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# Strains of the egg parasitoid *Trichogramma minutum* Riley

## II. Utilization for release against the spruce budworm

By S. M. Smith and M. Hubbes

**Abstract**

Strains of *Trichogramma minutum* Riley varied in their parasitism of spruce budworm eggs under field conditions. Biological indices (I) of these strains when reared under different conditions in the laboratory predicted their performance in the field whereas their respective rates of increase ($r_i$) did not. The electrophoretic relationship among the strains did not correspond with their biological relationship. To identify the strains of *T. minutum*, an electrophoretic key based upon estimated genotypic relationships was developed. When related to performance in the field, this key provided a simple means of identifying that strain of *T. minutum* best suited for inundative release against the spruce budworm, *Choristoneura fumiferana* (Clemens).

## 1 Introduction

Historically, the effectiveness of *Trichogramma* species in controlling pest populations has produced conflicting results. Failures have been mainly due to a lack of knowledge of the specific identity of the parasitoids and strains used and of their variable ecological potential (Kot 1968). Distinct strains are certain to develop in species of *Trichogramma* because of the tendency within this genus for submatings in isolated populations. The natural variability in these strains or ecotypes of *Trichogramma* has been linked by several authors with their relative effectiveness in the field (Kot 1979; Diehl and Bush 1984; Liying 1984; F. W. Quednau, pers. comm.). Accurate identification of these strains, therefore, will be critical to the success of future inundative releases with species of *Trichogramma*.

Berlocher (1980) has been instrumental in developing electrophoretic keys to aid in the identification of taxonomically controversial insect genera. Little use of such keys has been made, however, in the taxonomy of *Trichogramma*. Because they are based upon real, observable differences in isoenzyme bands, electrophoretic keys have a low probability of error. As such, they can be invaluable in the recognition of differences between insect populations whether at the generic or specific level. Few electrophoretic keys, however, have been applied to the identification of populations within a single species.

Life tables incorporating the effect of different environmental conditions upon rates of development, reproduction, and survival into an intrinsic rate of increase value ($r_m$) have been developed for several insect species (Birch 1948; Messenger 1964). Orphanides and Gonzalez (1971) used $r_m$ to differentiate between *T. pretiosum* Riley and *T. retorridum* Girault. Recently, Pak and Oatman (1982) developed life tables based upon fluctuating temperatures to

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compare the fitness of *T. brevicapillum* Pinto and Platner and *T. pretiosum* for mass release. In both cases, the rate of increase provided a reliable index for separating species of *Trichogramma*. To date, however, this value has not been used to describe intra-specific differences.

The present paper is the second in a two-part study to select a candidate strain of *Trichogramma minutum* Riley for biological control of the spruce budworm, *Choristoneura fumiferana* (Clemens). In our initial work, we characterized seven strains of *T. minutum* collected from different geographical regions, both biochemically and biologically (Smith and Hubbes 1986). In the present paper, our objective has been to develop a simple method for identifying these strains of *T. minutum* and relating their biological performance in the laboratory to their parasitism or efficacy in the field. This approach could then be used to provide a rapid method of selecting that strain of *T. minutum* best suited for inundative release against the spruce budworm.

## 2 Materials and methods

### 2.1 Electrophoretic studies

Details on the collection and identification of the seven strains *T. minutum* as well as biological studies and the electrophoretic techniques for their description have been reported previously (Smith and Hubbes 1986). Because electrophoretic patterns for individual *T. minutum* could not be resolved in this study, the actual frequencies of alleles or genotypes were not determined. There was little value, therefore, in examining this data further with standard principle coordinate analysis (Howard 1983; Pashley and Rai 1983). In order to describe the interrelationship of the strains, however, we estimated the genetic frequencies of the presumed alleles. The presence or absence of 32 bands from 13 hyphothetical loci and seven enzyme systems was described by Smith and Hubbes (1986) based upon their degree of similar migration and findings of previous authors (Jardak et al. 1979; Pintureau and Babault 1981, 1982; Berge et al. 1982; Klausnitzer et al. 1983). In the present paper, to estimate gene frequency for each strain of *T. minutum*, we considered each isoenzyme band from our initial study to be the product of one allele (monomeric). Where it was obvious that more than a single band was present for a particular presumed locus (*i.e.* ACPH3, EST5, and EST6), a frequency was assigned to each band based upon its staining intensity relative to other allelic bands at that locus (table 1). Average linkage hierarchical cluster analysis (SAS User Guide 1982, pp. 416–432) was then used to provide a visual summary of the electrophoretic relationship between the strains.

### 2.2 Biological studies

In our initial studies, the parasitoid strains reared at 13, 15, 17, 20, 23 and 27 °C and on either *C. fumiferana* or the Mediterranean flour moth, *Ephestia kuehniella* (Zeller) were compared biologically in terms of their developmental rate, fecundity, longevity, emergence, sex ratio of the progeny, and the proportion of non-fecund females produced (Smith and Hubbes, in litt.). In the present paper, we have summarized these data for each strain in rates of increase ($r_i$) and biological indices (I). Although the values were obtained from experiments conducted at different times, for statistical purposes, each test was considered a replicate because the test conditions were the same varying only in time.

Biological indices were calculated as described by Russo and Voegele (1982) where I = F + (%E)/10 + (T.S.)/10 − [(%M)/10 + (%St.)/2] and F = total fecundity per female, E = emergence, T.S. = proportion of female progeny, M = proportion of females living seven days, and St. = proportion of non-fecund females. Because this value required a measurement of female sterility (%St.), I was determined only from those experiments on different rearing hosts where the proportion of non-fecund females was measured.

Rates of increase were derived from life tables using daily age intervals. The accurate method of Birch (1948) was used to compute the rate of increase from the formula, $(\Sigma e^{-r_i x} \mid {}_x m_{x r_j} \rightarrow 1)$. It should be noted that the term, rate of increase ($r_i$) is used synonomously with Birch's term intrinsic rate of natural increase ($r_m$), however, $r_m$ is defined under optimal conditions. Because some of the experimental conditions described in the present study did not meet these criteria (*i.e.* no food

supplied to the adult parasitoids) and true fecundity was not measured but only estimated from the number of parasitized host eggs, we have equated $r_i$, here with Birch's $r_m$. To provide a means of relating these biological data with the electrophoretic data obtained earlier, the rates of increase and developmental rates (SMITH and HUBBES 1986) for each geographical strain of *T. minutum* were ranked and also subjected to hierarchical cluster analysis (SAS User Guide 1982, pp. 416–432).

*Table 1.* Estimated frequency of alleles for seven strains of *Trichogramma minutum* based upon observed differences in isoenzyme patterns in seven enzyme systems as described by SMITH and HUBBES (in litt.)

| Presumed Locus | Allele | Frequency of Allele by *T. minutum* strain | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Grundy | Maine | Plummer | Rogers | Strong | Vineland | Field | Total |
| ACPH3[a] | a) | 0.45 | 0 | 0 | 0.50 | 0 | 0 | 1.0 | 0.279 |
| | b) | 0.45 | 0 | 1 | 0.25 | 1 | 1 | 0 | 0.529 |
| | c) | 0.10 | 1 | 0 | 0.25 | 0 | 0 | 0 | 0.193 |
| ACPH4 | a) | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 0.571 |
| | b) | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0.286 |
| | c) | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0.143 |
| AK2 | a) | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0.286 |
| | b) | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0.571 |
| | c) | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0.143 |
| ∝GPDH5 | a) | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0.143 |
| | b) | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 0.714 |
| EST2 | a) | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0.143 |
| | b) | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0.857 |
| EST4 | | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0.857 |
| EST5 | a) | 0.15 | 0 | 0 | 0.45 | 0 | 0 | 0.75 | 0.193 |
| | b) | 0.65 | 0 | 0.7 | 0.45 | 0.45 | 0.8 | 0.25 | 0.471 |
| | c) | 0.15 | 0.75 | 0.3 | 0.1 | 0.1 | 0.2 | 0 | 0.229 |
| | d) | 0 | 0.25 | 0 | 0 | 0 | 0 | 0 | 0.064 |
| | e) | 0 | 0 | 0 | 0 | 0.45 | 0 | 0 | 0.064 |
| EST6 | a) | 0.2 | 0.1 | 0.4 | 0.4 | 0.45 | 0.45 | 0.45 | 0.350 |
| | b) | 0.8 | 0.7 | 0.4 | 0.4 | 0.45 | 0.45 | 0.45 | 0.521 |
| | c) | 0 | 0.2 | 0.2 | 0.2 | 0.1 | 0.1 | 0.1 | 0.129 |
| G6PDH | a) | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0.286 |
| | b) | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0.857 |
| IDH1 | a) | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0.286 |
| | b) | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0.714 |
| IDH2 | a) | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0.286 |
| | b) | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0.714 |
| IDH3 | a) | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0.286 |
| | b) | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0.714 |
| 6GPDH | a) | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0.143 |
| | b) | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0.857 |

[a] ACPH, acid phosphatase; AK, adenylate kinase; ∝GPDH, ∝-glycerophosphate dehydrogenase; EST, esterase; G6PDH, glucose-6-phosphate dehydrogenase; IDH, isocitrate dehydrogenase; 6GPDH, 6-phosphogluconate dehydrogenase.

### 2.3 Outdoor cage studies

Sleeve cages on the branches of white spruce trees were used to compare parasitism of spruce budworm eggs by strains of *T. minutum* in the field. Tree branches of similar size, condition, and location in the canopy were selected in Hearst, Ontario, Canada (50°N, 84°W). The cages were constructed of chicken wire rolled into a cylinder, 30 cm long and 32 cm in diameter, and covered with fine-mesh, nylon organdy. The material was glued together, longitudinally with approximately 8 cm of extra material left on either end. This cylinder was then stapled to a 30 cm length of 6 by 6 cm lumber. Predators and spruce budworm egg masses laid naturally on each selected branch were removed and the cage placed over the branch. Each cage was then tied onto the branch at its proximal end; the other end of the cage was closed with elastics. Under each branch a wooden stake was driven into the ground and the cage attached to it for support.

Strains of *T. minutum* were compared using three sentinel spruce budworm egg masses and 15 newly-emerged female parasitoids per cage. Based upon the average expected surface area of the branch in each cage, these values represented a severe infestation of spruce budworm (over 200 egg masses/9.29 $m^2$ of branch surface) and *ca.* 20.6 million female *T. minutum*/ha. Sentinel egg masses were obtained from spruce budworm moths reared in the laboratory on artificial diet in the early instars and on buds of white spruce in later instars. Adults obtained in this manner were provided with twigs of balsam fir for oviposition. These twigs were collected daily, cooled, and sent to the study area every three days. The three twigs were then attached to each branch within the cage by twist ties.

Parasitoids from parasitized eggs of *Sitotroga cerealella* (Olivier) were shipped weekly to the field from the Biological Control Laboratory at Guelph, Ontario. Within six hours of emergence, the 15 female *T. minutum,* were isolated into individual 0.5-dram vials. These vials were then opened, set on the bottom of each cage, and the cage closed with elastics. After four days, the cage was removed and measurements of the length and width of each branch taken to calculate surface area (Sanders 1980).

In 1983, four strains, Maine (M), Plummer (P), Strong (S), and Vineland (V), each replicated six times, were compared. In 1984, four separate experiments, each comparing seven different strains were conducted; Grundy (G), M, P, Rogers (R), S, V, and Field (F). Because there was a limited number of parasitoids of the same age, only five of the seven strains were selected for each experiment based upon those strains emerging simultaneously. The results from the four experiments were combined and treated together in a random block design, each experiment considered as a block.

## 3 Results

The expected frequency of heterozygosity (H) in randomly mating populations can be described where $H = 1 - (f_1^2 + f_2^2 + \ldots + f_n^2)$ for 'n' alleles with frequencies $f_1, f_2, \ldots, f_n$ (Ayala 1978). Based upon frequencies of the 13 presumed alleles in the present study and the 42 monomorphic loci from the remaining enzyme systems described by Smith and Hubbes (1986), the estimated average heterozygosity for *T. minutum* was 0.105 (+/− 0.028). In our study, heteromeric bands were considered as alleles and this, to some extent, may have affected our calculation of heterozygosity. Ayala (1978) stated that allelic frequencies at about 20 gene loci are necessary in order to determine the average heterozygosity of a population and reported the greatest degree of genetic polymorphism in an insect population to be 0.309. The level of heterozygosity and thus, genetic variation we obtained, however, for populations of *T. minutum,* although lower than many insect species, was not unexpected.

Cluster analysis of the electrophoretic data indicated that all strains of *T. minutum* belonged to a single group (fig. 1). Within this group, strains P and V had the greatest association although they were also closely linked with strains G and R while strains M, S, and F had the least relationship. These findings suggest that, electrophoretically, the strains represent one species of *T. minutum* with P and V closely associated and M, S, and F relatively distant.

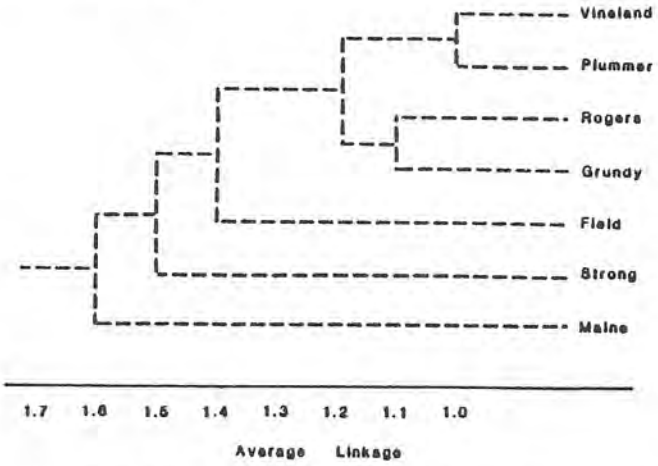


*Fig. 1.* Dendrogram obtained from electrophoretic studies showing the average linkage between seven strains of *Trichogramma minutum*

The mean biological indices (I) calculated for the strains of *T. minutum* reared under different host conditions suggested that strains P, G, and S, in decreasing order, had the greatest biological potential (table 2). Conversely, based upon I, strains R, M, and V appeared to have the least potential. The low ranking of strain R may be inappropriate here, however, because the value of I was derived from a single experiment. In this experiment, I ranged from −18.2 for strain M to 32.0 for strain G and strain R at 20.5 ranked second after strain G.

The rates of increase ($r_i$) for the strains of *T. minutum* were used to compare their potential for population growth at the six rearing temperatures and on the two different hosts (table 2). The mean value of $r_i$ for all strains increased as the rearing temperature increased; 0.04, 0.07, 0.11, 0.12, 0.21, and 0.21 at 13, 15, 17, 20, 23, and 27 °C, respectively. This undoubtedly reflected the longer developmental time associated with lower rearing temperatures. The rates of increase suggested that strains G and M had higher optimal rearing temperatures (27 °C) than the remaining strains (23 °C). Over all temperatures, however, the biological performances of the strains were relatively similar. Although P and S strains had slightly higher values ($\bar{x}$ = 0.14 females/female/day) and V strain the lowest value ($\bar{x}$ = 0.11 females/female/day), the differences between the strains were slight. The higher overall values for strains P and S may have been due to their relatively higher $r_i$'s at the lower temperatures (less than 17 °C) compared to the other strains.

All strains of *T. minutum* performed significantly better ($P \leq 0.05$) when reared on eggs of *C. fumiferana* ($r_i$ = 0.25 females/female/day) than on eggs of *E. kuehniella* ($r_i$ = 0.14 females/female/day). No significant differences in $r_i$ were seen for parasitoids reared on *C. fumiferana* and provided with *E. kuehniella* eggs although strains R and G had the highest values. When the strains were reared on *E. kuehniella* and provided with eggs of *E. kuehniella*, $r_i$ for strain V was significantly less than that for M or S strains. The opposite relationship occurred when strains of *T. minutum* were reared on *E. kuehniella* and provided with eggs of *C. fumiferana;* V strain had a

*Table 2.* Summary of the biological indices and rates of increse ($r_i$) for six strains of *Trichogramma minutum* reared at six different temperatures and on either *Ephestia kuehniella* or *Choristoneura fumiferana* and provided with eggs of either *E. kuehniella* or *C. fumiferana*

| Strain | Biological Index (I)[a] | Rearing temperature (°C)[b] | | | | | | | Host origin:Host provided[c] | | | | Overall Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 13 | 15 | 17 | 20 | 23 | 27 | Mean | SBW:MFM[d] | MFM:MFM | MFM:SBW | Mean | |
| Grundy | 35.5 | 0.05 | 0.07 | 0.11 | 0.13 | 0.20 | 0.24 | 0.13 | 0.27a[e] | 0.12ab | 0.19a | 0.19 | 0.15 |
| Maine | 24.2 | 0.01 | 0.07 | 0.11 | 0.12 | 0.20 | 0.21 | 0.12 | 0.24a | 0.22a | 0.05b | 0.17 | 0.13 |
| Plummer | 38.6 | 0.05 | 0.09 | 0.12 | 0.14 | 0.23 | 0.22 | 0.14 | 0.21a | 0.17ab | 0.07b | 0.13 | 0.14 |
| Rogers | 20.5 | 0.05 | 0.07 | 0.09 | 0.13 | 0.22 | 0.18 | 0.12 | 0.28a | 0.14ab | 0.11ab | 0.18 | 0.14 |
| Strong | 31.0 | 0.05 | 0.09 | 0.12 | 0.12 | 0.23 | 0.22 | 0.14 | 0.24a | 0.22a | 0.12ab | 0.19 | 0.16 |
| Vineland | 25.8 | 0.01 | 0.02 | 0.12 | 0.11 | 0.20 | 0.18 | 0.11 | 0.24a | 0.06a | 0.17a | 0.16 | 0.12 |
| Mean | | 0.04 | 0.07 | 0.11 | 0.13 | 0.21 | 0.21 | 0.13 | 0.25 | 0.16 | 0.12 | 0.17 | 0.14 |

[a] Each value represents a mean of three replicates except Rogers where the number of non-fecund females was determined in only one experiment.
[b] Host origin:host provided was *E. kuehniella:E. kuehniella*.
[c] Rearing temperature was 25°C.
[d] SBW = spruce budworm, *C. fumiferana;* MFM = Mediterranean flour moth, *E. kuehniella*.
[e] Means followed by the same letter within each column are not significantly different at $p \leq 0.05$ (Duncan's New Multiple Range Test).

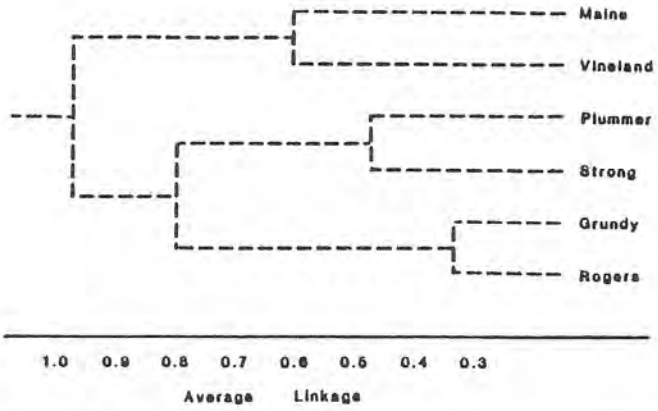


*Fig. 2.* Dendrogram obtained from cluster analysis of the rate of increase values ($r_i$) and developmental rates for six strains of *Trichogramma minutum*

significantly higher $r_i$ than M or P strains. Under these conditions, the G strain also had a significantly higher $r_i$ than the remaining strains.

Cluster analysis of the rates of increase and development for each geographical strain of *T. minutum* indicated that the parasitoid strains belonged to a single group (fig. 2). Biologically, the closest relationships were shown for strains M and V, strains P and S, and strains G and R. Of these, the only relationship that was also found in the electrophoretic study was that between strains G and R. In both studies, strain M separated out early and, therefore, was more distinct than the remaining strains. In general, however, the relationship of the strains based upon the cluster analysis of electrophoretic data did not correspond to that based upon biological data.

The effectiveness of the seven geographical strains of *T. minutum* in parasitizing eggs of the target host, *C. fumiferana,* was compared in sleeve cages under field conditions (table 3). No significant differences were observed between the strains in the proportion of female progeny produced. Fewer progeny, however, emerged from host eggs parasitized by M strain than either P, R or S strains. Strain V parasitized a significantly lower proportion of both eggs and egg masses than P strain. Strains M, P, R, and F parasitized the greatest proportion of egg masses while G, P, and R strains parasitized the greatest proportion of eggs.

## 4 Discussion

Similarities in the mean values of $r_i$ for the six strains of *T. minutum* (0.13 and 0.17 females/female/day for rearing temperature and host respectively) suggest that all these parasitoids were the same species. ORPHANIDES and GONZALEZ (1971) found the value of $r_m$ to be 0.32 and 0.28 females/female/day for *T. pretiosum* and *T. retorridum,* respectively, when reared on *S. cereallela* at 25 °C. PAK and OATMAN (1982) reported values for *T. brevicapillum* and *T. pretiosum* at 25 °C (+/− 5.0 °C) on *Trichoplusia ni* (Hübner) eggs of 0.33 and 0.34 females/female/day, respectively, while NAGARKATTI and NAGARAJA (1978) reported a $r_m$ of 0.30 females/female/day for *T. confusum* Viggiani reared on *Corcyra cephalonica* St. The mean for $r_i$ in the present study, for all

*Table 3.* Parasitism of spruce budworm eggs by strains of *Trichogramma minutum* in field cages in Hearst, Ontario, Canada

| Year | Strain | n (No. of egg masses) | Branch surface area ($cm^2$) | Eggs parasitized (%) | Egg masses parasitized (%) | Progeny/ parasitized egg | Female progeny (%) |
|---|---|---|---|---|---|---|---|
| 1983 | Maine | 18 | 1023a[a] | 44.0a | 60.0a | 1.6a | 85.6a |
| | Plummer | 18 | 904a | 10.6a | 16.7a | 0a | — |
| | Strong | 18 | 1088a | 21.9a | 25.0a | 1.2a | 46.2a |
| | Vineland | 18 | 904a | 13.7a | 25.0a | 1.4a | 72.4a |
| 1984 | Grundy | 36 | 1404a | 46.8a | 54.3ab | 1.8ab | 78.1a |
| | Maine | 36 | 1321a | 33.8ab | 66.7a | 1.8b | 73.9a |
| | Plummer | 48 | 1377a | 45.1a | 74.0a[1b] | 2.3a | 67.0a |
| | Rogers | 36 | 1226a | 45.3a | 66.8a[1] | 2.3a | 70.4a |
| | Strong | 24 | 1381a | 22.6b | 52.1ab[2] | 2.3a | 63.6a |
| | Vineland | 36 | 1476a | 18.7b | 38.8b[2] | 1.8ab | 82.4a |
| | Field | 24 | 1416a | 38.6ab | 62.6a | 2.5ab | 67.4a |

[a] Means followed by the same letter within each column by year are not significantly different at $p \leq 0.05$ (Duncan's (1951) multiple range test).

[b] 1. Consistently high in all replicates.
2. Consistently low in all replicates.

strains reared on *S. cereallela* at 25 °C would be 0.21 females/female/day. ORPHANIDES and GONZALEZ (1971) considered $r_m$ consistent enough to aid in differentiating species of *Trichogramma*. The lower $r_i$'s we found for all strains of *T. minutum* may be due to biological differences between the species. It is more likely, however, that the lack of food supplied to the adult parasitoids upon emergence and the associated reduction in their longevity had a more significant impact.

At different rearing temperatures, strains P and S had the highest $r_i$'s while

V strain had the lowest. In the experiments with different rearing hosts, however, on average, P strain had the lowest $r_i$ while strains G and S had the highest. When the overall average $r_i$ values were examined, strains S and G ranked the highest whereas strains V and M ranked the lowest (table 2).

In the field, female parasitoids of strains P and R appeared to be the most effective, consistently parasitizing the greatest proportion of both host eggs and egg masses; females from strain V were the least effective. Generally, the effectiveness of the strains corresponded to their biological indices (I); strain P had a high value whereas strain V was relatively low. In contrast, the laboratory based values of $r_i$ corresponded with parasitism in only one case; V strain. Based upon the value for $r_i$, V strain was generally selected as the worst strain in both experiments. Under the same conditions, however, strains P and R were never the best candidates for release. It appears then that the value of I is much more indicative of effective strains under field conditions than that of $r_i$. This may be due to the fact that $r_i$ is dependent upon the proportion of female progeny produced and this is not of prime importance in inundative releases. Improvements in the predictive value of biological indices might be made if other attributes such as host searching and behaviour were included in the assessment of parasitoid quality (Pak and Van Heiningen 1985).

The results from the preceeding studies may reflect, to some extent, the various lengths of time the strains were reared under laboratory conditions. By 1984, strain V had been maintained for approximately 254 generations whereas strains P and R had been maintained for only 146 and 102 generations, respectively (Smith 1984). A reduction in effectiveness for laboratory-reared *Trichogramma* has been shown by Ashley et al. (1973) and Nagarkatti and Nagaraja (1978). In addition, strain V was the only strain isolated from southern Ontario and not collected from eggs of *C. fumiferana*. This factor is probably more important than the number of generations reared in the laboratory because although the F strain had only been reared for 54 generations, it did not perform better than P or R strains in the field.

Generally, electrophoresis underestimates the amount of genetic variation in a species; not all nucleotide differences result in different protein composition and not all amino acid substitutions in a protein result in different electrophoretic mobilities (Ayala 1978). As Berlocher (1984) points out, however, the fact that electrophoresis fails to discriminate all polypeptides does not make detectable differences any less real. Our work shows that species of *Trichogramma* can be characterized enzymatically and that there are consistent electrophoretic differences between strains of *T. minutum* (Smith and Hubbes 1986). In many insect species, electrophoretic differences can be related directly to morphological and biological differences (Berlocher 1980; Pashley and Rai 1983) and our data suggest that this relationship may also hold true for strains within a single species.

The future feasibility of using inundative releases of *T. minutum* will depend to a large extent upon the ability and speed with which 'superior' strains can be identified and screened from mixed populations collected in the field. The application of isoenzyme techniques to this problem provides a reproducible and precise method for monitoring genetic traits and thus, identifying 'diagnostic' patterns for strains of *T. minutum*. Enzymatically, strains P and V had the closest relationship, however, these two strains had very different levels of parasitism in the field. Despite this inconsistency, an electrophoretic key which reflects the relationship of parasitism in the field can

*Table 4.* Electrophoretic key for strains of *Trichogramma minutum* corresponding to their effectiveness ranked in field cages

| | | |
|---|---|---|
| 1. | 6GPDH;[a] | |
| | Same mobility as standard[b] | 2 |
| | Slower mobility than standard | Vineland (poor performance) |
| 2. | ∝GPDH5; | |
| | Same mobility as standard | 3 |
| | Slower mobility than standard | 4 |
| 3. | IDH 1.3; | |
| | Same mobility as standard | 5 |
| | Slower mobility than standard | Grundy (intermediate) |
| 4. | IDH 1.3; | |
| | Same mobility as standard | Maine |
| | Slower mobility than standard | Strong (intermediate) |
| 5. | G6PDH; | |
| | Same mobility as standard | Plummer |
| | Faster mobility than standard | Rogers (good performance) |

[a] Systems run in 12.4% starch gel 0.04M citric acid buffer (pH = 6.7), 200 volts, until front measures 10 cm from origin (Smith 1984). 6GPDH, 6-phosphogluconate dehydrogenase; ∝GPDH, ∝-glycerophosphate dehydrogenase; IDH, isocitrate dehydrogenase; G6PDH, glucose-6-phosphate dehydrogenase.

[b] The P strain of *T. minutum* was used as the standard. Branches of the key were determined according to the relative mobility of the common allele of the P strain versus the unknown strain. Differences in mobility must be greater than, or equal to, one millimeter.

be constructed (table 4). Such a key requires only four enzyme systems to classify and predict the efficacy of an unknown strain of *T. minutum.*

Strain F was used to test the ability of our electrophoretic key in predicting parasitism by strains of *T. minutum* under field conditions. This strain was collected from the field in 1983 following the release of strain P. Strain F was considered an unknown strain, however, because it had been collected in an area where the native population was strain R. Both strains P and R were rated as having good performances under field conditions and were identified by the same couplet in the electrophoretic key. Using this key, strain F would be classified with strain P. Parasitism by strain F in the field was not significantly

different from that of either strains P or R (table 3). Although this tends to confirm the usefulness of the key and suggests that such an approach can be used to quickly identify and screen good strains of *T. minutum*, further trials using other unknown strains of *T. minutum* are needed. In future work, it will also become increasingly necessary to be able to correlate these observed genotypes with biological characteristics such as I for successful strains of parasitoids.

## 4 Conclusions

Strains of *T. minutum* from different geographical regions will have different biological and electrophoretic parameters and this intraspecific variability will affect levels of parasitism in the field. In our study, the performance of *T. minutum* strains in the field generally corresponded to their biological indices, but could not be linked with their rates of increase or their tolerance to changes in rearing temperature and host in the laboratory. This difficulty in translating laboratory results to the field makes further investigations of prime importance for continued successful releases.

In the future, we must consider strains of *T. minutum* to be dynamic. As biological organisms, they can be modified to improve their efficacy in the field. BABI and PINTUREAU (1984) found that the number of eggs parasitized by *T. maidis* Pintureau and Voegele had a high degree of heritability ($h = 0.45$ by the mother-daughter regression method) and that improved fecundity could be selected in the laboratory. If these results could be translated to the field, we would be one step closer to engineering the type of biological agent we require. *Trichogramma* has been criticized for its polyphagous nature. With identification, selective breeding, and biological engineering we may be able to improve upon such biological factors as host selection, host discrimination, and searching efficiency and, thereby, develop a relatively specific parasitoid for improved use in individual pest situations. Biochemical markers are available for identifying strains of *T. minutum;* what we now need is the ability to use these markers and correlate them more precisely with biological characteristics of parasitoids and their relative efficacy under field conditions.

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## Zusammenfassung

*Stämme des Ei-Parasitoiden, Trichogramma minutum Riley. II. Verwendung gegen den Tannenwickler, Choristoneura fumiferana (Cl.)*

Stämme von *Trichogramma minutum* variierten unter Freilandbedingungen in ihrer Parasitierungsquote bei Eiern von *Choristoneura fumiferana*. Die biologischen Merkmale dieser Stämme, die unter verschiedenen Bedingungen im Labor gezüchtet wurden, erlaubten eine Vorhersage ihrer parasitischen Tätigkeit im Freiland, während ihre Vermehrungsraten (v) diesbezüglich keine

brauchbaren Angaben lieferten. Die elektrophoretisch meßbaren Merkmale und Beziehungen der Stämme untereinander entsprachen nicht denjenigen der biologischen Merkmale.

Zur Bestimmung der einzelnen Stämme von *Trichogramma minutum* wurde an Hand von Isoenzymprofilen ein Bestimmungsschlüssel entwickelt, der auf den genotypischen Beziehungen basiert. Hinsichtlich der Anwendung unter Freilandbedingungen bietet der Bestimmungsschlüssel den einfachsten Weg zur Bestimmung desjenigen Stammes von *T. minutum*, der zur Bekämpfung von *C. fumiferana* am besten geeignet ist.

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