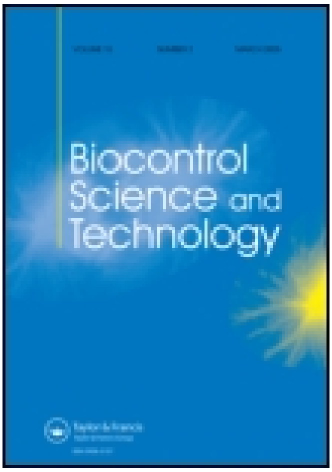

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An entomopathogenic fungus and nematode prove ineffective for biocontrol of an invasive leaf miner Profenusa thomsoni in Alaska

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RESEARCH ARTICLE

An entomopathogenic fungus and nematode prove ineffective for biocontrol of an invasive leaf miner *Profenusa thomsoni* in Alaska

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A non-native invasive sawfly, the amber-marked birch leaf miner *Profenusa thomsoni* (Konow), was first detected in south-central Alaska in 1996 and is now widely distributed throughout urban and wild birch trees in Alaska. Impacts have been considered primarily aesthetic because leaf miners cause leaves of birch trees (*Betula* spp.) to senesce prematurely, but the leaf miners likely also reduce birch vigour and thereby increase susceptibility to diseases and other insects. We tested the ability of commercially available biological control agents to control *P. thomsoni*. The entomopathogenic fungus *Beauveria bassiana* (Bals.-Criv.) Vuillemin GHA strain and the entomopathogenic nematode *Steinernema carpocapsae* (Weiser) were applied in aqueous suspension to the soil/litter surface beneath infested birch trees in Alaska at one site in 2007 and 2008 and two sites in 2010. There was no evidence the fungus or nematode controlled *P. thomsoni*. Instead, there was evidence the fungus increased the density of this pest insect at two sites, likely by reducing its predators. As tested, *B. bassiana* and *S. carpocapsae* do not appear effective as biological controls of *P. thomsoni*.

Keywords: *Profenusa thomsoni*; *Beauveria bassiana*; *Steinernema carpocapsae*; biological control; invasive insect

1. Introduction

Non-native invasive species pose significant threats to native ecosystems (Chapin III et al., 2000). In North America, several species of leaf-mining sawflies have been introduced from Europe over the past 120 years (Goulet, 1992). *Profenusa thomsoni* (Konow), the amber-marked birch leaf miner, is a European sawfly (Hymenoptera: Tenthredinidae) (Pieronek & Soltyk, 1993) that is widely distributed in North America (Digweed & Langor, 2004; Drooz, 1985; Digweed, Spence, & Langor, 1997). The insect was first detected in Alaska in the south-central part of the state in 1996, although there is evidence it was present in Alaska as early as 1982 (Snyder, MacQuarrie, Zogas, Kruse, & Hard, 2007).

In recent years, *P. thomsoni* has caused severe defoliation of the native birch species: dwarf arctic birch (*Betula nana* L.), resin birch (*Betula glandulosa* Michaux), paper birch (*Betula papyrifera* Marshall), Alaska paper birch (*Betula neoalaskana*

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Sargent), western paper birch (*B. papyrifera* variety *occidentalis* Fernald) and Kenai birch (*Betula kenaica* W.H. Evans) in addition to non-native horticultural varieties (Digweed et al., 1997; Snyder et al., 2007). Most of the damage has occurred in and around the towns of Anchorage, Fairbanks, Haines and Skagway as well as parts of the Kenai Peninsula (USDA Forest Service, 2011). The insect is now spreading rapidly beyond the urban areas into the surrounding forests. *P. thomsoni* feed inside leaves of birch causing blotches of necrotic tissue to form on the leaves. Beyond this aesthetic damage, leaf miner feeding is hypothesised to reduce growth and weaken trees, leaving affected trees susceptible to diseases and other insects (Hoch, Zeldin, & McCown, 2000; Snyder, et al., 2007).

Like most leaf-mining sawflies, *P. thomsoni* is univoltine. Adults begin emerging from the soil in spring when leaves are forming and continue emerging for most of the summer. Females oviposit an average of nine eggs in slits made with a saw-like ovipositor in the upper surface of mature leaves (Drouin & Wong, 1984). Five larval instars feed and develop between the epidermal layers of the leaves (Digweed et al., 2009). Between August and October, the final instar larva drops from the leaf and overwinters as a pre-pupa in the litter near the soil surface (Digweed et al., 2009; MacQuarrie, 2008; Martin, 1960).

Insecticidal treatments such as emamectin benzoate (TREE-age™, Arborjet Inc., Woburn, MA) injected with the Quickjet™ system (Arborjet, Inc., Woburn, MA), a system that injects pesticides into the tree bole so they can be translocated throughout the tree, have been used to control *P. thomsoni* (Fettig, Burnside, Hayes, & Schultz, 2011; Fettig, Burnside, & Schultz, 2013). Compared to insecticides, microbial biocontrol agents are sometimes more sustainable for pest insect control, both because insecticides tend to be expensive and because of the risk target insects will develop resistance to insecticides (Devi, Vimala, Ranga Rao, Gopalakrishnan, & Sivakumar, 2012). One microbial biocontrol agent that is sometimes used for pest insect control is the fungus *Beauveria bassiana* (Bals.-Criv.) Vuillemin. This fungus occurs naturally in soils throughout the world and is known to cause disease in ~700 species of insects (Devi, Padmavathi, Rao, Khan, & Mohan, 2008; Glare & Milner, 1991; Goettel, Inglis, & Wraight, 2000; Humber, 1991; Li, 1988). *B. bassiana* has been widely tested as a biological control agent of a wide-range of pest insects with varying results. Insects tested include thrips (Thysanoptera) (Zahn & Morse, 2013), Colorado potato beetle (*Leptinotarsa decemlineata* (Say)) (Wraight & Ramos, 2002), house flies (*Musca domestica* (L.)) (Kaufman, Reasor, Rutz, Ketzis, & Arends, 2005), mosquitoes (*Anopheles stephensi* Liston) (Kanzok & Jacobs-Lorena, 2006) and many others. When spores of the fungus come into contact with susceptible insects under suitable environmental conditions, they germinate, and the fungus penetrates the insects, eventually killing them. Spores then develop on the surface of the cadaver to allow the fungus to spread.

Entomopathogenic nematodes such as *Steinernema carpocapsae* (Weiser) are also sometimes effective as insect biological control agents (Gaugler, 1988). *S. carpocapsae* juveniles infect hosts via the mouth, anus and spiracles (Smart, 1995). Juvenile nematodes enter the haemocoel where they release rapidly multiplying bacteria of the genus *Xenorhabdus*. Although the nematode produces a lethal toxin (serine protease; Toubarro et al., 2009), the bacteria are considered primarily responsible for insect mortality (Burman, 1982). Together the nematodes and bacteria feed on the host and reproduce for several generations inside the insect's body. Although many insect pests are susceptible to *S. carpocapsae*, there have been no adverse effects shown to non-target insects during short-term pest control studies (Dillon, Foster, Williams, & Griffin, 2012; Georgis, Kaya, & Gaugler, 1991). The majority of insects attacked by the nematode are beetles (Coleoptera) and flies (Diptera), although a total of 19 insect orders are susceptible to *S. carpocapsae* (Poinar & Thomas, 1984), including Hymenoptera, particularly sawflies (Bednarek & Mracek, 1986; Mracek, 1986; Vincent & Belair, 1992).

The objective of this study was to determine the feasibility of using *B. bassiana* and *S. carpocapsae* to control *P. thomsoni* in Alaska. Since the insect spends much of its life in the soil, we hypothesised that soil-applied *B. bassiana* and *S. carpocapsae* would reduce the number of insects completing pupation and emerging from the soil as adults.

2. Methods

2.1. Study sites

Our study had four field experiments: (1) a 2007–2008 experiment and (2) a 2008–2009 experiment both conducted at the Alaska Botanical Gardens (N 61.1781, W 149.7603) in Anchorage, AK, (3) a 2010–2011 experiment conducted at Javier de La Vega Park (N61.1672 W149.9171) in Anchorage, AK, and (4) a 2010–2011 experiment conducted at Eielson Air Force Base in central Alaska (N 64.6542, W 146.9907). Alaska birch (*B. neoalaskana* Sargent) was the most abundant tree species at each study site with alder (*Alnus* spp.) and spruce (*Picea* spp.) also present.

2.2. Pretreatment sampling

Within each experiment, eighteen 6 × 6-m plots (six untreated, six *B. bassiana*-treated and six *S. carpocapsae*-treated) were established beneath canopies of *P. thomsoni*-infested birch trees. To assess and control for plot-to-plot variation in *P. thomsoni* densities occurring prior to treatment, pretreatment sampling was conducted in all experiments, except the 2008–2009 experiment. Emergence trapping was used to collect the adult sawflies upon emergence from the soil using methods outlined by Southwood (1978). Six emergence traps consisting of a 0.5 × 0.5-m pyramidal polyvinyl chloride (PVC) pipe frame with a black vinyl cover around the sides and a fine mesh screen over the apex were randomly placed in each plot in early June. Emerging *P. thomsoni* ascended to the trap apex where they were caught on a 10 × 15-cm sticky card. *P. thomsoni* were counted and cards replaced every two weeks through the end of July.

2.3. Biological control treatments

Treatments were applied the second week of August (2007–2008 experiment and 2008–2009 experiment) and the first week of August (both 2010 and 2011 experiments) before leaf miners emerged from leaves and dropped to the litter/soil surface to overwinter. In the 2007–2008 experiment, a *B. bassiana* spore suspension called Mycotrol® (Laverlam International Corp. 117 South Parkmont, Butte, MT 5970) was applied at the manufacturer-recommended rate of 1.0 ml of spore suspension per plot, which delivered approximately 2.06 × 10^{10} *B. bassiana* spores per plot. In this same experiment, a *S. carpocapsae* product called NemAttack®

(Arbico Organics, Oro Valley, AZ) was applied at the manufacturer-recommended rate of 0.44 g per plot, which delivered approximately 1.2 million nematodes per plot. Within each treated plot, the fungus or nematode products were mixed with 7.6 L of water for application, and the fungus and nematode spray solutions were applied to the litter/soil surface of six randomly assigned plots with a hand-held garden sprayer SureSpray™ Select Sprayer – 2G Model #27020 (Chapin International, Inc. Batavia, NY 14021) with a cone nozzle. Due to insufficient *P. thomsoni* control in the 2007–2008 experiment, application rates of both biological agents were increased tenfold in the 2008–2009 and 2010–2011 experiments.

2.4. Post-treatment sampling

Post-treatment sampling was conducted using the same protocol described for pre-treatment sampling, except for the time periods the traps were left in place. Traps were placed in plots the first week of June in 2008 (2007–2008 experiment), 2009 (2008–2009 experiment) and 2011 (2010–2011 experiment at Anchorage) and the third week in June in 2011 (2010–2011 experiment at Eielson). *P. thomsoni* were counted and sticky cards replaced every two weeks through the end of July at Anchorage and on July 1 and July 28 at Eielson.

2.5. Soil temperature data

To help explain results of our Anchorage experiments, we relied on soil temperature data gathered with an Onset HOBO® (Bourne, MA) 2.8 km from the study plots near the US Department of the Interior Bureau of Land Management Office at Campbell Creek near Anchorage, Alaska. To help explain results of our Eielson experiment, we used soil temperature data gathered at the Bonanza Creek Long Term Ecological Research site located 51 km west of the study plots.

2.6. Verifying activity of biological controls against **P. thomsoni**

In August 2014, two experiments were conducted to verify that *B. bassiana* and *S. carpocapsae* were capable of attacking *P. thomsoni*. The fungus and nematode products tested were those used in the field experiments. Late instar *P. thomsoni* larvae were placed in 100-mm Petri dishes (first experiment: six larvae per dish treated August 14; second experiment: 10 larvae per dish treated August 21). Each experiment consisted of 15 Petri dishes (5 replicates of an untreated control, a *B. bassiana* treatment and a *S. carpocapsae* treatment). Treatments consisted of 1.0 ml of the *B. bassiana* product and 0.1 g of the nematode product mixed with 100 ml of water and sprayed on the larvae in the Petri dishes until they appeared saturated. After the insects were treated, the Petri dishes were placed outside for two days where night-time low temperatures were 4–10°C and daytime high temperatures were 10–13°C. Then the dishes were stored inside for five days at 23.4–24.5°C, and the larvae were examined for evidence of fungal or nematode attack. Fungal hyphae growing on dead *P. thomsoni* were considered evidence of fungal attack, and nematodes within bodies of dead *P. thomsoni* that were crushed and examined under a phase contrast microscope were considered evidence of nematode attack.

2.7. Data analysis

Data from the four field experiments were analysed with a regression model with the natural log of the total number of *P. thomsoni* trapped per plot over the trapping period as the response. Independent variables were indicators for the experiments, treatments (control, *B. bassiana*, *S. carpocapsae*) and experiment × treatment interactions. Except for the 2008–2009 experiment, pre-treatment trap counts (total *P. thomsoni* trapped per plot standardised to mean 0, standard deviation 1) were included as a covariate. The model was fit using Mathematica® 9, and inferences were based on 95% confidence intervals on the relevant parameters (WolframResearch, 2010).

3. Results and discussion

The two Petri dish experiments verified the biological control agents were capable of attacking *P. thomsoni*. In the first and second experiments, respectively, 60 and 100% of the Petri dishes contained larvae colonised by *B. bassiana*. Additionally, 100% and 60% of Petri dishes contained larvae attacked by nematodes in the first and second experiments, respectively. No untreated larvae showed evidence of fungal or nematode attack.

There was weak evidence *B. bassiana* may have partially reduced populations of *P. thomsoni* in 2008 (p = 0.14) (Figure 1). However, the point estimate suggested only a 50% reduction in *P. thomsoni* density (Figure 1). There was no evidence *S. carpocapsae* reduced *P. thomsoni* abundance in 2008.

The lack of adequate *P. thomsoni* control from the 2007–2008 experiment prompted us to increase the *B. bassiana* and *S. carpocapsae* application rates in the 2008–2009 and 2010–2011 experiments (see Materials and Methods). There was no evidence the higher rate of *B. bassiana* provided control of *P. thomsoni* in the 2008–2009 experiment. Instead, surprisingly, there was evidence that *P. thomsoni* density was slightly higher in plots treated with *B. bassiana* than in untreated plots (p = 0.06), with treatment means of insects trapped per plot being 4.3 and 2.5,

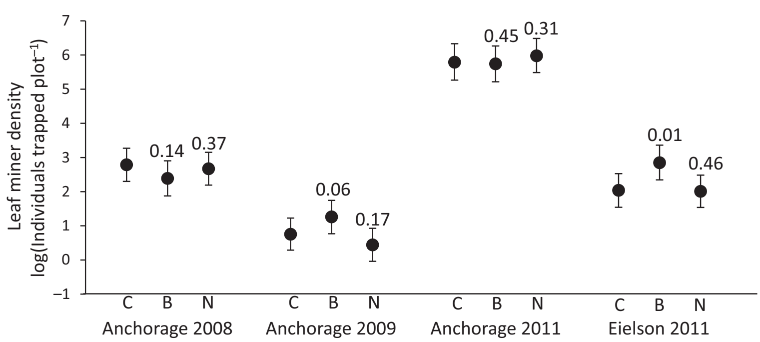

Figure 1. Point estimates (dots) and 95% confidence intervals (bars) from four experiments used to estimate leafminer densities in untreated plots (C), plots treated with the biocontrol fungi *B. bassiana* (B) and plots treated with the biocontrol nematode *S. carpocapsae* (N). Numbers above bars denote p-values for difference from controls. Surprisingly, data provide evidence *B. bassiana* increased leafminer densities at Eielson and Anchorage in 2009.

respectively. However, perhaps because summer conditions were cool and wet, densities of *P. thomsoni* were too low to get a clear indication of the effects of *B. bassiana* and *S. carpocapsae* in the 2008–2009 experiment.

The data from Eielson also provided evidence that *P. thomsoni* densities were higher in plots treated with *B. bassiana* ($p = 0.01$) (Figure 1). At this site, an average of 27 and 37 *P. thomsoni* were trapped from untreated and *B. bassiana*-treated plots, respectively. There was no evidence *B. bassiana* affected *P. thomsoni* density at the Anchorage site in 2011, and there was no evidence *S. carpocapsae* affected *P. thomsoni* density at either study site in 2011 (Figure 1).

Temperature affects *B. bassiana*'s germination, growth, survival and virulence (Dimbi, Maniania, Lux, & Mueke, 2004; Ekesi, Maniania, & Ampong-Nyarko, 1999; Kiewnick, 2006). According to data gathered 2.8 km from our study Anchorage study sites, soil temperatures from August 1 to October 15 averaged 8.7°C with a range of 3.5–24°C when *P. thomsoni* were dropping from trees and entering the soil/litter. Soil temperatures for the same period 51 km west of our Eielson research site averaged 7.3°C with a range from 1.0°C to 13°C. These temperatures are well below the 25–30°C optimum range of activity for *B. bassiana* (Dimbi et al., 2004; Ekesi et al., 1999; Kiewnick, 2006; Tefera & Pringle, 2003). Perhaps soil temperatures in our study were too low for *B. bassiana* to effectively reduce *P. thomsoni* populations.

Variation in temperature tolerance among *B. bassiana* isolates can be significant (Dimbi et al., 2004; Ekesi et al., 1999; Tefera & Pringle, 2003). Fernandes, Rangel, Moraes, Bittencourt, and Roberts (2008) found that *B. bassiana* isolates from cold regions were more active at cold temperatures than isolates from regions closer to the equator. Similarly, Ekesi et al. (1999) observed that *B. bassiana* virulence is linked to climatic conditions in its region of origin, suggesting fungal isolates should be chosen from a climatic region similar to where they will be applied as biological control measures. The *B. bassiana* fungal isolate used in our study is native to Central and South America (D. North, personal communication). Perhaps *B. bassiana* fungal isolates from cooler, northern climates would be more effective against *P. thomsoni* in Alaska.

P. thomsoni densities were sometimes greater where *B. bassiana* was applied. This suggests *B. bassiana* negatively impacted natural enemies of *P. thomsoni*, which is somewhat surprising given the fungus was not highly virulent to *P. thomsoni* in our study system. *B. bassiana* has been shown to reduce populations of overwintering ground beetles (Carabidae) and rove beetles (Staphylinidae) (Steenberg, Langer, & Esbjerg, 1995). It may be that, compared to *P. thomsoni*, these beetle predators of *P. thomsoni* were more susceptible to the fungus at our study sites.

As with *B. bassiana, S. carpocapsae* virulence is influenced by temperature (Saunders & Webster, 1999). In one study, survival and pathogenicity of *S. carpocapsae* was significantly higher at low (5–25°C) than high (35°C) soil temperatures (Kung, Gaugler, & Kaya, 1991). In another study, Grewal, Selvan, & Gaugler (1994) found the soil temperature range allowing establishment, infection and reproduction of *S. carpocapsae* to be 10–39°C. These authors also found that warmer temperatures were necessary for establishment and reproduction than survival and infection. The previously described soil temperatures near our study sites were within the range that allowed *S. carpocapsae* pathogenicity in these previous studies, so it is unclear why *S. carpocapsae* failed to control *P. thomsoni.*

A number of other factors influence *S. carpocapsae* virulence (Gaugler, 1988), and these factors may explain why *S. carpocapsae* failed to control *P. thomsoni*. Specifically, the virulence of *S. carpocapsae* has been shown to increase with increasing *S. carpocapsae* application rate (Brusselman et al., 2012), decrease with increasing soil depth (Toledo et al., 2009) and vary by application method (Jackson & Hesler, 1995) and soil type and texture (Barbercheck & Kaya, 1991).

In some studies, *B. bassiana* and *S. carpocapsae* have provided excellent control of wildland pests (Bugeme, Maniania, Knapp, & Boga, 2008; Kamali, Karimi, Hosseini, Campos-Herrera, & Duncan, 2013). In other studies, including our study, these agents have failed to control the targeted pest (Guzmán-Franco et al., 2012; Park et al., 2013; Vanninen, Hokkanen, & Tyni-Juslin, 1999; Williams et al., 2013). Further research is needed to identify the conditions (e.g. climate, pest species, geographic origin, host specificity of the variant) that allow *B. bassiana* and *S. carpocapsae* to consistently reduce insect pest populations. As tested, *B. bassiana* and *S. carpocapsae* were not effective as biological control agents of *P. thomsoni* in Alaska.

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