax-like material. These organs increase in size as a larva grows, and the fungus recontaminates the larva after each molt. The generally accepted hypothesis is that these organs are present in larvae to ensure fungal conservation. For example, even if the wood dries out so that fungal development stops and fewer larval instars are completed, the resulting small adult females that emerge will still carry the fungus (Gilmour 1965).

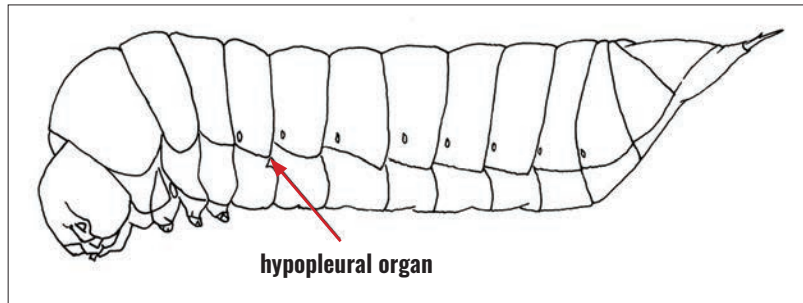


Figure 4. Location of hypopleural organ, where arthrospores of *Amylostereum areolatum* are retained in larval *Sirex noctilio* (illustration based on Boros [1968]).

But how is the fungus moved from the larval hypopleural organs into adult female mycangia? Before the molt that initiates pupation, the glands in the hypopleural organ produce an abundance of waxy secretion that encases the fungal cells. Once a larva pupates, the hypopleural organs on the molt skin, now containing fungal cells within wax, are evaginated (pushed outward from the molt skin). The molt skin

remains attached to the abdominal end of the adult female once she has eclosed and is ready to chew her way out of the tree (Gilmour 1965). It is thought that the movements of a female while she chews her way out of the wood, moving forward toward the bark, breaks up the wax plates containing the fungal cells and that the fungal cells then move up the ovipositor and fill the mycangia.

***Sirex*-*Amylostereum* interactions**

Amylostereum areolatum potentially influences *S. noctilio* egg eclosion by creating suitable wood moisture levels (i.e., conditioning the wood). This fungus is required for larval nutrition (see Chapter 5), and survival and development of immature *S. noctilio* have been found to be dependent on active growth of *A. areolatum*. The final size of *S. noctilio* adults, which can vary astonishingly, has been associated with the same conditions affecting fungal growth, which includes moisture content of the wood. Therefore, with optimal fungal growth, *S. noctilio* females are larger and will consequently be more reproductively successful (Madden and Coutts 1979; Madden 1981).

The presence of the fungus within a tree also influences the behavior of *S. noctilio* females and some of the associated parasitoids of *S. noctilio*. Mated female *S. noctilio* are attracted to volatiles from *A. areolatum* (Fernandez Ajo et al. 2015), but not *A. chailletii* (Sarvary et al. 2016), a white rot symbiont used by other woodwasps in Eurasia and North America (Table 1). This would assist these short-lived adults in finding a tree that is already infected with *A. areolatum*, and therefore suitable for oviposition. This also benefits the fungus in attracting its potential vector. Likewise, volatiles from this same fungus are used by *S. noctilio* parasitoids so that they can find and locate potential hosts (see Chapters 3 and 8).

In North America, other insects also attack weakened pines that *S. noctilio* prefers; these insects, in particular bark beetles, carry their own fungal symbionts. When pines have been colonized by other fungi, growth of *A. areolatum* is often impeded; subsequently *S. noctilio* larvae can starve. Studies have been conducted that demonstrate that *A. areolatum* is often outcompeted by ophiostomatid species of fungal symbionts associated with bark beetles (e.g., Ryan et al. 2011; see Chapter 10).

***Sirex*-symbiont fidelity**

Fidelity between the woodwasp *S. noctilio* and fungus *A. areolatum* was previously a source of disagreement, with arguments supporting some flexibility in symbiont association versus strict specificity (Talbot 1977). When *S. noctilio* and *A. areolatum* were first discovered in North America, it was assumed that *A. areolatum* was not native. However, *A. chailletii* was known to be native and was considered the only symbiont associated with native North American siricid species (Table 1). Subsequent studies revealed, however, that *A. areolatum* was sometimes also associated with the native siricids *S. nigricornis* and *S. nitidus* in areas where *S. noctilio* did not occur (Hajek et al. 2013; Olatinwo et al. 2013). The *A. areolatum* strains found in these circumstances differed significantly from the strains associated with (i.e., introduced with) *S. noctilio* in North America. Therefore, we learned that some *A. areolatum* strains are native and are unique to North America, while other strains arrived with *S. noctilio*. Also, some siricids, such as *S. nigricornis*, can be flexible about the species of fungal symbiont that they use while others, such as *S. noctilio*, are almost always associated only with one species of *Amylostereum*.

How do the different siricids acquire different symbionts? Associations of fungal symbionts with siricids are always via the mycangia of adult females. Females inoculate trees with fungal symbionts, and so in this case, transmission is vertical (from a parent). However, different species of siricids can co-infest the same trees; when this occurs, horizontal transmission of fungal material can occur (= environmental transmission, acquisition of the symbiont not from the mother) (Hajek et al. 2013; Castrillo et al. 2015). When *S. nigricornis*

Table 1. Fungi known to be vectored within mycangia of female siricids in northeastern North America¹

SIRICID SPECIES	NATIVE/ INTRODUCED	FUNGAL SYMBIONT		CITATIONS
		<i>Amylostereum areolatum</i>	<i>Amylostereum chailletii</i>	
<i>Sirex noctilio</i>	Introduced	Yes	(Yes) Extremely rare and of questionable use by larvae	Nielsen et al. 2009; Bergeron et al. 2011; Hajek et al. 2013; Wooding et al. 2013
<i>Sirex nigricornis</i>	Native	Yes	Yes	Nielsen et al. 2009; Hajek et al. 2013; Olatinwo et al. 2013; Wooding et al. 2013
<i>Sirex nitidus</i>	Native	Yes	Yes	Hajek et al. 2013
<i>Sirex cyaneus</i>	Native		Yes	Hajek et al. 2013
<i>Urocerus albicornis</i>	Native	(Yes) Rare	Yes	Stilwell 1966; Hajek et al. 2018
<i>Urocerus cressoni</i>	Native	(Yes) Rare	Yes	Hajek et al. 2018
<i>Urocerus flavicornis</i>	Native		Yes	Stilwell 1966

¹ Associations of fungal symbionts and siricids are based on the fungus being carried in the mycangia of adult females. However, many native siricid species have not been studied enough to understand the diversity of fungal symbionts that are carried.

and *S. noctilio* occupied the same tree, a *A. areolatum* genotype putatively introduced to North America with *S. noctilio* was subsequently found in *S. nigricornis* mycangia. We also found that a genotype of *A. areolatum* associated with *S. noctilio* (i.e., originating from Europe) was present in the mycangia of the native North American siricids *Urocerus albicornis* and *U. cressoni* (Hajek et al. 2018). *Urocerus albicornis* was previously only known to be associated with *A. chailletii*, and the symbiont of *U. cressoni* had not previously been reported. However, the majority of *U. cressoni* collected carried *A. chailletii* and not *A. areolatum*.

A survey by Wooding et al. (2013) in Canada revealed that 3.5% of *S. noctilio* females carried *A. chailletii* (2 out of 55), and the rest carried *A. areolatum*. In other studies of symbionts of *S. noctilio* in North America, researchers have only found *S. noctilio* associated with *A. areolatum* (Table 1). Whether *S. noctilio* larvae can survive using *A. chailletii* as a symbiont is unknown, but this fungus appears to rarely be vectored by *S. noctilio*. Therefore, this invasive woodwasp seems to be less promiscuous about the symbiont it will use, compared to some of the native siricids (Table 1). Regardless, many native woodwasps have not been studied enough to know whether they regularly use only one or multiple symbiont species.

Bacterial symbionts

The majority of research on symbionts associated with *S. noctilio* has focused on *A. areolatum*. However, bacteria are also often important mutualists for wood-inhabiting insects. Studies of the guts, ovipositors, and mycangia of *S. noctilio* from New York State found 1–4 taxa of cellulose-degrading bacteria associated

with each individual host. Among these, 94% of the insects had *Streptomyces*, bacteria that are associated with cellulose degradation, and 88% had gamma-Proteobacteria, probably associated with hemicellulose degradation (Adams et al. 2011). We do not know whether bacterial symbionts of *S. noctilio* are specific and, if so, whether specific bacterial species or strains might be obligate symbionts. Bacterial symbionts of *S. noctilio* have not been studied in other areas. However, at present it is thought that the contributions of the fungal and bacterial symbionts to *S. noctilio* nutrition are complementary.

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**CHAPTER
5****Nutritional ecology of *Sirex noctilio*****Daniel S. Gruner and Brian M. Thompson**Department of Entomology, University of Maryland, College Park, Maryland 20742 USA

Abstract

In pine wood, xylophagous *Sirex noctilio* larvae utilize the most abundant organic polymers on earth: the broad class of refractory compounds known as lignocellulose. Accordingly, wood-boring species are limited not by the quantity of energy in carbon molecules, but by its quality. Moreover, mineral elements such as nitrogen and macromolecules such as amino acids, sterols, and vitamins, are essential for insect survival, development, and reproduction, but in short supply or entirely absent in wood. *Sirex noctilio*, in partnership with the white-rot fungus *Amylostereum areolatum* and other microbial symbionts, employs behavioral, morphological, and physiological adaptations to overcome structural and chemical defenses of pine wood (*Pinus* spp.) and acquire nutrition to complete their development. Here, we review adaptations for physical consumption and intake, degradation and digestion of lignocellulose in wood, and provisioning of scarce and essential elements and macronutrients, such as sterols and nitrogen. Collectively, multipartite microbial associations are essential to understanding *Sirex noctilio* larval nutrition and development in pines.

Introduction

Herbivorous insects face a gauntlet of physical, biochemical, and nutritional challenges to completing their life cycles consuming plants. The mechanical and biochemical properties of cell walls, vascular bundles, leaves, and secondary chemical defenses, together with a scarcity in essential compounds needed for growth, provide suboptimal diets to consumers. Herbivores require behavioral, morphological, and physiological adaptations, along with symbiotic partnerships with fungi and bacteria, to overcome plant defenses and achieve nutritional balance on suboptimal resources (Simpson et al. 2015).

Plants provide food that is incomplete and often well-defended. Elemental nutrients (e.g., nitrogen [N]) and macromolecules (e.g., amino acids, sterols) found in plants are necessary for insect growth, survival, and reproduction. However, these nutrients are in low concentration in plant tissue and are

imbalanced with respect to insect nutritional needs. Nutrients and macromolecules that insects can directly consume, assimilate, metabolize into energy, and repurpose into new insect biomass are scarcely available (Mattson 1980; Fagan et al. 2002; Simpson et al. 2015). For example, leaf nitrogen content is typically an order of magnitude lower than levels in insect bodies, while carbon concentrations are 10- to 100-fold higher (Fagan et al. 2002). Most of this carbon bonanza is in the form of inert structural compounds such as lignin, cellulose, and hemicellulose. Plant leaves and woody tissues laced with lignocellulose are tough and resistant to chewing or penetration by insect mouthparts. These compounds are not easily converted to simple carbohydrates, and most insects lack the digestive enzymes to exploit these compounds as food. Moreover, plant tissues are rife with chemical toxins or digestibility reducers, particularly in saps, resins, or latexes that flow through injured tissues.

Compared to the insect taxa that can consume the relatively more nutritious leaves, flowers, fruits, and seeds, the relative paucity of true wood feeders, such as the *Sirex noctilio* woodwasp, highlight the special barriers to consumption that wood presents. The majority of subcortical insects that exploit living trees and shrubs feed within the thin phloem and vascular cambium tissues between the bark and the wood. These layers are the sites of active cell growth and differentiation, where carbon fixed through photosynthesis in the canopy is translocated to the roots. As such, concentrations of limiting elemental nutrients and macromolecules, such as simple carbohydrates, are at higher levels in the layers just beneath the bark, although still well below the average levels in leaves, flowers, and fruits (Meerts 2002).

Xylem specialists, such as cerambycid beetles and woodwasps, feed within the sapwood and heartwood of living or recently killed trees. Feeding larvae must breach the outer gauntlet of sticky saps, latexes, and resins that carry secondary compounds and chemical defenses. Ovipositing females, in the case of *Sirex* woodwasps, accomplish this task by drilling into the sapwood where they lay eggs alongside fungal mutualists and a venom laden with plant toxins (Coutts 1969; Bordeaux et al. 2014; see Chapters 2 and 4). These xylophagous species must then extract energy and satisfy nutritional requirements by feeding within the sapwood, which features exceedingly dilute, aqueous solutions that translocate minerals from the roots to the canopy and the dead heartwood layers (typically <0.2% N dry weight) (Mattson 1980; Meerts 2002). To persist on a diet of inert cell walls and xylem vascular bundles, *S. noctilio* has a suite of organismal adaptations and microbial partnerships to extract nutrition and manufacture essential compounds.

Here, we review these adaptations and known microbial associations that together describe the fascinating nutritional ecology of *S. noctilio*. Because adult siricids do not feed, their nutritional ecology is understood through study of the larval stages and symbionts inside the xylem of pine trees (*Pinus* spp.; Fig. 1). We review existing knowledge of i) physical food intake (consumption), as constrained by toughness, low water content, and lignocellulose cell walls and vascular bundles; ii) degradation and microbial digestion



Figure 1. *Sirex noctilio* larva feeding within the xylem of *Pinus resinosa* exhibits trademark 'packed' frass, characteristic of Siricidae. Loose frass is more typical in species 'eating' wood (image by M.P. Ayres).

of lignocellulose; and provisioning of scarce and essential macromolecules and elemental nutrients, with a focus on iii) sterols and iv) nitrogen. Most of what we understand about the nutritional ecology of *S. noctilio* is tightly bound with the ecology of its white-rot basidiomycete mutualist, *Amylostereum areolatum* (see Chapter 4). This and other microbial partnerships are critically important to *S. noctilio* nutritional ecology, and there is still much to learn.

Physical consumption

Woody plant secondary cell walls, which keep trees upright, are composed of lignocellulose: predominantly cellulose, with hemicelluloses, pectin, and lignins interwoven to provide strength, durability, and a matrix resistant to biological degradation. The large polysaccharide cellulose is the most abundant organic polymer on earth and the dominant fraction in woody plant tissue (Gibson 2012). Although large in molecular size, its chemical composition is simple: hundreds to thousands of glucose molecules are bound together in a helix, interwoven with hydrogen bonds in crystalline fibers or microfibrils. The hemicelluloses are smaller-chained polysaccharides, with polymers made up of various 5- and 6-carbon sugars bound into xyloglucans, xylans, glucomannans, and galactoglucomannans (Gibson 2012). In turn, lignins are the most abundant class of aromatic organic polymers; they are not polysaccharides but are more variably composed of different phenolic building blocks to generate diverse and complex motifs (Weng and Chapple 2010). As with plant polysaccharides, the energy content of lignin is high. However, of the major constituents in wood, its structural heterogeneity, the aromaticity of phenolics, and extensive carbon-carbon crosslinking all serve to make it the most stubborn challenge to microbial exploitation (Geib et al. 2008). Overall, this matrix of cellulose, hemicellulose, and lignins gives wood its high mechanical toughness, which as a composite, is perhaps ten-fold tougher than the sum of its component parts (Lucas et al. 2000).

Wood-boring insects such as *S. noctilio* are mandibulate chewers with behavioral and morphological adaptations in larval stages to overcome the physical toughness of wood (Hochuli 2001; Chiappini and Aldini 2011). Insect mouthparts typically are the first point of interaction with food substrates and are under direct selection for form and function. Mandibles are sclerotized and hardened with elements such as magnesium and zinc, and molting allows for replacement of worn structures with new larval instars. Compared to related species whose larvae do not feed within wood, mandibles of wood-borers are specialized and much reduced in size relative to the head and body, and smaller mouthparts are correlated with the smaller size of food particles created (Watanabe and Tokuda 2010). Asymmetric mandibles, with complementary rather than additive functions, are more regularly featured in xylophagous taxa within the Blattodea, Lepidoptera, and Coleoptera (Deligne 1999; Chiappini and Aldini 2011). Chisel-shaped mandibles with a small and mostly untoothed contact surface are common among wood-feeding Coleoptera larvae that feed upon living or otherwise sound wood. Additionally, larvae of many cerambycid beetles have a raised molar plate on the occlusal surface of mandibles, which helps to grind and pulp woody material to small particles for ingestion (Chiappini and Aldini 2011).

By comparison, the mandibles of *S. noctilio* larvae are more similar in form to those described from termites, which Deligne (1999) likened to the analogy of a carpenter's plane designed to shave, rather than to grind and pulp. As with many termites, but unlike most Hymenoptera overall, left and right mandibles are asymmetric (Deligne 1999; Thompson et al. 2014). The right mandible in *S. noctilio* rests within a pocket created by the larger, strongly decurved left mandible (Fig. 2). This form is consistent with a shearing function initiated by the (outer) left mandible, which slides across and over the right mandible, such that thin wood shavings can be pressed between them.

Fracturing, shearing, and pulping the wood serves to break down wood into smaller particles that can be processed and ingested. However, without chemical breakdown by digestive enzymes, wood pulp and fragments still have the recalcitrant chemical structure of lignocellulose. As such, cerambycid and

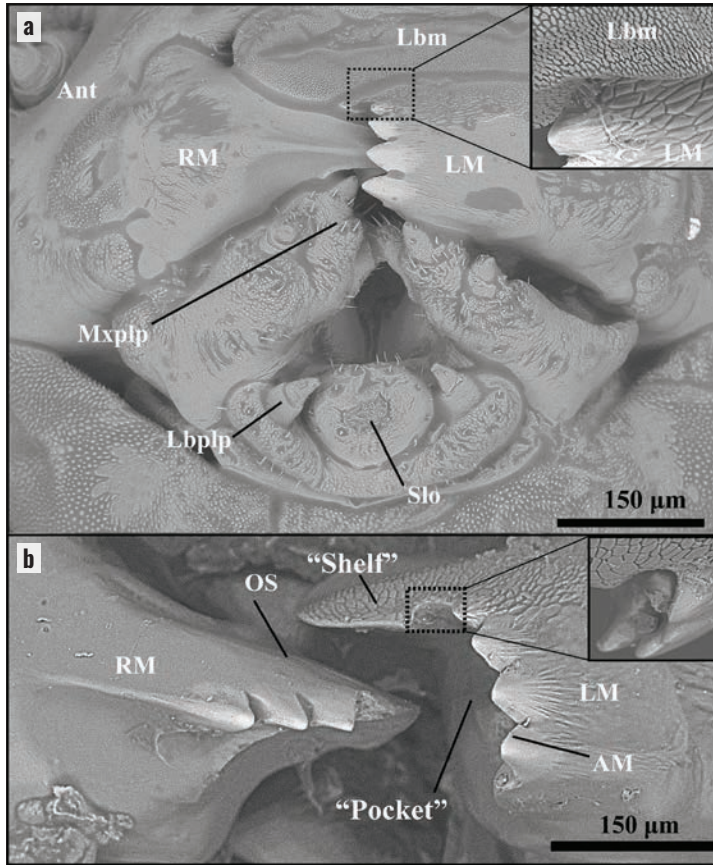


Figure 2. Scanning electron micrograph of the feeding appendages of larval *Sirex noctilio* with mandibles (a) occluded and (b) open. Right and left mandible (RM and LM, respectively) are asymmetrically aligned along the mesal margin. The mandibles are bordered by the maxillary (Mxplp) and labial (Lbplp) palps, the salivary orifice (Slo) from below, and a highly textured labium (Lbm; inset a). The top of the left mandible extends as a “shelf” above the occlusal surface (OS) of the right mandible. The “pocket” created by the “shelf” of the left mandible holds wood shavings cut by the apical margin (AM) and the “carpenter’s plane” (inset b), where it is pressed, releasing fluid fractions that flow in channels towards the oral orifice (images from Thompson et al. [2014]; reprinted with permission of the Entomological Society of America).

termite guts also possess ‘mycetomes’, which are invaginations and expanded side channels of the gut that house microbial symbionts for fermentative digestion (Grünwald et al. 2010). These chambers slow the passage of food intake to harbor and increase interaction with gut-associated yeasts and bacteria, some of which may be involved in amino acid or vitamin supplementation (Grünwald et al. 2010; Brune and Ohkuma 2011). However, in sharp contrast to the intestinal tract morphology of the cerambycid arrowhead borer *Xylotrechus sagittatus*, *S. noctilio* has a simple, linear alimentary canal lacking mycetomes (Fig. 3).

In fact, foraging *S. noctilio* larvae do not ingest or internally process bulk xylem tissue at all (Morgan 1968; Thompson et al. 2014). Processed shavings are manipulated by the mandibles and labia, expelled from the oral cavity without ingestion, passed along the underside of the larvae via peristaltic undulation, and packed to the rear of the feeding gallery (Fig. 1). This process is evident in the microstructure of ‘frass’ extracted from respective galleries of *S. noctilio* and *X. sagittatus* (Fig. 3). While the cerambycid produces a fine frass composed of processed wood fragments mixed with feces, *S. noctilio* ‘frass’ is little more than expelled wood shavings still preserving the microstructure of xylem vascular elements. Thus, *S. noctilio* larvae use their asymmetric mandibles to shave xylem tissue and to press shavings to extract liquid fractions (Thompson et al. 2014). No xylem tissue was recovered from gut dissections in the study of Thompson et al. (2014). Returning to the mandibular structure of *S. noctilio*, the occlusal surface of the left mandible has a prominent sulcus, or furrow, which we hypothesize allows liquid fractions pressed by the mandibles to drain towards the oral cavity. In the next section, we turn to evidence for extra-oral digestion in *S. noctilio* larvae and the ever-expanding role of microbial symbionts in its nutritional ecology.

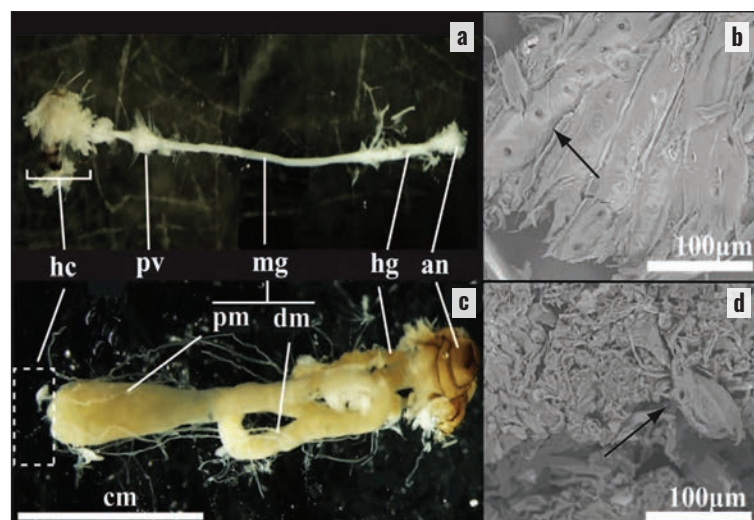


Figure 3. Gut dissections showing the intestinal tracts (fat bodies and malpighian tubules removed) and SEM images of frass at 600x magnification from co-occurring larval woodborers (a, b) *Sirex noctilio* and (c, d) *Xylotrechus sagittatus* (Cerambycidae) isolated from *Pinus resinosa* in Pennsylvania, USA. The *S. noctilio* digestive tract (a) is simple and lacking fermentation chambers, and (b) frass presents as unmanicured shavings of pinewood xylem with original wood architecture evident (arrow); whereas the *Xylotrechus* digestive tract (c) has two clear midgut sections with high volume ‘mycetomes’—chambers that house fermentative microbes, and (d) frass shows extensive manipulation and degradation. Head capsule (hc); proventriculus (pv); midgut (mg); proximal midgut (pm); distal midgut (dm); hindgut (hg); anus (an); and tracheids (arrows) (images from Thompson et al. [2014]; reprinted with permission of the Entomological Society of America).

Microbial digestion of lignocellulose

Wood digestion in insects is facilitated by both physical disruption of lignocellulose and by diverse enzymes to digest lignocellulose (e.g., lignases, cellulases, etc.) into byproducts that can be assimilated or metabolized. Until quite recently, the prevailing hypothesis was that digestion of cellulose could not occur in animalian guts without persistent symbiotic relationships with microbes (Martin 1991), such as those first discovered within termites (Breznak and Brune 1994). The endogenous capacity to synthesize enzymes that degrade plant polysaccharides into simple carbohydrates is now understood to be widespread in the genomes of diverse Fungi, Bacteria, Archaea, viruses, and even higher plants and invertebrates (Davison and Blaxter 2005; Watanabe and Tokuda 2010; Lombard et al. 2013). Specifically, enzymatic degradation of cellulose is accomplished through the action of glycoside hydrolases, carbohydrate esterases, polysaccharide lyases, and other carbohydrate-binding modules. Kukor and Martin (1983) isolated cellulolytic enzymes targeted to plant polysaccharides including cellulose, xylan, pectin, and amylose, finding similar fractions from the midguts of *Sirex cyaneus* larvae as in the surrounding matrix of xylem tissue infested with *Amylostereum chailletii*. This finding suggested that enzymes from *A. areolatum* are present in the wood surrounding *S. noctilio* larvae and are also ingested by larvae. Recent research has uncovered additional agents in the woodwasp-microbial complex, such as *Streptomyces* bacteria (Adams et al. 2011; Takasuka et al. 2013; Book et al. 2014). Their enzymatic weapons include a so-called microbial ‘power tool’ for breakdown of lignin fractions: lytic polysaccharide monooxygenases (Johansen 2016; Kracher et al. 2016) (Fig. 4).

As was apparent in early studies of the *Sirex*-*Amylostereum* mutualism (Cartwright 1938; Francke-Grosmann 1939; Parkin 1942; Talbot 1977), *A. areolatum* is critically important to *S. noctilio* nutrition, and cultured larvae invariably die without it (Madden 1981). The same is true with other well-known, obligate insect-fungus nutritional mutualisms. Fungal tissues contain limiting resources in higher concentrations than in wood; a recent meta-analysis of fungal nutrient contents estimated median dry N content for Basidiomycetes was between 3.5 and 4%, and phosphorous was similarly high at approximately 0.5% (Zhang and Elser 2017). This raises the question: does *A. areolatum* serve as a direct food source that provides the quantity and quality of nutrition needed for *Sirex noctilio* development, or does it serve principally to pre-digest xylem lignocellulose?

Multiple lines of evidence support the alternative hypothesis, that *A. areolatum* provisions *S. noctilio* nutrition, but is not consumed in bulk as a direct resource by larvae. That is, larvae obtain nutrients indirectly from plant material via extracellular digestive enzymes, derived from *A. areolatum* and other

microbial sources, consistent with the ‘external rumen hypothesis’ (Swift et al. 1979). *Sirex noctilio* has well-developed salivary glands (Maxwell 1955) that excrete substances to rapidly destroy the mycelial networks of *A. areolatum* in wood. For the *Sirex cyaneus*-*Amylostereum chailletii* system, Kukor and Martin (1983) noted that because biomass of the fungal hyphae appeared to be insubstantial, the fungus provides external digestion services but not bulk food resources. Indeed, the biomass of *A. areolatum* within the xylem remains very low relative to that of wood in actively infested areas within the tree bole (Thompson et al. 2013) (Fig. 5). This stands in contrast to leafcutter ants (Attini: *Atta* and *Acromyrmex*), ambrosia beetles (Scolytinae: Xyloborinae), and some termites (Macrotermitinae) which graze fungal production for direct nutrition (Mueller et al. 2005). These fungicultural systems are characterized by complex insect behaviors to propagate cultivars, maintain high standing biomass, and apply ‘auxiliary’ microbes to suppress disease, or even to fix atmospheric nitrogen within gardens, in the case of attine leafcutter ants (Pinto-Tomas et al. 2009). As discussed in turn, additional evidence that *Sirex noctilio* does not graze fungal gardens for direct nutrition comes from biochemical studies of sterols, a class of lipids essential for nutrition.

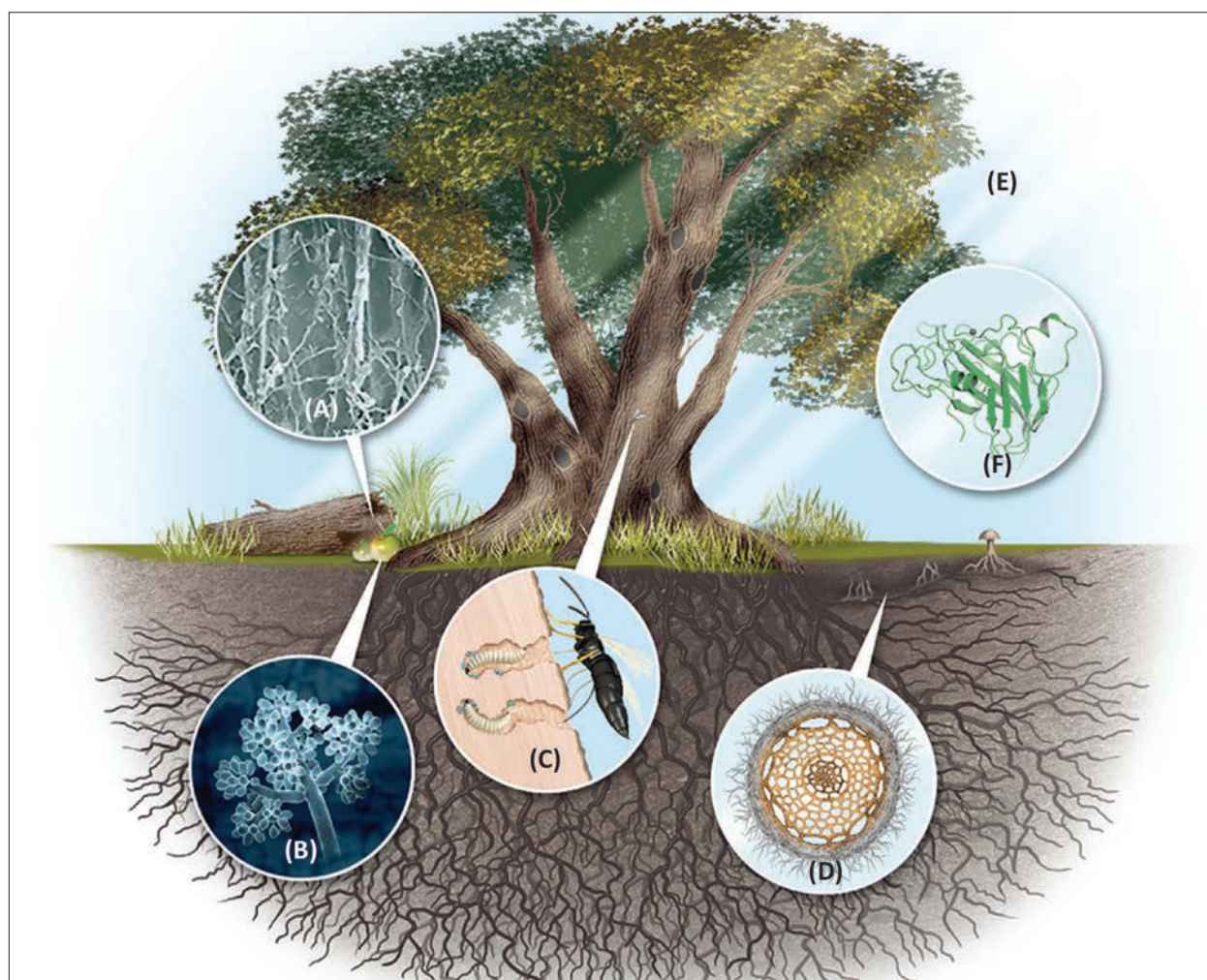


Figure 4. Examples of plant-microbe interactions which involve lytic polysaccharide monooxygenases (LPMOs: copper-enzymes that catalyze oxidative cleavage of glycosidic bonds). (A) Saprophytic fungi degrading a log of wood. (B) Degradation of fruits by grey mold. (C) Larvae of a *Sirex* woodwasp feeding on a tree assisted by microbial symbionts. (D) Cross-section of a root with ectomycorrhizal fungi involved in degrading soil organic matter. (E) Sunlight drives the storage of energy and CO₂ in photosynthetic plant tissue, and may also modulate destructive processes through photoinduced electron transfer. (F) A representative LPMO molecule (figure from Johansen [2016]; reprinted with permission from Elsevier).

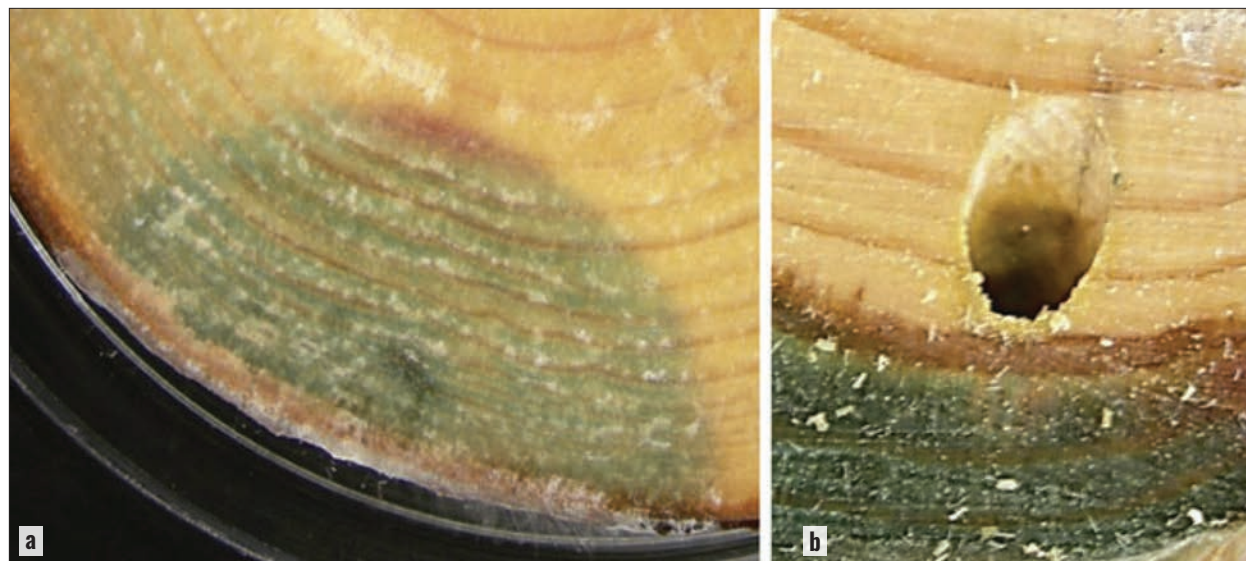


Figure 5. (a) Laboratory cultures of *Amylostereum aerolatum* grown on sterilized *Pinus resinosa* sections stained blue for the presence of laccase, a peroxidase-driven indicator reaction using ABTSTM (Sigma-Aldrich). (b) Section of *P. resinosa* from a tree with an active *Sirex noctilio* infestation, showing a larval feeding gallery marginal to active *A. aerolatum* growth. Statistical analyses showed that larvae concentrated foraging near the sites of highest fungal enzyme activity at the borders of fungal growth in pine xylem (images from Thompson et al. [2013]; reprinted with permission from Springer).

Sterol provisioning

Thompson et al. (2013) used the biochemistry of essential sterols, such as cholesterol and their metabolic intermediates, to trace their dietary sources (Bentz and Six 2006; Janson et al. 2009). To our knowledge, insects are incapable of producing sterol molecules *de novo*, despite the integral role of sterols in cell membrane fluidity, permeability, and signaling. Thus, insects must acquire and metabolize sterols from external sources (Behmer and Nes 2003). Predatory and parasitic insects may obtain cholesterol directly from food items, but herbivores must either synthesize cholesterol by modifying the chemical structure of ingested phytosterols or, less commonly, use alternate sterols to maintain homeostasis (Behmer and Nes 2003). Fungi uniquely synthesize ergosterol as the basic unit, and this molecule and its secondary byproducts definitively distinguish fungal from plant sources of sterol nutrition.

Using gas chromatography and mass spectrometry analyses, ergosterol and derivatives were present in *Amylostereum*-infested wood, absent from all environmental samples that lacked fungus, and absent from all but one life stage of *S. noctilio* (Thompson et al. 2013). Ergosterol was isolated only from adult females, which carry fungal cultures in their mycangia—a pair of specialized in-folded sacs between body segments linked by ducts to the base of the ovipositor (Parkin 1942). By contrast, the dominant sterol in all life stages was cholesterol, and both larvae and adults showed strong signals of the plant sterols campesterol, sitosterol, or stigmasterol. The lack of ergosterol and its associated metabolites in *S. noctilio* larvae, coupled with a strong phytosterol signal and their associated metabolites, align with other evidence for nutrition derived primarily from plant but not fungal tissues. Altogether, the hypothesis is well supported that *S. noctilio* larvae use the extensive mycelial network of the white-rot fungus as an external gut for digestion of recalcitrant lignocellulose in pine wood.

Based on shotgun metagenomic sequencing, the metabolic pathways associated with the *S. noctilio* microbiome appear optimized for metabolism of carbohydrates, such as starch and sugar moieties (Thompson 2013). Notably absent from the *S. noctilio* metabolome were genes for digestion of the majority of cellulose, lignin, and hemicellulose bonds ubiquitous to woody plant tissues (Thompson 2013). A recent

genomic analysis of *A. areolatum* filled these gaps with at least 580 carbohydrate-active enzymes (CAZymes), including many that are active against cellulose and lignin (Fu et al. 2020). The analysis also identified many virulence-factor genes, putatively linked to disabling tree defense mechanisms and metabolizing terpenoid chemistry, but also linked to intracellular signaling and carbohydrate transport. As discussed next, the microbial powerhouse for metabolizing lignocellulose into simplified lipids and carbohydrates is critically important to the provisioning of nitrogen, an essential and highly limited building block for life.

Nitrogen provisioning

Even while *A. areolatum*, *Streptomyces*, and other microbial associations solve the problem of refractory carbon, pine xylem tissues are deficient in other important ways. Most elemental nutrients and macromolecule resources essential to insect growth are present, but in vanishingly low concentrations. This is a problem of ratios with structural carbon but also of absolute abundances. For example, N content of wood can be as low as 0.03% by dry mass, and xylem sap N content is 2–3 orders of magnitude lower than phloem sap (Mattson 1980; Meerts 2002). Insect body tissues, on average, are closer to 10% N by dry mass (Fagan et al. 2002). Although low in concentrations, some essential amino acids and sterols are present and can be metabolized to different forms. Other amino acids, B vitamins, and sterols are essential for insect herbivore growth and development, cannot be synthesized *de novo*, and are not dietarily available in sufficient quantities (Douglas 2015).

Although dinitrogen (N_2) gas is by far the dominant molecular fraction in the atmosphere at 78% by mass, organisms cannot use or metabolize nitrogen in this inert form—it must be ‘fixed’, or captured, into biologically available inorganic forms, such as ammonia (NH_3) or nitrate (NO_3). Internal N-fixation is now known from numerous wood-feeding insects (mostly termites), but evidence exists within five coleopteran families and for *S. noctilio* as the lone hymenopteran (Thompson 2013; Ulyshen 2015). The *A. areolatum* genome does not include genes coding for a nitrogenase enzyme, which is not surprising (Fu et al. 2020). All known biological systems for atmospheric dinitrogen fixation operate anaerobically because oxygen (O_2) is more reactive and competitive than dinitrogen for the binding sites in nitrogenase enzymes. Activity of the nitrogenases for N-fixation therefore occurs within organs that are internally isolated from ambient air, such as within chambers of the termite gut (Brune and Ohkuma 2011), or possibly within salivary glands. Nitrogen fixation would not be likely within the ‘external rumen’ inside the xylem of a tree, exposed as it would be to ambient oxygen. This process is also energetically intensive, requiring 12–24 ATPs to reduce each triple-bonded N_2 molecule to ammonia (NH_3) (Stam et al. 1987). Given the powerful genomic capacity for the white-rot fungus to digest and metabolize lignocellulose (Floudas et al. 2012; Fu et al. 2020), *A. areolatum* may instead provide the fuel for an internal N-fixation engine driven by microbial actors not yet identified. Potential sites for N-fixing microbes could be within specialized cells of the fat bodies, called oenocytes, around the tracheae of *S. noctilio* (Thompson 2013), or within the guts or salivary gland tissues as in termites and beetles (Ulyshen 2015).

Even without the ‘smoking gun’ identifying the microbial symbiont(s) likely responsible, multiple lines of evidence indicate internal dinitrogen fixation in *S. noctilio*. Rough calculations for a nitrogen budget, based on volume of displaced xylem in galleries of woodwasps completing their development, places the total N-content in a typical pine xylem gallery at approximately 10% of total needs (M.P. Ayres et al. unpublished data). Allowing for nitrogen provisioning by transport in mycelial networks, and allowing the unrealistic assumption that 100% of dietary nitrogen can be assimilated, these calculations suggest an immense N shortfall for larvae to complete their development. Additionally, if nitrogen is highly limited, there should be strong natural selection for efficient capture, use, and recycling of nitrogen. By contrast, frass discarded by larvae has a higher nitrogen content than the surrounding xylem, either infested or uninfested by *A. areolatum* (Thompson 2013).

The final lines of evidence come from stable (non-radioactive) isotope ratios of the heavy nitrogen isotope ^{15}N , to the dominant lighter isotope, ^{14}N (Hood-Nowotny and Knols 2007). As suggested by their name, stable isotopes do not decay, but instead maintain constant ratios in the absence of biological processes. The ratio of $^{15}\text{N}/^{14}\text{N}$ in the atmosphere is so inert and stable that it is used as the scientific standard for all other comparisons. Evidence from both natural patterns of $^{15}\text{N}/^{14}\text{N}$ in *S. noctilio* body tissues and from lab experiments using N_2 gas enriched with ^{15}N reflect atmospheric, not dietary, sources of nitrogen in *S. noctilio* body tissues (Thompson 2013; Ulyshen 2015). Altogether, these lines of evidence resolve the important question as to how *S. noctilio* and its microbial partners can subsist and thrive in an exceptionally nitrogen-limited system inside pine wood.

Conclusions

We have reviewed evidence that the woodwasp *Sirex noctilio* uses a battery of behavioral, morphological, and physiological adaptations, along with essential fungal and bacterial mutualisms, to overcome structural and chemical defenses of pine wood (*Pinus* spp.) and to acquire nutrition to complete their development entirely on this most intransigent of biological resources.

Despite the wealth of information documented on the nutritional ecology of *Sirex noctilio* over nearly a century of research, there is much we do not know. For example, we do not know how *S. noctilio* provisions essential amino acids and vitamins that are scarce in pine xylem, or the detailed pathways by which *S. noctilio* and its microbiome detoxifies and metabolizes pine chemical defenses. Keys to improving our understanding undoubtedly lie with more research into the composition and function of the *S. noctilio* microbiome. The powerful nutritional provisioning capabilities that we understand thus far suggest that the microbiome may contribute to the pest status of this species. Microbial provisioning of offensive traits has been suggested to explain the greater polyphagy of the Asian long-horned beetle, *Anoplophora glabripennis*, compared to related species (Scully et al. 2014). A comparative approach, examining other siricid species and xylophagous lineages and their microbial symbionts more generally, may be particularly rewarding, as it has been in exploring morphological and physiological adaptations for wood-feeding across taxa.

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**CHAPTER
6****Natural enemies: Biology of parasitic nematodes attacking *Sirex noctilio* and history of their use for biological control in the Southern Hemisphere**Ann E. Hajek¹ and E. Erin Morris²¹Department of Entomology, Cornell University, Ithaca, New York 14853-2601 USA²Department of Biological Sciences, University of New Hampshire, Durham, New Hampshire 03824 USA**Abstract**

Siricids from around the world were collected in the 1960s and 1970s, and seven species of the dimorphic nematode *Deladenus* were described from these hosts or from siricid associates. Among these nematodes, *Deladenus siricidicola* was chosen and developed for biological control of *Sirex noctilio* in Australia. Dimorphic *Deladenus* spend part of their life feeding on the fungal symbiont used by siricids and part of their life as a parasite of siricids. The strain of *D. siricidicola* developed for biological control sterilizes females of the genotype of *Sirex noctilio* present in Australia. Eggs of infected female *S. noctilio* are not viable and are filled with nematodes that are vectored to new trees when female *S. noctilio* oviposit. Mass production, storage, and release methods have been developed for successful use of this nematode in Australia. Presently, *D. siricidicola* is re-released for re-establishment when localized populations of *S. noctilio* begin to increase from low, non-damaging densities. The same strain of this nematode has been released to control *S. noctilio* in Brazil, Argentina, Uruguay, Chile, and South Africa, but control in these countries has been variable. Among the many factors implicated are genetic homogeneity of *D. siricidicola*, presence of genotypes of *S. noctilio* differing from the Australian genotype in susceptibility to sterilization, wood moisture conditions, and loss of nematode virulence. In addition, the presence of sapstain fungi in the wood can negatively influence nematode growth by outcompeting the *S. noctilio* fungal symbiont that is needed as food by the fungal-feeding form of the nematode.

Introduction

Throughout the 1960s and in the early 1970s, natural enemies of siricids from around the world were collected for potential use in biological control, in response to economic levels of damage being caused by *Sirex noctilio* in pine plantations in Australia. During this time, seven species of *Deladenus* parasitic to or associated with *Sirex* species were discovered. The species showing the most promise for biological control was *Deladenus*

(formerly *Beddingia*) *siricidicola*, first described in 1967, which sterilizes female *S. noctilio* (Bedding and Akhurst 1978). *Deladenus siricidicola* was used extensively for biological control efforts in Australia and New Zealand and, as *S. noctilio* was introduced in and caused outbreaks to new continents and countries in the Southern Hemisphere, *D. siricidicola* was also introduced to South America (Brazil, Uruguay, Argentina, and Chile) and South Africa (Hurley et al. 2007; Hajek et al. 2016). This same nematode species was also found in northeastern North America, after the 2004 discovery that *S. noctilio* had become established. However, genotypes within the *D. siricidicola* species can have very different effects on different woodwasp hosts. The *D. siricidicola* strain non-intentionally introduced to North America does not sterilize the *S. noctilio* genotype evaluated in the northeast (see Chapter 7) and, therefore, does little toward control of *S. noctilio* populations.

This chapter will describe the general biology, ecology, and use to date of *D. siricidicola* for biological control. Among the dimorphic species of *Deladenus*, we know by far the most about this species, and information from this nematode can inform the potential use of *Deladenus* to control *S. noctilio* in North America (see Chapter 7). First, we discuss the general biology and factors used to select the biological control strain. Next, we discuss how the life history of the nematode was exploited for mass culture, the methods to release the nematodes in the field, and how these methods have been updated to preserve nematode virulence. Finally, we will discuss the history and effectiveness of *D. siricidicola* used for biological control of *S. noctilio* in the Southern Hemisphere.

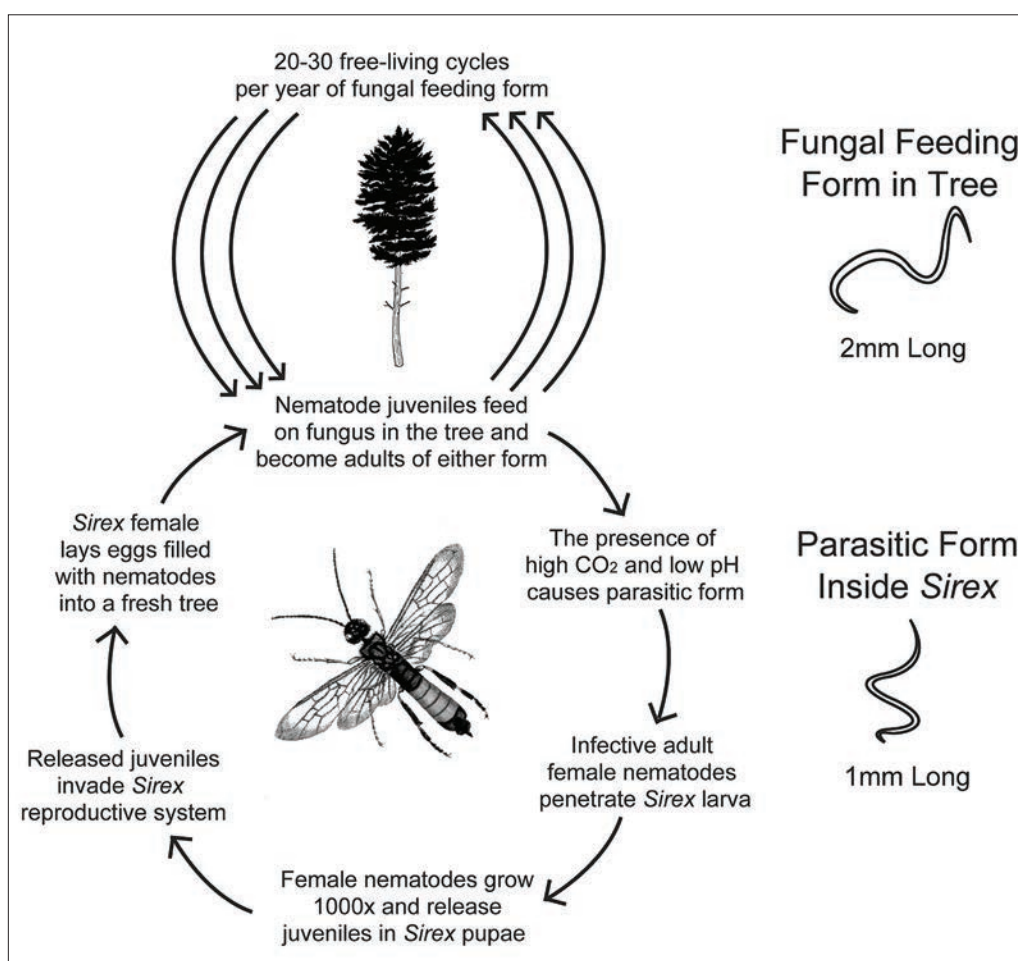


Figure 1. Life cycle of *Deladenus siricidicola* Kamona associated with *Sirex noctilio* and its symbiotic fungus *Amylostereum areolatum* (diagram from Hajek [2004]; reprinted with permission).

General biology of *Deladenus*

The biology of dimorphic *Deladenus* is complicated, and little is known specifically about the species and strains present in North America (see Chapter 7). Therefore, the description presented below is largely based on the Sopron/Kamona strain of *Deladenus siricidicola* used for biological control of *S. noctilio* in the Southern Hemisphere (Bedding 1972). This general biology is assumed to be similar for all dimorphic *Deladenus*.

Some species of *Deladenus* are dimorphic, and only these are important to our discussion here. For dimorphic species, one form is parasitic on woodboring insects, and the other form is free-living and fungus-feeding (Fig. 1). The two forms are remarkably different in their morphologies. The more common mycophagous form is found within trees inhabited by *S. noctilio*; mycophagous *D. siricidicola* feeds specifically on *Amylostereum areolatum*, the fungal symbiont used by *S. noctilio*. The mycophagous forms are larger, with adult females approximately 2 mm long (Bedding 1972) (Fig. 2). Adult females of the parasitic form are closer to 1 mm long, and, when observed with a microscope, they have a spear-shaped stylet within their mouths that is used for boring into woodwasp larvae (Fig. 3).

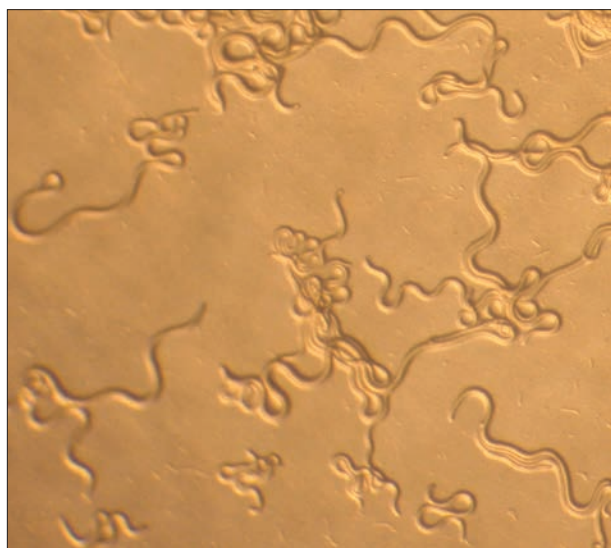


Figure 2. Petri plate culture of the mycophagous form of *Deladenus siricidicola* (image by E.E. Morris).



Figure 3. An infective adult female of the parasitic form of *Deladenus siricidicola*. The arrow indicates the stylet used for piercing the larval cuticle to enter a *Sirex noctilio* larva (image by J. Henry).

How do the nematodes switch from one form to another? During woodwasp oviposition, the fungus is inserted into the trees along with *S. noctilio* eggs (see Chapters 2, 4, and 5). As the woodwasp eggs hatch and larvae begin to grow, the fungus grows as well. When woodwasp eggs containing nematodes are inserted into trees, the early-stage nematodes inside of eggs are the mycophagous form. The mycophagous form then hatches and disperses through xylem tracheids within trees, reproducing (Figs. 4, 5) and feeding on the growing tips of the fungus, and usually remaining within the outer 5 cm (2 in) of the sapwood. There is at least one mycophagous generation (although it is thought that there are usually many) before a switch to the parasitic phase. Eggs and young juvenile nematodes produced by mycophagous females in proximity to *S. noctilio* larvae are influenced by the locally increased CO₂ and low pH to grow into parasitic forms. Once these nematodes become adults, the parasitic males and females mate, and the mated females (now called ‘infectives’) then bore into a *S. noctilio* larva. The mated female nematode then sheds her outer skin for faster nutrient absorption and increases in size up to 1000x in volume and up to 20 mm (0.75 in) in length.

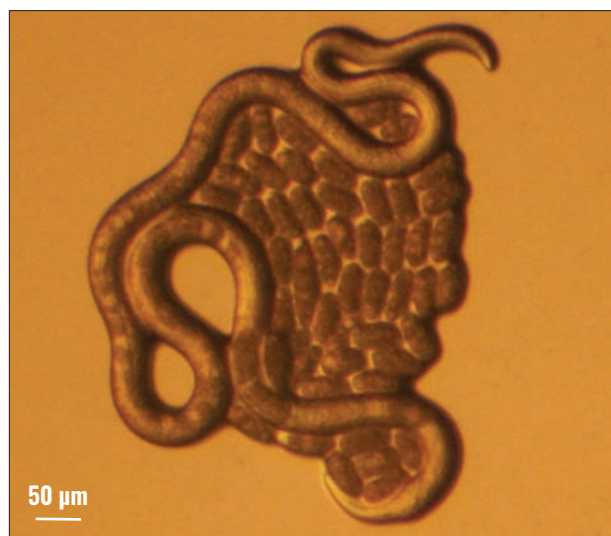


Figure 4. A female mycophagous *Deladenus siricidicola* Kamona surrounding her eggs (image by E.E. Morris).

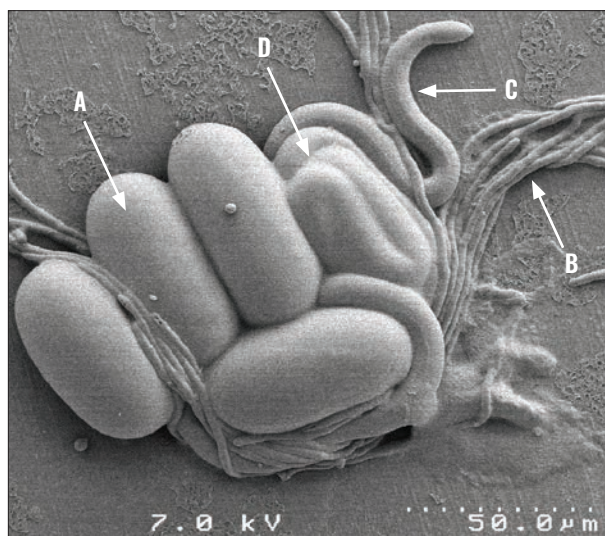


Figure 5. Scanning electron micrograph of (A) eggs of the *Deladenus siricidicola* Kamona mycophagous form surrounded by (B) fungal hyphae and (C) juveniles that have hatched. (D) Nematodes develop into first stage juveniles within the eggs and molt to the second juvenile stage before they exit the eggs. Eggs are approximately 55 x 27 µm (image by E.E. Morris).

However, the nematode's reproductive system temporarily stops developing, until the host *S. noctilio* larva nears pupation. Only then are nematode eggs produced in abundance within the mother nematode and fertilized with the sperm stored from the time of nematode mating, before the female nematode entered the *S. noctilio* larva.

Nematode eggs hatch in just a few days, and the juvenile nematodes leave the mother's body. The juvenile nematodes then migrate within the woodwasp's body to the ovaries or testes. Testes of parasitized *S. noctilio* males become enlarged with nematodes, but this is a biological dead end for the nematodes because parasitized males do not pass on nematodes to females when mating. In female *S. noctilio*, parasitized ovaries are smaller than normal and often contain fewer eggs when compared with healthy woodwasps. Within the ovaries, the nematodes can penetrate *S. noctilio* eggs before the eggshells harden. The contents of the woodwasp eggs are no longer viable, and the female *S. noctilio* is thus sterilized because her eggs contain nematodes and not her own offspring. This sterilization is characteristic of the biological control strain of *D. siricidicola* used against *S. noctilio*. The parasitized *S. noctilio* females are not killed; they continue with their normal behavior, which turns out to be beneficial for the nematodes because they are vectored to a new tree. After emerging from the tree, nematode-parasitized *S. noctilio* females disperse to lay eggs (which now only contain nematodes) into new trees.

Reports from 1972 (Bedding 1972) with the Sopron strain of *D. siricidicola* stated that usually all eggs were sterilized in this way. But if it is true that all of the *S. noctilio* eggs are sterilized, how do the nematodes find new hosts to eventually travel to a new tree? This high level of egg sterilization works for the nematodes because numerous *S. noctilio* often infest the same trees, increasing chances that both healthy *S. noctilio* eggs and later stage larvae would be present in the same tree where nematode-filled eggs were deposited in the xylem by females attempting to lay eggs of their offspring. So, because many *S. noctilio* from different mothers often develop within the same tree (see Chapter 2), the nematodes can find a healthy larva to parasitize, eventually leading to nematode dispersal. To summarize, the nematodes within woodwasp eggs deposited in a new tree are the mycophagous form, and it is thought that they survive and reproduce in that tree for numerous generations until they have moved within the tree to encounter a healthy *S. noctilio* larva, at which time they switch to the parasitic form.

Biological control using *Deladenus* in the Southern Hemisphere

Differences among *D. siricidicola* strains evaluated for biological control

During the 1960–1970s, the Commonwealth Scientific and Industrial Research Organisation (CSIRO) and associated agencies collected natural enemies of woodwasps around the world. In total, seven species of *Deladenus* were found infecting numerous species of siricids or siricid parasitoids (Bedding and Akhurst 1978). When choosing which *Deladenus* to focus on developing for biological control, it was important to choose a nematode species that was parasitic on siricids (one species mostly parasitized siricid parasitoids) and fed on the same fungus utilized by *S. noctilio* as a symbiont; *Deladenus* species are thought to be highly specific regarding which species of fungal symbiont their mycophagous stage will feed on (Bedding and Akhurst 1978; but see Morris et al. 2014). Some of the *Deladenus* species parasitizing siricids utilized *Amylostereum chailletii*, but only *D. siricidicola* used the *S. noctilio* symbiont (*A. areolatum*) exclusively, and so this species was chosen (Bedding and Iede 2005).

Deladenus siricidicola was found infecting numerous species of siricids in Europe, North Africa, and Asia (Turkey and Japan), including *Sirex noctilio*, *S. juvencus*, *S. nitobei*, *S. cyaneus* (which is now called *S. torvus*), and *Xeris spectrum*. However, these species names are based on taxonomy that might have changed since 1978 (Bedding and Akhurst 1978; Schiff et al. 2012). In addition, a European wood-inhabiting beetle, *Serropalpus barbatus*, commonly associated with siricids, was parasitized by *D. siricidicola*. This seeming lack of host specificity was not an issue because there were no native siricids or siricid-associated insects in Australia, where pines are also not native.

A strain of *D. siricidicola* from Japan did not sterilize the *S. noctilio* genotype present in Australia and was removed from further consideration (Bedding 1972). Among nematode strains that did cause woodwasp sterilization when injected into wood infested by the *S. noctilio* strain in Australia, four strains (from Greece, Corsica, Hungary, and New Zealand) achieved nearly 100% parasitism and were tested further in limited field trials in Tasmania. Flight capacity of adult *S. noctilio* infected by these four different strains was evaluated. While parasitism did not affect flight directly, it negatively influenced flight indirectly because parasitism decreased female *S. noctilio* size, and larger females flew further; i.e., very large *S. noctilio* could fly up to 200 km (124 mi) while very small females only flew about 2 km (1.2 mi) (Bedding and Iede 2005). The strain from Hungary, originally isolated from *S. juvencus* and named “Sopron” based on the collection location, was chosen as the strain to develop for biological control. *Sirex noctilio* parasitized by the Sopron strain were larger compared with those infected with the other strains. This would allow greater dispersal potential as well as greater egg production, both of which would enhance the potential for *S. noctilio* to become established and vector these nematodes to new trees (Bedding 1993).

Development and use of *D. siricidicola* Sopron and Kamona for control

Methodology

Deladenus siricidicola can be grown relatively easily in the free-living, mycophagous phase that feeds on the *S. noctilio*-symbiotic fungus, *A. areolatum*, and this created the opportunity for mass production of the nematodes (Bedding 2009). Methods were developed to release the nematodes in pine forests: i) “trap trees” were created by weakening pines via injection with low doses of herbicides (see Chapter 12), which in turn attracted *S. noctilio* and led to oviposition in these trees during the *S. noctilio* flight season; ii) these *S. noctilio*-infested trees were felled in early fall, and *D. siricidicola* from mass-produced cultures were injected into the trap trees; and iii) the next summer, nematode-infested *S. noctilio* emerged on their own and then vectored the nematodes to other infested trees in the area when ‘nemapositing’ (= laying nematode-filled eggs). Numbers of trees that needed to be

inoculated per area of infested forest were determined, and it was later found that spatial arrangement of inoculated trees can also affect nematode parasitism early after releases (Corley et al. 2014). Because nematode-parasitized females do not usually fly as far as healthy females, a more dispersed arrangement of releases results in better spread of nematodes.

These methods worked well initially, but a few issues arose over time. First, as *S. noctilio* spread in Australia, the mycophagous form of *D. siricidicola* continued to be mass produced repeatedly in the lab. Over time, the Sopron strain lost much of its ability to produce the infective form in the presence of *S. noctilio* larvae. The resulting decrease in sterilization in the field resulted in limited control of *S. noctilio* populations and catastrophic levels of pine mortality. Upon this discovery, researchers returned to a forest named Kamona in Tasmania where the Sopron strain had first been released, and they re-isolated *D. siricidicola* from that site. The resulting virulent strain of *D. siricidicola* (producing both mycophagous and parasitic phases) that is now used for control is the re-isolate, named after the Kamona site. The Kamona strain was released throughout Australia and Brazil as a replacement in places where the weakened Sopron strain had been released. Methods for freezing living nematodes were developed in order to prevent future loss of parasitism via mass production. Today, *Deladenus siricidicola* Kamona can be purchased from an Australian company that mass produces and sells the Kamona strain for control of *S. noctilio* populations. A new aliquot of frozen nematodes is thawed each year and tested for its ability to produce the infective form, prior to mass production, in order to maintain virulence (Bedding and Iede 2005).

Use of *D. siricidicola* for biological control in the Southern Hemisphere

In 1962, *D. siricidicola* was found parasitizing *S. noctilio* on the North Island of New Zealand, but it had not been purposefully introduced there. This strain was subsequently moved to the South Island, resulting in parasitism levels of >75% in uninoculated trees (Zondag 1979). *Sirex noctilio* is now considered a minor pest in New Zealand, based on activity of the nematodes, parasitoids, and use of better silvicultural practices. This was the initial example in the Southern Hemisphere that suggested *D. siricidicola* could provide significant assistance in controlling *S. noctilio* (Bain et al. 2012).

In 1970, approximately 50 female *S. noctilio* parasitized by the Sopron strain were released in Tasmania in one corner of a forest. Two years later, nematodes were present within 92% of the *S. noctilio*-infested trees in the 12 ha (30 ac) closest to the release site, and usually >70% of the individuals that emerged from the wood were parasitized (Bedding and Akhurst 1974). After initial releases, it was found that nematode populations declined coincident with *S. noctilio* population decline. Then, if and when the *S. noctilio* population increased again, the nematodes responded too slowly on their own to prevent pine losses. Also, *D. siricidicola* did not spread from forest to forest fast enough to prevent damage in plantations where *S. noctilio* was increasing. For these reasons, biological control of *S. noctilio* with *D. siricidicola* in Australia remains augmentative, with surveillance conducted to detect new areas of pine mortality, where low density *S. noctilio* populations are potentially beginning to increase (Carnegie and Bashford 2012). The density, health, and distribution of the *S. noctilio* population is assessed when determining the number and distribution of nematode releases needed to prevent a potential outbreak (Carnegie and Bashford 2012).

In South America, *D. siricidicola* has been considered the primary biological control agent for use against *S. noctilio* and has been released in all *S. noctilio*-infested countries, including Brazil, Argentina, Uruguay, and Chile (Hajek et al. 2016). Overall in South America, the impact of *D. siricidicola* Kamona has been highly variable across regions and time (14–90% parasitism) (Corley et al. 2019). *Deladenus siricidicola* Kamona has also been released in South Africa with variable success, and its use for control of *S. noctilio* there is under study (B. Hurley personal communication).

Some patience is necessary in evaluating success of releases, as time since release of *D. siricidicola* can strongly affect results. In Brazil, around Cape Town, South Africa, and in southern Chile, levels of

parasitism were initially low after releases. However, 3–4 years after first release, during which time nematodes were often released again, parasitism built to >90% (Fig. 6).

In more recent years, a problem has developed with the nematode inoculation procedures, caused by establishment of another invasive insect species in Australia, the pine engraver beetle *Ips grandicollis* (Yousuf et al. 2018), which is native to North and Central America. This bark beetle attacks the trap trees used for inoculation with Kamona, limiting *S. noctilio* colonization and subsequent spread of the nematodes throughout the forest. The beetle carries its own symbiotic fungus, *Ophiostoma ips*, a fast-growing sap-staining fungus that competes with *A. areolatum* within pines. Presence of *O. ips* therefore disrupts nematode parasitism of *S. noctilio* (Yousuf et al. 2018). Adjustment in the timing of and amount of herbicide application used to create trap trees can minimize *I. grandicollis* colonization, and this change has improved biological control with Kamona in some locations (Clarke et al. 2016).

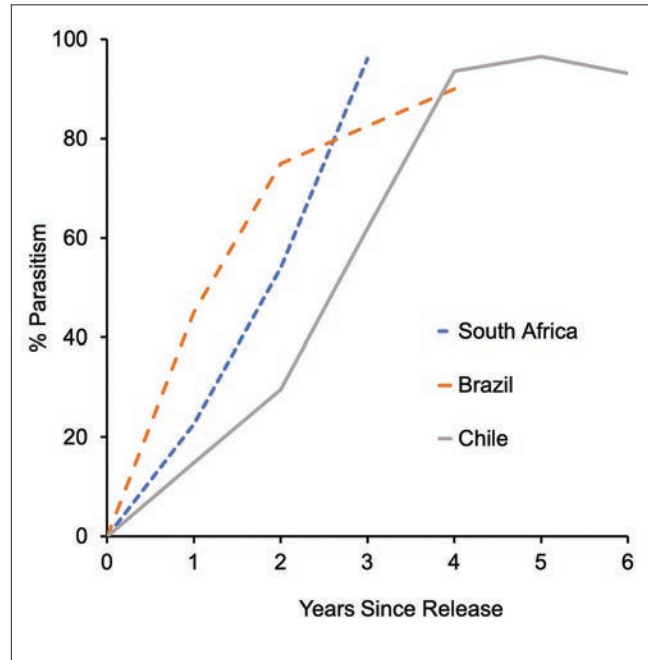


Figure 6. Percent parasitism of *Sirex noctilio* by *Deladenus siricidicola* Kamona by years after initial release in Encruzilhado do Sul, Brazil (released in 1990–1993; Bedding and Iede 2005), around Cape Town, South Africa (released in 1995–1996; Tribe and Cillie 2004), and in southern Chile (released in 2010–2016; Castillo et al. 2018).

Factors influencing *D. siricidicola* parasitism levels

Many different and often interacting factors can impact the success of *D. siricidicola* in providing control, including *S. noctilio*, *D. siricidicola*, or fungal symbiont strains, competition, environmental conditions, and methods for release. The genotype of *S. noctilio* matched with specific strains of *D. siricidicola* can impact whether female sterilization occurs or not (Bedding 1972). As an example of the influence of woodwasp genotype, *D. siricidicola* from Hungary sterilized virtually all eggs in the genotype of *S. noctilio* in Australia, but a Japanese strain of *D. siricidicola* did not sterilize eggs of Australian *S. noctilio*. As an example of the influence of nematode strain, while *D. siricidicola* from Belgium did not sterilize eggs of Belgian *S. noctilio*, they sterilized eggs of the genotype of *S. noctilio* introduced to Australia. These differences in the occurrence of sterilization have been attributed to the relative developmental timing of woodwasp and nematode.

From 1 to 100 adult female *D. siricidicola* can develop and reproduce within one *S. noctilio* larva (Bedding 1972; Bedding and Akhurst 1974). Although it is not known how the density of *D. siricidicola* within a tree impacts transmission to healthy *S. noctilio* larvae within that tree, it seems logical that with more nematodes in a tree, there are greater chances of their finding and parasitizing healthy larvae. Regardless of the number of adult female nematodes initially penetrating a *S. noctilio* larva, studies have reported a trend that *S. noctilio* adults parasitized by Kamona are smaller in size than healthy adults (Eskiviski et al. 2004). As first reported by Bedding, smaller size of nematode-parasitized *S. noctilio* adults negatively influences flight capacity, and consequently nematode dispersal (Bedding and Iede 2005). However, in Argentina, the spread of the *S. noctilio* population into new areas was not slowed after releases of *D. siricidicola*, in part because without 100% parasitism, healthy

individuals continued to fly the longer distances, so populations of *S. noctilio* continued to spread (Corley et al. 2014).

The strains of *A. areolatum* that are fed on by the mycophagous stages of *D. siricidicola* can influence the growth of *D. siricidicola* (Morris et al. 2012, 2014). Competition between *A. areolatum* and other fungi within pines can also impact control by *D. siricidicola* (see previous section). Different strains of *A. areolatum* grow at different rates. In the laboratory, the fast-growing strains can parasitize and kill eggs and adults of mycophagous *D. siricidicola*, decimating nematode colonies (Morris and Hajek 2014). The extent that this turn-about (i.e., the fungus eating the nematode rather than the nematode eating the fungus) occurs within trees needs further study.

Several environmental factors potentially impacting *D. siricidicola* have been identified, including temperature, tree species, and conditions within trees, such as moisture. In Australia and North America, it was found that the impact of *D. siricidicola* can be altered by the species of tree infested by *S. noctilio* (Nahrung et al. 2016; Williams and Hajek 2017). In addition, the wood moisture content can influence the activity of *D. siricidicola* within trees; nematode parasitism is greater in the bases compared with the middles and tops of pines in South Africa (Hurley et al. 2008). When reared at constant temperatures $> 24^{\circ}\text{C}$ (75°F), which could occur under climate change scenarios in Australia, nematode sterilization and *S. noctilio* size and egg production decreased, which suggests that the activity of Kamona could be compromised in warmer regions (Yousuf et al. 2014).

Summary

Our knowledge about the complex biology and ecology of *Deladenus* species is based on numerous studies of *D. siricidicola*, the species that has been used extensively for *S. noctilio* control in the Southern Hemisphere. Notably, this control is not due to the host woodwasp being killed, but due to it being sterilized. While this nematode is considered very effective in Australia, in other countries where it has been released, results have not always been as positive (e.g., northeastern South Africa). Regardless, we can use the information learned from *S. noctilio* and *D. siricidicola* Sopron and Kamona strains as a springboard toward potential use of other species and strains of *Deladenus* to control invasive populations of *S. noctilio* in North America.

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**CHAPTER
7****Natural enemies: Potential use of *Deladenus* for control of *Sirex noctilio* in North America**Ann E. Hajek¹ and E. Erin Morris²¹Department of Entomology, Cornell University, Ithaca, New York 14853-2601 USA²Department of Biological Sciences, University of New Hampshire, Durham, New Hampshire 03824 USA**Abstract**

Three different *Deladenus* species and/or strains are of interest relative to the potential biological control of *Sirex noctilio* in North America. After *S. noctilio* was first found in North America, long-term studies were initiated to evaluate whether the commercially available *D. siricidicola* Kamona (see Chapter 6; hereafter “Kamona”), used for biological control in the Southern Hemisphere, would be appropriate for release in North America. These trials were conducted for six years and included injecting Kamona into 464 pines infested with *S. noctilio*. Parasitization and sterilization levels that resulted were low, and non-target insects were also affected. It is hypothesized that one of the reasons results were so poor was that a strain of *D. siricidicola* was already established in *S. noctilio* populations in North America (INA = Introduced North American strain). It is assumed that *D. siricidicola* INA was introduced with *S. noctilio*. The problem with the INA strain is that it does not sterilize *S. noctilio* and appears to often outcompete *D. siricidicola* Kamona when the latter is inoculated into trees. However, limited evidence suggests that this non-sterilizing strain may reproduce with the Kamona strain to yield a hybrid strain that can sterilize, although more research is necessary to understand the potential for these interactions. Efforts have now focused on a native species, *Deladenus proximus*, which normally inhabits pines where it parasitizes the native *S. nigricornis*. This nematode, which can cause high levels of sterilization in *S. nigricornis*, occurs throughout the eastern US, also parasitizes *S. noctilio*, and can use the same fungal symbiont as *S. noctilio*. The extent that *D. proximus* will be able to compete with *D. siricidicola* INA, either on its own or when mass produced and purposefully released, remains to be seen, when or if *S. noctilio* requires control.

Introduction

In considering the Southern Hemisphere model of using *Deladenus siricidicola* to control *Sirex noctilio*, it is important to note key differences between the biotic communities associated with conifers in North

America versus the Southern Hemisphere. Pines (*Pinus* spp.) are not native to the Southern Hemisphere, so the majority of organisms associated with conifers there have been introduced. In contrast, pines are native in North America and are associated with 28 native species of woodwasps in the family Siricidae (Schiff et al. 2012). None of these North American native woodwasps are considered pests, so knowledge about these species and their associated biotic communities is much more limited than knowledge about invasive *S. noctilio* and its associated communities in the Southern Hemisphere.

In North America, five species of *Deladenus* are known to be associated with native siricids and their fungal symbionts (Morris et al. 2013, 2018) (Table 1). Of these five species, each has mostly been found to be associated with one native species of *Sirex*. In a worldwide survey conducted in the 1960s and 1970s, *D. siricidicola* was found in many sites in Eurasia, but it was not detected in North America (Bedding and Akhurst 1978). However, *D. siricidicola* was eventually collected in North America in 2007–2008, when it was first found infesting *S. noctilio* populations that were established in Ontario (Yu et al. 2009). It is assumed that *D. siricidicola* in North America had been introduced by accompanying invasive *S. noctilio*. Notably, this introduced strain of *D. siricidicola* is significantly different from the Kamona or Sopron strains that have been used for biological control in the Southern Hemisphere. It differs genetically, but it also does not sterilize the strains of *S. noctilio* in North America (Yu et al. 2009). This invasive strain of *D. siricidicola* has been referred to as the “non-sterilizing” strain in the literature, but we now refer to it as the “Introduced North American” strain (INA) (Morris et al. 2020).

This chapter will describe and discuss the extensive experimental trials with the Kamona strain of *D. siricidicola* in North America, as well as what is known about the INA strain of *D. siricidicola* assumed to have been introduced with *S. noctilio*. We will then discuss the native *D. proximus*, which parasitizes the native pine-specific *S. nigricornis* but is also known to parasitize *S. noctilio*, and the potential for use of this species for biological control of *S. noctilio* in North America.

Table 1. Species of *Deladenus* with parasitic and mycophagous forms known from North America. Tree species are those with which these *Sirex* species are usually associated.

NEMATODE SPECIES	PARASITIZED INSECT HOST	ASSOCIATED FUNGI	DISTRIBUTION	CITATIONS
<i>D. beddingi</i>	<i>Sirex californicus</i> (in pines)	<i>Amylostereum chailletii</i>	U.S. (Washington)	Morris et al. 2018
<i>D. canii</i>	<i>Sirex cyaneus</i> (in firs)	<i>Amylostereum chailletii</i>	Canada (New Brunswick)	Bedding, 1974
<i>D. nevexii</i>	<i>Xeris spectrum</i> (in firs)	<i>Amylostereum chailletii</i>	U.S. (Nevada)	Bedding, 1974
<i>D. proximus</i>	<i>Sirex nigricornis</i> (in pines) and <i>Sirex noctilio</i> (in pines)	<i>Amylostereum chailletii</i> and <i>Amylostereum areolatum</i>	Eastern US and Canada	Bedding 1974; Yu et al. 2011; Morris et al. 2013; Zieman et al. 2015; Hartshorn et al. 2017
<i>D. siricidicola</i>	<i>Sirex noctilio</i> (in pines) and <i>Sirex nigricornis</i> (in pines)	<i>Amylostereum areolatum</i>	US, Canada, Europe, Asia, South America, Africa, Oceania	Bedding 1968; Bedding and Akhurst 1978; Yu et al. 2009; Kroll et al. 2013; Hajek and Morris 2014
<i>D. wilsoni</i>	<i>Rhyssa</i> spp. (in wasp parasitoids of siricids)	<i>Amylostereum areolatum</i> and <i>Amylostereum chailletii</i>	SE and SW US, Canada, Europe, Asia	Bedding 1968; Bedding and Akhurst 1978

Experiments with *Deladenus siricidicola* Kamona in North America

After *S. noctilio* was collected in North America in 2004 and found to be established, an initial question was whether to introduce *D. siricidicola* Kamona for biological control. This nematode had been effective at controlling *S. noctilio* in Australia, where it sterilizes females by entering and killing woodwasp eggs (Fig. 1a) and then is vectored by female *S. noctilio* to new trees (see Chapter 6). *Deladenus siricidicola* was readily available from a commercial Australian firm (Williams et al. 2012). Researchers tested whether Kamona would provide control but also whether it would survive the climate in the *S. noctilio*-infested area of North America, where winters are colder than areas where *D. siricidicola* is known to provide effective control in the Southern Hemisphere. Another initial goal was to determine whether Kamona would negatively impact non-target species.

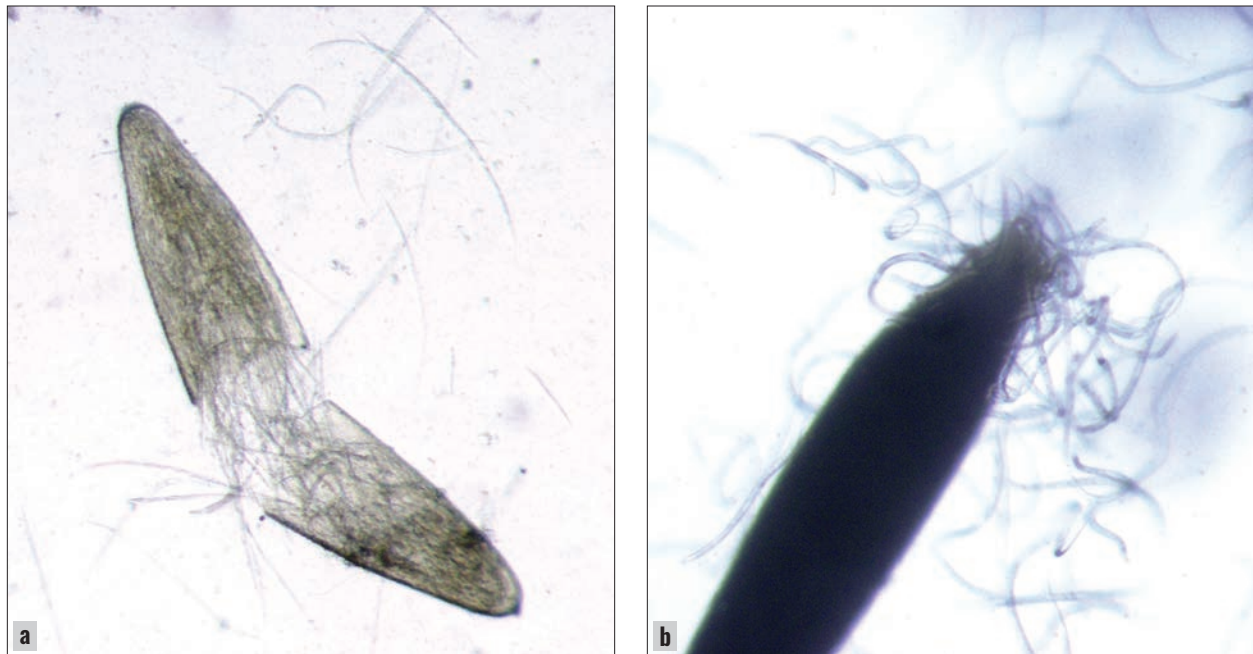


Figure 1. *Deladenus siricidicola* in and surrounding *Sirex noctilio* eggs. (a) Juveniles of the Kamona strain within, and effectively sterilizing (i.e., killing) *S. noctilio* eggs. The nematodes are vectored in this way by the parasitized female woodwasp during oviposition. (b) Juveniles of the INA strain that does not enter and sterilize eggs but is vectored by parasitized female woodwasps (images by C.L. Crook).

Experimental controlled release studies were conducted, injecting Kamona into *S. noctilio*-infested pines in New York and Pennsylvania. To carry out these experiments, Kamona was purchased from Australia and mass produced in a quarantine facility in the US (Fig. 2a). *Sirex noctilio*-infested pines were created or located, and felled, mostly in early fall. Felled trees were then treated via injection of Kamona (Fig. 2b,c), using methodology developed in Australia (Williams and Hajek 2017). In spring, well before *S. noctilio* emergence, three bolts 1 m (3.2 ft) long were cut from each tree and taken to a quarantine facility, and the insects were reared out. The remainder of the nematode-inoculated pines in the field were chipped. The chipped wood was left on site because *S. noctilio* that had been within this wood would have been destroyed, and any nematodes that were present would not survive; *Deladenus* can only live within trees that are either infested by *Sirex* or that are supporting growth of the fungal symbiont.

These experimental studies, spanning from 2007 to 2012, were begun prior to the discovery of the INA strain of *D. siricidicola* in North America (Yu et al. 2009). As the existence and abundance of the INA strain



Figure 2. Methodology for releasing *Deladenus siricidicola* developed in the Southern Hemisphere. (a) Mass production of *D. siricidicola* Kamona in a quarantine laboratory. (b) Creating holes in felled trees infested by *Sirex noctilio*. (c) Injection of gel containing nematodes into the felled trees (images by D.W. Williams).

became known, methods for its detection were developed and incorporated. Throughout this study testing the efficacy of Kamona, the INA strain was present at all study sites. To evaluate results from Kamona releases, both molecular methods and data on egg sterilization were used to identify Kamona vs INA. However, data on egg sterilization (all years) is only possible to collect for female *S. noctilio*, and molecular methods (2008–2012, both sexes) could only be used for a subsample (21%) of specimens. Overall, 464 pines over this six-year period were treated with Kamona, and *S. noctilio* were reared from this experimental wood (Williams and Hajek 2017). *Sirex noctilio* emerging from many of the treated pines were not infected with Kamona and therefore were not sterilized. An average of 28.1% of emerging *S. noctilio* contained nematodes (either INA or Kamona). In three of those years, no emerging females were sterilized, and so all parasitism is assumed to have been due to INA. In the other three years, sterilization (i.e., due to Kamona and not INA) ranged from 13.1 to 50.0% (although the 50.0% was for 2011, when a total of only 10 females emerged and were evaluated). Although *S. nigricornis*, the native woodwasp that co-infests pines with *S. noctilio*, was not abundant during these studies (e.g., Hajek et al. 2017), a few females with sterilized eggs were reared from Kamona-injected trees, suggesting that *S. nigricornis* is also sterilized, presumably by Kamona as INA does not sterilize *S. nigricornis*.

In summary, results from this project led to the hypothesis that the genotypes of *S. noctilio* present in North America (or at least in these study areas) were predominantly not very susceptible to parasitism and sterilization by Kamona. Molecular studies help explain this lack of susceptibility; genotypes of *S. noctilio* present in North America are very different from those in Australia that were used when choosing Sopron/Kamona as the best *Deladenus* for *S. noctilio* control (Bittner et al. 2017a; see Chapter 6). In addition, the INA strain present in *S. noctilio* in North America could complicate the efficacy of Kamona by outcompeting it. The potential extent and overall impact of hybridization between these strains is unclear at this time (but see next section).

It should be noted that Figure 6 in Chapter 6 shows that releases of Kamona in the Southern Hemisphere did not lead to high levels of parasitism the first year of release, but parasitism levels increased over time. However, the controlled release study conducted from 2007 to 2012 in North America was only conducted for serial one-year periods so that Kamona was not being released into the forests, principally due to concerns regarding lack of knowledge about the potential for non-target effects if Kamona became established. This study, therefore, could not evaluate whether levels of parasitism would increase over time after releases, as occurred in South America and South Africa (see Chapter 6, Fig. 6).

The *Deladenus siricidicola* strain naturalized in North America (INA)

In 2007–2008, it was discovered that *D. siricidicola* was already infesting *S. noctilio* in Ontario, Canada (Yu et al. 2009), and in 2011, it was found in New York state (Kroll et al. 2013). Molecular techniques demonstrated that the strain already present was very similar to *D. siricidicola* Kamona but differed genetically (Morris et al. 2013; Fitza et al. 2019; Morris et al. 2020). Importantly, the strain of *D. siricidicola* present in North America did not sterilize *S. noctilio* females—nematodes were found within the bodies of *S. noctilio* but were not inside of the eggs (Fig. 1b). The INA strain is vectored because it is transmitted in accessory fluids that accompany healthy siricid eggs during *S. noctilio* oviposition. Whether or not parasitism by *Deladenus* results in female sterilization is a function of both the strain of the nematode and the strain of the woodwasp host (Bedding 1972). The INA strain has previously been referred to as “non-sterilizing,” but because lack of sterilization could be caused by the strain of nematode and/or the genotype of host, this name is not appropriate (e.g., this nematode strain might sterilize other genotypes of *S. noctilio* although it does not sterilize the *S. noctilio* genotype introduced to North America). Therefore, this strain is now being referred to as the “Introduced North American strain” or “INA”.

Deladenus siricidicola was not found in North American samples of *Sirex* collected from 1963 to 1970 (Bedding and Akhurst 1978). After *S. noctilio* was found to be established in North America, this nematode was found parasitizing both *S. noctilio* (Yu et al. 2009) and the native *S. nigricornis* (Morris et al. 2013; Haavik et al. 2016). It is hypothesized that parasitism of *S. nigricornis* was initially due to horizontal transmission occurring when these *Sirex* species co-inhabited the same trees. All indications suggest that this strain of *D. siricidicola* is alien to North America (see Introduction).

While the INA strain of *D. siricidicola* does not sterilize *S. noctilio*, it still has a negative impact on *S. noctilio*; parasitized adults have a somewhat decreased size and carry fewer eggs (Kroll et al. 2013). (However, one study found that adult male *S. noctilio* parasitized by this strain were larger than healthy individuals [Gaudon et al. 2016; Haavik et al. 2016]). As with Kamona (see Chapter 6), INA parasitism can negatively affect flight in *S. noctilio* when parasitism reduces adult size (Gaudon et al. 2016). The INA strain of *D. siricidicola* is also similar to the Kamona strain in that it exclusively uses *Amylostereum areolatum* when mycophagous, and it parasitizes other siricid species. Notably, the native *S. nigricornis* parasitized by this strain are also not sterilized. Additionally, this strain parasitizes the native North American beetle *Serropalpus substriatus*, frequently found in association with siricids (Bittner et al. 2017b). During one study conducted in upstate New York, the INA strain was found in 44.0% of trees sampled and in 27.9% of emerging *S. noctilio* (Kroll et al. 2013). More female *S. noctilio* were parasitized than males, and parasitized adults emerged from trees earlier than non-parasitized adults.

In some cases, the INA strain was already present in the pines injected with the Kamona strain (Williams and Hajek 2012; study described in previous section). During these trials where *S. noctilio* adults were caged on stressed trees that were subsequently injected with Kamona (Fig. 3), we found evidence of co-infection



Figure 3. Experimental cage used for studies of parasitism of *Sirex noctilio* by *Deladenus siricidicola* Kamona and INA. The tree was stressed by injection with weak herbicide after which *S. noctilio* adult females were placed within cages during summer to oviposit. Some of these females were already naturally parasitized by INA. In fall, the tree was felled and *D. siricidicola* Kamona was injected into the caged portion. When *S. noctilio* emerged, some were parasitized by *D. siricidicola* that appeared to be hybrids between Kamona and INA (Bittner et al. 2019) (image by I.A.L. Caetano).

within the same *S. noctilio* individuals by both strains of *D. siricidicola* (INA and Kamona); in these cases, both nematode strains must have entered and developed within the woodwasp larvae in the sapwood. Moreover, experiments using *S. noctilio* challenged with both INA and Kamona resulted in hybridization between the nematode strains (Bittner et al. 2019). This would have occurred due to mating between parasitic forms of the two nematode strains before an infective female nematode entered a *S. noctilio* larva. Although sample sizes were limited, in trees containing both Kamona and INA, we found *S. noctilio* eggs that were killed but were filled with nematodes that were identified as INA. Thus, sterilization had occurred, but it was associated with the nematode strain that had not been found to sterilize eggs previously, suggesting that hybridization between Kamona and INA could influence whether sterilization occurs.

Native *Deladenus proximus*

The dimorphic native North American nematode *D. proximus* reproduces within pines, where it parasitizes *S. nigricornis* throughout the eastern and southeastern US (Zieman et al. 2015; Hartshorn et al. 2016a). In the worldwide search for biological control candidates in the 1960s–1970s, *D. proximus* was rejected due to its reported obligate association with *A. chailletii*, a fungus previously thought to be the only fungal symbiont associated with *S. nigricornis*; this fungus was not known to be associated with *S. noctilio* (Bedding and Iede 2005). However, it has since been discovered that *S. nigricornis* can use *A. areolatum* (see Chapter 9); it has also been discovered that *D. proximus* will feed on strains of *A. areolatum* associated with *S. noctilio* and *S. nigricornis* (Morris et al. 2014). This nematode has been experimentally reared in North America, and it naturally parasitizes *S. noctilio* as well as *S. nigricornis* in field-collected pines (Morris et al. 2013), but further empirical studies of the impact of *D. proximus* on *S. noctilio* in the field in North America have not been conducted.

Female *S. nigricornis* infected by *D. proximus* are smaller and lay fewer eggs compared with healthy *S. nigricornis*. One study found variable levels of sterilization of *S. nigricornis* eggs by *D. proximus*, with sterilization ranging from 0–100%, although > 50% of females had 80–100% sterilization (Fisher et al. 2017) (Fig. 4). Another study reported averages of 78–85% sterilization by *D. proximus*, with some females being fully sterilized (Hartshorn et al. 2016b). Higher percent sterilization was inverse to the body size and number of eggs carried by females.

Deladenus proximus seems promising as a native biological control agent with potential for use against *S. noctilio*. Further studies are needed that challenge *S. noctilio* with *D. proximus*. It is unknown to what extent



Figure 4. (a) Eggs of *Sirex nigricornis* (approximately 1 mm long), and (b) sterilized egg of *S. nigricornis* containing *Deladenus proximus* (images by D.C. Steinkraus in Fisher et al. [2017]; reprinted with permission).

D. proximus would naturally enter and infest *S. noctilio* populations (this has been reported only rarely in the northeastern US [Morris et al. 2013] where, as *S. noctilio* has become established, *S. nigricornis* co-exists with it [Hajek et al. 2017]). However, there is also definitely the potential to mass produce and release this nematode using methodology developed for *D. siricidicola*, although the outcome of competition between *D. proximus* and *D. siricidicola* INA (that we assume will accompany *S. noctilio* as it spreads) remains to be seen.

Summary

The introduced strain (INA) of *D. siricidicola* that does not sterilize *S. noctilio* in North America is established throughout the *S. noctilio* distribution in North America and will spread with *S. noctilio*. It is assumed that INA will compete with, co-parasitize, and potentially hybridize with *D. siricidicola* Kamona. Both Kamona and the INA *D. siricidicola* are known to parasitize the native *S. nigricornis*, and the INA strain will also parasitize the *Sirex*-associated beetle *Serropalpus substriatus*. *Deladenus siricidicola* is not native to North America, but *D. proximus* is, and *D. proximus* has been found parasitizing *S. noctilio*. Therefore, at present, it makes most sense to focus further studies on *D. proximus*. Moving ahead, researchers should keep in mind that in Australia and South America (see Chapter 6, Fig. 6), it has taken at least several years after introductions of the nematode *D. siricidicola* Kamona for parasitism and sterilization to increase to high levels.

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**CHAPTER
8****Natural enemies: The parasitoid complex attacking
Sirex noctilio in North America****Christopher J. Foelker¹ and Dylan Parry²**¹ Wisconsin Department of Agriculture, Trade and Consumer Protection, Madison, Wisconsin 53718 USA² College of Environmental Science and Forestry, State University of New York, Syracuse, New York 13210 USA**Abstract**

As *Sirex noctilio* has expanded its invasive range in North America, it has encountered a diverse assemblage of native hymenopteran parasitoids. The most common is *Ibalia leucospoides ensiger* (Ibaliidae), an egg/early instar endoparasitoid that has a similar flight period as *S. noctilio*. In addition, a suite of rhyssine ectoparasitoids (Ichneumonidae: genera *Rhyssa* and *Megarhyssa*) attack late instar siricid larvae and emerge the following year. Another species, *Pseudorhyssa nigricornis* (Ichneumonidae: Poemeniinae), is a cleptoparasitoid, attacking early instar rhyssines and then consuming the siricid host. These species collectively parasitize 15–25% of the *S. noctilio* population, although this can vary considerably by year and location. There has been limited research on their life history and impact in North America, although several of these species have been well-studied in the Southern Hemisphere where they were released for biological control of *S. noctilio*. Their complex phenology and subcortical lifecycles have hampered our understanding of their roles in attenuating the *S. noctilio* invasion in North America.

Introduction

Parasitoids are a ubiquitous component of mortality in woodboring insects, although their relative importance varies widely across species. In its native range of Eurasia and North Africa, *Sirex noctilio* has a suite of hymenopteran parasitoids that attack during the egg, larval, and pupal life stages (Spradbery and Kirk 1978). After its introduction to the Southern Hemisphere, there were sustained efforts to establish hymenopteran parasitoids from both Europe and North America as classical biological control agents (i.e., non-native natural enemies introduced for permanent establishment and control of a target species). These introductions varied in establishment success and efficacy, and subsequent biocontrol efforts shifted to the parasitic nematode (see Chapter 7). Most invaded areas of the Southern Hemisphere have two or more established parasitoid species, and their presence has become a component of overall *S. noctilio* management in this region (Cameron 2012).

Unlike in the Southern Hemisphere, when *S. noctilio* invaded North America it encountered a diverse community of native hymenopteran parasitoids associated with native siricids (Long et al. 2009; Eager et al. 2011; Ryan et al. 2012; Foelker et al. 2016). These parasitoids readily adopted *S. noctilio* as a host, although they did not share a coevolutionary history with *S. noctilio*. Unfortunately, prior to the advent of *S. noctilio* as an invasive species in North America, relatively little research had been conducted on these parasitoids, and an understanding of their biology was cursory. There is still limited knowledge of their host range and fidelity, life history, and voltinism. Complicating understanding further, *S. noctilio* itself varies in voltinism with a majority of a given cohort having a one-year generation time (Fig. 1), and a small percent (often 1–10%) having a two- or three-year generation time (Ryan et al. 2012; Foelker et al. 2016; Hajek et al. 2017). Additionally, voltinism of these parasitoids can be variable and is not well understood; the majority are univoltine (95%), but a small percent have at least a two-year life cycle (Foelker et al. 2016). Trees can also be co-colonized by the native *Sirex nigricornis*, itself one of many potential native siricid hosts for the same suite of generalist (yet siricid-specific) parasitoids, making accurate host-parasitoid linkages challenging to determine. Definitive host-parasitoid linkages have been established between *S. noctilio* and native parasitoids through molecular methods; however, this is a laborious and costly process (Foelker et al. 2015). Identification of these siricids and parasitoids is an additional complexity. Based on morphology, none are readily identifiable to species in their immature form, and distinguishing traits can be variable or inconsistent even in adults (Schiff et al. 2012; Standley et al. 2012). Thus, quantifying parasitism in the field (or from field collections) is challenging because within the same tree there can be multiple cohorts of different siricids and different parasitoids, neither of which have species-level identifying characteristics as larvae, and both of which can have variable voltinism.

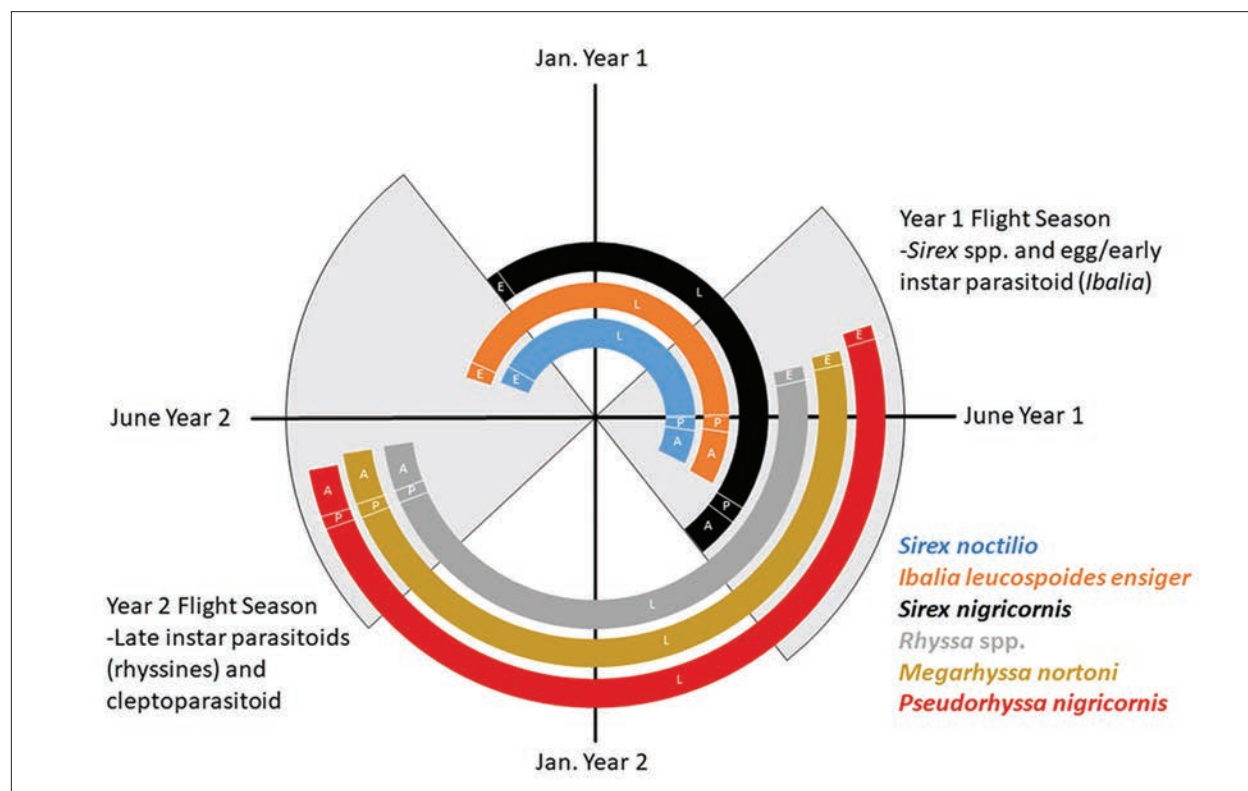


Figure 1. Life cycle of a single cohort of siricids and associated parasitoids in northeastern North America, assuming a one-year generation time for all species. Note the offset between the *Sirex* spp. and rhyssine parasitoids, with adult rhyssines emerging the year after the siricid cohort being parasitized. Letters indicate insect life stage: Egg (E), Larval (L), Pupal (P), Adult (A) (figure by C.J. Foelker).

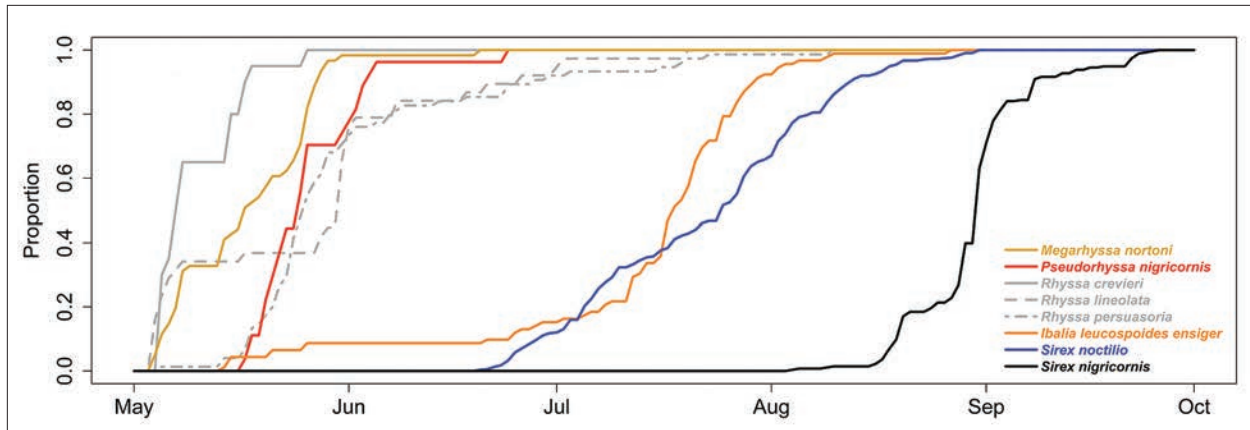


Figure 2. Cumulative emergence phenology for *Sirex* spp. and parasitoids in upstate New York. *Sirex* spp. and parasitoids are pooled across pine host species, site, and sex. Emergents were reared from *Pinus resinosa* (n=15 trees) and *P. sylvestris* (n=15) collected at two field sites in 2010 (figure from Foelker et al. [2016]; reprinted with permission).

The parasitoid complex

Ibalia leucospoides ensiger (Hymenoptera: Ibalidae)

This solitary species is a koinobiont endoparasitoid, meaning a single parasitoid feeds internally within a living and developing host. The flight period largely overlaps with that of *S. noctilio* in North America, with adults emerging and mating in July–August (Fig. 2). Female *I. l. ensiger* use the same drill shaft as *S. noctilio* and deposit a single egg within or very near the egg or an early instar larva of *S. noctilio* (Fig. 3). Female *I. l. ensiger* locate suitable oviposition sites through chemical cues derived from the fungal symbiont of *S. noctilio*, *Amylostereum areolatum* (Madden 1968; see Chapter 3). *Ibalia l. ensiger* is actually an endoparasitoid only until its third instar, at which point it ruptures and kills the developing siricid host and begins feeding externally on the siricid cadaver until pupation (Fig. 4). The lifecycle of *I. l. ensiger* is synchronous with *S. noctilio* (Fig. 1). When both *I. l. ensiger* and the siricid host are univoltine, adults of *I. l. ensiger* emerge during the same year and season as the siricid host cohort they have parasitized.



Figure 3. Adult female *Ibalia leucospoides ensiger* (image by C.J. Foelker).



Figure 4. *Ibalia leucospoides ensiger* larvae emerge from their siricid host during the third instar and begin feeding externally (image by C.J. Foelker).

In eastern North America, *I. l. ensiger* appears to be the most ubiquitous parasitoid attacking *S. noctilio* and has been recovered at most sites and in all studies conducted to date (Long et al. 2009; Eager et al. 2011; Ryan et al. 2012; Foelker et al. 2016). Levels of parasitism are less variable than with other parasitoid species, with 15–20% parasitism consistently reported, although up to 40% has been found. Parasitism by *I. l. ensiger* has tended to be greater in *S. noctilio* feeding in *P. sylvestris* than in either *P. resinosa* or *P. banksiana* and is also greater in the mid to upper portions of pines (Ryan et al. 2012; Foelker 2016; Foelker et al. 2016). This may be due to thinner bark on the upper bole of trees, particularly in *P. sylvestris*, which may enhance the access of *I. l. ensiger* females during oviposition. *Ibalia l. ensiger* is the smallest of the major *S. noctilio* parasitoids; it has a comparatively shorter ovipositor, which it coils within the abdomen when not in use.

Rhyssines (Hymenoptera: Ichneumonidae: Rhyssinae)

Rhyssa spp.

Rhyssa spp. as a group are solitary idiobiont ectoparasitoids, meaning a single larva feeds externally on a siricid host (Fig. 5) that has been paralyzed by the adult female (Fig. 6). Adult *Rhyssa* spp. emerge in May–June (Fig. 2), and females can be noticed when flying due to their long ovipositors. Female *Rhyssa* attack late instar siricids by boring through the bark and xylem tissue with their ovipositors (Fig. 6). Host location by adult females is accomplished by a combination of chemical sensory cues from *A. areolatum* and ovipositor probing to locate siricid galleries (Spradbery 1970). Adult females lay an individual egg and inject a toxic venom that paralyzes and eventually kills the siricid host. The generational life cycle of *Rhyssa* spp. is staggered from that of its host (Fig. 1). In the spring, *Rhyssa* adult females attack late instar siricids that have been developing since the previous year or years, depending on voltinism. Subsequently, adult siricids and rhyssines present in the same field season represent two different generations, which makes accurately quantifying parasitism challenging.

Several *Rhyssa* species use *S. noctilio* as a host in North America. These include *R. persuasoria*, *R. lineolata*, and *R. crevieri* (Table 1). All three *Rhyssa* species appear to be widespread throughout the invaded region of *S. noctilio* and generally across eastern North America. Among these three species, *R. persuasoria* and *R. lineolata* are most often recovered of all rhyssines, with *R. crevieri* being considerably less common (Long et al. 2009; Ryan et al. 2012; Standley et al. 2012; Foelker et al. 2016). *Rhyssa crevieri* is frequently noted as being smaller, and it emerges slightly earlier than the other two *Rhyssa* spp. (Fig. 2). Definitive adult morphological traits among these three *Rhyssa* species are subtle (see Standley et al. 2012 for key to rhyssines commonly associated with *S. noctilio*).



Figure 5. Late instar rhyssine larva (bottom) and siricid cadaver (top) (image by C.J. Foelker).



Figure 6. Adult female *Rhyssa persuasoria* drilling in a pine tree (image by C.J. Foelker).

Table 1. Major parasitoids attacking or associated with *Sirex noctilio* in northeast North America (adapted from Coyle and Gandhi 2012).

PARASITOID	POTENTIAL OR ACTUAL HOST	LIFE STAGE ATTACKING	SOLITARY/ GREGARIOUS	FEEDING HABIT
<i>Ibalia leucospoides ensiger</i>	<i>Sirex noctilio</i> , <i>Sirex nigricornis</i> , and other conifer-infesting siricids	Egg/ 1 st instar siricid	Solitary	Endoparasitoid until 3 rd instar, then ectoparasitoid
<i>Rhyssa persuasoria</i>	<i>Sirex noctilio</i> , <i>Sirex nigricornis</i> , and other conifer-infesting siricids	Late instar/pupa siricid	Solitary	Ectoparasitoid
<i>Rhyssa lineolata</i>	<i>Sirex noctilio</i> , <i>Sirex nigricornis</i> , and other conifer-infesting siricids	Late instar/pupa siricid	Solitary	Ectoparasitoid
<i>Rhyssa crevieri</i>	<i>Sirex noctilio</i> , <i>Sirex nigricornis</i> , and other conifer-infesting siricids	Late instar/pupa siricid	Solitary	Ectoparasitoid
<i>Megarhyssa nortoni</i>	<i>Sirex noctilio</i> , <i>Sirex nigricornis</i> , and other conifer-infesting siricids	Late instar/pupa siricid	Solitary	Ectoparasitoid
<i>Pseudorhyssa nigricornis</i>	Rhyssines (<i>Rhyssa</i> and <i>Megarhyssa</i> genera) attacking <i>Sirex noctilio</i> , <i>Sirex nigricornis</i> , and other conifer-infesting siricids	1 st instar rhyssine → Late instar/pupa siricid	Solitary	Ectoparasitoid

Megarhyssa nortoni

The biology of this species is very similar to *Rhyssa* spp. It is a solitary ectoparasitoid idiobiont attacking late instar siricid larvae. Adults of *M. nortoni* are distinct and striking due to their large size and yellow, red, and black coloration (see key in Pook et al. 2016). Adult females have long, noticeable ovipositors, reaching 7 cm (2.8 in) in length (Fig. 7).

Their phenology in North America is also similar to *Rhyssa* spp. (Fig. 2). Adults emerge May–June and attack developing larvae within the tree by drilling through the bark and xylem tissue to pierce the siricid host. An advantage that *M. nortoni* has over other parasitoids is that its exceptionally long ovipositor can penetrate deep into the tree bole, likely allowing it access to a greater proportion of larvae than *Rhyssa* spp. Host range with *M. nortoni* has not been well investigated, but it appears to parasitize numerous siricids that colonize conifers (Coyle and Gandhi 2012). Parasitism by *M. nortoni* was commonly low (0–5%) in studies of natural enemies of *S. noctilio* (Long et al. 2009; Ryan et al. 2012; Standley et al. 2012; Foelker et al. 2016), perhaps because of its broader host range.



Figure 7. Adult female *Megarhyssa nortoni* (image by Vicky Klasmer, Bugwood.org).

***Pseudorhyssa nigricornis* (Hymenoptera: Ichneumonidae: Poemeniinae)**

This species has a fascinating biology as it is a cleptoparasitoid, “stealing” or usurping the host of a primary parasitoid. *Pseudorhyssa nigricornis* emerges synchronously with the rhyssines it cleptoparasitizes, from May–June in northeastern North America (Fig. 2). Adult female *P. nigricornis* seek out drill shafts created by rhyssines and use these to deposit an egg near the unhatched or early instar rhyssine larva (Fig. 8). First instar *P. nigricornis* have oversized mandibles which they use to kill and consume the smaller rhyssine larva, as well as the siricid host paralyzed by the rhyssine adult female. Similar to rhyssines, *P. nigricornis* adults emerge the following year in early summer (Fig. 1).

Relatively few studies have detected *P. nigricornis* in northeastern North America (Standley et al. 2012; Foelker et al. 2016). It appears to be uncommon and is not consistently recovered among sites, even when sampled in the same year and in close proximity. When it was found, it had cleptoparasitized 20–30% of the rhyssine population at a site. Though it is believed to be able to attack both *Rhyssa* spp. and *Megarhyssa* spp., most studies in North America and elsewhere have found it closely associated with *Rhyssa* spp. The much longer oviposition drill shaft of *M. nortoni* may act as an impediment to successful egg laying by *P. nigricornis* as it would be unable to place its progeny in close proximity to the host larva.



Figure 8. Adult female *Pseudorhyssa nigricornis* using a rhyssine drill shaft to reach an egg/early instar rhyssine and paralyzed siricid host (image by C.J. Foelker).

Summary

Several existing native parasitoids in North America have readily adopted *S. noctilio* as a host, but these do not appear to be a major regulator of its population dynamics (see Chapter 10). Two commonalities across all of these parasitoids are i) a fairly wide host range, attacking multiple siricid species in a variety of coniferous host species and ii) a solitary life history, meaning each siricid host produces only a single parasitoid (Table 1). This fairly wide host range and limited reproductive capacity may dampen their ability to play a significant role in siricid population dynamics. Other relevant biological attributes, such as parasitoid foraging time and dispersal ability, have been identified as potential limiting factors on *S. noctilio* biocontrol in the Southern Hemisphere (Fischbein and Corley 2015). Collectively, parasitism of *S. noctilio* by the suite of associated Hymenoptera averages 15–25% in North America. In comparison, parasitism of *S. noctilio* in the Southern Hemisphere is generally higher, although this varies considerably among regions and with population densities of *S. noctilio*. A recurrent theme in North American studies is very high temporal and spatial variability in parasitism, a pattern not easily explained with our existing knowledge of these species and their interactions. High spatial heterogeneity, and the fragmented nature of suitable habitat in eastern North America for *S. noctilio* and alternate siricid hosts alike, may drive some of the variability in the response of the shared suite of parasitoids—a hypothesis deserving of further investigation.

Our knowledge of hymenopteran parasitoids of siricids lacks balance. On one hand, there is a wealth of research on parasitoid ecology, biology, and population dynamics derived from decades of classical biological control efforts for *S. noctilio* in the Southern Hemisphere. However, the applicability of this information to *S. noctilio* in North America remains to be seen. Ecologically, relative to the Southern Hemisphere, invaded

areas of North America comprise fundamental differences in the structure, composition, and complexity of forests, forest management practices, climates, and the diversity of competitors and natural enemies. Before *S. noctilio*'s invasion in North America, little was known about native siricids or their parasitoids, largely because these siricids were not considered pests.

In northeastern North America, *S. noctilio* is now the most abundant siricid colonizing pines and has almost undoubtedly impacted native insect communities to some degree, with both positive and negative direct and indirect effects possible. For example, a higher population density of *S. noctilio* relative to native siricids may inflate the abundance of shared parasitoids, resulting in a reduction of the less common native siricid species. This type of interaction, often called 'hyperpredation', is well-documented in a number of systems where a shared natural enemy is supported at high population densities by an abundant invader (Holt 1977). Alternatively, *S. noctilio* may increase the abundance of host trees for native species by killing or weakening healthy trees that otherwise would be unavailable to native siricid species that rely on trees that are dying or are recently dead. While speculative, these topics might be fruitful avenues of future research.

Fundamental and complex questions exist relative to parasitoids attacking *S. noctilio* in North America. The taxonomy and host fidelity (of both siricids and parasitoids) have only recently received attention (Coyle and Gandhi 2012; Schiff et al. 2012; Standley et al. 2012). There are even inconsistencies with the basic life cycle of insects in this system, as many studies using field-infested materials found rhyssines, *Sirex* spp., and *I. l. ensiger* could emerge from the same tree in the same season, which is at odds with the generally accepted staggered emergence of *Sirex* and rhyssines (Fig. 1). Potential explanations may be one or both of: i) trees being successfully colonized over multiple years by *Sirex* spp. and parasitoids, ii) variable voltinism of *Sirex* spp. and parasitoids. There is suggestive evidence from European studies (Spradbery and Kirk 1978) and North American insect collections that rhyssines may have two generations per year—an interesting facet that warrants further investigation.

The cryptic nature and morphologically similar larval stages of siricids and parasitoids makes research on their population dynamics challenging to execute in a field setting. Manipulative studies that might provide insight on population-level processes are prohibitively difficult for subcortical-feeding insects at relevant spatial and temporal scales. However, a better understanding of *S. noctilio* and the dynamics of its parasitoid complex will require going beyond the observational and survey study designs that have been used to date. Investigating these relationships and interactions will provide useful insights into how *S. noctilio* has affected forest insect communities in the northeast and what may be expected when it invades other regions of North America.

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**CHAPTER
9**

Native *Sirex nigricornis* and potential for interactions with *S. noctilio*

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Abstract

Sirex nigricornis is the only native woodwasp in eastern North America that uses pine as its exclusive host, and it overlaps with *Sirex noctilio* in the northeastern US. Therefore, it is also likely to significantly overlap and interact with *S. noctilio* when *S. noctilio* reaches the southeastern US. *Sirex nigricornis* shares many characteristics with *S. noctilio* in that it varies greatly in size, fecundity, and abundance. However, unlike *S. noctilio*, *S. nigricornis* is unable to successfully attack healthy trees, making future interactions between the two species uncertain and difficult to predict. *Sirex nigricornis*, and its mutualistic fungal symbiont, *Amylostereum*, also interact with a complex of subcortical insects including competitors, facultative predators, and parasitoids, as well as parasitic nematodes. A study conducted in the northeastern US hypothesized that *S. nigricornis* populations in the Southeast could decline following the introduction of *S. noctilio*, although these predictions were made based on limited observational data. Currently, many questions exist regarding how *S. noctilio* will affect *S. nigricornis* in southeastern pine stands. In this chapter, I examine the biology and ecology of *S. nigricornis* and attempt to address these questions.

Species description and natural history

Fourteen *Sirex* species have been recorded in the Western Hemisphere, and several native Siricidae exist in eastern North America (Schiff et al. 2012; Hartshorn et al. 2015; Zieman et al. 2015; see Chapter 1, Table 1). The range of *S. nigricornis* covers the eastern US and from Quebec to Alberta in Canada (Fig. 1), overlapping with other native species, such as *S. cyaneus* and *S. nitidus*. While these other *Sirex* species commonly infest fir (*Abies*) and spruce (*Picea*), respectively, *S. nigricornis* is the only native *Sirex* in the eastern US that develops almost exclusively in pine (*Pinus*) (with incidental collections from *Picea*). The abundance of *S. nigricornis* varies greatly by region, in part due to the regional distribution and abundance

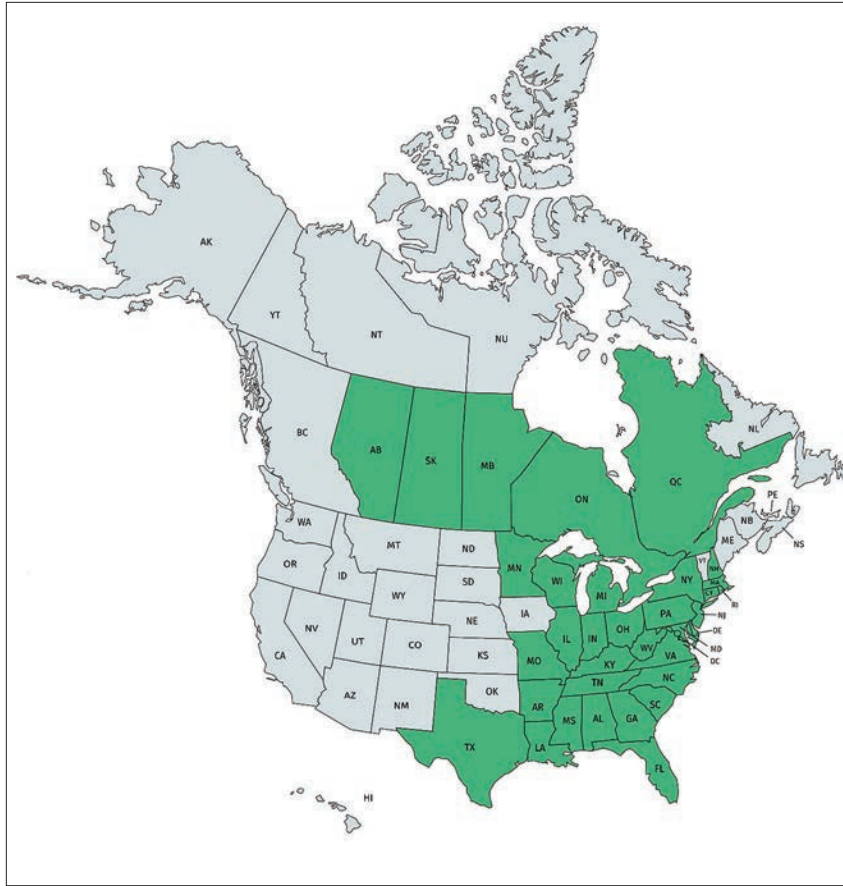


Figure 1. Range of *Sirex nigricornis* in North America. Shaded states and provinces are those with confirmed *S. nigricornis* collections (Schiff et al. 2012; Hartshorn et al. 2015; Zieman et al. 2015).

of suitable host pines. *Sirex nigricornis* was formerly considered two separate species, *S. nigricornis* and *S. edwardsii*. However, through the use of genetic analyses, we now know that these two are simply two color forms of female *S. nigricornis* (Schiff et al. 2012) (Fig. 2).

Several western species, such as *S. areolatus*, *S. longicauda*, and *S. varipes*, have been collected incidentally in the eastern US, likely through the transportation of pine, fir, and spruce lumber (Schiff et al. 2012). *Sirex areolatus* and *S. varipes* have also been detected at ports outside the US, emphasizing the importance of controlling international transport in order to limit the introduction and spread of non-native species, especially cryptic species like *Sirex* that can be easily missed during inspections (see Chapter 1). However, these species are likely not established in eastern North America or abroad (Schiff et al. 2012). Other non-native siricid species, such as *Eriotremex formosanus*, are established in the eastern US and regularly encountered (e.g., Johnson et al. 2013), but they are not currently causing ecological or economic damage to North American forests.

Adult *S. nigricornis* vary greatly in size, with females typically larger than males and female size positively correlating with potential fecundity; that is, larger females contain more eggs (Hartshorn et al. 2016a). The head and thorax are typically dark blue to black, and their abdomen can range from a pale orange color to black (Fig. 2). Both fore- and hind-wings are darkly tinted. Unlike other Hymenoptera (i.e., ants, bees, wasps, and parasitic wasps), adult siricids do not have constricted “waists,” and are generally robust and cylindrical in shape. Siricid larvae look nearly identical across species, are cylindrical and pale in color, and can vary greatly in size during development. The identifying characteristic for siricid adults is the dark “horntail” at the tip of their abdomen, which also gives them the common name of “horntails” (Schiff et al. 2012) (Fig. 2).

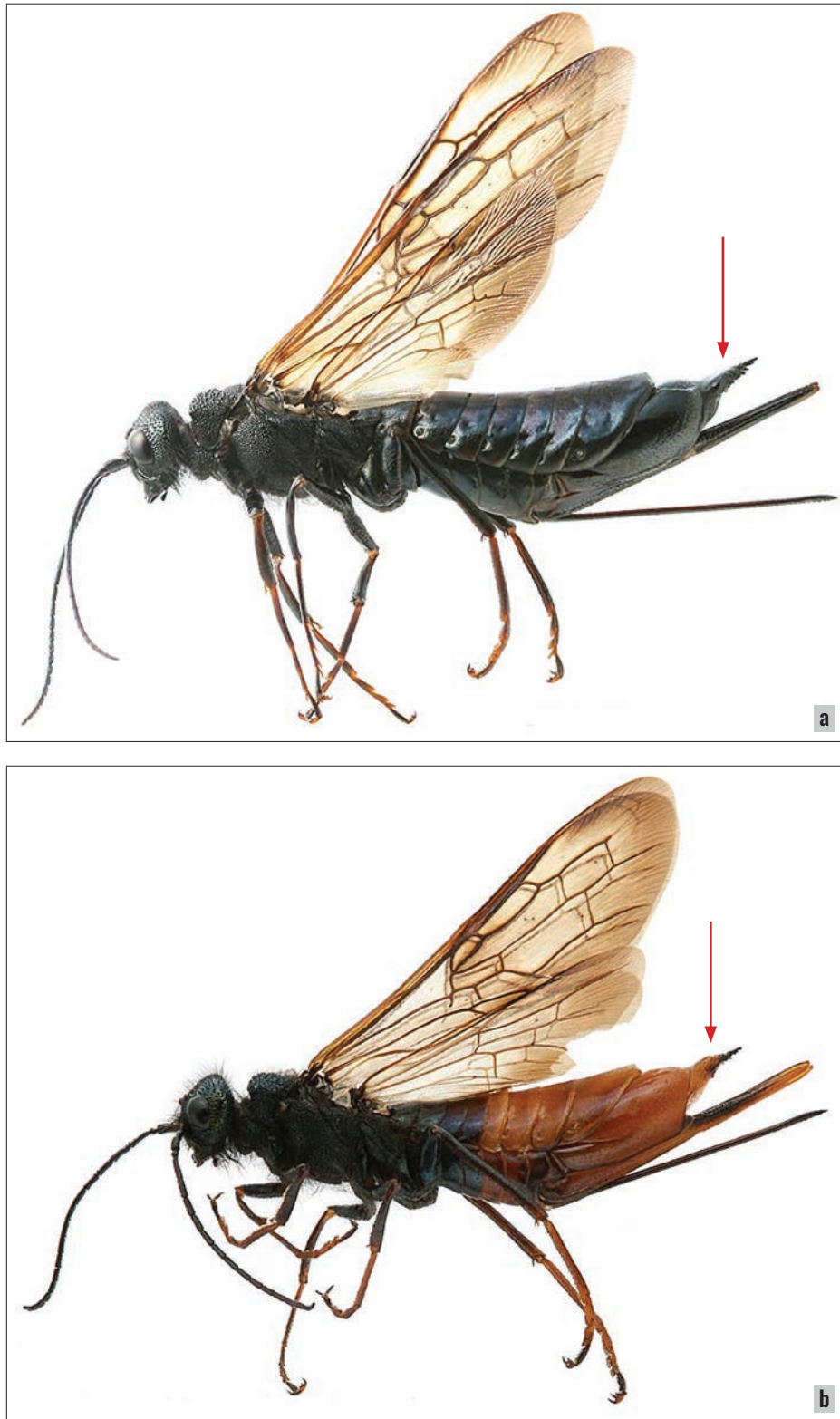


Figure 2. Color variation of female *Sirex nigricornis*. Under previous species descriptions, the dark color form (a) was identified as *S. edwardsii* and the pale color form (b) was identified as *S. nigricornis*. The “horntail” is identified with a red arrow (images from Schiff et al. [2012]; reprinted with permission).

Following the initial collection of *S. noctilio* in Fulton, New York (Oswego County) in 2004, the USDA Forest Service funded work on the fundamental biology and ecology of native North American Siricidae in order to form a baseline before *S. noctilio* could significantly impact the environment. *Sirex nigricornis*, in particular, was studied due to its abundance as well as its overlap in host and geographic range with *S. noctilio* in the eastern US. Therefore, the biology and potential interactions discussed in this chapter will refer to *S. nigricornis* (e.g., Johnson et al. 2013; Barnes et al. 2014; Chase et al. 2014) and not to other native *Sirex* species. Additionally, the different host tree ranges, and therefore life histories, of other native *Sirex* suggest that interactions will principally be between *S. noctilio* and *S. nigricornis*.

***S. nigricornis* life history, biology, and chemical ecology**

Fecundity of *S. nigricornis* varies greatly, and females may contain anywhere from a few eggs to more than 600 eggs (Kroll et al. 2013; Hartshorn et al. 2016a), which they oviposit by drilling through the bark and into the xylem of pines. As with *S. noctilio*, female *S. nigricornis* have mycangia at the base of their ovipositor that are filled with arthrospores of the symbiotic white rot fungus *Amylostereum*. Much of what we know about *S. nigricornis* oviposition behavior and development is based on their assumed similarity to *S. noctilio*. For example, when ovipositing, we suspect that the first drill made by a female may be used to determine if the tree is acceptable for oviposition and, if it is not, the female primes the tree by inserting fungal spores and a phytotoxic venom that will reduce tree defenses. If, instead, the female chooses the site for oviposition, the first drill contains the first egg laid by the female. Females then partially remove their ovipositor and re-insert it into the xylem, laying eggs singly or doubly in additional drills, with an apparent maximum of five drills as six drills per oviposition event have not been recorded (Hartshorn et al. 2016b) (see Chapter 2, Fig. 6). The final drill contains only fungal spores and phytotoxic venom. Like egg abundance, venom gland weight is positively correlated with female size, with larger females containing larger venom glands (Hajek et al. 2017). However, *S. nigricornis* females contain venom glands nearly half the size of those in *S. noctilio* females, possibly helping to explain the inability of *S. nigricornis* to infest healthy trees (Hajek et al. 2017).

Eggs hatch in approximately 10–14 days, and larvae begin tunneling into the wood, creating long, winding galleries in the xylem. *Sirex noctilio* larvae do not feed directly on *Amylostereum*, rather the fungal symbiont degrades wood just in front of developing larvae, which allows the larvae to squeeze the decayed wood using specialized mandibles and then imbibe the resulting nutrients (Thompson et al. 2014; see Chapter 5). *Sirex noctilio* also have bacterial gut symbionts with activities hypothesized as complementary to the symbiotic fungus (Thompson et al. 2014; see Chapters 4 and 5). Similar processes have also been identified for several bark and woodboring beetles (e.g., Delalibera et al. 2007; Morales-Jiménez et al. 2013) and, considering the poor nutritional quality of dead wood and the close relationship between *S. nigricornis* and *Amylostereum*, it is likely that this mode for feeding occurs in this native woodwasp larvae as well.

Sirex nigricornis has been found to be associated with either of two species of *Amylostereum*: *A. chailletii* or *A. areolatum* (Nielsen et al. 2009; Hajek et al. 2013) (Chapter 4, see Table 1). This is in contrast to *S. noctilio* that almost always carries only *A. areolatum*. Most native northeastern siricids are historically known for their association only with *A. chailletii* (Gilbertson 1984), and for many years it was thought that each coniferous siricid species was associated with only one species of *Amylostereum*. The biological significance of this flexibility in association with fungal symbionts for *S. nigricornis* is not known, but certainly this flexibility would assist *S. nigricornis* in the use of an ephemeral resource (dying trees). In the Northeast, many *S. nigricornis* adults emerge after *S. noctilio* (e.g., Ryan et al. 2012), and it has been common for *S. nigricornis* to emerge from the same trees as *S. noctilio* (but in much lower numbers). In some cases, *S. nigricornis* then carried the same invasive *Amylostereum areolatum* strain as *S. noctilio* (Nielsen et al. 2009); it is assumed that in these cases, *S. nigricornis* acquired this symbiont via close association with *S. noctilio*.

when developing within the same trees. So, the ability of *S. nigricornis* to use both fungi suggests that the presence of *A. areolatum* already established within trees infested by *S. noctilio* would not deter *S. nigricornis* from using the same trees.

After developing for at least a year, larvae then move toward the outer bark to pupate. Adults wait under the outer bark, emerging only when temperatures begin to decline in the late summer and fall (Hartshorn et al. 2015, 2016c). Emergence of *S. nigricornis* then continues throughout the fall and winter across the southeastern US (Johnson et al. 2013; Chase et al. 2014; Hartshorn et al. 2015). While a small proportion of *S. noctilio*, and up to half of *S. nigricornis*, have been recorded as having a two-year life cycle in northeastern North America (Ryan 2011; Hajek et al. 2017), a one-year life cycle is much more common for *S. nigricornis* in the southeastern US (Hartshorn et al. 2015). Emerged females can lay eggs whether mated or unmated; unmated females produce only male offspring, and mated females produce either males or females. Adult females are not alive for very long, sometimes dying during the process of oviposition while still attached to the tree (J.A. Hartshorn personal observation).

Like *S. noctilio*, only female *S. nigricornis* are attracted to host volatiles, namely α - and β -pinene as well as ethanol (Coyle et al. 2012). These compounds indicate the presence of a stressed tree (see Chapter 3) in which females may oviposit. Trapping, therefore, focuses on capturing females, and it can be done using a wide array of techniques, such as Lindgren funnel traps, intercept panel traps, and trap trees (Johnson et al. 2013; Barnes et al. 2014). The type of trap is not important in capturing either *S. nigricornis* or *S. noctilio* (Haavik et al. 2014), although *S. nigricornis* are most attracted to traps containing freshly cut pine material as a lure (Barnes et al. 2014). When females are collected through the use of trap trees, the trees are weakened with herbicide (e.g., Dicamba) or girdled with a chainsaw (Johnson et al. 2013). One particularly useful technique for attracting adult female *S. nigricornis* is to cut trees into 1-m (3.2-ft) logs and stack them in a “Lincoln log” style, creating a concentrated plume of volatiles (J. Meeker personal communication) (Fig. 3).



Figure 2. *Pinus taeda* tree cut into several 1-m (3.2-ft) sections and stacked in a “Lincoln log” style designed to release volatiles that attract female *Sirex nigricornis* that have been used for rearing studies (image by J.A. Hartshorn).

Evidence suggests that the fungal symbiont, *Amylostereum*, also releases volatiles that attract *S. noctilio*, although these volatiles also attract associated parasitoids, namely *Ibalia leucospoides* (Bryant 2010). While these experiments have been done solely with *S. noctilio* (Sarvary et al. 2016), the attraction of the fungus to native parasitoids, as well as the association between *Amylostereum* and *S. nigricornis*, suggests that fungal volatiles may also be attractive to *S. nigricornis*. However, additional research in this area is warranted.

***S. nigricornis* interactions with native North American insects and fungi**

Phenology and competition

One key factor to the success of any invasive species is its potential to outcompete native species for an ephemeral resource (e.g., space within suitable hosts for wood borers). For woodboring organisms like *S. nigricornis*, this potential lies with each species' ability to gain access to an acceptable host before that host is colonized by another wood borer, and this is largely dependent on each species' phenology. *Sirex nigricornis* inhabits the same hosts as bark and ambrosia beetles, as well as longhorned beetles and other associates (Ryan 2011; Lynn-Miller 2012), and these interactions may be very important in the establishment and impacts of *S. noctilio* throughout pine-growing regions in the US.

While *Sirex* larvae are buffered from environmental conditions by developing inside host trees, female emergence of both *S. noctilio* and *S. nigricornis* across the entire eastern US appears to coincide with the onset of lower minimum temperatures (Hartshorn et al. 2016c). In Canada, where both *Sirex* species are present, *S. noctilio* consistently emerges up to three months earlier than *S. nigricornis* (Haavik et al. 2013; Hartshorn et al. 2016c). With this temporal partitioning, *S. noctilio* often finds stressed pines first, colonizing these trees and therefore reducing available resources prior to *S. nigricornis* emergence. Conversely, *S. noctilio* may decrease defenses of healthy or slightly weakened trees and introduce appropriate *S. nigricornis* fungal symbionts, actually increasing the availability of resources for *S. nigricornis*. Observed trends are that *S. noctilio* more frequently inhabits pines before *S. nigricornis*, outcompeting the native for this ephemeral resource. These impacts increase with time since initial *S. noctilio* establishment (Hajek et al. 2017). Additionally, bark and ambrosia beetles emerge throughout the growing season and can potentially inhabit suitable hosts prior to both *S. noctilio* and *S. nigricornis*, possibly altering the interactions among these two species. Relating to *S. noctilio*, the presence of bark beetles within trees colonized by *S. noctilio* affects the population density, size, and fecundity of *S. noctilio* as well as their associated parasites (Yousuf et al. 2014).

Competitors

The potential for competition between *Amylostereum* and *Ophiostoma*, as well as other common tree-infesting decay fungi, is significant and under-studied. *Sirex noctilio* larvae are unable to develop without *Amylostereum* (and we assume this is also true of *S. nigricornis*), and *Amylostereum areolatum* is unable to move to a new host tree without *Sirex* (see Chapter 4). While this fungal/siricid mutualism was initially thought to be species-specific, based on the frequency with which *S. nigricornis* females carry *Amylostereum* species, we assume that *S. nigricornis* larvae can develop using either of two *Amylostereum* species present in eastern North America (see previous sections and Chapter 4).

We can predict some fungal interactions among native wood borers, including *S. nigricornis* and *S. noctilio*, by examining other areas of *S. noctilio* introduction. For example, in Australia, where *S. noctilio* invaded more than a century ago, *Ips grandicollis*, a widespread and abundant bark beetle species native to the eastern US, has been more recently introduced along with its associated bluestain fungus (*Ophiostoma ips*) (Yousuf et al. 2014). This fungus has had a negative effect on *S.*

nigricornis development in the southeastern US (Hartshorn et al. 2020) and on *S. noctilio* biological control efforts in Australia by limiting the efficacy of *Deladenus siricidicola*, a parasitic nematode that also requires *Amylostereum* for development (Hurley et al. 2012; Yousuf et al. 2014; see Chapter 6). Fungal competition experiments have shown variable competitive ability among *Amylostereum* and *Ophiostoma*, as well as other native fungi such as *Diplodia*. In laboratory experiments, water potential and media played important roles in hyphal growth and competition of *Amylostereum areolatum*, *Ophiostoma ips*, and *Diplodia pinea* (Hurley et al. 2012), suggesting that environmental factors such as soil moisture will play a significant role in these interactions.

While current knowledge on fungal competition relies solely on studies done with *S. noctilio*, there is the potential for bark beetles to limit *S. nigricornis* populations as well because *S. nigricornis* also relies on *Amylostereum*, which may be outcompeted by bark beetle-associated fungi (Yousuf et al. 2014; Hartshorn et al. in press). Native bark beetles and their fungal associates frequently occur in the same trees as *S. nigricornis* (Lynn-Miller 2012). *Ips* bark beetles in the Southeast are limited substantially by host defenses of healthy trees, but they are capable of producing 6–10 generations every year when a consistent food source is present, typically infesting trees prior to *S. nigricornis* emergence (Coyle et al. 2016). For example, in Arkansas alone, more than 30 bark and woodboring beetle species across 11 families were collected in *S. nigricornis* traps; in laboratory experiments, female *S. nigricornis* limited their oviposition in logs colonized by these subcortical insects. In addition, *S. nigricornis* larval mortality was highest in these co-colonized logs (Lynn-Miller 2012).

The most aggressive bark beetle in the southeastern US, the southern pine beetle (SPB) *Dendroctonus frontalis*, requires live trees for successful development and has historically caused significant pine mortality through periodic outbreaks (Coulson and Klepzig 2011). These outbreaks frequently move faster than management, leaving thousands of hectares of dead and dying pines that are susceptible to an array of bark and woodboring insects, including *S. nigricornis*. Recently, potentially as a result of climate change, unprecedented outbreaks of SPB have been recorded as far north as New York and Massachusetts (previously not considered as being in the range of SPB). This now poses a significant threat to naïve pine forests in the northeastern US (Dodds et al. 2017; Lesk et al. 2017). These SPB outbreaks co-occurring in areas with current *S. noctilio* infestations may result in interactions with impacts to native insects like *Ips* bark beetles, *S. nigricornis*, and other competitors and natural enemies. Similar to SPB and other woodboring pests, *S. nigricornis* abundance is significantly related to competitive stand conditions and individual tree stress, namely high basal area, small diameter at breast height, and shorter tree height (Chase et al. 2014). This indicates that management to mitigate SPB outbreaks may also reduce *S. nigricornis* abundance.

Natural enemies

In North America, native *Sirex nigricornis* do not reach pest status for multiple reasons; they are not very aggressive insects and, as mentioned previously, their relatively small venom glands likely contribute to their inability to successfully attack healthy trees. In addition, there is a suite of native parasitoids, nematodes, and predators that prey on and parasitize *S. nigricornis*, and these natural enemies can have significant impacts on *S. nigricornis* populations (see Chapter 8). There are several native parasitoids in North America that attack *S. nigricornis* along with other woodwasps, including *S. noctilio* (Coyle et al. 2012). Associated parasitoids lay eggs on or in *S. nigricornis* eggs or larvae within the wood, and they feed either internally (endoparasitoids) or externally (ectoparasitoids) until they fully consume the larvae (Coyle et al. 2012). Generalist parasitoids, like those found among *S. nigricornis* and associated wood borers, emerge throughout the growing season and have many additional hosts, allowing them to remain in the environment and ready to parasitize *Sirex*. Additionally, increases in *S. noctilio* populations likely support higher parasitoid populations, which may then parasitize native *Sirex* at a higher rate.

The parasitic nematode *D. siricidicola* (Kamona strain), originating in Hungary, has been used for decades for biological control of *S. noctilio* in the Southern Hemisphere where it sterilizes females (see Chapter 6). However, the native North American nematode, *D. proximus*, is also capable of parasitizing larval *S. noctilio* (Morris et al. 2013). Both *D. proximus* and *D. siricidicola* (INA strain) have been recovered from both *S. noctilio* and *S. nigricornis* in North America (Morris et al. 2013); however, *D. siricidicola* (INA strain) is not regulating *S. noctilio* populations and reducing spread, mostly because it does not sterilize females (Yu et al. 2009; see Chapter 7). The impact of *D. proximus* on fitness and spread of *S. nigricornis* is also inconsistent (Zieman et al. 2015). In North America, *D. proximus* has significant effects on *S. nigricornis* in the form of smaller female size, lower egg load, and decreased egg viability (Hartshorn et al. 2016a). However, sterilization by native nematodes is highly variable; infection rates in individual females range from 0% (no infected eggs; only mycangia infected) to 100% (no viable eggs present) (Fisher et al. 2017), and it is difficult to predict how they will interact with *S. noctilio* upon its spread into the southeastern US. Due to the effects of native nematodes on size and fecundity of *S. nigricornis*, there is interest in investigating the use of southeastern-collected *D. proximus* as biological control agents of *S. noctilio* in the northeastern US (see Chapter 7).

Sirex nigricornis also co-exists alongside many other insects that inhabit dead and dying pine trees, such as longhorned beetles, flat-headed woodboring beetles, checkered beetles, and carpenter ants, many of which are exclusively or facultatively predatory. The effects of the native natural enemies on *S. nigricornis* have not been quantified, but we do know something about their impacts on other woodboring species. Some of these species have been speculated as mitigating or contributing to the collapse of outbreaks of native bark beetles (Schoeller et al. 2012; Clarke and Hartshorn 2020); however, the feeding habits of these common natural enemies and competitors keep them mostly in the cambium and phloem of trees, rather than moving throughout the xylem where *S. nigricornis* larvae occur (Dodds et al. 2001). Currently, these factors make it difficult, or nearly impossible, to predict their effects on a xylem-boring woodwasp.

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**CHAPTER
10****Biotic resistance to *Sirex noctilio*:
Tree resistance, natural enemies, and competitors****Christopher J. Foelker¹ and Laurel J. Haavik²**¹ Wisconsin Department of Agriculture, Trade and Consumer Protection, Madison, Wisconsin 53718 USA² USDA Forest Service, Forest Health Protection, Missoula, Montana 59804 USA**Abstract**

The limited impact *Sirex noctilio* has had on pine forests in northeastern North America is largely due to biotic resistance: the ability of a native community to keep populations and damage by an exotic invader in check. For *S. noctilio*, this has manifested in three ways: tree resistance, natural enemies, and competition from other pine-colonizing insects and their associated fungi. Tree resistance probably plays a dominant role for two important reasons: i) observational and manipulative life tables indicate that most mortality occurs during initial tree colonization, between the egg and first larval stages; and ii) there is correlative evidence that the availability of suitable pines at a site limits the *S. noctilio* population. Observational evidence that healthy trees generally have not been colonized by *S. noctilio* also supports this latter notion. Natural enemies and competitors appear to play a role as well because they were recovered wherever *S. noctilio* was found, and *S. noctilio* numbers were greater in their absence. The interaction between fungal associates of competing pine-colonizing insects and *S. noctilio* and its obligate fungal mutualist is also important, particularly during establishment and development. However, the identity of the most important agents, and the quantitative contributions of all three forms of biotic resistance, have been difficult to specify with consistency.

Introduction

The invasion of *Sirex noctilio* in northeastern North America is markedly different from its invasion of regions in the Southern Hemisphere. A key difference is the presence in North America of a large assemblage of native pine-colonizing insects and their natural enemies. Additionally, 66 native pine species exist (see Chapter 2) and appear both in natural and plantation settings, whereas in the Southern Hemisphere all pines are non-native and occur almost exclusively in plantations, often at extremely high densities. In totality, the ecological context and relationships encountered by *S. noctilio* in North America are analogous to conditions in the native Eurasian range of *S. noctilio*, where this insect is not a pest. Given the potential for the native

community to limit the impact of *S. noctilio* in North America, multiple studies were initiated after its initial detection to census co-colonizing insects, identify important mortality factors, and quantify whether biotic resistance was affecting *S. noctilio* population growth.

Tree resistance

Tree resistance appears to be the dominant biotic factor limiting *S. noctilio* in northeastern North America. Occurrence of *S. noctilio* has been almost exclusively limited to overstocked, unmanaged Scots (*Pinus sylvestris*) or red pine (*Pinus resinosa*) stands on poor growing sites. Even within unmanaged stands, *S. noctilio* is typically found colonizing suppressed, intermediate, or injured pines. *Sirex noctilio* prefers trees with low bark moisture and low osmotic pressure in the phloem (Madden 1974), and it alters conditions beneath the bark by injecting a toxic venom and its fungal symbiont prior to and while laying eggs (see Chapters 2, 3, and 4). When this is prior to oviposition, this primes (or conditions) the tree for later oviposition. One study in Ontario found that *S. noctilio* primes a substantial portion of poorly defended trees in this way more than once, sometimes in multiple successive years (i.e., the trees do not die after being primed in the first year), before they are apparently suitable for oviposition (Haavik et al. 2018). Several studies observed that healthy, co-dominant trees were rarely attacked, and even less often successfully colonized by *S. noctilio* (Dodds et al. 2010; Ayres et al. 2014; Haavik et al. 2016a; Haavik et al. 2018). Further, there is correlative evidence among several stands that *S. noctilio* attacked (primed) and colonized (successful oviposition and subsequent tree mortality) more trees when more stressed or sub-dominant pines were available (Haavik et al. 2016a). Similar observations were made during a *S. noctilio* outbreak in Australia, where suppressed trees were killed at the beginning of the outbreak when *S. noctilio* population density was increasing. Larger, co-dominant trees were attacked in one or more years prior to being successfully colonized and killed in later years of the outbreak, when *S. noctilio* population densities were greater (Madden 1975).

Sirex noctilio faces the greatest resistance from trees when in the egg/neonate stage. In manipulative and observational studies, Haavik et al. (2015) found substantial mortality (44–94%) during this early life stage, likely from induced and/or constitutive tree defenses. This is not unexpected, as tree defenses are a critical factor limiting insects colonizing live trees. Generally, pines have complex, well-adapted defense mechanisms against subcortical insect colonizers. Specifically, resin floods *S. noctilio* oviposition drill sites when they attack, and defensive polyphenols, some with antifungal properties, are quickly produced. These polyphenols are thought to restrict establishment and growth of larvae and the obligate fungal mutualist, *Amylostereum areolatum* (Coutts and Dolezal 1966; Hillis and Inoue 1968). All but the most unhealthy, stressed pines are likely well equipped to defend themselves from low *S. noctilio* population levels. Even in the Southern Hemisphere, where baseline *S. noctilio* populations are greater than in North America, population outbreaks are associated with acute drought stress that has compromised tree defenses (Madden 1988; Lantschner et al. 2019).

Pine host species appears to play a role in *S. noctilio* fitness, although the underlying mechanisms remain unclear. Most field studies concluded that *S. noctilio* has had the greatest impact in Scots pine stands (Dodds et al. 2010; Foelker et al. 2018), and larvae occur in greater densities and reach a larger larval size in Scots pine (Foelker 2016) (Fig. 1), which is a naturalized pine in North America and an ancestral host of *S. noctilio*. It is uncertain if this affinity for Scots pine is due to preference/recognition or general condition of Scots pine on the landscape, as this tree faces a multitude of predisposing stressors in northeastern North America, such as poor site quality and unmanaged plantation settings.

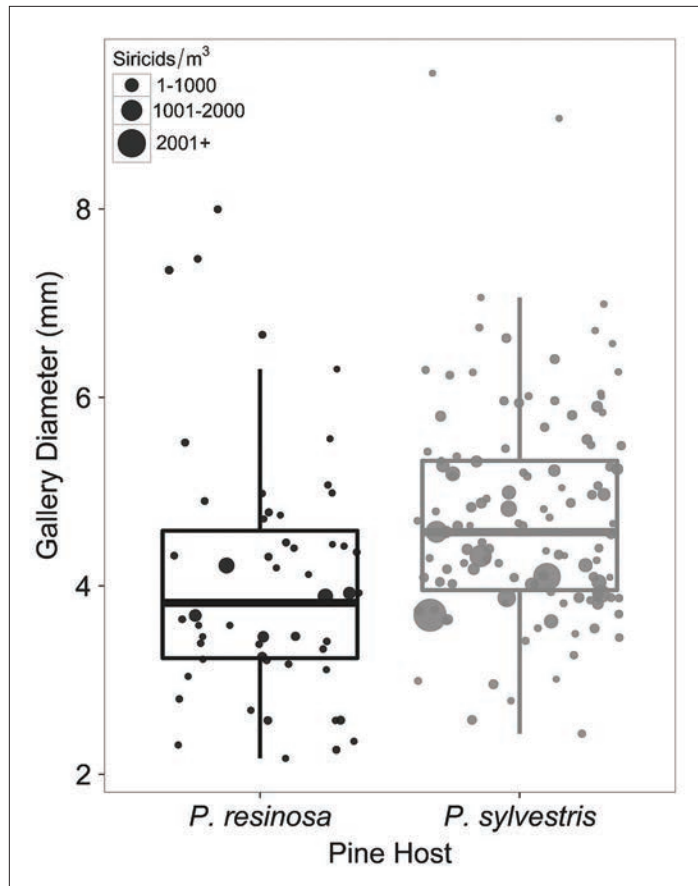


Figure 1. Effects of pine host species on gallery diameter (mm) (a surrogate for woodwasp size) and colonization density. Each dot represents an infested log with at least one larva, and circle size indicates larval density (figure from Foelker [2016]; reprinted with permission).

Natural enemies

There are two notable groups of hymenopteran parasitoids that attack *S. noctilio*: *Ibalia leucospoides ensiger*, an egg/early larval instar parasitoid, and species in the Rhyssinae (genera *Rhyssa* and *Megarhyssa*), late larval instar parasitoids (see Chapter 8). Parasitism by *I. l. ensiger* is typically 10–20%, and most studies have found this to be relatively consistent among sites and across multiple years (Long et al. 2009; Ryan et al. 2012; Zylstra and Mastro 2012; Foelker et al. 2016; Haavik et al. 2016a). *Ibalia l. ensiger* is common wherever *S. noctilio* has been found, and there is limited evidence that it exhibits a density-dependent response, but the response does not appear to be strong enough to be a major limiting factor for *S. noctilio* in the North America populations (Haavik et al. 2015; Thompson et al. 2019). The contribution of *I. l. ensiger* to biological control programs elsewhere in the Southern Hemisphere has been variable (Fischbein and Corley 2015). Possible reasons for this limited effect include a long handling or foraging time during oviposition and that it is a solitary parasitoid (i.e., only one parasitoid per siricid host instead of multiple) (Fischbein and Corley 2015).

Rhyssine parasitism is more variable and considerably less common than *I. l. ensiger* parasitism. Rhyssine parasitism is typically less than 10%, but can vary greatly among trees, sites, and years (Ryan et al. 2012; Zylstra and Mastro 2012; Foelker et al. 2016; Haavik et al. 2016a;). Parasitism by rhyssines has been difficult to properly measure, because of their phenology and the challenges of experimental design. Rhyssines search for hosts in late spring, when most studies had already removed *Sirex* spp.-infested trees from pine forests (see Chapter 8). There has been some evidence of rhyssines exhibiting a positive response

to *S. noctilio* density (Foelker 2016). *Rhyssa* spp. showed a delayed density-dependent response to *S. noctilio* in Australia prior to the introduction of parasitic nematodes (Taylor 1978), which suggests that they have the capacity to limit *S. noctilio* populations. Consequently, they should not be ignored as a potentially important mortality factor. There has been no evidence thus far that *I. l. ensiger* or rhyssines have regulated growing or spreading populations of *S. noctilio* in North America. Studies in the Southern Hemisphere have similarly found that hymenopteran parasitism alone does not provide population regulation, but it is a component of management strategies for *S. noctilio* (Hurley et al. 2007).

The parasitic nematode *Deladenus siricidicola*, likely introduced to North America at the same times as the woodwasps, has also been found in *S. noctilio* in North America (see Chapter 7). It has a complex life cycle with free-living and parasitic forms. The free-living form inhabits the xylem of pines, feeding on *A. areolatum*, the fungal mutualist of *Sirex* spp. The parasitic form infests the reproductive organs of both sexes of *Sirex* spp. The strains of *D. siricidicola* present in North America do not sterilize the female's eggs (see Chapter 7). The indirect effects of parasitism, such as smaller body size and decreased fecundity, limit adult dispersal distance and egg-laying capacity in females (Kroll et al. 2013; Haavik et al. 2016b). These effects could impact population growth of *S. noctilio* to some extent. There is a sterilizing strain of this nematode that was developed for use in Australia against the strain of *S. noctilio* introduced there. While it is currently being used as a highly effective biological control agent in Australia and South America, it has not been released in North America, and no plans are under way to do so (see Chapters 6 and 7).

Predation from woodpeckers has also been noted as a mortality factor, although there has been limited investigation as to its general importance. Woodpeckers have been observed foraging for siricid larvae, often in late winter/early spring. In a manipulative study that excluded natural enemies and competitors based on size and examined the resulting *S. noctilio* brood, woodpeckers were found not to have an effect when excluded (Haavik et al. 2020). Woodpeckers are unlikely to play a major role in regulating *S. noctilio*, and their impact is likely highly variable. Nonetheless, foraging activity by woodpeckers can be a useful indicator for locating siricid-infested trees during field surveys (Fig. 2).

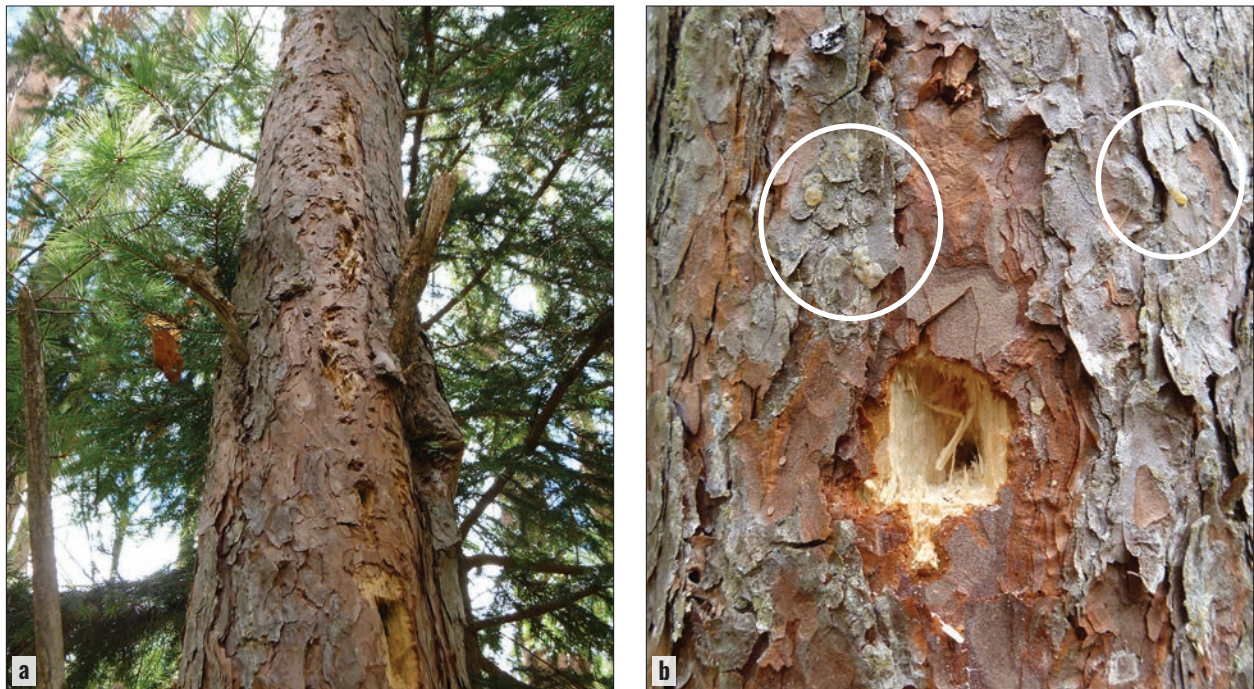


Figure 2. (a) Evidence of woodpecker foraging on siricid larvae along the main bole of a red pine in upstate New York. Note the resin beads on the bark (circled in white) around the (b) foraging site (images by C.J. Foelker).

Competitors

Sirex noctilio's interaction with native co-colonizers may be the most challenging to fully understand because throughout the growing season there is a diverse insect community attacking pines in variable health conditions. Furthermore, these interactions may be indirect and mediated through fungal associates.

Ips spp., pine engraver beetles, are the most prominent of many insects competing for resources that would be used by *S. noctilio*. Additionally, there is a diverse community of woodborers and other bark beetles attacking red and Scots pine in northeastern North America, including the non-native pine shoot beetle, *Tomicus piniperda*. The interactions with woodborers are least understood, although there is evidence that cerambycid (round-headed borer) larvae will consume (i.e., facultative predation) larvae of *Sirex* spp. if encountered in the wood (Thompson et al. 2019). Cerambycid larvae are also known to consume bark beetle larvae in the same context (Dodds et al. 2001). In a field study, Foelker et al. (2018) found co-occurrence of symptoms of *S. noctilio* attack (resin beading) and other pine-colonizing insects to be neutrally (bark beetles) or negatively associated (woodborers) with *S. noctilio*. In another study, pines co-colonized by *S. noctilio* and woodborers and bark beetles produced fewer *S. noctilio* than pines with no beetle co-colonizers (Ryan et al. 2012).

An important component to all these interactions is the role of fungal associates of pine co-colonizing insects, which can interact with developing *S. noctilio* larvae and/or its obligate fungal mutualist, *A. areolatum*. Bark beetles introduce bluestain fungal species (*Ophiostoma* spp.) that can outcompete *A. areolatum* and rapidly degrade wood tissue. Multiple studies have demonstrated that bark beetle bluestain fungal associates are superior competitors to *A. areolatum*; they have faster growth rates and reduce moisture content below an optimal level for *A. areolatum* colonization in laboratory growth assays (Ryan et al. 2011; Yousuf et al. 2014; Thompson et al. 2019). Bluestain fungal colonization also has been associated with smaller *S. noctilio* larval body size and lower survivorship (Foelker 2016). Female *S. noctilio* avoid depositing eggs in wood already colonized by bluestain fungi (Hajek et al. 2018).

Sirex noctilio also encounters a native pine-colonizing siricid in North America, *Sirex nigricornis*. There was limited baseline knowledge about *S. nigricornis* before the introduction of *S. noctilio* because it is a secondary insect, only colonizing highly stressed or recently dead trees (Schiff et al. 2012; see Chapter 9). The frequency at which these congeners co-occur likely varies by local populations, invasion history, and host conditions. Some studies have observed co-occurrence in over 25% of pines symptomatic for *S. noctilio* colonization (i.e., resinosis, see Chapter 11, Fig. 1; Foelker et al. 2016), while other studies have found very limited numbers of *S. nigricornis* (Long et al. 2009; Ryan et al. 2012; Foelker 2016; Hajek et al. 2017). In almost all instances of co-colonization, *S. noctilio* greatly outnumbers *S. nigricornis*. An interesting pattern in survey studies that examined the same sites in successive years is the decrease over time in the percent of co-colonized pines and the total number of *S. nigricornis* collected (Ryan et al. 2012; Foelker et al. 2016; Hajek et al. 2017). It is unlikely that *S. nigricornis* has any meaningful competitive interaction with *S. noctilio*; it colonizes trees later in the growing season than *S. noctilio* and typically attacks pines in a more degraded health condition (Schiff et al. 2012).

There is a complex temporal component to pine colonization in North America that is absent in the Southern Hemisphere. Stressed or weakened trees in the Southern Hemisphere have had very few mortality agents and can remain in a compromised state throughout the early growing season until *S. noctilio* emergence and attack. In contrast, pines in North America are challenged with bark and woodboring beetles early and regularly throughout the growing season. As examples, pine engraver beetles (*Ips* spp.) have several generations throughout the year, and *T. piniperda* begins colonizing pines as early as March. This has the potential to greatly reduce or degrade the pool of suitable hosts by the time *S. noctilio* emerges in midsummer.

Conclusion

A diverse array of limiting biotic factors appears to have relegated *S. noctilio* to the status of secondary pest in its invaded region of northeastern North America. The strongest limitations appear to be host factors, which may provide more resistance to damage and/or outbreaks than in the Southern Hemisphere. Biotic resistance will likely limit *S. noctilio* as it continues to spread across North America. Regions dominated by native pines (western North America and the southeastern US) have similarly complex native communities of co-colonizing insects and parasitoids. They also have prominent primary bark beetles (*Dendroctonus* spp.) that can cause widespread damage and mortality. *Dendroctonus* spp. are an aggressive component of the subcortical pine community absent in the Southern Hemisphere and historically absent in northeastern North America.

There are additional host and environmental components that could alter how biotic resistance operates when *S. noctilio* spreads into new regions. Host species composition, continuity, and condition are all considerably different in the southeastern US and western North America where pine species are often large components of forests. In the invaded region of northeastern North America, pines are a comparatively small component of the forest composition, with hardwoods being much more prevalent. Climate will also play a role. Climate matching models indicate conditions in the southeastern US are favorable, while conditions in western North America are only marginal (Ireland et al. 2018; but see Chapter 1). However, drought stress has been closely tied with *S. noctilio* population outbreaks in the Southern Hemisphere (Lantschner et al. 2019), and hot, dry conditions are becoming increasingly common in western North America.

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**CHAPTER
11****Forest impacts, management,
and monitoring of *Sirex noctilio*****Laurel J. Haavik¹ and Christopher J. Foelker²**¹USDA Forest Service, Forest Health Protection, Missoula, Montana 59804 USA²Wisconsin Department of Agriculture, Trade and Consumer Protection, Madison, Wisconsin 53718 USA

Abstract

Initial concerns that *Sirex noctilio* would have serious impacts in northeastern North American pine forests subsided once it was realized that this woodwasp was not killing pines in healthy stands. Thus far, *S. noctilio* damage has mostly been limited to stressed pines and overtopped crown classes, primarily in unmanaged stands. Economic and ecological impacts have been difficult to delineate and quantify for several reasons: i) measured impacts were not investigated on a consistent or systematic basis; ii) they were limited spatially and temporally; and iii) they were biased towards unmanaged and overstocked stands where *S. noctilio* could easily be found. Ecologically, *S. noctilio* is apparently the most abundant and aggressive woodwasp of pines throughout its introduced range in North America. There are no continuous organized efforts to monitor its impact, or track or limit its spread. Active management throughout the rotation in pine stands and monitoring for symptoms of *S. noctilio* will be key in reducing future impacts in vulnerable forests. *Sirex noctilio* has the potential to be a more aggressive pest in North America when it spreads to regions of the southeastern and western US with vulnerable forests, or within its introduced range in northeastern North America if environmental conditions change (e.g., more frequent and severe droughts, increasing temperatures, and longer growing seasons).

Introduction

The arrival of *S. noctilio* in North America caused justifiable alarm. This insect has been the most damaging pest of pine plantations in several areas of the Southern Hemisphere for decades. Widespread forest impacts in North America were possible, given the woodwasp's pestilence in climatically similar areas of the Southern Hemisphere (see Chapter 1) and the amount of unmanaged pine on the North American landscape. Pine mortality from *S. noctilio* in the northeastern US and eastern Canada occurred in stands growing on poor or unmanaged sites (e.g., abandoned Christmas tree plantations, windbreaks, sandy soils, and replanted strip

mines). Particular concern arose for the southeastern US, which has greater abundance, homogeneity, and continuity of economically valuable native pines than the Northeast. In the southeastern US, pines grow in dense conditions, either planted for commercial purposes or occurring through natural regeneration in the absence of natural fire regimes. Though western North America is of further distance from the initial introduction of *S. noctilio*, similar concerns have arisen for its vast acreages of dense, unmanaged pine forests in the context of chronic drought, rapidly rising summer temperatures, and increasing severity and frequency of wildfire. Additionally, both southeastern and western North America contain pine species which, when grown in plantation settings in the Southern Hemisphere, have known susceptibility to *S. noctilio* (see Chapter 2).

***S. noctilio* impacts 2004–2020**

More than a decade following detection of *S. noctilio* in northeastern North America, its impact is best described as limited at the landscape scale, and context-dependent at the stand scale, being most common in unmanaged forests, and usually restricted to trees in weakened conditions. Pine mortality from *S. noctilio* appears to be greatest and most noticeable in stands early in the invasion cycle, and then declines to a low, background level in subsequent years. This reduction in pine mortality rate likely occurs after suitable and susceptible pine host material is exhausted and is perhaps partially related to a time lag in response from local parasitoid populations (see Chapter 10). Mortality estimates range from 1 to 17% of pine basal area in single-year studies conducted soon after invasion (2–4 years since detection; Dodds et al. 2010; Ayres et al. 2014), and 3–48% of pine basal area in a five-year study conducted at sites invaded at different times (study began six years since detection at some sites and during the year of detection at other sites; Haavik et al. 2018). *Pinus sylvestris* and *P. resinosa* were most studied, most common on the landscape, most often reported killed and/or attacked, and some studies have also suggested that *P. sylvestris* was more impacted than *P. resinosa* (Ayres et al. 2014; Foelker et al. 2018; see Chapter 10). Such comparisons have been difficult because tree and stand conditions are often variable, and these observations may have been influenced by the greater abundance of *P. sylvestris* in a weakened condition on the landscape. *Sirex noctilio* was found attacking and killing *P. banksiana* considerably less often and was even less frequent on *P. strobus*.

The impact of *S. noctilio* has been highly dependent on site-level variables, some of which are unknown, though pine density and basal area, species composition, and management history are important, with more *S. noctilio* activity found in unmanaged, pine-dominant, and/or overstocked stands (Dodds et al. 2010; Ayres et al. 2014). Site quality or abiotic factors (e.g., aspect, soils, slope, and drainage) may also contribute, though these have not been empirically disentangled from the influence of stand structure and composition. In all studies, pine mortality was usually restricted to suppressed and intermediate crown classes and damaged or dying trees (Dodds et al. 2010; Ayres et al. 2014; Haavik et al. 2018). Of important note is that all of these studies were limited in spatial and temporal scope with constrained sample sizes. There are no long-term data on the impact of *S. noctilio* on northeastern pine forests, and research on this insect in North America has progressively slowed following its initial discovery.

Sirex noctilio appears to be the most abundant and aggressive woodwasp attacking pine in northeastern North America, particularly when compared with the native *Sirex nigricornis*. *Sirex nigricornis* has historically not been considered a pest, probably because it colonizes trees in such a weakened condition as to not be economically important. Whether *S. noctilio* competes with or facilitates the less aggressive *S. nigricornis* is somewhat unclear, though co-infestation is common (Ryan et al. 2012; Hajek et al. 2017; see Chapters 9 and 10). *Sirex nigricornis* is much less abundant than *S. noctilio* in trees that are co-colonized, although surveys targeted *S. noctilio*, so these efforts may have been biased. This has also been difficult to examine carefully because *S. noctilio* attacks and colonizes trees in midsummer, while *S. nigricornis* does not attack until early fall. It is unclear whether external symptoms of attack (resin beading, Fig. 1) are the same for both



Figure 1. Several examples of diagnostic resin beading along the boles of different pine species attacked by *Sirex noctilio* (a, c–e) *Pinus sylvestris*; (b) *P. resinosa*; (f) *P. banksiana*. These can be visible for up to a year or two following attack (images by L.J. Haavik).

woodwasp species; many have suggested that substantially weakened pines suitable for *S. nigricornis* do not have the resistance capacity for resin flow to produce diagnostic beading on the bark surface.

Native hymenopteran parasitoids have readily adopted *S. noctilio* as a host (see Chapter 8). The addition of *S. noctilio* to this existing native siricid-parasitoid community has the potential to affect the population dynamics of *S. noctilio*, *S. nigricornis*, and their parasitoids (see Chapter 10). In northeastern North America, *S. noctilio* appears to occupy a similar ecological niche as it does in its native range in Europe, where it is the most aggressive colonizer of pines, but it is still limited to stressed, damaged, or dying trees that it attacks in concert with a suite of other subcortical pine insects and their associated microbes. Interactions with these native co-colonizers are complex and context-dependent, but they may contribute to *S. noctilio*'s limited impact to date in northeastern North America (see Chapter 10).

It is difficult to accurately and precisely measure the impacts of *S. noctilio* in North America for several reasons. First, identification of *S. noctilio*-caused mortality is challenging. Unless a tree died recently, in which case there could still be diagnostic resin beading on the bole (Ryan et al. 2013) (Fig. 1), it is not possible to discern if *S. noctilio* attacked the tree and/or was solely responsible for its death without destructively sampling the wood—a time and labor intensive task. An additional symptom of successful

S. noctilio colonization is the presence of emergence (exit) holes. Adult emergence holes appear perfectly circular and are most often perpendicular with the bark surface. On average, they are the diameter of a pencil, but they can have a wide range in diameter (2.5–7.5 mm or 0.1–0.3 in) (Foelker 2016). Trees are most heavily colonized near mid-bole (Ryan et al. 2012; Foelker 2016). Emergence holes cannot be used as absolute evidence of *S. noctilio* colonization because they are similar in size, shape, and pattern to other woodboring insects, including *S. nigricornis* and longhorned beetles in the genus *Monochamus*, both of which co-colonize pines with *S. noctilio*. Emergence holes of hymenopteran parasitoids attacking both *Sirex* species are also indistinguishable from *S. noctilio* emergence holes (Dodds et al. 2010) (Fig. 2). Second, an effective lure and trap combination for detecting *S. noctilio* in new areas has not yet been developed (see Chapter 3). Black panel or funnel traps baited with a synthetic blend of host volatiles (70:30 blend of alpha:beta pinene) are the standard trapping method, though there is little confidence in them for early detection (see Chapter 3). Third, forests within the range currently occupied by *S. noctilio* are heterogeneous and highly fragmented on the landscape, limiting or preventing spatial and temporal replication within and among studies regarding host species, stand conditions, management histories, and consistent *S. noctilio* population densities. Studies that have been conducted sought *S. noctilio* where it could be easily found and was abundant, resulting in sampling of unmanaged stands with varying site characteristics, which created bias in reporting the total and average impacts of *S. noctilio*.

Monitoring and management recommendations

Knowledgeable monitoring and active forest management are critical to reducing *S. noctilio* impacts. As of 2020, there are no government-sponsored monitoring or survey/detection programs, quarantine, or movement regulations for *S. noctilio* in the US or Canada. This limits knowledge of its distribution and spread to records from initial delimitation surveys (see Chapter 1) and scattered, ongoing research projects. The actual distribution of this insect is probably more extensive than has been documented (see Chapter 1, Fig. 2). Consequently, forest managers will need to voluntarily monitor for *S. noctilio* presence and population



Figure 2. A downed *Pinus sylvestris* with some bark missing/decayed. Insect emergence holes are varied in size and could be from *Sirex noctilio*, parasitoids or other members of the subcortical pine community (image by L.J. Haavik).

growth, especially as pine stands near intermediate harvest dates and during and after drought. Particular emphasis will need to be placed on informing a wide audience of forestry professionals on recognition of symptoms of *S. noctilio* attack and colonization.

Stand-level monitoring for unexpected increases in mortality is best accomplished through ground survey for symptoms of *S. noctilio* attack (see Chapter 12 for a survey protocol). The key diagnostic symptom in response to *S. noctilio* attack and oviposition is scattered resin beading at approximately mid-bole, although in heavily attacked trees resin beading can be visible throughout the length of the bole (Fig. 1). If attack was successful (sometimes it is not; see Chapter 10), the fungal symbiont establishes and *S. noctilio* larvae develop, the tree dies, and needles fade to red. Time to death ranges from a few weeks to nearly a year following successful attack (Ryan et al. 2013). In the summer following successful attack, circular emergence holes will be visible on the bole, although as discussed in the previous section, these could be created by other insects and should not be relied upon as sole confirmation of *S. noctilio*. Resin beading from current-year *S. noctilio* attack is first visible once the adult flight period has begun, approximately between mid-July and September, and can sometimes be visible for one or two years following attack. Survey for *S. noctilio* attack should occur in the fall, after the adult flight period has ceased, and before damage from winter storms could potentially dislodge fragile resin beads from the bark surface. For these walking surveys, examination of suppressed or stressed trees and those with fading canopies should be prioritized, and binoculars are often helpful to identify resin beads from a distance or on taller trees.

Alternatively, monitoring with traps or trap trees girdled by herbicide (with or without traps hung in them) may detect *S. noctilio* (Zylstra et al. 2010; Dodds et al. 2012; see Chapters 3 and 12), but these approaches are more likely to result in a false negative than properly timed, annual ground surveys that target suppressed trees. In addition, traps and trap trees can be more costly than ground surveys. At the stand level, once *S. noctilio* has been confirmed in the area, continued monitoring efforts will be necessary to determine whether attack was successful, which crown classes are affected, and if the population is growing quickly enough that co-dominant, healthy trees could be killed. If co-dominant trees are successfully colonized, then harvest or sanitation/salvage treatments should be planned in the short term (see Chapter 12 for specifics).

Well-managed stands have so far been affected little by *S. noctilio* (Fig. 3a vs. b). Research has been limited in North America, but conclusions have clearly demonstrated less *S. noctilio* damage in stands that have been thinned (Ayres et al. 2014; Dodds et al. 2014). This is consistent with *S. noctilio* behavior in invaded regions of the Southern Hemisphere, where active stand management throughout the rotation has been a critical factor in reducing pine mortality (e.g., Haugen 1990; Hurley et al. 2007). In general, silvicultural practices that avoid growing trees on sites to which they are not adapted (i.e., off-site), reduce overall tree stress, and improve tree vigor will increase stand and tree resistance to *S. noctilio*. Important management practices include intermediate harvests with thinnings from below to remove suppressed, poor growth form, or genetically inferior trees. In *P. resinosa* and *P. sylvestris* stands in New York, thinning to a basal area of 14–18 m²/ha (60–80 ft²/ac) reduced *S. noctilio* attack by 75% (Dodds et al. 2014). If *S. noctilio* is present in a stand, avoiding harvests during drought and scheduling them well outside of the adult July–September flight period (harvest between November and April; see Dodds et al. 2007), limiting mechanical damage to residual trees, and promptly removing slash following harvest will provide additional stand-level resistance. Although *S. noctilio* has not been found in pine slash in North America, slash has been colonized in other invaded regions and is a potential source of population build-up. From experience both in the Southern Hemisphere and northeastern North America, vulnerable stands are those that are overstocked, unmonitored, affected by disturbance (e.g., severe or prolonged drought, or winter storms), and with poor growing conditions. Stands heavily impacted by *S. noctilio* were often compromised by chronically stressful conditions (e.g., sandy soils, xeric topography, or replanted strip mines). Stands most at risk are those managed to maximize biomass (e.g., pulpwood) or where regular thinning is deferred, at times because it is not economical.



Figure 3. *Pinus resinosa* stands in Ontario, Canada that were (a) thinned versus (b) unthinned. Thinned stands were not affected by *Sirex noctilio* because they provided little or no suitable habitat. Unthinned stands that provided ample habitat for *S. noctilio* were severely affected (images by L.J. Haavik).

Future *S. noctilio* impacts

Future impacts of *S. noctilio* in North America are unclear and will depend on environmental conditions, monitoring, and forest management. In the 16 years since first detected in northeastern North America, *S. noctilio* has behaved mostly as a secondary pest, killing trees that are already stressed. It could continue to behave as such, or it could become a more serious pest, both as it spreads to vulnerable areas of the southeastern and western US, and as climate conditions become more stressful for trees. Based on decades of experience in the Southern Hemisphere where pines and *S. noctilio* are non-native, environmental conditions that are unfavorable for trees, especially drought (e.g., Rawlings and Wilson 1949; Lantschner et al. 2019), can produce an abundance of stressed trees suitable for *S. noctilio* colonization. This situation facilitates rapid increase of *S. noctilio* population densities, which then allows the woodwasp to become an aggressive pest. *Sirex noctilio* outbreaks associated with drought in Patagonia and New Zealand resulted in 12–75% (Lantschner et al. 2019) and 25–30% (Rawlings and Wilson 1949) pine mortality, respectively. Given that *S. noctilio* interacts mostly with native pines in North America, it is difficult to speculate how influential similar climate scenarios in North America will be on *S. noctilio* population dynamics.

The potential for continued spread of *S. noctilio* within North America is very high, demonstrated by its rapid movement between and within multiple continents in the Southern Hemisphere (see Chapter 1). *Sirex noctilio* has the capacity for long-range natural dispersal (see Chapter 2), inadvertent human-assisted dispersal due to its development deep in the pine wood, and rapid population growth in stressed trees, due to high fecundity and haplodiploidy (i.e., females do not need a mate to infest trees and produce a new brood of males). If rapid population growth occurs and is not detected, and management actions are not taken, then a *S. noctilio* outbreak that causes substantial losses in pine basal area could develop.

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**CHAPTER
12****Recommendations for land managers
concerned about *Sirex noctilio*****Laurel J. Haavik¹ and Ann E. Hajek²**¹USDA Forest Service, Forest Health Protection, Missoula, Montana 59804 USA²Department of Entomology, Cornell University, Ithaca, New York 14853-2601 USA

This chapter is intended as a guide to assist private and public land managers in detection, monitoring, and management of *Sirex noctilio*. These recommendations were developed based on knowledge from *S. noctilio* management programs in the Southern Hemisphere, as well as research and observations of the *S. noctilio* invasion in northeastern North America (2004–2020).

The landscape in northeastern North America consists of highly fragmented forests, often with low abundance of pines (*Pinus* spp.). The northeastern native pine component (*P. resinosa*, *P. strobus*, and *P. banksiana*), along with non-native *P. sylvestris*, consists of small acreages of non-contiguous stands, growing naturally, as windbreaks, or in plantations, often mixed with other species (hardwoods or conifers). Overcrowded stands are common, resulting from fire suppression throughout the 20th century. These overstocked stands are most susceptible to *S. noctilio*, though usually only suppressed and intermediate trees are killed. Stands that are properly managed have thus far not been affected by *S. noctilio*. Due to this limited impact (see Chapter 11), specific practices for management of *S. noctilio* in northeastern North America have not yet been developed or implemented. Based on existing research and observations, we suggest an approach to identification, monitoring, and mitigation of *S. noctilio* in similar forest types.

Proper identification of *S. noctilio* by an entomologist or a trained forester is critical because there are several other pine-associated insects and diseases throughout North America that kill weak pines. Some of these insects (e.g., southern pine beetle, mountain pine beetle) can undergo landscape-scale outbreaks, which often cause substantial pine losses. *Sirex noctilio* has not yet demonstrated this capacity in North America.

This guide begins with a brief explanation of specific and general symptoms associated with *S. noctilio* infestation. It then describes three methods to survey for *S. noctilio* presence, one of which also monitors the level of *S. noctilio* impact. Finally, it lists management considerations and techniques to mitigate damage from *S. noctilio* according to the manager's level of concern (i.e., severity of the infestation).

Evidence of *S. noctilio* infestation

Specific evidence

Diagnostic symptoms on trees

- The key diagnostic symptom is scattered resin beading along the main bole (see Chapter 11, Fig. 1 and Chapter 2, Fig. 5a,b for example images). Resin beads are typically less than the width of a pencil eraser, but larger than a pen tip (approximately 3 mm in diameter). Sometimes, enough resin has been produced by the tree to cause dripping.

Correctly identified adult woodwasp specimens

- Adult woodwasps can be difficult to collect because adults live only a short time in summer/early fall (approximately 1–2 weeks). Occasionally females die while laying eggs and are still attached to the tree bole.
- Specimens collected could be compared with photos of *S. noctilio* (Fig. 1). Images of other species and keys for identification are available in Schiff et al. (2012).
- For identity confirmation, especially if *S. noctilio* is not known from your area, send specimens to specialists. Diagnostic labs of extension offices, state universities, and state departments of agriculture employ entomologists who can accurately identify specimens. Siricids can be challenging to identify to species level on your own; we strongly recommend confirmation of identification by an expert.

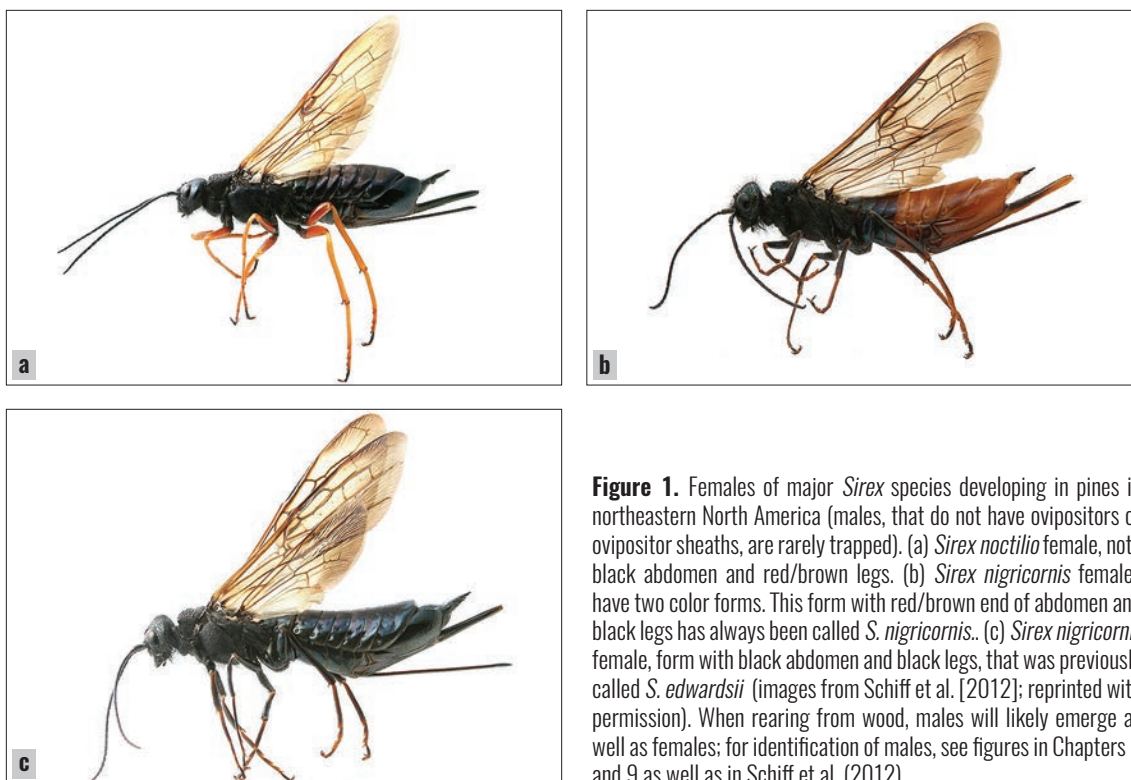


Figure 1. Females of major *Sirex* species developing in pines in northeastern North America (males, that do not have ovipositors or ovipositor sheaths, are rarely trapped). (a) *Sirex noctilio* female, note black abdomen and red/brown legs. (b) *Sirex nigricornis* females have two color forms. This form with red/brown end of abdomen and black legs has always been called *S. nigricornis*. (c) *Sirex nigricornis* female, form with black abdomen and black legs, that was previously called *S. edwardsii* (images from Schiff et al. [2012]; reprinted with permission). When rearing from wood, males will likely emerge as well as females; for identification of males, see figures in Chapters 2 and 9 as well as in Schiff et al. (2012).

General evidence in trees and stands

- Level of pine mortality seems unusual or has increased recently, especially among suppressed or intermediate trees. Mortality could be due to native insects and/or pathogens, natural senescence, or *S. noctilio*.
- Dead pines with many round emergence holes of varying sizes on the main bole (see Chapter 2, Fig. 5d,e and Chapter 11, Fig. 2 for example images), although these can be the same size and shape as holes created by native wood borers, including the native woodwasp *S. nigricornis* and parasitoids of all of these insects (see Chapter 11).
- Tree crown can turn red in recently killed pines (Fig. 2). This can occur weeks, months, or even a year after *S. noctilio* attacks and colonizes the tree. This also occurs when the tree is killed by other agents, especially bark beetles.



Figure 2. Red crown of dead *Pinus sylvestris* (center) in September 2014, attacked and colonized by *Sirex noctilio* in summer 2014 (image by L.J. Haavik).

Methods for detecting and monitoring *S. noctilio*

Ground surveys

Annual ground surveys are ideal because they can serve to initially detect *S. noctilio* and, if present, monitor its level of impact over time. They involve walking through stands and searching for evidence of *S. noctilio* attack. To reduce survey time, focus efforts on suppressed and intermediate trees.

Survey considerations:

- Conduct walking surveys, ideally in fall (October through November), although damage may be visible at other times of the year.

- Survey more carefully as stands approach intermediate harvest dates, or in portions of stands that have higher than average stocking.
- Survey especially in years when precipitation is below normal.

Survey protocol:

- Survey pine stands. All pines are probably susceptible to *S. noctilio*, although *P. strobus* is rarely attacked and does not need to be surveyed (see Chapter 2).
- On each tree, examine the main bole for scattered resin beads (see ‘diagnostic symptoms on trees’ in previous section) which indicate that the tree has been attacked by *S. noctilio*. Resin beads are usually visible at mid-bole but can occur along the entire length of the bole, especially in heavily attacked trees. Binoculars may be useful for examining tall trees, or for observations at a distance.
- If resin beading is observed, note if the crown has changed color to red, an indicator that *S. noctilio* has killed the tree, although this may not occur until the spring following attack. It is also possible that *S. noctilio* attack was unsuccessful, and the tree will survive. This occurs when the tree is healthy and vigorous enough to resist colonization by *S. noctilio* and its fungal symbiont (see Chapters 2 and 4).
- Pay careful attention to suppressed, intermediate, stressed, or poor growth-form trees, as these are likely to be attacked and killed first upon initial arrival of *S. noctilio* in an area.
- Examine all recently dead trees for symptoms (see ‘general evidence in trees and stands’ in previous section) and note canopy positions of these trees. If *S. noctilio* adults emerged from a tree in previous years or the current year, emergence holes will be visible at the time of fall ground surveys.

Trap trees

Trap trees, with or without traps hung in them, are effective for detection but can be costly, labor-intensive, and tricky to create. This involves properly timed herbicide injection into a tree to weaken it, which makes it an attractive bait for *S. noctilio* attack (Zylstra et al. 2010). Weakening trees should be done up to a month before, or at the start of, the adult flight period (early June or early July for eastern Canada and the northeastern US, but see model projections for the eastern US by Myers et al. [2014]). The timing of herbicide injection is very important because it is easy to stress the tree too quickly, which may kill it. After the flight period, trap trees must then be examined for evidence of *S. noctilio* attack (i.e., resin beading or positive identification of adult females if a trap is hung in the tree).

- Use only hard pine species (*P. sylvestris*, *P. resinosa*, or *P. banksiana*), not soft pines (*P. strobus*) as trap trees. Choose trees you are willing to sacrifice. Suppressed or intermediate trees in overstocked stands are ideal. Create a group of 3–5 trap trees. To maximize attractiveness to *S. noctilio*, these trees should be as close to one another as possible.
- Create trap trees by injecting them with herbicide. The goal is to significantly weaken the tree and not kill it quickly.

- Drill holes at a 45° downward angle, 10 cm (4 in) apart around the circumference of the bole, 30–60 cm (1–2 ft) from the base of the tree.
- Holes should be 1.25 cm (0.5 in) in diameter and drilled ≤ 5 m (2 in) into the sapwood, depending on the diameter of the tree. Take care not to drill holes through the bole to the opposite side.
- Inject 1 mL of a 20% solution of Dicamba into each drilled hole. Be careful not to inject too much herbicide; too little is better than too much.
- See Zylstra et al. (2010) and Dodds et al. (2012) for more details on creating trap trees.
- If hanging a trap for detection of adult woodwasps, follow the instructions for traps stated in the next section.
- At the end of the adult flight season (late August/early September in eastern Canada and the northeastern US), inspect trap trees for resin beads (see ‘diagnostic symptoms on trees’ in previous section).
- If a trap is not hung in the tree, no resin beading is visible, and confirmed presence or absence of *S. noctilio* is desired, then the trap tree must be examined closely for emerging adult woodwasps. Do this after winter, but prior to the next adult flight season (before June). Fell and section trap trees, and place the sections in sealed containers (window screening lids are recommended). Carefully open containers weekly throughout the summer, collect adult woodwasps that emerge from log sections, and identify to species (see instructions on page 100).

Traps

Semiochemical-baited traps are least preferable of the three detection methods because they may not necessarily capture *S. noctilio*, even if it is present in an area.

- Just prior to adult flight (late June for eastern Canada and the northeastern US, but see model projections for the eastern US by Myers et al. [2014]), place black intercept panel traps (Chapter 3, Fig. 2) or multiple funnel Lindgren traps in stands of interest in or near suppressed or intermediate trees, at least 1.8 m (6 ft) above the ground. These traps only attract females, and each female only lives for approximately one week, so timing is important.
- Bait traps with a commercial *Sirex* woodwasp lure (alpha- and beta-pinene, e.g., AlphaScents, Synergy Semiochemicals) and place a killing agent (propylene glycol is often used) in collection cups fitted to traps. Lures may need to be replaced mid-season.
- Inspect traps weekly or bi-weekly throughout the adult flight season, collect adult woodwasps, and identify to species (see instructions on page 100).
- Traps can be removed at the end of September.

Level of concern: I do not have *S. noctilio* on my property yet, but I want to monitor for its presence.

Determine *S. noctilio* invasion status in your area

Find out if *S. noctilio* is in your state or county and if it is known to be in your area (see Chapter 1, Fig. 2 for known distribution as of 2020). Online websites that report annual detections of invasive insects by county can be helpful (e.g., [Pest Tracker](#), [National Agriculture Pest Information System](#)). Keep in mind that *S. noctilio* may occur in areas not reported by these websites, because tracking the spread of *S. noctilio* is not presently the objective of any government survey programs.

Monitor for *S. noctilio* presence and damage

For all three of the detection methods described in the previous section, one or both specific symptoms (resin beading and properly identified *S. noctilio* adults) are needed to confirm *S. noctilio* presence. If you suspect you have collected *S. noctilio* adults, or have recognized infested trees based on the presence of resin beading, contact your local extension office.

Level of concern: I now have *S. noctilio* on my property. What do I need to do to modify my monitoring system? What management options should I be thinking about, and how can I best implement them?

- Evaluate initial considerations for management action (below).
- Use yearly ground surveys to estimate whether the *S. noctilio* population is increasing and which canopy classes are affected to determine if and how soon management action should occur.
- Implement planned management (as described on pages 106–107) between November and April. Sanitation treatments to reduce *S. noctilio* population growth as quickly as possible can occur anytime, ideally prior to late June.

Initial considerations for management action

- Consider developing a management plan if one does not already exist.
- Proximity to intermediate harvest dates or rotation age may affect management decisions.
- Determine if stands have characteristics that make unacceptable tree mortality likely. For example, advancing stand age and/or high basal area.
- Determine an acceptable level of elevated mortality.

- Unexpected or increasing levels of mortality beyond that acceptable threshold may warrant management, regardless of whether trees were killed by *S. noctilio* and/or some other agent(s).
- Consider whether growing conditions in the current or previous year may have affected mortality levels or *S. noctilio* population growth. Drought may cause trees that are usually healthy to become vulnerable to *S. noctilio*, especially if the population appears to be increasing.

Conduct annual ground surveys in fall (October through November)

- Locate, mark, and count the number of attacked (confirmed by resin beading) and killed (confirmed by red crown) trees according to the survey protocol described earlier on page 102.
- Note the canopy position of attacked and killed trees (co-dominant, intermediate, or suppressed).

Estimate whether the *S. noctilio* population is increasing

- Relative to the previous year's survey,
 - if the number of attacked and killed trees is approximately the same or less, then the population is stable or declining.
 - if the number of attacked trees is greater, but the number of killed trees is similar, then the population may be increasing.
 - if the number of killed trees is greater, then the population is increasing.
- If a previous annual survey is not available for comparison,
 - and more trees are attacked than killed, and only suppressed trees are killed, and no co-dominant trees are attacked, then the population is probably stable or declining.
 - and more trees are attacked than killed, and only suppressed trees are killed, but some intermediate and/or co-dominant trees are attacked, then the population may be increasing.
 - and more trees are attacked than killed, and some intermediate and/or co-dominant trees are killed, then the population may be increasing.
 - and the mortality level has increased unexpectedly, or all attacked trees are killed, then the population is increasing.
- Keep in mind that time between *S. noctilio* attack and tree mortality (needle change from green to red) is variable (a few weeks to a year), so fall surveys may underestimate the amount of total mortality. A late spring check of attacked trees to evaluate mortality may be necessary.

Determine the need for management

- If the population is or may be increasing, consider which canopy classes are attacked and killed and whether treatment is warranted based on management objectives.
- If only suppressed trees are killed, then *S. noctilio* may be acting as a biological thinning agent, and treatment may not be necessary.
- If some intermediate trees are killed, and this level of mortality is approaching or beyond an acceptable threshold, then a sanitation treatment that removes recently attacked and killed trees prior to adult flight (which begins late June) may be warranted. Where removals occur, growing space will improve for surrounding live trees, effectively thinning portions of the stand.
- If co-dominant trees are attacked, but not killed, it is possible *S. noctilio* is conditioning them to be colonized and killed in the future (see Chapters 2, 4, and 10), and a sanitation treatment that removes recently attacked and killed trees prior to adult flight (which begins after late June) may be warranted. Where removals occur, growing space will improve for surrounding live trees, effectively thinning portions of the stand.
- If co-dominant trees are killed, the *S. noctilio* population may be capable of killing a larger number of co-dominant trees the following year. Depending on management objectives and proximity to planned harvest dates, thinning, sanitation, or salvage is likely necessary in this scenario to prevent substantial basal area loss.

Management implementation

- Reduce basal area to $\leq 14\text{--}18\text{ m}^2/\text{ha}$ or $60\text{--}80\text{ ft}^2/\text{ac}$, or other similar species-specific recommended optimal density. Options include:
 - Regeneration harvest if near rotation age. Remove the entire overstory as appropriate for the pine species.
 - Intermediate harvest if near commercial or pre-commercial thin dates. Thin from below to remove suppressed, intermediate, and poor growth-form trees to reduce available host material for *S. noctilio* population growth in the stand and to free up resources for co-dominant trees.
 - Sanitation harvest to remove all attacked trees. To reduce *S. noctilio* population growth and mitigate damage, destroy all *S. noctilio*-attacked trees prior to late June when adults begin to emerge, fly, and attack new trees. Do not move infested trees off site: chip or burn them.
 - Salvage harvest to remove all recently killed trees and reduce timber loss. Do not send timber to mills in areas where *S. noctilio* has not yet been detected.
- For any management action, take the following precautions:
 - Schedule harvests between November and April. If immediate action is desired to reduce *S.*

noctilio population growth, sanitation can occur outside this time period, preferably before *S. noctilio* flight begins in late June.

- Avoid harvesting during drought.
- Avoid mechanical damage to remaining trees.
- Promptly remove slash, or pile and burn, directly following harvest or before June as *S. noctilio* adults may emerge from this material to infest surrounding live trees or will disperse further away.

Level of concern: If I suddenly have an outbreak of *S. noctilio*, what are the best options for management and to negate future outbreaks?

Immediate options

- To reduce *S. noctilio* population growth and mitigate damage, implement a sanitation harvest that removes and destroys all *S. noctilio*-attacked trees prior to late June. Do not move infested trees off site: chip or burn them.
- Implement a salvage harvest to remove all recently killed trees and reduce timber loss. Do not send timber to mills in areas where *S. noctilio* has not yet been detected.
- Implement a regeneration harvest to establish a low site hazard for *S. noctilio* and other mortality agents for the next several decades.
- Promptly remove slash, or pile and burn, directly following harvest or before June as *S. noctilio* adults may emerge from this material to infest surrounding live trees or disperse further away.

Future considerations

- Plant pine species and cultivars adapted to growing conditions at the site.
- Develop and implement a stand management plan.
- Conduct pre-commercial and commercial thinning on schedule to remove suppressed or poor growth-form individuals.
- Maintain a basal area according to optimal species-specific recommendations (e.g., 14–18 m²/ha or 60–80 ft²/ac for *P. sylvestris* and *P. resinosa*; see Dodds et al. 2014).
- Avoid harvests during drought, and schedule them between November and April.
- Limit mechanical damage during harvests.

References

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Glossary

TERM	DEFINITION
Alightment	Descent and settling upon a surface
Basidiomycete fungus	Division of filamentous fungi composed of hyphae that typically reproduce sexually via the formation of specialized club-shaped cells from which spores are produced.
Cellulose	The most abundant organic polymer on Earth and the main constituent of plant cell walls. It is a polysaccharide consisting of chains of glucose monomers that is indigestible to most animals without microbial enzymes.
Chemoreceptor	Specialized sensory receptor cell that transduces (i.e., converts) a chemical substance to generate a biological signal
Cleptoparasitoid	Parasitoids that lay their own eggs near or within other parasitoids. Once hatched, the cleptoparasitic larva often kills the primary parasitoid larva and may consume it; the cleptoparasitoid then consumes the resources that would have been used by the primary parasitoid (the host larva)
Complete metamorphosis	Type of insect development that includes all four stages of egg, larva, pupa, and adult, which differ greatly in morphology
Cuticular waxes	On the surfaces of insects, there can be a layer of wax which act as a protectant against desiccation. Embedded within this layer can be chemicals that are used by other insects for recognition.
Diploid species	Species with two sets of chromosomes within each cell
Drill	The narrow and relatively short excavations made by female woodwasps to deposit eggs, venom, or fungus within sapwood
Eclose	When an insect emerges as an adult from the pupa or as a larva from the egg
Ectoparasitoid	A parasitoid with larvae developing externally on a host
Endoparasitoid	A parasitoid with larvae that develop within the body of a host
Frass	Feces of an insect. For example, fine powdery refuse defecated by woodboring insects
Gallery	A passage made in wood by an insect
Hemicellulose	Substances in the cell walls of plants that are polysaccharides of simpler structure than cellulose
Hypopleural organs	External pockets formed by folds of the skin on the dorsum of each side of the larval body, between the first and second abdominal segments
Idiobiont	Parasitoids that prevent further development of the host after initially immobilizing it
Infectives	Producing or capable of producing or transmitting infection
Introduced North American strain (INA)	The introduced strain of <i>Deladenus siricidicola</i> that is established in North America but does not sterilize the genotype of <i>Sirex noctilio</i> present in North America
Koinobiont	Parasitoid that allows the host to continue its development while feeding upon it
Lek	Aggregated group of males associated with mating behavior
Lignin	A class of complex organic polymers in the secondary cell walls of woody plants that is much more resistant to break-down than cellulose
Lignocellulose	Structural carbon, composed of interlinked cellulose, hemicellulose, pectin, and lignin, that gives wood its strength, durability, and resistance to biological degradation
Macromolecules	Very large molecules composed of thousands of covalently bonded atoms
Microbial symbiont	Microorganism living in association with another organism. Usually these terms are used to describe relationships where both microbe and host benefit (= mutualism)
Microbiome	Genetic material of all the microbes—bacteria, fungi, protists and viruses—that live on and inside an organism
Monoculture	Cultivation of a single species in a given area

TERM	DEFINITION
Monoterpene	Class of terpenes that consist of two isoprene units and have the molecular formula $C_{10}H_{16}$
Mutualistic fungi	Fungi that have a relationship with another organism which is mutually beneficial for both partners
Mycangia	Special structures in or on the body of an animal that are adapted for the transport of fungi
Mycetomes	Specialized organs in a variety of animal species which house that animal's symbionts, isolating them from the animal's natural cellular defense mechanisms and allowing persistence and sustained, controlled symbiotic growth
Mycophagous	Feeding on fungi
Natal tree	The tree within which a woodboring or bark feeding insect completes development, and from which it later emerges as an adult
Obligately associated	Associated out of necessity; typically where one organism cannot live without the association with another
Olfaction	Smell
Optomotor anemotaxis	Upwind flight whereby an insect assesses its progress relative to the ground through optical feedback
Osmotic potential	The potential of water molecules to move from a hypotonic solution (more water, less solutes) to a hypertonic solution (less water, more solutes) across a semi-permeable membrane
Oviposit	Lay eggs (term commonly used for insects)
Parasitoid	An insect whose larvae live as parasites, consuming resources from the host insect, and eventually killing the host
Phloem	Vascular tissue in plants that conducts sugars, nutrients, and other metabolic products throughout a plant
Resin beads	Drops of tree resin exuded by a tree upon damage by an invading organism. The drops eventually harden to form a protective seal over the site of injury
Saprotrophic decay fungus	One ecological group of fungi that decays plant tissues
Semiochemical	A chemical or mixture released by one individual that affects the behavior of another
Sensilla	Arthropod sensory organ protruding from the cuticle of an exoskeleton, or sometimes lying within or beneath it. These often appear as small hairs or pegs over an organism's body, and inside each are two to three sensory neurons
Sexually dimorphic	Condition where the two sexes of the same species exhibit different phenotypic characteristics beyond the differences in their sexual organs
Silviculture	The growing and cultivation of trees
Solitary parasitoids	A single individual parasitoid develops from each host individual
Sterols	A group of naturally occurring unsaturated steroidal alcohols, typically waxy solids, that insects require but cannot synthesize
Subcortical insects	Insects that feed beneath the bark of trees
Symbiotic fungi	Fungi living in association with another organism; often this refers to a mutualistic relationship where both partners benefit
Transmission, horizontal	Acquisition of a microbe from the environment rather than from a parent
Transmission, vertical	Acquisition of a microbe from a parent, usually the mother
Volatile organic compound (VOC)	Organic chemicals that evaporate easily and are emitted as gases from many solids and liquids
Xylem	Vascular tissue in plants that conducts water and solutes upward from the root and which also helps to form the woody element in the stem
Xylophagous species	Species that feed on wood

