

A LARVAL BLACK FLY CONTROL FIELD TRIAL USING MERMITHID PARASITES AND ITS COST IMPLICATIONS^{1, 2}

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ABSTRACT. Treatment of a small stream with *Neomesomeris flumenalis* preparasites resulted in black fly larval infection rates of up to 71.4%. The preparasite density and black fly infection rate decreased markedly and progressively below the point of introduction indicating poor preparasite dispersion. *Simulium venustum* larvae were more readily parasitized than *S. vittatum*. A substrate density of 0.7 preparasites/cm² was required to achieve a PR₅₀ (parasitism rate) for *S. venustum*.

By extrapolating beyond the parasitism rate achieved, it was calculated that 10 preparasites/cm² would be required for a PR₅₀. Maintenance of these high preparasite densities in a typical black fly producing stream (e.g. 2.5 meters wide) would require application of approximately 3.6 x 10⁹ preparasites per mile of stream. Cost for production of such large quantities of preparasites is at present too great even for intermediate stage field trials.

INTRODUCTION

The possibility that mermithid nematodes can be used for the biological control of black flies has recently received considerable attention (Anonymous 1972, Gordon et al. 1973, Jamnback 1973, Nickle 1974). In the northeastern United States and adjacent areas of Canada, research efforts have concentrated primarily on *Neomesomeris flumenalis* (Welch), a mermithid commonly found parasitizing black flies (Bailey et al. 1974, Ebsary and Bennett, 1973, 1975, Ezenwa 1973, 1974). The laboratory feasibility of controlling larval black flies by application of these preparasites has been demonstrated (Molloy and Jamnback 1975). The present report describes a trial in a small stream using the same technique to evaluate its feasibility under field conditions and to provide a

basis for estimating the costs of black fly control with this parasite.

MATERIALS AND METHODS

N. flumenalis postparasites were collected from streams in the Rensselaerville, New York area throughout April 1975. Rocks containing large numbers of infected *Prosimulium* spp. larvae were placed in a large metal funnel (height - 53 cm, end diameters—7 cm and 36 cm). Water was poured through to detach black fly larvae. These were strained from the washwater by a screened cylinder located beneath the funnel. Allowing the rocks to air-dry for 5 min prior to washing facilitated detachment of the larvae. They were then transferred onto pieces of organdy draped across aquaria. The water above the organdy was aerated and the aquaria held at 12°C. Emerging postparasites crawled down through the organdy mesh and sank to the bottom. They were then pipetted into round plastic dishes (35 mm x 28 mm) containing sterilized streamwater underlaid by 10 mm of coarse sand and incubated at 12°C. About 8,000 nemas were processed in this manner with rearing dishes holding an average of 4 males

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and 4 females. The majority of females oviposited during the summer months with their eggs hatching in early fall. The stream test was run when large numbers of newly hatched preparasites became available.

A very small stream on the property of the New York State Science Service Laboratory for Biological Control in Cambridge, N.Y. was selected as the site for the field trial. Five stations in the stream were sampled: the 1st was 2 m upstream from the treatment point, the 2nd immediately below the treatment point, and the next 3 stations, 5, 10 and 20 m, respectively, downstream from the treatment point. The stream at these stations averaged 21 cm (15–25 cm) in width, 5.5 cm (3.5–8.0 cm) in depth and 33.4 cm/sec (25.0–41.0 cm/sec) in current velocity. Since only small populations of larval black flies would normally have been present within the stream in mid-October when the trial was carried out, the stream was heavily stocked with *S. venustum* Say and *S. vittatum* Zett. eggs during the 40 days preceding. At the beginning of the trial, larvae of all instars were present throughout the stream. Active laboratory-reared *N. flumenalis* preparasites were siphoned into the stream between 11 and 11:30 a.m. on each of 4 consecutive days, October 14–17, 1975. A total of ca. 1.5 million preparasites was added to the stream (estimated from aliquot samples). During the test period, the streamwater remained close to 10.6°C., unusually constant because it issues from a spring-fed pool. The stream contained abundant vegetation, primarily *Spirogyra*, *Vaucheria* and trailing grasses. These were used as attachment sites by black fly larvae and provided a suitable substrate for the preparasites seeking their hosts.

Black flies were collected at each station twice daily (1 hr before and 2 hr after each treatment). An additional sampling was also made on October 18,

1975, approximately 23 hr after the final treatment. Substrates (primarily trailing grasses) were collected at each station and taken to the laboratory where all black fly larvae were removed under a stereomicroscope. The larvae were dissected and examined for the presence of nemas within their hemocoels using a compound microscope at 150X. The number of active preparasites crawling about on the sampled vegetation was also noted and their density determined. An additional sampling of stream substrates for preparasite density data only was conducted at all stations immediately following each daily treatment. Preparasite densities, black fly infection rates and superparasitism ratios (average number of nemas per infected larva) for each station were recorded (Table I).

RESULTS AND DISCUSSION

INFECTION RATES. The highest infection rate, 71.4% (35/49), was recorded immediately below the treatment point in early instar *S. venustum*. At all stations infection rates were consistently higher in *S. venustum* than in *S. vittatum* larvae. Since both species were almost always present together on the sampled vegetation, these data imply a lower susceptibility of *S. vittatum* to *N. flumenalis* infection. Preparasite densities, infection rates, and superparasitism ratios all decreased markedly below the treatment point indicating poor preparasite dispersion (Table I). Since preparasites live only about 48 hr. (12–120 hr) in the laboratory and are very active only for about the 1st half of this period (unpublished data), there probably is no appreciable subsequent dispersion and infection.

Probit analysis indicates that an average preparasite density of 0.7 preparasites/cm², maintained for 4 days on stream substrates was required to achieve a PR₅₀ (50% parasitism rate) in

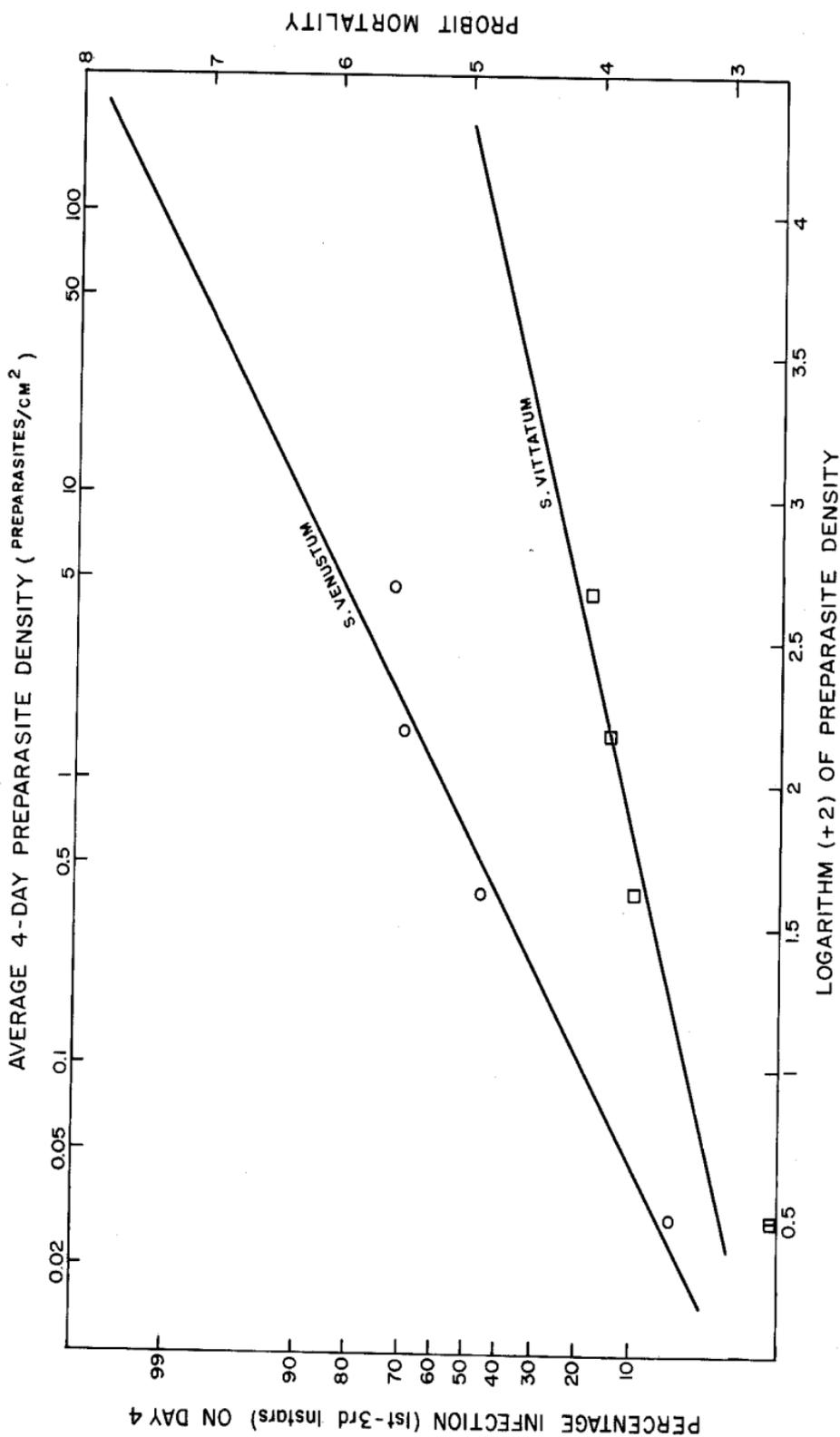


Fig. 1. Relation of infection rates of *S. venustum* & *S. vittatum* larvae (Instars I-III) to the density of preparasites of *N. fluminalis*.

Table 1. Density of *N. flumenalis* preparasites and blackfly larval infection and superparasitism rate at various locations in a stream following an experimental release.

Distance from point of application (M)	Average preparasite density/cm ² ^a	Percentage of hosts infected after 4 days ^b		Average number of nematodes per infected host ^a	
		<i>Simulium</i>		<i>Simulium</i>	
		<i>venustum</i>	<i>vittatum</i>	<i>venustum</i>	<i>vittatum</i>
-2	0	0	0	0	0
0	4.7	71.4	20.1	4.3	2.0
5	1.5	68.7	14.0	2.0	1.5
10	0.4	44.2	10.1	1.8	1.6
20	.03	5.5	1.2	1.1	1.0
No. larvae examined	565	1990	189	318

^a Averages for 4 days of releases.

^b Data on 1-3rd instar hosts only.

1st-3rd instar *S. venustum* larvae (Figure 1). Extrapolation of this probit line beyond the parasitism rate achieved indicates that 10 preparasites/cm² maintained for 4 days would have been necessary to obtain a PR₉₀. The infection rate achieved for *S. vittatum* was too low for meaningful probit analysis.

The high superparasitism ratios in this field trial are believed to be a consequence of the intensive method in which the stream was treated. Application of the same number of preparasites over a longer time period would probably have more closely simulated natural conditions and resulted in lower superparasitism ratios.

COST ANALYSIS. If the calculations of this small field trial are applied to a "typical" New York *S. venustum*-breeding stream 2.5 m wide by 0.3 m deep, (about 5.0 m x 0.3 m considering the normally irregular streambottom and sides as a simple one layer surface), about 3.6 x 10⁹ preparasites would have to be applied per mile to achieve a theoretical PR₉₀. For purposes of this preliminary study, the effects of varying stream conditions (e.g., current velocity, turbulence, type substrate, water temperature) on preparasite performance and inoculum quantity require-

ments were not evaluated. Since the price of chemical larvicides to treat one mile of this "typical" black fly stream would be about 40¢ for methoxychlor or \$2.00 for Abate® (both at 0.1 ppm/20 min), preparasite production costs would have to be reduced from our present \$300/million to approximately 15¢/billion to be competitive. Even assuming that major advances in *in vitro* and/or *in vivo* rearing of these parasites may occur, it is doubtful whether preparasite production costs could ever be reduced to this figure.³

Stocking streams with *N. flumenalis* in stages other than the preparasitic would introduce variables of synchronization of worm and host development that would require large quantities of inoculum to produce equivalent numbers of preparasites when black flies are in the susceptible 1st to 3rd larval instars. Even if high rates of infection were achieved by mermithid application, retreatment on

³ After 10 years of research and development on the mosquito mermithid *Romanomermis culicivorax* Ross and Smith (= *Reesimermis nielseni* Tsai and Grundmann), they are now commercially available (*in vivo* production) at \$15/million preparasites (D. Chittick, Fairfax Biological Laboratory, Inc., Clinton Corners, N.Y.).

a regular basis would probably be required for most streams. Natural populations of *N. fluminalis* typically exert only minor control (less than 20% infection) of black fly larval populations, and thus effective long-term control levels are not likely to persist from a single artificially created epizootic.

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