Effects of host-egg age on the parasitism by *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae), an egg parasitoid of the diamondback moth

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Abstract

Eggs of *Plutella xylostella* (DBM), 0, 1, 2, 2.5 and 3 days old, were offered to female *Trichogramma chilonis* in a laboratory experiment. Females oviposited on host eggs of all ages tested. However, the percentage of oviposition was highest on the 1-day-old host eggs, and decreased as the host egg became younger or older than the 1-day-old host. Parasitized host eggs did not hatch. The emergence rate was high on the 1-day-old eggs but did not differ among other host ages, except the 3-day-old host. A *T. chilonis* progeny failed to complete development on the 3-day-old DBM eggs. Hind tibia length of the emerging parasitoid female progeny also tended to decrease as the host egg became older than 2 days. Host age had no significant effect on development time. A method of utilization of the parasitoid in a biological control program against DBM is also discussed based on the effect of DBM egg age on the parasitism of the parasitoid.

Key words: *Trichogramma chilonis*, diamondback moth, host acceptance, host suitability, biological control

INTRODUCTION

The diamondback moth (DBM), *Plutella xylostella* (L.), is a noted pest of numerous cruciferous crops in many areas of the world. Its resistance to many commonly used insecticides makes it difficult to manage the pest (Ankersmit, 1953; Liu et al., 1982), and alternatives that do not depend on toxic agents are required including those of biological control. *Trichogramma chilonis* Ishii is an important solitary egg parasitoid of DBM (Iga, 1985; Okada, 1989), hence, we are studying the utilization of *T. chilonis* as a biological control agent of the moth (Miura, 1992; Miura and Kobayashi, 1993, 1997; Miura et al., 1994).

Most biological control projects which include the use of *Trichogramma* can be characterized as “trial and error” per van Lenteren (1980), so that Waage and Hassell (1982) stated that preceding and follow-up research before and after release of *Trichogramma* is important. The parasitization process after *Trichogramma* release is generally divided into five steps: (1) host habitat location; (2) host location; (3) host acceptance; (4) host suitability; and (5) host

regulation (e.g., Douthett, 1964; Vinson, 1976). For the third and fourth steps, the relationships between the ages of DBM eggs and host acceptance behavior or host suitability of *T. chilonis* females are virtually unknown. van Lenteren et al. (1980) and van Lenteren (1981) also criticized that most researchers employing biological control determined the effect of host age on host suitability merely by recording the number of parasitized (black) eggs. Here we report the effects of DBM age on host acceptance and host suitability of *T. chilonis* from several aspects, oviposition behavior, survival and development time of progeny, and size of females emerging, and comment on the utilization of *T. chilonis* for DBM in biological control.

MATERIALS AND METHODS

The *T. chilonis* used was part of the stock culture at the Chugoku National Agricultural Experiment Station, which was derived from the original colony introduced from Taiwan (Hirashima et al., 1990) and has been reared on eggs of *Ephestia kuehniella* Zeller under conditions of 24°C and 16L–8D. Larvae and pupae of

DBM were collected from cabbage fields in Kume, Okayama Prefecture, in 1991. A stock culture of DBM was continuously maintained in a cabinet controlled at 24°C, and cabbage leaves were supplied as food for the larvae. Humidity and photoperiod were not controlled.

To obtain DBM eggs for experiments, moths were released for 1 h into a rearing cage (34 cm × 34 cm × 26 cm) containing a piece of Sealon film® (Fuji Photo Film Co., Ltd.) as a substratum for eggs, and were supplied with honey solution (20%) as food (Murai's method, personal com.). DBM eggs on the film were transferred to a petri dish (9 cm in diameter, 2 cm in height) and incubated at 24°C and 16L-8D until the following experiments. The eggs require incubation for 3 days at this temperature.

Experiments to determine the effects of host egg age on parasitism were carried out at 24°C using female wasps mated within 24 h after emergence which had not oviposited before the experiments. One DBM-egg 0-, 1-, 2-, 2.5- or 3-days-old, and one female parasitoid were placed in a petri dish, and the behavior of the wasp was observed under a binocular microscope. An egg onto which the female oviposited and came in contact with five times, was regarded as "accepted." Twenty females which did not escape from the petri dish were individually observed at each host age. Parasitized host-eggs were reared at 24°C and 16L-8D and daily DBM hatching and parasitoid emergence were recorded to determine the percentage of emergence (=no. of wasps emerged/no. of parasitized host-eggs), developmental period of the wasp, and DBM larvae hatching rate. To estimate the survival rate of unparasitized eggs of DBM, 34 other intact eggs were incubated at 24°C and 16L-8D, and the number of larvae hatching were recorded daily. The size of emerging female wasps was represented by the hind tibia length which was individually measured after mounting in a drop of balsam on a slide glass.

RESULTS

The process of oviposition by *T. chilonis* females after coming into contact with host eggs could be divided into four stages: drumming stage, drilling stage, ovipositing stage and host

Fig. 1. Relationship between host ages and percentage of oviposition by *T. chilonis* females. Vertical bars indicate 95% CI.

feeding stage. The four steps were almost the same as those on eggs of *Papilio xuthus* described by Suzuki et al. (1984). However, host-feeding behavior on the 3-day-old host was not observed. Figure 1 shows the percentage of oviposition by *T. chilonis* females exposed to different ages of DBM eggs. Females oviposited on host eggs of all ages tested, with the highest percentage of oviposition being observed on the 1-day-old host. We conducted a regression analysis to clarify the changes of oviposition behavior with host egg development. The relationship between host age (X) and the percentage of oviposition (Y) is represented as $Y = -20.2X^2 + 45.5X + 73.1$ ($r^2 = 0.98$, $p < 0.01$), and the data suggested that the relationship between percentage of oviposition and host age is unimodal. Therefore, the percentage of oviposition was highest on the 1-day-old host, and decreased as the host egg became younger or older.

Almost all unparasitized host eggs hatched (97.1%), whereas none of the parasitized host eggs hatched.

Table 1 shows the host suitability among different host ages. The emergence rate was highest on the 1-day-old host, and did not differ among the other ages except the 3-day-old host (Tukey-type multiple comparison test by Zar, 1984). *T. chilonis* progeny failed to complete development on the 3-day-old DBM eggs. Because no adult wasps emerged from 3-day-old hosts, no measure of host suitability is available except for the emergence of parasitoids.

Table 1. Parasitism of *T. chilonis* exposed to host eggs of different ages

Host age (days)	Emergence rate (%)	<i>n</i>	Length of female hind tibia (mm)	<i>n</i>	Female development time (days)	<i>n</i>
0	0.467 ab*	15	0.130 ab**	6	9.5 a**	6
1	0.778 a	18	0.132 ab	13	9.2 a	13
2	0.500 b	16	0.118 bc	6	9.7 a	6
2.5	0.615 b	13	0.111 c	8	9.8 a	8
3	0 c	5	—	—	—	—

*Values in a column with the same letter are not significantly different at the 5% level (Tukey-type multiple comparison test).

**Values in a column with the same letter are not significantly different at the 5% level (Scheffe's multiple comparison test).

Hind tibia length of emerging parasitoid female progeny also tended to decrease as the host eggs became older than 2 days. Host age had no significant effect on development period (Scheffe's test, $p > 0.05$).

DISCUSSION

Trichogramma is known to show great flexibility in parasitizing developed hosts (Pak, 1986). In some *Trichogramma*, oviposition is restricted to the earlier stages of the host eggs within the first one-sixth or one-half of the host incubation period (Hiehata et al., 1976; Juliano, 1982). In some species, however, the percentage parasitism is not strictly restricted but is less than 50% during half the host incubation period (Houseweart et al., 1982; Pak and Oatman, 1982; Navarajan, 1978/1979). *T. chilonis* was able to attack all ages of DBM eggs, including the oldest eggs which had developed to the stage just prior to hatching. The percentage of oviposition was also over 60% on most host ages within the first five-sixths of the incubation period. *T. chilonis* is believed to have good parasitization capability on DBM eggs, because they can parasitize eggs of all developmental stages and their oviposition percentage is high during most of the host incubation period as compared with other *Trichogramma* species. The oviposition behavior of *T. chilonis* accounts for the mortality of DBM eggs.

Pak (1986) classified the relationships between host age and parasitism by *Trichogramma* spp. into six basic types. The percentage of oviposition of *T. chilonis* females was

influenced by the age of the host; they prefer the intermediate ages of the DBM eggs, so that host selection appears to occur in this phase of parasitization behavior. According to Pak's classification, oviposition of *T. chilonis* on DBM eggs of a certain age might belong to "Vib type," in which the intermediate age host is the most often attacked of all host ages. There are no reports of *Trichogramma* not ovipositing in an egg immediately after it was laid by a host moth female, and the percentage of oviposition was lower in the oldest host eggs. The type Vib relationship for attack or oviposition has not been found in any other species.

No host larvae hatched from DBM eggs in which *T. chilonis* females oviposited, even if the parasitoid offspring failed to develop in these eggs. Marston and Ertle (1969) and Hawlitzky and Boulay (1982) suggested that *Trichogramma* spp. might inject a chemical substance into a host during oviposition, thus causing host necrosis and ultimate death. In *T. chilonis*, this capacity for killing of host eggs is not influenced by the age of a host; it might also be attributed to mortality of the host eggs that were unable to sustain the parasitoid offspring development.

There is a positive relationship between fecundity of female *T. chilonis* and its hind tibia length (Miura and Kobayashi, 1995). Pak and Oatman (1982) and Waage and Ng (1984) also reported that large females of *Trichogramma* produced more progeny than small females. The size of *T. chilonis* females identified by hind tibia length gradually decreased as the host egg became older than 2 days. The emergence rate was less than 61.5% in most host ages except the

1-day-old host. These results show that host ages within only a short term of incubation are suitable for *T. chilonis*. The population growth is affected by the emergence rate and fecundity of emerged females, and that *T. chilonis* after release would be strongly influenced by their developing hosts.

Miura and Kobayashi (1995) stated that with regard to the reproductive properties of *T. chilonis*, an inundative method would be better than inoculative release to control DBM by mass rearing *T. chilonis* in the laboratory. The present results also support the same utilization method, because *T. chilonis* can parasitize or kill host eggs of all developmental stages, although the emergence rate was less than 61.5% in almost host ages.

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