EFFECTS OF A SIMULATED AERIAL SPRAY SYSTEM ON A MERMITHID PARASITE OF MOSQUITOES[1]

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ABSTRACT. A simulated aerial spray system was designed to evaluate effects of pressure and nozzle impact on the preparasitic (infective) stage of the mermithid nematode *Reesimermis nielseni* Tsai and Grundmann. Spray tests against field-collected and laboratory-reared *Culex pipiens quinquefasciatus* Say larvae indicated that a TeeJet ® nozzle assembly can effectively be used for aerial dissemination of the preparasitic stage of *R. nielseni* at an average spray pressure of 27 psi with no apparent loss of viability, infectivity, or life cycle development.

Effective suppression of natural populations of several species of freshwater mosquitoes has been demonstrated in small plot field tests with the mermithid nematode *Reesimermis nielseni* Tsai and Grundmann[2] (Petersen et al. 1972, Petersen and Willis 1972b, Petersen et al. 1973, Petersen and Willis 1974); however, no large scale testing has been conducted to determine the applicability of *R. nielseni* as a biological control agent for use in an integrated mosquito control program. A practical method of dissemination aimed at large area control has not been demonstrated, even though adequate mass production procedures have been developed (Petersen and Willis 1972a). Present methods of field application have been by hand introduction of postparasites and sand cultures containing eggs and adults and/or spraying preparasitic (infective stage) nematodes into the larval habitat by use of a compressed air hand sprayer (Petersen et al. 1972, Petersen and Willis 1972b, Petersen et al. 1973, Mitchell et al. 1974, Petersen and Willis 1974).

The present research was aimed at evaluating the effect of two spray system

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[1] In cooperation with the USDA, ARS, Gulf Coast Mosquito Research Laboratory, Lake Charles, Louisiana 70601.

[2] Editor's note: After this article was set in type it was learned that *Reesimermis nielseni* Tsai and Grundmann has been synonymized with *Romanomermis culicivorax* Ross and Smith.

characteristics, viz. pressure and impact, on the preparasitic (infective) stage of *R. nielseni* to determine if a potential exists for large scale aerial application, particularly via helicopter.

Methods and Materials. A standard (7.4 l) B&G [3] compressed air sprayer was adapted with a pressure gauge and one TeeJet® spray nozzle (FloodJet® Tip No. TK20) (Fig. 1). Similar nozzles are currently used on our District helicopters for aerial application of mosquito larvicides.

Preliminary experiments were conducted using this system at an average spray pressure of 27 psi to simulate nozzle delivery at a spray pressure comparable to the pressure (i.e. approximately 25 psi) produced within a Simplex low profile aerial spray system used on one of our Bell 47G helicopters. Effects of an average 27 psi spray pressure and nozzle design on preparasitic nematodes were investigated to determine if the nozzle assembly would impair viability, infectivity and normal development of *R. nielseni*.

The following criteria were used to evaluate the test results: *Test 1* (t_1 and t_2)—grams (g) postparasitic *R. nielseni* collected per 1000 *Culex pipiens quinquefasciatus* Say larvae; *Test 2* (t_a and t_b)—percent 4th instar *Cx. p. quinquefasciatus* larvae parasitized by *R. nielseni*. A 4:1 and 10:1 ratio of preparasites to *Cx. p. quinquefasciatus* larvae were used for *Test 1* and *Test 2*, respectively (i.e. approximately 4260 (*Test 1*) and 10000 (*Test 2*) preparasites per 1000 *Culex* larvae.

Cx. p. quinquefasciatus larvae were collected from a sewage settling tank on Sanibel Island, Lee County, Florida (*Test 1*) or obtained from nematode host colonies maintained in our laboratory (*Test 2*). First and second instar larvae were placed in plastic buckets in 2 liters of well water (purified by a reverse osmosis (RO) filtration system) that was dechlorinated with 5% sodium thiosulfate (one drop/liter). A few hours prior to this procedure a sand culture containing *R. nielseni* eggs was flooded with dechlorinated RO water (*Test 1*=3 hr; *Test 2*= 6 hr) to induce hatching of the preparasitic nematodes (Petersen and Willis 1972a).

Dechlorinated water (3.7 liters) containing the desired number of prepara-

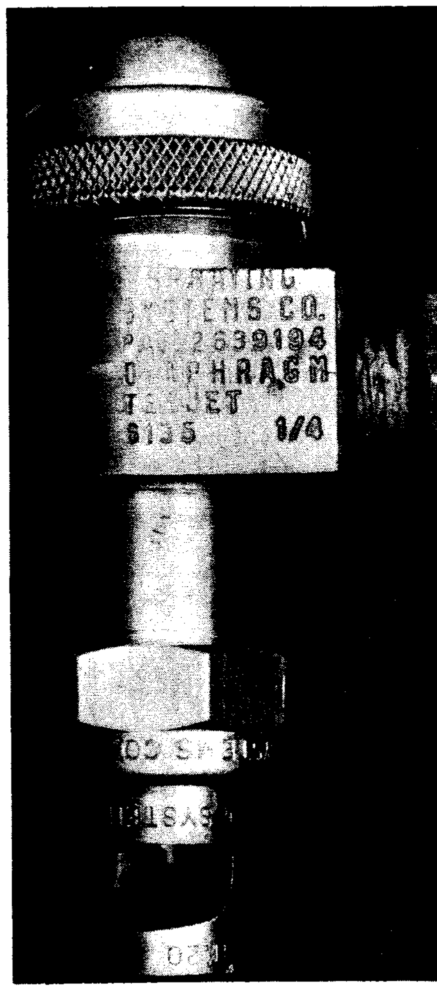

Fig. 1. TeeJet ® spray nozzle used for disseminating the preparasitic stage of *Reesimermis nielseni*.

[3] Mention of a brand name or proprietary product does not constitute a guarantee or warranty by Lee County Mosquito Control District, and does not imply its approval to the exclusion of other products that may also be suitable.

sitic nematodes was added to the spray tank, and the system was pumped to 50 psi 10–15 min before spraying. The nozzle assembly was held approximately 15–21 cm from the water surface. Preparasites were initially applied at 32 psi by several intermittent spray bursts until the pressure in the spray tank had dropped to 30 psi (i.e. 22 psi spray pressure), thereby producing an average spray pressure of 27 psi (32–22 psi) with an average container pressure of 40 psi (50–30 psi). This procedure was then repeated until the spray tank was emptied to deliver the desired number of preparasites. Preparasites used for controls were decanted directly from a flask and not passed through a spray system.

Experiments with nematode-free water indicated that the spray tank could not be satisfactorily drained, thereby leaving numerous preparasites within the tank. To alleviate this, the siphon tube was removed and a longer tube was adapted which provided almost complete drainage from the system.

Test and control containers were each filled with 6 liters of dechlorinated RO water, and *Culex* larvae and nematodes reared according to procedures modified from Petersen and Willis (1972a). Room temperature (26° C) and relative humidity (80%) were constant (±10%) throughout the experiment.

RESULTS AND DISCUSSION. Random sampling of several *Culex* larvae 72 hr post-treatment (*Test 1*) indicated that nematode infection had occurred in both tests and control. Approximately 2 weeks after inoculation of the preparasites via the spray system, postparasites were collected and weighed according to the methods described by Petersen and Willis (1972a). Results indicated that 1.50 g of postparasites were recovered from the control and an average of 1.46 g of postparasites were obtained from the tests (t_1=1.55 g and t_2=1.37 g). In addition, F_1 preparasites obtained from culturing t_1, t_2, and control postparasites (Petersen and Willis 1972a) showed no significant differences in infectivity to *Culex* larvae when compared to controls.

Data obtained from sampling 4th instar *Culex* larvae for *R. nielseni* parasitism (i.e. 55 larvae/bucket, *Test 2*) indicated that an average of 99.09% of the larvae examined in the tests (t_A=100% and t_B=98.18%) were infected with *R. nielseni* compared to 98.18% infection in the control.

In general, our spray tests against field-collected and laboratory-reared *Cx. p. quinquefasciatus* larvae have indicated that a TeeJet® nozzle assembly can be used effectively for aerial dissemination of the preparasitic stage of *R. nielseni* at an average operational spray pressure of 27 psi with no apparent loss of viability, infectivity, or life cycle development.

Tests at the Lee County Mosquito Control District are presently in progress to evaluate the actual helicopter system on *R. nielseni* growth and development as well as control potential against several species of mosquitoes under field conditions. Field tests with *R. nielseni* are presumed to be quite safe to nontarget aquatic organisms and mammals which might accidentally be exposed to the introduced nematode (Ignoffo et al. 1973, Ignoffo et al. 1974).

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