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

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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 ± 3.2% in the controls to 27.4 ± 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

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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