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Isoenzyme patterns and biology of *Trichogramma minutum* as influenced by rearing temperature and host

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Abstract

Females of *Trichogramma minutum* Riley reared at temperatures below 17 °C had lower percent emergence, lower fecundity on the first day of emergence, and a reduced rate of population increase (r_i) than those reared at temperatures above 17 °C. Parasitoids were more fecund and lived longer when raised on *Choristoneura fumiferana* (Clemens) compared to *Ephestia kuehniella* (Zeller). The parasitoids performed better when parasitizing the same species of host on which they had been reared. Isoenzyme patterns were not affected when *T. minutum* was reared at 13 or 27 °C but additional bands appeared when parasitoids were reared on *Sitotroga cerealella* (Olivier) compared to *C. fumiferana*. No correlation could be made between biological and biochemical changes in *T. minutum*. Isoenzymes have the potential to assess the quality of parasitoids during mass production.

Introduction

In recent years, electrophoretic studies on a number of insect species have been conducted, however, relatively few have dealt with members of the Trichogrammatidae. Because of the minute size of these chalcid wasps, where such studies have been carried out, the main role of isoenzyme analysis has been to aid in taxonomic identification (Jardak *et al.*, 1979; Pintureau & Babault, 1981; Klausnitzer *et al.*, 1983). Little or no attempt has been made to measure genetic changes when these parasitoids have been subjected to long periods of mass production.

Two important factors in mass-rearing *Trichogramma* are temperature and species of host. The effect of rearing temperature on species of *Trichogramma* has been discussed by Schread & Garman (1934), Lund (1938), Quednau (1957), and Stinner *et al.* (1974a, 1974b). While not host specific, members of the Trichogrammatidae do show certain preferences in the species of host egg they attack (Quednau, 1956; Taylor & Stern, 1971; Curl & Burbutis, 1978; Benoit & Voegele, 1979). In addition, different host species will influence the bionomics of *Trichogramma* so that the biology or quality of the parasitoids will vary depending upon the species of host in which they have been reared. Under conditions of mass production, *Trichogramma* species are reared in eggs of stored product pests such as the Angoumois grain moth (AGM), *Sitotroga cerealella* (Olivier), or the Mediterranean flour moth (MFM), *Ephestia kuehniella* (Zeller). Their size, longevity, and fecundity, however, will usually be superior in host eggs of larger size (Marston & Ertle, 1973; Lewis *et al.*, 1976; Brower, 1983).

In view of the success in several countries with inundative releases of *Trichogramma* in the field and the need to develop alternatives to chemical control in the forest ecosystem, a project was initiated in Ontario, Canada during 1982 to assess the potential of using releases of *Trichogramma minutum* for control of the spruce budworm (SBW), *Choristoneura fumiferana*. In order to meet the demand of these inundative releases, mass-rearing of *T. minutum* under controlled laboratory conditions

is required. This necessitates guidelines or standards to measure the quality of the parasitoids and ensure their continued effectiveness under field conditions. The aim of the present study, therefore, was to determine the biochemical criteria associated with effectiveness of *T. minutum* reared at different temperatures and on different hosts. The main objective was to correlate observed biochemical changes with biological differences and thereby, provide a suitable means of measuring quality of *T. minutum* during mass production.

Materials

Biological studies. Laboratory experiments were conducted to compare the fecundity, longevity, percent emergence, sex ratio, and proportion of nonfecund females of *T. minutum* reared at different temperatures (i.e. 13, 15, 17, 20, and 27 °C) and on different hosts (i.e. SBW and MFM or AGM). These values were summarized in rate of population increase values (r_i). It should be noted that although the term fecundity is used, only the observed number of parasitized host eggs was recorded and not the inherent fecundity. In addition, r_i was computed using the method of Birch (1948) for calculating r_m where $\sum_{x=0} e^{-r_i x} l_x m_{xr} - 1$ and x = the daily age interval, 1_x = the fraction of the initial sample still alive, and m_{xr} = the fertility rate per female. Because r_m is defined under optimal conditions, however, and some of these conditions were not met in the present study (i.e. food supply to the adult parasitoids), we have defined the rate of population increase here as r_i.

Irrespective of the factor under study, vials containing the parasitized eggs were removed after the conditions of the experiment had been met and placed in the dark at 17 °C one day before the estimated time of emergence. This synchronized the emergence of a large number of parasitoids of similar age. After 4 days at this temperature, they were then transferred to an incubator and maintained at 25 °C and LD 16:8 until the parasitoids emerged. Unless stated otherwise, all parasitoids were fed with honey and maintained in ventillated vials at 25 °C, 75% rh and LD 16:8 photoperiod after emergence.

Electrophoretic studies. Isoenzyme patterns were compared for populations of *T. minutum* which had been reared at either 13 or 27 °C and on either AGM or SBW. The rearing conditions were the same as those described previously except no honey was supplied to the adult parasitoids. Parasitoids were reared under each condition in ventilated, 2 by 9 cm glass vials. Upon emergence, all parasitoids, irrespective of the factor under study, were maintained at 25 °C, 75% rh, and LD 16:8 for 24 h. These were then frozen, 'en masse' in glass vials (more than 200 *T. minutum*/vial), at −20 °C and stored for 2 to 3 months.

Specimens of *T. minutum* were prepared for electrophoresis by weighing parasitoids from sample vials held at −20 °C. Samples of a given weight ('x' mg) were placed in 400 µl, disposable centrifuge tubes and suspended in an ice bath. The samples were then homogenized with a Caframo Stirrer (R2R1) for 20 seconds in 10'x' µl of cold sample buffer (0.06 Tris/20% glycerol; adjusted to pH 6.8) and centrifuged at 15000 to 20000 rpm for 10 min. To absorb the supernatant, 'x' number of Whatman filter papers (No. 1), cut to 3 by 10 mm, were added directly to each tube. The tubes were then frozen and stored at −20 °C until electrophoresis the following morning. Based upon a protein assay using bovine chrystallized albumine as a standard, the concentration of protein in the samples was 5.65 ± 0.15 mg/ml (Bio-Rad protein assay kit, Bio-Rad Laboratories).

Electrophoresis was performed within 24 h of grinding. The most successful system for analyzing zymograms of *T. minutum* was horizontal starch gel. For electrophoresis, filter papers were inserted into 12.4% gels (4.1% electrostarch; 8.3% Connaught Laboratories starch) and subjected to 200 volts for ca. 2.5 h in a refrigerator set at 4 °C. When the tracking dye had migrated to the end of the gel, they were removed and the front notched. The gels were then sliced into six parts (each 1.5 mm thick), stained, and fixed (Shaw & Prasad, 1970; Siciliano & Shaw, 1976). The mobility of the bands was defined as the distance travelled from the origin divided by the distance travelled by the front (R_f).

Methods and results

Rearing temperature. Parasitoids were exposed to

cards with fresh eggs of MFM for 24 h at 25 °C. These cards were then placed in 2 by 9 cm vials, inside waterproof, glass jars and submerged under water in aquaria at four constant temperatures: 15, 17, 20, and 25 °C (± SE = 0.5 °C). Water baths were used in place of standard incubators because the latter were unavailable. In a preliminary experiment, the percent emergence, longevity, fecundity, and sex ratio of parasitoids reared in these baths at 25 °C did not differ significantly from those maintained in an incubator at the same temperature.

Estimates of emergence and sex ratio were made using samples (N greater than 100) of parasitized eggs and adults collected from each vial. To measure longevity, a total of twenty parasitoids were divided equally among four 2-dram vials and provided with a drop of 50% honey solution. These vials were examined daily until all the parasitoids had died. Fecundity was measured by isolating thirteen, mated females from each temperature into separate 0.5-dram vials and providing each with a 50% honey solution. The greatest number of eggs are laid by *T. minutum* during the first few days of emergence (Yu, 1981; Houseweart *et al.*, 1983). A minimum of 100 fresh MFM eggs attached to cards with water, therefore, were supplied to each female on day 1 of emergence, 50 eggs on day 2, and 20 eggs on each day thereafter. The cards were notched to allow easy access by the female to all areas within the vial. After 24 h, the cards were removed and stored in the dark at 25 °C until the parasitized eggs could be counted (ca. 5 to 7 days). The cards were then placed into individual vials and the emergence and sex ratio of the progeny determined.

As the rearing temperature of *T. minutum* increased, the percent emergence, longevity of males, and fecundity on the first day of emergence were increased (Table 1). The proportion of females emerging, however, decreased with increasing temperatures while the longevity of females and total fecundity were unaffected by rearing temperature. Males were always shorter-lived than females. The average longevity for egg-laying females was approximately 20 days; for females not laying eggs, approximately 8 days; and for males, approximately 3 days. Both the total and the daily number of eggs produced by each female were not affected by those temperatures studied. Each female, on average, laid a total of 110.8 eggs in her lifetime with 5.8 eggs laid each day. The number of eggs laid on the first day after emergence, however, was significantly less at 15 and 17 °C than at either 20 or 25 °C.

On average, female parasitoids reared at different temperatures produced progeny in a ratio of 1:1 male:female. In all cases, however, more females were produced than males when the female parent was younger (Fig. 1). Eggs laid on day one of emer-

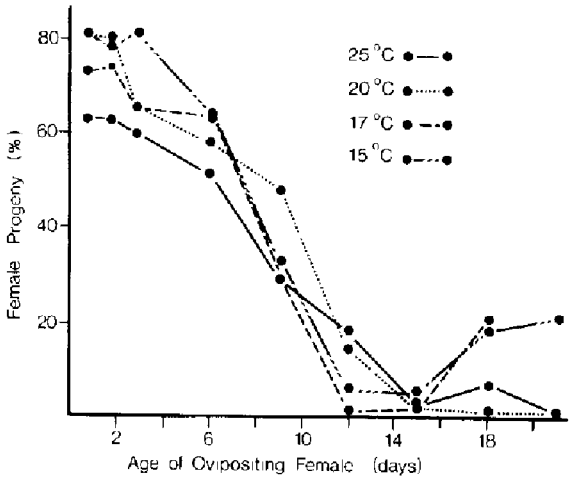

Fig. 1. Percent progeny emerging from eggs laid by *T. minutum* supplied with honey and reared from *E. kuehniella* at 15, 17, 20, and 25 °C, 75% r.h., and LD 16:8 photoperiod.

Table 1. Per cent emergence, sex ratio, adult longevity, and fecundity of *T. minutum* supplied with honey and reared from *E. kuehniella* at 15, 17, 20, and 25 °C, 75% r.h., and LD 16:8.

Temperature (°C)	Emergence (%)	♀♀ %	Longevity (days)			Fecundity (No./♀)	
			Non-egglaying ♀♀	Egglaying ♀♀	♂♂	First day	Total
15	73.7a*	69.3a	5.7a	16.9a	1.9a	8.8a	110.0a
17	77.1ab	54.5b	10.6a	21.1a	3.0b	11.9ab	95.2a
20	80.4b	38.4c	5.8a	19.0a	4.4b	25.4bc	92.2a
25	82.5b	36.4c	9.7a	21.0a	3.8b	30.8bc	128.0a

* Means followed by same letter within each column not significantly different at $p \leq 0.05$ (Duncan's (1955) multiple range test).

Table 2. Population growth statistics for *T. minutum* supplied with honey and reared from *E. kuehniella* at 15, 17, 20, and 25 °C, 75% r.h., and LD 16:8 photoperiod.

Growth*	Rearing temperature (°C)			
	15	17	20	25
GRR (eggs/♀)	110.0	95.2	92.2	128.0
R_0 (♀♀/♀)	41.4	38.1	36.0	36.9
Developmental time (days)**	25.0	20.0	15.0	8.0
r_i (♀♀/♀/day)	0.07	0.13	0.18	0.32
T (days)	28.8	24.8	18.4	11.7
λ (♀♀/♀/day)	1.07	1.14	1.20	1.37
Doubling time (days)	2.80	2.63	2.50	2.19

* GRR, gross reproduction rate; R_0, net reproduction rate; r_i, rate of population increase; T, generation time; λ, finite rate of increase.

** From Yu (1981).

gence were 80% female. By day 12, less than 10% were female. Day 6 appeared to be the critical day for this switch with rearing temperature having no effect. After day 6, the female may have depleted her supply of sperm because she had access to males only for 4 h immediately following emergence. A similar drop in the percentage of female progeny later in life was observed by Houseweart *et al.* (1983) and F. W. Quednau (pers. comm.) for *T. minutum* reared on SBW.

The influence of temperature is readily apparent from the life table (Table 2). As temperature decreased, the reproductive power of the parasitoid (GRR) was reduced from 128.0 eggs/♀ at 25 °C to 110.0 eggs/♀ at 15 °C. The number of females produced by each female (R_0), however, increased from 36.9 ♀♀/♀ at 25 °C to 41.4 ♀♀/♀ at 15 °C, thus offsetting the reduction in the GRR. Both developmental times and generation times (T) increased as rearing temperatures were reduced. This significant increase was reflected in the reduction of the rate of increase (r_i) from 0.32 ♀♀/♀/day at 25 °C to 0.07 ♀♀/♀/day at 15 °C.

No differences in isoenzyme patterns of acid phosphatase (ACPH), adenylate kinase (AK), α-glycerophosphate dehydrogenase (αGPDH), glucose-6-phosphate dehydrogenase (G6PDH), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), malic enzyme (ME), phosphoglucomutase (PGM), and 6-phosphogluconate dehydrogenase (6PGDH) were observed for *T. minutum* reared at 13 or 27 °C. The patterns obtained at each temperature were the same and will be dealt with in a subsequent paper as they relate to the general isoenzyme pattern for this species (Smith & Hubbes, 1986).

Rearing host. Fresh eggs of MFM and SBW were exposed for 24 h to female *T. minutum* and allowed to develop until emergence. Eggs of MFM and SBW were obtained from colonies reared in the laboratory as described by Yu (1981) and Grisdale (1970), respectively. To ensure freshness, eggs of both hosts were collected daily and stored in the dark at 4 °C for a maximum period of 3 weeks. MFM eggs were sterilized after collection by freezing at −20 °C for 48 h. This did not affect parasitism by *T. minutum* (Yu, 1981). In a preliminary experiment, susceptibility of SBW egg masses to *T. minutum* also was not affected by storage under these conditions. Within 4 h of emergence, a minimum of twenty females from each rearing host were provided daily with host eggs of either MFM or SBW. To exceed the maximum daily rate of fecundity, more than either 80 MFM eggs and 45 SBW eggs were given daily to each female. The method of exposure was similar to that described for the rearing temperature except no honey solution was supplied to the adult parasitoids. Three separate, no-choice experiments were conducted with parasitoids reared from: (1) MFM or SBW and provided with MFM; (2) SBW and provided with MFM or SBW; and (3) MFM and provided with MFM or SBW.

Parasitoids reared on the target host (SBW) were superior to those reared on the production host (MFM) (Table 3, Exp. 1). The total fecundity of females from eggs of SBW was three times higher and longevity twice as long as that for females from eggs of MFM. The remaining biological parameters were not significantly different at $p \leq 0.05$.

When *T. minutum* was reared on SBW and allowed to parasitize eggs of MFM and SBW, no significant differences ($p \leq 0.05$) were observed, with two exceptions (Table 3, Exp. 2). Females reared on SBW lived 2.6 days and produced 23.8 progeny/♀ when exposed to eggs of SBW while those exposed to eggs of MFM lived only 2.0 days and produced only 16.0 progeny/♀. Because more than one parasitoid egg can be laid in each egg of SBW, the most appropriate parameter for comparing fecundity between the two host species is the number of progeny. Although not significant, all other

Table 3. Summary of biological parameters for *T. minutum* not supplied with honey and reared from and provided with different host eggs.

Biological parameter	Host origin:Host provided					
	Exp. 1		Exp. 2		Exp. 3	
	MFM:MFM*	SBW:MFM	SBW:MFM	SBW:SBW	MFM:MFM	MFM:SBW
N (No. ♀♀)	25	25	20	20	20	20
First Day Fecundity (No. Parasi. Eggs/♀)	10.5	18.2**	21.0	26.5	13.0	6.4**
Total Fecundity (No. Parasi. Eggs/♀)	10.8	31.3**	30.6	35.1	14.5	7.4**
Longevity (days)	1.0	2.2**	2.0	2.6**	1.5	1.5
Emergence (%)	82.2	78.0	77.6	88.1	72.3	48.1**
Progeny (No./♀)	–	–	16.0	23.8**	1.6	0.9**
♀ Progeny (%)	63.6	53.2	55.0	67.4	15.9	67.7**
Non-Fecund ♀♀ (%)	36.0	32.0	16.0	0.0	33.0	20.0
Rate of Increase (r_i)	0.22	0.35	0.21	0.30	0.12	0.06

* MFM, *E. kuehniella*; SBW, *C. fumiferana*.

** Means within each experiment significantly different at ± 2.01 and 2.02, d.f. = 99 and 39, $P \leq 0.02$ (Duncan's (1955) multiple range test).

parameters were higher for parasitoids on eggs of SBW and this resulted in rates of increase on MFM and SBW of 0.21 and 0.30 ♀♀/♀/day, respectively. These data suggest that the rate of increase depends not only on the rearing host, but also the host to which the parasitoid is exposed.

Table 3 also shows the results for the reverse relationship; parasitoids reared on eggs of MFM and exposed to eggs of MFM and SBW (Exp. 3). In this case, fecundity on the first day of emergence, total fecundity, and the number of progeny/♀ were approximately twice as high for parasitoids exposed to MFM than SBW. In addition, emergence was 1.5 times greater from eggs of MFM than SBW. The proportion of female progeny, however, was 4.3 times higher from eggs of SBW than MFM while the remaining parameters were not significantly different ($p \leq 0.05$).

Unlike temperature, the host in which *T. minutum* was reared did affect the isoenzyme pattern (Fig. 2). When parasitoids were reared in eggs of AGM, additional bands appeared in the standard patterns for ACPH, AK, and PGM. Populations of *T. minutum* collected from different geographical areas were variable with some populations also showing additional bands for IDH, MDH, and ME when reared on this host. These additional bands for AGM could not be attributed directly to the host egg because unparasitized eggs of AGM were run simultaneously with the samples of *T. minutum* from each host. Although isoenzyme bands were also observed for eggs of AGM alone, these bands never coincided with those of the parasitoid reared in eggs of AGM.

Discussion

Lund (1938) found that longevity of adult *T. evanescens* Westw. was reduced when immatures were reared at 15 °C. He attributed this reduction to the increased activity of adult parasitoids reared at this temperature compared to higher temperatures. Similarly, adults reared at 15 °C in the present experiment, jumped and flew more readily when they were maintained at 25 °C than those reared at higher temperatures. The physiological explanation for this activity might be that those insects reared at 15 °C were more restless at a temperature that was higher than that at which they had been reared. In our study, only the longevity of male parasitoids was affected by this increased activity when immatures were reared at lower temperatures and this would have little consequence for parasitism in the field. In addition, we found that the emergence of adult parasitoids was reduced when reared at tem-

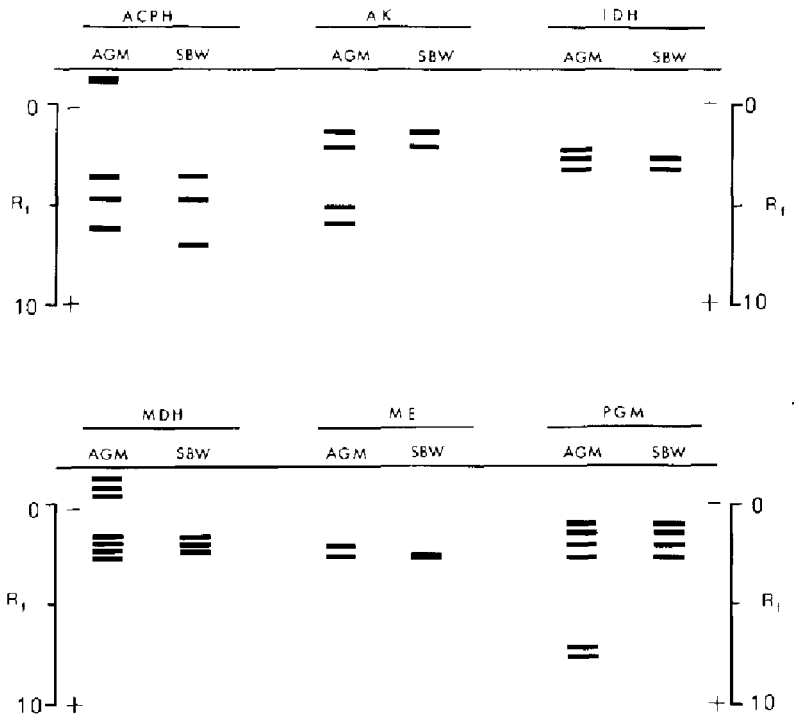

Fig. 2. Isoenzyme patterns for *T. minutum* not supplied with honey and reared from either *S. cerealella* (AGM) or *C. fumiferana* (SBW). ACPH, acid phosphatase; AK, adenylate kinase; IDH, isocitrate dehydrogenase; MDH, malate dehydrogenase; ME, malic enzyme; and PGM, phosphoglucomutase. Patterns of IDH, MDH, and ME only observed in some populations.

peratures below 17 °C. Stinner *et al.* (1974a) found an emergence rate of 93% for adult *T. pretiosum* Riley reared at 16.7 °C for 4 to 10 days. They did not observe, however, any detrimental effects on emergence for these parasitoids even when the immatures were maintained at 15 °C for 6 days. The low emergence seen in our work may have been due to the longer rearing time of 24 days at 15 °C. This might result in higher mortality in the immature stages.

When *T. pretiosum* was reared for varying lengths of time at reduced temperatures, Stinner *et al.* (1974a) reported no change in the sex ratio. Schread & Garman (1934), however, found an increase in the production of male parasitoids of both *T. minutum* and *T. pretiosum* when immature stages were exposed to 10 °C for 12 to 40 days. These authors also observed wing deformities directly proportional to the length of refrigeration and considered both the shift in the sex ratio and these deformities to be indicators of general weakening of the parasitoids. In the present experiment, wing deformities (i.e. wrinkled wings) were observed only at 15 °C and would be indicative of inferior parasitoids. The increased number of females observed at lower temperatures, however, improves the 'quality' of this species although the reason for this increase is not apparent. Crozier (1977) suggested that in haploid-diploid reproduction, characteristic of Hymenoptera, lower temperatures could promote fusion of nuclei and thus, increase the proportion of diploid offspring (females).

Stinner *et al.* (1974b) found no difference in total fecundity when *T. previosum* was reared from 4 to 10 days at 16.7 °C, however, they did not report daily fecundity. In the present study, although the production of eggs was compensated for later in life (i.e. the total fecundity being similar) fecundity on day one after emergence was reduced at lower rearing temperatures. Similar results, in terms of ovipositional rates for a number of *Trichogramma* species, were reported by Pak & Van Heiningen (1985). The reduction in the number of eggs laid on the first day of emergence that we observed as well

as the overall reduction in the rate of increase for female parasitoids when reared at lower temperatures will have a significant effect in the field. Birch (1948) stated that a population cannot be expected to persist and increase in numbers in environments where r_m for each generation has a value less than or equal to zero. Survival in the laboratory under the conditions of the present experiment was optimal and yet r_i values, although positive, were approaching zero as the temperature was reduced. In the field, these values would undoubtedly be reduced even further because of lower survival rates. If no food was obtained in the field, these parasitoids reared at lower temperatures would probably die before they could compensate for their initial loss in egg production and this would reduce their overall fecundity and thus, effectiveness.

The parameter of non-fecund females was included in our work because early experiments indicated that there could be a large number of female parasitoids not laying eggs. Frequently up to 50% of the females in a given experiment did not parasitize hosts even though they lived for several days and were given a fresh supply of host eggs each day. Yu (1981) also described this phenomenon but provided no explanation for it. Zalavskiy & Kvi (1982) stated that 'refusal' by part of the females to parasitize eggs had the greatest effect on total fecundity and that such females probably did not use any of their egg complement at emergence. They suggested that this phenomenon was probably based upon the behaviour of the females. Further work, examining the physiological and behavioural state of such females is required. Accounting for this individual variability in fecundity for *T. minutum* will significantly improve our understanding and prediction of parasitism and thus, efficiency with inundative releases.

In general, SBW is a better rearing host for *T. minutum* than MFM. In experiment one (Table 3), it is apparent that although slightly more female progeny are produced by females from MFM eggs, more eggs are parasitized and more progeny produced by females from SBW. Smith *et al.* (1986) and F. W. Quednau (pers. comm.) found a similar reduction in the number of parasitized eggs for female *T. minutum* reared from MFM compared to SBW. The rate of increase values (r_i) for parasitoids reared from SBW averaged 0.29 ♀♀/♀/day while those of parasitoids from MFM averaged 0.14 ♀♀/♀/day. This suggests that populations reared from SBW have higher growth rates than those from MFM and that different rearing hosts will influence the species value for r_i.

Our results also indicate that parasitoids tend to perform better on host eggs similar to those on which they were reared as reflected by their higher r_i values (Table 3, Exp. 2 and 3). This is in contrast with work reported by Houseweart *et al.* (1983). They found that although the daily production of progeny by a 'Maine strain' of *T. minutum* was higher for females using SBW than those using AGM, female parasitoids lived longer when they were provided with eggs of AGM than SBW. Vinson (1976) has pointed out that parasitoids with wide host ranges, such as *Trichogramma*, will often prefer the host species from which they have emerged. Work by Taylor & Stern (1971) with *T. semifumatum* (Perkins) and by Navarajan *et al.* (1981) with *T. chilonis* Ishii and *T. exiguum* Pinto & Platner, support our findings.

As a monitoring program for quality control during mass production, Bush & Neck (1976) proposed the use of electrophoresis. They suggested that this approach could be used to measure genetic changes in proteins which would be of ecological importance to the successful implementation of introduced insects (i.e. flight metabolism, oogenesis, etc.). Oliveira *et al.* (1984) reported a modification in esterases when *T. evanescens* and *T. nagarkattii* Voegele were maintained for one month at 3 °C. The results in the present study show no enzyme response to changes in rearing temperature between 13 and 27 °C. This may be due to the comparatively higher minimum temperature used (13 °C) or to a difference in the metabolic response to temperature for *T. minutum*. Both the size and the quality of the host egg play an important role in the subsequent quality of parasitoids. The fact that we saw changes in enzyme patterns for parasitoids reared on different hosts suggests that qualitative differences in eggs of SBW are at least as important, if not more so than quantitative differences.

Our work has shown that electrophoretic analysis can detect changes in parasitoids as a direct result of rearing conditions and thus, provide a means of 'quality' control for their production. It also suggests that for *T. minutum*, the rearing host (nutritional diet) will have more of an impact upon

the isoenzyme pattern and consequently, the metabolic processes, than the rearing temperature.

The relationship between structural variation of enzymes or isoenzyme patterns and metabolic activity is not clear. In some cases, isoenzymes may be neutral and may have no relationship, while in others, there may be strong effects on physiology. Singh (1984) reported that in the screwworm fly, the high constant temperatures used in mass production to speed development exerted a selective force favouring one form of αGPDH over another. This, in turn, affected the flight capabilities of these individuals since the amount and type of αGPDH present in flight muscle was directly related to flying ability (Bush & Neck, 1976). In the present study, there was no obvious correlation between observed biological changes and enzymatic patterns. Although biological parameters of *T. minutum* changed according to the temperature they were reared at, no corresponding differences in isoenzyme patterns were observed over the range examined. In addition, the initial biological studies on rearing hosts were conducted with MFM and SBW. Later enzymatic work made use of *T. minutum* from AGM not MFM. Lower fecundity and longevity for parasitoids reared on a standard rearing host (MFM) compared to SBW might be linked with extra bands in the ACPH, AK, IDH, MDH, ME, and PGM enzyme systems. Verification using genetic frequencies obtained from individual parasitoids would be required, however, before a direct correlation could be established.

Conclusions

Some rearing conditions, such as low temperature, which are in standard use for synchronizing the emergence of *Trichogramma* species in mass production adversely affect parasitism in the field. Lower fecundity on the first day of emergence, lower percent emergence, and reduced rates of increase will all be associated with parasitoids reared at temperatures below 17 °C. Because of the short life span of these parasitoids in the field, the effects of reduced temperatures should be weighed carefully against their benefits in synchronizing emergence. *Trichogramma* mass produced on unnatural hosts (AGM or MFM) are inferior to those produced on the target or natural host (SBW) and this information can help us to improve the timing of future inundative releases. If *T. minutum* are released early enough in the ovipositional period of SBW, there will be a second generation of active, 'superior' parasitoids from SBW when fresh SBW eggs are still present and susceptible for attack later in the ovipositional period of the host.

An important, future aspect of mass-rearing *Trichogramma* will be quality control. Generally, the quality of laboratory-reared insects is defined and measured in terms of how well the insects fulfill their intended role in the field. We have shown that gel electrophoresis of enzymatic proteins can be used to monitor genetic changes and, as expressed in enzyme systems, can provide a relatively simple and convenient means of detection. In the present study, however, specific biological changes could not be correlated with observed enzymatic changes. Caution must be exercised, therefore, in selecting the most appropriate biological parameters to measure and in correctly interpreting the results from these enzymatic studies.

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Zusammenfassung

Einfluss von Zuchttemperatur und Wirt auf Isoenzymmuster und Biologie von Trichogramma minutum *Riley*

Weibchen von *Trichogramma minutum*, welche bei 17 °C gezüchtet wurden, hatten eine geringere Schlüpfrate, eine geringere Fruchtbarkeit am ersten Tag nach dem Schlüpfen sowie ein reduziertes Populationswachstum (r_i) im Vergleich zu Tieren wel-

che sich bei Temperaturen über 17 °C entwickelt hatten. Wurden die Parasitoiden auf *Choristoneura fumiferana* gezogen, so war ihre Fruchtbarkeitsrate wie auch Lebensdauer grösser als wenn *Ephestia kuehniella* als Wirt diente. Die parasitische Fähigkeit von *T. minutum* war immer auf derjenigen Wirtsart am grössten auf der die Schlupfwespen vorher gezogen wurden. Aufzuchttemperaturen von 13 °C oder 27 °C hatten keinen Einfluss auf den Isoenzymbändern wenn die Tiere statt auf *C. fumiferana* auf *Sitotroga cerealella* gezogen wurden. Es konnte aber kein Zusammenhang zwischen den biologischen und biochemischen Veränderungen gefunden werden. Hingegen besteht die Möglichkeit während der Massenproduktion die Qualität der Parasitoiden anhand von Isoenzymprofilen zu überwachen.

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