# *Effect of Two Bark Beetle-Vectored Fungi on the On-Host Search and Oviposition Behavior of the Introduced Woodwasp* Sirex noctilio *(Hymenoptera: Siricidae) on* Pinus sylvestris *Trees and Logs*

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# Effect of Two Bark Beetle-Vectored Fungi on the On-Host Search and Oviposition Behavior of the Introduced Woodwasp *Sirex noctilio* (Hymenoptera: Siricidae) on *Pinus sylvestris* Trees and Logs

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**Abstract** *Sirex noctilio's* fungal symbiont, *Amylostereum areolatum*, is required for its offspring's development. The symbiont is a weak competitor with bark beetle-vectored fungi so it would be beneficial to the woodwasp if it avoided ovipositing in substrate colonized by these competitors. The response of *S. noctilio* to the presence of two beetle-vectored fungi, *Leptographium wingfieldii* and *Ophiostoma minus*, inoculated into living trees, and to *L. wingfieldii* and *A. areolatum* inoculated into cut logs was investigated. The wasp avoided areas with *L. wingfieldii*; there were fewer signs of oviposition activity and drilling in these zones. There was no significant response to *O. minus* or *A. areolatum*. Female woodwasps can detect the presence of some fungi and make choices about oviposition sites that benefit their offspring.

**Keywords** Woodwasp · fungi · oviposition · resource competition

## Introduction

*Sirex noctilio* (Fabricius) is a woodboring wasp native to Eurasia and northern Africa, and was recently discovered in eastern North America (Hoebeke et al. 2005; de Groot et al. 2006). In the southern hemisphere, where this insect is an introduced invasive pest, it is capable of causing extensive economic loss and ecological impact, yet in its native range it is of little ecologic or economic concern (Hall 1968). With the recent

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discovery of *S. noctilio* in North America, the nature and impact of the native bark and woodboring insect community on the survival and population dynamics of *S. noctilio* are unknown. It is important to investigate this interaction as it could have significant implications for pest management, in fact, it may determine whether *S. noctilio* will become a pest at all in this new range.

In its native range, *S. noctilio* shares trees with curculionids, cerambycids, buprestids, and other wood-inhabiting insects that also favor and colonize weakened and stressed trees (Wermelinger et al. 2008). In North America there is a rich and diverse community of bark and woodboring insects that also colonize *Pinus* spp. along with *S. noctilio*, the most frequent being *T. piniperda* (L.), *Pissodes nemorensis* Germar and *Ips grandicollis* (Eichhoff) (Ryan et al. 2011b). Curculionids are well-documented as vectors of various species of fungi, primarily ophiostomatoid (blue stain) species. All of the most frequent beetles that co-habit with *S. noctilio* do vector fungi at times; examples include *Leptographium wingfieldii* Morelet, *L. lungbergii* Lag. & Melin, *Ophiostoma minus* (Hedg.) H. & P. Syd., *O. ips* (Rumb.) Nannf. and *Sphaeropsis sapinea* sensu lato (*T. piniperda*) (Hausner et al. 2005), *O. ips* (*I. grandicollis*) (Whitney 1982) and *L. procerum* (Kendr.) Wingf. (*P. nemorensis*) (Nevill and Alexander 1992).

*Sirex noctilio* is highly dependent on its fungal symbiont; growth of *A. areolatum* is essential for both woodwasp egg eclosion (Madden 1981) and larval development (Coutts and Dolezal 1965; King 1966) and its symbiont, *Amylostereum areolatum*, is known to be a weak competitor with some ophiostomatoid fungi including *L. wingfieldii* and *O. minus* (King 1966; Ryan et al. 2011a). Therefore, this species could be negatively affected by competing fungal species vectored by other insects. Given the poor competitive abilities of *A. areolatum*, it would be of reproductive benefit to *S. noctilio* if it could detect the presence of competing fungi in the tree and avoid them. The avoidance of oviposition in substrate colonized by harmful fungi has been recently documented in other insects systems (e.g. Lam et al. 2010). Hanson (1939) and Spradbery and Kirk (1978) speculate that *S. noctilio* detects and avoids areas of trees colonized by the beetle-vectored-fungi but this has never been investigated.

In this study we tested the hypothesis that *S. noctilio* avoids ovipositing in wood already colonized by ophiostomatoid fungi by conducting two experiments; in Experiment 1, living *Pinus sylvestris* were inoculated with two ophiostomatoid fungi (*O. minus* and *L. wingfieldii*) and female woodwasps were caged on trees to evaluate the wasp's behavior and oviposition drilling. In Experiment 2, *L. wingfieldii* and *A. areolatum* were tested in a similar fashion on cut sections of wood (bolts), to further assess wasp behavior in the presence of these fungi.

## Methods

*Fungi* Fungal strains were obtained from the Canadian Forest Service culture collection (accession numbers: *A. areolatum* SSM 075 7011, *L. wingfieldii* SSM 025 7010 and *O. minus* SSM 075 7007). Prior to producing them for use in Experiments 1 and 2, they were inoculated onto, and re-isolated from, sterile *Pinus sylvestris* wood chips in order to counteract any effects of long-term storage in culture. Each of the three

fungal species was then grown in sterile, climate-controlled conditions on potato dextrose agar (PDA) for use in the field.

*Insects* *Sirex noctilio* were reared from infested pines (*P. sylvestris* or *P. resinosa*) that were obtained from eight pine plantations located in southern Ontario, Canada. Trees were felled in the spring or early summer of each year, prior to the onset of *S. noctilio* emergence, and cut into bolts, which were stored in either screened tents or enclosed tubes. Female wasps were collected from the rearing containers several times a day, before mating could occur. Each female was placed in an individual glass vial, labeled with the emergence date. The vials were stored in a refrigerator at 5–8°C, for a maximum of 2 weeks, until the insects were used in the following experiments.

*Sirex noctilio Behavior* A preliminary trial of *S. noctilio* oviposition behaviour was conducted in 2008 to develop categories of insect oviposition behaviour. Fourteen females were caged onto stems of *S. noctilio*-favourable *P. sylvestris*; half were placed on an untreated tree, the other half on a tree that had been inoculated 3 weeks earlier with the two fungal pathogens *L. wingfieldii* and *O. minus* (treatment and caging are described the following section). The female wasps exhibited three types of behaviour: 1) tapping antennae over the bark while walking, hereafter referred to as searching; 2) insertion of the tip of the ovipositor only, for a short duration (< 60 s), termed probing; and 3) insertion of most of the ovipositor into the tree for >60 s, referred to as drilling. An examination of the drills revealed two distinct patterns: shallow drill scars extending into the phloem only (corresponding to probing behavior), and deep drill scars extending into the sapwood (corresponding to drilling behavior). Although there was no unequivocal evidence of eggs in the deep drill scars in these test trees, dissection of the insect's ovaries revealed fewer eggs than expected for a female of a given size implying some oviposition occurred. The wasps exhibited similar behaviour regardless of the tree treatment and these behaviours were comparable to those observed in wild populations. These behavior types were used to evaluate female *S. noctilio* activity in Experiments 1 and 2.

*Field Sites* Four *P. sylvestris* plantations near Barrie, Ontario were selected for Experiment 1. *Sirex noctilio* favors *Pinus* with declining crowns (≤25% residual foliage) and those that are suppressed or intermediate in dominance (Ryan 2011), so sites with an abundance of declining or suppressed *P. sylvestris* were selected. One of these plantations was the source of logs used in Experiment 2.

*Experiment 1: Effect of L. wingfieldii and O. minus on S. noctilio Behaviour in Live Pinus sylvestris Trees* An in-vivo study was conducted in 2008 and 2009 to test the ability of *S. noctilio* to detect, and alter its searching and oviposition drilling patterns, in the presence of certain ophiostomatoid fungi. In 2008, 12 *S. noctilio*-favourable *P. sylvestris* trees were selected from each of the four field sites (48 trees total). In some sites, there were insufficient trees meeting the selection criteria available, so trees with a slightly greater proportion of residual foliage (30–35%) were selected and the crowns pruned to 20–25% residual foliage; five trees of the 48 were pruned. In 2009, 36 trees were selected in a similar manner; 12 from each of three of the four field sites used in 2008; five trees were pruned. To further stress each of the study trees and to

make them more attractive to the woodwasp for oviposition (Spradbery and Kirk 1981), they were girdled in mid-June of each year. To do this, a 30-cm wide section of bark and cambium was removed from around the entire circumference of each tree stem, the lower end extending to breast height, and a 1-cm wide strip of outer sapwood was excavated to a depth of 5–10 mm in the centre of the girdled area.

Twenty-four trees were selected for fungal inoculation treatment in each year, six per site in 2008 and eight per site in 2009 (i.e. there were six untreated trees per site in 2008 and four per site in 2009). To insure that trees selected for the treated and untreated groups were of a similar condition and favourability to the wasps, we used a hierarchical process to assign them to groups. For each site, we ranked each of the 12 trees according to *S. noctilio* favourability using percentage of residual foliage (least to most), dominance class and lastly diameter at breast height. From this ranked list of trees, every second tree in the list was selected for the untreated group in 2008 and every third tree in 2009.

On each tree, a 90-cm length of stem, approximately 30 cm above the girdled area, was divided longitudinally into equal one-third sections starting from due north (0° compass bearing) and using indelible ink to mark the sections. On the treated trees, sections were randomly selected for inoculation with the two test fungi (*L. wingfieldii* or *O. minus*) and the third section was left as an untreated control. Within each treatment section, bark plugs were cut to the phloem-sapwood interface with a sterile #4 cork borer and the resulting cavities inoculated, mycelium side in, with a #3 cork borer plug of fungus. Fungal plugs were taken from the colonized surface layer of the growing edge of the colony. All bark plugs were re-inserted after inoculation. This procedure was repeated every 1.5 cm in a circumferential pattern around the stem in rings 10 cm apart. A 2 cm buffer zone between treatments was maintained. In the control section, the holes were bored in a similar manner but not inoculated with fungus. Fungal inoculation took place in early July in both study years, 2 weeks after the trees were girdled. Both fungal species grow rapidly in live *Pinus*, >15 mm in 2 weeks (Långström et al. 1993). In the untreated trees the sections were marked, but had no further treatment.

The 90 cm marked section of each treated and untreated tree was caged immediately after the fungus inoculation treatment took place in order to prevent colonization by other insects. Cages were constructed of nylon screen held above the bark with cylindrical polyethylene foam approximately 7 cm in diameter (commonly, pool noodles); one side of the foam was bevelled so that it could be snugly attached to the tree stem. The screen was held in place at each end with wire, and the opening was overlapped, rolled and secured with metal clips.

Between late July and mid-August of each year, female *S. noctilio* were added to the cages. Two active but host naïve females were randomly selected from the pool of specimens, placed in each cage during the afternoon and, after an initial 5 min acclimatization period, were monitored for a 20-min period. Each of the two insect's movements over the stem was mapped; the number of search visits, probes and drills were tallied for each one-third section of the tree. The insects were observed in an identical fashion on days two and three (i.e. three consecutive days of 20 min observations), and removed from the cages on the fourth day after a 72 h period of time in the cage. In 2009 insects were monitored on days two and three only. The number of observation days was reduced to two in 2009 because we found in 2008

that the wasps tended to be less active on day one. Woodwasps that died, or were injured or compromised, were replaced. The cages remained in situ on the tree until autumn.

In the fall of each year, all of the study trees were felled and the previously caged sections were removed to the lab. From each of the trees (treated and untreated), samples were taken for gravimetric wood moisture measurement; a 1 cm×1 cm sample was extracted from the outer 1 cm of sapwood immediately adjacent to the formerly-caged area. Using a draw-knife, we peeled the bark from each of the tree sections leaving the phloem intact. In the fungus-treated trees, four fungal cultures were taken from each of the fungus-treated sections and cultured on PDA to re-isolate the inoculated species.

Shallow drill scars, those extending into the phloem layer only, were tabulated per one-third section for each of the treated and untreated trees. The phloem layer was then peeled away, and the deep drill scars, those into the sapwood, were tabulated for each section. Finally, in each of the fungus-treated trees, we identified the location of each of the boreholes used for fungal inoculation, and measured the length to the nearest mm of each response zone (i.e. the resin-infiltrated area of active host response to injury), and the vertical extent of fungal growth (when present) in the sapwood above and below each point. These response variables were then averaged per one-third section for each tree. There was never evidence of lateral growth from the fungal inoculation points, so fungal growth was not measured in this direction.

In 2008, one of the treated trees could not be safely felled and one of the untreated trees was killed by girdling, resulting in excessive growth of adventitious ophiostomatoid fungi (those not purposely inoculated): both trees were removed from the analyses.

*Experiment 2: Effect of L. wingfieldii and A. areolatum on S. noctilio Behaviour on P. sylvestris Logs* A second study was conducted in 2009 in an outdoor insectary using cut bolts to extensively evaluate the woodwasp behavioral responses to fungi, without the effects of weather and tree health. In this experiment, *L. wingfieldii* was selected for further testing since the woodwasp demonstrated a response to this pathogen in 2008 (Experiment 1), and the wasp's own symbiont, *A. areolatum*, was tested as a negative control.

Bolts 30 cm in length were cut from freshly felled *P. sylvestris* and bolt ends were immediately sealed with paraffin to prevent desiccation and introduction of adventitious fungi. The treatments consisted of inoculating one side of each bolt with *L. wingfieldii* or with *A. areolatum*; the other half of the bolt was left untreated. The third and final treatment consisted of control bolts in which the boreholes were left empty on treated side of the bolt. Inoculation techniques were the same as described for the previous experiment. All bolts were left in the insectary for 2 weeks before the growth experiment began; both fungi are known to grow quickly in freshly cut wood *A. areolatum* up to 14 mm per day, and *L. wingfieldii* up to 10 mm (King 1966; Uzunović and Webber 1998).

Each bolt was placed upright in a small plywood and screen cage. Two inexperienced, host naïve female *S. noctilio* were placed on the transition zone of each log, i.e. on the border between the treated and untreated sections, and was observed for 10 min. The number of search visits, probes and drill attempts (as previously defined) per section were recorded. The insects were kept caged with the bolt for 2 days; observations were repeated on day two. There were 30 replicate bolts for each treatment and the control (90 bolts in total).

Within 2 weeks of the experiment, the bolts were peeled, drill scars were counted per treatment and control zone, and the linear extent of fungal growth in the sapwood above and below each inoculation point were measured in the same manner as described for Experiment 1. Fungi were cultured from the fungus-treated zones.

Growth of adventitious ophiostomatoid fungi became a problem in this experiment despite all efforts to prevent it. In addition, wasp activity was low on many of the bolts and upon peeling them drill scars were absent, so there were several zeros in this data set. These issues thwarted the original research objectives, so data collection and analysis methods were modified and used to augment those from Experiment 1. We rated the amount of adventitious fungal growth on a scale of 0 to 3, with a rating of 0 indicating no contamination; 1 corresponding to trace amounts (< 1% of the area colonized); 2 indicating 1–50% of the area was colonized by these fungi, and a rating of 3 corresponding to >50% colonization. The three response variables, searching, drilling and drill scars, were converted to binary variables for subsequent statistical analyses.

*Data Analysis* In Experiment 1, the drill scar data from the untreated trees, as well as the insect behaviour data from treated and untreated trees, could not be transformed to meet the assumptions of parametric testing. Therefore, a G-test was used to compare data between treated and untreated trees (drill scars and behaviour types), and within the treated trees to compare the number of observed occurrences of each of the three behaviour types per treatment section. This test was based on the null hypothesis that the frequency of the occurrence of each event would be equally distributed between treated and untreated trees, or between treatment sections. To test the effect of treatment and field site on the number of drill scars in the treated trees, we used a nested Generalized Linear Model using the explanatory variables Treatment and Site (Treatment), with a Tukeys HSD post hoc test. To meet the assumptions of normality and homoscedasticity, drill scar data were transformed with $\log_n +1$ for the GLM. Removing the pruned trees from the analysis did not affect the statistical results so pruned trees were retained in all of the presented results. Mean reaction zone length ($\log_n$ transformed) per treatment was compared in the treated trees using a GLM with Tukeys HSD. Wood moisture was compared between treated and untreated trees with a pooled-variance *t*-test. Experiment 1 analyses were conducted in SYSTAT 13.0 (SYSTAT Software Inc, Chicago) (GLM, *t*-test), or calculated in Microsoft Excel (2003) (G-test). In Experiment 2, the probability of occurrence of searching, and drilling behaviours, and the presence of drill scars in response to the three explanatory variables fungal treatment (*A. areolatum*, *L. wingfieldii*, or control), bolt section (treated or untreated) and presence of adventitious fungi was tested with a multiple logistic regression model using R 2.10.1 (R-Foundation, Vienna). The alpha value was set to $p<0.05$ for all tests.

## Results

*Experiment 1* There were more deep drill scars found in treated trees than in untreated ones in both years (Tables 1 and 2). Within the treated trees, the number of deep drill scars made by *S. noctilio* females differed significantly between the fungal treatments in both years, but were unaffected by site (Table 2). There were

**Table 1** Mean (±SE) number of *Sirex noctilio* drill scars counted, and searching and oviposition behaviours observed, per section of fungus-treated and untreated *Pinus sylvestris* stem

| | Untreated Trees | | Treated trees | |
|---|---|---|---|---|
| Event | 2008 ($n$=23 trees) | 2009 ($n$=12) | 2008 ($n$=23) | 2009 ($n$=24) |
| Deep drill scars | 2.2±0.4 | 5.3±2.6 | 7.3±1.2 | 11.0±2.0 |
| Shallow drill scars | 3.4±0.4 | 0.8±0.2 | 4.8±0.6 | 3.1±0.4 |
| Searches | 3.9±0.4 | 1.8±0.5 | 6.0±0.4 | 2.0±0.2 |
| Probes | 1.0±0.2 | 0.2±0.1 | 0.5±0.1 | 0.3±0.1 |
| Drills | 1.0±0.2 | 0.1±0.1 | 1.8±0.2 | 1.1±0.2 |

fewer deep drill scars in the sections treated with *L. wingfieldii* than in sections treated with *O. minus* or left as untreated controls (Fig. 1). There was no effect of the aspect of the section (i.e. direction) on the number of deep drill scars in either year (Table 2).

When shallow drill scars were analyzed, a pattern similar to the deep drill scars was apparent. That is, there were more shallow drill scars in the treated than the untreated trees in both years, and within the treated trees there was a significant difference in the number of shallow drill scars between treatment sections in both years but no effect of site (Tables 1 and 2). There were fewer shallow drill scars in *L. wingfieldii* treated sections than either of the other treatment sections in 2009; in 2008 there were fewer scars in the *L. wingfieldii* treated section than the control (Fig. 2). There was no effect of the aspect of the section on shallow drill scars in either year (Table 2).

There were more searching visits in treated than in the untreated trees in both study years (2008: $G$=32.31, $P$<0.001; 2009: $G$=32.66, $P$<0.001) (Table 1). Within the treated trees, there were no differences in the number of visits observed per treatment section in either year (2008: $G$=1.86, $P$=0.39; 2009: $G$=3.32, $P$=0.20) (Fig. 3a–b). The number of probes was less in treated than untreated trees in 2008 ($G$=11.57, $P$<0.001) and more in 2009 ($G$=12.51, $P$<0.001) (Table 1). Fungus treatment had a significant effect on probing activity in 2008 ($G$=11.72, $P$=0.003) but not in 2009 ($G$=2.99, $P$=0.22) though there were few probing events witnessed in either year (Fig. 3a–b). There was more drilling activity observed in treated than untreated trees in both years (2008: $G$=18.62, $P$<0.001; 2009: $G$=77.28, $P$<0.001) (Table 1); in the treated trees drilling activity was not affected by fungus treatment in 2008 ($G$=3.71, $P$=0.16) but was in 2009 ($G$=9.83, $P$=0.007) (Fig. 3a–b).

Mean growth of *L. wingfieldii* was 6.0±1.9 mm in 2008 and 10.0±1.8 mm in 2009, and that of *O. minus* was 10.4±5.1 mm and 20.7±5.1 mm respectively. The inoculated fungal species were re-isolated from most of the fungus-treated sections in 2008; in 2009 *O. minus* was re-isolated from about half of the trees but *L. wingfieldii* was rarely re-isolated. In the treated trees, the mean reaction zone length in the *O. minus* treatment (43.0±2.6 mm) exceeded that in the *L. wingfieldii* (26.0±3.1 mm) and control sections (18.2±1.6 mm) ($F_{2,66}$=22.1, $P$<0.001). Results were similar in 2009: mean reaction zone length in the *O. minus* treatment (55.3±4.2 mm) exceeded that in the *L. wingfieldii* treatment (24.0±1.8 mm) which in turn exceeded the reaction zones in the control section (15.8±1.2 mm) ($F_{2,69}$=52.2, $P$<0.001). There

**Table 2** Statistical results (G-test and Nested GLM) for the number of *Sirex noctilio* drill scars found on live *Pinus sylvestris* in southern Ontario experimental sites. Comparisons between trees treated with inoculation of ophiostomatoid fungi and untreated trees; between fungus inoculation treatments in the treated trees (*Leptographium wingfieldii*, *Ophiostoma minus* or control); and between aspects of the treatment section (0–120°, 120–240°, 240–360° compass bearing)

| Response | Year | Treated vs. untreated trees[a] | Treatment section (Treated trees) | | Aspect of section (Treated trees) | |
|---|---|---|---|---|---|---|
| Deep drill scars (into sapwood) | 2008 | $G$=204.71, $P$<0.001 | Treatment | $F_{2,57}$=8.63, $P$=0.001 | Direction | $F_{2,57}$=0.50, $P$=0.95 |
| | | – | Site (Treatment) | $F_{9,57}$=1.97, $P$=0.06 | Site (Direction) | $F_{9,57}$=1.88, $P$=0.07 |
| | 2009 | $G$=394.65, $P$<0.001 | Treatment | $F_{2,63}$=22.29, $P$<0.001 | Direction | $F_{2,63}$=0.91, $P$=0.41 |
| | | – | Site (Treatment) | $F_{6,63}$=0.67, $P$=0.67 | Site (Direction) | $F_{6,63}$=0.42, $P$=0.86 |
| Shallow drill scars (into phloem) | 2008 | $G$=15.99, $P$<0.001 | Treatment | $F_{2,57}$=3.81, $P$=0.03 | Direction | $F_{2,57}$=0.87, $P$=0.42 |
| | | – | Site (Treatment) | $F_{9,57}$=2.04, $P$=0.05 | Site (Direction) | $F_{9,57}$=2.51, $P$=0.02 |
| | 2009 | $G$=177.73, $P$<0.001 | Treatment | $F_{2,63}$=12.15, $P$<0.001 | Direction | $F_{2,63}$=0.24, $P$=0.78 |
| | | – | Site (Treatment) | $F_{6,63}$=1.45, $P$=0.21 | Site (Direction) | $F_{6,63}$=1.21, $P$=0.31 |

$n$=23 treated trees in 2008 and 24 in 2009; 23 untreated trees in 2008 and 12 in 2009

[a] Treated exceeded untreated in all cases

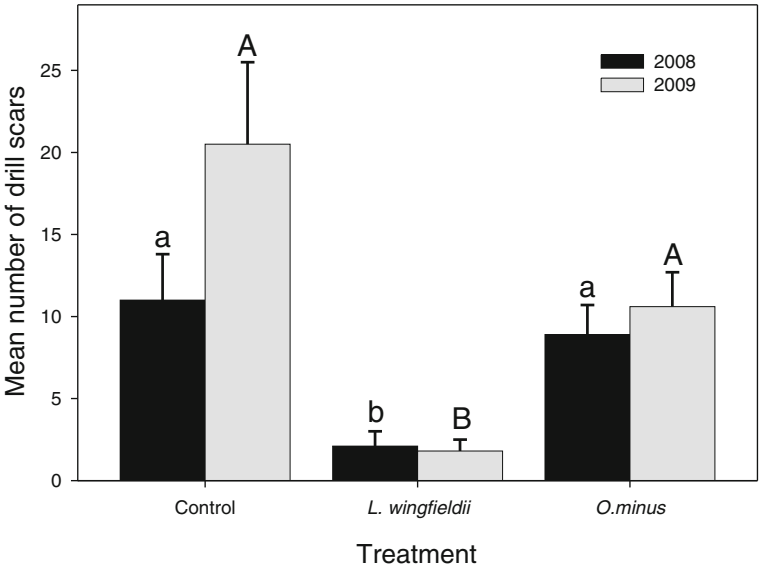


**Fig. 1** Mean number (+SE) of *Sirex noctilio* drill scars extending into sapwood (deep drill scars) per treatment section (untreated control, *Leptographium wingfieldii*, *Ophiostoma minus*) in living *Pinus sylvestris*. Results of Tukey's HSD test: lower case letters indicate significant differences between treatment groups in 2008 and upper case in 2009. $n$=23 trees in 2008 and 24 trees in 2009

was no significant difference in wood moisture between the treated (2008: 85%; 2009: 47%) and untreated trees (2008: 95%; 2009: 51%) in either year (2008: $t_{44}$= 1.43, $P$=0.16; 2009: $t_{44}$=0.86, $P$=0.39).

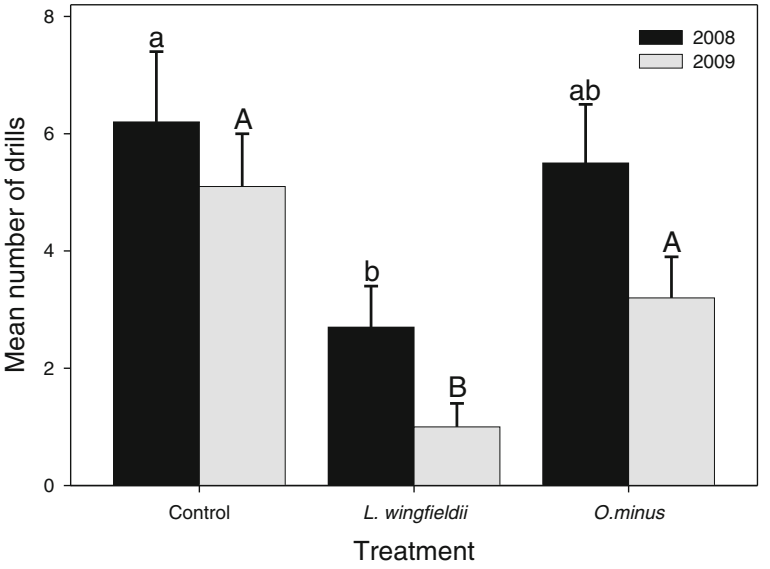


**Fig. 2** Mean number (+SE) of *Sirex noctilio* drill scars extending into phloem (shallow drill scars) per treatment section (untreated control, *Leptographium wingfieldii*, *Ophiostoma minus*) in living *Pinus sylvestris*. Results of Tukey's HSD test: lower case letters indicate significant differences between treatment groups in 2008 and upper case in 2009. $n$=23 trees in 2008 and 24 trees in 2009

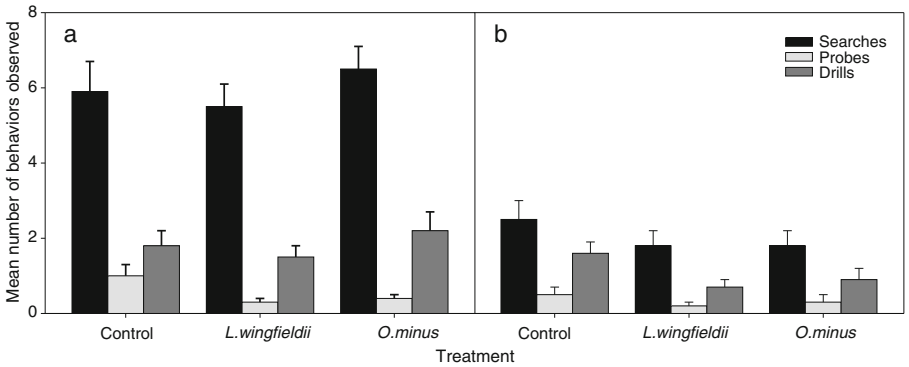


**Fig. 3** Mean number (+SE) of search visits, probes or drills by *Sirex noctilio* per treatment section (untreated control, *Leptographium wingfieldii*, *Ophiostoma minus*) of living *Pinus sylvestris* trees in a) 2008 and b) 2009. $n$=23 trees in 2008 and 24 trees in 2009

*Experiment 2* The presence of *L. wingfieldii*, treated side of the bolt (vs. control), and the degree of adventitious fungal colonization all affected the probability of the presence of drill scars in the logistic regression model (Table 3); all three variables

**Table 3** Logistic regression results for the effect of inoculated fungus (*Amylostereum areolatum*, *Leptographium wingfieldii* or control) ($n$=30 bolts each treatment), treated vs. control side of log, and the degree of adventitious fungal growth, on *Sirex noctilio* searching and drilling behaviours, and drill scars on *Pinus sylvestris* bolts; significant results bolded

| Behaviour | Model term | Estimate | Std. error | z value | *P* | Odds ratio | Confidence interval |
|---|---|---|---|---|---|---|---|
| Search | Intercept | −0.72 | 0.47 | −1.53 | 0.13 | 0.49 | 0.19–1.21 |
| | Fungus *A. areolatum* | 0.45 | 0.38 | 1.17 | 0.24 | 1.56 | 0.74–3.33 |
| | Fungus *L. wingfieldii* | 0.03 | 0.39 | 0.07 | 0.94 | 1.03 | 0.48–2.21 |
| | Treated | 0.19 | 0.31 | 0.61 | 0.54 | 1.21 | 0.66–2.21 |
| | Adventitious fungus | 0.41 | 0.21 | 0.94 | 0.05 | 1.50 | 1.00–2.29 |
| Drill | Intercept | −0.36 | 0.53 | −0.67 | 0.50 | 0.70 | 0.24–1.98 |
| | Fungus *A. areolatum* | 0.36 | 0.43 | 0.85 | 0.40 | 1.44 | 0.62–3.38 |
| | Fungus *L. wingfieldii* | −1.42 | 0.53 | −2.67 | **0.008** | 0.24 | 0.08–0.66 |
| | Treated | 0.77 | 0.38 | 2.01 | **0.04** | 2.16 | 1.03–4.68 |
| | Adventitious fungus | −0.66 | 0.26 | −2.60 | **0.009** | 0.52 | 0.31–0.84 |
| Drill scar | Intercept | 1.28 | 0.52 | 2.47 | **0.01** | 3.61 | 1.33–10.34 |
| | Fungus *A. areolatum* | −0.11 | 0.40 | −0.26 | 0.79 | 0.90 | 0.41–1.98 |
| | Fungus *L. wingfieldii* | −1.64 | 0.47 | −3.50 | **<0.001** | 0.19 | 0.07–0.47 |
| | Treated | −0.87 | 0.35 | −2.50 | **0.01** | 0.42 | 0.21–0.82 |
| | Adventitious fungus | −0.72 | 0.24 | −3.00 | **0.003** | 0.49 | 0.30–0.77 |

were associated with the absence of drill scars. The size of the treatment effect was greatest for *L. wingfieldii*; bolts treated with this fungus were one-fifth as likely to have drill scars as those having the other treatments. Treatment sections and bolts with adventitious fungi were about half as likely to have them as control sections and bolts without adventitious fungi. There were no shallow drills found on the bolts.

Searching was not predicted by any of the tested variables although the presence of adventitious fungi approached significance (Table 3). The presence of *L. wingfieldii* and adventitious fungi both had a similar negative relationship and magnitude of effect on drilling activity as it did on drill scars (Table 3). In contrast to the drill scar results, treatment had a positive relationship with drilling activity, which was twice as likely to occur on treated sections of the bolt as on untreated ones. There was no probing activity evident on the cut bolts.

Inoculum growth varied between the two fungal species. There was minimal growth of the *A. areolatum* inoculum, on average 2.1±0.3 mm per inoculation point. Mean *L. wingfieldii* growth was 83.0±4.4 mm. *Amylostereum areolatum* was never re-isolated from the bolts and *L. wingfieldii* was only occasionally re-isolated. Mean colonization by adventitious ophiostomatoid fungi was classified as 1–50% in the *A. areolatum* and control bolts, and <1% in the *L. wingfieldii* treated ones.

## Discussion

Experiments 1 and 2 showed similar results; there were fewer *S. noctilio* drill scars in sections of trees or bolts inoculated with *L. wingfieldii* than other sections. Both probing and drilling activity, though not always significant, followed similar patterns. This is of considerable reproductive benefit to the wasp because *L. wingfieldii* outcompetes the wasp's symbiont, *A. areolatum*, for resources (Ryan et al. 2011a). Thus if the female selected these areas for oviposition it could inhibit the development of the wasp offspring.

The lack of effect of *O. minus* on the number of wasp drill scars in Experiment 1 in contrast to its avoidance of *L. wingfieldii* was surprising especially since there was a lack of wasp drilling associated with the presence of adventitious fungi in Experiment 2. Similar to *L. wingfieldii*, *O. minus* outcompetes the wasp's symbiont *A. areolatum* (Ryan et al. 2011a). The wasp is likely to have interacted with both fungal species in its native range in Eurasia since they are both regular associates of *T. piniperda* there (reviewed in Kirisits 2004), and so have had the opportunity to evolve the ability to detect both species. One explanation is that there may have been volatiles emitted from the extensive defensive reaction zones in the *O. minus*-inoculated-sections that overwhelmed volatiles emitted by *O. minus* itself, thereby masking volatiles from the fungus. There are a number of volatile metabolites associated with ophiostomatoid fungi (reviewed in Hanssen 1993) and they differ from those associated with pine response to injury (Cheniclet 1987). Wound response associated compounds include alpha-pinene and 3-carene (Cheniclet 1987; Manninen et al. 2002) and both are known attractants to *S. noctilio* (Simpson 1976). In pines inoculated with fungal pathogens, terpenes associated with the wound accumulate in especially large amounts and are persistent (Cheniclet 1987) and therefore could be expected to affect the wasp's oviposition behavior at the time of the experiment. Since Experiment 2

was conducted on cut bolts and there was no associated resinous reaction, the adventitious fungi should be more readily detected by the wasps than the *O. minus* in Experiment 1.

The wasps showed a preference for treated over untreated trees for searching, probing and drilling, and there was a corresponding pattern in drill scars. Though the treated trees contained a fungal species that was avoided by the female wasps, these trees were probably more stressed than the untreated ones because of the inoculations of fungal pathogens, and were emitting stress volatiles that were attractive to the wasp (Madden 1968). In addition, attractive volatiles emitting from the extensive reaction zones at the inoculation sites may have stimulated an increase in searching and drilling activity.

Fungal growth was more limited than expected in Experiment 1. The density of inoculations was expected to overwhelm the tree's defenses and result in growth of the inoculated fungi from most of the inoculation points. Weather conditions during the two study periods were generally favorable (Environment Canada n. d.), so environmental stress is expected to have been relatively low during the experiment, enhancing the tree's ability to resist fungal colonization (Schoenweiss 1981; Smalley et al. 1993). The extensive reaction zones in the fungus-inoculated treatment sections suggest that the trees were able to respond to, and contain, the pathogen at most of the inoculation sites. Re-isolation of these fungi in 2008 suggests that they were present in a quiescent state and therefore could have been detectable by the wasps; conifers are known to sequester viable fungal inoculum within reaction zones (Raffa and Smalley 1988). Although fungi were rarely re-isolated in either experiment in 2009, overall results (reaction zones, insect behavior and drilling) were similar to 2008 when the inoculated fungi were re-isolated so it is expected that the lack of re-isolation was not due to the absence of the fungi.

This study is the first to demonstrate that *S. noctilio* avoids areas of trees colonized by at least one species of bark beetle-vectored fungus and clearly shows that interactions between *S. noctilio* and bark beetles could occur via interaction with fungal associates of beetles. These interactions could influence the selection of oviposition sites by the woodwasp and limit its colonization in areas of the tree previously colonized by beetles carrying these fungi. Competition with beetles for uncolonized host resources could be a factor in helping to limit *S. noctilio* populations in its native Eurasian range and, given the well established populations of bark and woodboring beetles in its introduced range in North America, could do so here as well.

Investigation of the reproductive success of *S. noctilio* that do oviposit in the presence of ophiostomatoid fungi is necessary to clarify the effect of subcortical beetles on the population dynamics of the woodwasp. A more extensive survey of the response of *S. noctilio* to other ophiostomatoid species such as the fungal associates of *Dendroctonus ponderosae* and *Dendroctonus frontalis* is also warranted. As the woodwasp moves into new ranges in North America, interactions with these species may have a significant affect on the wasp's population dynamics. The investigation of the volatile profiles of repellant fungal species may also provide more insight into the interaction of the wasp with the woodborer community. The extent of the woodwasp's response to these fungi would provide insight into the effect that this

phenomenon may have on wasp populations, i.e. does the wasp abandon the whole tree if a repellant fungus is present?

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