Evaluation of *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) Against Two Pyralid Stemborers of Texas Sugarcane[1]

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Abstract The role of *Trichogramma minutum* Riley (reared on *Helicoverpa zea* [Boddie] eggs or on an artificial diet) and *T. pretiosum* Riley (reared on *H. zea* eggs) as parasitoids of the Mexican rice borer, *Eoreuma loftini* (Dyar) and sugarcane borer, *Diatraea saccharalis* (F.), was assessed under laboratory and greenhouse conditions. The laboratory results showed that *T. minutum* reared on *H. zea* eggs parasitized 52.5% of Mexican rice borer eggs, compared to 50.3% for *T. pretiosum*. Parasitization of Mexican rice borer eggs by *T. minutum* reared on the artificial diet (62.6%) was not significantly different compared to those reared on *H. zea* eggs (52.5%). *Trichogramma minutum* reared on *H. zea* eggs parasitized 42.0% of sugarcane borer eggs, compared to only 12.6% by *T. pretiosum*. About 43.4% of sugarcane borer eggs were desiccated, despite 80 to 85% RH. Percentages of emergence (83.0%), female progeny (67.0%), deformed females (3.3%), and development time (8.2 d) in *T. pretiosum* reared from Mexican rice borer eggs did not differ significantly from those reared on *H. zea* eggs (91.3%, 72.5%, 2.5%, 8.3 d, respectively). However, *T. pretiosum* females reared from Mexican rice borer had greater longevity (2.5 d) and body length (0.385 mm) than females reared on *H. zea* (1.6 d, 0.326 mm, respectively). In the greenhouse, *T. pretiosum* parasitized an average of 55.3% of the Mexican rice borer eggs (egg masses were distributed on the sugarcane plants). About 21.0% of the eggs were dessicated, probably due to puncturing by the parasitoid, compared with 4.5% in the control, where egg masses were distributed on the plants without release of *Trichogramma*. Our results show the potential for use of *Trichogramma* spp. to control sugarcane stalk borers in a field IPM program.

Key Words *Trichogramma*, Mexican rice borer, sugarcane borer, biological parameters

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Trichogramma spp. attack more than 400 pest species, mostly lepidopterans, and some 32 × 10^6 ha of agricultural and forest land are treated with *Trichogramma* spp. annually (Li 1994). In South America and Southeast Asia, *Trichogramma* spp. are used against several species of sugarcane borers (Hassan 1984, 1990). High levels of parasitism (>90%) by *T. atopovorilia* Oatman and Platner and *T. pretiosum* Riley on *Diatraea muellerella* Dyar and Heinrich eggs on corn and *D. grandiosella* Dyar eggs on sugarcane were commonly observed in several locations in Mexico (Rodriguez-del-Bosque and Smith 1991, 1995). Field releases of *T. pretiosum* reared commercially under artificial conditions have been used for controlling *D. saccharalis* (F.) and *D. indigenella* Dyar and Heinrich in the Cauca Valley, the largest sugarcane growing area in Colombia (Gomes 1990). In Vietnam, overall parasitism by *T. chilonis* Ishii was 40.0% on *Sirpophaga nivella* F., and 79.3% on *Chilo sacchariphagus indicus* (Kapur) in the field after releases of 500,000 individuals per ha (Singh and Jalali 1993, Pham et al. 1995). In China, treatment of sugarcane with *T. chilonis,* which were reared on artificial diet, resulted in 75.4 to 93.2% control of stemborers. When reared on oak silkworm eggs, *T. chilonis* resulted in 67.4 to 82.8% control while treatment with conventional pesticides resulted in 12.2 to 34.3% control (Liu et al. 1995). The first attempt to evaluate eight species of *Trichogramma* for biological control of stemborers on sugarcane in the Lower Rio Grande Valley of Texas was conducted by Browning and Melton (1987). Low rates of parasitization by *Trichogramma* spp. prevented the authors from recommending the use of these parasitoids in the field. However, positive experiences in other parts of the world with the use of *Trichogramma* against pest species, including stemborers on sugarcane, encouraged us to re-evaluate this problem.

The objective of these studies was to determine the efficiency of *T. minutum* Riley (exotic) and *T. pretiosum* (indigenous) on egg masses of the Mexican rice borer, *Eoreuma loftini* (Dyar) and sugarcane borer, *D. saccharalis* (F.) under laboratory and greenhouse conditions. This allows an evaluation of the possibility of using *Trichogramma* spp. to control sugarcane stalk borers in a field IPM program. Because the Mexican rice borer is the dominant pest in sugarcane in the Lower Rio Grande Valley and *T. pretiosum* is an indigenous parasitoid, our studies were primarily focused on these two insects.

Materials and Methods

Insects. The *T. pretiosum* used in this experiment were originally collected from Texas in 1995 from Noctuidae in cotton. *Trichogramma minutum* used were originally collected in Canada and obtained in 1995 from Beneficial Insectary, Guelph, Ontario, Canada, where they were reared on *Ephestia kuehniella* Zeller eggs. In the Beneficial Insects Research Unit of the Subtropical Agricultural Research Center, Weslaco, TX, the *T. minutum* and *T. pretiosum* were maintained on irradiated (25kr, CS 137 source, 641 sec.) *Helicoverpa zea* (Boddie) eggs. Rearing was conducted in 3 × 15 cm glass tubes at 26 ± 1°C, 75 ± 5% RH, and 14 L:10 D photoperiod. The *H. zea* eggs were attached to paper strips (2.5 × 12.5 cm) with chicken egg white. Two cardboard strips with up to 0.66 g (approximately 7,260 eggs) of unparasitized *H. zea* eggs were inserted into a vial and later taped with another vial containing *Trichogramma* spp. approximately 24 h from adult emergence. The latter was covered with black paper to encourage movement of emerging parasitoids into the uncovered vial containing *H.*

zea eggs. A ratio of 1 parasitoid female: 10 host eggs was maintained, and the exposure period was 24 h. *Trichogramma minutum* also was reared on an oligidic diet in wax artificial eggs as described by Nordlund et al. (1997).

The egg masses of both borers used in these experiments were originally obtained from the Texas Agricultural Experimental Station where the Mexican rice borer had been reared on artificial diet (Martinez et al. 1988) during 6 months and sugarcane borer during 4 years after they were collected from sugarcane plants in Texas.

Laboratory experiments. In the laboratory experiments, paper cards with a known number of Mexican rice borer or sugarcane borer eggs were exposed to 1-day-old adult *T. pretiosum* or *T. minutum* (a ratio of approximately 1 parasitoid female:1 host egg) in a 12 × 8 × 2 cm plastic container (two 96 well tissue culture plate covers taped together with masking tape; Anchor Continental, Columbia, SC) for 24 h under the conditions described above. The high host:parasitoid ratio was used because only 30% or less of female *Trichogramma* spp. reared for a long time on one host (in our case approximately 30 generations on *H. zea* eggs) will oviposit on hosts of a different species (Greenberg et al. 1996). The parasitization rate, adult emergence rate (from black eggs), development time, longevity, sex ratio, percentage of deformed females, and female body size (from the frons to the tip of the abdomen) were recorded for the treatment *T. pretiosum* and *T. minutum* reared on borer eggs, and the control group reared on *H. zea* eggs. To determine the parasitization rate, approximately 400 eggs from each treatment were randomly selected from a cardboard strip after exposure to the parasitoids and held under the conditions described above until the parasitized eggs turned black. The eggs were then counted and classified as parasitized, desiccated or hatched. To determine the emergence rate, parasitized (blackened) eggs (approximately 300) were selected and held for adult emergence. After complete emergence and natural mortality of the adults, the emergence rate was determined by counting emergence holes. The sex ratio and percentage of deformed individuals was then determined by examining the dead individuals under a microscope. Longevity was measured by holding 40 adults, individually, in a 1 × 3 cm glass vial with no food, until death. Mortality was checked daily. Female body size was determined by measuring body length of 30 females with an Olympus dissecting microscope filled with a micro disk (10 mm/100xy).

Greenhouse experiments. In the greenhouse, *T. pretiosum* was evaluated against the Mexican rice borer. Egg masses (approximately 2 to 3 egg masses/plant) were placed on cards and into cages (3 × 2 × 2 m) with 15 sugarcane plants. The cards with egg masses were attached to the leaves by entomological pins. The egg mass distribution was similar to what we found under natural conditions. A ratio of approximately 5 parasitoid females: 1 host egg was maintained and the exposure period was 24 h because the condition for *Trichogramma* was complicated by necessity to search host eggs. After this time, the egg masses were collected and allowed to continue development in an environmental chamber (26 ± 1°C, 75 ± 5% RH, and 14L:10D photoperiod). Numbers of parasitized, desiccated and hatched eggs in each egg mass were counted and recorded. The parameters measured in the laboratory experiment were also evaluated in the greenhouse. This experiment was replicated three times (56, 37, 41 egg masses were distributed with an average of 58.7 eggs per egg mass).

Statistical analyses were conducted using the Independent *t*-test function of SYSTAT (Wilkinson et al. 1992).

Results and Discussion

Laboratory experiments. The mean percentage of parasitization of Mexican rice borer eggs by the two parasitoid species reared on *H. zea* eggs was not significantly different: 52.5% for *T. minutum* and 50.3% for *T. pretiosum* (t = 0.593, df = 26, P = 0.558). However, the parasitization rate by *T. minutum* was more variable (range = 38.5 to 80.4%) than the parasitization rate by *T. pretiosum* (range = 42.8 to 55.6%) (Table 1). When *T. pretiosum* or *T. minutum* attacked Mexican rice borer eggs, 22.4 ± 3.7% and 16.6 ± 1.3% (mean ± SEM) of the eggs were desiccated (possibly the parasitoids punctured them, but did not oviposit), respectively, compared with only 7.8% in the control group (t = 4.1, df = 7, P = 0.005; t = 3.8, df = 12, P = 0.003, respectively). Such desiccation increased total mortality (parasitized eggs plus desiccated eggs with correction on control using Abbott's [1925] formula) of Mexican rice borer to 66.1% when attacked by *T. pretiosum* and to 62.0% when attacked by *T. minutum.* The remaining eggs successfully eclosed (30.9% when Mexican rice borer were attacked by *T. minutum*, 27.3% when attacked by *T. pretiosum* and 92.2% in the control group).

The mean parasitization rate of sugarcane borer eggs by *T. minutum* was 42.0% (range = 0 to 91.7%) but was only 12.6% (range = 0 to 22.1%) with *T. pretiosum* (t = 2.686, df = 18, P = 0.015) (Table 1). We observed many dry host eggs (approximately 43.4%) in control and treatment groups even though the humidity was high (about 80 to 85% RH) because the optimum humidity for development sugarcane borer eggs is about 100% RH. However, the *Trichogramma* spp. used are not very effective at such high humidity rates.

Trichogramma minutum females reared on artificial diet parasitized 62.6% of Mexican rice borer eggs. This was not significantly different compared with parasitoids reared on *H. zea* (52.5%) eggs (P > 0.05).

The main biological parameters of *T. pretiosum* reared on Mexican rice borer or *H. zea* eggs are presented in Table 2. The mean emergence rate of *T. pretiosum* from Mexican rice borer eggs was 83.0% and from *H. zea* eggs was 91.3%, which were not significantly different (t = 1.515, df = 4, P = 0.204). The development time of *T. pretiosum* in Mexican rice borer eggs (8.2 d) was not significantly different from those on *H. zea* eggs (8.3 d) (t = 0.405, df = 58, P = 0.687). However, the mean longevity of *T. pretiosum* unfed females from Mexican rice borer eggs (2.5 d) was significantly

Table 1. Parasitization rate by *Trichogramma* spp. on sugarcane stemborer*.

Stemborers species	Percentage	Range	Percentage	Range
	T. minutum		*T. pretiosum*	
Mexican rice borer	52.5 ± 2.5a**	38.5-80.4	50.3 ± 1.3a**	42.8-55.6
	62.6 ± 1.2†	59.3-66.7		
Sugarcane borer	42.0 ± 9.6a	0-91.7	12.6 ± 2.9b	0-22.1

* Means (±SEM) in each row followed by different letters are significantly different at the 5% level as determined by Independent t-Test function of SYSTAT (Wilkinson et al. 1992).

** *T. minutum* or *T. pretiosum* reared on *H. zea* eggs.

† *T. minutum* reared on artificial diet.

Table 2. Comparative analyses of the biological parameters of *T. pretiosum* reared on different hosts*.

Parameters	reared host	
	H. zea	Mexican rice borer
Developmental time, d	8.3 ± 1.0a	8.2 ± 0.9a
Emergence rate, %	91.3 ± 4.5a	83.0 ± 8.3a
Longevity, d	1.6 ± 0.8a	2.5 ± 1.0b
Female progeny, %	72.5 ± 3.2a	67.0 ± 10.7a
Deformed females, %	2.5 ± 1.4a	3.3 ± 2.3a
Body length of females, mm	0.326 ± 0.009a	0.385 ± 0.011b

* Means (±SEM) in each row followed by different letters are significantly different at the 5% level as determined by Independent *t*-Test function of SYSTAT (Wilkinson et al. 1992).

longer than that for females from *H. zea* eggs (1.6 d) (t = 3.750, df = 58, P < 0.05). The percentage of female and deformed female parasitoids was not significantly different among hosts (P = 0.595 and P = 0.771, respectively). When *T. pretiosum* were reared on Mexican rice borer eggs, 67.0% of the adults were females (range = 45.6-78.8%) compared to 72.5% for parasitoids reared on *H. zea* eggs (range = 65.0-80.0%). The percentage of deformed females was 3.3% (range = 0 to 7.7%) for *T. pretiosum* reared on Mexican rice borer eggs compared with 2.5% (range = 0 to 5.0%) on *H. zea* eggs. The body length of *T. pretiosum* females reared on Mexican rice borer eggs (0.385 mm) was significantly greater than that of females reared on *H. zea* eggs (0.326 mm) (P < 0.05).

Greenhouse experiments. The mean parasitization rate of *T. pretiosum* on Mexican rice borer eggs in the greenhouse was 59.3% (range = 32.4 to 87.9%) for the first release, 56.0% (range = 20.0 to 87.3%) on the second release, and 50.5% (range = 0 to 85.7%) on the third release. In the greenhouse, we observed that, overall, 21.0% of Mexican rice borer eggs were desiccated, while in the control group it was only 4.5% (Table 3). It is possible that eggs, which were punctured by *T. pretiosum* but not parasitized, desiccated more rapidly. Such desiccation increased total mortality of Mexican rice borer to 72.6%. The emergence rate of *T. pretiosum* was 84.2 ± 2.4% (range = 67.1 to 97.2%), the number of parasitoid female progeny—79.7 ± 2.4% (range = 71.2 to 87.1%), deformed females—1.5 ± 0.4% (range = 1.0 to 3.6%), and the mean female body length was 0.373 ± 0.004 mm.

In summary, Mexican rice borer is the dominant pest in sugarcane in Lower Rio Grande Valley (Spurgeon et al. 1997). Under laboratory conditions *T. minutum* and *T. pretiosum* reared *in vivo* parasitized 52.5% and 50.3% of Mexican rice borer eggs, respectively. The parasitization rate of *T. minutum* reared *in vivo* was not significantly different compared to those reared *in vitro*. The main biological parameters of *T. pretiosum* reared on Mexican rice borer eggs did not differ significantly compared to parasitoids reared on bollworm eggs, except for longevity and body length of parasitoid females which were greater when reared on Mexican rice borer eggs. The average parasitization rate of *T. pretiosum* on Mexican rice borer eggs, artificially

Table 3. Results of greenhouse evaluations of *T. pretiosum* against Mexican rice borer on sugarcane plants.

	Mean percentage of Mexican rice borer eggs (±SEM)		
Treatments	parasitized	desiccated	hatched
1st release	59.3 ± 1.5	19.8 ± 1.0	21.0 ± 1.3
	(32.4-87.9)	(0-33.8)	(0-45.4)
Control	0	5.3 ± 1.5	94.7 ± 1.5
		(2.9-8.0)	(92.0-97.1)
2nd release	56.0 ± 3.4	18.4 ± 2.0	25.5 ± 2.1
	(20.0-87.3)	(0-42.2)	(8.2-80.0)
Control	0	3.7 ± 2.4	96.3 ± 2.4
		(0-8.3)	(91.7-100)
3rd release	50.5 ± 3.2	24.9 ± 2.7	24.5 ± 2.5
	(0-85.7)	(0-58.5)	(8.0-100)
Control	0	4.5 ± 3.4	95.5 ± 3.4
		(0-11.1)	(88.9-100)
	Over-all results		
Releases	55.3 ± 2.7	21.0 ± 1.9	23.7 ± 2.0
	(17.5-87.0)	(0-44.8)	(5.4-75.1)
Controls		4.5 ± 2.4	95.5 ± 2.4
		(1.0-9.1)	(90.9-99.0)

Numbers in parentheses indicate minimum and maximum values

distributed on sugarcane plants in greenhouse, was 55.3%. The percentage of desiccated Mexican rice borer eggs tended to increase the total host mortality induced by *Trichogramma,* possibly because of host-feeding or puncturing of the host eggs.

Analysis of available information and our results demonstrate that *Trichogramma* spp. have potential as effective agents for control of sugarcane pests in Texas. However, the wide variability in data from experimental and practical applications of *Trichogramma* presents a challenge for scientists and demands critical re-evaluation, improvement and development of existing or new production and application techniques, including making releases of parasitoid economically competitive with conventional management techniques. Many factors contribute to the effectiveness of *Trichogramma,* including selection of an appropriate species for release, the genetic consequences of mass rearing on the production culture, the quality of the parasitoids, the density of the pests, plant phenology, weather conditions and others. The systems approach allows development of techniques and methods for management of populations of mass reared beneficial insects and improvement of their performance under different ecological conditions.

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