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AN INJECTION METHOD FOR SPRAYING BIOLOGICAL CONTROL AGENTS AND A MONOMOLECULAR SURFACE FILM FOR CONTROL OF IMMATURE MOSQUITOES[1]

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Biological control agents against immature mosquitoes are replacing a significant percentage of conventional chemical toxicants at many mosquito control districts with little, or no, modification of the established methods for dispensing chemicals. However, the standard method for mixing the biological agents with water in spray tanks causes problems when the entire contents of the tank are not used the same day. For example, the larvicidal material tends to settle to the bottom of the tank, allowing contamination by proteinaceous anaerobic bacteria which often produce noxious odors and rapidly decrease the larvicidal potency. An attempt to avoid these problems by the use of a liquid chemical injection valve is described below.

Tests with biocontrol agents were conducted using a Dema[2] Model #203 chemical injection valve and tests with a monomolecular surface film were performed with Dema Model #202C. The injector was installed on a small trailer on the pressure side of the pumping mechanism (Fig. 1). The pumping mechanism was a John Bean Pump (Model R–5) capable of delivering 5 G.P.M., driven by a 4 hp Briggs and Stratton engine.

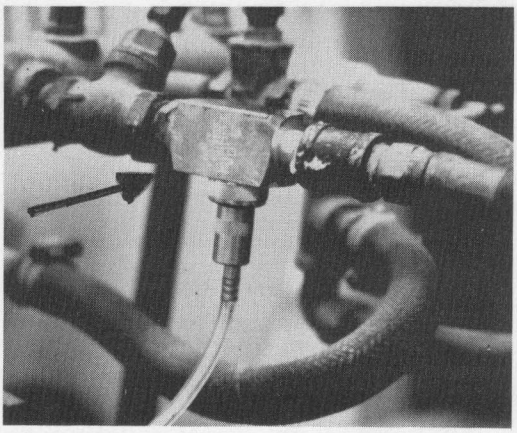

Fig. 1. A close-up view of the Dema injection valve installed.

The water volume sprayed from the nozzle was measured with a 50 liter jug and timed by a stopwatch. Output in liters/min. were measured at 5- and 10-minute intervals. One hundred ml of larvicidal concentrate in a graduated cylinder was sucked into the injection valve and timed by a stop-watch. Small changes in the suction rates were corrected by manipulation of the metering screw on the injection body capable of increasing or decreasing the flow rate 10-fold.

Test formulations were *Bacillus sphaericus,* strain 1593 (B.s. 1593) consisting of prespore and sporulating bacterial cells of ca. 2×10^9 c/ml; *Bacillus thuringiensis,* H–14 used as a 5% aqueous suspension of a slurry product provided by Sandoz, Inc.[3], yielding ca. 1.5×10^9c/ml; *Metarhizium anisopliae* var. *anisopliae* was used as a spore suspension of ca. 6.2×10^9

[1] 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.

[2] A series of Dema liquid chemical injectors are manufactured by Dema Engineering Company, 10020 Big Bend Boulevard, St. Louis, MO 63122.

[3] Sandoz, Inc., Crop Protection, 480 Camino Del Rio South, San Diego, CA 92108.

[4] Arosurf® MSF (=ISA-20E=Arosurf 66–E2) is a product of Sherex Chemical Company, Inc., Post Office Box 646, Dublin, OH 43017.

spores/ml; *Romanomermis culicivorax* was used as a concentrated suspension containing 1200 nematodes per ml; and the monomolecular surface film Arosurf® MSF was injected at a volume approximating the field dose rate of 0.3 gallon of film per acre.

The chemical injection valve Dema model #203 was adjusted to provide a 1:1000 dilution of the larvicidal bacilli suspensions to yield approximately 1×10^6c/ml at the spray nozzle. The bacterial concentration at the spray nozzle remained constant (Table 1). The high dilution rate is maintained only when the injection valve is installed on the pressure side of the pumping mechanism (Fig. 1). Bioassays which were conducted reflected the high dilution factor as well as the uniformity of the injection process.

Table 1. Larvicidal activity of *Bacillus thuringiensis* (H-14) and *B. sphaericus* (1593) using the Dema injection valve system.

	B. thuringiensis (strain H-14)[a]	*B. sphaericus* (strain 1593)[b]
Cell count of spray samples (per ml)	A) 9.00×10^5	1.35×10^6
	B) 6.5×10^5	1.00×10^6
	C) 1.1×10^6	1.4×10^6
Bioassay[c]		
Original culture	$10^{-5.9}$	$10^{-6.0}$
Spray samples	A) $10^{-2.65}$	$10^{-2.50}$
	B) $10^{-2.59}$	$10^{-2.65}$
	C) $10^{-2.66}$	$10^{-2.93}$

[a] 4.33 ml/min a.i. plus 5.0 l/min water, final dilution = 1:1154.

[b] 5.01 ml/min a.i. plus 5.0 l/min water, final dilution = 1:1000.

[c] Dilution factor of original material to produce an LC_{50} value using *Cx. quinquefasciatus*, 2nd instar; all tests in triplicate.

The fungal spores of *Metarhizium anisopliae* collected according to the method of Roberts (1969) were suspended evenly in water containing a 0.1% non-ionic wetting agent and stirred vigorously to prevent clumping. In this study, fungal spores ingested by the larvae appear to collect in the upper gut to form a plug, with death occurring between 24–72 hr due to blockage of the gut rather than an infection (Table 2).

The larvicidal nematode, *Romanomermis culicivorax*, supplied as a concentrate of infectious preparasites by R. Levy (Levy and Miller 1977) was diluted 1:85 in the injection system due to the relatively low numbers in the experimental concentrate (Table 3). The choice of this relatively large and delicate parasite was to determine its survival through the shearing action of the valve system. Approximately 33% of the nematodes survived passage through the injection valve and exhibited parasitic activity on 100% of the susceptible mosquito larvae at a nematode-larvae ratio of 14:1. Selection of a different valve model may increase the survival rate at the nozzle above the 33% level.

To accommodate the viscosity of Arosurf® MSF (64–70 C.P.S. at 22°C), a Dema injection valve #202C was selected. Pupae and 4th instar *Culex quinquefasciatus* Say exposed to the monomolecular film Arosurf MSF became disoriented and showed considerable morbidity within 30 min after application, similar to the descriptions by Levy et al. (1982). After 4 hr all pupae were dead and the larvae began to show high morbidity. All of the pupae and larvae were dead at 24 hr posttreatment (Table 4).

The installation of the injection system allowed conservation of an agent because the unused portion can be removed from the spray vehicle at the end of the day in the original jug

Table 2. Larvicidal activity of the fungus *Metarhizium anisopliae* using the Dema injection valve system.

	Metarhizium anisopliae[a]
Cell count of spray samples	A) 1.1×10^6
	B) 9.3×10^5
	C) 1.2×10^6
Bioassay[b]	
Original culture	$10^{-6.2}$
Spray samples	A) $10^{-2.01}$
	B) $10^{-2.23}$
	C) $10^{-2.19}$

[a] 4.69 ml/min a.i. plus 5.0 l/min water, final dilution = 1:1066.

[b] Dilution factor of original material to produce an LC_{50} value using *Cx. quinquefasciatus*, 2nd instar; all tests in triplicate.

Table 3. Survival and larval infectivity by *Romanomermis culicivorax* after passage through the Dema injection valve system.

	Romanomermis culicivorax[a]
Parasite count of spray samples (per ml)	A) 4.8
	B) 5.0
	C) 4.3
Bioassay b/(percent infected)	
Original culture	100
Spray samples	A) 100
	B) 90
	C) 100
Control, larvae only	D) 0

[a] 60 nematodes/min a.i. 5.1 l/min water, final dilution 1:85.

[b] Infection rate determined by larval examination for internal parasitic nematode using *Cx. quinquefasciatus*, 2nd instar; all tests in triplicate. Nematode-host ratio = 14:1.

Table 4. Larvicidal and pupicidal activity of monomolecular film using the Dema injection valve system.

	Arosurf® MSF[a]
Mortality (24 hr)[b] (larvae and pupae)	A) 100%
	B) 90%
Control (water only)	C) 0%
	D) 0%

[a] 11.5 ml/min a.i. 5.1 l/min water, final dilution 1:443.

[b] Test organism consisted of *Cx. quinquefasciatus*, pupae and 4th instar larvae in a ratio of approximately 1:1.

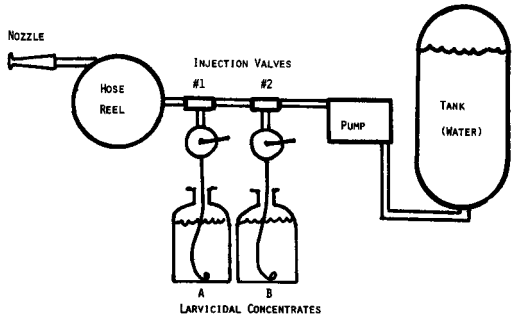

Fig. 2. Dema injection valve system.

containing the concentrate and refrigerated, if necessary. It also permits the application of water-borne Arosurf MSF without high speed agitation.

These tests with the Dema injection system indicate that a spray truck can carry two or more larvicidal concentrates and switch from one to another as the conditions and requirements change (Fig 2). The use of the injection system can achieve proper dosage rates of the test formulation that will kill mosquito larvae.

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ISOLATION OF CALIFORNIA ENCEPHALITIS SEROTYPE FROM MOSQUITOES COLLECTED IN MANITOBA, CANADA

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Three California (CAL) serogroup viruses, snowshoe hare, Jamestown Canyon and trivittatus (Artsob 1983), have been isolated from Canada, and CAL serogroup virus activity has been demonstrated serologically in all 10 Canadian provinces as well as the Yukon and Northwest Territories. This communication reports the isolation and identification in Canada of a fourth CAL serogroup member.

A pool of 8 *Culiseta inornata* (Williston) collected on July 16, 1979 from Selkirk/Oak Hammock, Manitoba yielded an isolate, Mn 296–300, which was identified in complement fixation tests as a CAL serogroup virus. Neutralization tests employing a Tissue Culture Infective $Dose_{50}$ method (Artsob et al. 1983) and enzyme-linked immunosorbent assay (ELISA) typing (Artsob et al. 1984) of the isolate were undertaken using hyperimmune mouse ascitic fluid. The ELISA typing method has been shown to successfully differentiate CAL serogroup members isolated in North America.

Isolate Mn 296–300 was shown to be closely related or identical to California encephalitis (CE) serotype (Table 1). This identification was confirmed using a serum dilution, plaque neutralization test (Lindsey et al. 1976) with single dose hamster sera (Karabatsos and Mathews 1980) (Table 2).

This marks the first identification of CE serotype in Canada. The serotype was first iso-

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