Laboratory Virulence of Entomopathogenic Nematodes to Two Ornamental Plant Pests, Corythucha ciliata (Hemiptera: Tingidae) and Stethobaris nemesis (Coleoptera: Curculionidae)

Authors: Shapiro-Ilan, David I., and Mizell, Russell F.

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LABORATORY VIRULENCE OF ENTOMOPATHOGENIC NEMATODES TO TWO ORNAMENTAL PLANT PESTS, *CORYTHUCHA CILIATA* (HEMIPTERA: TINGIDAE) AND *STETHOBARIS NEMESIS* (COLEOPTERA: CURCULIONIDAE)

David I. Shapiro-Ilan[1*] and Russell F. Mizell III[2]
[1]USDA-ARS, SE Fruit and Nut Tree Research Laboratory, Byron, GA 31008 USA

[2]North Florida Research and Education Center, University of Florida/IFAS, Quincy, FL 32351 USA

*Corresponding author: E-mail, David.Shapiro@ars.usda.gov

Abstract

Ornamental flowers, shrubs and trees are economically important commodities in the US and around the globe. In this study we evaluated the potential of entomopathogenic nematodes to control two important ornamental pests: 1) *Corythucha ciliata* (Say) (Hemiptera: Tingidae), a native lace bug that attacks the foliage of sycamore trees, and 2) the recently described exotic pest, *Stethobaris nemesis* (Coleoptera: Curculionidae), a weevil that attacks amaryllis leaves and bulbs. In the laboratory, the virulence of six entomopathogenic nematode strains (comprising five species) was evaluated on sycamore leaf discs for potential to control *C. ciliata*, and four nematode species were evaluated for control of *S. nemesis*. *Heterorhabditis indica* (HOM1) exhibited higher virulence to *C. ciliata* than *H. bacteriophora* (Baine and Oswego strains), *H. georgiana* (Kesha), and *Steinernema riobrave* (355); *S. carpocapsae* (All) virulence was not statistically separated from the other nematodes with the exception of *H. bacteriophora* (Oswego) (which exhibited the lowest virulence). Additionally, among the six nematodes tested, *H. indica* (HOM1) produced the highest level of infective juveniles in *C. ciliata*. *Steinernema carpocapsae* (All) exhibited the highest virulence to control *S. nemesis* (in well-plates containing soil), yet both *S. carpocapsae* (All) and *S. feltiae* (SN) exhibited high virulence after only 1d post-treatment. The other nematodes tested for *S. nemesis* suppression, *H. bacteriophora* (Hb) and *H. indica* (HOM1), also showed high levels of virulence particularly at 3 d post-treatment. Our results indicate that several entomopathogenic nematodes offer potential for control of *C. ciliata* and *S. nemesis* and thus additional research, e.g., field studies, is warranted.

Key Words: amaryllis weevil, biological control, *Heterorhabditis*, *Steinernema*, sycamore lace bug

Resumen

Las flores, arbustos y árboles ornamentales son productos de importancia económica en US y el resto del mundo. En este estudio evaluamos el potencial de nematodos entomopatógenos para controlar dos plagas importantes de plantas ornamentales: 1) *Corythucha ciliata* (Say) (Hemiptera: Tingidae), un chinche de encaje nativo que ataca el follaje de arboles de sicomoro, y 2) una plaga exótica recientemente descrita, *Stethobaris nemesis* (Coleoptera: Curculionidae), un picudo que ataca bulbos y hojas de amaryllis. Bajo condiciones de laboratorio se evaluó la virulencia de seis cepas de nematodos entomopatógenos (abarcando cinco especies) sobre discos de hojas de sicomoro para el control de *C. ciliate,* y cuatro especies de nematodos para el control de *S. nemesis*. *Heterorhabditis indica* (HOM1) mostro mayor virulencia en *C. ciliata* que *H. bacteriophora* (cepas Baine y Oswego), *H. georgiana* (Kesha), y *Steinernema riobrave* (355); la virulencia de *S. carpocapsae* (All) no fue significativamente diferente a la de los otros nematodos, con la excepción de *H. bacteriophora* (Oswego) (el cual mostro la virulencia más baja). Entre los seis nematodos evaluados, *H. indica* (HOM1) produjo el nivel más alto de juveniles infectivos en *C. ciliata*. *Steinernema carpocapsae* (All) mostro mayor virulencia para controlar a *S. nemesis* (en platos con pozos llenos de suelo), aunque ambos *S. carpocapsae* (All) y *S. feltiae* (SN) mostraron alta virulencia 1 d después del tratamiento. Los otros nematodos evaluados para suprimir *S. nemesis*, *H. bacteriophora* (Hb) y *H. indica* (HOM1), también mostraron niveles altos de virulencia particularmente 3 d después del tratamiento. Nuestros resultados indican que varios nematodos entomopatógenos ofrecen potencial para el control de *C. ciliata* y *S. nemesis,* en consecuencia más investigaciones e.g., estudios de campo, están garantizados.

Palabras Clave: picudo del amaryllis, control biológico, *Heterorhabditis*, *Steinernema*, chinche de encaje del sicomoro

Production and maintenance of ornamental plants is an important industry in the US. The value of combined floriculture and nursery production in the US is estimated at more than $14 billion (Daughtrey & Benson 2005). Two important groups of ornamentals in the Southeastern US are sycamore (*Platanus* spp.) and amaryllis (*Amaryllis* spp.). Both of these groups are attacked by insect pests that limit profitability and aesthetic value (Filer et al. 1977; Thomas 2005). Two important pests of sycamore and amaryllis are the sycamore lace bug, *Corythucha ciliata* (Say) (Hemiptera: Tingidae), and the weevil *Stethobaris nemesis* Prena & O'Brien (Coleoptera: Curculionidae), respectively.

Corythucha ciliata attacks a variety of trees in the genus *Platanus* and is particularly damaging to the American sycamore, *Platanus occidentalis* L. (Proteales: Platanaceae), (Halbert & Meeker 2001; Ju et al. 2011). This pest is native to North America and has been introduced into Europe and Asia (Horn et al. 1983; Halbert & Meeker 2001; Ju et al. 2011). The lace bugs feed on the underside of the leaves damaging the foliage and causing premature senescence (Horn et al. 1983; Halbert & Meeker 2001). A single female can lay more than 250 eggs and in the southern US the insects can have several generations per yr. *Corythucha ciliata* overwinter as adults under loose bark, or in nearby cracks and crevices (Horn et al. 1983; Halbert & Meeker 2001). At high infestation levels *C. ciliata* can kill trees, especially when associated with pathogenic fungi such as *Ceratocystis fimbriata* Ellis and Halst. (Ophiostomatales: Ophiostomataceae) and *Apiognomonia veneta* (Sacc. and Speg.) (Diaporthales: Gnomoniaceae) (Halbert & Meeker 2001).

Options for control of *C. ciliata* rely on chemical insecticides, yet in many populated urban areas where the pest occurs, application of chemicals is problematic due to safety issues (Halbert & Meeker 2001). Currently, no biological control option is available for suppression of *C. ciliata*. Entomopathogenic nematodes (EPNs) in the genera *Steinernema* and *Heterorhabditis* (Rhabditida: Steinernematidae and Heterorhabditidae) may have potential as alternative control agents for *C. ciliata* management (Tarasco & Triggiani 2006). These nematodes are commercially available biocontrol agents that kill insects with the aid of symbiotic bacteria (steinernematids are associated with *Xenorhabdus* spp. and heterorhabditids with *Photorhabdus* spp.) (Lewis & Clarke 2012). EPNs are used to control a wide variety of economically important pests (Grewal et al. 2005). Overwintering *C. ciliata* (found on the tree trunk and surrounding area) may be amenable to control with EPNs in a similar approach taken for suppression of overwintering codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) (Lacey & Unruh 1998; Lacey & Shapiro-Ilan 2008). There have been only a few reports of EPNs as biocontrol agents in association with insects in the order Hemiptera (Leite et al. 2005, Cuthbertson et al. 2007, 2008; de Paula Batista & Auad 2010) and prior to this study there has been only one report on EPN pathogenicity to *C. ciliata* (Tarasco & Triggiani 2006). Our research expands on that of Tarasco and Triggiani (2006) because the prior study only considered two EPN species as potential control agents whereas we tested six EPNs in the present study.

The recently described exotic weevil, *S. nemesis* is a pest of various plants in the family Amaryllidaceae including *Amaryllis* L. spp., *Hippeastrum* Herb. spp., spider lily *Hymenocallis* Salisb. spp., swamp lily *Crinum* L. spp., and Amazon lily *Eucharis* × *grandiflora* Planch. & Linden (Thomas 2005; Epsky et al. 2008; Prena & O'Brien 2011). Adult insects feed primarily on the foliage, though they may also feed on the surface of bulbs (Thomas 2005). After adults lay eggs on the foliage, the larvae hatch and travel downward to the bulb to feed. Larval feeding hollows out the bulb, which can kill the plant. Larvae are believed to pupate in the surrounding soil and emerge as adults (Price 2009). The life cycle from oviposition to adult eclosion lasts approximately 47.4 ± 3.7 d and adults may live up to two years (Epsky et al. 2008; Price 2009). In the southern US several generations of *S. nemesis* occur and the weevils may occur throughout the yr (Epsky et al. 2008; Thomas 2005).

Control tactics for *S. nemesis* are limited to chemical insecticides or removal of infested plants (Price 2009). Given that EPNs have been reported to control numerous weevil pests in soil (Shapiro-Ilan et al. 2002; Grewal et al. 2005), biocontrol using EPNs may be an option for *S. nemesis* as well. Other than this study, there have been no reports on the susceptibility of *S. nemesis* to EPNs. Thus, the objective of this study was to estimate the potential for EPNs to control *C. ciliata* and *S. nemesis* by determining pathogenicity, virulence and reproductive capacity under laboratory conditions.

Materials and Methods

Insects and Nematodes

Overwintering adult sycamore lace bugs were collected from sycamore trees in north Florida near Quincy. Late instar *S. nemesis* were obtained from infested amaryllis bulbs collected in south Georgia near Tifton. All insects were used in experiments within 2 to 3 d. Nematodes were reared on commercially obtained last instar greater wax moth, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) at 25 °C according to procedures described in Kaya & Stock (1997). The source of all nematodes was the laboratory culture collection

of Dr. Shapiro-Ilan (USDA-ARS, Byron, Georgia). Following harvest, nematodes were stored at 13 °C for less than 2 wk before experimentation.

Susceptibility of *C. ciliata* to EPNs

The experiment consisted of 6 nematode treatments and an untreated (water-only) control. Nematode treatments included were two strains of *Heterorhabditis bacteriophora* Poinar (Baine and Oswego strains), *H. indica* Poinar Karunakar and David (HOM1 strain), *H. georgiana* Nguyen, Shapiro-Ilan, and Mbata (Kesha strain), *S. carpocapsae* (Weiser) (All strain), and *S. riobrave* Cabanillas, Poinar and Raulston (355 strain). Experimental arenas consisted of Petri dishes (90 mm) that were half-filled with 2% agar, contained three sycamore leaf discs (approximately 3 cm diam., bottom side facing up), and filter paper (Whatman No. 1) lining the lid of dish; leaves were collected from mature trees. Approximately 1600 infective juvenile nematodes (IJs) were applied in 1 ml of tap water to the agar side and to the lid of each dish using a spray bottle (applications were made to both sides because some insects moved to the lid). The rate of application (approximately 25 IJs per cm^2), was equivalent to standard minimum recommendations for most insect pests (Shapiro-Ilan et al. 2002, 2006). After the Petri dishes were allowed to dry in a bio-safety cabinet until there was no excess moisture, 10 adult *C. ciliata* were added to each dish. The dishes were incubated at 25 °C and insect mortality was determined at 2 d post-treatment. There were five replicate dishes per treatment (35 total), and the experiment was repeated once in time (2 trials).

Nematode reproductive capacity was also assessed in *C. ciliata.* Three infected *C. ciliata* cadavers (from the virulence experiments described above) were placed on a White trap (Kaya & Stock 1997) and the number of IJs produced per insect was determined 28 d post-treatment. There were 5 replicate White traps per treatment.

Susceptibility of *S. nemesis* to EPNs

Susceptibility of *S. nemesis* to EPNs was assessed based on procedures described for evaluation of other species of weevils in soil (Shapiro-Ilan et al. 2003, 2011). Treatments included four nematodes *H. bacteriophora* (Hb strain), *H. indica* (HOM1 strain), *S. carpocapsae* (All strain), and *Steinernema feltiae* (Filipjev) (SN strain), and an untreated (water-only) control. Experimental arenas consisted of well plates (12 wells per plate, Costar®) with each of 10 wells containing 2.5 g dry autoclaved soil and one late instar *S. nemesis*. The soil (obtained from a pecan orchard in Byron, Georgia) was a loamy sand with the percentage sand: silt: clay = 84: 10: 6, pH = 6.1, and organic matter = 2.8% by weight. Approximately 300 IJs were pipetted onto the soil surface of each well in 0.2 ml of water so that the final moisture was standardized at field capacity (16%). A cover was placed over the well plate and parafilmed close. Plates were incubated at 25 °C and insect mortality was determined at 1, 2, and 3 d post-treatment. The experiment included two trials (conducted consecutively); in the first trial, there were four replicates of 10 insects per treatment and in the second trial there were four replicates of seven insects per treatment.

Statistical Analysis

Within each experiment, treatment effects were analyzed using ANOVA. If a significant *F*-test was detected, the Student-Newman-Keuls' (SNK) test was used to further elucidate treatment differences (SAS Version 9.1, SAS Institute, Inc., Cary, North Carolina). Data from repeated experiments (trials) were pooled and trial was considered as a block effect. Percentage data (insect mortality) were arcsine transformed and numerical data (nematode yield) were square-root transformed prior to analysis (Southwood 1978; Steel & Torrie 1980; SAS 2002); non-transformed means are presented in figures.

Results

Differences in virulence and reproductive capacity were detected among nematode treatments when using *C. ciliata* as the target host. Percentage insect mortality was lower in the control than in all treatments except *H. bacteriophora* (Oswego) (F = 10.63; df = 6, 56; P = 0.0001) (Fig. 1). *Heterorhabditis indica* (HOM1) caused higher mortality than all other treatments except *S. carpocapsae* (Fig. 1). Mortality in the *S. carpocapsae*

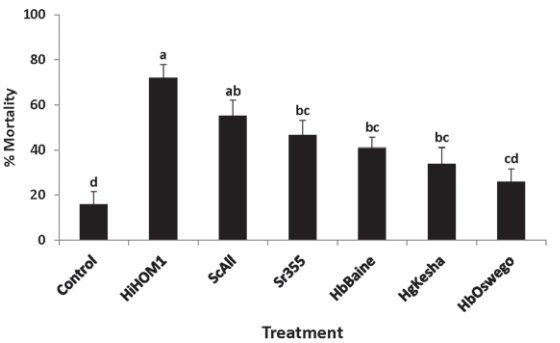

Fig. 1. Percentage mortality of *Corythucha ciliata* following exposure to entomopathogenic nematodes or a water-only control for 2 d. Nematode species names are abbreviated as follows: Hi = *Heterorhabditis indica*, Sc = *Steinernema carpocapsae,* Sr = *S. riobrave*, Hb = *H. bacteriophora*, and Hg = *H. georgiana*; strain designations are indicated following the species abbreviation. Different letters above bars indicate statistically significant differences (SNK test, α = 0.05).

treatment was higher than mortality caused by *H. bacteriophora* (Oswego) but was not different from other nematode treatments (Fig. 1). Reproductive capacity was higher in the *H. indica* (HOM1) treatment than other treatments (F = 6.87; df = 5, 24; P = 0.0004) (Fig. 2). No other differences in reproductive capacity were detected (and no reproduction was detected in *S. riobrave* [355]) (Fig. 2).

Differential virulence to *S. nemesis* was also detected among EPN treatments (Fig. 3). One d after treatment, all nematode treatments caused higher mortality than the control, and *S. carpocapsae* and *S. feltiae* caused higher mortality than the two heterorhabditids (F = 26.46; df = 4, 34; P = 0.0001) (Fig. 3). Two d after treatments, *S. carpocapsae* (All) caused higher mortality than *H. indica* (HOM1), and the other two treatments, *S. feltiae* and *H. bacteriophora* (Hb), were intermediate. (F = 91.86; df = 4, 34; P = 0.0001). After three d no differences were detected among treatments (F = 63.26; df = 4, 30; P = 0.0001) (Fig. 3).

Discussion

Biocontrol potential against both pests, *C. ciliata* and *S. nemesis,* was observed using entomopathogenic nematodes. Based on virulence and reproductive capacity our results indicate *H. indica* (HOM1) showed the highest potential for *C. ciliata* suppression. *S. carpocapsae* also showed high potential. Our results can be compared with reports of EPN virulence to other hemipterans. Similar to our study, Cuthbertson et al. (2008) reported high levels of *S. carpocapsae* virulence to the sweetpotato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae). Additionally, significant virulence among steinernematids and heterorhabditids has been reported for control of spittlebugs (Hemiptera: Cercopidae) in the genus *Mahanarva* (Leite et al. 2005; de Paula Batista & Auad 2010).

Tarasco & Triggiani (2006) conducted a field test comparing *S. carpocapsae* and *H. bacteriophora* for control of *C. ciliata*. In that study, pathogenicity was not detected in either nema-

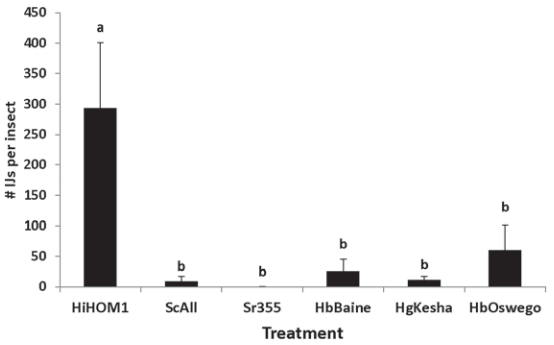

Fig. 2. Number of infective juvenile nematodes (IJs) produced per infected *Corythucha ciliata* cadaver. Nematode species names are abbreviated as follows: Hi = *Heterorhabditis indica*, Sc = *Steinernema carpocapsae,* Sr = *S. riobrave*, Hb = *H. bacteriophora*, and Hg = *H. georgiana*; strain designations are indicated following the species abbreviation. Different letters above bars indicate statistically significant differences (SNK test, α = 0.05).

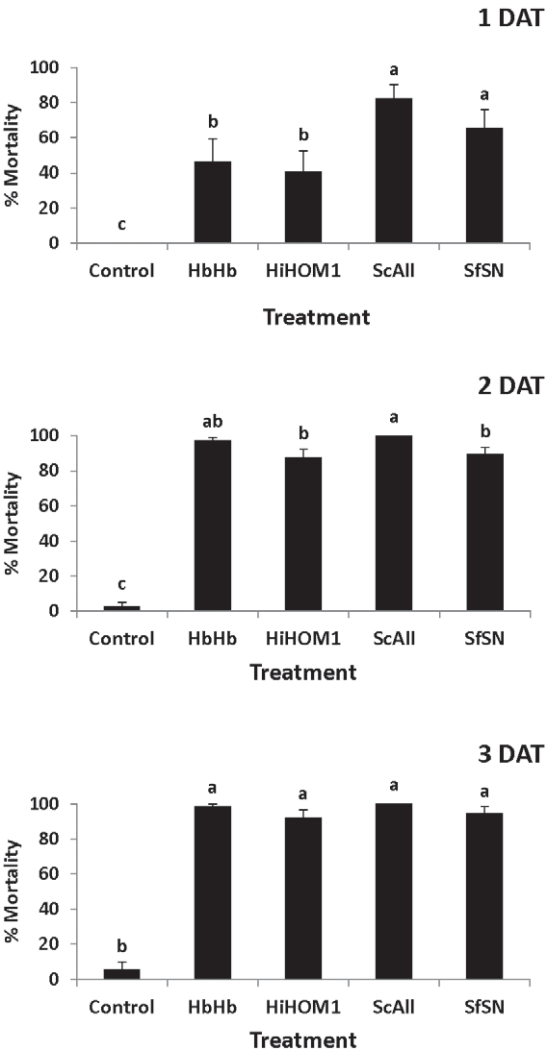

Fig. 3. Percentage mortality of *Stethobaris nemesis* following exposure to entomopathogenic nematodes or a water-only control for 1, 2, or 3 d after treatment (DAT). Nematode species names are abbreviated as follows: Hb = *Heterorhabditis bacteriophora*, Hi = *H. indica*, Sc = *Steinernema carpocapsae,* and Sf = *S. feltiae*; strain designations are indicated following the species abbreviation. Different letters above bars indicate statistically significant differences within each DAT (SNK test, α = 0.05).

tode (though they did mention a preliminary non-refereed report in which pathogenicity was detected). Thus, this is the first refereed report to indicate EPN pathogenicity and virulence to *C. ciliata* (and to the family Tingidae); this is also the first report of EPN reproduction in *C. ciliata*.

A challenging aspect of using entomopathogenic nematodes for control of *C. ciliata* is that the overwintering insects occur aboveground. Aboveground applications of EPNs can be limited due to the biocontrol agent's sensitivity to ultraviolet light and desiccation (Shapiro-Ilan et al. 2006). Possibly, environmental conditions associated with aboveground applications were the cause of failed biocontrol applications in the study of Tarasco & Triggiani (2006). The efficacy of EPN aboveground applications, however, may be enhanced by using improved formulations or post-application covers that protect the nematodes from environmental stress (Shapiro-Ilan et al. 2012). For example, the use of firegel (an environmentally friendly polymer based gel) or wood foam (based on hardwood fiber and a Celvol /starch mix) improved control of lesser peachtree borer, *Synanthedon pictipes* (Grote & Robinson) (Lepidoptera: Sesiidae) and *C. pomonella* (Shapiro-Ilan et al 2010; Lacey et al. 2010). Conceivably, these improvements in formulation and application may facilitate control of *C. ciliata* as well.

Among the nematodes tested, *S. carpocapsae* (All) may have the highest potential to control *S. nemesis* because it was the only one in the top tier of virulence on all sample dates. The high rate of mortality observed only 1 d post-treatment when applying *S. carpocapsae* (All) or *S. feltiae* (SN) may be particularly suitable for control of *S. nemesis* because amaryllis growers may find the quick reduction in damage attractive. The other nematodes tested, i.e., *S. feltiae* (SN), *H. bacteriophora* (Hb) and *H. indica* (HOM1), also showed high levels of virulence particularly 3 d post-treatment.

Application of EPNs for control of soil dwelling weevil pests has been successful in various systems (Grewal et al. 2005; Lacey & Shapiro-Ilan 2008). Similar to our study, *S. carpocapsae* has exhibited high levels of virulence to certain curculionid larvae such as the banana root borer, *Cosmopolites sordidus* Germar (Treverrow et al. 1991). In contrast, in a number of cases *S. carpocapsae* virulence to curculionid larvae has been reported to be inferior to other EPN species, e.g., when targeting Diaprepes root weevil, *Diaprepes abbreviatus* (L.) (Shapiro & McCoy 2000), plum curculio, *Conotrachelus nenuphar* (Herbst) (Shapiro-Ilan et al. 2011), or pecan weevil, *Curculio caryae* (Horn) (though *S. carpocapsae* is highly virulent to the adult stage) (Shapiro-Ilan et al. 2003).

Conceivably one might argue *S. carpocapsae* is not appropriate for *S. nemesis* control because the nematode is an ambush forager and thus tends to remain near the soil surface (Lewis & Clarke 2012). However, the search strategy is unlikely to be a major hindrance because the nematodes will not need to forage deep in the soil to infect *S. nemesis*. Furthermore, success in comparable situations has been observed when using *S. carpocapsae* to control other soil dwelling pests that bore into plant, e.g., when targeting peachtree borer, *Synanthedon exitiosa* (Say) (Lepidoptera: Sesiidae) (Shapiro-Ilan et al. 2009).

Applications of EPNs for *S. nemesis* control is promising due to the high degree of virulence observed in this study and bolstered by the history of success in using EPNs to control soil-dwelling weevils. Additionally, certain aspects of amaryllis culture, such as the need for moist soil conditions (Jauron & Nelson 2007) are also conducive to the use of EPNs (Shapiro-Ilan et al. 2006), and thus the compatibility of the biocontrol application is enhanced. Moreover, the relatively high crop value per unit area will likely make use of EPNs economically feasible.

Due to varying biotic or abiotic factors, EPN efficacy in laboratory studies is not equivalent to field efficacy (Shapiro-Ilan et al. 2012). Also, for the same reasons, the EPN species or strains with the highest laboratory virulence may not turn out to be the most efficacious under field conditions (Shapiro-Ilan et al. 2012). Therefore despite the potential shown in these laboratory studies for control of *C. ciliata* and *S. nemesis*, the promising EPN treatments observed in this study need to be tested for efficacy under field conditions prior to adoption as a control tactic.

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