BIOLOGICAL CONTROL 3, 135–139 (1993)

Host Acceptance and Parasitoid Size as Predictors of Parasitoid Quality for Mass-Reared *Trichogramma minutum*

R. S. BOURCHIER,[1] S. M. SMITH, AND S. J. SONG

Faculty of Forestry, University of Toronto, Toronto, Ontario, Canada, M5S 3B3

Received September 24, 1992; accepted April 26, 1993

Parasitoid size and host acceptance were examined as quality control parameters for *Trichogramma minutum* Riley (TM) reared on a factitious host, the Mediterranean flour moth, *Ephestia kuehniella* Zell. (MFM), and on their target host, the spruce budworm, *Choristoneura fumiferana* (Clemens) (SBW). Wing length was a better predictor of potential fecundity of TM reared on SBW and MFM than hind-tibial length. SBW-reared TM were larger and had higher potential fecundities than MFM-reared TM. When given MFM eggs, SBW-reared TM did not realize their potential fecundity whereas MFM-reared parasitoids realized their potential fecundity as would have been predicted based on their body size. When given SBW eggs, both SBW-reared TM and MFM-reared TM realized their potential fecundity as predicted by their body size. When attacking SBW eggs in the laboratory, parasitoid size is a good predictor of realized fecundity for both MFM-reared and SBW-reared TM. The mean proportion of time spent on the egg mass during a 2-h host acceptance assay was a good predictor of the mean-realized lifetime fecundity of TM attacking SBW eggs. Lab assays that predict field performance of mass-released parasitoids will allow for the modification of release rates in response to measured reductions in parasitoid quality in the rearing facility. Increasing release rates may, in many cases, be an adequate and probably easier adjustment than modifying mass-production techniques to account for fluctuations in quality of rearing stock.
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KEY WORDS: Insecta; *Trichogramma minutum; Choristoneura fumiferana; Ephestia kuehniella; Sitotroga cerealella;* biological control; inundative release; release rates.

INTRODUCTION

The spruce budworm, *Choristoneura fumiferana* (Clemens) (SBW) is a serious pest of spruce-fir forests in northeastern North America (Carrow, 1990). Recently it was demonstrated that inundative releases of the egg parasitoid *Trichogramma minutum* Riley (TM) resulted in egg parasitism rates of up to 79% in treatment plots and reductions of larval populations of the spruce budworm by 80% in the following year (Smith *et al.*, 1990). These encouraging results have led to increased interest in the mass production of *T. minutum* for larger-scale use against the budworm.

As with most mass-rearing programs for parasitoids, the use of a factitious host for production of the parasitoid is desirable because of potential reductions in cost associated with the mechanization of production (Vinson *et al.*, 1988). An immediate concern associated with the use of factitious hosts is the quality of parasitoids being produced, relative to the quality of parasitoids that emerge from the target host (Bigler, 1989; Noldus, 1989). A formal definition of parasitoid quality is the subject of active discussion in the scientific community (Bigler, 1991); however, "the aim of quality control should be to determine whether a natural enemy is still in a condition to properly control the pest" (van Lenteren, 1991). Attributes that are important for mass-reared parasitoids include: (1) can they find the target host (Yu *et al.*, 1984), (2) do they accept the target host (Kaiser *et al.*, 1989; Hassan and Gu, 1991), and (3) do they develop in the target host (Bergeijk *et al.*, 1989), as successfully as parasitoids that are reared from the target host. Changes in these parasitoid attributes will affect the realized fecundity of mass-reared parasitoids and ultimately their ability to control pests.

In this study, we examined the quality of *T. minutum* produced on a factitious host, the Mediterranean flour moth *Ephestia kuehniella* Zell. (MFM), for use against the spruce budworm. Our objective was to assess parasitoid size and one measure of host acceptance as quality-control parameters for mass-reared TM attacking the spruce budworm. These parasitoid attributes are only two of the many quality characteristics that have been examined for other mass-reared parasitoids (see Bigler, 1989; Noldus, 1989 for review of factors influencing parasitoid quality) and are viewed as a first step for the

[1] Current address: Forest Pest Management Institute, Box 490, Sault Ste Marie, Ont. CANADA, P6A 5M7.

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development of a comprehensive quality index for *T. minutum*. For parasitoid size, we examined whether body measurements could be used to predict the potential fecundity of TM; we then determined whether parasitoids could realize their predicted potential fecundity on either the spruce budworm or the Mediterranean flour moth. For host acceptance we relate the time spent on a spruce budworm egg mass during a 2-h behavioral assay to realized fecundity on spruce budworm eggs.

MATERIALS AND METHODS

Parasitoid Colonies

Trichogramma minutum were reared at 25°C with a 16L:8D photoperiod on either spruce budworm or Mediterranean flour moth eggs. Parasitoids were collected from SBW eggs near Vermilion Bay, Ontario (UTM: VF7222) in July 1991. Parasitoids reared on MFM eggs were from a colony that had been collected near Vermilion Bay in July 1990 from SBW, and reared in the laboratory for more than 30 generations on MFM.

Relationship between Body Size and Potential Fecundity

Body size of female *T. minutum* was assessed by measuring the length of the hind tibia and the length of the forewing under a Wild M3 stereomicroscope equipped with a Wild MMS 235 Digital-Length Measuring Set. For measurement, the hind leg of the parasitoid was removed and placed flat on a microscope slide; tibiae were measured from the point of attachment to the femur to the top of the first tarsal segment. To ensure that wing measurements were consistent, wings were laid flat on a microscope slide and the principal vein was aligned to a cross-hair present in the ocular micrometer. Wings were measured from point of articulation with the body to the distal edge (excluding the hairs) of the end of the wing.

Egg complement as an index of potential fecundity of adult parasitoids was determined by dissection of female wasps. Females were placed in a saline solution, with dish soap added to break the surface tension, and their abdomens removed. The ovaries were then separated from the abdomen and split to free oocytes for counting.

Relationship between Body Size and Realized Fecundity

We define realized fecundity to be the number of progeny produced by a female wasp that survive to emerge as adults. Realized fecundity was estimated by placing a single female parasitoid in a 60 × 15-mm glass vial with either a SBW egg mass or approximately 80 irradiated MFM eggs. Host eggs were prepared by gluing either the MFM eggs or a fir needle with a single SBW egg mass (containing 15–20 eggs) on it to a 20 × 10-mm paper card that was inserted into the vial. Sixteen days after exposure to female wasps the number of emerged adult parasitoids was counted. Thirty females were tested for each of the following four treatments: (1) parasitoids reared from MFM eggs and given MFM eggs to attack; (2) parasitoids reared from MFM eggs and given SBW eggs to attack; (3) parasitoids reared from SBW eggs and given MFM eggs to attack; and (4) parasitoids reared from SBW eggs and given SBW eggs to attack.

Host Acceptance of SBW by Trichogramma minutum Produced on MFM

Acceptance of SBW eggs by MFM-reared TM was determined using a behavioral assay. Newly emerged female wasps were placed individually in 60 × 15-mm glass vials with a single budworm egg mass. The behavior of each female was checked every 5 min during a 2-h observation period. Females were scored as (1) on the egg mass or (2) off the egg mass. The proportion of time spent on the egg mass was calculated by dividing the total number of times the female was observed on the egg mass during the 2-h assay by 25 (the maximum number times that a female could be observed on the eggs in 2 h). After completion of the observation period, females were left in the vials until death to allow measurement of their realized fecundity. Each time the assay was performed using 25–30 insects, a single data point relating the mean number of time intervals spent on the egg mass with mean realized fecundity (based on the mean number of adult wasps emerging in each vial) was obtained.

Statistical Analysis

Mean fecundities and body size measurements were compared using analysis of variance in the MGLH module of Systat (Wilkinson, 1990). Relationships between fecundities, body size, and host acceptance measurements were assessed using linear regressions. Data on proportion time spent on the egg mass was arcsin ($\sqrt{}$) transformed to ensure a normal distribution (Sokal and Rohlf, 1981).

RESULTS

Relationship between Body Size and Potential Fecundity

There were significant positive relationships between potential fecundity, as determined by the number of oocytes, and tibial and wing lengths of female *T. minutum* reared on both hosts (Table 1). Using the criterion of explained variance, wing length was a better predictor of potential fecundity than tibial length, for TM produced from both MFM and SBW (Table 1).

T. minutum reared on the spruce budworm were significantly larger and had a higher potential fecundity

TABLE 1

Simple Linear Regressions for Potential Fecundity (as Measured by the Number of Eggs in the Ovaries) on Tibial and Wing Length of Female *T. minutum* Produced on MFM and SBW

Rearing host	*N*	Model log (# eggs in the ovaries) =	Equation	*P*-value	R^2
MFM	28	log (wing length)	$Y = 8.4 + 6.9X$	<0.001	0.64
MFM	28	log (tibia length)	$Y = 7.4 + 2.1X$	0.002	0.30
SBW	28	log (wing length)	$Y = 5.6 + 3.1X$	<0.001	0.73
SBW	28	log (tibia length)	$Y = 7.4 + 2.0X$	<0.001	0.58

than parasitoids produced from the Mediterranean flour moth (Fig. 1a). Wing lengths (mean ± SE) were 0.565 mm ± 0.011 and 0.469 mm ± 0.003 and potential fecundities (mean ± SE) were 48.5 ± 3.2 eggs and 26.9 ± 1.5 eggs for TM reared from the spruce budworm (n = 28) and the flour moth (n = 28), respectively.

Relationship between Body Size and Realized Fecundity

When presented with the eggs of the factitious host MFM, SBW-reared parasitoids did not realize the potential fecundity as predicted by their body size, even though host eggs were available to attack (Fig. 1b; location of predicted fecundity regression line relative to circles). MFM-reared parasitoids realized their potential fecundity as predicted by their body size (Fig. 1b; mean (±SE) number of emerging wasps (actual) = 19.1 ± 1.4; mean (±SE) number of emerging wasps (predicted) = 22.0 ± 1.1; t test, means not significantly different, $P = 0.116$; location of predicted fecundity regression line relative to triangles).

When given SBW eggs, SBW-reared TM realized their potential fecundity as predicted by their body size (Fig. 1c, location of predicted fecundity regression line relative to circles). TM produced on the factitious host MFM, also realized their predicted fecundity (Fig. 1c, location of regression line relative to triangles); thus when attacking SBW eggs, parasitoid size is a good predictor of realized fecundity in vials for both MFM-reared and SBW-reared TM (Fig. 1c). TM reared on the flour moth were unable to take advantage of the larger available host and lay as many eggs as SBW-reared TM, even though the host was capable of supporting more parasitoid eggs (Fig. 1c, location of triangles relative to location of circles).

Host Acceptance of SBW by Trichogramma minutum Produced on MFM

The mean proportion of time spent on the egg mass during the 2-h host acceptance assay was a good predictor of the mean-realized fecundity of the parasitoids on SBW eggs in vials (Fig. 2). The estimate of mean-realized fecundity includes both laying and nonlaying TM.

DISCUSSION

Southard *et al.* (1982) reported a significant reduction in the size of the parasitoids produced when SBW-reared TM were offered Angoumois grain moth eggs (*Sitotroga cerealella* (Olivier)), but this size reduction could be reversed after one generation on a larger host. Our results suggest that when TM are collected from SBW eggs in the field for use in a mass-rearing program, the realized fecundity of the parasitoids is reduced in the first generation attacking the smaller factitious host

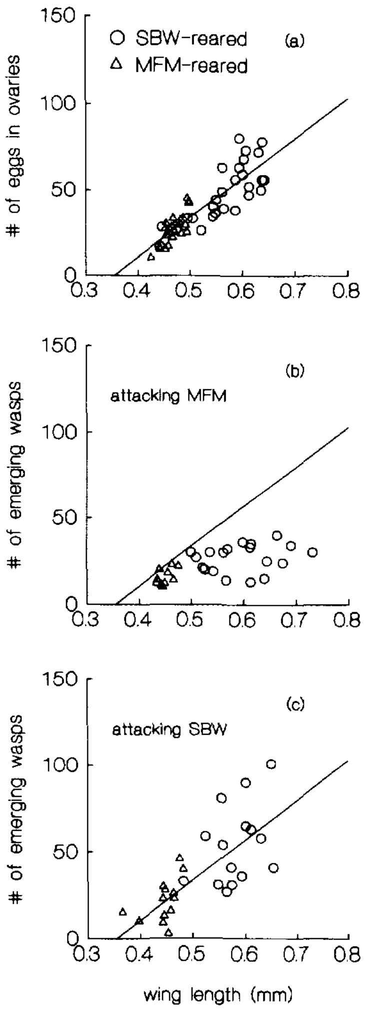

FIG. 1. (a) Relationship between potential fecundity and wing length for TM reared on SBW and MFM. $Y = -82.1 + 231.1\,X$; $R^2 = 0.77$; $P < 0.001$, $N = 56$. (b) Realized fecundity and wing length for TM reared on SBW and MFM attacking MFM eggs; regression line is for predicted potential fecundity from Fig 1a. (c) Realized fecundity and wing length for TM reared on SBW and MFM attacking SBW eggs; regression line is for predicted potential fecundity from (a).

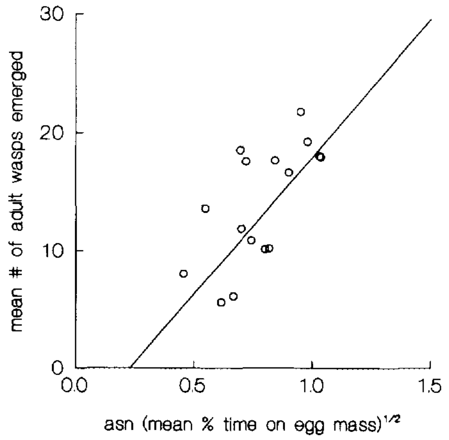

FIG. 2. Relationship between the mean lifetime fecundity (number of parasitoids that emerged from a SBW egg mass) and the proportion of time spent on the egg mass during the host acceptance assay which ran for 2 h. Each point is the mean response of a group of TM and is weighted by 1/(variance of adult emergence). $Y = -5.379 + 23.31\ X$; $R^2 = 0.68$, $P = 0.001$, $N = 16$.

(MFM) in the lab (Fig. 1b); regardless of the parasitoid's size, the maximum fecundity of SBW-reared TM when attacking MFM eggs is far below their predicted potential. This reduction in fecundity that occurs when transferring *T. minutum* to a factitious host is maintained while the parasitoid is on the factitious host, as demonstrated by the inability of MFM-reared TM to take advantage of larger hosts when available and increase their realized fecundity (MFM-reared TM attacking SBW eggs in Fig. 1c); this situation is analogous to what MFM-reared TM would encounter when they are released into the field to attack budworm. The ability of *T. minutum* to increase body size in subsequent generations in response to a larger host will only be important if release strategies are employed that allow time for a second generation of TM on the budworm (Smith and You, 1990).

Parasitoid size most likely influences important behavioral traits such as host acceptance or host searching ability; this interaction may diminish our capability to predict realized fecundity by only measuring parasitoid size. Abdomen size was found to be an indicator of fecundity for *T. minutum* but only for individuals emerging from and attacking the same host species; females that had the same size abdomens emerging from differing hosts had significantly different realized fecundities (Marston and Ertle, 1973). Similarly, in our switching experiment, the usefulness of parasitoid size as a predictor of realized fecundity of *T. minutum* was dependent on both the source and the target host (Fig. 1).

The host acceptance assay was designed to measure the response of parasitoids reared on the factitious host (MFM), when attacking the target host (SBW). The realized fecundity estimate resulting from our host acceptance assay is a summation of performance by MFM-reared parasitoids, incorporating a physiological component associated with parasitoid size and host suitability, and a behavioral component associated with host acceptance. Offspring that are counted for the fecundity estimate are parasitoids that found the host egg suitable for development and survived to emerge. In addition, the estimate is calculated using both individuals that attacked and accepted the host eggs and individuals that chose not to attack the host eggs. As the host rejection rate rises the number of zero values contributing to the mean rises and the overall mean realized fecundity declines. The measurement of a single important parasitoid attribute (host acceptance) provides an estimate of the product of a series of parasitoid behaviors, realized fecundity.

A critical factor for the development of any pest-control strategy is the ability to predict the efficacy of a given treatment in the field. Larger *T. pretiosum* reared from *Heliothis zea* were "on average" 1.96 times more effective at parasitizing *Trichoplusia ni* eggs than small female parasitoids reared from *S. cerealella* (Kazmer and Luck, 1991). There was however more variation in the performance of the large wasps than in the performance of the small wasps, which would not have been predicted from simple measurements of female size (Kazmer and Luck, 1991). Using a predictability criterion implies a possible tradeoff between highest performance (i.e., realized fecundity) and predictability in order to achieve standardized release rates. In order to determine release rates of parasitoid lines with high realized fecundities, but that also may have higher variation in performance, release rates should be based on the minimum level performance of the released parasitoid strain as determined in laboratory assays, rather than the mean performance. This ensures a minimum level of efficiency that compensates for possible variations in performance not predicted by laboratory assays of quality.

Quality control tests must be simple in order to be easily incorporated into a mass-rearing program. Measurement of variables such as realized fecundity in vials, parasitoid size, and host acceptance assays such as the one described in this paper, can be easily incorporated into a mass-rearing program. The response of a group of insects is measured simultaneously and the tests do not require automated recording equipment. The use of these measurements alone or in combination with other techniques (walking speed assays, Bigler *et al.*, 1988; host acceptance tests, Bergeijk *et al.*, 1989) will allow for the modification of release rates in response to measured reductions in parasitoid quality in the rearing facility. Increasing release rates may be easier than modifying mass-production techniques to account for fluctuations in quality of rearing stock.

ACKNOWLEDGMENTS

We thank E. Forsse, V. Nealis, P. Newton, J. Roland, and D. Wallace for their comments on the manuscript and J. Farnsworth and J. Proud for technical assistance. This research was supported by a NSERC operating grant to S.M.S. and a NSERC postdoctoral fellowship to R.S.B.

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