2004 BELTWIDE COTTON CONFERENCES, SAN ANTONIO, TX – JANUARY 5-9

EVALUATION OF FOUR TRICHOGRAMMATIDS AS BIOLOGICAL CONTROL AGENTS FOR *PECTINOPHORA GOSSYPIELLA* (SAUND.) IN EGYPT

Alia Abd El-Hafez
Plant Protection Research Institute
Agriculture Research Center
Dokki, Giza, Egypt

Abstract

Field studies were conducted at Sharkia Governorate in 2000 and 2001 cotton seasons to evaluate the augmentative release of four trichogrammatids namely, *Trichogramma embryophagum* Hartig, *Trichogramma brassicae* Bezdenko, *Trichogrammatoidea bactrae* Nagaraja, and *Trichogramma evanescens* Westwood in controlling pink bollworm (*Pectinophora gossypiella* Saund.). Five releases of each parasitoid were conducted in each season with a rate of 60,000 ♀♀/ release/ feddan. This rate was determined from laboratory and field estimations to the quality of the aforementioned wasps. The four *Trichogramma* species were significantly able to reduce pink bollworm infestation than control. Infestation of pink bollworm in green cotton bolls in the released area was below that of control by 87.00- 71.68% in the first season and 86.25-71.93% in the second one. Regarding the four species, *T. embryophagum* and *T. bactrae* were more effective in reducing pink bollworm infestation than the other two species.

Introduction

Practical uses of trichogrammatid egg parasitoids occur worldwide against many lepidopterous pests on several key crops (Smith *et al*. 1986; Tuhan *et al*. 1987; Newton 1993; Smith 1994; Zandigiacome & Greatti 1997 and Lundgren & Heimpel 2003). Although *Trichogramma spp.* are well known as parasitoids having wide range of host species, especially those belonging to Lepidoptera, the different species of the parasitoid vary in their potentiality in controlling different pest species; i .e, each species is better to be used for controlling a target pest than the others. Hassan and Guo (1991) stated that selection of the strain showing the higher efficiency against a target pest in a given ecosystem is an important step for achieving an efficient biological control program. Therefore, successful biological control is a clear indication of adequate parasitoid quality. Preliminary laboratory studies were done in our laboratory (Abd El-Hafez 2001) to compare the quality of four trichogrammatids namely *Trichogramma embryophagum* Hartig, *Trichogramma brassicae* Bezdenko, *Trichogrammatoidea bactrae* Nagaraja, and *Trichogramma evanescens* Westwood when reared on *Pectinophora gossypiella* (Saund.) eggs. The obtained data suggested that *T. embryophagum* demonstrates quite good qualities of a parasitoid, including a high net reproductive rate, a high intrinsic rate of natural increase, a high finite rate of increase and a short population doubling time. The present work was carried out on the same trichogrammatids to evaluate their efficacy on controlling pink bollworm, *Pectinophora gossypiella* (Saund.) under field conditions. The Account Supporting Field And Applied Research For Agricultural Pest Control funds it.

Materials and Methods

Rearing Technique

Trichogramma evanescens Westwood, (a native parasitoid), *Trichogrammatoidea bactrae* Nagaraja, (imported from USA in 1992), *Trichogramma embryophagum* Hartig and *Trichogramma brassicae* Bezdenko, (imported from Iran in 1998), were reared on angoumois grain moth, *Sitotroga cerealella* (Oliv.) eggs according to the method described by Hassan (1993 and 1995). For efficient mass rearing of each of the four parasitoid species, *S. cerealella* eggs (< 24 hr old) were glued to paper cards (10X15 cm.) and exposed to *Trichogramma* adults in glass jars (2-liters capacity) provided with 10% sucrose solution for nutrition and covered with cloth-wrapped cotton kept in position by rubber band. Egg sheets were renewed daily to avoid super-parasitism and the parasitized egg sheets were kept in clean glass jars. Rearing took place at constant temperature of 25 ± 1°C and 80 ± 5 %R.H.

The Releasing Cards

Trichogramma spp. were released into the field using a release card that protect them from predators and unfavorable weather. For each parasitoid species, the release card consists of thick paper (6 x 8cm) folded to make a closed container of 4 x 6 cm. Three strips of grain moth eggs that contain parasitoid (about 500 eggs/ each) at three different stages of development (1, 3 & 5 days before emergence) were glued in this container. Thus, the total number of parasitoids/ card was about 1500 parasitoids. Six waves of *Trichogramma* adults emerge from each card with a rate of two waves per each stage. The emergence of parasitoid's waves begins within 12-24h after release, and continue through six days. On this way, *Trichogramma* adults cover a control period ranged between 8-10 days according to the longevity of the emerged adults (Abd El-Hafez 2001). Seventy cotton plants/ feddan were selected to serve as release points. The distance between the release points was 7m, and started 3.5 m from the edges of the field. Cards were hand-placed (before the sunset) on a 0.5m above soil surface.

Ten days after release, 5-cards/ feddan were selected at random, recovered and brought to the laboratory. The percentage of broken eggs was calculated to determine the level of parasitoid emergence. The obtained data reveal optimal levels of emergence, which are similar to those recorded under laboratory conditions (90- 97%). Also, high percentage of females in progeny (60.5- 70.0% females) was calculated when samples from released cards were kept to check in the laboratory. The number of *Trichogramma*/ release/ feddan was selected according to these estimations in addition to our previous study in cotton fields (Abd El-Hafez & Nada 2000; Abd El-Hafez *et al.* 2002 and Shalaby *et al.* 2002). The target number of parasitoids/ release/ feddan was 60,000 ♀♀ (a rate of 20,000♀♀ from each released stage of *Trichogramma*). Accordingly, about 100,000 parasitoids (unsexed)/ feddan were released in each application. The releasing cards were transported to the field in a cooling box to avoid the adverse effect of hot weather during transportation.

Field Application

The study was conducted in 2001 and 2002 cotton seasons at Sharkia Governorate to evaluate the efficacy of the four aforementioned trichogrammatids in suppressing pink bollworm population when introduced in the integrated pest management (IPM) of cotton program. At each season, an area of 25 feddan was selected and planted with the variety of Giza 85 cotton seeds. In the two seasons, the planting date of the cotton seeds in the selected area was relatively late (April 26th and may 2nd, respectively). Accordingly, the fruiting stage of those plants started lately and the growing season expanded until the end of September. The area was divided into five parts, 4 feddan/ each, while 1 feddan was left between each two parts to avoid dispersal of the different species. Four parts were selected for parasitoid treatments while the fifth was used as control. One recommended insecticide treatment was done in control and release area, 7 days before starting the release of parasitoids. Five releases with parasitoids were applied in the two seasons. These releases were distributed in a way to protect most of the green cotton bolls during the growing season (on July 23rd, August 6th; August 20th; August 31st and September 10th). At the two seasons, one insecticide application with Karate (Lambda- cyhalothrin) was done in the four parasitoid treatments (August 13th). While two recommended insecticide applications with Karate (Lambda- cyhalothrin) and Curacron (Profenofos) were done in the control area (July 30th and August 13th, respectively).

The efficacy of the four parasitoid species against pink bollworm was determined through estimation of the ability of those parasitoids to reduce the infestation of the green cotton bolls. The sequential sampling method was used to evaluate this infestation. Therefore, three replicates (50-100 green bolls/ each) were collected randomly from treatment and control area at 7 days intervals, dissected and the number of infested bolls was recorded. The reduction in the infestation with the target pest was determined by using Henderson and Tilton equation (1955). Static differences between the four parasitoids were determined by an analysis of variance (ANOVA), while Duncan's multiple range test was used to separate the means.

Results and Discussion

2001 Cotton Season

The levels of infestation for each inspection were listed separately in Table 1. The first release of the four trichogrammatids started on July 23rd; at that time, the pink bollworm infestation ranged between 6.0-7.33% in all the experimental area, while this area was free from spiny bollworm infestation. Seven days later (July, 30th), pink bollworm infestation became more twice (14.67%) than the first inspection in control area. While some decrease in infestation occurred in all *Trichogramma* treatment to be ranged between 3.67 - 6.0%. Accordingly, releasing of the four trichogrammatids indicating 72.19- 54.53% reduction in the percentage of infested bolls. At that time, an insecticide recommended treatment with Karate was applied in control area.

The second release of *Trichogramma* occurred on August 6th. At that time, the percentage of infestation with pink bollworm slightly decreased to 13.33% in control area, while it ranged between 2.67- 6.00% in the area that received the four parasitoids, indicating 81.77- 52.71% reduction than control. In the 4th inspection (August 13th) the pink bollworm infestation ranged between 3.33 - 6.0% in the four treatments, opposed to 18.33% in the control. Therefore, the reduction in infestation averaged 79.80 - 63.61%. At that time, a recommended insecticide treatment was done in control and treated area.

On August 20th (7 days after insecticide treatment), an obvious decrease in pink bollworm infestation was achieved in *Trichogramma* treatments as it ranged between 1.0- 4.67% in the four releases area opposed to 20.00% in control area. Accordingly, high reduction in infestation occurred in released area to be below than that of control by 95.54, 80.94, 74.04 and 75.93% in treatments that received *T. embryophagum, T. brassicae, T. bactrae* and *T. evanescens*, respectively. At that time the 3rd release of *Trichogramma* was conducted.

On August 27th (the 6th inspection), percentage of infestation increased to 28.67.0% in control area while it averaged 1.67-8.00% in the four treatments, indicating 94.45-73.41% reduction in pink bollworm infestation than control. The 4th release of the parasitoids was applied on September 3rd when infestation rate averaged 2.33- 10.33% in *Trichogramma* treatments and 36.33% in control. Accordingly, the reduction in *P. gossypiella* infestation ranged between 93.16- 72.91%. On September 10th, more increase in the percentages of infestation occurred, being 4.0-14.67% in treatments opposed to 54.0% in control. The indicating reduction in infestation caused by the four aforementioned parasitoids averaged 93.26- 74.11%. Another re-

lease of *Trichogramma* occurred at that time to avoid the natural increase in infestation which always occurred at the end of the season and subsequently reduce the number of bollworms which enter diapause and cause infestation in the next season. At the last inspection (September, 17th), the majority of the green cotton bolls in control area became infested with pink bollworm, showing 70.00% infestation, while infestation in the releases area ranged between 8.33-17.33% indicating 89.17-76.41% reduction than control.

The seasonal mean percent of infestation with pink bollworm for all inspections reached 29.11% in the control area, while it ranged between 4.07–8.63% in *Trichogramma* treatments. The mean reduction percentage in the four treatments was below that of control by 87.00-71.68%. Statistically, the four trichogrammatids caused significant reduction in infestation than control (F value= 4.20). *T. embryophagum* was significantly more effective than *T. evanescens* (the local species) and *T. brassicae*, while the efficacy of this parasitoid did not differ significantly than that of *T. bactrae.* On the other hand, there is no significant difference between the reduction of pink bollworm infestation in the area received *T. brassicae, T. bactrae and T. evanescens* (LSD= 10.47).

Season 2002

The rate of infestation by *P. gossypiella* to cotton bolls at the time of the 1st release (July 23rd) ranged between 5.67-7.00% in all the experimental area (Table 2). Seven days later (July, 30th), pink bollworm infestation increased to 12.67% in control area and accordingly a recommended insecticide treatment with Karate was done in this area. On the other hand, infestation in *Trichogramma* treatments (4.33-4.67%) decreased to be below than that of control by 69.84-64.81%.

The 3rd inspection (August 20th) reveal that *T. embryophagum* was the most effective parasitoid followed by *T. bactrae,* since area received *T. embryophagum* became free from pink bollworm infestation and that received *T. bactrae* became below than control by 87.72%. While those received *T. brassicae* and *T. evanescens* showed approximately the same percentage of reduction (47.46 and 46.20%). On August 13th, the percentage of infestation increased in control to reach 17.00% opposed to 0.67- 7.33% in *Trichogramma* treatments, showing 96.79- 62.80% reductions than control. At that time an insecticide treatment was applied at control and *Trichogramma* released area. On August 20th (the time of the third release), the percentage of infestation increased in control area to reach 22.00%, while it averaged 2.0- 8.00% in the four treatments. These percentages were below that of control by 91.88-65.65%.

On August 27th, more increase occurred in the percentage of pink bollworm infestation at control area to reach 32.00%, while it ranged between 4.0- 11.33% in the four parasitoid treatments. Therefore, 86.81- 60.47% reduction in pink bollworm infestation was achieved in area that received *Trichogramma*. An obvious increase in pink bollworm infestation occurred in control area to reach 48.0% on September 3rd opposed to 8.0% in *T. embryophagum* treatment, 12.00% in *T. brassicae* treatment, 9.67% in *T. bactrae* treatment and 9.33% in *T. evanescens* treatment. These percentages were below that of control by 82.42, 72.09, 81.78 and 79.49 %, respectively. On September 10th, most of the cotton bolls entered the mature stage and accordingly the pink bollworm has concentrated its infestation in the few numbers of acceptable green bolls. So 64.0 % of the green bolls in the control area became infested by pink bollworm opposed to 8.67- 11.33% in the four treatments, indicating 85.71-78.49% reduction. At the last inspection (September 17th), the percentage of infestation increased in the four-*Trichogramma* treatments to reach 16.33-17.67% while it decreased slightly to 61.33% in control. Accordingly, these percentages were below that of control by 75.50-69.60%.

In this season, the seasonal mean of infestation percentages through the period of application reached 32.30% in control area, while it ranged between 5.70- 9.48% in parasitoid treatments indicating a whole mean of 84.83- 65.34% reduction than control. Statistically, the four *Trichogramma* species were able to reduce pink bollworm infestation than control (F value= 5.359). Regarding the reduction in pink bollworm infestation at the all inspections of the four treatments, *T. embryophagum* was more effective in reducing pink bollworm infestation than the other three species although it differed insignificantly than *T. bactrae.* While, the efficacy of these two species was significantly more than that of *T. brassicae.* On the other hand, there is no significant difference between the efficacy of *T. evanescens* (the local parasitoid) and *T. brassicae* or *T. bactrae* (LSD, $P<0.05$= 10.656). According to the statistical analysis of the data of the two seasons, it could classified the four parasitoids into two categories as follows; *T. embryophagum* and *T. bactrae* at the first one while *T. brassicae* and *T. evanescens* at the second (LSD, $P<0.05$= 7.346). Regardless the species of the released *Trichogramma*, reduction in pink bollworm infestation which reflects the efficacy of the released parasitoids did not differ significantly through the two cotton seasons (F value= 0.895).

According to these results, the use of *Trichogramma spp*. to control pink bollworm is possible and could be used as biocontrol agent in integrated pest management (IPM) program in cotton fields. It is important to note that *T. embryophagum* demonstrates quite good qualities of a parasitoid followed by *T. bactrae* when compared with the other two species under laboratory conditions (Abd El-Hafez 2001). These qualities including a high net reproductive rate, a high intrinsic rate of natural increase, a high finite rate of increase and a short population doubling time. It is recommended to recover these species from fields after releasing to establish a laboratory colony. Moreover, seasonal survey must be conducted to determine their adaptability to environmental conditions. Hassan and Guo (1991) mentioned that species differ in their searching capability, host preferences and adaptability to environmental conditions.

As for *T. evanescens* (the native parasitoid), it can be chosen for controlling pink bollworm because it is supposedly better adapted to local environmental conditions. Efficacy of this parasitoid in cotton fields can be optimized by developing suitable strategies i.e., development of inexpensive effective mass production system; improving the quality of the produced wasps; planting cotton in a suitable date to escape from the 1st generation of pink bollworm emerged after diapausing; starting release program early before cotton boll formation and reducing the period between releasing to 7-10 days. Some investigators reported similar suggestions. Tuhan *et al.* (1987) found that release of *Trichogramma brasilience* at a rate of 20 000 newly emerged adults/acre per week in combination with sprays of carbaryl, dimethoate and monocrotophos, significantly, reduced the damage caused to cotton by *Earias insulana*, *E. vittella* and *Pectinophora gossypiella*. Releases at the same rate but at intervals of 15 and 30 days were less effective. Also, Scholz and Murry (1995) found high natural incidence of *T. bactrae* in cotton fields and mentioned that it may have some potential as biological control agent, particularly if it can be established in cotton earlier in the season. In China, Chao *et al.* (1996) released *Trichogramma flavum* in 1993-95, in Nanpi County, to control cotton bollworms (Noctuidae) and found that the release of *T. flavum* was less costly than chemical sprays. They added that, a small release of *T. flavum* was sufficient to control the pests' population during the year of moderate/light incidence of Noctuidae. However, during an outbreak year, both releases of *T. flavum* and spraying chemicals were necessary.

In Egypt, Abd El-Hafez and Nada (2000) reported similar results when they introduced *T. bactrae* in the IPM program in cotton fields and when this parasitoid was used in combination with insecticides at Sharkia Governorate. More recently, Shalaby *et al.* (2002) released *T. bactrae* for four times against pink and spiny bollworms in cotton fields at Qalyubia Governorate. The experimental area was free from any insecticidal treatments, where they found that *T. bactrae* was able to minimize the total infestation as well as the percentage of crop losses. Also, Abd El-Hafez *et al.,* (2002) released the four aforementioned parasitoids after the termination of the recommended insecticide program to determine their efficacy in protecting the new-formed green cotton bolls from infestation with bollworms and subsequently to reduce the number of bollworms which enter diapause and cause infestation in the next season. They reported that all the 4 parasitoid species were able to suppress the pink bollworm population in the four treatments to below that of control by 64.0- 80.0%.

References

Abd El-Hafez, Alia 2001. A comparison of quality attributes and life table parameters of four trichogrammatids (Hymenoptera: trichogrammatidae) reared from *Pectinophora gossypiella* (Saund.) eggs. Arab Univ. J. Agric. Sci., 9: 411-421.

Abd El-Hafez, Alia and M. A. Nada 2000. Augmentation of *Trichogrammatoidea bactrae* Nagaraja in the IPM programs for control of pink bollworm, *Pectinophora gossypiella* (Saund.) in Egypt. 2000 Beltwide cotton conferences, cotton Insect research and Control Conference, 1009-1014.

Abd El-Hafez, Alia, F.F. Shalaby, E.F. El-Khayat and Manal A.A. El-Sharkawy 2002. Efficiency of late season releasing of four trichogrammatid species in suppressing infestation with *Pectinophora gossypiella* (Saund.) in cotton fields at Sharkia Governorate. 2nd International Conference, Plant Protection Research Institute, Cairo, Egypt, 21-24 Dec., 1: 605-610.

Chao-Yuzhi, Liu-JinHua, YZ-Chao and JH-Liu 1996. The induction of adversity-resistant *Trichogramma flavum* and its use in the experimental control of bollworms. China-Cottons, (10): 23-26.

Hassan, S.A. 1993. The mass rearing and utilization of *Trichogramma* to control Lepidopterous pests: achievements and outlook. Pesticide sci., 37 (4): 387-391.

Hassan, S.A., 1995. Improved method for the production of the angoumois grain moth, *Sitotroga cerealella* (Oliv.). Trichogramma and other egg parasitoids conference, Cairo, Egypt, October 4-7, 1994, ed INRA, Paris, 157-160.

Hassan, S. A and M. F. Guo. 1991. Selection of effective strains of egg parasites of the genus Trichogramma (Hymenoptera - Trichogrammatidae) to control the European corn borer *Ostrinia nubilalis* Hb. (Lepidoptera: Pyralidae). J. App. Entomol. 111: 335-241.

Henderson, C. F. and E. W. Tilton 1955. Test with acaricides against the brown wheat mite. J. Econ. Ent., 48: 157-161.

Lundgren, J. G. and G. E. Heimpel 2003. Quality assessment for three species of *Trichogramma* and the report of a commercially-produced strain of *Trichogramma.* Biological Control. 26: 68-73.

Newton, P.J. 1993. Increasing the use of trichogrammatids in insect pest management: a case study from the forests of Canada. Pestic sci. Essex: Elsevier App. Sci. 37: 381-386.

Scholz B. C. G. and D. A. H. Murray 1995. Evaluating egg parasitoids for integrated pest management in Australian rain-grown cotton. *Trichogramma* and other egg parasitoids Cairo (Egypt), 4-7 October 1994. 133-136.

Shalaby, F.F., Alia Abd El-Hafez, E.F. El-Khayat and Manal A.A. El-Sharkawy 2002. Applied control of *Pectinophora gossypiella* (Saund.) and *Earias insulana* (Boisd.) in cotton fields by releasing *Trichogrammatoidae bactrae* Nagaraja. 2nd International Conference, Plant Protection Research Institute, Cairo, Egypt, 21-24 Dec., 1: 402-411.

Smith, S. M. 1994. Methods and timing of releases of *Trichogramma* to control Lepidopterous pests. "Biological control with egg parasitoids". Wajnberg E. & S. A. Hassan (eds.). CAB International. 113 – 144.

Smith, S. M., M. Hubbes and J. R. Garrow. 1986. Factors affecting inundative releases of *Trichogramma minutum* Ril. Against the spruce budworm. J. App. Entomol. 101: 29-39.

Tuhan, N. C.; A. D. Pawar and R. S. Arora. 1987. Use of *Trichogramma brasiliensis* Ashmead against cotton bollworms in Srigangangar, Rajasthan, India. Journal-of Advanced Zoology. 8: 131-134.

Zandigiacome, P. and M. Greatti. 1997. Biological control of the European corn borer *Ostrinia nubilalis* using *Trichogramma brassica* (Zea mays). Informatore- Fitopatologico (Italy). 47: 21-27.

Table 1. Efficiency of treatment with four trichogrammatids on suppressing infestation of green cotton bolls with pink bollworm in Sharkia Governorate at 2001 cotton season.

Date of		*T. embryophagum*		*T. brassicae*		*T. bactrae*		*T. evanescens*	
inspection	**Control**	**Infestation**	**Reduction**	**Infestation**	**Reduction**	**Infestation**	**Reduction**	**Infestation**	**Reduction**
23/7	6.67	7.33		7.00		6.00		7.00	
30/7	14.67	5.00	68.99	6.00	61.03	3.67	72.19	6.00	54.53
6/8	13.33	2.67	81.77	6.00	57.11	3.00	74.98	5.67	52.71
13/8	18.33	4.33	78.50	4.33	77.49	3.33	79.80	6.00	63.61
20/8	20.00	1.00	95.45	4.00	80.94	4.67	74.04	4.33	75.93
27/8	28.67	1.67	94.70	8.00	73.41	3.67	85.77	3.00	88.37
3/9	36.33	2.33	93.16	10.33	72.91	2.67	91.83	6.33	80.63
10/9	54.00	4.00	93.26	14.67	74.11	4.00	91.77	8.00	83.53
17/9	70.00	8.33	89.17	17.33	76.41	8.33	86.77	10.67	83.06
Mean	29.11	4.07	87.00[a]	8.63	71.68[b]	4.37	82.14[ab]	6.22	72.80[b]

Means with the same letter are not statistically different (LSD, P<0.05= 10.47).

Table 2. Efficiency of treatment with four trichogrammatids on suppressing infestation of green cotton bolls with pink bollworm in Sharkia Governorate at 2002 cotton season.

Date of		*T. embryophagum*		*T. brassicae*		*T. bactrae*		*T. evanescens*	
inspections	**Control**	**Infestation**	**Reduction**	**Infestation**	**Reduction**	**Infestation**	**Reduction**	**Infestation**	**Reduction**
23/7	6.33	6.00		5.67		7.00		6.00	
30/7	12.67	4.67	64.81	4.33	65.47	4.67	69.84	4.33	67.37
6/8	14.00	0.00	100	8.00	47.46	4.00	87.72	8.67	46.20
13/8	17.00	0.67	96.79	7.33	62.80	4.67	80.80	3.33	84.03
20/8	22.00	2.00	91.88	8.00	65.65	5.33	81.46	2.67	89.17
27/8	32.00	4.00	86.81	11.33	60.47	5.33	84.94	9.33	69.24
3/9	48.00	8.00	82.42	12.00	72.09	9.67	81.78	9.33	79.49
10/9	64.00	8.67	85.71	12.33	78.49	12.00	83.04	13.33	78.83
17/9	61.33	17.33	70.19	16.33	70.27	16.67	75.42	17.67	69.60
Mean	32.30	5.70	86.25[a]	9.48	65.34[c]	7.70	79.50[ab]	8.30	72.89[bc]

Means with the same letter are not statistically different (LSD, P<0.05= 10.656).