treatment removed evidence of either a blood meal or a gravid condition.

Chloral hydrate required ca.2 wk to clear the abdomens sufficiently and phenol did not clear them at all. Since chloral hydrate is much slower acting than potassium hydroxide, it would be of use to an investigator who could not closely monitor the more rapid clearing action of potassium hydroxide.

Specimens cleared for parity determinations by either potassium hydroxide or chloral hydrate were not suitable for repinning but could easily be mounted on microscope slides. If it were essential to preserve wing patterns (colorations) then the wings would have to be removed and held separate prior to clearing.

This is the first technique known to this author for determining parity of pinned specimens. It permits parity investigations to be made on pinned or dried collections that have potential epidemiological significance in addition to the taxonomic value for which they may have been first prepared. This technique will probably remain limited in usefulness to parity determinations in *Culicoides* unless external pigmentation changes related to oogenesis are also found to occur in other ceratopogonids or mosquitoes.

Parity data from the pinned specimens used in this technique study will be reported elsewhere as part of a study on the parity of *C. variipennis* captured in bait traps and from collections of biting midges attracted to animals.

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SOME FACTORS AFFECTING THE USE OF *NEOAPLECTANA* SP. FOR MOSQUITO CONTROL

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There are several strains and species of *Neoaplectana*, some of which are the most easily and economically mass producible nematodes pathogenic to insects. As the infective stage of the nematode is resistant to a wide variety of environmental conditions, it is likely that the nematodes could survive in a range of mosquito habitats. The infective stage can be dispersed by all common spray methods used for insecticides. The drawback to its general use (against forest and agricultural pests) has been its susceptibility to desiccation. This would not pose a problem in the aquatic habitat of mosquitoes.

A range of culicids has been found to be susceptible to invasion by the DD-136 strain of *Neoaplectana carpocapsae* (Table 1). These laboratory results indicated the possibility of utilizing this parasite for controlling certain mosquito species. However, irrespective of the natural larval habitat(s) of these species all infectivity tests have been carried out in smooth-bottomed containers. Such results make no provision for the length of time the nematode would normally be available to the

Table 1. Mosquitoes susceptible to invasion by the DD-136 strain of *N. carpocapsae.*

Mosquito Species	References
**Aedes abserratus*	Finney and Harding (unpublished)
Ae. aegypti	Welch and Bronskill (1962)
**Ae. atropalpus*	Finney and Harding (unpublished)
Ae. communis	Welch and Bronskill (1962)
Ae. intrudens	Welch and Bronskill (1962)
Ae. sierrensis	Poinar (1979)
Ae. stimulans	Welch and Bronskill (1962)
Ae. togoi	Welch and Bronskill (1962)
Ae. trichurus	Welch and Bronskill (1962)
Culex pipiens	Poinar and Leutenagger (1971)
Cx. p. molestus	Skierska and Szadziewska (1976)
Cx. restuans	Welch and Bronskill (1962)
Culiseta inornata	Webster (1973)

* Field collected 3rd and 4th instars tested at R.U.V.P.

host under field conditions due to two interrelated factors: the rate of settling of the nematode to the bottom of the mosquito habitat and, as Welch (1960) pointed out, roughness of the habitat substrate where uneveness would decrease the chances of grazing larvae encountering the nematodes. These factors were investigated in the present study.

MATERIALS AND METHODS. A culture of the DD-136 strain of *N. carpocapsae* was provided by R. Gaugler, New York State Science Service. It was subsequently reared in *Galleria mellonella.* All experiments used *Aedes aegypti* (Linnaeus) from R.U.V.P. stock culture. In order to determine the rate of settling of the nematodes at different temperatures, 8 × 500 ml measuring cylinders were filled with water to the 500 ml mark. Two cylinders were placed at each of 5, 10, 15 and 25°C to equilibrate. After 3 hours, 500 infective stage *N. carpocapsae* were carefully placed in each of the cylinders at the 500 ml mark and the cylinders returned to their respective temperatures. After 30, 60 and 120 minutes 5 × 1 ml samples were taken from each cylinder at the 500 ml (Depth = 0 cm), 250 ml (13.5 cm) and 0 ml (27.0 cm) marks and the number of nematodes per ml counted.

Substrate investigations were carried out in glass, flat-bottomed containers (10 × 8 cm) without substrate, with sand to 0.5 cm depth or a 1 cm layer of leaves (2 replicates of each) at room temperature (25±2°C). The dishes were set up with 250 ml water containing approximately 150 nemas/ml and the nematodes allowed to settle before addition of 50 4th instar *Ae. aegypti* to each dish. Control dishes contained substrate/no substrate plus mosquito larvae in 250 ml water only. Mortality was counted after 48 hours.

RESULTS AND DISCUSSION. The depth to which the nematodes settled in 30, 50 and 120 min. at different temperatures is shown in Table 2. Within 30 min., at all temperatures the majority would have settled to the bottom of a pool or container 13.5 cm. deep. On a smooth, impervious substrate, the nematodes, upon reaching the bottom would be freely available to the bottom browsing mosquitoes,

Table 2. Settling of the DD-136 strain of *N. carpocapsae* (doses of 500 nemas in 500 ml of water—2 replications).

Temperature (C.)	Depth (cm)	Nemas recovered/ml at indicated time (min)		
		30	60	120
5°	0	0.90 ± 0.74	0.30 ± 0.48	0
"	13.5	1.00 ± 0.67	0	0
"	27.0	6.40 ± 2.63	7.70 ± 3.06	13.7 ± 4.32
10°	0	0.70 ± 0.82	0.10 ± 0.32	0.10 ± 0.32
"	13.5	0.30 ± 0.67	0.30 ± 0.48	0
"	27.0	7.00 ± 4.64	13.10 ± 4.86	13.46 ± 6.35
15°	0	0.30 ± 0.48	0	0
"	13.5	1.00 ± 1.33	0.10 ± 0.32	0
"	27.0	5.70 ± 2.58	13.70 ± 4.95	16.60 ± 4.14
25°	0	0.40 ± 0.89	0.20 ± 0.45	0.40 ± 0.55
"	13.5	1.20 ± 0.84	1.20 ± 1.10	0.60 ± 0.89
"	27.0	12.80 ± 1.92	21.60 ± 4.62	29.40 ± 3.65

their uptake by the mosquito larvae limited only by the larval instar exposed (Dadd 1971) and the temperature (Webster 1973). However, the results shown in Table 3 quite clearly

Table 3. Effect of substrate on nematode uptake by 4th instar *Aedes aegypti.*

Test condition	% 48 h mortality[1]
No substrate	61
Sand	3
Leaves	15
Controls	0

[1] 50 larvae exposed to 37,500 nemas in 250 ml of water (2 replications).

indicate that the availability of the nematodes to the host is markedly decreased when a substrate is present. So that although smooth-bottomed containers can be used successfully for susceptibility testing of mosquito larvae, care must be taken in the extrapolation of laboratory results for the determination of effective field doses for mosquito control. Obviously special attention will have to be paid to the nature of the natural habitat(s) of the target species. Economic use of the nematode may be limited to artificial containers or rock pools as it will be necessary to vastly increase the dose of nematodes applied to a pool with a high degree of substrate uneveness or porosity. Alternatively, the nematode could be applied in a formulation that would ensure that it remained available to the mosquito for a sufficiently lengthy period of time by decreasing the settling rate.

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AN ARTIFICIAL ATTACHMENT MEDIUM AND SUBMERGED AIR SOURCE FOR LARVAE AND PUPAE OF *MANSONIA* AND *COQUILLETTIDIA*.

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Three species of mosquitoes, *Mansonia titillans* (Walker), *Ma. dyari* Belkin, Heinemann and Page, and *Coquillettidia perturbans* (Walker), occurring in Florida are characterized by modified air-tubes which allow their insertion into stems and roots of aquatic plants through which they obtain air. While air may be obtained at the water surface or from that dissolved in the water, successful development requires air from cells in submerged plant parts. Thus, in nature, there is always an association between these species and plants having submerged parts with air cells that may be penetrated by the modified larval siphon and the pupal trumpets.

Because of these specialized habitats, the developmental stages of *Mansonia* and *Coquillettidia* have been difficult to locate and retrieve under natural conditions and even more difficult to manage in the laboratory. Thus, there are gaps in our knowledge of the basic biology of this group of mosquitoes, especially in terms of measuring natural populations and in determining life cycles and seasonal histories. Development of an understanding of the population dynamics of these species for improved control will require new methodology for precise observations of field and laboratory populations.

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