Reproductive Properties of *Trichogramma chilonis* Females on Diamondback Moth Eggs

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The effects of temperature on the reproductive properties of *Trichogramma chilonis* females developed in *Plutella xylostella* eggs (TC-DBM) were compared with those developed in *Ephestia kuehniella* eggs (TC-MFM) by rearing with diamondback moth (DBM) eggs at constant temperatures of 20°C, 24°C, 28°C and 32°C under 16L–8D. For both TC-MFM and TC-DBM, the daily egg production peaked on the first day after emergence, and adult longevity was reduced as the temperature increased from 24°C to 32°C. The number of eggs laid throughout the lifetime was a maximal at 24°C. Despite a lack of significant difference in the properties mentioned above between TC-DBM and TC-MFM which developed at 24°C, the number of matured eggs in the ovary at emergence as well as the number of eggs laid on the initial day after emergence of TC-DBM was significantly smaller than for TC-MFM. The method of utilization of the parasitoid in a biological control program against DBM is also discussed based on the reproductive properties of the parasitoid.

Key words: *Trichogramma chilonis*, *Plutella xylostella*, reproductive property, biological control

INTRODUCTION

The diamondback moth (DBM), *Plutella xylostella* (L.), is a serious pest of cruciferous crops in many countries. Development of alternative control methods including biological control is needed to cope with recent development of insecticide resistance of DBM. *Trichogramma chilonis* ISHII (TC) is an important solitary egg parasitoid of DBM (IGA, 1985; OKADA, 1989), thus we have been investigating the possibility of a biological control program against DBM using TC (MIURA, 1992; MIURA and KOBAYASHI, 1993; MIURA et al., 1994). However, the problem of how to utilize TC against DBM still remains. There are two categories of biological control using mass-reared natural enemies to obtain biotic reductions of a pest: (1) inundative release, and (2) inoculative release (DEBACH and HAGEN, 1964). Inundative release is made to control a pest, largely, if not entirely, by the natural enemies released, not by their progeny (FLANDERS, 1951). The host searching efficiency of the natural enemies selected may hold the key to the success of the inundative program (WAAGE and HASSELL, 1982). On the other hand, control from the use of inoculative release is dependent upon progeny being produced for more than one generation following the colonization of individuals of the natural enemies (DEBACH and HAGEN, 1964). Natural enemies that have a synchronization with host and a high reproductive potential should be adopted for inoculative release as compared with inundative release (VAN LENTEREN, 1986). We, thus, had to clarify the properties of natural enemies and then determine how to utilize them.

Mass rearing of *Trichogramma* species, which are polyphagous egg parasitoids, for a

biological control program has been generally conducted using alternative host species which differ from target pest species simply because of accessibility. Since host species should strongly affect developmental rate, survival rate and reproductive properties of the emerging wasp (LEWIS et al., 1976; HASSAN et al., 1978; BIGLER et al., 1987; KAZMER and LUCK, 1991), it is necessary to understand the reproductive properties of *Trichogramma* species developed in both alternative and target host species for determining how to best utilize them against target host species.

Mass rearing of TC has been also conducted using eggs of the Mediterranean flour moth (MFM), *Ephestia kuehniella* ZELLAR as an alternative host in the laboratory (HIRASHIMA et al., 1990). Here, we present data on the effect of temperature on the reproductive properties of TC developed in MFM or DBM eggs, and discuss a possible improvement for method of release.

MATERIALS AND METHODS

The population of TC used in the present study was part of a stock culture at the Chugoku National Agricultural Experiment Station, which was derived from the original colony introduced from Taiwan (HIRASHIMA et al., 1990) and reared with MFM eggs under 24°C and 16L-8D conditions. Larvae and pupae of DBM were collected from cabbage fields in Kume, Okayama Prefecture, in 1991. The stock culture of DBM was continuously maintained in a cabinet controlled at 24°C and cabbage leaves were supplied as food for the larvae. Moths just after emergence were released 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 oviposition and honey solution (20%) as the food source. Eggs were collected daily from the rearing cage.

Mated parasitoid females which developed in the MFM eggs (TC-MFM) and those which developed in the DBM eggs (TC-DBM) were prepared. TC-MFM represents females mass-produced and reared under the same conditions as those of the routine mass rearing of TC mentioned above, while TC-DBM represents the progeny of adult parasitoids which were released in a field, and then reared with the DBM eggs at 20°C, 24°C, 28°C and 32°C under 16L-8D. These females were individually placed into glass vials (3.7 cm in diameter and 18 cm length) immediately after emergence. Experimental temperatures were the same as those at which TC-DBM was reared. For each vial, a sufficient number of DBM eggs which were deposited within 24 h and a piece of filter paper infiltrated with a drop of honey solution (20%) were supplied daily until the parasitoid female died. The DBM eggs were removed from the vials and reared at 24°C under 16L-8D. The numbers of parasitoid adults emerging and parasitized host eggs which turned black were recorded daily. The numbers of TC-MFM females at 20°C, 24°C, 28°C and 32°C were 12, 11, 12 and 12, respectively. The numbers of TC-DBM females at the respective temperatures were 10, 12, 11 and 17.

In the present study, the number of DBM eggs parasitized by a single female was regarded as the fecundity of the parasitoid for both TC-MFM and TC-DBM. Because, as mentioned above, TC is a solitary parasitoid and self-superparasitism seldom occurs in the parasitoid (MATSUDA and MIURA, unpublished), the discrepancy between the number of the DBM eggs parasitized and the total number of eggs laid by a parasitoid female under conditions where a sufficient number of DBM eggs was provided may be negligible.

In TC-DBM, tables of the age-specific survival rate (l_x , proportion of females alive at

Fig. 1. Daily oviposition per surviving female (—) and survivorship curve (.....) for *T. chilonis* females, which were developed in *E. kuehniella* eggs, at various temperatures.

Fig. 2. Daily oviposition per surviving female (—) and survivorship curve (.....) for *T. chilonis* females, which were developed in *P. xylostella* eggs, at various temperatures.

age x) and oviposition rate [m_x , $m_x = (\text{proportion of females}) \times (\text{age-specific oviposition})$] were constructed. The parameters, the net reproduction rate (R_0), mean length of generation (T) and intrinsic rate of natural increase (r_m) were calculated from the table according to the methods of BIRCH (1948). The values of R_0 and T were estimated by $\sum l_x m_x$ and $\sum x l_x m_x / \sum l_x m_x$, respectively. The r_m was estimated by selecting values of r that satisfied the expression $\sum e^{-rx} l_x m_x = 1$. The values of developmental period and survival rate from egg to adult emergence at 20°C, 24°C, 28°C and 32°C were based on MIURA and KOBAYASHI (1993).

The portion of TC-MFM and TC-DBM females that developed at 24°C was dissected immediately after emergence. The number of matured eggs in the ovaries was counted, and the length of the hind tibiae measured as an index of body size. Twelve eggs each of MFM and DBM which had been deposited within 24 h were weighed with a micro balance (Sartorius 4503 MP6).

RESULTS

1. Effects of temperature on fecundity and longevity of TC females

The daily oviposition per surviving female and the survivorship curve for TC-MFM and TC-DBM at various temperatures are shown in Figs. 1 and 2, respectively. Daily egg production peaked on the first day after emergence, irrespective of the provenance of the parasitoid and temperature exposed.

Figures 3 and 4 show the effect of temperature on adult longevity and the number of

Fig. 3. Number of eggs laid throughout the life-time (—) and adult longevity (.....) for *T. chilonis* females, which were developed in *E. kuehniella* eggs, at various temperatures. Vertical lines indicate standard errors.

Fig. 4. Number of eggs laid throughout the life-time (—) and adult longevity (.....) for *T. chilonis* females, which were developed in *P. xylostella* eggs, at various temperatures. Vertical lines indicate standard errors.

Table 1. Net reproductive rate (R_0), mean length of a generation (T) and intrinsic rate of natural increase (r_m) of *T. chilonis* developed in *P. xylostella* eggs (TC-DBM)

Temperature (°C)	R_0	T	r_m
20	10.50	14.60	0.163
24	21.53	10.10	0.315
28	3.49	8.19	0.154
32	0.11	6.00	— ^a

^a Not calculated because R_0 was less than 1.

eggs laid by a female throughout her lifetime. For both TC-MFM (Fig. 3) and TC-DBM (Fig. 4), adult longevity tended to be longer at lower temperatures, and the total number of eggs laid by a female was maximal at 24°C. The number of eggs deposited by the TC-MFM females was evidently larger, however, than that by the TC-DBM females, despite a slight difference in adult longevity between the two (see next section).

The values for net reproduction rate (R_0), mean length of a generation (T) and intrinsic rate of natural increase (r_m) calculated for TC-DBM are given in Table 1. The values of T became larger as the temperature decreased, while those of R_0 and r_m were largest at 24°C. It should be noted that the value of R_0 was less than 1 at 32°C.

2. Effects of host species on reproductive properties of TC females

The weight of the DBM egg (= 0.02 mg) was significantly less than that of the MFM egg (= 0.03 mg) ($p \leq 0.01$, t -test). Table 2 shows the effects of host species on female size and reproductive properties of the TC females. The mean length of the hind tibia of TC-MFM was significantly longer than that of TC-DBM ($p < 0.05$, t -test). The numbers of matured ovarian eggs at emergence and eggs laid on the first day after emergence were also significantly larger for TC-MFM than those for TC-DBM ($p < 0.05$, t -test), but there were no significant differences in the number of eggs laid throughout the lifetime and adult

Table 2. Effects of host species on female size and reproductive properties of *T. chilonis* reared at 24°C under 16L-8D

Host species	Hind tibia length (mm) (mean $\pm$ SE)	No. of matured ovarian eggs at emergence (mean $\pm$ SE)	No. of eggs laid in a lifetime (mean $\pm$ SE)	No. of eggs laid on the first day after emergence (mean $\pm$ SE)	Adult longevity (mean $\pm$ SE)
MFM	0.216 $\pm$ 0.004 a (16)	32.6 $\pm$ 1.3 a (16)	62.5 $\pm$ 7.9 a (11)	31.3 $\pm$ 2.7 a (11)	5.0 $\pm$ 1.0 a (11)
DBM	0.172 $\pm$ 0.005 b (13)	14.9 $\pm$ 1.1 b (13)	43.6 $\pm$ 5.1 a (11)	21.4 $\pm$ 1.7 b (11)	6.0 $\pm$ 0.9 a (11)

MFM: *E. kuehniella*, DBM: *P. xylostella*.

Numerals in parenthesis refer to the number of females examined.

Means in a column with the same letter are not significantly different at the 5% level (*t*-test).

longevity between TC-DBM and TC-MFM ($p > 0.05$, *t*-test).

DISCUSSION

LEWONTIN (1965) devised a simple method for evaluating the relative importance to *r* of variations in total natality, the shape of the $l_x m_x$ function and the proportion of life-span concerned with laying eggs or giving birth. He indicated that a change in development time always had a greater effect on *r* than a similar change in fecundity. Also, in regard to the $l_x m_x$ curve, when the age at first reproduction and last reproduction remain constant and peak reproduction changes, he showed that the earlier peak of reproduction results in an increase of the *r* value. Thus, pro-ovigenic species, where the ovaries are already developed at the time of emergence as in TC (Fig. 1) may be more *r*-selected than synovigenic species because the average age at reproduction in pro-ovigenic species is earlier.

SALT (1940) and BIGLER et al. (1987) stated that the adult size of *Trichogramma* species is strongly influenced by volume of the host egg. Fitness components such as fecundity, longevity, rate of search and flight ability of parasitoid wasps generally decline as their body size decreases (SALT, 1940; BIEVER, 1972; MARSTON and ERTLE, 1973; STINNER et al., 1974; CHARNOV and SKINNER, 1984; WAAGE and NG, 1984; KAZMER and LUCK, 1991). Since the weight of the DBM egg was two thirds smaller than that of the MFM egg, the size of TC-DBM was smaller than that of TC-MFM; hence, the number of matured eggs in the ovaries at emergence of TC-DBM was smaller than of TC-MFM (Table 2). Consequently, the number of eggs laid on the first day after emergence was significantly smaller in TC-DBM than in TC-MFM, although the potential ability of TC-DBM and TC-MFM was almost equivalent in relation to the number of eggs laid by a female throughout the lifetime and adult longevity (Table 2). In fact, longevity of TC females in the field should be shorter than those in the laboratory, because environmental conditions in the laboratory may be generally considered to be better for insects; for example, longevity of adults of *Pieris rapae crucivora* (OSADA and ITÔ, 1974; KOBAYASHI and TAKANO, 1978; SUZUKI, 1978) and DBM (SARNTHOY et al., 1989; SIVAPRAGASAM et al., 1988), in the field is shorter than that in the laboratory. The reproductive capacity of TC within a few days after emergence, therefore, would be more important than that throughout the lifetime to evaluate the reproduction of the parasitoid in the field. As an important component of fitness, the oviposition rate of TC-DBM within a few days just after emergence in TC-DBM is considered to be less than that in TC-MFM.

The number of eggs laid by TC females was smaller at higher temperatures (Figs. 1 and 2). It is likely that, because at higher temperatures longevity of females was shorter, there was not enough time for females to search for and oviposit in hosts. In particular, the number of eggs laid by TC-DBM was notably smaller at higher temperatures. This might be related to the smaller size (Table 2) and a markedly shorter longevity at higher temperatures (Figs. 1 and 2) in TC-DBM females when compared with TC-MFM females. The fecundity of TC-DBM at high temperatures during summer, or in greenhouses, might be small.

Next, we compared the population growth of DBM with that of TC-DBM. The life-history parameters of DBM in Japan have been reported by several authors (UMEYA and YAMADA, 1973; NAKAGOME and KATO, 1975; YAMADA and KAWASAKI, 1983; NEMOTO et al., 1984; SARNTHOY et al., 1989). Although the calculation method of the increase rate (r_m) differed among these authors, SARNTHOY et al. (1989) gave the highest r_m values. According to SARNTHOY et al. (1989), the r_m of DBM increased with temperature from 0.116 at 17.6°C to 0.256 at 28.9°C. Unfortunately, a correlation between the r_m values by SARNTHOY et al. (1989) of DBM and the r_m values of TC-DBM in the present study (Table 1) was difficult to determine because temperatures and photoperiods in these experiments differed. It is obvious, however, that the r_m of TC-DBM is larger than that of DBM at lower temperatures (below 24°C), and smaller at higher temperatures. TC-DBM is not considered to have a reproductive potential much higher than that of DBM, although TC would have the character of the r -selected parasitoid. In the field, the population density of TC is high during spring and early summer, but decreases rapidly in mid-summer (IGA, 1985). A possible reason for this decrease may be the low fecundity and r value of the TC-DBM females at higher temperatures. The low rate of adult emergence from DBM eggs at higher temperatures (MIURA and KOBAYASHI, 1993) and the decrease of DBM egg density (IGA, 1985) may also affect seasonal fluctuation.

Here, we consider the release method of TC for control of DBM. The aim of inundative release is primarily to control pests by the action of individuals released, not by their progeny. On the other hand, control using inoculative release comes from cumulative action of progeny produced over several generations following the release of a relatively small number of natural enemies as compared to the host. With regard to the reproductive properties of TC, the inundative method would be better than inoculative release for control of DBM using TC-MFM in warmer temperate zones for the following three reasons. First, parasitoid females developed in DBM eggs in the field should show a lower fecundity than those developed in MFM eggs in the laboratory. Second, TC-DBM did not have a markedly higher reproductive capacity. Third, under conditions of high temperature, a smaller percentage of emergence of the progeny (MIURA and KOBAYASHI, 1993), associated with low fecundity would be expected. However, in cooler zones the inoculative method can also be expected because the r of TC-DBM was larger than that of DBM at lower temperatures.

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