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Multifaceted effects of host plants on entomopathogenic nematodes

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ABSTRACT

The success of parasites can be impacted by multi-trophic interactions. Tritrophic interactions have been observed in parasite-herbivore-host plant systems. Here we investigate aspects of multi-trophic interactions in a system involving an entomopathogenic nematode (EPN), its insect host, and host plant. Novel issues investigated include the impact of tritrophic interactions on nematode foraging behavior, the ability of EPNs to overcome negative tritrophic effects through genetic selection, and interactions with a fourth trophic level (nematode predators). We tested infectivity of the nematode, *Steinernema riobrave*, to corn earworm larvae (*Helicoverpa zea*) in three host plants, tobacco, eggplant and tomato. Tobacco reduced nematode virulence and reproduction relative to tomato and eggplant. However, successive selection (5 passages) overcame the deficiency; selected nematodes no longer exhibited reductions in phenotypic traits. Despite the loss in virulence and reproduction nematodes, first passage *S. riobrave* was more attracted to frass from insects fed tobacco than insects fed on other host plants. Therefore, we hypothesized the reduced virulence and reproduction in *S. riobrave* infecting tobacco fed insects would be based on a self-medicating tradeoff, such as deterring predation. We tested this hypothesis by assessing predatory success of the mite *Sancassania polyphyllae* and the springtail *Sinella curviseta* on nematodes reared on tobacco-fed larvae versus those fed on greater wax moth, *Galleria mellonella*, tomato fed larvae, or eggplant fed larvae. No advantage was observed in nematodes derived from tobacco fed larvae. In conclusion, our results indicated that insect-host plant diet has an important effect on nematode foraging, infectivity and reproduction. However, negative host plant effects, might be overcome through directed selection. We propose that host plant species should be considered when designing biocontrol programs using EPNs.

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1. Introduction

Entomopathogenic nematodes (EPNs) in the genera *Steinernema* and *Heterorhabditis* are insect parasitic organisms that live in soil ecosystems (Lewis and Clarke, 2012). Relationships between the plant and insect herbivore, and between the nematodes and their insect hosts have been extensively explored (Cory and Hoover, 2003; Lewis and Clarke, 2012; Stam et al., 2014). However, there is a relative dearth of knowledge concerning relationships between plants (first trophic level) and EPNs (third trophic level) (Rasmann et al., 2005; Ali et al., 2010, 2011; Hiltpold et al., 2010, 2011, 2015).

The infective juvenile (IJ) stage of EPNs penetrates the host insect through natural openings (mouth, spiracles, anus) or thin areas of the host's cuticle. The IJs then release their mutualistic bacteria (*Xenorhabdus* spp. for steinernematid nematodes and *Photorhabdus* spp. for heterorhabditids) into the insect's hemocoel. The mutualistic bacteria propagate and produce toxins and antimicrobial substances that kill the host in 48–72 h and protect the cadaver from colonization by other microorganisms. The nematodes initiate their development by using the food supplies provided by the bacterial biomass and the metabolized insect tissues and reproduce 1–3 generations depending on host size (Hazir et al., 2003; Lacey et al., 2015).

Several studies have shown that EPNs can be affected directly or indirectly by plants and that these interactions are potentially important in driving nematode foraging behavior and infection decisions. For example, phytochemicals produced in the host plant

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may have a direct effect on the insect immune system (Ojala et al., 2005; Klemola et al., 2007). Therefore, host plants may alter the insects' susceptibility to EPNs or their symbiotic bacteria (Kunkel et al., 2004; Thoden et al., 2007; Gassmann et al., 2010). Several studies assessing host plant effects on EPNs have been conducted with larvae of the southern corn rootworm, *Diabrotica undecimpunctata howardi* (Barbercheck, 1993; Barbercheck et al., 1995, 2003; Barbercheck and Wang, 1996). Differential tri-trophic effects among host plant species can reduce the fitness of EPNs that infect insects feeding upon these plants. Likewise, other studies have demonstrated that insect mortality can vary up to 50-fold when entomopathogens infect insects that feed on different host plants (Duffey et al., 1995; Kouassi et al., 2001; Ali et al., 2004).

Most of the prior studies involving EPNs such as those by Barbercheck (1993) and Barbercheck et al. (1995, 2003) focused on tri-trophic interactions associated with plant roots and associated root-based compounds. However, relative to the roots, most of the phytochemicals are found in plant leaves (phylloplane); insects feeding on leaves are relevant to soil entomopathogens (such as EPNs) because most of these insects will go on to pupate or overwinter in the soil. Therefore, the phylloplane is likely to be a major site for direct effects of the plant on entomopathogens (Cory and Hoover, 2003). Leaf phytochemicals have been observed to synergize or antagonize the fitness of other entomopathogenic viruses, fungi and bacteria (Cory and Hoover, 2003). For example, biologically activated phytochemicals can bind to viral occlusion bodies in the larval midgut and reduce subsequent infectivity to host insects (Felton and Duffey, 1990). Thus, it is critical to explore relationships between aboveground plant structures and natural enemies (e.g., EPNs); accordingly, objective 1 was to compare EPN virulence and reproduction when the nematodes are exposed to hosts fed on different host plants.

Additionally, we explored several other novel aspects of multitrophic interactions. Once a detrimental tri-trophic effect was detected, we explored the ability of the nematodes to adapt to the system through directed selection (objective 2). Another area that has not been addressed is tritrophic effects on nematode foraging. Prior studies have focused on the impact of tritrophic interactions after the nematodes have invaded the insect host (e.g., success of the infection, fecundity, etc.). In this study we examine the impact of host plant on EPN attraction behavior (objective 3). Moreover, to go beyond immediate tri-trophic effects, we investigated the impact of plant host-insect-nematode interactions on a fourth trophic level (nematode predators) (objective 4).

2. Material and methods

2.1. Host plants, insects and nematodes

Three solanaceous host plants were included in the study: tomato *Solanum lycopersicum* (Beefsteak Heirloom variety), eggplant *Solanum melongena* (Black Beauty Heirloom variety) and tobacco *Nicotiana tabacum* (Virginian Gold variety). The plants were grown in a greenhouse at the USDA-ARS Research Station in Byron, Georgia, USA and the leaves of the plants were used to feed the insect larvae. We chose three plants in the same family, Solanaceae, to allow for some evolutionary similarity among the host plants, but also with the understanding that the plants produce substantially different secondary compounds (in quality and quantity) (Pomilio et al., 2008; Yan et al., 2015).

Corn earworm (CEW) *Helicoverpa zea* (Lepidoptera: Noctuidae) eggs were obtained from the USDA-ARS CPRMU laboratory in Tifton, Georgia, USA. The insect eggs were divided into three groups and each group of eggs (approximately 200 eggs) was placed separately on leaves of a different host plant. The leaves were kept in a plastic container (10 × 20 × 15 cm) and incubated at 25 °C with a 14:10 light-dark cycle. When eggs hatched, the first instars were transferred to 30 ml plastic cups (WNA Chelmsford, MA) individually to prevent cannibalism and fed with their respective host plant leaves until they were used in experiments. The insect larvae were fed with the same plant from the egg stage to 5th instar.

The entomopathogenic nematode, *Steinernema riobrave* (isolate 355) was used in the experiments. Based on preliminary studies comparing several nematode species (unpublished data) as well as previous studies, we chose *S. riobrave* because it is highly virulent against *H. zea*, has been considered as a biocontrol tool for the target pest, and the two species (nematode and host) overlap in their natural distributions (Cabanillas and Raulston, 1996). The nematodes were reared in greater wax moth *Galleria mellonella* (Lepidoptera: Pyralidae) larvae according to Kaya and Stock (1997). Collected IJs were rinsed three times in sterile distilled water before being stored at 10 °C and used in experiments within two weeks.

2.2. Virulence experiments

Experiments were conducted in 30 ml plastic cups (4 cm diam., 3.5 cm deep) (Bioserv Inc., Frenchtown, NJ) filled with 5 g oven-dried sand (Shapiro and McCoy, 2000). Fifty *S. riobrave* IJs were pipetted in 0.5-ml distilled water onto the surface of sand in each cup. *S. riobrave* persists naturally in soils with high sand content (e.g., >97%) (Duncan et al., 2003) and thus the use of sand as a medium was justified. The total moisture level of sand was 10% (w/v). A single 5th instar *H. zea* was added to each cup immediately after nematode inoculation. Note, *H. zea* may enter soil to pupate at the 5th instar hence this insect stage was chosen (Reitz and Nettles, 1994). A piece of host plant leaf (approximately 4 cm^2) was added to each container to feed the larva. Three controls were included consisting of *H. zea* reared on the three different host plants, but not receiving nematode application. There were 3 replicates of 10 cups per treatment and control. The cups were incubated at 25 °C and larval mortality was recorded after 5 days. The experiment was conducted twice on different dates.

2.3. Reproduction experiments

Insect weight can significantly affect nematode progeny production (Barbercheck, 1993); therefore, each nematode-killed larva was weighed and then placed individually on White traps (White, 1927), which were stored at 25 °C. Infective juveniles that emerged from each cadaver were collected and counted using a microscope and nematode counting chamber. Reproduction was determined as IJs produced per mg of insect tissue (Barbercheck et al., 1995). There were 3 replicates of 10 cups per treatment and the experiment was conducted twice.

2.4. The impact of multiple generations for adaptation to host plant

Tomato and tobacco host plants were used for this experiment. The nematodes were reared for five passages consecutively on *H. zea* larvae fed on tomato or tobacco leaves. After the five passages, putatively adapted nematodes were assessed for virulence and reproduction in comparison with nematodes reared only on *G. mellonella* (these nematodes are hereafter referred to as 0-generation). Assays to measure virulence and reproduction using *H. zea* reared from egg to 5th instar on the different host plants were as described above.

2.5. Frass attraction experiments

Nematode attraction assays were based on procedures described by Grewal et al. (1997) and Shapiro-Ilan et al. (2009). Insect frass was obtained from each larval group dieting on the different host

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