

Sirex noctilio flight behavior: toward improving current monitoring techniques

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Abstract

Sirex noctilio Fabricius (Hymenoptera: Siricidae), an invasive woodwasp from Spain and North Africa, has an expanding global presence in pine forests. Although their populations are managed in most parts of the world, the success rate of existing prevention and control strategies has been historically variable, and there is consensus that monitoring techniques could be improved with increased knowledge about the behavioral biology of this insect. We conducted descriptive field studies in pine plantations in northern Patagonia to establish the flight behavior of *S. noctilio*. Specifically, the aim was to characterize male and female vertical flight distributions to assess ideal trap placement in pest monitoring programs. Our results indicated that males fly higher than females. A post-hoc analysis revealed two distinct flight patterns of females; when males are present, females fly higher than when males are absent. This behavioral variance is likely linked to (1) oviposition substrate location and (2) mate location. Results suggest that the most effective trapping and monitoring strategies for females should be deployed at 1.8 m height, whereas monitoring of males or females that are searching for mates would occur at greater heights. Results also suggest that if future techniques involving pheromones are developed, trap placement should be reassessed.

Introduction

The woodwasp *Sirex noctilio* Fabricius (Hymenoptera: Siricidae) is the only species in the Siricidae family capable of killing trees (Spradbery, 1973) and, as such, is a major threat to pine forests throughout the world. Native to Europe and northern Africa, in the past century it has invaded Oceania, Africa, South America, and more recently North America. Tree mortality occurs when female wasps lay eggs in stressed pines (e.g., crowded, suppressed, or damaged) together with a phytotoxic mucus and spores of a symbiotic fungus. Larvae initially feed on the fungal hyphae and at later stages also feed on degraded wood (Madden & Coutts, 1979). Pupation generally occurs after 1 or 2 years, with developed males emerging from trees slightly before females during the flight season (Morgan & Stewart,

1966). Once emerged, females may or may not mate but begin flight in search of suitable oviposition substrates (i.e., new trees). Adult wasps do not feed, relying entirely on reserves accumulated during larval development (Ryan & Hurley, 2012).

Management of *S. noctilio* generally involves (1) prevention of establishment, and (2) wasp population suppression. The best means to prevent establishment usually involves silvicultural practices which minimize the proportion of stressed trees (= available hosts) within a stand (Villacide & Corley, 2012). The use of a parasitic nematode (*Deladenus siricidicola* Bedding) and parasitoids (*Ibalia leucospoides* Hochenwarth and *Megarhyssa nortoni* Cresson) are the basis of biological control techniques used to suppress established *S. noctilio* populations. Despite the variety of measures applied, *S. noctilio* continues to be a significant threat and cause of economic losses to pine plantations worldwide. For instance, due to a combination of different factors (e.g., environment and rearing techniques), the use of the nematode has had limited success in some South African regions (Hurley et al., 2008). In

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Argentina, despite widespread establishment of the nematode and parasitoids, outbreaks of *S. noctilio* continue to be observed. One challenge to effective management is our limited ability to detect low-density *S. noctilio* populations, such as those found in endemic populations or in recently invaded stands. Control of incipient *S. noctilio* populations would likely be more efficient than controlling them once they have reached outbreak levels, but monitoring low-density populations is difficult. In this sense, by expanding our knowledge of *S. noctilio* ecology and behavior, the potential exists for improving monitoring techniques (Rodríguez-Saona & Stelinski, 2009; Witzgall et al., 2010; Nadel et al., 2012).

Although many aspects of *S. noctilio* biology and ecology are well known (Slippers et al., 2012), further knowledge of wasp behavior, especially flight patterns, could improve current monitoring techniques. For instance, monitoring and control traps (baited or unbaited) need to be positioned in such ways that capture rates are maximized (Byers, 2011, 2012); this is critical for detecting rare insects, which would be expected for incipient populations.

We conducted descriptive field assays to establish the flight behavior of *S. noctilio*. In this species, flight is primarily associated with two activities: (1) mate location and (2) finding suitable oviposition sites (females only). To assess flight patterns, unbaited sticky traps were placed in vertical transects in the forest to determine the flight height of male and female wasps within *Sirex*-infested plantations. We hypothesize that female flight will be evenly, vertically distributed when searching for oviposition sites as this would match resource availability (i.e., the whole length of the tree bole, which is known to be utilized for oviposition; Madden, 1974). We also predict that males will be found higher in our traps than females, as previous studies showed that males aggregate in the upper canopy, forming leks for mating (Madden, 1988; Cooperband et al., 2012). Previous field observations suggest that copulation takes place in the upper canopy (Madden, 1988; Cooperband et al., 2012), therefore we predict that females searching for a mate will fly higher than those searching for oviposition sites.

Materials and methods

Study sites and trapping technique

The study was conducted in three Patagonian pine plantations ranging from 10 and 14 ha in size, each with trees between 25 and 35 years old, and consisting of mixed *Pinus contorta* Douglas and *Pinus ponderosa* Douglas (Pinaceae) affected by *S. noctilio* (site A: 42°32'28.32"S, 71°26'17.16"W; site B: 41°12'49.68"S, 71°13'27.12"W; site

C: 41°0'42.12"S, 71°7'59.88"W). Two types of unbaited traps were deployed from February through April 2013, during the *S. noctilio* flight period. Traps were inspected for captures every fortnight for the duration of the sampling period.

Flight behavior: sticky traps

A total of 28 sticky traps (0.4 m wide, 6 m high; Figure 1) were placed in the three sites (10 traps at site A; nine traps each at sites B and C) to determine wasp vertical flight patterns. Traps were made of black plastic netting (1 cm mesh) covered with sticky material (Tute; Envasadora Caseros, Buenos Aires, Argentina). Traps were randomly distributed within each plantation with a minimum distance of 20 m between traps and 20 m from the plantation edge. Each trap was hung at 6 m height in between two trees separated by ca. 2 m (Figure 1). In this way flying wasps would be intercepted by the hanging structure and individuals would stick to the trap. Branches along the main stems were trimmed so that traps hung freely. To avoid being moved by wind, traps were secured to trees with ropes. A pulley was secured on each tree so the trap could be lowered and checked for captured insects and then raised again. The stickiness of the material was such that wasps remained in the initial position where the interception occurred. The following variables were recorded for each *S. noctilio* captured: trap ID number, date, height of capture, sex, and size (thorax width).

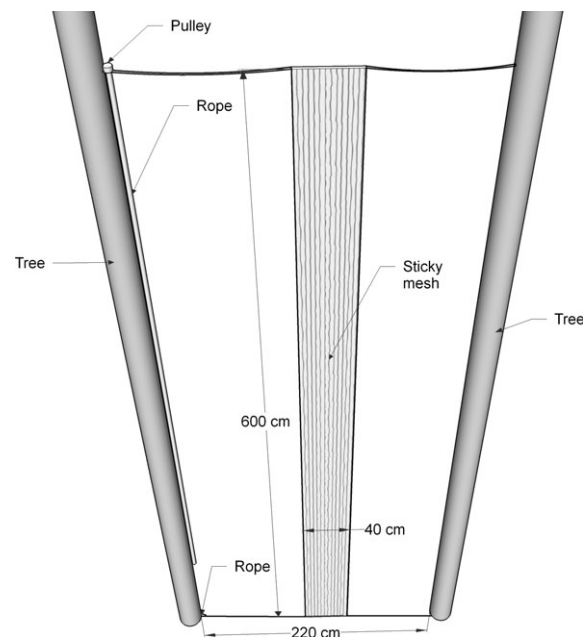


Figure 1 Schematic representation of sticky traps used to assess the vertical flight of *Sirex noctilio* males and females.

Sex ratio measurements: emergence traps

To obtain a sex ratio estimate of emerging wasps, which represent the population of flying wasps that could be caught in the sticky traps, a second type of trap was designed and deployed (Figure 2). At each site, 10 trees that had been attacked the previous year (indicated by fresh resin beads on the trunk) were selected at random and branches removed between 1 and 2.2 m from the ground. This section of the main stem was surrounded with a 1.2-m wide screen (2 mm mesh) separated from the trunk by eight iron nails (length 12.7 cm) positioned at regular intervals on the circumference of the stem, with a piece of wire joining each nail head and the tops and bottoms secured against the trunk with wire. In this way, a 1-m section of the trunk was 'sealed' and all wasps emerging from the tree stem were trapped in the space between the trunk and the mesh. For each wasp that emerged, we recorded capture date and sex.

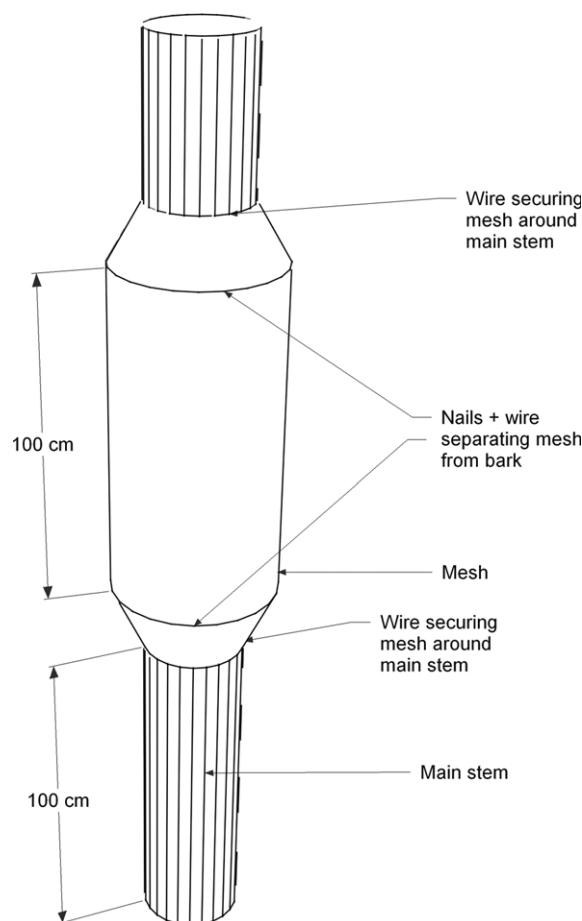


Figure 2 Schematic representation of emergence traps placed on trees to assess *Sirex noctilio* sex ratio.

Data analysis

Data for all traps within a site were combined and summed across the season of sampling. Response variables were flight height and number of captured wasps, and explanatory variables were study site, date, sex, size, and presence of males in traps. As vertical flight data were not normally distributed, data were analyzed using non-parametric statistics. Contrasts within capture heights and number of captures were compared via Mann–Whitney–Wilcoxon tests (one-way test with χ^2 approximation). Multiple comparisons were made with Z statistics from the Wilcoxon method.

A post-hoc analysis revealed an effect of captured males on female flight height. Therefore, we compared capture heights of females in traps with and without males by using the mean capture height of all females collected in each trap per fortnight (R programme, version 3.0.1; R Foundation for Statistical Computing, Vienna, Austria).

Results

Across all sites, a total of 10 males and 183 females (sex ratio = 0.05) were captured in 27 of the sticky traps (one trap did not capture any individuals). The mean (\pm SE) number of captures throughout the period was 7 ± 2 wasps per trap per fortnight. In emergence traps, a total of 39 males and 22 females were captured (sex ratio = 1.77).

The average flight height for females was lower than for males (females: 249 ± 10 cm, males: 394 ± 26 cm; $\chi^2 = 10.1$, d.f. = 1, $P < 0.01$; Figure 3). Female flight height

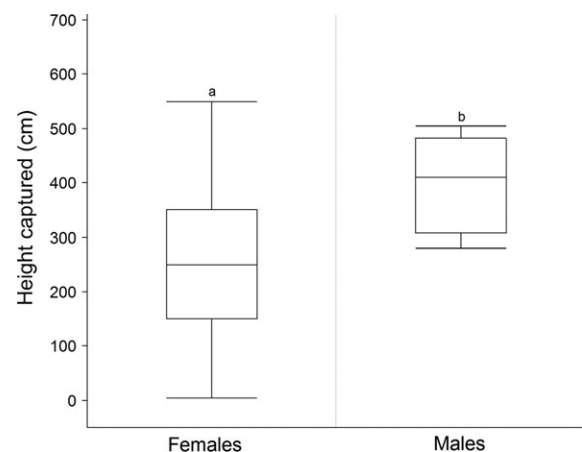


Figure 3 Capture height for all female and male *Sirex noctilio* in sticky traps. Females were captured throughout the height of the trap while males were captured higher. For each box, the top and bottom indicate the interquartile range, and the line across the middle indicates the median value. Error bars indicate the range of the data. Different letters above the boxes denote a statistical difference between the sexes (one-way Mann–Whitney–Wilcoxon test with χ^2 approximation: $P < 0.01$).

was not different between the three study sites ($\chi^2 = 3.58$, d.f. = 2, $P > 0.05$). Male flight pattern was not compared between sites because of low capture numbers.

In sticky traps where both males and females were captured, a marginally higher flight was observed for females captured in traps together with males than in traps with no males present (279 ± 30 vs. 223 ± 15 cm; $Z = 1.84$, $P = 0.066$; Figure 4). In addition, on average, 4× more females were captured in traps that contained males than in traps where no males were present (9 ± 2.8 vs. 2.2 ± 0.3 ; $\chi^2 = 14.2$, d.f. = 1, $P < 0.001$).

Female flight height was concentrated at lower heights and best fitted a generalized β distribution. This special type of β distribution has two additional parameters assigned as endpoints to the interval sampled (we used a scale parameter of 600 and lower threshold of 0). The

observed distribution depended on the presence of males. Distribution parameters for all females captured (Figure 5A) were: $\alpha = 1.39$, $\beta = 1.75$, Kolmogorov's goodness-of-fit test: $D = 0.084$, mode = 205 cm, $P > 0.14$ (where $P > 0.05$ indicates a good fit). When females were captured in traps without males (Figure 5B), the flight distribution moved to lower altitudes: $\alpha = 1.69$, $\beta = 2.63$, $D = 0.088$, mode = 178 cm, $P > 0.25$.

Throughout the sampling period, there was no temporal variation in female flight height in either traps with or without males (all females captured: $\chi^2 = 8.5$, d.f. = 10, $P > 0.05$; females captured without males: $\chi^2 = 8.44$, d.f. = 10, $P > 0.05$). In addition, no correlation was found between flight height and wasp size for females ($y = 386 - 0.38x$, $R^2 = 0.05$) or males ($y = 339 + 0.18x$, $R^2 = 0.03$).

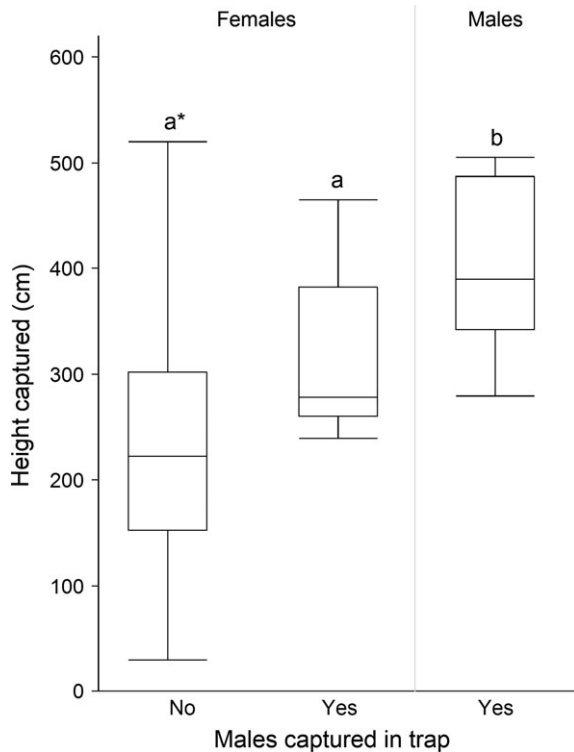


Figure 4 Flight height of female *Sirex noctilio* captured in traps that did or did not have males present. Female wasps were captured slightly higher in traps with males present than in traps with no males. Male flight was higher than female flight. For each box the top and bottom indicate the interquartile range, and the line across the middle indicates the median value. Error bars indicate the range of the data. Different letters above the boxes denote statistical differences between treatments (Wilcoxon Z statistics: $P < 0.05$; the asterisk marks a marginal statistical difference: $P = 0.07$).

Discussion

This is the first report, to our knowledge, that quantifies vertical flight paths for both male and female *S. noctilio*. Sticky trap captures resulted in a relatively high number of individuals captured, albeit female biased. Male flight tended to be higher in the canopy than female flight, and when males were present in traps, female flight was higher than when males were absent. It is likely that the sticky traps failed to capture many higher flying males, as evidenced by the higher proportion of males found in emergence traps (sex ratio = 1.77) compared to sticky traps (sex ratio = 0.05). These findings agree with previous field observations, which reported that after emergence from tree trunks, males fly to the upper can-

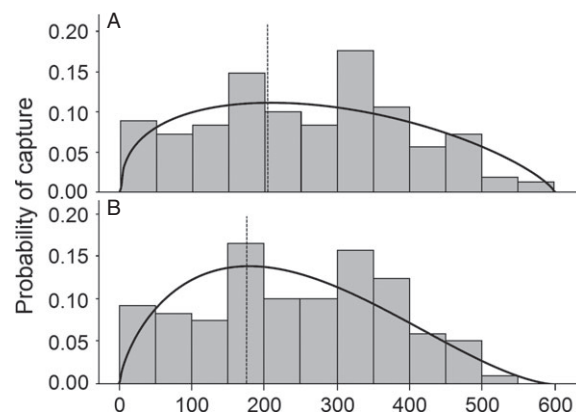


Figure 5 Frequency distribution of capture heights of (A) all females ($n = 183$) and (B) females in traps without males ($n = 122$). Both distributions are right-skewed and best represented by a generalized β distribution. Continuous lines represent the distributions and dotted lines the modes.

opy to mate, suggesting that most males do not perform horizontal flights (at least within the 0–6 m vertical layer) (Madden, 1988). It is also worth noting that the sex ratio calculated from our emergence traps probably underestimates the proportion of males, because past studies suggest that *S. noctilio* sex ratio can vary with tree height, with proportionally more males emerging above 2 m (Spradbery & Kirk, 1978).

Results also indicate that females fly across the vertical space sampled following an asymmetrical distribution, with more females found to be flying at lower altitudes than higher ones. Previous studies have analyzed the vertical flight distribution of other insect species and observed that generally a normal distribution best fits vertical flight patterns (Byers, 2011). But in these cases individual baited traps were placed at discrete heights, hence the vertical space was not covered continuously, offering less resolution than this study.

Interestingly, there is a marginal difference in the flight pattern of females that were captured on traps with males, when compared to females found in traps without males. This difference, albeit small, could be attributed to two distinct behaviors occurring at different flight layers: one related to the search of oviposition substrates and a second one influenced by males (i.e., probably via pheromones; Böröczky et al., 2009; Cooperband et al., 2012). When the flight pattern is analyzed only for those females captured in traps without males, the right-skew becomes even more pronounced (i.e., more females flying even lower), with the distribution reaching the maximum frequency at ca. 1.8 m (Figure 5B). Previous work has indicated that females oviposit over most of the tree height, suggesting once arriving at a suitable tree, females walk upwards, thus covering the entire substrate that is available (Madden, 1974). These results suggest that traps targeting females should be placed at 1.8 m to maximize captures. Although not tested here, one aspect to consider is that the flight pattern observed in this study could be affected by habitat structure, as observed in the lesser peachtree borer, *Synanthedon pictipes* Grote & Robinson, where the efficiency of traps varied with tree height (Cottrell et al., 2008). In the case of *S. noctilio*, the flight distribution parameters could shift with plantation characteristics (i.e., tree height, density, pruning). Coincidentally, the height of 1.8 m is in agreement with the ‘*Sirex noctilio* trapping protocols’ manual (Forest Service US Department of Agriculture, 2008) that recommends placing baited traps at least at this height, although no explanation as to why this height should be used is provided, and it may have been selected for ease of hanging.

The increased number of captures and higher flight of those females that were found in traps with males could be

the result of males releasing a pheromone eliciting an attraction response in females. *Sirex noctilio* mating behavior has been described as males and females, upon emergence, meeting in groups at the top of trees where mating leks form on the higher branches (Madden, 1988). The main mechanism proposed, until recently, was through a photopositive response, but recently a short-chained alcohol with electrophysiological and behavioral activity in both males and females, has been identified and is thought to mediate the lekking behavior (Cooperband et al., 2012). The exact mechanism behind *S. noctilio* mate location remains unknown, but it is possible that location is regulated via a male-produced pheromone. With respect to this behavioral observation, our results are not conclusive, mainly because of the low number of males captured in our sticky traps, rendering weak statistical power for comparisons, but this study would suggest that a male-produced pheromone could attract females to leks. It is important to note that although males tend to emerge ahead of females, there still could have been females arriving to our sticky traps ahead of males, thus increasing the variance of these data. To arrive at concluding results in this respect, further field and laboratory bioassays are needed.

This study suggests that focusing trapping and any behavioral modification strategies toward females could be more fruitful than toward males, as male behavior is primarily restricted to the top of the canopy, and females fly across the whole vertical section with a bias toward the lower sections of the forest, where monitoring traps would be placed with a minimal effort and cost. In addition, when utilizing traps baited with volatile compounds that mediate oviposition site searching behavior, such as pine volatiles, a height of 1.8 m is recommended (although forest structure could affect ideal trap height). If future techniques that involve sex pheromones are developed, trap placement should be re-evaluated carefully because flight related to mating seems to occur higher and could affect ideal trap placement.

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