Effects of a novel entomopathogenic nematode-infected host formulation on cadaver integrity, nematode yield, and suppression of *Diaprepes abbreviatus* and *Aethina tumida*

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**Abstract**

An alternative approach to applying entomopathogenic nematodes entails the distribution of nematodes in their infected insect hosts. Protection of the infected host from rupturing, and improving ease of handling, may be necessary to facilitate application. In this study our objective was to test the potential of a novel method of formulating the infected hosts, i.e., enclosing the infected host in masking tape. *Tenebrio molitor* L. cadavers infected with *Heterorhabditis indica* Poinar, Karunakar and David or *Steinernema carpocapsae* (Weiser) were wrapped in tape using an automatic packaging machine; the machine was developed to reduce labor and to standardize the final product. The effects of the tape formulation on the ability to protect the cadavers from mechanical damage, nematode yield, and pest control efficacy were tested. After exposure to mechanical agitation at 7-d-post-infection, *S. carpocapsae* cadavers in tape were more resistant to rupture than cadavers without tape, yet *H. indica* cadavers 7-d-post-infection were not affected by mechanical agitation (with or without tape), nor was either nematode affected when 4-d-old cadavers were tested. Experiments indicated that infective juvenile yield was not affected by the tape formulation. Laboratory experiments were conducted measuring survival of the root weevil, *Diaprepes abbreviatus* (L.), or the small hive beetle, *Aethina tumida* Murray, after the application of two *H. indica*-infected hosts with or without tape per 15 cm pot (filled with soil). A greenhouse experiment was also conducted in a similar manner measuring survival of *D. abbreviatus*. In all experiments, both the tape and no-tape treatments caused significant reductions in insect survival relative to the control, and no differences were detected between the nematode treatments. Fifteen days post-application, the infected host treatments caused up to 78% control in *A. tumida*, 91% control in *D. abbreviatus* in the lab, and 75% in the greenhouse. These results indicate potential for using the tape-formulation approach for applying nematode infected hosts.

1. **Introduction**

Entomopathogenic nematodes in the genera *Steinernema* and *Heterorhabditis* are important biological control agents for a variety of economically important pests (Grewal et al., 2005). The nematodes can be mass-produced using *in vivo* or *in vitro* methods (Shapiro-Ilan and Gaugler, 2002). Commercial entomopathogenic nematodes are generally applied as infective juveniles (IJJs) in aqueous suspensions using various irrigation systems, sprayers, or injection techniques (Shapiro-Ilan et al., 2006). Entomopathogenic nematodes can also be applied in infected insect cadavers (Creighton and Fassuliotis, 1985; Jansson et al., 1993; Shapiro-Ilan et al., 2003; Bruck et al., 2005; Del Valle et al., 2008). In this approach, nematode-infected cadavers are disseminated and pest suppression is subsequently achieved by the progeny IJJs that exit the cadavers. Laboratory studies indicate that nematode application in infected hosts can be superior to application in aqueous suspension (Shapiro and Glazer, 1996; Shapiro and Lewis, 1999; Perez et al., 2003). Additionally, pest control trials have indicated that cadaver application can be superior in efficacy to aqueous application (Shapiro-Ilan et al., 2003).

A potential problem with the cadaver application, however, is that the infected hosts can rupture or stick together during transport or distribution (Shapiro-Ilan et al., 2001). Shapiro-Ilan et al. (2001) reported that the problem can be overcome in soft bodied hosts such as the greater wax moth, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae), by coating the cadavers with a powder such as clay. Similarly, cadaver coatings were developed for application...
of Heterorhabditis baujardi Phan et al. (Del Valle et al., 2009). Another approach is to use hard-bodied insects, such as the yellow mealworm, Tenebrio molitor L. (Coleoptera: Tenebrionidae), for which the harder cuticle can naturally resist rupture and prevent cadavers from sticking together (Shapiro-Ilan et al., 2008). Consequently, current commercial pursuit of the cadaver application approach in USA is focused on use of T. molitor. Yet, use of hard-bodied infected hosts may still result in some rupturing during shipping or (perhaps more so) handling upon application. Furthermore, use of cadavers in certain markets, such as home gardens, may be limited due to aversion of humans to touching insects (including dead ones) (Kellert, 1993). Thus, a formulation that protects cadavers and allows ease of handling would be beneficial for hard and soft bodied hosts. Additionally, a formulation that is amenable to mass production and standardization will facilitate successful commercialization.

In this study our objective was to determine the potential of using a new method of formulating and packaging the infected hosts, i.e., wrapping the infected hosts in masking tape. Nematode-infected cadavers were wrapped in masking tape using an automatic packaging machine (Morales-Ramos et al., 2008); the machine was developed to facilitate mass production, reduce labor in the formulation process, and standardize the final product. To address our objective, we determined the effects of the tape formulation on the ability to protect the cadavers from mechanical damage, nematode yield, and pest control efficacy.

For the experiments focusing on protection against mechanical damage and nematode yield, we chose one nematode from each of the entomopathogenic nematode genera, i.e., Heterorhabditis indica Poinar, Karunakar and David and Steinernema carpocapsae (Weiser), and used T. molitor as the “model” host. For assessment of pest control efficacy, we chose the Diaprepes root weevil Diaprepes abbreviatus (L.) (Coleoptera: Curculionidae) and the small hive beetle, Aethina tumida Murray (Coleoptera: Nitidulidae) as target pests, and used T. molitor infected with H. indica as the treatment. T. molitor is amendable to, and currently used in, in vivo mass production of entomopathogenic nematodes (Shapiro-Ilan and Gaugler, 2002; Shapiro-Ilan et al., 2002a). H. indica is a commercially available nematode that is virulent to a variety of economically important pests (e.g., Mbata and Shapiro-Ilan, 2005; Dolinski et al., 2006) including D. abbreviatus (Shapiro et al., 1999) and A. tumida (Tedders, unpublished). D. abbreviatus is a major citrus pest and an important commercial target for entomopathogenic nematodes in North America (Shapiro-Ilan et al., 2005), and A. tumida is an important pest of honey bees, Apis mellifera L. (Ellis and Delaplane, 2008), and has shown susceptibility to entomopathogenic nematodes (Cabanaillas and Elzen, 2006; Tedders, unpublished).

2. Materials and methods

2.1. Nematodes, insects, and formulation of cadavers

T. molitor (9th–10th instar weighing 70–90 mg each) were supplied by Southeastern Insectaries, Inc. (Perry, GA). D. abbreviatus larvae (40–60 d old) were obtained from the Florida Department of Plant Industries (Gainesville, FL), and last instar A. tumida were provided by J.D. Ellis (University of Florida, Gainesville). Prior to experiments, the nematodes H. indica (Hom 1 strain) and S. carpocapsae (All strain) were cultured in G. mellonella (obtained from Webster’s Waxie Ranch, Webster, WI) according to Kaya and Stock (1997). For all experiments, H. indica and S. carpocapsae infected cadavers were produced on filter paper (Whatman No. 1) based on procedures described by Shapiro-Ilan et al. (2003); insects were inoculated with 200 IJs per insect for S. carpocapsae, and 800 (yield experiments) or 600 (efficacy experiments) IJs per insect for H. in-

dica. All nematode and insect culturing was conducted at approximately 25 °C.

Infected cadavers were formulated using a mechanized tape-packaging machine (Fig. 1) (Morales-Ramos et al., 2008). The packaging material used was making tape, which is a pressure-sensitive tape made of easy-to-tear paper, and an easily released adhesive. In the packaging machine, the cadavers are placed in a holding container that is slightly agitated. Individual cadavers are then picked up via vacuum suction and placed at the juncture between extended pieces of masking tape that are automatically dispensed. The tape strips are then clamped together enclosing the cadaver and the joined tapes are advanced at a predetermined interval. Subsequent cadavers are placed at evenly spaced intervals between the tapes. The finished tape strips with cadavers are then formed into a roll for packaging, storage, or direct use. The cadavers in a roll can then be cut out into single or multiple units as needed. A programmable logic controller enables the machine to be completely automatic and seal cadavers between two pieces of tape without any manual intervention except turning on and off the power switch.

2.2. Effects of tape formulation on the ability to protect the cadavers from mechanical damage and nematode yield

The ability of the tape formulation to protect infected cadavers from rupturing was evaluated based on procedures described by

Fig. 1. An automatic formulation and packaging machine for enclosing nematode infected hosts in tape. The top photograph is an overview. The bottom photograph is a close up with labeled parts including: (A) tape dispenser, (B) cadaver positioning device, (C) cadaver wrapper, (D) moving piston, and (E) cadaver holding bowl.
Shapiro-Ilan et al. (2001). Using manual shake tests, the goal was to simulate potential mechanical agitation during shipping or application. The tendency to rupture was evaluated in tape-formulated and non-formulated cadavers at 4 and 7 d post-inoculation. Five formulated or non-formulated cadavers were placed in a 90 mm Petri dish. The two Petri dishes (one from each treatment) were stacked on top of each other in random order and shaken vigorously for 20 s; the rate of shaking was equivalent to approximately 2.7 swing actions up and down per second. The cadavers from each nematode species and time of formulation were treated separately. After shaking, the cadavers in each Petri dish were examined and the percentage ruptured was recorded; any breakage in the cuticle was considered a rupture. To determine yield, after the shaking was complete, cadavers from each Petri dish were placed on White traps and the numbers of IJs emerging was determined after 21 d post-inoculation (Kaya and Stock, 1997). Additionally, for inclusion in the yield comparisons, an equivalent set of T. molitor were inoculated but not shaken. There were four replicates (Petri dishes) of each treatment and time of formulation and all experiments were repeated once in time (two trials). Note that in the second trial the cadavers were wrapped in tape manually as the packaging machine was temporarily out of order.

2.3. Pest control efficacy of tape-formulated and non-formulated cadavers

The effects of formulated and non-formulated cadavers on survival of D. abbreviatus were evaluated based on procedures described by Shapiro-Ilan et al. (2003). Experimental arenas consisted of 13 cm square pots (18 cm depth) filled with potting soil (Metro-Mix 360, Sun Gro, Inc. Bellevue, WA, USA) and with metal screens on bottom to prevent insect escape. Five D. abbreviatus larvae were introduced approximately 2 cm below the soil surface along with a piece of carrot (ca. 1 cm diam. × 3 cm long) as food source, which was placed vertically directly below the insect. One day after adding the D. abbreviatus larvae, one third of the pots received two H. indica infected cadavers with the tape covering, one third received two cadavers without tape, and the last third was left as an untreated control; cadavers used in pest control experiments were not previously shaken. Cadavers, which had been exposed to H. indica 7 d earlier, were each inserted into test pots approximately 1 cm below the soil surface. At 10 d post-treatment, half of the pots were dismantled and the number of surviving larvae was determined for each pot; the second half of the pots was evaluated at 15 d post-treatment. There were five replicate pots for each treatment and control and for each sampling date (30 pots total). The experiment was conducted in the laboratory and repeated once in time with a fresh batch of cadavers (two trials). A single trial (30 pots) was also conducted in the greenhouse. Temperatures were monitored during the experiments and ranged from 22.8 °C to 25.6 °C in the first laboratory trial, 21.1–22.2 °C in the second laboratory trial, and 22.7–30.6 °C in the greenhouse trial.

The effects of the tape-formulated versus non-formulated cadavers on the survival of A. tumida was conducted in the laboratory in a similar manner to the D. abbreviatus evaluation except that the arenas consisted of plastic containers (11 cm top diam., 8 cm bottom diam., 8 cm depth, with approximate 0.5 mm holes on bottom) containing 10 A. tumida each, and the arenas did not contain carrots. The containers were lidded during the experiment (to prevent insect escape). Each trial contained four replicate containers for each treatment and each assessment date (10 and 15 d) resulting in 24 containers total. All other parameters were the same as those described for the D. abbreviatus evaluation. The experiment was repeated once in time (two trials); the first trial was conducted simultaneously with the first D. abbreviatus laboratory trial (using the same batch of T. molitor cadavers), whereas the second trial was conducted separately. Temperature was monitored during the experiment and ranged from 22.8 °C to 25.6 °C in the first laboratory trial, and 21.1–22.2 °C in the second laboratory trial.

The number of IJs emerging from cadavers was also estimated in the pest control efficacy tests. In the D. abbreviatus greenhouse efficacy test, reproductive yield from tape-formulated and non-formulated cadavers was statistically compared using the same batch of cadavers (an excess of cadavers were produced for this purpose); methods were as described above for assessment of yield. There were four replicate White traps containing five cadavers each. The White traps were set up on the day that cadavers were applied to pots containing D. abbreviatus; the number of IJs emerged was determined 14 d later (21 d post-inoculation). Additionally, we recorded average yield per cadaver in inoculations made for the first laboratory test (which included both A. tumida and D. abbreviatus trials) as well as the second D. abbreviatus laboratory trial. These estimates for the laboratory tests were based on two White traps totaling 196 and 31 cadavers, respectively. The intent was not to compare yield in tape versus no-tape, since this was done in separate experiments as indicated above, but rather simply to verify that a reasonable number of IJs were emerging from the cadavers in these experiments (thus giving an estimate of how many IJs were emerging into each pot).

2.4. Data analysis

Differences in percentage of cadavers rupturing, percentage survival of target insects in the pest control efficacy experiments, and IJ yields were detected through ANOVA; the Student–Newman–Keuls’ test was used to elucidate treatment effects when a significant F value (P < 0.05) was detected (SAS, 2001). Data from the 10 d and 15 d assessments were analyzed separately. Percentage data were arcsine transformed and numerical data (nematode yield) were square-root transformed prior to analysis (Southwood, 1978; Steel and Torrie, 1980; SAS, 2001). In all experiments with multiple trials, interactions between trial and treatment effects were not significant (P > 0.05); thus, data from trials repeated in time were combined and variation among trials was accounted for as a block effect.

3. Results

3.1. Effects of tape formulation on the ability to protect cadavers from mechanical damage and nematode yield

Following mechanical agitation (shaking), tape-formulated S. carpocapsae cadavers were more resistant to rupture than cadavers without tape at 7-d-post-infection (F = 16.38; df = 1, 13; P < 0.0014); 37.5 ± 11.0% of the unformulated cadavers ruptured, whereas 0% rupturing was observed in the tape-formulated cadavers. However, no rupturing was detected in any H. indica cadavers 7-d-post-infection, nor was rupturing detected in cadavers infected with either nematode species 4-d-post-infection.

No differences in IJ yield per insect were detected in cadavers formulated in tape and non-formulated cadavers, except in one instance (S. carpocapsae 4-d-post-infection) the no-tape treatment that was shaken yielded more IJs (21,888 ± 5418 per insect) than the other treatments including the no-shake/no-tape control; the range of yield in the other treatments was 4036 ± 1453 to 7438 ± 2083. In the experiment testing S. carpocapsae at 7-d-post infection, yields ranged from 30,176 ± 6386 to 33,938 ± 7844. For H. indica, the yield of IJs per insect varied among the experiments from 31,024 ± 8616 to 63,750 ± 11,564. Also, estimated yields of IJs from cadavers inoculated for use in other experiments (not statistically compared) varied and were (mean ± SD) 29,965 ± 10,231
and 66, 308 ± 25,890 in the first laboratory and second *D. abbreviatus* laboratory trial, respectively. These yields, however, are all within expected ranges based on prior studies (Shapiro-Ilan and Gaugler, 2002; Shapiro-Ilan et al., 2002a, 2003).

3.2. Pest control efficacy of tape-formulated and non-formulated cadavers

Data indicated tape-formulated cadavers are efficacious in reducing insect pest survival at a level similar to that of non-formulated cadavers. In the laboratory evaluation of *D. abbreviatus*, both the formulated and non-formulated infected *T. molitor* cadaver applications resulted in lower insect survival than the control at 10 d and 15 d post-application (*F* = 29.32; *df* = 2, 32; *P* < 0.0001 and *F* = 37.95; *df* = 2, 32; *P* < 0.0001, for the 10 d and 15 d assessment dates, respectively), and there were no differences detected between the two treatments (Fig. 2). If Abbott's formula (Abbott, 1925) is applied to the *D. abbreviatus* survival data in the laboratory, the level of control at 15 d post-application was 75.0% and 90.9% in the tape and no-tape treatments, respectively. Similar to the laboratory trials, in the greenhouse test, no differences in *D. abbreviatus* survival were detected between the formulated and non-formulated cadavers at 10 d and 15 d post-application, and both treatments caused lower *D. abbreviatus* survival than the control (*F* = 8.94; *df* = 2, 12; *P* = 0.004, and *F* = 16.92; *df* = 2, 12; *P* = 0.0003 for the 10 d and 15 d assessment dates, respectively) (Fig. 3). Based on Abbott's formula, the level of control at 15 d post-application was 70.8% and 75.0% in the tape and no-tape treatments, respectively.

The pest control efficacy results in the *A. tumida* experiment were similar in that the *D. abbreviatus* experiments in that the infected cadaver applications resulted in lower insect survival than the control at 10 d and 15 d post-application, and there were no differences detected between the tape and no-tape treatments (*F* = 15.03; *df* = 2, 23; *P* < 0.0001, and *F* = 24.55; *df* = 2, 23; *P* < 0.0001, for the 10 d and 15 d assessment dates, respectively) (Fig. 4). Based on Abbott's formula, the level of control at 15 d post-application was 78.0% and 67.8% in the tape and no-tape treatments, respectively.

4. Discussion

Our results indicate the novel formulation of tape-enclosed cadavers has potential for use in application of entomopathogenic nematodes. We did not detect any detrimental effects of the tape formulation on pest control efficacy of *D. abbreviatus* and *A. tumida*, overall IJ yield was also not affected, and in some circumstances the tape formulation provided protection from rupture. Furthermore, the approach has promise because the mechanized packaging machine facilitates mass production of the final product.

In terms of IJ yield, in four out of five comparisons no differences were detected among all tape and no-tape treatments. The only difference detected was in one comparison (*S. carpocapsae* 4-d-post-infection) where the no-tape treatment that was shaken yielded more IJs than other treatments, including the no-shake/no-tape control. We cannot explain why that single treatment resulted in a higher yield. If the difference was due to the tape acting as an impediment, then the no-tape/no-shake treatment should also have exhibited a higher yield. The yields of other treatments (besides no-tape/shake) in the 4-d-post-infection experiment with *S. carpocapsae* were more than 3-fold lower than the 7-d-post-infection experiment; possibly, a handling error occurred in the 4-d-post-infection experiment. Nonetheless, given that tape versus no-tape was not conclusively indicated as the cause of the IJ yield difference (in the one experiment where a difference was detected), and in all experiments yields in no/shake tape and no-shake/no-tape treatments were similar, we conclude that overall the tape formulation does not appear to impede IJ yield. Appar-
ently the small openings on the side of the tape enclosure are sufficient for nematode escape.

To simulate potential mechanical agitation during shipping or application, and determine if the tape formulation can protect the cadavers from damage, we conducted manual shake tests. The tape formulation protected *S. carpocapsae* cadavers from rupturing in the latter stages of infection (7 d). However, in the earlier stage of *S. carpocapsae* infection (4 d), and in all *H. indica* tests, no differences were detected because none of the treatments exhibited any rupturing. Conceivably, a greater amount of mechanical agitation would have caused ruptures in all treatments; but the shaking approach used in this study was vigorous, and it is unlikely that normal shipping or handling procedures would inflict greater force. Furthermore, the shaking method we used was sufficient in a prior study to readily cause rupturing in non-formulated *G. mellonella* cadavers regardless of the stage of infection (Shapiro-Ilan et al., 2001). Thus, our results support prior studies suggesting that *T. molitor* cadavers are more resistant to rupturing than soft-bodied larvae such as *G. mellonella* (Shapiro-Ilan et al., 2008).

Additionally, our results indicate that in *T. molitor* hosts, *H. indica* infected cadavers are more resistant to rupturing than *S. carpocapsae* infected cadavers, and for *S. carpocapsae* late stage infections are more susceptible than earlier stage infections. Similarly, Shapiro-Ilan et al. (2001) observed an increased tendency for *G. mellonella* infected with *Heterorhabditis bacteriophora* Poinar to rupture as the age of infection increased, which is likely due to increased digestion of tissues by the symbiotic bacteria and nematodes over time. For *T. molitor* cadavers infected with *S. carpocapsae*, the tape formulation could offer significant advantages for shipping and application relative to the use of non-formulated cadavers. The timeline within infection process that we tested was appropriate from a commercial perspective because it would be necessary to ship cadavers within 4-d-post-infection (when signs of infection are apparent), and apply them within 7 d so that IJs do not start emerging prior to application. Even though we did not detect protective advantages of tape when testing *H. indica* cadavers, the tape formulation may still be beneficial due to increased ease of handling.

High levels of efficacy were observed using nematode-infected cadavers for suppression of *D. abbreviatus*. *D. abbreviatus* has been a major commercial target for entomopathogenic nematodes for more than a decade (Shapiro-Ilan et al., 2002b, 2005); entomopathogenic nematodes have been shown to be effective in controlling this insect under field conditions (Duncan and McCoy, 1996; Shapiro-Ilan et al., 2002b) and greenhouse conditions (Shapiro and McCoy, 2000). Efficacy against *D. abbreviatus* has also been demonstrated previously using *T. molitor* infected hosts in the greenhouse (Shapiro-Ilan et al., 2003). For unknown reasons, the level of efficacy observed in the cadaver treatment by Shapiro-Ilan et al. (2003) 14 d after application (approximately 95% after correction with Abbott’s formula) was slightly higher than the levels observed in the greenhouse in the present study (71% [tape] to 75% [no-tape], after correction with Abbott’s formula). Nematode strain, temperature, soil, and other environmental conditions were the same or similar between the studies (Shapiro-Ilan et al., 2003, unpublished data). The discrepancy in efficacy may have been due to attenuation of the nematode strain over time (Bai et al., 2005) or perhaps simply random variation among experiments. Nonetheless, the cadaver treatment provided significant levels of suppression in our study at 15 d post-application, and we expect that the level of control would have increased with time due to recycling within the hosts and additional opportunity for the nematodes to cause infection (Shapiro et al., 1999; Shapiro-Ilan et al., 2003). Thus, we suggest that tape-formulated cadaver application can be an efficacious approach to controlling *D. abbreviatus* in potted plants. Although application of the cadavers in orchard settings presents the problem of achieving greater dispersion of IJs than is needed in closed containers, prior research indicates that this may also be feasible (Del Valle et al., 2008).

High levels of efficacy were also observed using nematode-infected cadavers for suppression of *A. tumida* indicating potential for control under field conditions. In a relatively recent laboratory study, *A. tumida* was found to be susceptible to several entomopathogenic nematode species, *S. carpocapsae*, *Steinernema riobrave* Caba-
nillas, Poinar and Raulston, and *Heterorhabditis megidis* Poinar, Jackson and Klein (with *S. riobrave* and *H. megidis* showing higher virulence than *S. carpocapsae*) (Cabanillas and Elzen, 2006). Our study now establishes *H. indica*’s pathogenicity and virulence to *A. tumida*. Based on the level of suppression observed in our soil cups, there is potential to cause substantial mortality in *A. tumida* larvae under field conditions as well; indeed preliminary field tests with *H. indica* cadavers support this premise (Tedders, unpublished data). When *A. tumida* larvae mature they leave the hive (where they have been feeding on pollen and brood) and enter the soil to pupate (Torto et al., 2007). Thus, these larvae occupy a relatively densely concentrated area in the soil, which would be amenable to cadaver application because relatively little dispersion of IJs would be required compared with applications over larger areas (e.g., turf).

In summary, we have developed a novel formulation and packaging system for entomopathogenic nematode infected hosts, and have demonstrated potential for using this new formulation in pest control applications. In prior research, powder-coated formulations were demonstrated as a protective mechanism to enhance storage and application of cadavers (Shapiro-Ilan et al., 2001). The formulation that was the subject of this study is different from those investigated in the prior study in that here we place the cadavers in masking tape. The tape method has the advantage of mass production facilitated by an automatic packaging machine (though it is conceivable the powder formulation could also be mechanized such equipment does not exist currently). Additionally, the tape formulation offers an option for hand application (such as for home-owners or small garden use) that avoids touching a dead insect. In this study, we demonstrated the use of the novel formulation using *T. molitor* and *H. indica* as the model host and nematode, and we tested pest control efficacy and IJ yield under controlled conditions. Additional research is required to determine if the formulated cadavers can be stored prior to application (e.g., under refrigeration or partial desiccation) without loss of IJ yield or efficacy. Furthermore, studies are required to determine efficacy under field conditions and the applicability of the approach to other hosts (such as *G. mellonella*) and nematode species.

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References


