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Three dimensional study of wounded plant roots recruiting entomopathogenic nematodes with Pluronic gel as a medium

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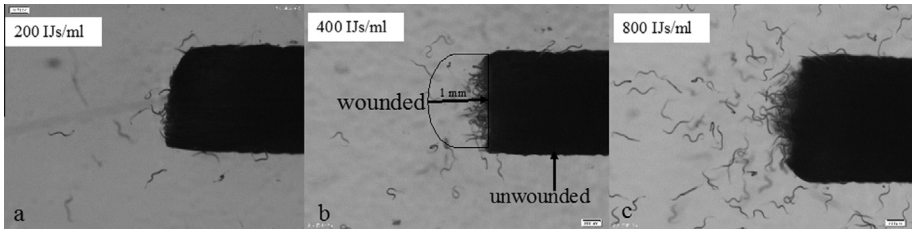
HIGHLIGHTS

- Pluronic gel system is a useful medium to study EPN host habitat finding behavior.
- Mechanically damaged roots greatly improve seeking ability of EPNs.
- Host habitat root exudates plays an important role in directing EPN localization.
- Storage duration and species/strains of EPNs influence nematode response to roots.

GRAPHICAL ABSTRACT

Attraction of *Heterorhabditis bacteriophora*-HBN (Hb-HBN) to wounded and unwounded chive root in pluronic gel with three concentrations at 4 h after assay initiation.



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ABSTRACT

Pluronic F-127 is a non-toxic and thermoreversible transparent copolymer that allows nematodes to move freely inside the gel and be observed under a microscope. Pluronic gel has previously been shown to be a useful medium for three dimensional study of plant parasitic nematode host-finding behavior, but not for host-habitat finding of entomopathogenic nematodes (EPNs). In the current study, we used Pluronic gel for the first time to investigate the foraging behavior of EPNs, which are natural enemies of root-feeding insect pests, and to investigate the effect of storage duration of EPNs on attraction to wounded roots. We first tested one isolate of EPN, *Heterorhabditis bacteriophora* HBN (Hb-HBN), from northeastern China that is known to be an effective bio-control agent against Chinese chive gnat (*Bradysia odoriphaga*). The nematodes aggregate around wounded root parts, indicating that the nematodes are attracted to compounds released from freshly wounded root tissue, where insect hosts are more likely to be present. Among species/strains of EPNs that were tested, Hb-HBN showed the strongest attraction to Chinese chive roots. Surprisingly, attraction to a Chinese chive root following storage duration of up to 30 days was much less than for Hb-HBN that were stored longer, with an optimal attraction observed for Hb-HBN that were stored 75–90 days. This might be associated with phased infectivity. This study provides a better understanding of EPN foraging behavior and introduces the Pluronic gel system as a useful medium to study EPN attraction.

1. Introduction

During the past 60 years entomopathogenic nematodes (EPN) (Rhabditida: Heterorhabditidae and Steinernematidae) have received considerable attention as biological control agents of soil insect pests (Campos-Herrera et al., 2012). EPNs are obligate insect parasites; the infective juveniles (IJs) of the third larval instar penetrate their hemocoel, release their symbiotic bacteria, and kill insects within 24–48 h (Chaston and Goodrich-Blair, 2010; Kaya and Gaugler, 1993). During the infection process, host-habitat finding (finding the habitat that favors presence of the insect host),

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might be one of the most important steps. Insect herbivore damaged plants have been shown to release chemical compounds (plant exudates) that recruit the natural enemies of their pests, and some plant exudates have been shown to attract EPNs resulting in more efficient bio-control (Ali et al., 2010, 2011; Aratchige et al., 2004; Choo et al., 1989; Gassmann et al., 2010; Hiltpold and Turlings, 2008; Jagdale et al., 2009; Rasmann et al., 2005; Turlings et al., 2012; Wang and Gaugler, 1998; Van Tol et al., 2001). Root exudates not only play a role as attractants, signal molecules, and stimulants to beneficial organisms, but also as inhibitors and repellents to soil pathogens or pests, which form mutualistic associations in the rhizosphere including root–root, root–microbe and root–insect interactions (Badri et al., 2009; Baetz and Martinoia, 2014; Bais et al., 2006). Identification and characterization of these unknown chemical compounds would help to elucidate the direct or indirect effect on plant defenses and the soil microbial community.

Although great progress has been achieved toward understanding interactions among insects, EPNs and symbiotic bacteria of EPNs, relatively less is known about how plants interact with EPNs. Lack of efficient methods or tools/medium to observe nematode behaviors below ground might be a major obstacle. Previous studies were based on either two-dimensional agar plates or non-transparent sand or soil filled olfactometers. So far, a below ground six-arm olfactometer has proven to be useful in studying EPN attraction (Rasmann et al., 2005) although it remains difficult to observe nematode movement and behavior in real time. Recently, a non-toxic and thermo-reversible transparent copolymer, Pluronic F-127, widely used in medical, pharmaceutical and cosmetic fields (Barichello et al., 1999; Farrugia et al., 2014; Marsh et al., 2003; Morishita et al., 2001; Zhang et al., 2010), has received more attention and proven to be a useful medium to study interactions between plant-parasitic nematodes and plants, including nematode host-seeking behavior and nematode chemotaxis (Feng et al., 2014; Fudali et al., 2013; Sasaki-Crawley et al., 2012; Wang et al., 2009a,b, 2010). A 23% solution of Pluronic is a semisolid gel at room temperature but a liquid at 15 °C and below. A stable gradient could be formed in the Pluronic gel in the presence of roots or chemicals (Feng et al., 2014; Fudali et al., 2013; Sasaki-Crawley et al., 2012; Wang et al., 2009a,b, 2010). The transparency and semisolid states allow nematodes to move freely in the gel fostering three-dimensional assay and allowing direct and real time observation of interactions between nematodes and the root system. Clearly, the plant cues were used by plant pathogenic nematodes for direct host finding. While the plant cues were used by EPNs for host-habitat finding. Therefore, whether or not the Pluronic gel system is suitable for the study of host-habitat finding behavior is unclear.

In our group, local isolates of a species of *Heterorhabditis bacteriophora* Poinar (Hb) from Harbin, China, had been shown to tolerate relatively lower soil temperatures and to be efficient against the root gnats, *Bradysia odoriphaga* Yang et Yang (Diptera: Sciaridae) on Chinese chive (*Allium tuberosum* Rottle ex Spreng) (Asparagales: Amaryllidaceae) (Li et al., 2011, 2013), a favored vegetable of Asians. In this study, we used the Hb-HBN strain and Chinese chive root in Pluronic gel, to test the system for a host-habitat location study and then the system was used to answer some fundamental questions. Our hypothesis is that the Pluronic gel should work for the EPN-host-habitat finding study since both plant pathogenic nematodes and EPNs use chemical cues for establishing either host or host-habitat location. Our objectives were to determine (1) if Pluronic gel can be used to study EPN host habitat finding behavior, (2) if storage time has any effect on the ability of host-habitat location, (3) if the same nematode species respond the same to different plant species and (4) if different nematode species or strains respond to the same plant roots differently.

2. Materials and methods

2.1. Nematodes

H. bacteriophora-HBN (Hb-HBN) was isolated from the soil around a pine tree at the Northeast Institute of Geography and Agroecology (IGA) of the Chinese Academy of Sciences, Harbin (45°45′N/126°39′E), Heilongjiang Province, China (Li et al., 2011). Two other isolates, *H. bacteriophora*-CD-11 (CD-11) and *H. bacteriophora*-NT-82 (NT-82), were kindly provided by Dr. Shulong Chen from the Institute of Plant Protection, Hebei Academy of Agriculture and Forestry Science in China. One isolate of *H. bacteriophora*-NJ (NJ) and four species of *Steinernema carpocapsae* (Weiser) – All (Sc-all), *Steinernema glaseri* (Steiner) (Sg), *Steinernema feltiae* (Filipjev) (Sf) and *Steinernema riobrave* Cabinillas, Poinar and Raulston (Sr), were obtained from Dr. Randy Gaugler's lab (Rutgers University, USA). *Steinernema litorale* Yoshida (Sl) was obtained from Dr. Kuijun Zhao, Northeast Agricultural University, Harbin, China. All EPN species and isolates were cultured in the last-instar of the great wax moth, *Galleria mellonella* L. (Lepidoptera: Pypalididae), at room temperature for 7–10 days (Kaya and Stock, 1997). IJs emerged from insect cadavers into White traps (White, 1927) and were collected and stored in shallow water in transfer flasks at 10 °C for up to three months; the specific storage time period is referred as "storage duration". Prior to experiments, IJs taken from storage were acclimated to room temperature for 1 h and their viability on the basis of movement was checked under a stereomicroscope with a hair prob.

2.2. Plant root preparation

All plants were grown and maintained in the experimental field of IGA. Chinese chive (*A. tuberosum* Rottle ex Spreng) was grown for more than 2 years. Shallots (*Allium cepa* L. var. aggregatum) (Asparagales: Amaryllidaceae) were planted in the field for 2 months during the growing season, garlic (*Allium sativum* L.) (Asparagales: Amaryllidaceae), tomato (*Lycopersicon esculentum* Mill.) (Solanales: Solanaceae) cv. Qiyanaifen and soybean [*Glycine max* (L.) Merrill] (Fabales: Fabaceae) cv. Hefeng 25 were planted in the greenhouse for one month before use. Approximately 0.5 cm of washed roots, where insects dwell, were excised from 2 to 2.5 cm of the bulb base of chive, shallot and garlic and of the stem of tomato and soybean. The transverse sections of all the cut roots from each plant were approximately 0.1 cm in diameter. The segments from 6 to 6.5 cm and 12 to 12.5 cm of the bulb base of Chinese chive roots were also excised for use to compare nematode response to different parts of wounded roots.

2.3. Pluronic gel preparation

Gel preparation and attraction assays were conducted according to Wang et al. (2009b). Approximately 23% (wt/vol) Pluronic F-127 gel (NF Prill Poloxamer 407, BASF, Mt Olive, NJ, USA) in 10 mM Tris–MES (morpholino-ethanesulfonic acid) buffer (Sigma–Aldrich) was made under refrigeration at 4 °C and with continuous stirring overnight. The dissolved gel was stored at 4 °C and aliquots were dispensed for experiments.

2.4. Nematode concentration and Heterorhabditis bacteriophora-HBN response to wounded and unwounded Chinese chive roots

To optimize the system, nematode Hb-HBN attraction to Chinese chive roots was tested with variable concentrations (200, 400 and 800 IJs/mL) based on the response of root-knot nematodes