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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.
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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 Agulera-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

stores in Manhattan, KS, USA between April and November 2002.

All tests were conducted in glass Petri dishes that contained *P. interpunctella* eggs distributed on paper disks. Three treatments were conducted in 10-cm dishes: an empty dish, one that contained a thin layer (5 g) of flour approximately 1 mm thick, and one that contained a thin layer (7.5 g) of millet consisting of a single layer of millet granules approximately 1 mm thick. Granules of millet were approximately 1 mm in diameter; those of flour typically <0.1 mm. Millet and flour were used to create substrates of two different textures for *Trichogramma* movement compared to the empty dish. The fourth treatment consisted of an empty 15-cm dish to comprise an increase in simple surface area without a change in habitat structure. Approximate surface areas of the 10-cm empty, 10-cm flour, 10-cm millet, and 15-cm empty dishes were calculated as 204, 201, 380, and 424 cm^2, respectively. Surface areas of the empty and flour-filled dishes were calculated as the total area of the top, sides, and bottom, whereas surface area for the 10-cm millet dish was calculated as the total dish surface area plus the approximate total surface area of the millet granules as determined by weight. The surface area calculation for millet did not account for tangential contacts between individual grains and the arena floor and should therefore be considered an approximate measurement of total possible surface area.

Pairs of host eggs (<18 h-old) were attached to 1-cm diameter cardstock disks using Traganth natural glue (Merck, NJ, USA). Ten disks were then placed in each dish in a regular pattern of two concentric rings. In the 10-cm dishes, ring diameters measured 3 and 6 cm. In the 15-cm dishes, the ring diameters were 4.5 and 9 cm. Four disks were placed on the inner ring, one at each of the cardinal compass points (N, E, W, S) and six disks were placed on the outer ring, one on each of the N, S, NE, NW, SE, and SW points.

In each trial, a single female parasitoid was released in the center of a dish and allowed to forage for 2 h, after which the parasitoid was removed and the egg disks collected. For each experimental run, 100–140 non-experimental eggs (4 eggs per disk) were kept to assess egg mortality unrelated to parasitoids. Both the control and experimental egg disks were kept in a chamber set at 26 ± 1 °C and $60 \pm 7\%$ RH for 7 days, at which time egg status was assessed. Experimental runs in which control egg mortality exceeded 20% were excluded from the experiment. Based on this rule, a total of four replicates were repeated for *T. deion* and *T. ostriniae*, while six were repeated for *T. pretiosum*.

2.3. Data collection

Seven days after each experimental run, egg state was recorded as hatched, parasitized, or dead following examination under a stereo microscope (110–140× magnification). Parasitism was recognizable by a dark coloration of the egg. Percentages of parasitism and total mortality were computed. Data were corrected for control mortality using Abbot's formula (Abbott, 1925) as follows: corrected mortality = (observed total mortality − control mortality)/(100 − control mortality). Sources of mortality related to parasitoid behavior that did not result in successful parasitism were probing without oviposition (host killing), probing and host feeding, and unsuccessful parasitism.

In addition to egg parasitism and total corrected mortality, behavioral observations were made on a subset of six replicates of each species and treatment. Observations were made at 150 s intervals over the 2 h experimental duration using a 10× hand lens. Behavioral data included parasitoid location in the arena (i.e., egg, disk, floor, or wall and/or ceiling), identification of specific disks and eggs visited, and the behavior exhibited (interacting with the host, walking, still, or unknown). *T. deion* was readily identifiable in all four arenas due to its dark coloration. However, the lighter-colored *T. ostriniae* and *T. pretiosum* were often hard to locate when on millet, resulting in incomplete data for these two species. Because the wasps were readily observed only on the egg disk, egg, ceiling, or wall of the arena, periods of unobserved activity were recorded as "unknown" behavior on the floor substrate.

2.4. Data analyses

2.4.1. Percentage parasitism and total corrected mortality

Data were analyzed using the 'Proc Mixed' procedure (SAS Institute, 2000). Proportions were transformed using the arcsine transformation (arcsine($x^{0.5}$)) prior to analysis to achieve a normal distribution. Replicates with zero parasitism were excluded from the analysis to reduce variability. Complete factorial mixed model ANOVAs were run for 'percent parasitism' and 'percent corrected total mortality'. Because each species was run in a separate series of trials, the fixed factor 'species' was nested within experimental run as a random factor in order to account for covariance due to time. Additional three-way ANOVAs were run to compare parasitism and total corrected mortality within each arena, and four-way ANOVAs were run to compare arena effects within each species. A mixed model was used for species comparisons, using species nested within experimental run for the random factor, while no random factor was used for arena comparisons, because all arenas were represented within each run for each species. Multiple comparisons were made using the PDIFF option ($\alpha = 0.05$) and a Tukey–Kramer adjustment for multiple comparisons was used to minimize type I error.

2.4.2. Behavioral observations

Four types of behavioral responses were analyzed: the percentage of disks and eggs visited, the percentage of observations a parasitoid was spotted on a given substrate (egg, egg disk but not on egg, floor or other (ceiling or wall)), the percentage of observations during which a par-

asitoid exhibited a given behavior (interacting with host, walking, or still), and the duration of the first host contact versus the mean duration of subsequent events. All data were analyzed using the 'Proc Mixed' procedure (SAS Institute, 2000). Percentages were transformed using the arcsine transformation (arcsine($x^{0.5}$)), while duration data were left untransformed prior to running analyses. Complete 3×4 mixed model ANOVA as well as a three-way ANOVA among species by arena were run. Because each species was run in separate series of trials, the species fixed factor was nested within experimental run as a random factor in order to account for covariance due to time. A four-way ANOVA among arenas by species were run for all but three of the responses as described above. Two exceptions were for the percentage of observations walking or still in millet arenas where the relatively high number of unknown observations for *T. ostriniae* and *T. pretiosum* necessitated the removal of the small/millet arena from the analysis (leaving a 3×3 ANOVA). The third exception was for comparisons between the duration of the first host encounter and the mean duration of subsequent visits. These tests were performed using an LSMEANS statement for a 3×4 mixed model ANOVA for the difference of first and subsequent visits. In this analysis, a significant difference between duration of the first host encounter and the mean duration of subsequent visits was indicated by a significant model term deviation from zero.

3. Results

3.1. Percentage parasitism and total corrected mortality

There were significant differences among species in both the percentage of eggs parasitized ($F = 13.41$, df = 2, 30.4, $P < 0.0001$) and total corrected percentage egg mortality ($F = 8.01$, df = 2, 32.3, $P < 0.001$). Microhabitat also significantly affected percentage parasitism ($F = 24$, df = 3, 214, $P < 0.0001$) and percentage egg mortality ($F = 30$, df = 3, 215, $P < 0.001$). However, there were no significant ($P > 0.05$) species × microhabitat interactions. *T. deion* parasitized a significantly greater percentage of eggs than did *T. ostriniae* or *T. pretiosum* in the small empty dishes (22% and 19% greater, respectively; $F = 11.22$; df = 2, 32.9; $P = 0.0002$), large empty dishes (12% and 11% greater, respectively; $F = 10.94$; df = 2, 54; $P = 0.0001$), and a significantly greater percentage of eggs than *T. ostriniae* in the small flour-filled dishes (11% greater; $F = 4.32$; df = 2, 27.6; $P = 0.0234$; Fig. 1). In addition, the percentage total corrected mortality of *P. interpunctella* eggs was significantly greater with *T. deion* than with *T. ostriniae*, both in small empty dishes (24% greater) ($F = 8.97$; df = 2, 32.7; $P = 0.0008$), and in small flour-filled dishes (11% greater) ($F = 4.71$; df = 2, 23.7; $P = 0.0189$) (Fig. 1).

All three species tended to parasitize more hosts, and cause greater overall mortality, in empty dishes than in those that contained grain. However, this difference was the most significant in *T. deion*, which parasitized and killed nearly twice as many hosts in empty dishes ($F = 12.52$; df = 3, 79; $P < 0.0001$; Table 1).

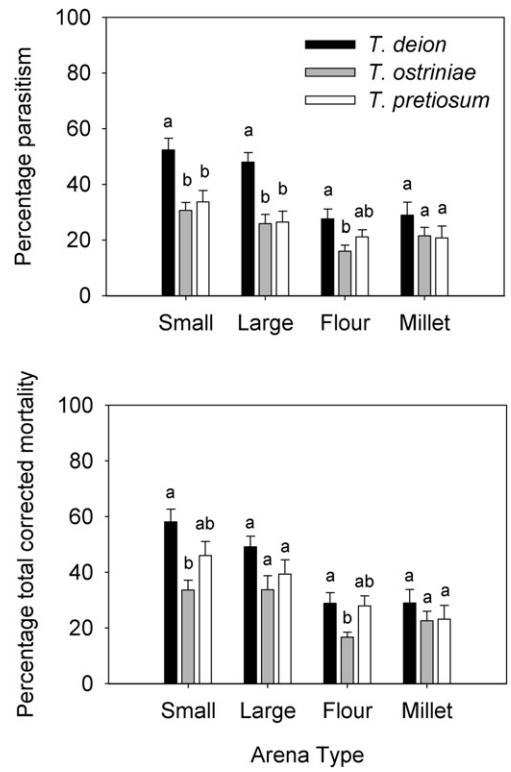

Fig. 1. Mean (±SEM) percentage parasitism and total corrected mortality by *Trichogramma* species and arena type. Bars within the same arena type with different letters are significantly different (LSD with Tukey–Kramer correction $P = 0.05$).

3.2. Behavioral observations

Both the percentage of egg disks and the number of eggs visited were significantly affected by microhabitat ($F = 6.61$; df = 3, 54; $P = 0.001$). However, there were no significant differences among species ($P > 0.05$) with respect to the effects of different microhabitats on host finding.

Table 1
Percentage of parasitism and total corrected mortality among arenas by the three *Trichogramma* species

	T. deion Mean ± SEM	*T. ostriniae* Mean ± SEM	*T. pretiosum* Mean ± SEM
Percentage parasitism			
Small/empty	52.39 ± 4.14a	30.58 ± 2.94a	33.70 ± 4.06a
Large/empty	47.89 ± 3.53a	25.79 ± 3.39ab	26.43 ± 3.86ab
Small/flour	27.50 ± 3.59b	15.93 ± 2.21b	21.09 ± 2.53b
Small/millet	28.89 ± 4.70b	21.43 ± 3.04ab	20.67 ± 4.34ab
Percentage total corrected mortality			
Small/empty	57.47 ± 4.29a	32.70 ± 2.97a	41.46 ± 4.41a
Large/empty	49.61 ± 3.58a	30.96 ± 3.91a	34.61 ± 4.35ab
Small/flour	29.54 ± 3.54b	18.50 ± 1.94b	25.60 ± 3.16b
Small/millet	29.84 ± 4.70b	24.07 ± 3.10ab	22.19 ± 4.32b

Means in the same column followed by a different letter are significantly different (LSD with Tukey–Kramer adjustment $P < 0.05$).

For all three parasitoids, the percentage of egg disks and total eggs visited tended to be highest in the small empty dishes followed by the large empty dishes, the small dishes with flour, and lowest in the small dishes with millet. However, the differences were only significant for *T. deion* ($F = 8.82$; df = 3, 18; $P = 0.0008$), which found two to three times as many egg disks in small empty dishes compared to the other treatments (Table 2). *T. deion* also found significantly more host eggs in small empty dishes than in large dishes or small dishes with millet ($F = 5.12$; df = 3, 18; $P = 0.0098$; Table 2).

The type of microhabitat had a significant effect on the relative amount of time *Trichogramma* females spent on individual eggs ($F = 4.32$; df = 3, 54; $P = 0.0084$), egg disks ($F = 9.38$; df = 54; $P < 0.0001$), and the sides or lids of the dishes ($F = 4.16$; df = 3, 54; $P = 0.0101$), but not on the floor. Microhabitat influence on time allocation was not significantly different among species, except for relative time on the floor ($F = 11.58$; df = 2, 6; $P < 0.0001$). Similarly, the only significant microhabitat × species interaction was found for relative time on the floor ($F = 3.43$; df = 2, 54; $P = 0.0056$). *T. ostriniae* was observed on the floor of the small empty dishes about twice as often as were the other two species ($F = 6.97$; df = 2, 15; $P = 0.0072$), and six times more often than *T. deion* in the millet dishes ($F = 26.87$; df = 2, 15; $P < 0.0001$). In addition, *T. pretiosum* was observed about four times more often on eggs in the flour-filled dishes than was *T. ostriniae* ($F = 6.28$; df = 2, 6; $P = 0.0338$; Fig. 2).

Within species, there were significant differences among microhabitats in the time spent on substrates within dishes. For example, *T. deion* spent almost twice as long on egg disks in small empty dishes as in dishes with millet ($F = 3.42$; df = 3, 20; $P = 0.0372$). *T. ostriniae* spent twice as long on egg disks in small empty dishes compared to large empty dishes or millet dishes, and four times as long in flour dishes compared to large empty and millet-filled dishes ($F = 6.75$; df = 3, 20; $P = 0.0025$; Table 3). There were significant differences in the percentage of floor observations for both *T. deion* and *T. pretiosum*. *T. deion* was observed four times more frequently on the floor in the large empty and small flour dishes compared to the small millet dishes ($F = 5.94$; df = 2, 20; $P = 0.0046$) and *T. pretiosum* was observed three times more frequently on the floor of small millet dishes compared to the small empty dishes ($F = 4.12$; df = 2, 20; $P = 0.02$; Table 3). Similarly, *T. deion* and *T. ostriniae* showed significant differences among arenas in the percentage of time they spent on the walls and ceiling, with frequencies five times higher in the small millet dishes compared to the small empty dishes for *T. deion* ($F = 3.54$; df = 3, 20; $P = 0.0332$). *T. ostriniae* spent significantly more time on the walls and/or ceiling in large empty and small millet dishes—3 and 4 times more, respectively, than in small flour dishes ($F = 5.04$; df = 3, 20; $P = 0.0092$; Table 3).

Averaged over all microhabitats, *T. ostriniae* spent a greater percentage of time walking than did *T. deion* or *T. pretiosum* ($F = 14.07$; df = 2, 6; $P < 0.0001$). The species × microhabitat interaction was not significant ($F = 2.09$; df = 6, 54; $P = 0.0981$) and species differences were influenced mainly by differences in activity in empty dishes (Fig. 3). Comparing species within dishes, *T. ostriniae* was observed walking approximately 1.5 times more often in the small empty dishes than *T. deion* or *T. pretiosum* ($F = 13.0$; df = 2, 15; $P = 0.0005$) and 1.5 times more often than *T. pretiosum* in the large empty dishes ($F = 5.46$; df = 2, 15; $P = 0.0165$). Likewise, *T. deion* was observed walking 1.5 times more frequently than *T. pretiosum* in the large empty dishes ($F = 5.46$; df = 2, 15; $P = 0.0165$).

A significant difference was found for *T. deion* between the amount of time spent during the first egg encounter and the mean of subsequent egg encounters in the small dish with flour. Approximately twice as much time was spent on the first egg visit than the mean of subsequent encounters (Fig. 4). For *T. pretiosum*, the difference between the duration of the first egg encounter was four to five times longer than the mean of subsequent encounters in all four arenas (Fig. 4), No significant differences were detected for *T. ostriniae* for the duration of time spent on the first egg compared to the mean of subsequent egg encounters in any microhabitat (Fig. 4).

Table 2
Percentage of sentinel egg disks and eggs found in the behavioral trial among arenas by the three *Trichogramma* species

	T. deion Mean ± SEM	*T. ostriniae* Mean ± SEM	*T. pretiosum* Mean ± SEM
Percentage disks found			
Small/empty	83.33 ± 4.22a	65.00 ± 11.47a	60.00 ± 14.83a
Large/empty	50.00 ± 10.65b	48.33 ± 15.37a	50.00 ± 12.91a
Small/flour	43.33 ± 3.33b	43.33 ± 9.19a	35.00 ± 5.63a
Small/millet	26.67 ± 10.22b	36.67 ± 6.15a	46.67 ± 8.03a
Percentage eggs found			
Small/empty	65.83 ± 4.90a	31.67 ± 10.54a	39.17 ± 12.48a
Large/empty	25.83 ± 12.21b	21.67 ± 8.43a	34.17 ± 11.36a
Small/flour	20.83 ± 6.51ab	11.67 ± 5.58a	26.67 ± 4.59a
Small/millet	15.83 ± 11.14b	11.67 ± 5.58a	15.83 ± 9.70a

Means in the same column followed by a different letter are significantly different (LSD with Tukey–Kramer adjustment $P < 0.05$).

4. Discussion

Trichogramma deion performed better than the other two species tested, and therefore may be the best-suited for use as an augmentative biological control agent for *P. interpunctella* in stored product environments where microhabitat complexity is limited (e.g., packaged products in warehouses and retail stores). Overall, *T. deion* parasitized nearly twice as many *P. interpunctella* eggs as either of the other *Trichogramma* species in empty dishes, and slightly more than *T. ostriniae* in flour. However, none of the species were effective in finding eggs in millet, which

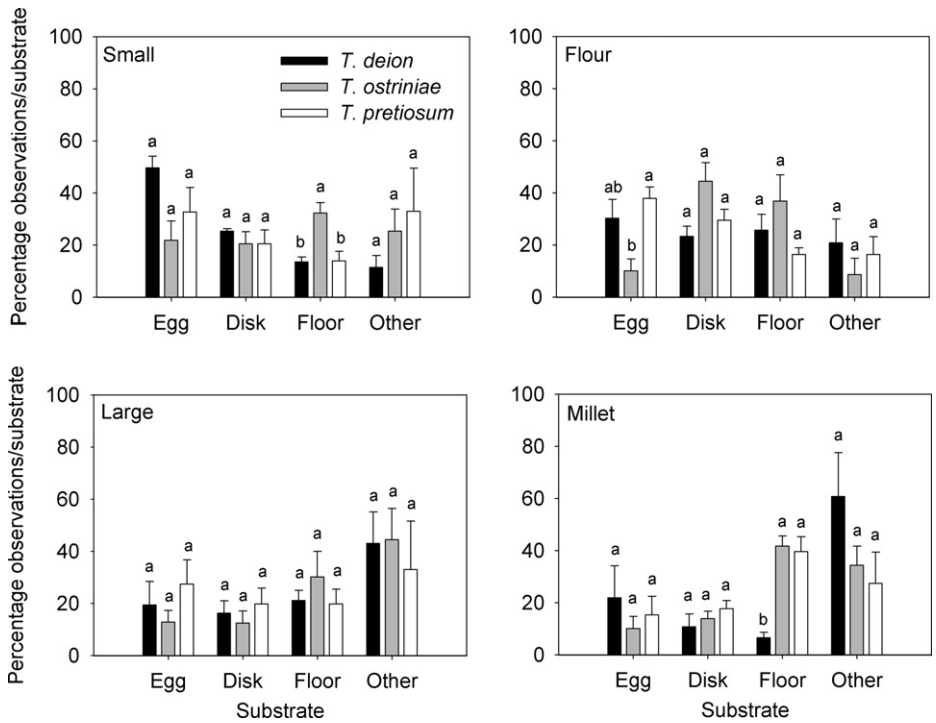

Fig. 2. Mean (±SEM) percentage observations on each substrate by arena type and species. Bars within the same chart and arena type with different letters are significantly different (LSD with Tukey–Kramer correction $P = 0.05$).

Table 3
Percentage of observations per substrate (host egg, disk, floor, and wall or ceiling) among the three *Trichogramma* species by arena

	T. deion Mean ± SEM	*T. ostriniae* Mean ± SEM	*T. pretiosum* Mean ± SEM
On eggs			
Small/empty	49.65 ± 4.45a	21.88 ± 7.35a	32.64 ± 9.48a
Large/empty	19.44 ± 8.96a	12.85 ± 4.51a	27.43 ± 9.28a
Small/flour	30.21 ± 7.33a	10.07 ± 4.58a	37.85 ± 4.42a
Small/millet	21.88 ± 12.35a	10.07 ± .70a	15.28 ± 7.13a
On disk			
Small/empty	25.35 ± 0.99a	20.49 ± 4.61a	20.49 ± 5.39a
Large/empty	16.32 ± 4.70ab	12.50 ± 4.63b	19.79 ± 6.08a
Small/flour	23.26 ± 3.97ab	44.44 ± 7.11a	29.51 ± 4.15a
Small/millet	10.76 ± 4.91b	13.89 ± 2.88b	17.71 ± 3.17a
On floor			
Small/empty	13.54 ± 1.92ab	32.29 ± 3.98a	13.89 ± 3.75b
Large/empty	21.18 ± 3.89a	30.21 ± 9.81a	19.79 ± 5.74ab
Small/flour	25.69 ± 6.05a	36.81 ± 10.06a	16.32 ± 2.60ab
Small/millet	6.60 ± 2.04b	41.67 ± 3.92a	39.58 ± 5.77a
On wall or ceiling			
Small/empty	11.46 ± 4.52b	25.35 ± 8.43ab	32.99 ± 16.58a
Large/empty	43.06 ± 12.07ab	44.44 ± 11.96a	32.99 ± 18.57a
Small/flour	20.83 ± 9.10ab	8.68 ± 6.24b	16.32 ± 6.92a
Small/millet	60.76 ± 16.83a	34.38 ± 7.31a	27.43 ± 12.02a

Means in the same column and location followed by a different letter are significantly different (LSD with Tukey–Kramer adjustment $P < 0.05$).

has larger granules and therefore is structurally more complex (Fig. 1).

The fact that parasitism and total host mortality caused by *T. deion* was similar in small and large empty dishes, but significantly lower in the small dishes of millet, which had approximately the surface area as the large empty dishes, suggests that differences in surface area were not solely responsible for the reduced host-foraging efficiency observed in millet (Table 1). Furthermore, the consistent trend among species for reduced parasitism in dishes with grain as compared to empty dishes supports the hypothesis that microhabitat complexity has the potential to interfere with *Trichogramma* host foraging. Additional experiments comparing the foraging success of the same three species of *Trichogramma* on retail shelves and packages in room-sized arenas showed that *T. deion* parasitized a greater percentage of host eggs compared to the other two species (Grieshop et al. 2006b). Furthermore, an experiment assessing the ability of *T. deion* to mitigate the infestation of either bulk or packaged corn meal indicated that the presence of packaging was essential to the success of biological control (Grieshop et al. 2006a). Thus, while none of the three species is likely to provide adequate parasitism in situations with high levels of microhabitat complexity (e.g., grain spillage, bulk products), *T. deion* may be well suited to protecting products that provide minimal habitat complexity (e.g., packaged products).

Trichogramma ostriniae was observed walking more frequently than *T. deion* and *T. pretiosum* (Fig. 3). However, *T. ostriniae* also was observed slightly less frequently on host eggs than *T. deion* and *T. pretiosum* (Table 2), and in no case parasitized the majority of eggs (Fig. 1). The fact that the relatively greater frequency of walking observed for *T. ostriniae* is not commensurate with increased host

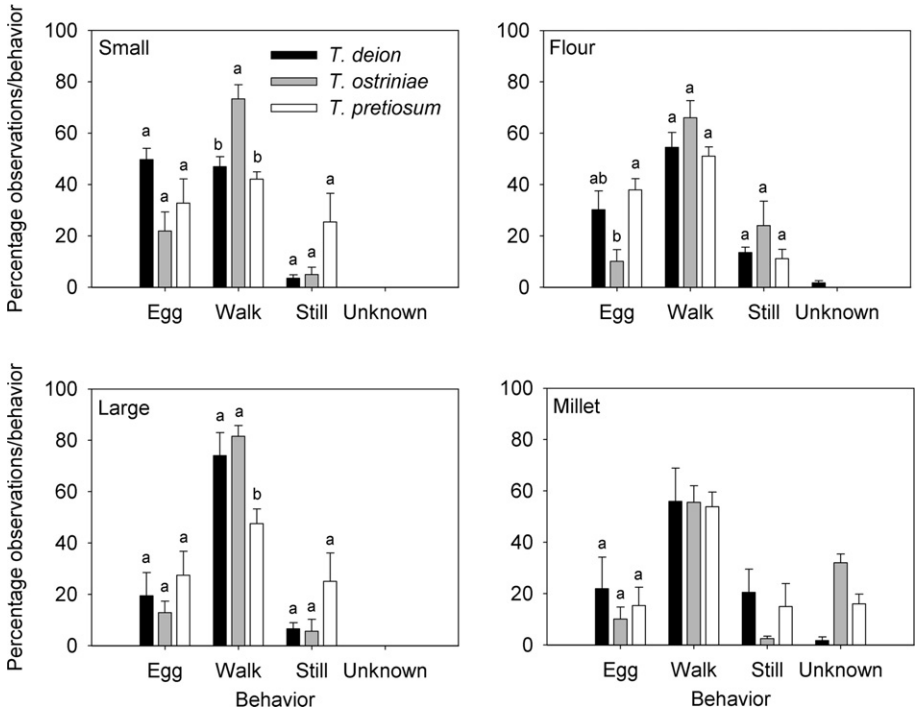

Fig. 3. Mean (±SEM) percentage observations for each behavior by arena type and species. Bars within the same chart and arena type with different letters are significantly different (LSD with Tukey–Kramer correction $P = 0.05$). Walking and still behaviors were removed from analysis in the millet chart due to the high number of unknown observations for *T. ostriniae* and *T. pretiosum*.

encounters and/or host parasitism is relevant to the evaluation of *Trichogramma* species because searching speed has been used to measure of *Trichogramma* quality (Cerutti and Bigler, 1994). Our data supports the alternative hypothesis that host acceptance (Van Hezewijk et al., 2000; Steidle et al., 2001), and not searching speed, may be a better single measure of the ability of *Trichogramma* to suppress host populations.

Another behavioral difference among the three species was that *T. pretiosum* remained longer (1.5–2 times) on the first egg compared to subsequent eggs, whereas *T. deion* and *T. ostriniae* spent equal time on all eggs (Fig. 4). The extra time spent by *T. pretiosum* on the initial host encountered might be related to host feeding, which we only observed in this species. Alternatively, because the females were naïve, the longer time spent on the first egg could have been due to priming (sensitization) after the first host encounter, resulting in the refinement of host recognition cues (Turlings et al., 1992). However, one would have expected this increase in host recognition to be exhibited by all three species but it was not. Regardless of the mechanism, the extra time spent on the first host would have the negative effect of reducing time available for *T. pretiosum* to locate subsequent hosts. This may have had an important effect on the results of the experiment because foraging time was limited to 2 h and it may partially explain the significantly greater rate of parasitism observed for *T. deion* compared to *T. pretiosum* in empty dishes (Fig. 1). Moreover, because *Trichogramma* have an adult life span of only 2–5 days, and host encounters may be relatively rare, extra time spent on initial host eggs could translate to reduced foraging efficiency (in the field), thus making it necessary to release greater numbers of parasitoids. In contrast, should host feeding extend the lifetime or overall fecundity of *T. pretiosum*, the initial time spent on host eggs might not be an important factor to parasitoid success. However, in experiments comparing the same three species over a 2 day period in arenas with retail shelving units, *T. pretiosum* proved again to provide the least amount of parasitism and overall mortality compared with the other two species (Grieshop et al. 2006b).

The relatively greater parasitism rates for *T. deion* on substrates other than millet may be the result of greater preference for or efficiency on simple substrates, reduced preference or efficiency on more complex substrates like millet, or a combination of the two. The fact that *T. deion* spent significantly less time on egg disks and on the millet compared to egg disks and floors of empty dishes or dishes with flour suggests that females may avoid structurally complex microhabitats like those composed of millet granules (Table 3), alternatively it is possible that a non structural aspect of the millet may be discouraging to *T. deion*. Under field conditions, this might result in a tendency for *T. deion* to select less complex microhabitats for host foraging. However, the behavioral data for *T. deion* also indicate that when the parasitoid was not on the walls or ceiling it was most often interacting with a host or on the egg disk (Table 3) which may translate to a greater host-foraging efficiency in complex microhabitats when the parasitoid chooses to remain in them. The pattern observed

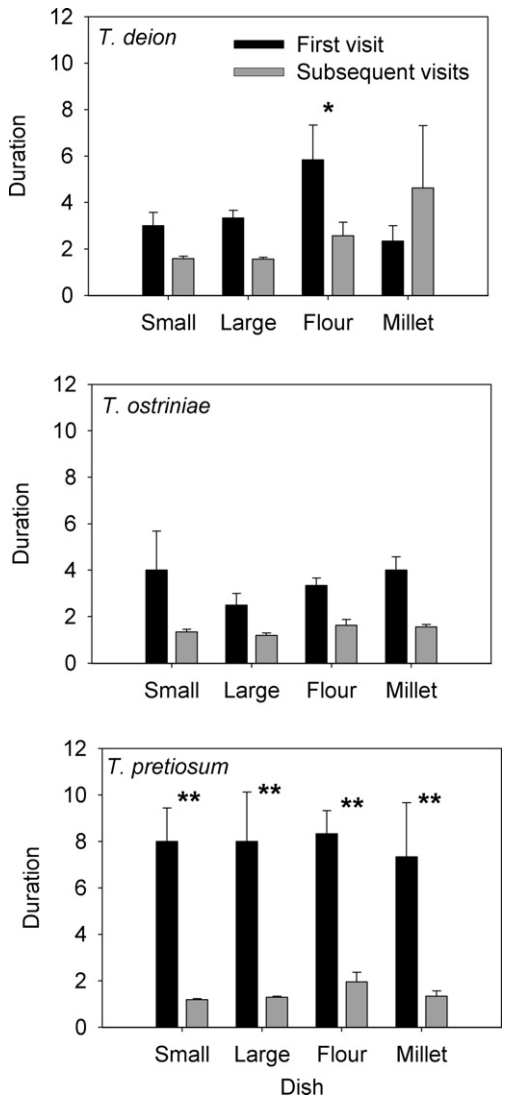

Fig. 4. Mean (±SEM) duration for first and mean of subsequent host interactions by species and arena type. Observations were taken at 150-s intervals. Asterisks indicate a significant difference (paired *t-test*) between bars within the same species and arena type ($^{*}P \leqslant 0.05$, $^{**}P \leqslant 0.01$).

for *T. deion* was not noted for the other two species. In fact, for *T. ostriniae* there was a trend for parasitism and overall host mortality to be lower on flour than on millet (Table 1).

None of the three species of *Trichogramma* performed satisfactorily in the more complex arenas, suggesting that increased microhabitat complexity may limit the host-foraging ability of *Trichogramma*. Other studies have also shown negative effect of increased microhabitat complexity on *Trichogramma* foraging in stored product environments. Schöller et al. (1996) showed that the ability of *T. evanescens* to locate hosts was greatly reduced by even a moderate depth of grain. Similarly, we have shown that *T. deion* was capable of controlling *P. interpunctella* eggs laid on the surface of packaged cornmeal, but not eggs laid on the cornmeal itself (Grieshop et al., 2006a). Thus, the consensus that can be drawn from the present and previous studies is that the use of *Trichogramma* spp. for stored product protection from *P. interpunctella* is best-suited to environments that limit microhabitat complexity; e.g. packaged products located in retail stores or warehouses.

In conclusion, *T. deion* appears to be the best-suited species of the three for management of *P. interpunctella* in stored product environments. However, future experiments assessing the relative impact of *T. deion* on field populations of *P. interpunctella* are needed to develop a better understanding of how augmentative releases of *Trichogramma* spp. could be best integrated into stored product moth management. In addition to parasitizing the highest percentage of hosts in both the small and the large arenas, rates of parasitism by *T. deion* were similar in both arenas, suggesting that increasing surface area alone may not pose a barrier to host foraging. However, increased fine-scale habitat complexity has the potential to disrupt foraging in all three species. An alternative parasitoid for use in these situations is the late larval parasitoid *Habrobracon hebetor* (Hymenoptera: Braconidae) (Say), which has been shown to forage equally well on packaged and bulk cornmeal (Grieshop et al., 2006a). Further comparisons of the three species by Grieshop et al. (2007) at the broader spatial scale of retail shelving also indicated that *T. deion* was best-suited to the management of *P. interpunctella*. However, a regular sanitation regime should probably remain the first line of defense against *P. interpunctella* and other stored product pests in retail stores and warehouses (Jones, 1991; Roesli et al., 2003). *Trichogramma* may be best-suited to situations that have reduced small-scale habitat complexity, such as packaged products, and the least well-suited for use in bulk products or in situations where there is grain spillage, such as in grain silos, processing facilities, or facilities with poor sanitation.

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