Note

Superior efficacy observed in entomopathogenic nematodes applied in infected-host cadavers compared with application in aqueous suspension

Entomopathogenic nematodes (genera *Steinernema* and *Heterorhabditis*) can control a wide variety of economically important pests (Shapiro-Ilan et al., 2002), and are generally applied in aqueous suspension through a variety of agricultural spray equipment or irrigation systems (Grewal, 2002). These nematodes have also been shown to be effective when applied in their infected-host cadavers (Creighton and Fassuliotis, 1985; Jansson et al., 1993). Compared with application in aqueous, laboratory studies have indicated application of infected cadavers can result in superior nematode dispersal (Shapiro and Glazer, 1996), infectivity (Shapiro and Lewis, 1999), and survival (Perez et al., 2003). There remains, however, a need to test further the infected cadaver approach under greenhouse and field conditions, and to directly compare this approach to aqueous application. In two greenhouse experiments, we compared the efficacy of entomopathogenic nematodes applied in aqueous suspension with application in infected cadavers; one experiment targeted the diaprepes root weevil, *Diaprepes abbreviatus* (L.) with *Heterorhabditis indica* Poinar, Karunakar, and David (Hom1 strain), and the other the black vine weevil, *Otiorhynchus sulcatus* (F.) with *Heterorhabditis bacteriophora* Poinar (Oswego strain).

Insects were obtained from laboratory-reared cultures, and nematodes were reared in vivo according to

![Graphs showing survival of insects](Fig. 1. Mean percentage survival of *D. abbreviatus* following application of *H. indica* (Hom1 strain) in aqueous suspension (from White traps or spray) or in infected *T. molitor* cadavers (A) 7, (B) 14, (C) 21, and (D) 28 days after nematode emergence began. Different letters above bars indicate statistical significance (Tukey’s test).)
Kaya and Stock (1997). Methods for greenhouse experiments were based on procedures described by Shapiro and McCoy (2000). Nematode cultures, maintained on Galleria mellonella L., were used to inoculate the host insect for application, Tenebrio molitor L.; T. molitor were infected on filter paper in Petri dishes (90 mm) with 600 (for H. indica) or 800 (for H. bacteriophora) infective juveniles (IJ$s) per insect. Seven days after inoculation the infected T. molitor were transferred to the greenhouse. Two infected T. molitor were either placed directly on soil in a 15-cm pot (the infected cadaver application), or on a White trap, which was adjacent to the corresponding pot designated for aqueous application. Thus, the nematode application rate was standardized at two nematode infected T. molitor worth of IJs per pot; previous studies indicated that the average (se) number of IJs produced per T. molitor infected under similar conditions was 58,250 (12,900) and 95,667 (9,765) for H. indica (Hom1) and H. bacteriophora (Owego), respectively (Shapiro-Ilan and Gaugler, 2002). The contents of White traps were poured into pots daily during the period of nematode emergence (up to 23 days post-inoculation for the D. abbreviatus experiment, and 21 days for the O. sulcatus experiment). Soil in each pot was kept at approximately the same moisture level throughout the experiment. Ambient and soil temperatures averaged (se) 26.8 (1.7) and 25.2 (1.1) °C in the D. abbreviatus experiment, and 23.7 (1.4) and 21.8 (0.8) °C in the O. sulcatus experiment. In the D. abbreviatus experiment, each pot contained 5 target hosts (ca. 7th–9th instar), and five baby carrots (ca. 5 cm long) for food. Pots in the O. sulcatus experiment each contained 10 insects and one medium-sized carrot as a food source. The number of live insects remaining was determined 7, 14, 21, and 28 days after IJs began to emerge in the D. abbreviatus experiment (i.e., 16, 23, 30, and 37 days post-inoculation), or 7, 14, and 28 days after IJs began to emerge in the O. sulcatus experiment. Pots for each sample date were organized separately in randomized block designs with 10 pots per treatment and a control (water only). In the D. abbreviatus experiment, an additional aqueous treatment was included consisting of nematodes harvested, stored and sprayed onto soil in the pots in a manner similar to commercial production and how a grower might apply them. Twenty infected T. molitor intended for the spray treatment were inoculated in parallel with other treatments, maintained on White traps in the laboratory, which were harvested daily, and stored at 10 °C until emergence ceased (23 days post-inoculation). The treatment was then applied from a plastic spray bottle at a rate of two infected T. molitor’s worth of IJs per pot and evaluated 14 days later. The pots receiving the spray treatment were placed and evaluated along with the other pots evaluated at 37 days post-inoculation. In each experiment, treatment effects were analyzed by sample date through ANOVA of mean percentage survival (transformed by arcsine of square root, α = 0.05), and Tukey’s multiple range test (SAS, 1999).

On all sample dates, survival of D. abbreviatus was lower in the infected cadaver treatment than the aqueous applications; all nematode applications caused reduced survival relative to the untreated control (Fig. 1). In the cadaver treatment, no D. abbreviatus survival was observed in the last sample date. Aqueous application in spray versus White traps did not appear to affect efficacy (Fig. 1). In the O. sulcatus experiment, the cadaver treatment caused lower insect survival than the aqueous treatment at the first sample date, and was the only treatment causing lower survival than the control on all sample dates (Fig. 2). By the third sample
date, control mortality reached high levels presumably
due to age of the larvae or other unidentified natural
mortality factors (Fig. 2). Several previous reports have
suggested that efficacy of the cadaver application ap-
proach is approximately equal to application in aque-
ous, but these studies were inconclusive or flawed due to
a complete lack of statistical methodology (Welch and
Briand, 1961), insufficient replication (Parkman et al.,
1993), or a lack of power to show differences among
treatments (even between any treatment and the control)
(Jansson and Lecrone, 1994). This study indicates that
entomopathogenic nematode application in infected
cadavers tends to be more efficacious than application in
aqueous. The increased efficacy observed in the cadaver
applications may have been due to additional physio-
logical stress in the aqueous application (during tem-
porary storage in water or upon application). Superior
efficacy in the cadaver application might also have been
due to compounds in the infected host cadaver that can
enhance nematode infectivity or dispersal (Shapiro and
Lewis, 1999; Shapiro et al., 2000). Further testing is
underway to verify the findings of this study.

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