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Strains of the egg parasitoid *Trichogramma minutum* Riley

I. Biochemical and biological characterization

By S. M. Smith and M. Hubbes

Abstract

The electrophoretic patterns and biological characteristics were compared for seven strains of *Trichogramma minutum* Riley collected from different geographical regions in eastern North America. Patterns of 16 enzyme systems from group extracts were described using starch gel electrophoresis; of these, seven could be used to differentiate the strains. General protein patterns did not show obvious differences between the strains. The mean developmental threshold for all

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strains, over all rearing temperatures, was 10.5 (± 1.7) °C. One strain had a significantly higher (166.2 °D) thermal constant than the remaining strains (X = 137.8 °D). Biological characteristics including the proportion of female progeny, longevity, and fecundity differed significantly between the strains when they were reared at either 13, 15, 17, 20, 23, or 27 °C and on either *Ephestia kuehniella* (Zeller) or *Choristoneura fumiferana* (Clemens). No one strain performed consistently better than the others under all rearing conditions examined. The identification of *T. minutum* strains has implications for future mass production and release in the field.

1 Introduction

Worldwide, the genus *Trichogramma* contains approximately 64 species (Voegele and Pintureau 1982). Because members of this genus feed exclusively on passive hosts (eggs), very little specialization of anatomical structures has been necessary, even in the immature stages (Nagarkatti and Nagaraja 1977). This has led to the proliferation of innumerable phenotypically identical sibling species. The taxonomy of this genus, consequently, cannot always be studied by conventional methods, particularly in view of the minute size of these chalcid wasps.

Electrophoretic studies have been invaluable in the taxonomic classification of several species of insects. Avise (1974) considered the analysis of isoenzyme patterns an extremely valuable tool for systematics because the relationships derived with this approach, in general, corresponded closely to previously recognized morphological relationships. Electrophoretic techniques have been used only recently to clarify the taxonomy of several species of *Trichogramma: T. cacoeciae* Marchal; *T. chilonis* Ishii; *T. embryophagum* Hartig; *T. euproctidis* Girault; *T. evanescens* Westwood; and *T. maidis* Pintireau and Voegele (Jardak et al. 1979; Pintureau and Babault 1981, 1982; Berge et al. 1982; Klausnitzer et al. 1983). Systematic work in the United States has concentrated on isoenzyme analyses of *T. pretiosum* Riley (A. C. F. Hung, pers. comm.). Little attempt has been made to examine intraspecific variability between populations of the same species of *Trichogramma* or to relate such variability in isoenzyme patterns to variable biological characteristics.

In contrast to electrophoretic studies, a great deal of information has accumulated on the biology and ecology of *Trichogramma* species. Available host records indicate that many species of *Trichogramma* have adapted to particular habitats; *T. evanescens* in fields, *T. embryophagum* and *T. minutum* in arboreal areas, and *T. semblidis* Surivillius in marshes (Flanders 1937). In the past, few of these ecological preferences were recognized and where such differences could be shown, the difficult taxonomy of this group prevented a complete understanding of their relationship to parasite biology or performance in the field following release. Because of the haplodiploid method of reproduction in this genus and the tendency for sibmating in isolated populations, the development of strains in species of *Trichogramma* is almost certain. The success of future inundative releases with these parasitoids, therefore, will depend to a large extent upon our ability to recognize and characterize strains that have superior parasitic ability.

In 1981, work was initiated by our group to assess the potential of *T. minutum* Riley as a biological control agent against the spruce budworm (SBW), *Choristoneura fumiferana* (Clemens). One of our main objectives was to examine populations of *T. minutum* that had been collected from different geographical areas to see if distinct strains could be identified. In the present

paper, we will deal solely with the recognition and characterization, biologically and biochemically, of such strains of *T. minutum*. Our attempt to correlate observed differences and to use them to identify and select that strain of *T. minutum* best suited for release in the field against the budworm will be discussed in a subsequent paper.

2 Material and methods

2.1 Parasitoid material

Strains of *T. minutum* were collected from five geographical areas in Ontario, Canada and one region in Maine, U.S.A. (table 1). The strains were maintained by the Biological Control Laboratory at the University of Guelph in 2 by 9 cm glass vials with fine-mesh corks under the following regime: 25 °C, 75 % R.H., and LD 16 : 8. Four days before the scheduled emergence, the parasitoids were placed in the dark at 17 °C to synchronize emergence. Under these conditions, the average generation time for all strains was two weeks.

From 1979 until 1983, the parasitoids were reared continuously on eggs of the Mediterranean flour moth, *Ephestia kuehniella* (Zeller) (MFM) (SMITH 1984). From September 1983, eggs of the Angoumois grain moth, *Sitotroga cerealella* (Olivier) (AGM), were used to rear the parasitoids. No food was supplied to the parasitoids during culture. Parasitoids were shipped to Toronto as required and maintained under similar conditions until emergence.

2.2 Electrophoretic studies

Parasitoids were reared on AGM at 25 °C, 75 % R.H., and a photoperiod of LD 16 : 8 until emergence. Within 24 hours of emergence, they were frozen 'en masse' and stored at 20 °C. Samples were prepared for electrophoresis as described by SMITH (1984). Although zymograms for individual *Trichogramma* could not be obtained, differences between patterns of almost all active isoenzymes could be resolved adequately with starch gel electrophoresis (SMITH 1984; SMITH and HUBBES in litt.). One enzyme system, the esterases, however, showed better resolution

Table 1. Summary of information on the strains of *Trichogramma minutum* collected from different geographical regions in eastern North America

Strain	Geographical origin	Latitude & longitude	Number of generations in culture[a]	Original host	Outbreak status of spruce budworm
Grundy (G)	Grundy Pk, Northern Ontario	46° N 81° W	146	SBW[b]	low
Maine (M)	Maine, U.S.A.	45° N 69° W	206	SBW	severe
Plummer (P)	Plummer Twp., Northern Ontario	46° N 84° W	146	SBW	severe
Rogers (R)	Rogers Twp., Northern Ontario	50° N 84° N	102	SBW	severe
Strong (S)	Strong Twp., Northern Ontario	46° N 80° W	146	SBW	severe
Vineland (V)	Vineland, Ont. Southern Ontario	43° N 80° W	254	Codling Moth	–
Field (F)	Rogers Twp., Northern Ontario	50° N 84° N	54	SBW	severe

[a] One generation every two weeks at 25 °C, 75 % RH, and LD16:8. – [b] SBW = *Choristoneura fumiferana*; Codling Moth, *Laspeyresa pomonella* (Linnaeus).

using a vertical polyacrylamide system (7.5 %; 0.377 M Tris-HCl; pH = 8.9). Electrophoresis with polyacrylamide gels was performed by loading 5 to 10 µl samples of *T. minutum* (5.65 mg/ml protein) on a Bio-Rad Protean Dual 16 CM Slab Cell in an electrode tank buffer of 0.052 M Tris-HCl (pH 8.9).

Gradient polyacrylamide gels were required to analyse the general protein pattern for strains of *T. minutum*. Extracts were prepared as before except that SDS (Sodium dodecylsulfate) (10 %) and mercaptethanol were added to the sample buffer. Each sample was heated for two minutes and stored at −20 °C for electrophoresis the following morning. Bio-Rad low molecular weight markers served as references. The gradient of the gel ranged from 8 % at the top to 15 % at the bottom with a stacking gel (0.06 M Tris-HCl; SDS 10 %; pH = 8.9) of approximately two centimeters on top (LAEMMLI 1970).

Isoenzyme samples in starch gels were run at 3 °C for ca 2.5 hr, those in polyacrylamide with the circulation of cold water for ca four hours, and samples to be stained for general protein, at room temperature for seven hours. All systems used a power supply with a constant voltage of 200 volts. The amperage varied during the time of running from 75 to 135 ma for starch gels and 45 to 15 ma for polyacrylamide gels. In all cases, when the tracking dye had migrated to the end of the gels (ca 10 cm), the gels were stained and fixed. The starch slab was trimmed and sliced into six gels while polyacrylamide gels were placed directly into the staining solutions (LAEMMLI 1970; SHAW and PRASAD 1970; SICILIANO and SHAW 1976). To verify the identified pattern, general proteins and each enzyme system were run under identical conditions a minimum of four times. Mobility of the isoenzyme bands was defined as the distance travelled from the origin divided by the distance travelled by the front (R_f).

2.3 Biological studies

Laboratory experiments were conducted to compare the biological response of the different geographical populations of *T. minutum* to rearing temperature and host. Fresh eggs of SBW and MFM were obtained from colonies reared in the laboratory while eggs of AGM were supplied by the Biological Control Laboratory at Guelph (SMITH 1984). Host eggs were exposed to individual females as described by YU et al. (1984) and SMITH (1984). Comparisons between the strains were made in terms of the fecundity, longevity, emergence, sex ratio of the progeny, and the proportion of non-fecund females. Fecundity was defined as the number of host eggs that were parasitized. Unless stated otherwise, all adult parasitoids were unfed and maintained in ventilated, 0.5-dram vials at 25 °C, 75 % R.H., and LD 16:8 photoperiod.

To examine the effect of rearing temperature, the seven populations of *T. minutum* were reared simultaneously on eggs of AGM at six different temperatures: 13, 15, 17, 20, 23, and 27 °C (± 0.5 °C). The eggs were attached to cards with water and maintained in ventilated, 2 by 9 cm, glass vials. One day prior to the estimated time of emergence (ca 42, 25, 20, 14, and 6 days, respectively), the parasitized eggs were placed in the dark at 17 °C to synchronize emergence. After four days, the vials were transferred to an incubator and maintained at 25 °C and LD 16:8 until emergence. Emergence and sex ratio were calculated approximately 12 days later for each strain at each temperature.

To estimate longevity and fecundity, 10, mated females from each strain and temperature were isolated into individual vials and each provided daily, until her death, with over 80 fresh AGM eggs. This experiment was replicated twice and treated as a two factor random block design with each replicate considered a block. Developmental rates were calculated by isolating a minimum of 50 parasitized eggs/strain at each experimental temperature into 10, 0.5 dram vials. The vials were maintained at the same six experimental temperatures as above and checked three times each day for emergence. When 50 % of the parasitoids had emerged, further emergence was recorded daily.

The populations of *T. minutum* from different geographical areas were also compared under three different host conditions: 1. reared on SBW and provided with MFM; 2. reared on MFM and provided with SBW; and 3. reared on MFM and provided with MFM. Strains reared on SBW and provided with SBW were not compared due to a shortage of SBW eggs. These experiments were conducted under the same conditions and examined the same biological parameters as the previous study on rearing temperature. To ensure an even age distribution of the parasitoids, the number of strains examined within each replicate varied depending upon the number emerging simultaneously within a period of four hours. A maximum of 50 individual parasitoids could be compared reasonably in this experiment at any one time. To provide an accurate estimate of longevity and fecundity, therefore, each experiment was replicated two or three times so that the minimum sample size would consist of 20 female parasitoids/strain/treatment. Each replicate was considered as a block for analysis.

3 Results and discussion

3.1 Electrophoretic studies

Specimens from the seven strains in this study were all taxonomically identified as *Trichogramma minutum* Riley (C. YOSHIMOTO, pers. comm.). The following work, therefore, considers all observed differences between the strains to be within-species differences.

Of 32 enzyme systems examined, only half provided patterns sufficiently consistent to be used in subsequent work (fig. 1). The standard enzyme pattern for *T. minutum* was comprised of up to 55 isoenzyme bands from 16 enzyme systems. Three enzyme systems produced anodal banding: acid phosphatase (ACPH), glutamate oxaloacetate transaminase (GOT), and malate dehydrogenase (MDH). Because each banding pattern represented a number of individuals (ca 400 to 4000 *T. minutum*/sample), bands were hypothetically assigned to loci based upon previous authors (JARDAK et al. 1979; PINTUREAU and BABAULT 1981; BERGE et al. 1982; KLAUSNITZER et al. 1983) and their degree of similar migration. To test such an allocation, controlled genetic crosses and the resolution of patterns for individual parasitoids would be required (WOOLING et al. 1978).

The protein patterns for all strains of *T. minutum* were similar (fig. 2). Although slight quantitative differences for specific bands were observed (i.e. at 105 K daltons), there were few apparent qualitative differences. This tended to verify the prior taxonomic identification and suggests that these populations do represent the same species of *Trichogramma*. In the isoenzyme studies, however, differences, both qualitative and quantitative, could be identified between the populations or strains of *T. minutum* (fig. 3). Consistent differences in patterns were observed for ACPH, adenylate kinase(AK), α-glycerophosphate dehydrogenase (αGPDH), catalase (CAT), esterase (EST), glucose-6-phosphate dehydrogenase (G6PDH), GOT, hexokinase (HEX), isocitrate dehydrogenase (IDH), 6-phosphogluconate dehydrogenase (6PGDH), and tetrazolium oxydase (TO).

The ACPH pattern for *T. minutum* consisted of four bands with considerable variation between the strains of *T. minutum*. Using polyacrylamide gels, BERGE et al. (1982) found only a single band for ACPH when examining patterns in *Trichogramma* other than *T. minutum*. By substituting β-naphthyl acid phosphate for the α-form the patterns might have been elucidated further (WOOLING et al. 1978). All strains of *T. minutum* showed similar activity for ACPH1 and ACPH2. A slower ACPH3 (R_f = 0.43) was present in G, R, and F, a medium moving band (R_f = 0.48) in G, P, R, S, and V, and a fast band (R_f = 0.54) in G, M, and R. The ACPH4 band was very poorly resolved and may have represented more than a single band. Slow variants (R_f = 0.58) for this band were observed for G, R, V, and F, medium variants (R_f = 0.63) for P and S, and a fast variant (R_f = 0.70) for M. For strains G and R, additional, indistinct bands, possibly associated with this band, were sometimes observed between ACPH3 and ACPH4.

Adenylate kinase showed variability in only one (AK2) out of a total of four bands. Slow bands of AK2 (R_f = 0.18) were found for strains G and R, a fast band (R_f = 0.20) for strain M, and medium bands (R_f = 0.19) for the remaining strains.

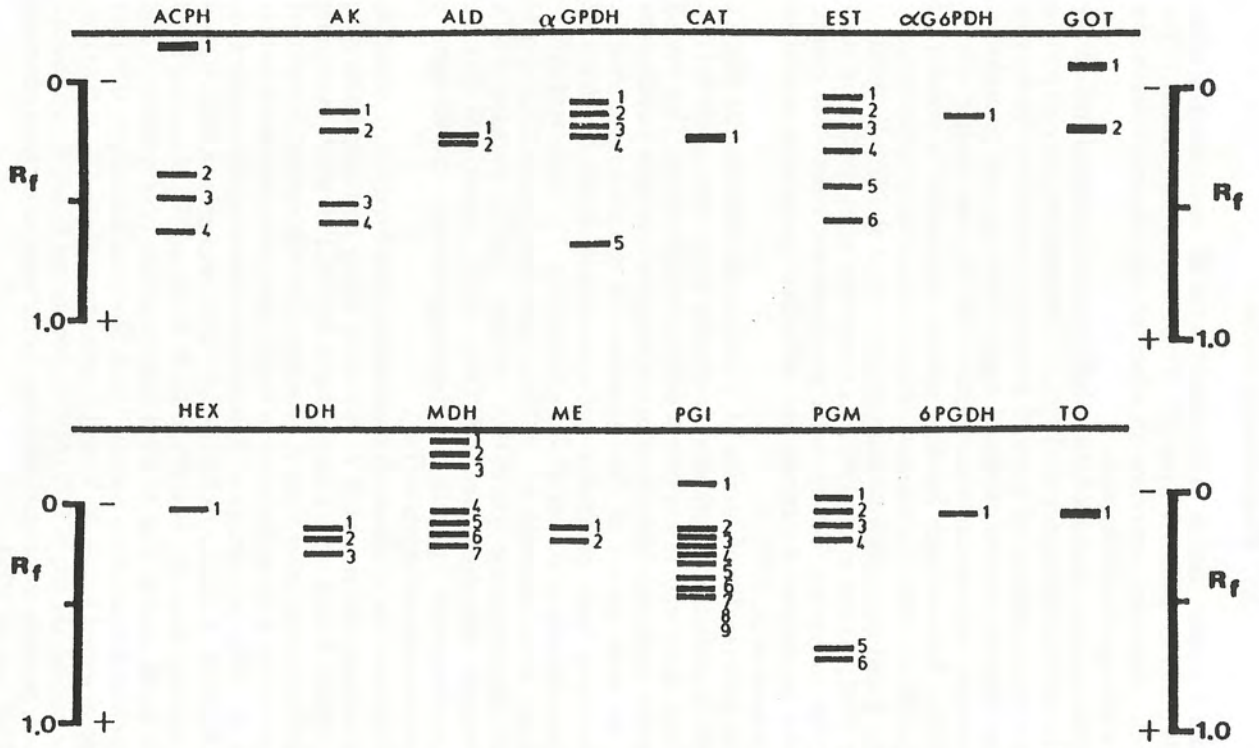

Fig. 1. General schematic isoenzyme pattern of *Trichogramma minutum*. ACPH = acid phosphatase, AK = adenylate kinase, ALD = aldolase, αGPDH = αglycerophosphate dehydrogenase, CAT = catalase, EST = esterase, G6PDH = glucose-6-phosphate dehydrogenase; GOT = glutamate oxaloacetate transaminase; HEX = hexokinase, IDH = isocitrate dehydrogenase; MDH = malate dehydrogenase, ME = malic enzyme, PGI = phosphoglucoisomerase, PGM = phosphoglucomutase; 6PGDH = 6-phosphogluconate dehydrogenase, TO = tetrazolium oxydase

Five isoenzyme bands for αGPDH were identified but differences showed in the mobility of only one band, αGPDH5. A large number of replicates were run for this enzyme due to the variable degree of staining intensity between samples and the importance of this enzyme in flight metabolism. Strains M and

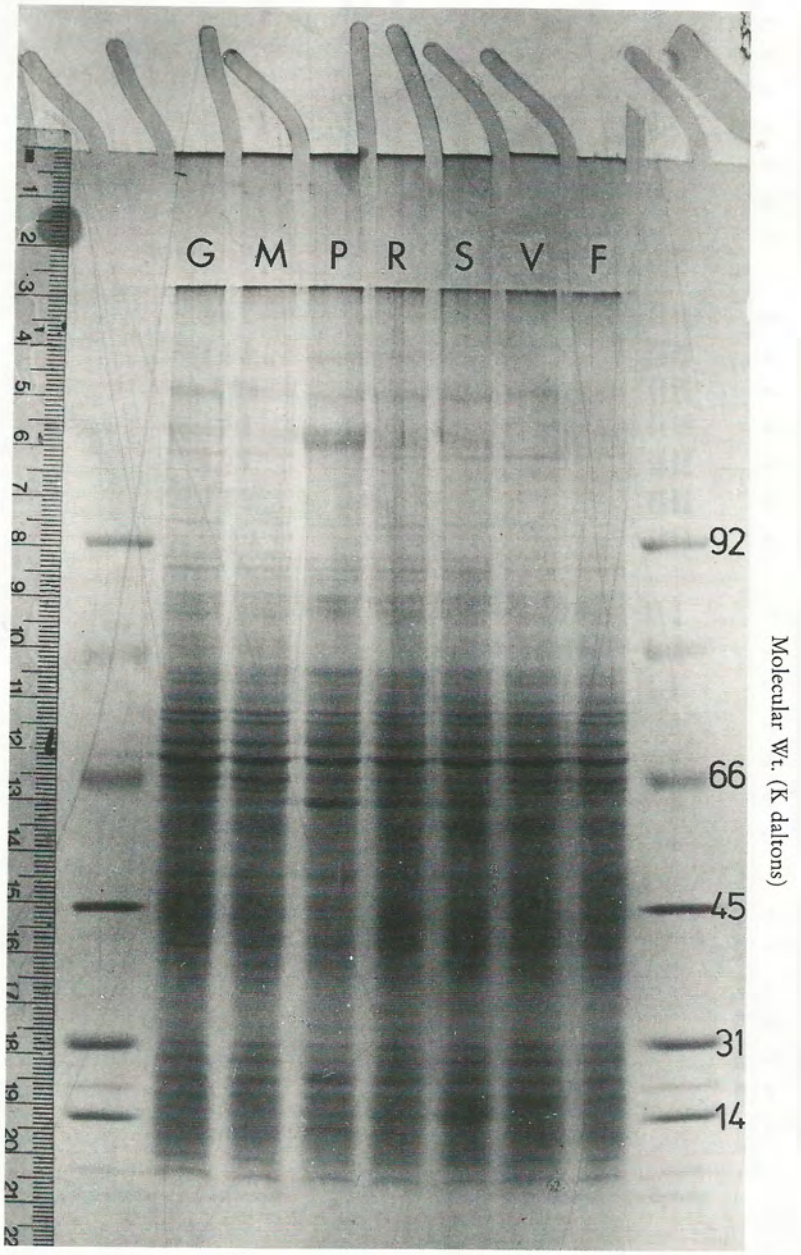

Fig. 2. General protein pattern for the seven strains of *Trichogramma minutum*. G = Grundy, M = Maine, P = Plummer, R = Rogers, S = Strong, V = Vineland, F = Field

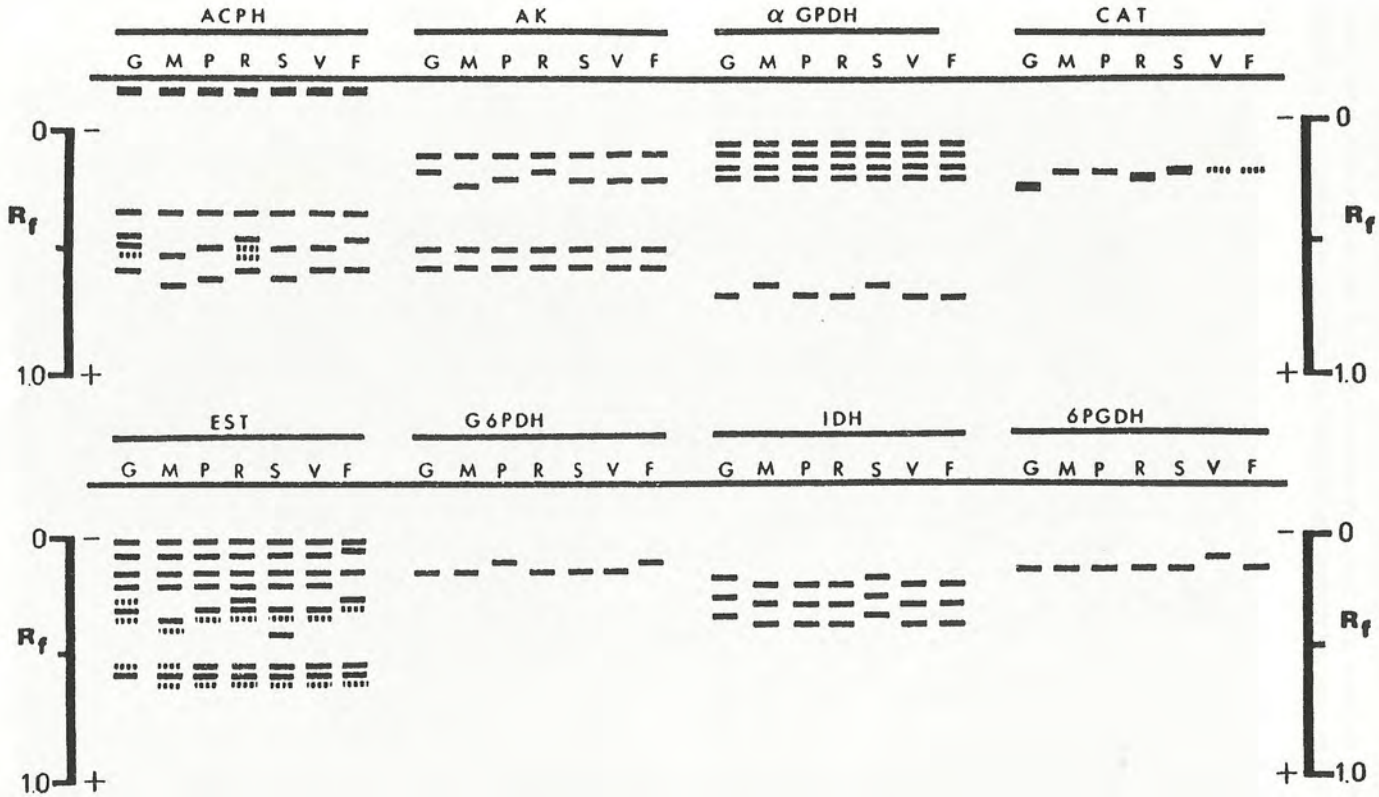

Fig. 3. Isoenzyme patterns for eight enzyme systems in seven strains of *Trichogramma minutum* reared from *Sitotroga cerealella.* ACPH = acid phosphatase, AK = adenylate kinase, αGPDH = αglucophosphate dehydrogenase, CAT = catalase, EST = esterase, G6PDH = glucose-6-phosphatedehydrogenase, IDH = isocitrate dehydrogenase, 6PGDH = 6-phosphogluconate dehydrogenase; G = Grundy, M = Maine, P = Plummer, R = Rogers, S = Strong, V = Vineland, F = Field. Dashed bars indicate presence of polymorphic loci

S showed a consistently slower moving band for αGPDH5 (R_f = 0.58) than the remaining strains (R_f = 0.65).

The esterase system of *T. minutum* consisted of six bands with five showing substrate affinity to α-naphthyl acetate and one (EST5) showing affinity to β-naphthyl acetate. Variation in bands EST2, EST4, EST5, and EST6 were observed among the strains. In particular, strains P, R, S, and V showed several distinct bands for EST5 and EST6. The relative proportion of these multiple bands will be discussed in a subsequent paper (SMITH and HUBBES in litt.). Esterase patterns similar to those described here (i.e. six bands) for other species of *Trichogramma* have been reported by JARDAK et al. (1979), PINTUREAU and BABAULT (1982), and KLAUSNITZER et al. (1983).

All strains of *T. minutum* displayed a fast moving band (R_f = 0.13) in the G6PDH system except P and F where a single band was found at R_f = 0.08. Three isoenzyme bands for IDH were each composed of two separate bands but these could not be consistently resolved. In strains G and S, all three bands were similar, slow migrants (R_f = 0.19, 0.25, and 0.30) while the remaining strains displayed faster moving bands (R_f = 0.24, 0.28, and 0.33). Similarly, for 6-PGDH, all strains displayed a fast moving band at R_f = 0.13 with the exception of strain V which had a single, slow band at R_f = 0.06.

Banding patterns of CAT, GOT (ATT), and HEX were inconsistent. For GOT and HEX, only 10 % and 33 % of the electrophoretic runs, respectively, produced the distinct patterns shown in fig. 3. As reported by BERGE et al. (1982), a single band for CAT was identified and this was very poorly resolved. These three although varying between the strains could not be consistently used for differentiating them.

The enzyme MDH was very active and stained readily (fig. 1). Each strain of *T. minutum* displayed seven distinct bands for MDH with no variation observed among any of the strains. These results contrast those reported by BERGE et al. (1982) and PINTUREAU and BABAULT (1981) where only three MDH bands were resolved for *T. maidis* and *T. evanescens* and considerable polymorphism was apparent within each species.

One of the most active systems and that with the greatest overall number of bands was PGI (fig. 1). The nine bands of this enzyme had the same intensity of staining in all the strains. Resolution of the bands PGI2 to PGI6 was optimal at low pH (< 7.0) and could not be improved by using polyacrylamide gels. A. C. F. HUNG (pers. comm.) found that PGI in *Trichogramma* had a single locus with four codominant alleles. Findings in the present study suggest that in populations of *T. minutum* a greater number of alleles are involved in the expression of PGI. Similarly, PGM was another active enzyme that could not be used to differentiate the strains in mass extracts. Six bands for PGM were identified with no intraspecific differences. BERGE et al. (1982) found no variation in PGM zymograms between samples of *T. maidis.*

In our study, we found the same single band of TO in all the strains of *T. minutum* (fig. 1). PINTUREAU and BABAULT (1981, 1982), however, described three extremely variable TO bands for *T. evanescens, T. maidis, T. rhenana* Voegele & Russo, *T. oleae* Voegele & Pointel, *T. embryophagum, T. nubilale,* and *T. dendrolimi.* With improved resolution, using buffer systems at lower pH (< 8.0) than those employed in the present study, it may be possible to differentiate more than one band for TO. As the New World species of *Trichogramma* have not yet been examined for this enzyme, this discrepancy may also represent natural variation within the family Trichogrammatidae.

In general, little electrophoretic variation was found among the strains of *T. minutum*. No obvious differences in protein patterns were observed and of 32 enzyme systems examined, only eleven showed variability. Of these, only seven were consistent enough to characterize the strains; ACPH, AK, αGPDH, EST, G6PDH, HEX, IDH, and 6PGDH. Considering all strains, only 13 out of 55 isoenzyme bands (24 %) showed variation indicating a low frequency of polymorphism. WOOLING et al. (1978) and SIMON et al. (1982) found 30 out of 32 and 21 out of 22 monomorphic loci, respectively, for populations of *Myzus persicae* (Scilz.) and *Acyrthosiphon pisum* (Harris). Such low genetic diversity was attributed, in both cases, to high levels of isolated, parthenogenetic reproduction. Generally, in most invertebrate populations, the proportion of monomorphic loci will range from 25 % to 75 % (AVISE 1974). This suggests that those populations of *T. minutum* in the present study have somewhat less genetic diversity than the average invertebrate species. This might be attributed to the length of time these strains have been mass-reared in the laboratory (table 1) or to the degree with which sibmating and thus, inbreeding occurs in this species.

3.2 Biological studies

Individuals from all strains of *T. minutum* survived until emergence when reared at each of the six temperatures (table 2). Only at 17 °C did all strains develop at a similar rate. At temperatures lower than 17 °C, G, P, R, and S

Table 2. Time for development (D), mean developmental rate, developmental threshold, and thermal constant for seven strains of *Trichogramma minutum* reared on *Sitotroga cerealella* at 75 % RH, LD 16:8, and six different temperatures

Biological parameter	Strain						
	Grundy	Maine	Plummer	Rogers	Strong	Vineland	Field
Time for development (days)							
at: 27 °C	8.42b[a] (51)[b]	8.51[b] (45)	8.33b (52)	8.34b (56)	8.34b (47)	8.33b (42)	9.33a (54)
23 °C	10.85bc (48)	10.95b (38)	10.56bc (64)	10.39c (46)	10.31c (45)	10.57bc (49)	12.16a (49)
20 °C	16.23b (40)	16.67ab (52)	16.65ab (54)	16.33b (66)	17.32a (57)	16.98ab (49)	17.26b (47)
17 °C	20.11a (54)	23.14a (44)	20.02a (50)	24.36a (42)	20.25a (49)	21.02a (53)	21.85a (54)
15 °C	31.58c (57)	35.06a (35)	30.23d (65)	29.66d (38)	30.65cd (60)	32.70b (56)	33.64b (25)
13 °C	47.33c (54)	53.11a (9)	49.14bc (51)	46.88c (57)	46.52c (50)	49.32b (38)	49.50b (24)
Mean developmental rate (1/D)	0.060	0.067	0.063	0.064	0.060	0.061	0.064
Mean developmental threshold (t) °C	10.4	11.2	10.5	10.2	10.5	10.8	9.8
Mean thermal constant (K) °D	140.3a	130.3a	137.5a	146.9a	137.5a	134.0a	166.2b

[a] Means for the strains within each row followed by the same letter are not significantly different at the $p \leq 0.05$ level (Duncan's [1951] multiple range test). – [b] Number of replicates given in parentheses.

developed relatively quickly while the M strain had the longest developmental time. Emergence for M strain was also reduced at 13 °C (n = 9) suggesting that this strain had higher temperature requirements than the remaining strains. The F strain, however, developed relatively quicker at lower temperatures and therefore, appeared to be better adapted to lower temperatures than the remaining strains.

Strains G, P, R, and S were collected from northern Ontario and might be expected to have relatively faster rates of development at lower temperatures. Strain M, collected in Maine, U.S.A. (45° N, 69° W), which would also be expected to develop relatively quickly under these conditions, however, did not. The F strain had been collected from the field in Rogers Township, Ontario in 1983 following release of strain P. Slower developmental times at higher temperatures and faster developmental times at lower temperatures may reflect a selection pressure for survival under varying temperature conditions in the field.

In addition to temperature, the species of host egg, the density of parasitoids, and the size of the host will affect developmental rates of *Trichogramma* (Calvin et al. 1984). Yu et al. (1984) reported the developmental time of *T. minutum* on eggs of MFM to be 6.9, 7.9, 14.7, 20.5, 25.7, and 43.3 days at 28, 25, 20, 17, 15, and 13 °C, respectively. These results are slightly lower than those derived for all strains of *T. minutum* reared on AGM in the present study.

The developmental threshold (t) and thermal constant (K) for each strain were determined by regressing individual developmental rates (D = 1/ developmental time) against temperature where $1/D = t + (1/K)x$ and x = degrees in Celsius (table 2). Although the fastest mean developmental rate was found for strain M followed by R, F, P, V, G, and S, these values were not significantly different. For all strains, the mean developmental threshold was 10.5 °C. Strain F, however, had a significantly higher thermal constant (166.2 °D) than the remaining strains ($\bar{X}$ = 137.8 °D), although, all were within an expected range for this genus. In their study, Yu et al. (1984) found the threshold temperature and thermal constant of *T. minutum* to be 10.2 °C and 128 °D, respectively, in eggs of MFM. Peterson (1931) reported the threshold temperature and day-degrees for the development of *T. minutum* to be 10 °C and 142.8 °D, respectively. Estimates of these variables for other species of *Trichogramma* range from 10.3 to 13.1 °C and 128.7 to 169.5 °D, depending upon the rearing host and temperautre (Butler and Lopez 1980; Pak and Oatman 1982; Goodenough et al. 1983; Calvin et al. 1984).

The slight difference in developmental thresholds between M and F, although not significant, supports earlier findings that the M strain requires a higher temperature for development (t = 11.2 °C) while F continues to develop at a lower temperature (t = 9.8 °C) than the remaining strains. The F strain also required a significantly higher number of day-degrees for development than the remaining strains. This suggests that although all strains were similar in development and could be considered members of the same species, they did exhibit distinct biological differences. In particular, some strains appeared to be better adapted to lower rearing temperatures and therefore, more suited to release in cooler environments than others. Similar results were obtained by Pak and van Heiningen (1985) examining behavioural responses of *Trichogramma* strains.

No effect of temperature or strain was seen in the percentage of parasitoids

emerging or in the proportion of female progeny produced (table 3). Over all temperatures, however, a greater proportion of female parasitoids from the G strain emerged successfully than from the R strain while the longevity of parasitoids from the M and R strains was significantly greater than that from the G and V strains. Among the different strains, M and F had significantly greater fecundity on the first day of emergence than strain V. Because similar results were obtained in the total number of eggs laid, these differences in fecundity were maintained throughout the adult life of the parasitoids. One exception was the G strain, where a low total number of eggs was also produced.

From previous studies, it was known that the key biological parameters of *T. minutum* affected by rearing temperature would be fecundity on the first day of emergence and longevity (SMITH and HUBBES in litt.). We examined these two biological variables, therefore, in more detail (table 4). Maximal fecundity on the first day of emergence occurred for all strains at 23 °C. Above or below this value, the number of eggs laid on the first day of emergence decreased with temperature.

No effect of rearing temperature on longevity was observed for parasitoids from the S strain with mean longevity averaging 2.5 days over the six temperatures. All strains of *T. minutum* lived for the same length of time at 20 °C. The poorest survival (1.9 days) for all strains, except G, was seen at 27 °C. Parasitoids from G strain lived the shortest time (1.3 days) at 13 °C. Longevity was greatest for M (3.8 days) and V strain (2.9 days) at 17 °C, for F (3.7 days) at 15 °C, and R (4.0 days) at 17 and 15 °C. Significant interaction between temperature and strains was observed for longevity in *T. minutum* ($p \leq 0.01$).

In general, all strains of *T. minutum* were relatively similar in their response to changes in temperature. No one strain performed (i.e. had high fecundity, emergence, and proportion of female progeny and low number of non-fecund females) consistently better than the rest for all parameters at all temperatures. M and F strains tended to have a higher fecundity than the remaining strains, however, R lived the longest at all temperatures. Based upon these results, P strain might be expected to achieve the highest levels of parasitism in the field. Not only does this strain perform better at lower temperatures (lower threshold temperature) but it also has one of the highest fecundities. By the

Table 3. Summary of biological characteristics for seven strains of *Trichogramma minutum* averaged over six different rearing temperatures

Strain	Emergence (%)	Females Emerging (%)	First day fecundity (no. eggs/female)	Total fecundity (no. eggs/female)	Longevity (days)	Female progeny F1 (%)
Grundy	99.3a[a]	66.3a	21.8ab	30.3b	2.4b	52.9a
Maine	100a	62.5ab	24.2a	38.9a	2.9a	53.6a
Plummer	100a	57.6ab	23.5ab	35.6ab	2.6ab	51.4a
Rogers	100a	56.4b	20.4ab	34.0ab	2.8a	50.9a
Strong	100a	61.3ab	21.7ab	35.6ab	2.5ab	59.5a
Vineland	100a	55.9ab	19.3b	27.2b	2.3b	58.5a
Field	–	–	24.2a	36.8a	2.6ab	59.0a

[a] Means followed by the same letter within each column are not significantly different at the ≤ 0.05 level (Duncan's [1951] multiple range test)

Table 4. Number of eggs parasitized (fecundity) on the first day of emergence and longevity in seven strains of *Trichogramma minutum* reared on *Sitotroga cerealella* at 75 % RH, LD 16:8 and six temperatures

Strain	Rearing temperature (°C)					
	27	23	20	17	15	13
First day fecundity (no. eggs/female)						
Grundy	21.8a[a]	27.7a	28.3a	21.9a	15.7b	15.6a
Maine	22.0a	30.7a	28.2a	23.5a	22.7a	18.0a
Plummer	24.1a	35.9a	28.7a	20.2a	13.0b	19.0a
Rogers	17.1a	32.4a	17.5b	19.8a	17.7ab	17.8a
Strong	17.3a	33.5a	26.6a	17.7a	18.0ab	17.3a
Vineland	19.5a	28.1a	25.2ab	22.8a	11.7b	7.4b
Field	22.4a	35.2a	24.0ab	21.4a	24.0a	18.4a
Longevity (Days)						
Grundy	2.6a	3.0ab	2.4a	2.1a	2.9bc	1.3b
Maine	2.2ab	3.0ab	3.0a	3.8b	3.2abc	2.4a
Plummer	2.0ab	3.2a	2.6a	2.9a	2.4c	2.5a
Rogers	1.7b	2.5bc	2.3a	4.0b	4.0a	2.3a
Strong	2.0ab	3.0ab	2.4a	2.5a	2.7bc	2.3a
Vineland	1.9ab	2.3c	2.2a	2.9a	2.6a	2.1ab
Field	1.6b	3.1ab	2.4a	2.7a	3.7ab	2.0ab

[a] Means followed by the same letter at the same temperature for each biological factor are not significantly different at the $p \leq 0.05$ level (Duncan's [1951] multiple range test).

same reasoning, strain V would probably show the least effectiveness in the field.

There was no difference in the total fecundity ($\overline{X}$ = 52.3 eggs/female), number of progeny (X = 50 parasitoids/female) or longevity (X = 3.5 days) among five strains of *T. minutum* reared on SBW and provided with MFM (table 5). On the first day of emergence, however, strains M and P parasitized a significantly greater number of eggs than strain V. Strains G, M, S, and V had better emergence than the P strain while parasitoids from G had a significantly higher proportion of female progeny than those females from M, P, or S. This suggests that the M strain performed the best when reared on SBW and provided with MFM because both emergence and fecundity on the first day of emergence were higher than for the remainig strains while the percentage of non-fecund females in this strain was lower. The V strain had the poorest overall record when reared on SBW with low fecundity on the first day of emergence and a high proportion of non-fecund females.

When the same five strains of *T. minutum* were reared on MFM and provided with eggs of MFM, no significant differences were seen in either longevity or emergence (table 5). Both fecundity on the first day of emergence and total fecundity were significantly greater for parasitoids of the P strain than those of the G or V strains. The V strain, however, had a greater proportion of female progeny than P strain under similar conditions while a greater proportion of non-fecund females was observed for both P and V strains than for the remaining strains. In general, V strain performed the worst. Although P strain had a high overall fecundity, the S strain would probably be considered superior to the other strains because fecundity was never significantly different between it and P strain and S generally had a low proportion of non-fecund females.

Table 5. Biological parameters for strains of *Trichogramma minutum* reared at 25 °C, 75 % RH, and LD 16:8 photoperiod

Host origin: Host provided	Strain	n (No. females)	Emergence (%)	First day fecundity (No. eggs female)	Total fecundity (No. eggs/ female)	Non-fecund females (%)	Longevity (days)	Female progeny F1 (%)
SBW:MFM	Grundy	30	82.6a[b]	28.9ab	52.4a	30a	3.6a	73.3a
	Maine	30	87.7a	33.8a	59.0a	10b	3.9a	58.9a
	Plummer	30	72.8b	35.0a	61.1a	10ab	3.4a	63.5b
	Strong	30	86.1a	28.4ab	44.2a	17ab	3.2a	61.7b
	Vineland	30	89.1a	20.2b	44.8a	30ab	3.5a	67.0ab
MFM:MFM	Grundy	30	68.8a	22.5b	27.4b	0a	1.9a	72.2a
	Maine	24	72.3a	22.2b	29.0ab	0a	2.2a	58.3ab
	Plummer	33	79.2a	30.1a	37.5a	6a	2.2a	49.4b
	Strong	20	79.4a	28.1ab	33.4ab	0a	2.0a	66.0ab
	Vineland	20	75.8a	14.4c	19.7b	6a	2.4a	75.8a
MFM:SBW	Grundy	20	46.8b	22.4a	28.9a	0a	2.7b	84.2a
	Maine	20	55.7ab	3.1c	4.1c	50b	2.0b	71.4a
	Plummer	20	47.8b	16.2ab	20.3ab	0a	2.3b	37.2b
	Rogers	20	82.0a	13.4b	20.3ab	10a	3.5a	69.7a
	Strong	20	50.5b	9.8b	12.9bc	0a	2.2b	65.6a
	Vineland	20	59.1ab	12.4b	17.8b	0a	2.7b	64.3a

[a] MFM = *Ephestia kuehniella*; SBW = *Choristoneura fumiferana*. – [b] Means followed by the same letter within each column by host origin: host exposed are not significantly different at $p \leq 0.005$ (Duncan's [1951] multiple range test).

Differences among six strains of *T. minutum* were found in all parameters when parasitoids were reared on MFM and provided with eggs of SBW (table 5). The G strain had significantly higher fecundity than either M, S, or V while the R strain lived significantly longer than all other strains and had higher emergence than G, P, or S. Compared to the remaining strains, M and R both had a high proportion of non-fecund females, while P produced fewer female progeny. The relationship between rearing host (MFM) and target host (SBW) presented here is the same as that which would occur during inundative release of *T. minutum*. The poorest strain under these conditions was M strain. Strains G, V, and R would probably be rated equally because strain G had high fecundity and strains R and V had high emergence and proportion of female offspring.

The performance of *T. minutum* strains under all host conditions examined was relatively similar. As with rearing temperature, no one strain performed consistently better than the others under all conditions suggesting that the differences observed here are due to intraspecific variation. In general, the P strain had high fecundity, a low proportion of female progeny, and poor emergence. The G strain, however, produced a high proportion of female progeny while the V strain had low fecundity and a high proportion of non-fecund females.

The longevity, fecundity, and sex ratio of *Trichogramma* species are of key importance in the biology of these parasitoids because they represent the mechanisms by which parasitoid densities are self-regulated and they determine the level of reduction of host eggs (Kot 1979). All three factors depend upon a number of variables including age of female, host species, hydrothermal conditions, and food supply. One of the most important factors affecting these

parameters and one which few studies have dealt with in the past is parasitoid ecotype or strain. Our work has shown that distinct differences, both biochemical and biological, albeit small, can be identified for isolated populations within the same species of *T. minutum*. These results are not unexpected because local ecological pressures would cause a natural selection of those biological and thus, biochemical characteristics of a population that are most advantageous to that particular niche. For species of *Trichogramma*, however, local inbreeding in comparatively isolated populations considerably enhances this selective pressure. Several studies have shown that the biological characteristics of *Trichogramma*, as with other artificially produced insect populations, can be rapidly changed through continued rearing and selection in the laboratory (ASHLEY et al. 1974b; NAGARKATTI 1979; BIGLER et al. 1982).

The identification of distinct biological and biochemical differences among populations of *T. minutum* has important consequences for future mass production and release. It is apparent that some strains will be better suited to the standard rearing host and thus, will be easier to mass produce than others. These same strains when reared on this standard host, however, may not respond as readily to the target host when released in the field. In addition, some strains of *T. minutum* will be less tolerant of cool environmental temperatures than others. Individual strains, therefore, when released in the field against spruce budworm will have variable effectiveness as control agents. The recognition of strains within species of *Trichogramma* and their associated characteristics will aid in our selection of desirable candidate strains and improve the probability of success following inundative release. This identification and selection of parasitoid strains will also allow us to direct the polyphagous nature of *Trichogramma* species more specifically against individual lepidopterous species. If the biological and biochemical differences we observed can be related to the relative effectiveness of *Trichogramma* strains under field conditions, then we will no longer have to rely upon the mass production of parasitoids with unknown and often variable effectiveness.

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Zusammenfassung

Stämme des Eiparasitoiden Trichogramma minutum Ril. I. Biochemische und biologische Charakterisierung

Biologische Merkmale und Elektrophorese-Profile von 7 *Trichogramma minutum* Ril.-Stämmen, die aus verschiedenen geographischen Regionen des östlichen Nordamerika stammen, wurden miteinander verglichen. Zuerst wurden 16 Enzymsysteme mit Hilfe der Stärkegelelektrophorese untersucht. Sieben der Enzymsysteme erwiesen sich als brauchbar, um die Stämme zu unterscheiden. Proteinprofile zeigten keine auffälligen Unterschiede, um die Stämme zu differenzieren. Der mittlere Schwellenwert für die Entwicklungstemperatur war 10.5 (± 1.7 °C). Ein Stamm besaß eine signifikant höhere (166.2 °D) Thermal-Konstante als die übrigen Stämme (X = 137.8 °D). Biologische Merkmale, wie Anteil weiblicher Nachkommen, Lebensdauer und Fruchtbarkeit, differierten signifikant zwischen den Stämmen, wenn sie bei Temperaturen von 13, 15, 17, 20, 23

oder 27 °C und auf *Ephestia kuehniella* oder *Choristoneura fumiferana* gezogen wurden. Die genaue Identifizierung der *T. minutum*-Stämme ist von großer Bedeutung für die zukünftige Massenproduktion und die Verwendung der Schlupfwespe im Freiland.

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