

# Efficacy of the entomopathogenic nematode *Steinernema riobrave* against the stored-product insect pests *Tribolium castaneum* and *Plodia interpunctella*

Olgaly Ramos-Rodríguez<sup>a,1</sup>, James F. Campbell<sup>b,\*</sup>, Sonny B. Ramaswamy<sup>a,2</sup>

<sup>a</sup> Department of Entomology, Kansas State University, Manhattan, KS 66506, USA

<sup>b</sup> Grain Marketing and Production Research Center, USDA ARS, 1515 College Ave., Manhattan, KS 66502, USA

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## Abstract

Persistence of stored-product insects in hidden refugia and their subsequent movement into stored commodities resulting in product infestation contributes to their pest status and represents a potential target for biological control agents. Entomopathogenic nematodes have not been previously tested against stored-product insects in environments such as empty grain bins or food processing and warehouse facilities, but their effectiveness at finding and infecting hosts in other cryptic habitats has been demonstrated. In laboratory bioassays, *Steinernema riobrave* reduced survival of red flour beetle, *Tribolium castaneum*, larvae, pupae and adults from  $77.9 \pm 3.2\%$  in the controls to  $27.4 \pm 2.5\%$  in treatments. Temperature (25 and 30 °C) and relative humidity (43, 56–57, 75, and 100%) did not significantly influence *S. riobrave* efficacy in this experiment. Field trials simulating empty grain bin treatments were conducted using red flour beetle and the Indian meal moth, *Plodia interpunctella*. Total survival of mixed stages (larvae, pupae and adults) of *T. castaneum* was 42% of that in the control and total survival of mixed stages of *P. interpunctella* was 27% of the control. Larval stages were the most susceptible to *S. riobrave* for both insect species with *P. interpunctella* larvae having 99% mortality and *T. castaneum* larvae having 80% mortality. *S. riobrave* shows promise as a biological control agent for stored-product insects, particularly Indian meal moth, but further studies looking at combinations of treatments may further enhance efficacy.

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## 1. Introduction

Bulk stored grain and processed food commodities can be negatively affected by stored-product insects during storage and processing (Hagstrum and Flinn, 1995). For example, the damage to bulk stored agricultural products is estimated to be between \$1.25 and 2.5 billion per year (Schöller et al., 2006). Stored-product pest management has

depended heavily on the use of chemical pesticides, but more emphasis is now being given to alternative control tactics (Subramanyam and Hagstrum, 2000). Management of stored-product insects can be targeted at two general areas: preventing and eliminating infestation of the stored-product, and eliminating sources of infestation. Stored-product insect populations can persist and increase on food that accumulates in inaccessible places, like cracks and crevices, under perforated floors, and inside machinery, and can move from these refugia into packaged and bulk stored products (Campbell et al., 2004). Stored-product insects may also originate from sources outside of food storage facilities and immigrate into these structures (Campbell and Mullen, 2004; Campbell and Arbogast, 2004). Pest management based on identifying these sources of infestation and

\* Corresponding author. Fax: +1 785 537 5584.

E-mail address: [james.campbell@gmprc.ksu.edu](mailto:james.campbell@gmprc.ksu.edu) (J.F. Campbell).

<sup>1</sup> Present address: University of Puerto Rico - Utuado, Department of Natural Sciences, Box 2500, Utuado, PR 00641, USA.

<sup>2</sup> Present address: College of Agriculture, Purdue University, 615 West State Street, West Lafayette, IN 47907-2053, USA.

targeting pest management there is an important component of less chemically intensive management programs. It is also potentially a better fit for biological control than applications targeted at preventing or eliminating infestations within the stored commodity (Schöller and Flinn, 2000).

Entomopathogenic nematodes are lethal endoparasites of insects (Gaugler and Kaya, 1990; Gaugler, 2002). They enter the host through natural body openings, penetrate into the hemocoel, release bacteria that kill the host within 24–48 h, and make the environment inside the insect suitable for nematode development. The only free-living stage, the infective juvenile (IJ), leaves a depleted host and searches for a new one. Entomopathogenic nematodes have a number of other characteristics that make them potentially good biological control agents for stored-product pests. They are safe to vertebrates (Bathon, 1996; Boemare et al., 1996; Kaya and Gaugler, 1993), are exempt from registration in the United States by the EPA (Kaya and Gaugler, 1993), are commercially available (Kaya and Gaugler, 1993), can be applied with conventional pesticide equipment (Hayes et al., 1999), tolerate many types of pesticides (Koppenhöfer et al., 2000; Nishimatsu and Jackson, 1998), have a wide host range (Capinera and Epsky, 1992; Gaugler et al., 1997), and have the ability to actively seek their host (Campbell and Lewis, 2002). Entomopathogenic nematodes are typically associated with soil, and have been widely used as biological control agents in this environment (Kaya and Gaugler, 1993).

Because entomopathogenic nematodes require a moisture film to prevent desiccation and in which to move, they appear a poor fit for the relatively dry stored-product environment and as a result have received very limited attention. While this is certainly true regarding their use as a bulk grain or processed commodity treatment, we would argue that they do have considerable potential as a treatment for hidden refugia and outside spillage or product accumulations. Entomopathogenic nematodes have already been used to control insects in cryptic environments outside the soil. For example, *Steinernema carpocapsae* (Weiser) has been used for controlling cockroaches (Appel et al., 1993), and the codling moth, *Cydia pomonella* (L.), in fruit bins (Lacey and Chauvin, 1999; Unruh and Lacey, 2001). Because chemical insecticides used as post-harvest surface, empty bin, and crack and crevice treatments are typically applied in an aqueous solution (Arthur and Phillips, 2003), if nematodes can be applied in a similar amount of liquid and this provides sufficient moisture for long enough to allow nematodes to find and infect the target, then they could be used as biological insecticides in these stored-product situations.

Previous studies have shown that entomopathogenic nematodes can be effective against some of the major pest families encountered in storage commodities, e.g., Pyralidae (Shannag and Capinera, 2000) and Curculionidae (Duncan and McCoy, 1996; Shapiro and McCoy, 2000). An early survey demonstrated the susceptibility of some stored-

product insects, including *Ephestia kuehniella* Zeller and *Tenebrio molitor* L., to a single high concentration of nematodes (Morris, 1985). Other studies demonstrated the susceptibility of the confused flour beetle *Tribolium confusum* Jacquelin du Val and the granary weevil *Sitophilus granarius* L. to *S. carpocapsae* while testing the effect of host size (Wójcik, 1986a) and host starvation (Wójcik, 1986b) on the intensity of infestation, and on nematode sex ratio, rate of growth, and morphometric relations with the stored-product host insects. Indian meal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), larvae and adults were found to be susceptible to various heterorhabditid species in laboratory experiments (Mbata and Shapiro-Ilan, 2005). Ramos-Rodríguez et al. (2006) compared the susceptibility of a range of stored-product pest species and stages to several *Steinernema* spp. under ideal infection conditions in the laboratory and concluded that *Steinernema riobrave* Cabanillas, Poinar, and Raulston showed the greatest potential. In this study, we investigated the use of *S. riobrave* as a control agent against two stored-product insect species under more realistic field conditions. First, the influence of temperature and relative humidity (RH) conditions present in the field on efficacy was tested in a laboratory bioassay simulating a crack and crevice application. Second, a field experiment was conducted in an empty grain bin.

## 2. Materials and methods

### 2.1. Insects

Last instar larvae, pupae and adults of the red flour beetle, *T. castaneum* (Herbst) (Coleoptera: Tenebrionidae), were used for the laboratory concrete arena and empty bin experiments. For this insect species, the larvae and pupae were highly susceptible, but adults exhibited intermediate susceptibility to *S. riobrave* (Ramos-Rodríguez et al., 2006). *P. interpunctella* was demonstrated to be highly susceptible to entomopathogenic nematodes (Ramos-Rodríguez et al., 2006), and was used in the empty bin experiments. Individuals of both insect species were taken from laboratory colonies maintained at the USDA ARS, Grain Marketing and Production Research Center, in Manhattan, Kansas.

### 2.2. Nematodes

The culture of *S. riobrave* used in these experiments was originally obtained from Harry K. Kaya at the University of California, Davis, and maintained on *Galleria mellonella* L. following the techniques described in Kaya and Stock (1997). Different nematode infection batches were used for each experimental block and only IJs younger than 2 weeks old were selected.

### 2.3. Concrete arena experiment

The first set of experiments involved the use of concrete arenas to simulate a concrete floor-wall junction and a

crack and crevice application. The concrete (made using concrete mix (Quikrete Companies, Atlanta, GA)) arenas consisted of two parts: the bottom part was a concrete cylinder (12 × 4 cm) and the upper part was another cylinder of the same size with an opening (8 cm diameter) in the center. The two parts were taped together with utility duct tape (48 mm wide) and 1.0 g of cracked wheat was added to the floor of the arena. Twenty individuals per stage of *T. castaneum* were placed in the arenas. A concentration of 300 IJs/cm<sup>2</sup> in 10 ml of water (treatment) or water alone (control) was pipetted into the arenas. The top of the arena was sealed with a size 40 mesh top to prevent insects from escaping.

Arenas were held under combinations of four RH and two temperatures. For each temperature-RH combination, five replications were performed and blocked in time with one replicate for each combination per block and each block performed on a separate day. A different nematode batch was used for each block. Relative humidities were created in plastic boxes (46 × 29 × 15 cm) with saturated aqueous salt solutions. Salts were selected to represent a range of humidities from low to high (Greenspan, 1977). Relative humidities tested were 43.2% (K<sub>2</sub>CO<sub>3</sub>), 56–57% (NaBr), 75% (NaCl) and 100% (H<sub>2</sub>O only). Two arenas (a treatment and a control) were placed in each humidity box and placed in an incubator at either 25 or 30 °C. HOBO data loggers (Onset Computer Corporation, Pocasset, MA) were placed inside each humidity box to monitor temperature and RH. After 72 h, all insects were removed from the arenas, number alive and dead was determined, and all dead insects were dissected to confirm presence of nematodes.

#### 2.4. Empty grain bin experiment

Two empty metal grain bins (6.6 m diameter, 4.2 m to eave height, and 6.0 m to peak height, full capacity of approximately 109,090 kg) with concrete floors containing Y-shaped aeration ducts (i.e., rectangular troughs in the cement floor) covered by sections (46 × 20 cm) of perforated (2 mm diameter holes) metal sheets were used to evaluate nematode efficacy. In each bin, one arm of the aeration duct was used for the nematode treatment application and the other for the control. A total of four replicates of each treatment were performed: two experimental blocks on different days were used with each block containing two replicates with each replicate in a separate bin. Treatment and control arms within a bin were switched between blocks.

Before starting experiments, each duct arm was cleaned and a layer of cracked wheat was added (approximately 2 cm thick layer) to the floor of ducts. Cages made from cylinders of 40 mesh (pore size 425 µm) metal screen, 65 mm long by 20 mm in diameter, and sealed on the ends with rubber stoppers were used to confine the test insects. Six cages per insect species (each containing 10 individuals/stage of *T. castaneum* or *P. interpunctella* and 0.5 g of cracked wheat) were placed in each arm prior to treatment.

The life-stages added to the cages were larvae, pupae and adults. Cages were arranged so that one cage for each insect species was under each of six sections of the metal perforated cover and in alternating order (two times on the left, right and center of the duct). After adding the cages the perforated covers were replaced over the arms. Treatments were applied using a Gilmour 7.6 liter sprayer (Gilmour Manufacturing Company, Somerset, PA). One liter of water was applied to each arm with the treatment arm receiving a rate of 300 IJs/cm<sup>2</sup>. Insect mortality and infection status were checked after 72 h. HOBO data loggers were placed in each arm of the bin to monitor temperature and RH during the experiment.

To evaluate *S. riobrave* persistence, 45 ml samples of the cracked wheat from the floors of each arm (one sample per area with cages) were collected in a 50 ml plastic graduated centrifuge tube (BD Falcon™) at 0, 12, 24, 48, 72 and 96 h after treatment application in both the treatment and control sides. Samples were taken to the laboratory and divided in half. One half was placed in a centrifuge tube and rinsed twice with distilled water by adding the water, shaking the tube and emptying the contents into a petri dish (100 × 15 mm) and the presence of live nematodes was determined. The other half of the sample was baited with *G. mellonella* to determine infectivity. Three larvae of *G. mellonella* were exposed to each sample in the centrifuge tube placed in an incubator at 25 °C and mortality was checked after 72 h. Dead larvae were dissected to check for nematode presence.

#### 2.5. Data analysis

The effects of temperature, RH and nematode treatment on mortality (concrete arena experiment) and nematode treatment (grain bin experiment) were tested using the ANOVA Procedure (SAS Institute, 2001). A significance level of  $P < 0.05$  was used for all comparisons. Data are presented as mean and standard error of the mean throughout the text.

### 3. Results

#### 3.1. Concrete arena experiment

For the total number of survivors (combining larvae, pupae and adults) (Table 1), the ANOVA model was significant ( $F = 9.76$ ;  $df = 15, 64$ ;  $P < 0.0001$ ). Nematode treatment was a significant factor ( $F = 139.73$ ;  $df = 1$ ;  $P < 0.0001$ ), but temperature ( $F = 0.02$ ;  $df = 1$ ;  $P = 0.8767$ ) and RH ( $F = 0.61$ ;  $df = 3$ ;  $P = 0.6132$ ) were not significant factors. There were no significant two-way or three-way interactions among factors. The average number of survivors, combining all temperature and relative humidities, in the controls was  $47 \pm 2$  individuals ( $77.9 \pm 3.2\%$  survival of original number added) and in the nematode treatment it was  $16 \pm 2$  individuals ( $27.4 \pm 2.5\%$  survival of original number added). Although RH was not a significant factor

in the model, at 25 °C there was a trend for larval survival to increase with decreasing RH (Table 1).

For the number of individuals in the larval stage at the end of the exposure (Table 1), the ANOVA model was significant ( $F=8.18$ ;  $df=15, 64$ ;  $P<0.0001$ ). Nematode treatment was a significant factor ( $F=113.83$ ;  $df=1$ ;  $P<0.0001$ ), but temperature ( $F=0.83$ ;  $df=1$ ;  $P=0.3666$ ) and RH ( $F=0.45$ ;  $df=3$ ;  $P=0.7202$ ) were not significant factors. There were no significant two-way or three-way interactions among factors. The average number of survivors, combining all temperature and relative humidities, in the controls was  $10 \pm 1$  individuals ( $48.4 \pm 3.8\%$  of original number of larvae added) and in the nematode treatment it was  $1 \pm 0$  individuals ( $4.4 \pm 1.3\%$  of original number of larvae added). The large decrease in the number of individuals in this stage in the controls results, in part, from some individuals transitioning into the pupal stage.

For the number of individuals in the pupal stage at the end of the exposure (Table 1), the ANOVA model was significant ( $F=3.23$ ;  $df=15, 64$ ;  $P=0.0005$ ). Nematode treatment was a significant factor ( $F=40.90$ ;  $df=1$ ;  $P<0.0001$ ), but temperature ( $F=0.04$ ;  $df=1$ ;  $P=0.8334$ ) and RH ( $F=1.15$ ;  $df=3$ ;  $P=0.3373$ ) were not significant factors. There were no significant two-way or three-way interactions among factors. The average number of survivors, combining all temperature and relative humidities, in the controls was  $15 \pm 1$  individuals ( $73.7 \pm 4.8\%$  survival of original number added) and in the nematode treatment it was  $6 \pm 1$  individuals ( $32.1 \pm 3.9\%$  survival of original number added). Changes in number of pupae after exposure to nematodes will have been influenced by transitions from larva to pupa and from pupa to adult.

For the number of individuals in the adult stage at the end of the exposure (Table 1), the ANOVA model was significant ( $F=4.80$ ;  $df=15, 64$ ;  $P<0.0001$ ). Nematode treatment was a significant factor ( $F=61.99$ ;  $df=1$ ;  $P<0.0001$ ), but temperature ( $F=0.72$ ;  $df=1$ ;  $P=0.3994$ ) and RH

( $F=1.11$ ;  $df=3$ ;  $P=0.3518$ ) were not significant factors. There were no significant two-way or three-way interactions among factors. The average number of survivors, combining all temperature and relative humidities, in the controls was  $22 \pm 1$  individuals ( $111.7 \pm 6.6\%$  survival of original number added) and in the nematode treatment it was  $9 \pm 1$  individuals ( $45.6 \pm 4.8\%$  survival of original number added). In the controls, the greater number of adults then at the start of the experiment is due to transitions from the pupal stage to the adult stage during the exposure interval.

### 3.2. Empty grain bin experiment

For *T. castaneum*, there was significantly lower survival for the combined life-stages, in the bin arms treated with nematodes than in the control arms (ANOVA:  $F=34.97$ ;  $df=1,6$ ;  $P=0.0010$ ) (Table 2). When considering each life-stage separately, the number of individuals in a larval stage at the end of the exposure period was significantly lower in the nematode treated arm compared to the control arm (ANOVA:  $F=58.66$ ;  $df=1,6$ ;  $P=0.0003$ ), but this was not true for the pupal (ANOVA:  $F=2.07$ ;  $df=1,6$ ;  $P=0.2002$ ) and adult (ANOVA:  $F=4.61$ ;  $df=1,6$ ;  $P=0.0754$ ) stages (Table 2). Percent survival for the combined stages was  $40.1 \pm 9.0\%$  in the nematode treatment compared to  $94.2 \pm 1.6\%$  in the controls. For *P. interpunctella*, there was significantly lower survival, when combining all the life-stages, in the arms treated with nematodes than in the control arms (ANOVA:  $F=53.74$ ;  $df=1,6$ ;  $P=0.0003$ ) (Table 2). When considering each life-stage separately, number of individuals in each stage at the end of the exposure period was significantly lower for the larvae (ANOVA:  $F=73.01$ ;  $df=1,6$ ;  $P=0.0001$ ), pupae (ANOVA:  $F=39.14$ ;  $df=1,6$ ;  $P=0.0008$ ) and adult (ANOVA:  $F=15.86$ ;  $df=1,6$ ;  $P=0.0073$ ) stages (Table 2).

Table 1  
Means ( $\pm$  SEM) of the number of *Tribolium castaneum* survivors (larvae, pupae, adults or total of all stages), initially 20 individuals of each stage per arena, after 72 h of exposure to water (control) or *Steinernema riobrave* IJs (300 IJs/cm<sup>2</sup>) at combinations of two temperatures and four relative humidities

Temperature	RH (%)	Treatment	Larvae	Pupae	Adults	Total
25 °C	100	Control	11.0 $\pm$ 1.9	14.6 $\pm$ 2.2	21.4 $\pm$ 3.6	47.0 $\pm$ 4.3
		<i>S. riobrave</i>	0.0 $\pm$ 0.0	2.8 $\pm$ 0.7	7.8 $\pm$ 1.5	10.6 $\pm$ 1.8
	75	Control	8.4 $\pm$ 1.8	16.4 $\pm$ 1.4	18.6 $\pm$ 3.8	43.4 $\pm$ 4.6
		<i>S. riobrave</i>	0.6 $\pm$ 0.4	8.0 $\pm$ 1.5	4.8 $\pm$ 1.4	13.4 $\pm$ 2.8
	56	Control	10.4 $\pm$ 2.7	15.6 $\pm$ 2.4	18.8 $\pm$ 3.9	44.8 $\pm$ 7.1
		<i>S. riobrave</i>	0.6 $\pm$ 0.6	8.6 $\pm$ 3.6	8.6 $\pm$ 3.4	17.8 $\pm$ 6.5
	43.2	Control	11.4 $\pm$ 2.1	13.8 $\pm$ 2.9	24.6 $\pm$ 2.6	49.8 $\pm$ 3.3
		<i>S. riobrave</i>	2.8 $\pm$ 1.6	6.0 $\pm$ 2.4	15.6 $\pm$ 3.9	24.4 $\pm$ 5.5
30 °C	100	Control	7.2 $\pm$ 1.4	15.6 $\pm$ 3.4	23.6 $\pm$ 4.4	46.4 $\pm$ 6.9
		<i>S. riobrave</i>	1.2 $\pm$ 0.6	5.6 $\pm$ 2.2	8.0 $\pm$ 2.6	14.8 $\pm$ 4.9
	75	Control	9.0 $\pm$ 2.6	14.4 $\pm$ 3.8	23.2 $\pm$ 4.4	46.6 $\pm$ 6.1
		<i>S. riobrave</i>	1.0 $\pm$ 0.8	4.6 $\pm$ 1.5	11.2 $\pm$ 2.7	16.8 $\pm$ 4.0
	57	Control	11.6 $\pm$ 2.4	15.6 $\pm$ 3.7	24.8 $\pm$ 2.3	52.0 $\pm$ 4.0
		<i>S. riobrave</i>	0.6 $\pm$ 0.2	10.0 $\pm$ 2.6	7.6 $\pm$ 2.3	18.2 $\pm$ 2.1
	43.2	Control	8.4 $\pm$ 2.6	12.0 $\pm$ 2.7	23.8 $\pm$ 5.6	44.2 $\pm$ 8.8
		<i>S. riobrave</i>	0.2 $\pm$ 0.2	5.8 $\pm$ 2.2	9.4 $\pm$ 2.2	15.4 $\pm$ 3.5

Table 2

Means ( $\pm$ SEM) of the number of survivors (larvae, pupae, adults and total of all stages) of *Tribolium castaneum* and *Plodia interpunctella* (initially 60 individuals of each species and stage per replicate) in empty grain bin arms after treatment with water (control) or *Steinernema riobrave* infective juveniles (300 IJs/cm<sup>2</sup>)

Insect	Treatment	Larvae	Pupae	Adults	Total
<i>T. castaneum</i>	<i>S. riobrave</i>	8.8 $\pm$ 2.1 a	25.5 $\pm$ 8.2 a	38.0 $\pm$ 14.6 a	72.2 $\pm$ 16.2 a
	Control	43.2 $\pm$ 4.0 b	50.7 $\pm$ 15.5 a	75.5 $\pm$ 9.5 a	169.5 $\pm$ 2.9 b
<i>P. interpunctella</i>	<i>S. riobrave</i>	0.2 $\pm$ 0.2 a	34.5 $\pm$ 4.4 a	6.5 $\pm$ 4.5 a	41.2 $\pm$ 6.8 a
	Control	28.8 $\pm$ 3.3 b	81.0 $\pm$ 6.0 b	43.8 $\pm$ 8.2 b	153.5 $\pm$ 3.7 b

Different letters in the same column for each insect species represent significant differences (ANOVA,  $P < 0.05$ ).

Evaluation of *S. riobrave* persistence in residue samples collected from the bins using *G. mellonella* larvae as bait insects, indicated that there was a difference in persistence between the two blocks. In the first block, live IJs were found up to 72 h after spraying, and there was 100% *G. mellonella* mortality in both arms out to 48 h and 50% mortality at 72 h. In the second block, at 12 h there were only a few IJs alive in the samples, but 100% *G. mellonella* larval mortality in both arms was observed. After 12 h, no live IJs and no dead *G. mellonella* larvae were found. None of the bait *G. mellonella* larvae from the control samples died, and dissections of the bait *G. mellonella* from the treated samples indicated that all the dead individuals contained nematodes. The mean temperature inside the bins for the first time period was 25.5 °C and the RH was 60.0%, while in the second block it was 23.4 °C and 48.3% RH.

#### 4. Discussion

All stages tested for both insect species, except *T. castaneum* adults, had significantly higher mortality than in controls. However, 100% mortality was not achieved in any of the treatments, except for *P. interpunctella* larvae that were highly susceptible ( $>$ mortality). In contrast, final larval instars of the Indian meal moth are generally more tolerant to insecticides than stored-product beetles (Yue et al., 2003). The susceptibility of *P. interpunctella* last instar, wandering stage larvae to nematodes shows the potential of integrating *S. riobrave* into management programs targeted at commodity residues. Indian meal moth is a serious problem in many types of food processing and storage facilities and feed on a wide range of materials including whole grain, cereal food products, dried fruits and nuts, and animal feeds. Sources of moths may be inside facilities, but immigration of moths from outside locations may also be an important source of infestation (Campbell and Arbogast, 2004). The red flour beetle is a cosmopolitan pest found in grain products in storage, processing and retail facilities. The lack of significant impact on *T. castaneum* adults in the empty grain bin experiment may be attributed to the lower susceptibility of adults (Ramos-Rodríguez et al., 2006), but pupae surviving and reaching adulthood may have also contributed. Thus, there is a wide range of potential targets for this biological control agent.

Combining entomopathogenic nematode applications with other treatments might increase pest mortality. Arthur (2004) tested the use of a combination of diatomaceous earth with conventional insecticides against *T. castaneum* showing the potential of combining treatments for the control of this pest. Diatomaceous earth increased the efficacy of *Beauveria bassiana* (Balsamo) Vuillemin against red flour beetle larvae (Akbar et al., 2004). Entomopathogenic nematodes have been tested in combination with various control tactics, including chemical (Koppenhöfer et al., 2000; Nishimatsu and Jackson, 1998) and other biological control agents (Koppenhöfer et al., 1999; Lacey et al., 2003; Shanag and Capinera, 2000). Different results have been obtained with these combinations, ranging from antagonistic (Barbercheck and Kaya, 1990; Kaya and Burlando, 1989) to synergistic interactions (Koppenhöfer et al., 1999, 2000), and in some cases both positive and negative outcomes were observed (Sher et al., 2000). In the stored product environment there are various control alternatives that could be explored, including biological control with parasitoids and other natural enemies. Another possibility is the use of insect growth regulators (IGRs) in combination with entomopathogenic nematodes. For example, hydroprene causes high molt failure in the red flour beetle (Arthur, 2003; Arthur and Hoernemann, 2004), and combining it with nematodes could result in better control.

Relative humidity (RH) was not a significant constraint in the current study, indicating that *S. riobrave* was applied with sufficient moisture to create favorable environmental conditions for infection, at least over a limited time period. This was also shown in the empty grain bin experiments where IJs persisted long enough to cause mortality even when persistence varied between blocks. Although IJ mortality under the stressful environmental conditions encountered during post-harvest releases may be high, the treatment of relatively small areas also makes it feasible to use higher nematode concentrations to compensate for this mortality. Applying this level of moisture to bulk grain and processed commodities is typically not feasible, but chemical insecticides used for crack and crevice and surface applications are applied using aqueous solutions (Arthur and Phillips, 2003), often until run-off. This shows the potential for using *S. riobrave*, and maybe other nematode species, in areas where sufficient moisture can be provided to ensure that nematodes survive long enough to seek and infect a potential host.

Environmental conditions inside empty grain bins during these experiments were consistent, but conditions vary during the year and have to be accounted for when developing protocols for using nematodes as control agents. Data records from other grain bins in the same area for almost a 4-month period (June 6–September 24, 2003) show temperatures varying from 34.0 (August 25) to 6.6 °C (September 19) and RH from 30.9 (September 19) to 87.1% (August 31, September 1 and 9). Conditions inside a flour mill during a year varied from a minimum of 13.7 °C and 23.4% RH to a maximum of 39.7 °C and 60% RH, with an average of 25.6 °C and 33.1% RH. The temperature and RH conditions used in these experiments encompass some of the environmental conditions measured in the field, but some conditions of even lower RH and both lower and higher temperatures can occur. It may be necessary to further evaluate extreme conditions or to avoid application when these conditions are present.

Georgis (1990) recommends field application concentrations of >2.5 billion nematodes/ha against insect pests of row crops, but sometimes higher concentrations (7–15 billion/ha) are required to achieve control (Loya and Hower, 2002). Our preliminary experiments showed that 300 IJs/cm<sup>2</sup> concentration was the most effective against *T. castaneum*. This is equivalent to 30 billion/ha, which is a much higher concentration than normally used, although some studies in the field have used up to 50 billion/ha. However, rates of nematodes used in targeted applications to non-soil environments are often much higher than field rates. Application to relatively small areas can make it economical to apply high IJ rates to compensate for low susceptibility of hosts and IJ mortality. Combining nematodes with other treatments or formulations with wetting agents could enhance feasibility. Some formulations used to enhance post-application survival include alginate capsules, gel-forming polyacrylamides, and baits (Grewal, 2002).

*Steinernema riobrave* has potential as a biological control agent for stored-product insects under certain conditions. Although environmental conditions in stored-product environments are generally not favorable for nematode persistence, we have shown that they can be manipulated (by applying nematodes with sufficient water) to enable nematodes adequate time to find and infect insects hidden in refugia. Entomopathogenic nematodes have seldom been considered as biological control agents for stored-product insects, but they would appear to have some potential for certain applications targeted at source and residual pest populations. Further testing is needed in other situations where conditions can be modified favorably for IJ survival: e.g., crack and crevice, surface treatments, and areas surrounding storage facilities where residue can accumulate. Tests with different formulations and treatment combinations might help to improve entomopathogenic nematode efficacy and help in developing a better management program using these natural enemies.

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