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Effects of fine-grain habitat complexity on egg parasitism by three species of *Trichogramma*

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Abstract

Effects of microhabitat complexity on host foraging by three species of *Trichogramma* (*T. deion*, *T. ostriniae*, and *T. pretiosum*) were evaluated under simulated stored product conditions. All three species have been considered as potential biological control agents for the Indianmeal moth, *Plodia interpunctella*, in retail stores and warehouses. Trials were conducted with single naïve female parasitoids in 10-cm Petri dishes that were either empty, contained flour, or contained millet. Empty 15-cm Petri dishes, which served as a surface area control, comprised a fourth treatment. Females were allowed to forage for sentinel egg disks for 2 h, after which percentages of parasitism and egg mortality were computed. In addition, behavioral observations were made on a subset of the trials. *Trichogramma deion* parasitized more eggs than the other two species in the empty dishes and in the dish containing flour. For *T. deion*, rates of parasitism and egg mortality were significantly greater in both the small and large empty dishes than in the small dishes containing flour or millet. Parasitism was consistently low for all three species in the grain-filled dishes. Among species, *T. ostriniae* spent the most time walking, while *T. pretiosum* was the most sedentary. In addition, *T. pretiosum* spent significantly more time on the first egg visited compared with subsequent eggs. *T. deion* may be the best-suited for use as a biological control agent for *P. interpunctella*. However, the potential negative effects of fine-grain habitat complexity should be taken into account when developing a release protocol for *Trichogramma* spp.

Keywords: *Trichogramma deion*; *Trichogramma ostriniae*; *Trichogramma pretiosum*; *Plodia interpunctella*; Habitat complexity; Stored products; Biological control; Spatial scale; Foraging behavior

1. Introduction

The Indianmeal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), is a serious pest of stored products in retail stores and warehouses where it attacks a wide range of products, including raw and processed cereals, dried fruit, pulses, and garlic (Perez-Mendoza and Aguilera-Pena, 2004). A single moth larva or adult may result in the total economic loss of the infested product, customer complaints, or even lawsuits (Subramanyam et al., 2001). Traditional pest management practices include insecticidal fogs, fumigants, and surface applications of insecticides (Cox and Bell, 1991). However, recent legislation has reduced the number and type of chemicals available for use on stored products (Arthur and Rogers, 2003), thus prompting a search for alternative tactics such as biological control.

The potential for using *Trichogramma* parasitoids as augmentative biological control agents for *P. interpunctella* in retail stores and warehouses has been suggested (Prozell et al., 1996). Previous stored product research has explored the use of trichogrammatids in bulk peanut storage (Brower, 1988), bulk wheat storage (Schöller et al., 1996), and bakeries (Prozell and Schöller, 1998; Steidle et al., 2001), as well as in warehouses and retail stores (Prozell et al., 1996). *Trichogramma* have several advantages over traditional insecticides in retail stores and warehouses. As egg

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parasitoids, they attack pests before they reach the damaging larval stages. They are also commercially available, relatively inexpensive, and because of their miniscule size (<0.5 mm) unlikely to be noticed by consumers.

Schöller et al. (1994) suggested that the selection of appropriate species of *Trichogramma* for stored product pests is one of the most critical issues facing their use as biological control agents. Previously, the selection criteria have consisted mainly of measuring rates of searching speed and/or host acceptance (Cerutti and Bigler, 1994; Van Hezewijk et al., 2000; Steidle et al., 2001). However, evaluating foraging success of *Trichogramma* in response to variation in habitat complexity may provide an even better measure of parasitoid suitability (Gingras et al., 2003). For example, habitat complexity has been demonstrated to reduce *Trichogramma* foraging efficiency both in the laboratory (Andow and Prokrym 1990; Lukianchuk and Smith, 1997; Gingras et al., 2002; Gingras and Boivin, 2002; Andow and Olson, 2003) and in the field (McCravy and Berisford, 1998). In retail stores and warehouses, as in more natural systems, habitat complexity may take multiple forms and affect *Trichogramma* foraging success over a range of spatial scales.

In their conceptual model of parasitoid foraging, Hassell and Wagge (1984) defined three functional spatial scales: microhabitat, macrohabitat, and ecosystem. With respect to *Trichogramma*, host searching is likely affected by variables operating at one if not multiple levels. The broadest scale, "ecosystem" would equate to an entire retail store or storage facility and influential factors might include the location of shelving units or other grain storage structures within the facility. "Macrohabitat" could be represented by individual shelving units, with factors contributing to habitat complexity at that level including shelf design as well as the presence of products on the shelves (Grieshop et al., 2006b, 2007). Finally, "microhabitat" would be defined by surfaces on which parasitoids walk (e.g., shelf, floor, or stored product) (Grieshop et al., 2006a). Specific features that might influence foraging ability or efficiency at this spatial scale would include the number and distribution of host eggs, spilled or bulk grain products, as well as textural and other micro-structural design differences in packages.

This study compared the foraging behavior and performance of three candidate species of *Trichogramma* in different microhabitats that varied in surface area (achieved by adjusting arena size) and matrix complexity (achieved by adding either flour or millet). The three species selected for comparison were *Trichogramma deion* Pinto and Oatman, *Trichogramma ostriniae* Pang and Chen, and *Trichogramma pretiosum* Riley. The specific strains of *T. deion* and *T. pretiosum* used in this study were selected based on host preference tests performed by Schöller and Fields (2002), that demonstrated that these two species readily parasitized the eggs of *P. interpunctella* under either choice and no-choice conditions. *T. ostriniae* has been extensively explored as a augmentative biological control agent for *Ostiniae nubilalis* (Hübner) in field and sweet corn (Wang et al., 1997, 1999; Hoffmann et al., 2002; Wright et al. 2002), and was shown capable of parasitizing the eggs of *P. interpunctella* in bulk wheat (Jeffery Gardener, personal communication).

2. Materials and methods

2.1. Insects

All insect colonies were maintained in a walk-in growth chamber set at 26 ± 1°C, 60 ± 5% RH and a 16:8 (L:D) photoperiod and located at the USDA-ARS Grain Marketing and Production Research Center (GMPRC) in Manhattan, KS, USA. All species of *Trichogramma* were reared on eggs (<1-day-old) of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) that had been sterilized with UV radiation. Both *E. kuehniella* and the experimental host, *P. interpunctella*, were reared on a standard diet of cracked wheat, wheat shorts, honey, water, and glycerin (McGaughey and Beeman, 1988). Colonies of *T. deion*, *T. pretiosum*, and *E. kuehniella* were initiated from stock provided by Beneficial Insectaries (Redding, CA, USA). The *T. ostriniae* colony was provided by Michael Hoffman, Cornell University. The *P. interpunctella* used in this study came from cultures maintained at the GMPRC. Specimens of all species used in the experiment were deposited into the Kansas State University Museum of Entomological and Prairie arthropod Research under voucher number 171.

The strains of *T. deion* and *T. ostriniae* used in this study were arrhenotokous with observed female:male sex ratios of 2:1 and 3:1, respectively. In contrast, the strain of *T. pretiosum* we tested was essentially thelytokous (sex ratio 24:1). Of the three species, only *T. pretiosum* was observed host feeding. All *Trichogramma* females used were less than 16 h old, naïve, and had been allowed access to both mates and a 1:1 honey:water solution for 4 h prior to testing. Individual females were collected by using a natural hair paintbrush to drive them onto a small strip of paper (0.25×4 cm), which was then placed in an empty shell vial.

2.2. Experimental design

We used a nested 3×4 factorial design, with three species of *Trichogramma* and four treatments related to microhabitat (described below), yielding 12 treatments, each of which was replicated 30 times. Each experimental run consisted of one to four replicates of the four microhabitat treatments for a single species. Experiments with *T. deion* were run between June and November 2003, those for *T. ostriniae* between January and June 2004, and those for *T. pretiosum* between June and August 2004. All experiments were conducted in a walk-in growth chamber set for 23 ± 1 °C and 45 ± 5% RH, under constant light conditions. Environmental conditions were selected based on temperature and humidity data collected at four retail