# 35

# Selection of Effective Species or Strains of *Trichogramma* Egg Parasitoids of Diamondback Moth

**U. Klemm, M. F. Guo[1], L. F. Lai[1] and H. Schmutterer[2]**

**Asian Vegetable Research and Development Center, P.O. Box 42, Shanhua, Tainan 74199, Taiwan, ROC; [1]Guangdong Entomological Institute, 105 Xingang West Road, Guangzhou, Guangdong, China; [2]Justus Liebig University of Giessen, Institute of Plant Pathology and Applied Zoology, Ludwigstr. 23, 6300 Giessen, Germany**

## Abstract

Twenty-seven *Trichogramma* and *Trichogrammatoidea* species or strains of different origin were tested on diamondback moth (*Plutella xylostella* (L.)) for host acceptance and host suitability. The experiments were conducted at $27 \pm 2°C$ and 75-90% RH. Depending on their initial parasitization activity, the number of offspring produced and emergence ratio, 18 *Trichogramma* species were identified as parasitoids of diamondback moth. Twelve *Trichogramma* species were compared by host preference experiments. None of the *Trichogramma* species showed host preference for diamondback moth over its laboratory rearing host *Corcyra cephalonica* Stainton, but differences between the species were found. The parasitization rate of diamondback moth eggs ranged between 0.47 for *Trichogramma semblidis* and 25.67 for *Trichogrammatoidea bactrae*. Seven out of twelve *Trichogramma* and *Trichogrammatoidea* species (*T. bactrae, Trichogramma* sp. (France), *T. principium, T. pretiosum, T. leptoparameron, T. chilonis, T. confusum, T. ostriniae*) showed promise for further investigation into selecting a biological control agent against diamondback moth. Three *Trichogramma* species were checked in cage experiments. *T. pretiosum* showed good searching capacity and percentage of parasitism (9.45/34.09%) compared to *Trichogramma* sp. (France) (2.5/19.26%) and *T. leptoparameron* (0.125/2.5%).

## Introduction

Trichogrammatids are egg parasitoids of a large number of insect species. *Trichogramma chilonis* Ishii was found on the eggs of diamondback moth (DBM) (*Plutella xylostella* (L.)) (Lepidoptera: Yponomeutidae) by Okada (1989) in Japan, and *Trichogrammatoidea bactrae* Nagaraja on the eggs of DBM in farmers' fields in Thailand. Both species were checked in the laboratory on DBM at AVRDC in Taiwan. Parasitization rate was satisfactory. Under high parasitoid density every DBM egg was parasitized. On plants and in the field, however, parasitism was not sufficient (Klemm, unpublished data). A selection of more efficient trichogrammatids, it was thought, might be promising in controlling DBM.

Pak (1988) tested 60 *Trichogramma* species/strains on five lepidopterous pests of cabbage. Hassan (1988) developed a laboratory glass tube method to select suitable *Trichogramma* species/strains to control the codling moth *Cydia pomonella* L., and two summer fruit tortrix moths *Adoxophyes orana* and *Pandemis heparana* (Lepidoptera: Tortricidae). Hassan and Guo (1991) used this method for selection of effective *Trichogramma* species or strains of egg parasitoids to control the European corn borer (*Ostrinia nubilalis* Hübner), and developed a simple cage test method to examine searching capacity.

The identification and selection of *Trichogramma* species/strains to control DBM had not yet been conducted. For this reason 27 *Trichogramma* species/strains were compared for their effectiveness in controlling DBM at Guangdong Entomological Institute (GEI), by utilizing laboratory glass tube and cage experiments. The identification of trichogrammatids as parasitoids of DBM was conducted by using a direct observation method, evaluating the initial parasitization activity, the ability for developing in DBM eggs and the emergence ratio of adult parasitoids. The host preference was compared by offering to a single trichogrammatid female the choice between eggs of two hosts, DBM and *Corcyra cephalonica* Stainton, respectively in a glass tube test. The parasitization rate was tested under 'no choice' conditions on DBM eggs. A cage experiment was conducted to evaluate the searching capacity of three *Trichogramma* species on DBM eggs on cabbage plants.

# Materials and Methods

## Insect cultures

The following 25 *Trichogramma* species/strains of exotic and indigenous origin were available at GEI: *T. agrotidis* (France), *T. cacoeciae* (USSR), *T. chilotraea* (Thailand), *T. confusum* (China), *T. cordubensis* (Iran), *T. deion* (USA), *T. dendrolimi* (China), *T. embryophagum* (France), *T. embryophagum* (Germany), *T. evanescens* (Iran), *T. japonicum* (China), *T. leptoparameron* (USSR), *T. maidis* (France), *T. nagarkatti* (France), *T. nubilale* (USA), *T. ostriniae* (China), *T. papilionis* (Japan), *T. pintoi* (USSR), *T. pretiosum* (USA), *T. principium* (USSR), *T.* sp. France (France), *T.* sp. USSR (USSR), *T. telengai* (USSR), and *T. trjapitzini* (USSR).

Two strains were provided by AVRDC: *Trichogramma chilonis* (Taiwan) and *Trichogrammatoidea bactrae* (Thailand).

A DBM culture was established from field-collected specimens. They were reared on Chinese cabbage (*Brassica campestris* ssp. *chinensis* L.) in 50 × 50 × 50 cm fine mesh nylon net cages. For DBM egg production, adults were confined in a container lined with filter papers dipped in cabbage leaf extract.

A culture of *C. cephalonica* was established at GEI. The sterilized eggs of this insect were used for rearing all *Trichogramma* and *Trichogrammatoidea* species/strains. Fresh eggs were continuously available. *Trichogramma* and *Trichogrammatoidea* species/strains were reared in 10 × 4 cm diameter glass tubes. All observations were conducted at 27±2°C, 75-90% RH.

## Direct observation method

Host acceptance was observed in the first monitoring after description of typical oviposition behavior described by Pak (1988) and determined by the number of oviposition acts within one hour of insect release. The host egg:parasitoid ratio was 1:10-15. DBM eggs (100-200/card) were replaced four times during 1 hour to avoid superparasitism. Up to five oviposition acts per replication were counted to judge host acceptance, i.e. the activity of parasitization on DBM eggs. After 1 hour, DBM eggs were replaced by eggs of *C. cephalonica* for 15 min, to ensure that *Trichogramma* laid eggs in them and thus exclude the influence of factors other than the host species themselves on parasitization. Five days later the second monitoring was conducted by counting parasitized DBM eggs. Parasitized eggs turn black indicating the development of parasitoids. The parasitoid adult emergence ratios were evaluated 10 days after oviposition in a third monitoring. The latter two parameters were used to evaluate the host suitability of DBM for *Trichogramma* species/strains tested, i.e. the ability of development and emergence of trichogrammatids in/from DBM eggs.

## Laboratory glass tube test methods

The host preference of *Trichogramma* was tested by offering the parasitoid the choice between eggs of DBM and *C. cephalonica* in glass tubes (Hassan 1988; Hassan and Guo 1991). A single *Trichogramma* female (12-24 hours after emergence) was released in a glass tube containing 80 eggs (2 × 40) of DBM and 80 eggs (2 × 40) of *C. cephalonica*.

Host eggs were glued on the surface of a small piece of paper (2 × 2 cm) and a drop of honey was placed at the center. To isolate a single female, *Trichogramma* adults were scattered on a smooth surface and an individual female was captured by placing an open end of a tube (50 × 9 mm diameter) around one individual. *Trichogramma* walked up over the tube walls and was easy to examine using a binocular. The single female is then transferred to the larger test tubes containing host eggs, by placing a suitable light source at the closed end of the large tube. The light stimulates the insect to walk towards the big tube. The adult female observed in the first monitoring was left with the host eggs until the second monitoring was conducted.

Host preference was monitored by (1) checking all tubes eight times during the first 6 hours of the experiment and recording the location of the parasitoid (on DBM or *C. cephalonica* or elsewhere); a minimum of 45 min elapsed between any two observations; (2) counting the number of parasitized eggs 5 days after release of females into the glass tubes, when the parasitized host eggs turned black.

Observations on the location of the parasitoid showed a preference by *Trichogramma* to search for, contact and remain on the eggs of its hosts. The number of parasitized host eggs/female (parasitization rate) shows the preference of the parasitoid female for laying eggs. The test should be repeated at least 30 times for each combination of *Trichogramma* and *Trichogrammatoidea* species/strain and host eggs.

The parasitization rate on DBM eggs (no-choice experiment) was tested in glass tubes. Separated *Trichogramma* females were provided by 80-100 DBM eggs on egg cards. Females were not fed. Five days later the parasitized eggs were counted.

## Cage experiments

The searching capacity of *Trichogramma* species/strains was tested by releasing 20 adult female parasitoids in cages (50 cm × 50 cm × 50 cm). Each cage contained five potted cabbage plants. The plants were exposed for one night for DBM egg-laying. DBM eggs were naturally distributed on the upper and lower surface of the plants. About 100 of these eggs per cage were marked. In addition, DBM eggs laid on paper were glued on the upper surface of the cabbage plant. Parasitized DBM eggs which had been previously marked were counted and percent parasitism was calculated. The number of parasitized eggs, divided by the number of released *Trichogramma* females, represents the searching capacity. The method was adapted from that of Hassan and Guo (1991).

# Results and Discussion

## Direct observation method

Nine of the tested species/strains did not show any oviposition behavior on DBM eggs, although these eggs were contacted at high frequency by *T. cacoeciae*, *T. chilotraea*, *T. cordubensis*, *T. embryophagum* (France), *T. embryophagum* (Germany), *T. evanescens* (Iran), *T. evanescens* (USSR), *T. maidis* and *T. papilionis*. Each of these species/strains readily parasitized eggs of *C. cephalonica*. Based on the criteria of host acceptance and suitability, 18 out of 27 tested *Trichogramma* species/strains were identified as parasitoids of DBM (Table 1). The 18 species were ranked after the first sequence of experiments, depending on their initial parasitization activity, number of eggs parasitized and emergence ratio of the offspring.

Distinct differences on these parameters were found among the 18 species. Eleven species showed high (more than 20 oviposition acts/hour), two species medium (11-20 oviposition acts/hour) and five species low (1-10 oviposition acts/hour) initial parasitization activity.

Whenever significant oviposition behavior was observed, the parasitoids were able to develop in, and to emerge from, DBM eggs. The emergence ratio ranged between 27.66% (*T. telengai*) and 100% (*T. agrotidis* and *T. nubilale*). A varying number of developing offspring was counted. Four species showed a high (more than 100 eggs parasitized), five species a medium (51-100 eggs parasitized) and nine species showed a low (1-50 eggs parasitized) level of parasitization of DBM eggs during 1 hour of observation. When parasitic activities and number of progeny produced were similar, the parasitoid species were ranked by the level of progeny emergence ratio.

Table 1. Ranking of DBM egg parasitoids according to parasitization activity, production of offspring and emergence ratio of adult parasitoids.

| Rank | Strain | Origin | Parasitism activity[a] | Production of offspring[a] | Emergence (%) |
|---|---|---|---|---|---|
| 1 | *T. bactrae*[b] | Thailand | 3 | 3 | 97.04 |
| 2 | *T. principium* | USSR | 3 | 3 | 96.24 |
| 3 | *T. sp.* (France) | France | 3 | 3 | 93.67 |
| 4 | *T. confusum* | China | 3 | 3 | 86.10 |
| 5 | *T. sp.* (USSR) | USSR | 3 | 2 | 94.10 |
| 6 | *T. leptoparameron* | USSR | 3 | 2 | 91.67 |
| 7 | *T. deion* | USA | 3 | 2 | 90.00 |
| 8 | *T. pintoi* | USSR | 3 | 2 | 76.04 |
| 9 | *T. chilonis* | Taiwan | 3 | 2 | 72.94 |
| 10 | *T. nagarkatti* | France | 3 | 1 | 61.90 |
| 11 | *T. telengai* | USSR | 3 | 1 | 27.66 |
| 12 | *T. pretiosum* | USA | 2 | 1 | 82.50 |
| 13 | *T. semblidis* | USSR | 2 | 1 | 81.25 |
| 14 | *T. nubilale* | USA | 1 | 1 | 100.00 |
| 15 | *T. agrotidis* | France | 1 | 1 | 100.00 |
| 16 | *T. trjapitzini* | USSR | 1 | 1 | 90.62 |
| 17 | *T. ostriniae* | China | 1 | 1 | 90.33 |
| 18 | *T. japonicum* | China | 1 | 1 | 47.05 |

[a]1 = low, 2 = medium, 3 = high. [b]belongs to the genus *Trichogrammatoidea*; all other species to the genus *Trichogramma*.

## Laboratory glass tube tests

The species ranked 1-9, 12, 13 and 17 were checked for their host preference. An ideal *Trichogramma* species should be active in searching for and showing parasitization preference for DBM eggs. None of the species was so ideal, but certain differences were observed.

The number of contacts per female on the eggs of DBM and *C. cephalonica* after 8 observations, as well as the resulting number of parasitized eggs after 5 days are shown in Fig. 1. None of the species showed host preference in searching for or parasitization of DBM eggs. This might be caused by the small size of DBM eggs (Pak 1991). Distinct preferences in searching for *C. cephalonica* were shown by *T. confusum* (4), *T. deion* (7), *T. pintoi*, (8), *T. chilonis* (9), *T. pretiosum* (12), *T. semblidis* (13) and *T. ostriniae* (17). *Trichogramma* sp. (France) (3) showed best results in searching for, and parasitization of, DBM eggs (1.6 contacts/female; 12.23 DBM eggs/female). The parasitization rate was 38.9 eggs/female. *Trichogrammatoidea bactrae* (1) and *T. pretiosum* (12) were less active in searching for DBM eggs (0.8 and 0.5 contacts of DBM eggs/female), and parasitized an average of 7.93 and 7.13 DBM eggs/female. *Trichogramma leptoparameron* (6), *T. principium* (2) and *T. pintoi* (8) had 1.53, 1.13 and 1.07 contacts/female on DBM eggs, but were less active in parasitization (4.3, 6.6 and 0.43 parasitized

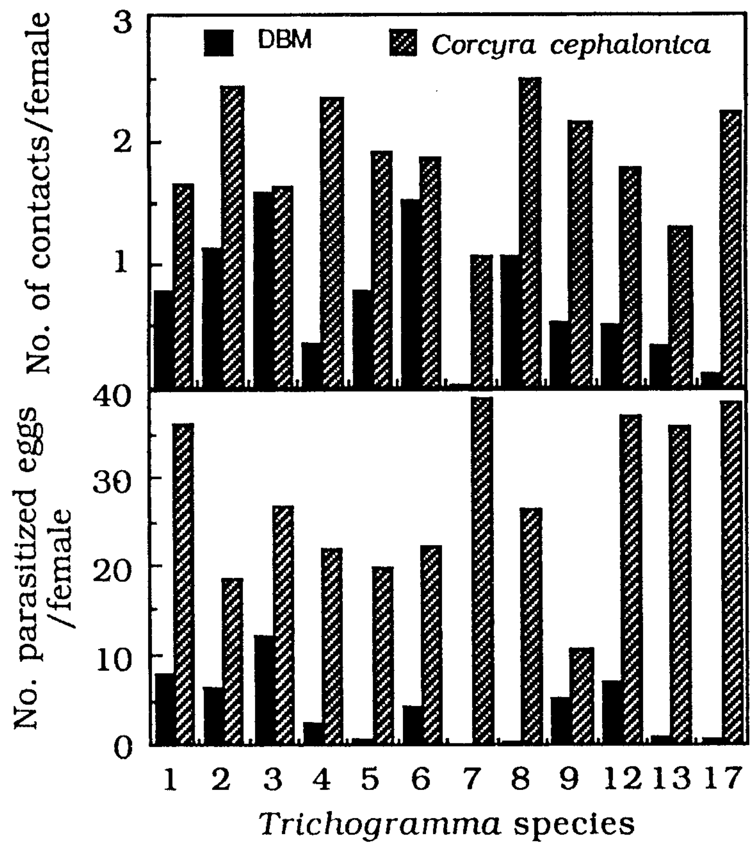


Fig. 1. Results of the laboratory glass tube test on host preference in searching for (No. of contacts/female) and parasitization (No. of parasitized eggs/female) of the eggs of DBM or *C. cephalonica* by various *Trichogramma* species. See Table 1 for identification of species.

DBM eggs/female). The number of parasitized host eggs was 26.5, 25.3 and 26.8 host eggs/female, respectively. The highest number of parasitized host eggs was presented by *T. bactrae* (1) (44.2) and *T. pretiosum* (12) with 44.03 eggs/female.

The correlation between the number of contacts/female of the tested species on DBM eggs and the resulting number of parasitized DBM eggs is shown in Fig. 2. Searching for DBM eggs leads to its parasitization by *Trichogramma* sp. (France) (3) and *T. principium* (2); *T. leptoparameron* (6) was searching for DBM eggs but parasitization was low. *Trichogrammatoidea bactrae* (1). *T. pretiosum* (12) and *T. chilonis* (9) showed less searching activity for DBM eggs than *T. pintoi* (8), but higher numbers of parasitized DBM eggs/female. The number 0.8 contacts on DBM eggs/female by *Trichogramma* sp. (USSR) (5) and *T. bactrae* (1) results in a difference of 7.3 parasitized DBM eggs in favor of *T. bactrae* (1).

The parasitization rate on DBM eggs (no choice experiment) is shown in Fig. 3. *Trichogrammatoidea bactrae* parasitized 25.67 DBM eggs/female, followed by *T. principium* (2) (24.27 eggs/female). *T. pretiosum* (12) (18 eggs/female), *T. ostriniae* (17) (12.5 eggs/female), *T.* sp. (France) (11.67 eggs/female), *T. chilonis* (9) (11.43 eggs/female) and *T. confusum* (4) (11.23 eggs/female), respectively. All other species parasitized less than 10 eggs/female.

Based on their performance on host preference and parasitization of DBM eggs (no choice experiment), 7 out of 12 tested species were identified as suitable for further investigations: *T. bactrae* (1) was confirmed to be one of the best species, as well as *Trichogramma* sp. (France) (3), *T. principium* (2), *T. pretiosum* (12), *T. chilonis* (9), *T. ostriniae* (17) and *T. leptoparameron* (6), respectively. To select the most efficient species for the control of DBM in farmers' fields, performance on searching capacity on cabbage plants was evaluated next.

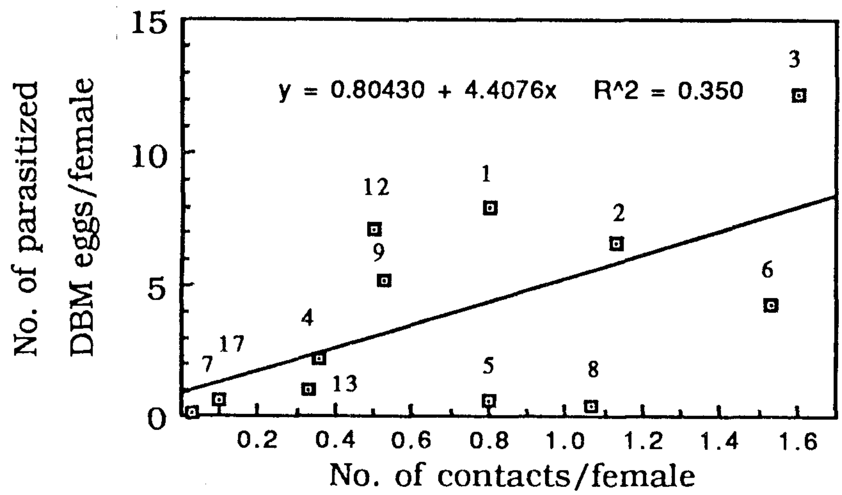


Fig. 2. Correlation between number of contacts of *Trichogramma* females with DBM eggs and number of eggs parasitized. Laboratory glass tube test on host preference. The numbers indicate the serial number of *Trichogramma* species listed in Table 1.

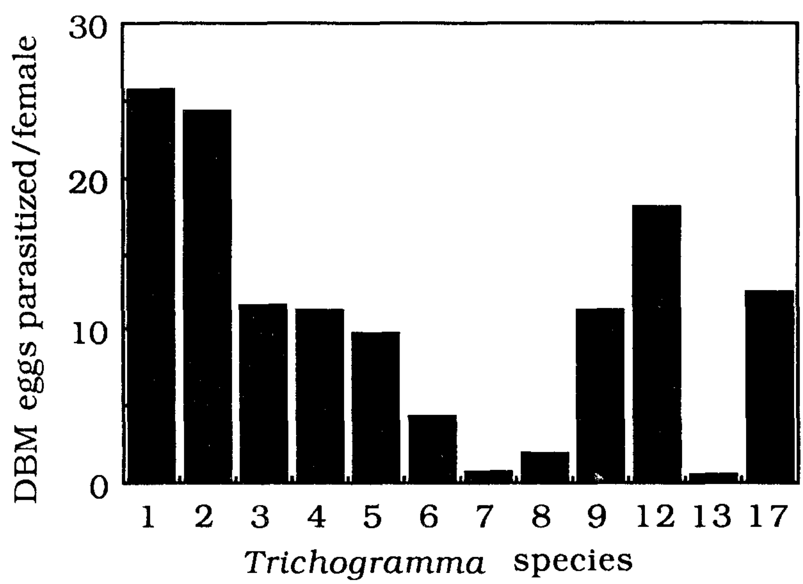


Fig. 3. Results of a laboratory glass the test on parasitization of DBM eggs by *Trichogramma* species. For species identification see Table 1.

## Plant cage experiments

Three out of 18 identified *Trichogramma*/*Trichogrammatoidea* species were compared using cage experiments for their ability to search for and parasitize DBM eggs on cabbage plants. *Trichogramma pretiosum* showed the highest percentage of parasitism (34.09%) of DBM eggs on cabbage plants and the highest searching capacity (9.45 eggs parasitized by one female) (Table 2). *Trichogramma* sp. (France) had 19.26% parasitism and 2.5 parasitized eggs per female. *Trichogramma leptoparameron* showed very low parasitism (2.48%) and an average number of only 0.125 parasitized DBM eggs per female. Marking DBM eggs on cabbage plants was suitable to judge the efficiency of parasitism by trichogrammatids.

Table 2. Searching capacity and parasitism by *Trichogramma* of DBM eggs on cabbage plants. Cage experiment.

| Species | No. of parasitized DBM eggs/female (±SD) | Parasitism % |
|---|---|---|
| *T.* sp. (France) | 2.5 ± 1.24b | 19.26 |
| *T. pretiosum* | 9.45 ± 3.47a | 34.09 |
| *T. leptoparameron* | 0.13 ± 0.25b | 2.48 |

Means followed by the same letter are not significantly different at the level of 5%, Duncan's Multiple Range Test (Duncan 1955).

## References

Duncan, D. B. 1955. New multiple range and multiple F-test. Biometrics, 11, 1-42.

Hassan, S. A. 1988. Selection of suitable *Trichogramma* strains to control the codling moth *Cydia pomonella* and the two summer fruit tortrix moths *Adoxophyes orana*, *Pandemis heparana* (Lep.: Tortricidae). Entomophaga, 33(4), 19-27.

Hassan, S. A., and Guo, M. F. 1991. Selection of effective strains of egg parasites of the genus *Trichogramma* (Hymenoptera, Trichogrammatidae) to control the European corn borer. Z. Angew. Entomol. (in press).

Okada, T. 1989. Parasitoids of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) species and seasonal changes of parasitism in cabbage fields. Jpn. J. Appl. Entomol. Zool., 33, 17-23.

Pak, G. A. 1988. Selection of *Trichogramma* for inundative biological control. Ph.D. Dissertation, The Agricultural University, Wageningen, The Netherlands, 224 p.

— 1991. Inundative release of *Trichogramma* for the control of cruciferous Lepidoptera: Preintroductory selection of an effective parasitoid. *In* Second International Workshop on the Management of Diamondback Moth and Other Crucifer Pests, 10-14 December 1990 Tainan, Taiwan, Abstract Volume.