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Entomopathogenic nematode infectivity enhancement using physical and chemical stressors

Ian M. Brown [a,*], David I. Shapiro-Ilan [b], Randy R. Gaugler [c]

[a] Department of Biology, Georgia Southwestern State University, Americus, GA 31709, USA
[b] USDA-ARS, SE Fruit and Tree Nut Research Lab, 21 Dunbar Road, Byron, GA 31008, USA
[c] Department of Entomology, Rutgers University, 93 Lipman Drive, New Brunswick, NJ 08901, USA

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Abstract

Entomopathogenic nematode *in vivo* production depends on a high level of successful infection during the inoculation process. Some host–nematode combinations do not provide sufficient or consistent infection levels and are, therefore, not suitable for *in vivo* production. Our objective was to determine whether infection levels could be improved through host or nematode exposure to physical and chemical stressors. Elevated stress may compromise host insect defenses, thus increasing its vulnerability to nematode infection. Specifically, we investigated the effects of stressors on *Heterorhabditis bacteriophora* infection of *Tenebrio molitor* larva. The stressors included temperature extremes, insecticidal oils and metal cations. Most chemical stressor–nematode combinations caused high host mortality and low infectivity rates. However, infective juvenile stimulation by the cation Mn^{2+} did enhance infectivity compared to the control. Dry heat (40 °C for 30 min) and hot water (60–70 °C for 1 s) treatments increased the host's susceptibility to *H. bacteriophora* infection with respect to unstressed controls, and the yields and virulence of infective juveniles produced in hosts stressed by these treatments were not affected. Thus, host stress methods can be used to successfully enhance infection levels for *in vivo* production of *H. bacteriophora*.

Keywords: *Heterorhabditis bacteriophora*; *Tenebrio molitor*; Infectivity; Entomopathogenic; Nematode; Production; Stressor; Temperature; Heat treatment; Oil; Magnesium; Manganese

1. Introduction

Entomopathogenic nematodes (Rhabditida: *Heterorhabditis* and *Steinernema*) exhibit virtually every attribute of an ideal biological control agent, including simple mass production. Nematodes are produced by large scale *in vitro* production (Friedman, 1990; Georgis, 2002; Shapiro-Ilan and Gaugler, 2002) and small-scale cottage industries employing *in vivo* methods (Gaugler and Han, 2002; Gaugler et al., 2000).

In vivo production depends on high levels of successful infections. Low infection levels waste insects and require the labor-intensive removal of non-infected hosts (Shapiro-Ilan et al., 2002). Most *in vivo* producers produce entomopathogenic nematodes in *Galleria mellonella* L. (Lepidoptera: Pyralidae) larvae using a modified White trap (Dutky et al., 1964). The advantages of *G. mellonella* are its susceptibility to most entomopathogenic nematode species, the high infective juvenile yields produced, and its commercial availability in the US (Gaugler et al., 2000).

Laboratory studies (Blinova and Ivanova, 1987; Shapiro-Ilan et al., 2002) suggest that *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) could be an alternative *in vivo* production host. Production of the yellow mealworm *T. molitor* is less expensive compared with *G. mellonella* and, therefore, promises to reduce nematode production costs. Current production costs are estimated at 0.2 ± 0.03 and 0.15 ± 0.02 US dollars/g of host tissue for *G. mellonella* and *T. molitor*, respectively (based on retail prices of seven US

* Corresponding author. Fax: +1 229 931 2734.
E-mail address: ibrown@canes.gsw.edu (I.M. Brown).

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companies who produce both species) (I. Brown 2005 unpublished results). Unfortunately, *T. molitor* is also less susceptible than *G. mellonella* to some nematode species, including *Heterorhabditis bacteriophora* Poinar. In short, the key advantage of *T. molitor* is reduced host production costs. However, this advantage cannot be exploited unless consistently high infection levels can be achieved.

Physical and chemical stressors could be used to compromise insect defenses in order to enhance nematode infection. Stresses induced by physical extremes such as temperature have been responsible for increased parasitism in a variety of plants and animals (Daane and Williams, 2003; Pickett et al., 2003; Shibata, 2000). Several studies (Hallman and Armstrong, 1994; Hansen et al., 1990; Jang, 1991; Sharp et al., 1991) demonstrated that physical heat treatments could be accurately fine-tuned to kill insects without compromising the fruit containing them. Based on these findings we examined the potential of sublethal heat treatments to compromise *T. molitor* defenses in order to increase *H. bacteriophora* infection.

Combinations of nematodes with chemicals and pathogens have been used to increase nematode efficacy in laboratory and field applications. Nematodes have been combined with pesticides (Head et al., 2000; Koppenhöfer et al., 2003; Rovesti et al., 1989; Zimmerman and Cranshaw, 1990), adjuvants (Baur et al., 1997), bacteria (Kaya et al., 1995; Koppenhöfer and Kaya, 1997; Koppenhöfer et al., 2000a; Thurston et al., 1993, 1994a), viruses (Agra Gothama et al., 1996) and fungi (Choo et al., 1996). Increased susceptibility to entomopathogenic nematode infection has also been demonstrated in several herbivorous insects exposed to plant and fungal secondary metabolites (Barbercheck and Kaya, 1991; Grewal et al., 1995). In addition to stressing the target hosts, infective juveniles can also be exposed to infectivity enhancing additives. Jaworska et al. (1997, 1999), demonstrated that manganese and magnesium cations enhanced *H. bacteriophora* infection in *G. mellonella* and the weevil *Sitona lineatus* (L.). Based on these findings, we examined the ability of certain chemical pesticides and metal cations to enhance the infection of *T. molitor*.

Specifically, our objective was to investigate the potential of host stressor regimes to produce acceptable levels of *H. bacteriophora* infection in *T. molitor* larvae. Shapiro-Ilan et al. (2002) observed poor infection levels when *T. molitor* larvae were immersed in suspensions of *H. bacteriophora* (55% infection after immersion in 28,000 infective juvenile/ml suspensions). The effectiveness of the immersion technique may be increased if host defenses are compromised, thus increasing the usefulness of *T. molitor* as a production host for *H. bacteriophora* and other important entomopathogenic nematode species.

2. Materials and methods

All experiments were conducted on final instar *T. molitor* larvae purchased from Southeastern Insectaries, Perry, Georgia. The *H. bacteriophora* (HB strain) used were from laboratory stock cultures held at the Fruit and Tree Nut Research Station, Byron, Georgia and were cultured in *G. mellonella* (Woodring and Kaya, 1988). Infective juveniles (IJ) used in all experiments were less than 7 days old.

Stress regime success was based on the percentage of successful patent infections. Only cadavers exhibiting patent signs of infection (i.e., color change to red) were counted as successful infections. Other dead larvae such as those that turned gray or black were contaminated with other non-symbiotic microbes, and less likely to produce nematode progeny (Woodring and Kaya, 1988). Infectivity was defined as the percentage of patent infections, whereas mortality is the percentage of dead larvae regardless of infection quality. This is a reasonable criterion since patent infections are most likely to result in normal infection and IJ yields, whereas contaminated cadavers are more likely to produce reduced yields.

2.1. Physical stressors

Tenebrio molitor larvae were exposed to temperature extremes and dehydration stresses to determine their effects on the host's susceptibility to *H. bacteriophora* infection. For all physical stress and yield experiments, 2 trials of 3 replicates (6 replicates total), each consisting of 20 larvae, were undertaken. All stressed larvae and positive controls were placed in a 3.2 mm sieve and immersed for 1 s in a 300 ml suspension of 21,000 IJ/ml of *H. bacteriophora* (see Shapiro-Ilan et al., 2002). After immersion the larvae were placed in 90-mm petri dishes. The petri dishes were incubated at 25 °C for 96 h, and *T. molitor* larval mortality and infection assessed. Mortality was assessed by gentle prodding with a blunt instrument; larvae showing no movement were considered dead. Infections were counted as successful only in cadavers exhibiting patent signs of infection.

Tenebrio molitor larvae were partially dehydrated by exposing them to dry heat prior to nematode infection. Larvae in glass petri dishes were exposed to dry heat at 35, 40, 45 or 50 °C for 30 min followed by immersion into a nematode suspension. In a second approach, larvae were partially dehydrated over ammonium sulphate desiccation crystals (Dryrite™, W.A. Hammond Co., Xenia, OH) for 24, 48, 72, 96, 120 or 144 h at 25 °C before immersion. In both dehydration experiments the controls were kept in petri dishes in incubators at 25 °C and approximately 95% RH prior to being exposed to nematodes. Humidity was maintained using petri dishes of water.

Temperature shock was applied to *T. molitor* using hot water. *T. molitor* larvae contained in a 3.2 mm sieve were immersed for 1 s in water heated from 50 to 80 °C in 5 °C increments. After immersion, the excess water was removed by pressing the sieve onto paper towels for 1 s. Larvae were then immersed in the nematode suspension for 1 s. Controls were exposed to nematodes only (i.e., positive control) since only infection improvement was being addressed. Negative controls (i.e., physical stressor only) were not carried out