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AERIAL APPLICATION OF *ROMANOMERMIS CULICIVORAX* (MERMITHIDAE:NEMATODA) TO CONTROL *ANOPHELES* AND *CULEX* MOSQUITOES IN SOUTHWEST FLORIDA

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ABSTRACT. An experimental release of preparasites of the mosquito nematode *Romanomermis culicivorox* Ross and Smith (=*Reesimermis nielseni* Tsai and Grundmann, auct., partim.) was made in 3 ponds via helicopter at the rate of ca. 4,629/m^2 of water surface to control natural populations of *Anopheles quadrimaculatus* Say, *An. crucians* Wiedemann, and *Culex erraticus* (Dyar and Knab) larvae. Mean levels of parasitism of 52.4, 66.7 and 43.8% of the 1st-4th instar *Anopheles* spp. and 39.2, 40.0, and 53.3% of 1st-4th instar larvae of *Cx. erraticus* from 3 ponds, respectively were obtained.

The aerial spray tests indicated that a helicopter equipped with a Simplex low profile spray system with TeeJet^R spray nozzles can be an effective means of disseminating the preparasitic stage of *R. culicivorax* in mosquito control operations.

Control of natural populations of mosquito larvae with a pathogenic agent applied by air has not been demonstrated. However, Levy et al. (1977) reported encouraging results in tests using a helicopter spray system to deliver the mosquito nematode *Romanomermis culicivorax* Ross and Smith. Tests were conducted at ground level with a Bell 47G helicopter equipped with a Simplex low profile aerial spray system having a boom fitted with TeeJet^R nozzles at 25 psi and no adverse effects on preparasite infectivity or nematode development were detected. These tests suggested the potential use of this aerial spray system for the dissemination of *R. culicivorax* in mosquito control operations.

The susceptibility of larvae of *Anopheles quadrimaculatus* Say, *An. crucians* Wiedemann and *Culex erraticus* (Dyar and Knab) to field parasitism by preparasites of *R. culicivorax* applied by conventional compressed air hand sprayer has been demonstrated by Petersen and Willis (1972; 1974). We are now reporting on the aerial application of preparasites of *R. culicivorax* via helicopter to control field populations of larvae.

METHODS AND MATERIALS

Three semipermanent ponds (30.5 × 15.2 × 0.5-1.0 m deep) constructed in 1976 at the Lee County Mosquito Control District were used as the sites for the tests (Fig. 1). The ponds contained rain and standing ground water (0.3-0.6 m deep) and vegetation scattered throughout but concentrated primarily around the edges. Pre-treatment sampling showed large populations of larvae and pupae of *An.*

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quadrimaculatus and *An. crucians* (1–30 per dip with a pint dipper) and lower populations of *Cx. erraticus* (0–5 per dip). Numerous insect predators (water scorpions, back swimmers, water scavenger beetles, predacious diving beetles, dragonflies, water striders, whirligig beetles, *Gambusia* spp. and tadpoles) were present in each pond.

Pre-treatment analyses of the ponds indicated that the water was low in salinity (1.0–7.5 mg/l Cl^-) and conductivity (117–163 μmhos/cm), and therefore was suitable for *R. culicivorax*. The water temperature of the ponds was 30° C at the time of aerial application.

Twenty to 24 hr prior to application 12 cultures containing eggs of *R. culicivorax* were flooded with ca. 800 ml of water purified by reverse osmosis (RO) filtration to induce egg hatching. Volumetric determinations of the preparasite population in the water from the combined cultures indicated that ca. 11,250,000 infective stage nematodes were available for the tests.

Bioassays to determine infectivity of unsprayed and sprayed preparasites in unfiltered well water and water from the 3 ponds were conducted in 400 ml glass beakers containing 300 ml of the test waters and 20 1st instar larvae of *Cx. quinquefasciatus* per container (3 replications/water type). In addition, one 18 × 30 cm enameled pan was filled with ca. 1,000 ml of RO water and placed at the south end of each of ponds 1 and 3 to catch preparasites sprayed during the aerial application (Fig 1,a). Preparasites sprayed through the helicopter spray system at ground level before and after the aerial spray tests were also bioassayed in RO water. Volumetric determination of the number of preparasites sprayed into the enameled pans in ponds 1 and 3 was determined to be ca. 0.25 per ml or about 250 preparasites per 540 cm^2 pan (4 replications); preparasites collected from the helicopter before and after the aerial application averaged ca. 15 and 31 ml, respectively. Preparasites in the pre-test sample were sprayed at ground level almost immediately upon additions of the preparasites to the helicopter spray tanks and apparently were not thoroughly mixed in the ca. 296 liters of well water in the tanks prior to spraying. However, preparasites taken in post-spray samples were thoroughly mixed from typical helicopter agitation during aerial application. Preparasites from the helicopter were bioassayed in 400 ml glass beakers containing 300 ml RO water and 20 hosts (3 replications). Preparasites sprayed into the enameled pans during the aerial tests were transferred to 1,000 ml glass beakers containing RO water for bioassay (1 trial of 20 and 100 hosts for ponds 1 and 3, respectively).

A 10:1 preparasite to host ratio was used in the bioassays and mosquito hosts were fed ground rabbit chow daily and maintained at ca. 20° C (ambient). Percentage parasitism of *Cx. quinquefasciatus* larvae was used as the criterion to determine the effects of the pond water and spray application of infectivity of preparasitic *R. culicivorax*. Infectivity was determined with a compound microscope and by emergence of postparasites. Larvae determined to be negative for nematode parasitism with the compound microscope were confirmed by dissection.

A Bell 47G helicopter equipped with a Simplex low profile aerial spray system fitted with 12 TeeJet[R] spray nozzles (FloodJet[R] Tip No. TK20) was used for the aerial application of preparasites (Levy et al. 1977). Helicopter spray tanks were filled with ca. 296 liters of unfiltered well water (conductivity= 720 μmhos/cm @ 25°C) while the rotor blades were operational. Then 7.6 liters of RO water containing preparasites were added directly into the spray tanks. The spray system was calibrated to spray 9.5 liters/min/nozzle or 114.0 liters/min at 25 psi. Preparasitic nematodes were ca. 30 hr old when sprayed.

Preparasites were applied from the helicopter at the rate of ca. 2,925,315 per pond at 32 kmph and at 2.5–3.0 m above the ground. The ponds were sprayed consecutively in a series of north to south and south to north spray passes. A west to east wind of ca. 10 kmph was recorded during the aerial application. About 25 liters of water containing preparasites (ca. 31 preparasites/ml) could not be sprayed and remained in the tanks and dump section of the Simplex system after the ponds were treated (Levy et al. 1977).

A total dosage rate of ca. 4,629 preparasites per m^2 per pond was determined from the average number of preparasites per ml of water recovered in enameled pans during aerial application (i.e. 0.25 preparasites per ml). A substantial number of preparasites were lost because of drift during the aerial application. Based on the preparasite dosage and water surface area per pond, each pond was considered a replicate even though the number of mosquito larvae per pond varied.

Each pond was sampled 24 and 48 hr post-treatment and the percentage parasitism by larval instar was determined (as previously described), to evaluate the effectiveness of the aerial application of preparasitic *R. culicivorax* against *Anopheles* spp. and *Cx. erraticus*.

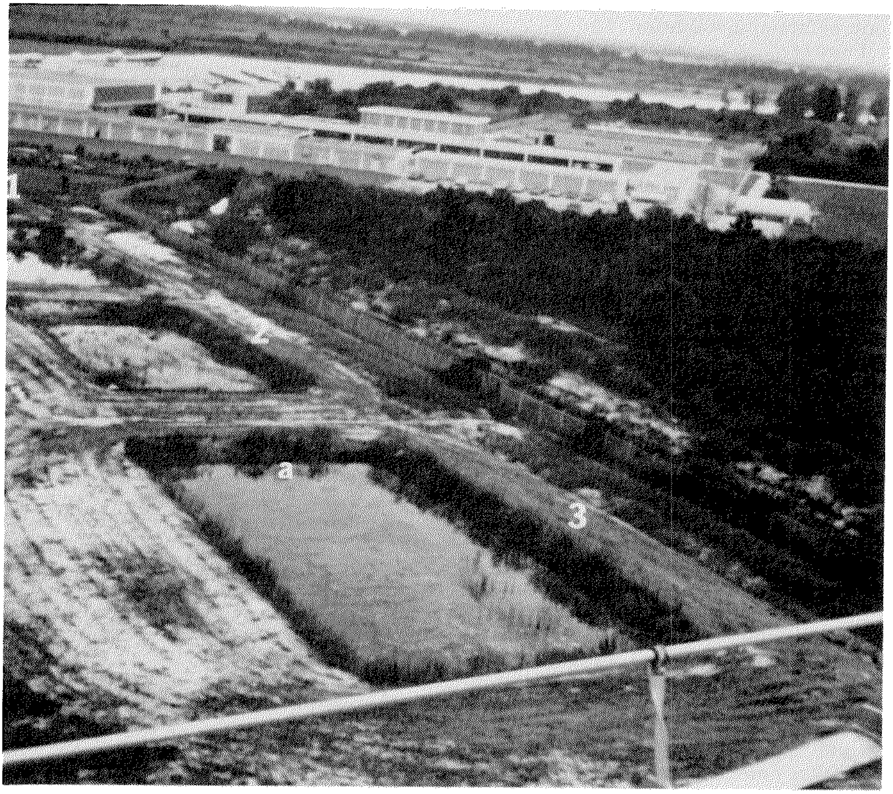

Figure 1. Experimental sites (Ponds 1–3) used for aerial application of *R. culicivorax* showing placement of enameled pans (a) used to catch preparasites at south end of ponds 1 and 3.

RESULTS AND DISCUSSION

Data collected from bioassay of unsprayed and helicopter sprayed preparasites of *R. culicivorax* in water from the 3 sources indicated that there were no significant differences in infectivity to *Cx. quinquefasciatus* larvae when preparasites were sprayed through the Simplex system compared with unsprayed preparasites in RO water (Levy et al. 1977). However, a 10–12% reduction in infectivity was observed when unsprayed preparasites were exposed to mosquito larvae in unfiltered well water (Table 1). In addition, all postparasites obtained from exposure of hosts to the sprayed and unsprayed preparasites in the various water types showed no observable morphological and behavioral differences when compared to postparasites recovered from the RO controls (Table 1).

The bioassay data indicated that little or no inhibitory effects on the infectivity and development of *R. culicivorax* could be expected as a result of the helicopter spray system or preparasite exposure to water in the 3 ponds. However, possible inhibition of the infectivity of preparasites exposed for 24 hr to unfiltered well water used in the spray tanks was observed. No effects were apparent in nematodes held in tanks for 30–45 min and reared in RO water, a condition similar to ponds.

Aerial application of preparasites of *R. culicivorax* to control mixed populations of larvae of *An. quadrimaculatus* and *An. crucians* in the 3 ponds resulted in mean parasitism ranging from 43.8–66.7% (Table 2). The mean parasitism of all 402 *Anopheles* spp. larvae sampled 24 and 48 hr post-treatment from the 3 ponds was 53.7% (100, 50.3, 59.5, and 43.4% parasitism of 2-1st, 117–2nd, 196–3rd, and 87–4th instar larvae, respectively).

Encapsulation and melanization of the nematode was observed in several 3rd and 4th instar *Anopheles* spp. larvae and indicated a certain degree of physiological resistance to this nematode (Petersen and Willis 1974). It should be noted that the majority of the *Anopheles* spp. larvae examined 24 and 48 hr post-treatment were infected with more than one *R. culicivorax*. Multiple parasitism may have resulted in some early host death prior to sampling. In addition, 100% postparasite emergence was observed from 25 *Anopheles* spp. larvae parasitized in the ponds by *R. culicivorax* and indicated the potential for nematode recycling in this type of habitat.

The parasitism of larvae of *Cx. erraticus* resulting from aerial application of preparasites of *R. culicivorax* is presented in Table 3. A mean parasitism of 41.9% was observed in the 86 *Cx. erraticus* larvae examined from the 3 ponds, i.e. 50, 35.9,

Table 1. Bioassays of infectivity of preparasitic *Romanomermis culicivorax* to 1st-instar larvae of *Culex quinquefasciatus* as a function of water quality and spray application.

Water Type Tested	Preparasites Tested	Percentage parasitism (No. larvae sampled)	Percentage postparasite emergence from infected 4th instar larvae (No. larvae sampled)
RO	Unsprayed	88.3 (60)	100 (29)
Well	Unsprayed	78.3 (60)	100 (30)
Ponds 1–3	Unsprayed	90.0 (60)	100 (30)
RO	Sprayed[a]	93.3 (15)	———
RO	Sprayed[b]	86.6 (15)	———
RO	Sprayed[c]	85.0 (120)	100 (60)

[a] Preparasites in well water sprayed from helicopter at ground level into buckets prior aerial test and added to beakers containing RO water.

[b] Preparasites in well water sprayed from helicopter at ground level into buckets after aerial test and added to beakers containing RO water.

[c] Preparasites in well water sprayed from helicopter into enameled pans containing RO water during aerial application.

Table 2. Parasitism of *Anopheles* spp. larvae resulting from aerial application of preparasites of *Romanomermis culicivorax* at a dosage rate of 4,629 nematodes/m².

Pond No.	Hr Post-Treatment	Percentage parasitism by instar (No. larvae sampled) First	Second	Third	Fourth	Combined Instar
1	24	100(1)	43.5(23)	48.4(31)	30.0(10)	44.6(65)
	48	—(0)	—(0)	79.0(19)	—(0)	79.0(19)
	Combined	100(1)	43.5(23)	60.0(50)	30.0(10)	52.4(84)
2	24	100(1)	75.0(28)	61.9(42)	75.0(4)	68.0(75)
	48	—(0)	33.3(3)	70.6(17)	50.0(4)	62.5(24)
	Combined	100(1)	71.0(31)	64.4(59)	62.5(8)	66.7(99)
3	24	—(0)	44.5(36)	60.4(53)	38.5(52)	48.2(141)
	48	—(0)	25.9(27)	42.9(34)	35.3(17)	35.9(78)
	Combined	—(0)	36.5(63)	54.0(87)	37.7(69)	43.8(219)

55.4, and 0% parasitism of the 4–1st, 48–2nd, 31–3rd, and 3–4th instar larvae, respectively. Little multiple parasitism was noted in larvae of this species. Two 4th instar *Cx. erraticus* larvae were found to have encapsulated and melanized *R. culicivorax* parasites, thus indicating possible physiological resistance to *R. culicivorax* or limited resistance due to weakened or damaged parasites dying after entering the host.

Although the preparasite dosage per m² appears high when considering the total percentage parasitism, one must consider the type of parasite application as well as the physical features of the test sites in relation to the orientation of parasites and hosts. Preparasites were evenly distributed over the 464 m² of water surface of each pond; however, the *Anopheles* spp. and *Cx. erratucus* larvae were unevenly distributed around the vegetative edges of each pond. Since preparasites are believed to swim only a few feet from their point of introduction (unpublished data), numerous preparasites sprayed in the central portions of each pond probably did not encounter hosts. In addition, preparasites were ca. 30 hr old when sprayed and 10–15% of the nematodes were assumed to have lost their infectivity (Petersen 1975). Some predation of infected mosquito hosts can also be expected to have occurred.

It is important to determine the most efficient and effective means of disseminating *R. culicivorax* preparasites (i.e. ground or aerial spraying) in the various types of fresh water mosquito habitats. In the test sites utilized in this study it is expected that the dosage per m² could have been reduced by as much as 50% if

Table 3. Parasitism of *Culex erraticus* larvae resulting from aerial applications of preparasites of *Romanomermis culicivorax* at a dosage rate of 4,629 nematodes/m².

Pond No.	Hr Post-Treatment	Percentage parasitism by instar (No. larvae sampled) First	Second	Third	Fourth	Combined instars
1	24	0(1)	39.1(23)	66.7(9)	—(0)	45.5(33)
	48	—(0)	36.4(11)	25.0(4)	0(3)	27.8(18)
	Combined	0(1)	38.2(34)	53.8(13)	0(3)	39.2(51)
2	24	—(0)	—(0)	50.0(2)	—(0)	50.0(2)
	48	—(0)	33.3(3)	—(0)	—(0)	33.3(3)
	Combined	—(0)	33.3(3)	50.0(2)	—(0)	40.0(5)
3	24	66.7(3)	57.1(7)	66.7(15)	—(0)	64.0(25)
	48	—(0)	0(4)	0(1)	—(0)	0(5)
	Combined	66.7(3)	36.3(11)	62.5(16)	—(0)	53.3(30)

the preparasites were applied with a conventional compressed air sprayer around the vegetative edges of each pond where sampling indicated the mosquito larvae were concentrated. Further, problems associated with drift during aerial spraying would have been greatly reduced or even eliminated. However, the potential for recycling that this nematode has exhibited in certain habitats (Petersen and Willis 1975) may justify the higher dosage rates when applying preparasites of *R. culicivorax* by helicopter over large areas.

Examination of samples of *Anopheles* spp. and *Cx. erraticus* larvae obtained 8 weeks post-treatment revealed no parasite activity and therefore indicated that natural recycling of *R. culicivorax* had not occurred in the test sites during this period. Eight weeks post-treatment, pond 2 was dry and the water in ponds 1 and 3 had receded from the vegetative perimeter where the infected larvae were originally sampled; larvae at this time were more evenly distributed in the central portions of the 2 ponds. It is expected that postparasite emergence from the *Anopheles* and *Culex* hosts and subsequent nematode egg laying occurred in the soil around the vegetative portions of each pond. Eleven soil samples per site obtained with a standard soil core from these areas and flooded with RO water for 24 hr indicated no preparasite activity at this time.

Test results suggest that a helicopter having a Simplex low profile aerial spray system equipped with TeeJet[R] spray nozzles can be an effective means of disseminating preparasites of *R. culicivorax* against natural populations of *An. quadrimaculatus*, *An. crucians* and *Cx. erraticus* larvae, as well as other species of mosquitoes in certain fresh water habitats.

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