LABORATORY TRANSMISSION OF MERMITHIDS PARASITIC IN BLACKFLIES

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ABSTRACT. Laboratory studies indicate that control of blackflies with mermithid parasites may be technically feasible. The sole method by which *Neomesomermis flumenalis* preparasites infected larvae of *Simulium vittatum* was by direct penetration of their integument. In tests in running water, simulating the natural habitat of immature blackflies, transmission rates of about 80% for first instar larvae, and 64% for second instar larvae were achieved. Infection rates were significantly lower for later instars and 0% for pupae. The presence of detritus was an important factor in obtaining high rates of parasitism as it allowed the preparasitic nemas to crawl about in search of blackfly larvae without being swept downstream.

A 1973 agreement between the governments of Dahomey, Ghana, Ivory Coast, Mali, Togo and Upper Volta and the World Health Organization to initiate a long term control program for onchocerciasis (river blindness) in the Volta River Basin has focused attention on this filarial disease and on the control of its vectors (family Simuliidae). Although current plans call for the use of the larvicide Abate®, there has also been considerable interest in the possible use of parasites for biological control since blackflies are commonly infected with protozoans, fungi and nematodes (Jamnback 1973).

The present report deals with laboratory studies on the technical feasibility of using mermithid parasites (Nematoda: Mermithidae) in such a control program. The primary objective of this study was the achievement of consistently high rates of mermithid parasitism under standardized laboratory conditions. Defining the exact mode of infection and the relative susceptibilities of individual blackfly life stages were critical aspects of this research.

Of the known blackfly pathogens, mermithids at present attract the most interest as potential biocontrol agents. Although the species found in blackflies are not found in other organisms, they appear to exhibit little host specificity among the species of Simuliidae. The mermithid *Neomesomermis flumenalis* (Welch), for example, has been reported parasitizing at least 14 species of blackflies in 3 genera.

High rates of mermithid parasitism are common in blackfly populations, and studies in Wisconsin (Phelps and DeFoliart 1964) and Russia (Welch and Rubtsov 1965) have found evidence of short-term eradication of individual simuliid species in localized areas. The infected larvae are killed when the nema emerges from the fly's hemocoele by boring out through its integument.

MATERIALS AND METHODS

Rocks covered with large numbers of late instar *Prosimulium mirtum* Syme and Davies larvae infected with *N. flumenalis* were removed from Becker Pond Outlet, Berne, New York between April 5 and 15, 1974 and placed in aerated aquaria maintained at about 12 degrees C. Postparasitic mermithids emerging during this time period were pipetted from the aquaria and transferred into round plastic dishes (35 mm x 10 mm) containing sterilized stream water underlaid by 5 mm of coarse sand. Dishes were incubated at 12 degrees C., and optimal temperature for rearing *N. flumenalis* postparasites (Ebsary and Bennett, 1973). Fully embryonated eggs and preparasites produced by these mermithids were used in laboratory transmission trials during September and October, 1974.

All larvae and pupae used in these tests were *Simulium vittatum* Zett. The eggs of this species had been field-collected from June through September, 1974 in the vicinity of Long Lake, New York and kept under moist conditions at 5 degrees C. When blackfly larvae were needed, the eggs were placed in water and depending on the maturity of the eggs and water temperature, first instar larvae hatched in 1 to 7 days.

Transmission tests were run both in troughs (running water) and dishes (still water). The amount of transmission was determined 24 hr after introduction of the parasites by dissection of the exposed blackflies.

TROUGH TESTS. The troughs, described elsewhere in some detail (Jamnback and Frempong-Boadu, 1966), were approximately 1 m long, 0.5 m wide and 15 cm deep. Water (9 to 10 degrees C.) from a spring-fed pool flowed in at one end and out the other over a 10 cm x 15 cm horizontal lip in a thin film (0.5 to 0.8 cm deep) at the rate of 21 cm/sec. Plankton and other microscopic food contained in the water were sufficient to permit normal feeding and rearing of blackflies from eggs to adults without daily attention. *S. vittatum* eggs were periodically hatched out on the upstream edge of the trough lip so that a wide variety of blackfly life stages were available during the testing period. Both preparasites and eggs containing nemas ready to hatch were used in the trough tests. The eggs and preparasites were carefully released with a micropipette so that they came into contact with the cephalic fans of the blackflies as well as the detritus adhering to the trough lip. Nemas used as inoculum in troughs were inserted directly into cocoons in tests of pupal susceptibility. The activities of the blackfly larvae and pupae, nema and nema eggs were observed through a stereomicroscope (figure 1). In all trough tests, the concentrations of preparasites listed in table 1 were maintained during the 24-hour testing period by hourly reapplications. The concentrations could be only roughly approximated since preparasites were often partially or completely concealed by detritus, and vision was obscured within the test area by the water's flow. Only a small delimited section of the trough lip was used in each test to facilitate observation of both blackflies and parasites.

In test no. 1 (table 1), larvae were removed from the lip during the test period as soon as they exhibited symptoms of infection. In all other trough tests, blackflies were removed from the lip only at the end of the 24-hour exposure period. Each trough test included a parallel check with an identical or nearly identical number of blackflies not exposed to nemas.

DISH TESTS. Blackfly larvae and pupae

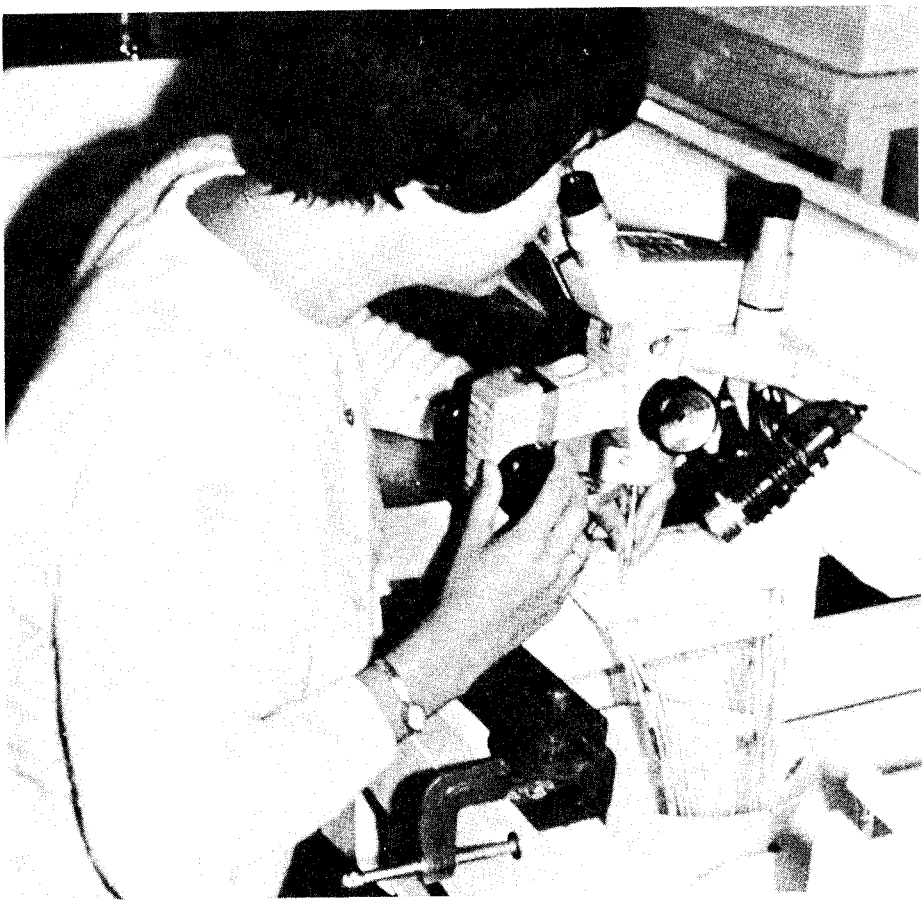

Fig. 1. Examining trough lip through microscope.

used in these tests were placed in round glass dishes (3 cm x 1 cm) containing 5 ml of sterilized stream water and 120 active preparasites (table 1). Duplicate series of tests with a single control were run with 1st instars, 3rd to 6th instars, and pupae. After holding for 24 hr at 12 degrees C., the blackflies were dissected and examined for infection.

RESULTS

TROUGH TESTS. Direct penetration of the blackfly larval cuticle was the sole mode of infection by preparasitic nemas of *N. flumenalis*. This is typically the case for mosquito larvae (Chapman 1974) and contrasts with the theory and belief that infection takes place when the preparasites are ingested and subsequently penetrate the gut wall to enter the hemocoele (Weiser, 1964; Welch, 1964). Penetration of the integument and passage of preparasites into the hemocoeles of 1st and 2nd instar larvae were witnessed in at least 50 instances. The average transmission rates were 1st instars—80% (3 trials); 2nd instars—64% (3 trials); 3rd to 6th instars—2% (2 trials); pupae—0% (1 trial) (table 1). Infected larvae

Table 1. Laboratory transmission of preparasitic nemas to *Simulium vittatum* larvae and pupae

Test no.	Blackfly larval instar or stage	Preparasitic Nemas/B'fly	Transmission rate after 24 hrs. No. parasitized/No. examined
		Trough tests[1]	
1	1st	1.6 (12.5cm²)	81/99 (81.8%)
	2nd		20/25 (80.0%)
2	1st	1.5 (7.5cm²)	21/26 (80.8%)
	2nd		8/13 (61.5%)
3	1st	1.7 (14 cm²)	8/12 (66.7%)
	2nd		6/15 (40.0%)
	3rd		0/15 (0.0%)
4	3rd-6th	1.0 (60 cm²)	2/80 (2.5%)
5	pupae	2/pupal cocoon	0/10 (0.0%)
		Dish tests[2]	
1	1st	4	9/30 (30.0%)
			5/30 (16.7%)
2	3rd-6th	3	0/40 (0.0%)
			0/40 (0.0%)
3	pupae	10	0/13 (0.0%)
			0/12 (0.0%)

[1] Test area size in parenthesis
[2] In 5 ml of water.

contained one or more nemas in their hemocoeles. No mermithid-parasitized blackfly larvae were found in any of the untreated control troughs. Phelps & De-Foliart (1964) reported only a 5% rate for *Gastromermis viridis* in *Simulium vittatum*.

In our tests, a significant proportion of the infected larvae did not survive. Of all 1st and 2nd instars infected in tests 2 and 3, 18 of 43 (41.9%) died or were moribund within 24 hours. (The data of test 1 are not included because the live larvae were removed as soon as they became infected.) These data suggest that reports of the overall mortality caused by mermithids in natural blackfly populations have been underestimated.

DISH TESTS. Transmission rates in these tests (still water) were appreciably lower than in troughs with running water (table 1). The major difference was that in running water the preparasites remained in close contact with the substrate; in still water they swam freely about with no indication of directional movement even when they came into accidental contact with blackfly larvae. In these tests 14 of 60 (23%) of the 1st instar larvae were parasitized, but none of the older larvae (3rd to 6th instar) or pupae became infected. A comparison of the susceptibility of 1st instar larvae (Dish Test 1) with 3rd to 6th instars (Dish Test 2) was hampered by the ingestion of almost all preparasites by the larvae in this latter test; dissection of these larvae indicated that ingestion destroyed the nemas. Blackflies were unparasitized in all parallel control dish tests.

DISCUSSION

The preparasites behave differently in still and moving waters. Lacking both a current and suitable substrate to crawl on, preparasites in still water wriggle aimlessly. In *moving* water preparasites have

directional movement. Active preparasites pipetted onto the detritus deposits on the trough lips were capable of crawling through the detritus along the surface of the metal trough lip. The long tapered tails of the preparasites (figure 2) were used to maintain themselves in flowing water.

The host-searching activity of preparasites on trough lips was not highly efficient. The posterior half of the worm remained in a fixed position, while the anterior half clumsily moved from side to side. If the preparasite failed to come in contact with a larva, the worm crawled to a new location and resumed its side-to-side movement. The direction of this host-seeking movement appeared to be almost random until the nema came within ca. 1 mm of a larva.

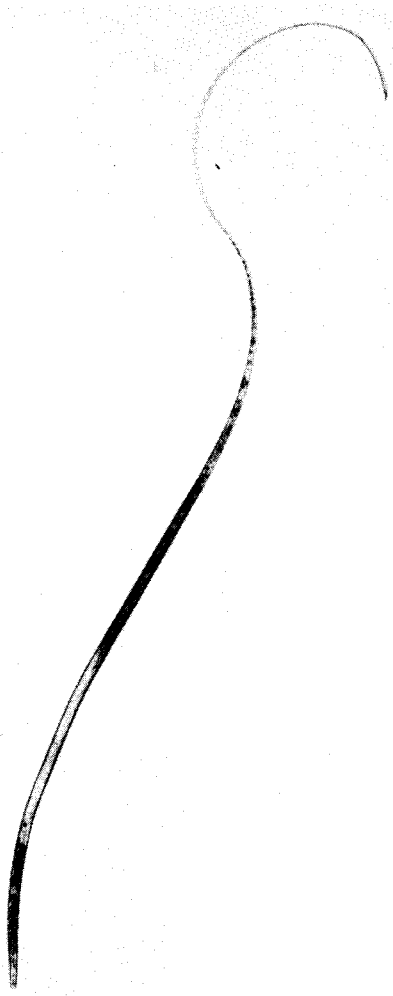

Fig. 2. Preparasitic nema.

The blackfly larvae often exhibited a vigorous defense against attacking nemas. When contacted by a nema, larvae responded quickly, attempting to avoid the worm or seize it with its mouthparts.

Succesful attacks on 1st and 2nd instar blackfly larvae were witnessed repeatedly and proceeded generally as follows: a nema wrapped itself tightly around either the blackfly's proleg or thorax while the larva moved in quick jerking movements, opening and closing its mandibles in a vain attempt to grasp the nema; the integument was quickly pierced by the nema's stylet, and within a few minutes the worm gradually disappeared from sight as it gracefully coiled into the fly's hemocoele. Occasionally the attempt to penetrate was unsuccessful and both nema and blackfly larva eventually died with the worm partly inside (figure 3). The thorax was the most frequent site of attack, but penetration of the integument was also observed in the abdomen and postgenal cleft. While the nema was entering, and for several minutes thereafter, the larva appeared partially paralyzed with its body often in an atypical contorted position. If the larva survived, normal movement and feeding gradually was resumed within an hour.

The presence of detritus is believed to be a key factor in the achievement of the high rates of parasitism. Early instar larvae which were attached to detritus deposits on the trough lips were almost always successfully parasitized, whereas larvae in clear areas remained uninfected during the tests.

Attempts to initiate infection in larvae through ingestion of mermithids proved unsuccessful. Preparasites and nema eggs were fed to individual blackfly larvae in

Fig. 3. Preparasitic nema entering first instar blackfly larva.

troughs using micropipettes to release the worms into the larval mouth. First and second instar larvae were unable to retain the preparasites within their cephalic fans in running water. Preparasites ingested by later instar blackfly larvae were found dead usually with their body walls rupturned when the host was dissected. Ingested, fully embryonated eggs were likewise found ruptured or unhatched in the digestive tracts of blackfly larvae.

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