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Parasitism and dispersal potential of *Sirex noctilio*: implications for biological control

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Abstract

1 *Sirex noctilio F.* (*Hymenoptera: Siricidae*) is a wood-boring wasp that attacks many pine species, including commercial trees planted throughout the world. Management of its populations is largely based on biological control using the nematode *Beddingia siricidicola*. Adult females are sterilized by the nematode, but are free to move and attack new trees, promoting nematode dispersal. Although generally successful, wasp management through nematode introductions has sometimes been inadequate.

2 We evaluated the effect of parasitism by *B. siricidicola* on flight performance of woodwasps under laboratory conditions. Using flight mills, we recorded a total of 46 flight trials over 23 h, obtained from infected and control (uninfected) females.

3 Although all wasps lost weight during flight, parasitized females were significantly smaller and suffered larger weight losses than uninfected females. In addition, total flight distance and velocity were lower in parasitized females.

4 Because nematode infection transmission relies on healthy wasps attacking trees previously visited by nematode-bearing females, differential dispersal capacity could limit biological control success.

Keywords *Deladenus siricidicola*, dispersal, entomophagous nematodes, flight mills, host-parasite interactions, insect flight performance.

Introduction

The woodwasp *Sirex noctilio* Fabricius (Hymenoptera: Siricidae) is a phytophagous insect that successfully attacks many pine species, including commercial trees planted throughout the northern and southern hemispheres. Woodwasp females lay eggs in the sapwood, usually of stressed trees, during the summer months and larvae grow and develop inside the wood throughout the rest of the year. The larvae create galleries in the sapwood and feed on decomposed wood and hyphae of a symbiotic fungus *Amylostereum areolatum* (Basidiomycotina: Corticiaceae) (Coutts, 1969).

Native to Eurasia and North Africa, *S. noctilio* has recently invaded several other regions of the world such as Australasia (Madden, 1988), South America (Iede *et al.*, 1988), South Africa (Tribe, 1995) and North America (Hoebeke *et al.*, 2005). Partly because *S. noctilio* is an invasive forest insect, it has become one of the most economically significant pests of softwood afforestation in most invaded areas (Madden, 1988; Hurley *et al.*, 2007).

Because *S. noctilio* populations can reach epidemic levels causing significant economic damage to pine plantations, much effort has been focused on successfully managing populations. Integrated pest management programmes have included timely silvicultural treatments of forests at risk and the introduction of biological control agents such as the parasitoids *Ibalia leucospoides* (Hymenoptera: Ibaliidae), *Rhyssa persuasoria* and *Megarhyssa nortoni* (Hymenoptea: Ichneumonidae), and the parasitic nematode *Beddingia* (=*Deladenus*) *siricidicola* (Tylenchida: Neotylenchidae) (Neumann & Minko, 1981; Haugen *et al.*, 1990; Haugen & Underdown, 1993; Villacide & Corley, 2006).

Beddingia siricidicola is the biocontrol agent most widely used in classical biological control aimed at reducing pest populations below damaging levels (Bedding & Akhurst, 1974; Lacey *et al.*, 2001). The nematode is cultivated on the fungus *A. aerolatum* in mass rearing laboratories and inoculated into selected, felled Sirex-infested trees in the field. In many cases, especially when wasps are at endemic population levels, artificially stressed trap trees are used to enhance

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and concentrate adult wasp attacks. Nematodes suspended in a gelatin solution are then introduced into trees through holes made by a punch mounted on a special hammer (Bedding & Iede, 2005).

The life history of *B. siricidicola* involves female dimorphism, associated with a free-living mycetophagus and a parasitic life cycle (Bedding, 1967). During oviposition, *S. noctilio* females inject spores of the symbiotic fungus on which juveniles and adult nematodes feed. However, some fertilized juvenile females may switch to a parasitic cycle and locate and penetrate woodwasp larvae. Within *S. noctilio* females, juvenile nematodes can suppress ovarian development, reducing wasp fecundity (Bedding, 1972, 1984). Parasitized *S. noctilio* females may still display oviposition behaviours. Thus, infected wasps will lay eggs filled with nematodes into new susceptible trees (Bedding, 1984; Bedding & Iede, 2005). Nematodes spreading within the tree may parasitize eggs laid by healthy females in the same tree, thus ensuring the transmission on the infection among wasps.

In addition to suppressing ovarian development, *B. siricidicola* can affect hosts in another two ways. First, mycetophagous nematodes can compete with host larvae for *A. areolatum*; when they occur simultaneously within the tree if nematode densities are high, competition may retard host development. Second, nematode infection can reduce adult host fat reserves and modify their metabolic function (Bedding, 1979, 1984; Bedding & Iede, 2005).

The impact of parasites on their host populations has been frequently evaluated in terms of its direct influence on host fitness (i.e. fecundity or survival; Tompkins & Begon, 1999). This is particularity important for the biological control of insect pests using entomophagous parasites. However, fewer studies have evaluated the influence of parasitic infection on other characteristics such as host behaviour or host dispersal potential (Bradley & Altizer, 2005). Note that individual insect movement is important in determining population dispersal and resulting spatial dynamics. For *S. noctilio* in particular, the effects of *B. siricidicola* parasitism on wasp dispersal and behaviour can affect the rate of spread of the infection within the host population and, consequently, success in managing populations.

In the present study, we evaluated the effects of parasitism by the nematode *B. siricidicola* on the flight capacity of its host, the woodwasp *S. noctilio*. In addition to affecting fecundity, the parasite may consume host resources during larval and adults stages; therefore, our working hypothesis was that parasitism will negatively affect the flight performance of *S. noctilio* females. As a result, we expected a reduction in flight velocity and a reduction in potential flight distance in parasitized females.

Materials and methods

To study the effects of parasitism by *B. siricidicola* on flight characteristics of *S. noctilio*, we used a set of five flight mills based on the model of Schumacher *et al.* (1997), fixed to a rectangular polypropylene tubular structure (30 × 15 cm). A vertical steel needle in the centre of this structure, supported by the magnetic field generated by two magnets, acted as the axle to which a short, light wooden horizontal rod was attached. Wasps were then attached to one end of the balanced wooden rod through the entomological pin. Each flight mill was mounted in a hardwood, square box with a Perspex transparent roof to minimize external odours and air fluxes and to allow overhead lighting. On the internal walls, white and black vertical stripes were drawn to stimulate flight. Overhead lighting was powered by a 40-W lamp, controlled by an analog timer set for an LD 16 : 8 h photoperiod.

The computer interface of the flight mill was an external board powered by a 4.5-V power battery. The circuit board had two inputs to connect optical sensors, and a parallel port output to plug into a computer. A specialized Python programme was designed to collect data from the interface board. A rotation of the wooden rod was recorded every time the beam of light generated by a light-emitting diode was interrupted at the end opposite to the one where the insect was held. For each flight mill, the programme recorded every rotation, calculated its speed and accumulated flight distance.

We collected 1-m long billets from several *Pinus contorta* var. *latifolia* trees recently attacked by *S. noctilio* (i.e. the previous flight season) from plantations located in north-west Patagonia (Argentina). At these sites, *B. siricidicola* (Encruzilhada do Sul strain) had been introduced repeatedly in the past and, therefore, all trees chosen for logging were naturally infected by nematodes. Billets were held for 3–4 months in individual, locker-type cages under semi-controlled laboratory conditions. For this study, we used recently emerged female *S. noctilio* wasps (24–48 h) collected from the cages.

Each female to be used was anaesthetized using CO_2 to allow fixing a pin to the back of the thorax. The head of a pin was first fixed to a plastic bead with a two component adhesive to increase its surface area and, once dry, attached to a wasp with fast-drying glue.

Before and after flight, each female was weighed (Scientech SA210; Scientech, Boulder, Colorado; to the nearest 0.0001 g.). We recorded the accumulated flight distance (km) and flight speed (m/s) over a period of 23 h. Infection status was determined after flight by dissecting wasps and inspecting their abdomen under a stereo-microscope. Body mass loss during flight was calculated as log (mass loss)/initial mass, where mass loss = initial mass – final mass (Bradley & Altizer, 2005).

We used one-way analysis of variance to test for differences in flight speed, total flight distance, initial body mass and weight loss between infected and uninfected wasps. General linear model (GLM) analysis was performed to evaluate the effects of initial body mass and infection status on flight parameters (Dytham, 2003).

Results

We recorded a total of 46 flights over 23 h, from 22 infected and 24 uninfected *S. noctilio* females. Infected *S. noctilio* females showed reduced flight performance compared with control wasps. The average maximum speed recorded for parasitized individuals was 0.79 ± 0.47 m/s, in contrast to 1.16 ± 0.41 m/s (mean ± SD, respectively) observed for uninfected females [*F*(1,44) = 8.33; *P* = 0.006; Fig. 1A]. Similarly, we observed a marked differences in the total flight distances displayed, between infected and uninfected females [infected wasps flew 16.1 ± 14.7 km; uninfected wasps flew 30.5 ± 15.8 km; *F*(1,44) = 10.23; *P* = 0.002; Fig. 1B].

Our results also show that parasitized females were significantly smaller [*F*(1,44) = 9.99; *P* = 0.003; Fig. 2] and suffered larger weight losses during flight, than uninfected females [*F*(1,44) = 7.03; *P* = 0.011]. Percent mean weight losses were 11.30 ± 0.88% and 10.84 ± 0.79% for infected and healthy wasps respectively. Because parasitism may affect flight performance through its effects on female initial body size, we applied GLM where flight parameters were analyzed considering the effects of parasitism on body size. These results suggest that parasitism reduces both flight velocity and flight distance through its effects on initial body size (Table 1).

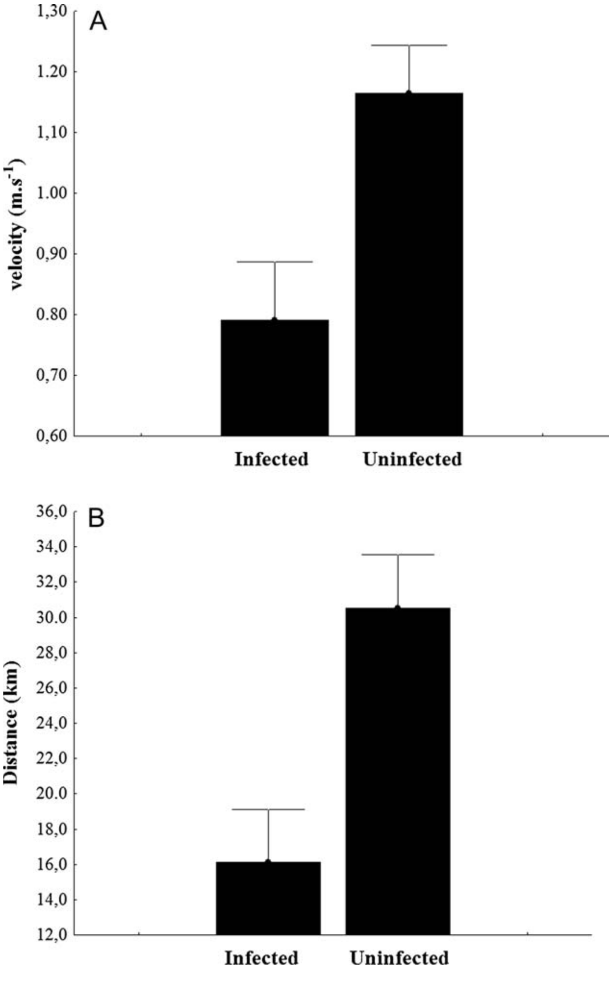

Figure 1 Differences in flight performance, measured as velocity (m/s) and total flight distance (km) between infected and uninfected *Sirex noctilio* females. Infected females were (A) slower [*F*(1,44) = 8.33; *P* = 0.006] and (B) had shorter flight distances [*F*(1,44) = 10.23; *P* = 0.002]. Error bars denote standard errors.

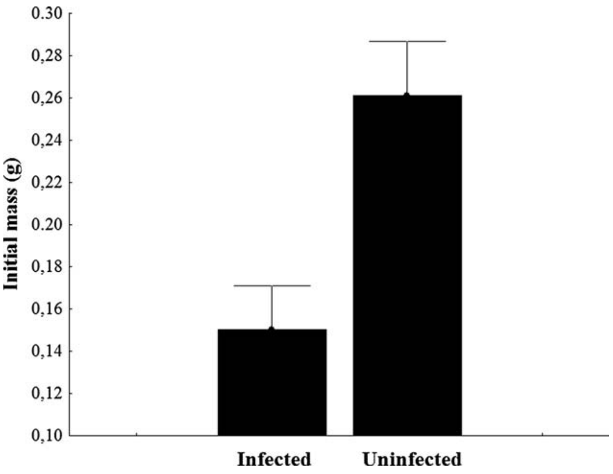

Figure 2 Differences in initial body mass after flight, between infected and uninfected *Sirex noctilio* females. Infected females were smaller than uninfected females [*F*(1,44) = 9.99, *P* = 0.003]. Error bars denote standard errors.

Discussion

Parasitism by *B. siricidicola* had significant consequences on flight performance of *S. noctilio* wasps through its effects on adult body size. Smaller, parasitized females displayed lower flight speeds and shorter flight distances than healthy (and larger) individuals.

The negative effects of parasites on insect host populations are well known. Many studies have focused on their consequences on host fecundity and longevity (Tompkins & Begon, 1999) and there has been some research on alterations in host behaviour induced by parasites (Moore & Gotelli, 1996; White *et al.*, 2000). Despite the diverse effects of parasitic infection on their hosts, the consequences of parasites and non-parasitic pathogens on insect dispersal capability has received less attention (Akbulut & Linit, 1999; Bradley & Altizer, 2005). However, understanding the potential consequences of the sub-lethal effects of parasites on their host and vectors is important from an evolutionary and applied perspective.

Many naturally occurring entomophagous parasites are significant regulators of several forest insect populations.

Table 1 Flight performance of *Sirex noctilio* females measured as flight speed and flight distance, as function of infection status and initial body mass

	d.f.	Mean square	*F*	*P*
Flight speed				
Initial mass	1	4.8847	58.91	< 0.001
Infection status	1	0.0363	0.44	0.51
Error	43	0.0829		
Flight distance				
Initial mass	1	4.4016×10^9	32.44	< 0.001
Infection status	1	2.3987×10^8	1.77	0.197
Error	43	1.3570×10^8		

GLM: flight speed (or flight distance) = initial mass + infection status.

Among these, nematodes are becoming increasingly popular in biological control (Lacey *et al.*, 2001). Some nematode species have an advantage in that they may be mass produced in laboratory conditions, stored for long periods and can achieve sustained host suppression (Lewis *et al.*, 1998; Lacey *et al.*, 2001).

During its free living stages, *B. siricidicola* feeds on the host symbiont *A. areolatum*, a species-specific basidiomycete fungus that, when dispersed by female wasps during oviposition, degrades cellulose and provides food for wasp larvae. In this sense, nematode presence within trees infested by *S. noctilio* implies competition for food between nematodes and developing wasps when fungus is limiting, which may bring about a reduction in adult wasp final body mass (Bedding, 1984). It should be noted that *S. noctilio* adults do not feed but rely entirely for movement and maintenance on resources gained during larval development.

According to our findings, the observed reduction in flight performance of parasitized females is probably not a direct consequence of individuals bearing the infection, as has been shown for other species (Bradley & Altizer, 2005). Parasitization of *S. noctilio* by *B. siricidola* nematodes is associated with a reduction in wasp body size, and smaller wasps are less capable of flying as fast and as far, than larger individuals (O. Bruzzone, J. Villacide, C. Bernstein & J. Corley, unpublished data).

Beddingia siricidicola has been generally recognized as an effective biological control agent of invasive *S. noctilio* populations, reaching 100% parasitism in a few host generations (Lacey *et al.*, 2001; Bedding & Iede, 2005). However, the outcome of woodwasp biological control programmes using this parasite is highly variable between different areas (Hurley *et al.*, 2007). In South America, for example, infection may reach 18–80% in Brazil (Iede *et al.*, 1988; Fenili *et al.*, 2000) and 2–60% in Argentina (Eskiviski *et al.*, 2003; Klasmer, 2004).

Several factors have been proposed to explain the observed variability in nematode infection (Bedding & Iede, 2005; Hurley *et al.*, 2007). Problems associated with laboratory culturing, such as loss of strain virulence or incompatibility between host and parasite development, have been reported as causes of failure in the field. In addition, bad timing of nematode introductions, inaccurate inoculation tools or protocols, or bad infested-tree selection can affect nematode establishment.

Another factor that may explain observed variable success in *S. noctilio* control through nematode introductions involves the natural rate of spread of the infection. To prevent wasp epidemics at the same time as optimizing introductions, nematode infection should naturally spread throughout the population (Lacey *et al.*, 2001). For this to occur, healthy wasps should attacks trees also attacked by infected wasps.

The results of the present study show that smaller infected female wasps display shorter flight distances than healthy ones. These differences in flight potential could lead to a reduction in the interaction between healthy and infected females in the field. Considering that during population build-up, *S. noctilio* wasps are highly clumped (Corley *et al.*, 2007). Differential dispersal of infected and uninfected female wasps may generate spatially segregated patches of newly-attacked trees, where *S. noctilio* populations would probably not be slowed by its natural enemy (Bedding & Arkhurst, 1974).

Because *B. siricidicola* is widely used in *S. noctilio* pest management programmes, understanding the mechanisms of nematode dispersal is important. Nematode introductions based on an inundative strategy rather than inoculative releases should be favoured. In addition, a wide distribution of trees for inoculation may improve nematode natural transmission and, consequently, may help increase effectiveness in woodwasp pest control.

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References

Akbulut, S. & Linit, M.J. (1999) Flight performance of *Monochamus carolinensis* (Coleoptera: Cerambycidae) with respect to nematode phoresis and beetle characteristics. *Environmental Entomology*, **28**, 1014–1020.

Bedding, R.A. (1967) Parasitic and free-living cycles in entomogenous nematodes of the genus *Deladenus*. *Nature*, **214**, 174–175.

Bedding, R.A. (1972) Biology of *Deladenus siricidicola* (Neotylenchidae) an entomophagus-mycetophagus nematode parasitic in siricid woodwasp. *Nematologica*, **18**, 482–493.

Bedding, R.A. (1979) Manipulating the entomophagous-mycetophagous nematode, *Deladenus siricidicola*, for biological control of the woodwasp *Sirex noctilio* in Australia. *Current Topics in Forest Entomology* (ed. by W. E. Waters), pp. 144–147. USDA-Forest Service Technical Report WO-8.

Bedding, R.A. (1984) Nematodes parasites of Hymenoptera. *Plant and Insect Parasitic Nematodes* (ed. by W. R. Nickle), pp. 755–795. Marcel Dekker, New York, New York.

Bedding, R.A. & Akhurst, R. (1974) Use of the nematode *Deladenus siricidicola* in the biological control of *Sirex noctilio* in Australia. *Journal of the Australian Entomological Society*, **13**, 129–135.

Bedding, R.A. & Iede, E.T. (2005) Application of *Bedingia siricidicola* for *Sirex* woodwasp control. *Nematodes as Biocontrol Agents* (ed. by P. S. Grewal, R. U. Ehlers and D. I. Shapiro-llan), pp. 385–400. CAB International, Wallingford, U.K.

Bradley, C.A. & Altizer, S. (2005) Parasites hinder monarch butterfly flight: implications for disease spread in migratory host. *Ecology Letters*, **8**, 290–300.

Corley, J.C., Villacide, J.M. & Bruzzone, O.A. (2007) Spatial dynamics of a *Sirex noctilio* woodwasp population within a pine plantation in Patagonia, Argentina. *Entomologia Experimentalis et Applicata*, **125**, 231–236.

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

Dytham, C. (2003) *Choosing and Using Statistics: A Biologist`s Guide*, 2nd edn. Blackwell Science, Oxford, U.K.

Eskiviski, E., de Coll, O., Faraldo, G.S. & Bennesch, M.A. (2003) *Efficiency of New Isolations of Deladenus siricidicola B. in the Biological Control of Sirex noctilio*. Jornadas tecnicas forestales y ambientales, FCF UNM – EEA Montecarlo, INTA, Argentina.

Fenili, R., Mendes, C.J., Miquelluti, D.J., Mariano-da-Silva, S., Xavier, Y., Ribas, H.S. & Burlan, G. (2000) *Deladenus siricidicola*, Bedding (Neotylenchidae) parasitism evaluation in adult *Sirex noctilio*, Fabricius, 1973 (Hymenoptera: Siricidae). *Revista Brasileira de Biologia*, **60**, 683–687.

Haugen, D.A. & Underdown, M.G. (1993) Reduced parasitism of *Sirex noctilio* in radiata pines inoculated with the nematode *Beddingia siricidicola* during 1974–1989. *Australian Forestry*, **56**, 45–48.

Haugen, D.A., Bedding, R.A., Underdown, M.G. & Neumann, F.G. (1990) National strategy for control of *Sirex noctilio* in Australia. *Australian Forest Grower*, **13**, 8.

Hoebeke, E.R., Haugen, D.A. & Haack, R.A. (2005) *Sirex noctilio*: discovery of a Palearctic siricid woodwasp in New York. *Newsletter of the Michigan Entomological Society*, **50**, 24–25.

Hurley, B.P., Slippers, B. & Wingfield, M.J. (2007) A comparison of control results for the alien invasive woodwasp, *Sirex noctilio*, in the southern hemisphere. *Agricultural and Forest Entomology*, **9**, 159–171.

Iede, E.T., Penteado, S.R. & Bisol, J.C. (1988) *Primeiro registro de ataque de Sirex noctilio em Pinus taeda no brasil. Circular técnica*. EMBRAPA–CNPF, Curitiva, Brasil.

Klasmer, P. (2004) Evaluación del nematode *Deladenus siricidicola* Bedding (Nematoda: Neotylenchidae) como potencial agente para el control de *Sirex noctilio* F. (Hymenoptera: Siricidae) en la Patagonia Argentina. *Investigación forestal al servicio de la producción II*, pp. 74–77. SAGPYA, Argentina.

Lacey, L.A., Frutos, R., Kaya, H.K. & Vail, P. (2001) Insect pathogens as biological control agents: do they have a future? *Biological Control*, **21**, 230–248.

Lewis, E.E., Campbell, J.F. & Gauler, R. (1998) A conservation approach to using entomopathogenic nematodes in turf and landscapes. *Conservation Biological Control* (ed. by P. Barbosa), pp. 235–254. Academic Press, New York, New York.

Madden, J.L. (1988) *Sirex* in Australasia. *Dynamics of Forest Insect Populations. Patterns, Causes, Implications* (ed. by A. A. Berryman), pp. 407–429. Plennum Press, New York, New York.

Moore, J. & Gotelli, N.J. (1996) Evolutionary patterns of altered behavior and susceptibility in parasitized hosts. *Evolution*, **50**, 807–819.

Neumann, F. & Minko, G. (1981) The *Sirex* wood wasp in Australian radiata pine plantations. *Australian Forestry*, **44**, 46–63.

Schumacher, P., Weyeneth, A., Weber, D.C. & Dorn, S. (1997) Long flights in *Cydia pomonella* L. (Lepidoptera: Tortricidae) measured by a flight mill: influence of sex, mated status and age. *Physiological Entomology*, **22**, 149–160.

Tompkins, D.M. & Begon, M. (1999) Parasites can regulate wildlife populations. *Parasitology Today*, **15**, 311–313.

Tribe, G. (1995) The woodwasp *Sirex noctilio* Fabricius (Hymenoptera: Siricidae), a pest of *Pinus* species, now in South Africa. *African Entomology*, **3**, 215–217.

Villacide, J.M. & Corley, J.C. (2006) Control de niveles poblacionales endémicos de la avispa de los pinos *Sirex noctilio* (Hymenoptera: Siricidae) mediante el raleo sanitario de hospederos atacados. *Revista de Investigaciones Agropecuarias*, **35**, 121–134.

White, A., Watt, A.D., Hails, R.S. & Hartley, S.E. (2000) Patterns of spread in insect–pathogen systems: the importance of pathogen dispersal. *Oikos*, **89**, 137–145.

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