APPLICATION OF A MERMITHID PARASITE OF MOSQUITOES WITH AN AERIAL SPRAY SYSTEM [1]

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ABSTRACT. Tests were conducted to determine if the preparasitic (infective) stage of the mermithid nematode *Romanomermis culicivorax* Ross and Smith (=*Reesimermis nielseni* Tsai and Grundmann, auct., partim.) could be applied from a helicopter via a Simplex low profile aerial spray system equipped with 12 TeeJet[R] spray nozzles. Results from ground spray application at 25 psi indicated that preparasites suffered no apparent adverse effects from impact within the spray system and nozzle assemblies when compared with unsprayed preparasites, and therefore indicated that this aerial spray system would be an effective means of disseminating the preparasitic stage of *R. culicivorax* for mosquito control.

Research aimed at determining the mosquito control potential of the mermithid nematode *Romanomermis culicivorax* Ross and Smith (=*Reesimermis nielseni* Tsai and Grundmann, auct., partim.) has indicated its effectiveness in suppressing natural populations of several species of mosquitoes in small plot field trials (Petersen et al. 1972, 1973; Petersen and Willis 1972a, 1974, 1975, 1976; Levy and Miller 1977 a,b). Although test results with *R. culicivorax* have indicated its usefulness in mosquito control operations, no practical methods for large scale field application have been demonstrated.

Levy et al. (1976) in a simulated aerial spray system, determined that a TeeJet[R] nozzle assembly could be used effectively for aerial dissemination of the preparasitic (infective) stage of *R. culicivorax* at an average operational spray pressure of 27 psi with no apparent loss of viability, infectivity, or life cycle development. In addition, high levels of parasitism (ca. 97%) were achieved when this delivery system was used against several species of mosquitoes breeding in a grassy field (Levy and Miller 1977b).

This research was conducted to determine if the preparasitic stage of *R. culicivorax* could be applied from a helicopter by means of a Simplex low profile aerial spray system with no significant loss of viability, infectivity, or life cycle development.

METHODS AND MATERIALS. Tests were conducted on the ground using a Bell 47G helicopter equipped with a Simplex low profile aerial spray system [spray boom with 12 TeeJet[R] spray nozzles (FloodJet[R] Tip No. TK20)].

Two sand cultures containing eggs of *R. culicivorax* were flooded with dechlorinated well water (Cl^- 20 ppm; conductivity= 180 umhos/cm) [purified by a reverse osmosis (RO) filtration system] for 24 hr to induce hatching of the preparasitic nematodes; the cultures produced about 1×10^6 preparasites as determined by volumetric dilution.

About 7.87×10^5 preparasites were added to the helicopter spray tanks which contained ca. 87 liters of unpurified dechlorinated well water (Cl^-= 65 ppm; conductivity= 620 umhos/cm). Plastic buckets (11.4 liters or 19.4 liters) were placed under each of the 12 spray nozzles and anchored to the spray boom to prevent the loss of spray containing preparasites due to the strong air currents generated by the main rotor blade of the helicopter and/or from the wide angle flat spray pattern which is characteristic of the FloodJet[R] nozzle tip.

Preparasites were sprayed directly into

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each of the 12 empty buckets at approximately 25 psi by intermittent activation of an electric motor-driven pump used to control spraying by the pilot. Intermittent spraying continued for 3-5 min until the spray tanks were emptied. The Simplex system was calibrated to spray 114.0 liters/min or 9.5 liters/min/nozzle.

The 12 buckets containing spray water (ca. 5250 ml/container) were returned to the laboratory and the average numbers (3 replicates/bucket) of actively swimming preparasites per ml of water were determined. Five thousand—40,000 first instar *Culex quinquefasciatus* Say larvae were added to each of the helicopter test and control containers (i.e. based on an average of 100 eggs/raft). To measure any loss of infectivity due to passage through the spray system as well as to determine the effects of parasite and host density on host parasitism, varying concentrations of mosquito larvae were added to the test buckets containing the sprayed preparasites. Water containing preparasites from the dump section of the spray system (ca. 8.6 preparasites/ml) and from the 2 flooded cultures (i.e. ca. 2×10^5 preparasites that were not added to the spray tanks) was used in controls. This water was poured directly into plastic buckets or stainless steel trays filled with RO or well water containing 1st instar *Culex* larvae. Water containing preparasites in several test and control containers was mixed to vary the infection ratio in the following manner: test containers No. 6, 7, 8 pooled; test containers No. 10,11,12 pooled; control containers No. 1,2 pooled. It should be noted that nematodes collected from the dump section of the Simplex system did not pass through the spray system and subsequently were not stressed by application pressures or impact. The volume of

Table 1. Results of helicopter ground tests to evaluate the effects of a Simplex low profile aerial spray system on viability and infectivity of the preparasitic stage of *R. culicivorax*.

Helicopter test (HT) and control (C) containers	No. actively swimming preparasites/ml	No. preparasites/host	No. larvae/cm² of surface area	Percentage parasitism of *Cx. quinquefasciatus* larvae[b]
HT–1	9.0	8.6	8.3	86
HT–2	7.3	7.0	8.3	62
HT–3	4.7	4.5	8.3	58
HT–4	7.7	8.1	7.6	98
HT–5	14.3	15.0	7.6	88
HT–6, 7, 8[a]	7.2	2.8	60.6	24
HT–9	8.3	13.2	5.0	74
HT–10, 11, 12[a]	7.3	2.9	60.6	16
Means	8.2	7.8	20.8	63
C–1, 2[a]	5.4	1.9	60.6	14
C–3	12.0	12.0	2.9	98
C–4	12.0	12.0	2.9	92
C–5	5.0	0.5	23.1	12
C–6	7.7	8.1	7.6	90
C–7	8.0	7.6	8.3	80
C–8	12.0	12.0	2.9	84
Means	8.9	7.7	15.5	67

[a] Preparasites pooled into one container.
[b] No significant statistical differences when HT compared with C.

water in all test and control containers was not equal; however, Petersen (1973) has indicated that dilution did not significantly affect parasitism of *Cx. quinquefasciatus* by *R. culicivorax*.

Mosquito larvae and nematodes were reared according to procedures modified from Petersen and Willis (1972b). Room temperature was 26°C throughout the experiment. Percentage parasitism of *Cx. quinquefasciatus* larvae (fifty 4th instar larvae sampled/container) was used as the criterion to evaluate the effects of the helicopter spray system on preparasitic *R. culicivorax*.

In addition, 15 g of postparasites collected from test and 15 g of postparasites from the control containers were cultured to determine any delayed inhibitory effects on general development as well as on F_1 preparasite infectivity.

Data were statistically analyzed using "z" and "t" tests and simple regression methods to evaluate the infectivity of sprayed vs. unsprayed preparasites. In addition, data were analyzed to determine the effect of several parasite to host ratios and host densities on percentage parasitism of *Cx. quinquefasciatus*.

RESULTS AND DISCUSSION. Analyses of the data presented in Table 1 indicated that infectivity of *R. culicivorax* preparasites when sprayed through the Simplex spray system (HT=63%) was not significantly different ($P<0.05$) from that of the unsprayed preparasites (C= 67%).

In addition, analyses were performed to determine if a relationship existed between the number of preparasites released per host and the resulting host parasitism (Petersen 1973). Regression analyses resulted in the model (Fig. 1), *percentage parasitism = 17.68 + 6.12 number preparasites released per host* ($r=0.85$; $P\leq0.01$). Based on this model, we have estimated that 5.3 and 11.8 preparasites per host are required to achieve 50% and 90% larval parasitism, respectively, at an average host density of 18.1 larvae per cm^2 of surface area.

Host density also appeared to affect parasitism (Petersen 1973). The average

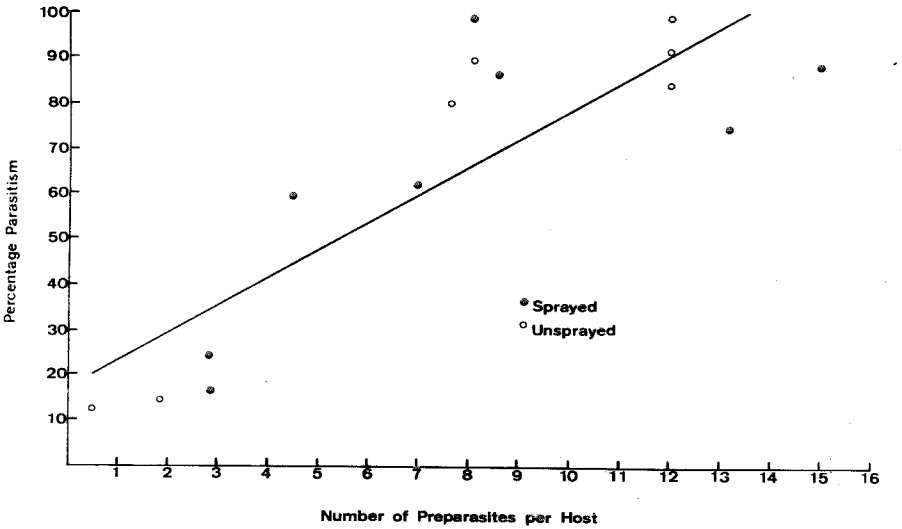

Fig. 1. Relationship between the number of preparasitic *R. culicivorax* per host and the percentage parasitism.

percentage parasitism (83%) for the lower host densities 2.9-8.3 for both HT and C was significantly greater (P=0.01) than the average percentage parasitism (18%) corresponding to the host density 60.6 (Table 1).

Our data also seem to indicate that plastic buckets may be satisfactory for rearing *R. culicivorax*. Current rearing of this parasite is in rectangular galvanized, stainless steel or plastic trays.

No observable adverse effects on viability and infectivity of preparasitic *R. culicivorax* resulted from exposure to the RO and well water, or from impact at 25 psi within the Simplex low profile aerial spray system and TK20 nozzle assemblies, as well as from impact in the collection buckets (Fig. 2). Furthermore, the spray application had no detectable effects on parasite development or on F_1 preparasite infectivity to *Cx. quinquefasciatus* larvae.

The Simplex system functioned effectively for the dissemination of the preparasitic stage of *R. culicivorax*. The average number of sprayed preparasites/ml of water/bucket was calculated to be 7.9 (4.7–14.3), i.e., an average of 41,518 (24,675–75,075) preparasites/bucket. A total of 498,225 sprayed and 211,048 unsprayed preparasites were estimated in the 63.0 liters of water in the test buckets and in the 24.4 liters of well water in the dump section of the spray system, respectively. Several thousand milliliters of water could not be collected from the dump section,

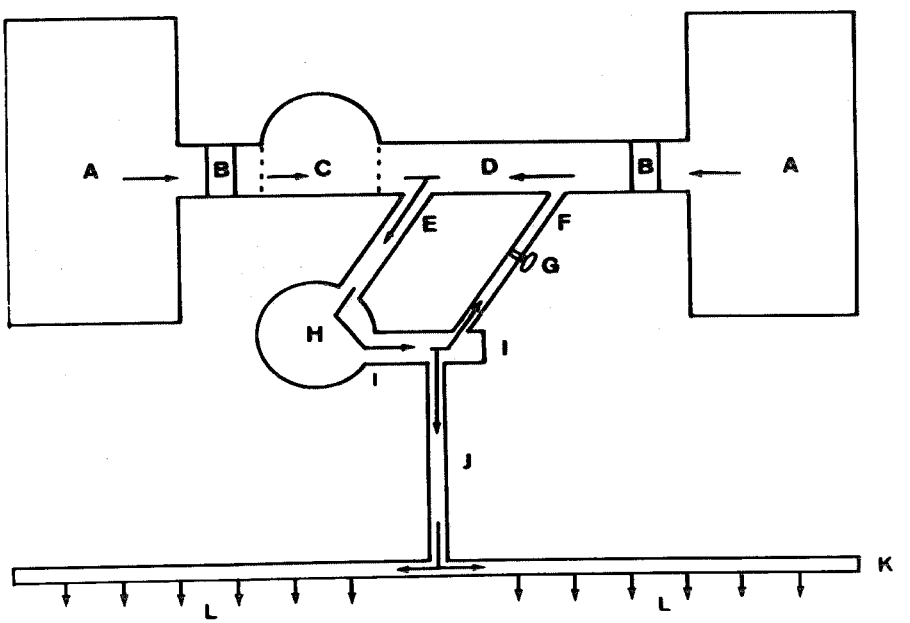

Fig. 2. Diagrammatic representation of Simplex spray system used to spray the preparasitic stage of *R. culicivorax*. A=209 liter tank; B=rubber connecting hose; C=dump chute; D=tank manifold; E=rubber hose (3.8 cm); F=rubber hose (2.5 cm); G=pressure regulation valve; H=electric pump; I=pump manifold; J=rubber hose (3.8 cm); K=spray boom; L=TeeJet® spray nozzles (Floodjet® Tip No. TK20). Arrows represent direction preparasites traveled during spraying.

which probably represents the additional 77,723 preparasites.

Numerous preparasites remained in the dump section of the Simplex system and therefore could not be sprayed in actual mosquito control operations. However, this system could be modified to reduce the volume of water trapped in the dump section and subsequently increase spraying efficiency. Also, unsprayed preparasites trapped in the dump section could be released from the Simplex system by pilot activation of the dump chute. (Fig. 2,C)

Although spray tests with the Bell 47G helicopter were conducted at ground level, the Simplex system was calibrated to spray 19 liters of water/acre at 40 mph. Presently, each Simplex system used on our Bell 47G helicopters is equipped with two 209 liter spray tanks which provide a carrying capacity of ca. 304 liters of water. With this capacity ca. 16 acres could be treated with preparasites in one control application.

In summary, our tests have indicated that a standard Simplex low profile aerial spray system having a boom equipped with 12 TeeJet^{R}/FloodJet^{R} (No. TK20) nozzle assemblies can effectively be used for disseminating the preparasitic stage of *R. culicivorax* at 25 psi via a Bell 47G helicopter. Furthermore, data from these tests also indicated that the number of preparasites released per host and the host density significantly affected the degree of host parasitism.

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