SUSCEPTIBILITY OF NON-TARGET ORGANISMS TO *Nosema algerae* VAVRA AND UNDEEN, A MICROSPORIDIAN PARASITE OF MOSQUITOES

By

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Nosema algerae Vavra and Undeen, a microsporidian parasite of mosquitoes, significantly reduces the longevity and egg-laying capacity of the adult female and adversely effects the developmental cycle of malarial parasites in *Anopheles albimanus* Wiedemann, a vector of the disease in Mexico and Central America.

If an organism such as *N. algerae* is to be released into the environment, in an effort to control malaria, information should be available as to the effect it may have on organisms other than mosquitoes. Therefore, laboratory studies were initiated to determine if various predators of mosquito larvae would become infected with *N. algerae* after having fed upon diseased larvae.

The 9 predators studied were: *Anax junius* (Drury) (Odonata); *Hydrophilus* sp., *Coptotomus interrogatus*

(Fabricius) (Coleoptera); Notonecta undulata Say, Belostoma fluminea Say, Ranatra australis Hungerford (Hemiptera); Chauliodes rastricornis Rambur (Megaloptera); Procambarus sp. (Decapoda); and Gambusia affinis (Baird and Girard) (Pisces).

Test animals were continuously fed infected mosquitoes containing 1×10^5 to 3.8×10^6 spores per larva for 14-24 days. Since some predators consumed up to 240 larvae, they were exposed to as many as 2.3×10^8 spores during the testing period.

Examination of the predators at an average of 10-14 days after feeding on infected larvae was discontinued revealed Notonecta undulata as the only susceptible host, with infection rates averaging 47.9%.

The following tissues were found to be infected in the adult notonectids: gut, muscle, fat body, Malpighian tubules, tracheal epithelium, testes, brain, hypodermis and ommatidia.

INTRODUCTION AND LITERATURE REVIEW

Potential for Control

In recent years, the problems involved with chemical control of insects, such as resistance of the target organism, persistence of some of the chemicals employed and the harmful effects on non-target organisms, have created considerable interest in the use of predators, parasites and pathogens as biological control agents. The reviews by Tanada (1959), Cameron (1963), Jenkins (1964), Gerberich and Laird (1968), Laird (1970), Weiser (1961, 1963, 1970), Burges and Hussey (1971), and Chapman (1974) amply illustrate the large amount of research that has been done in this area. These also point out that while considerable progress has been made, much of the work is still in the preliminary stages.

The impetus for the present study arose primarily from the work of Anthony *et al*. (1972). These investigators examined in the laboratory the effects of infection by *Nosema algerae* Vavra and Undeen on the

fecundity and longevity of Anopheles albimanus Weidemann, a vector of malaria in Mexico and Central America. Infection produced by continuous exposure of larval stages to a dosage of 5×10^4 spores/ml of rearing water reduced the longevity of female mosquitoes from 32 days to 16 days at the LT-90 level. Since the malaria parasite requires a minimum of 9 days to develop to the infective stage, the reduced longevity could have an adverse effect upon malaria transmission. Their data also indicated that even at low dosages, egg production, viability and, therefore, production of F_1 generation larvae were significantly reduced. With the aid of a population model designed to relate the effect of the nosematosis on malaria transmission, it was proposed that an infection which reduces the female LT-90 by one-half would cause a reduction of 85-97% in the number of infective females produced in a hypothetical population. Modeling was based on previous work with malaria by MacDonald (1952) and on various assumptions related to adult and immature survival values and rates of increase of natural populations of mosquitoes, as discussed more fully by Weidhaas (1974).

Although Reynolds (1972) reported that his experimental release of Plistophora culicis Weiser on the

Pacific island of Nauru did not adversely affect the natural population of Culex pipiens fatigans Wiedemann, it is felt that a similar release of N. algerae to control A. albimanus will be much more successful. Preliminary field tests in Panama during 1974 gave very promising results, where, in one plot 86% of A. albimanus present were found to be infected (Anthony, 1974).

Nomenclature and Life History

Some confusion exists in the literature regarding Nosema stegomyiae Marchoux, Salimbeni and Simond 1903 and N. algerae Vavra and Undeen 1970.

N. stegomyiae was first described from Aedes aegypti (Linn.) (Marchoux et al., 1903). Fox and Weiser (1959) reported N. stegomyiae from adults of Anopheles gambiae Giles and A. melas (Theobald) even though minor differences were observed between their material and that of Marchoux et al. N. lutzi Kudo (Kudo, 1929) is identical with N. stegomyiae Lutz and Splendore (Lutz and Splendore, 1908) which is also considered to be the same as N. stegomyiae of Marchoux et al. (Fox and Weiser, 1959). N. anophelis Kudo (Kudo, 1925a) which had also been synonymized with N. stegomyiae (Vavra and Undeen, 1970) was found in A. quadrimaculatus Say. This species is

now known as Parathelohania anophelis (Kudo) (Hazard and Anthony, 1974). Canning and Hulls (1970) reported a Nosema from a laboratory colony of A. gambiae from Tanzania. Although these authors report the Nosema as similar to N. stegomyiae as described by Fox and Weiser (1959), they preferred to call it N. algerae Vavra and Undeen. N. algerae was first reported from a laboratory colony of A. stephensi Liston reared at the Department of Zoology, University of Illinois but obtained from the National Communicable Disease Center, Savannah, Georgia (Vavra and Undeen, 1970). Hazard (1970) reported Nosema infections in two other laboratory colonies of mosquitoes. Nosema was found in A. albimanus, A. gambiae, A. quadrimaculatus and A. stephensi colonies at the University of Maryland and in A. quadrimaculatus, A. stephensi and A. balabacensis Baisas in colonies at the Walter Reed Army Institute of Research, Washington, D.C. The Nosema described by Vavra and Undeen (1970) was found in mosquitoes obtained from the NCDC but this colony was originally obtained from the London School of Hygiene and Tropical Medicine, as was the colony maintained at WRAIR. The staff at WRAIR and at the University of Maryland often exchanged mosquitoes when one of the insectories was not producing sufficient numbers of mosquitoes for malaria studies. With all

this in mind, Hazard (1970) considered the Nosema in the WRAIR and University of Maryland colonies to be identical to the Nosema reported by Fox and Weiser (1959), Vavra and Undeen (1970), Canning and Hulls (1970) and Hazard and Lofgren (1971).

The material for the present study was originally obtained from WRAIR and is considered to be N. algerae.

The following description of the life cycle of N. algerae is taken from Vavra and Undeen (1970) and their studies with A. stephensi. At 26° C the first spores were usually found in the third instar about 4 days after spores had been fed to newly hatched larvae. Infection could be produced by feeding spores to any larval instar. In larvae the following tissues were infected: gut, Malpighian tubules, hypodermal cells, muscle, fat body, tracheal epithelium, pericardial cells and testes. Heavily infected larvae usually died before, during or after pupation. Lightly infected larvae pupated normally. In the adult mosquito, the infection spreads further, with the gut being the most heavily infected. The Malpighian tubules, intersegmental muscles, thoracic muscles, gonads, hypodermal cells, fat bodies, tracheal epithelium and pericardial cells were also invaded. The first detected stages of the parasite are round or ovoid cells with 2 nucleii.

As the organism grows, its nucleii divide, resulting in a 4-nucleate plasmodium. Usually the whole cell then divides, giving rise to 2 daughter cells, each containing 2 nucleii. These apparently repeat the cycle. Sometimes the cell division is delayed and the 4 nucleii divide again producing an 8-nucleate plasmodium, although this stage is rare. The binucleate cells eventually produced from the 4 or 8-nucleate stages either repeat the whole cycle or change into sporoblasts. Each sporoblast then develops into a single spore, which always has 2 nucleii. The mature spores are ovoid, with one pole more pointed than the other. The extruded polar filaments were about 70μ long, although complete extrusion was not confirmed. The spores average 4.34μ x 2.71μ in larvae and 4.41μ x 2.80μ in adults.

In laboratory studies by Hazard and Lofgren (1971), the following tissues of A. quadrimaculatus were found to be infected with N. algerae: accessory glands, brain, fat body, gut, Malpighian tubules, muscle, nerve ganglia, rectum, ventral diverticula, and ventriculus. In Culex pipiens quinquefaciatus Say, fat body, gut and Malpighian tubules were infected. In Culex salinarius Coquillett, fat body, gut, Malpighian tubules and muscle were infected. In Aedes aegypti, only the brain and nerve ganglia were invaded. While A. quadrimaculatus was heavily

infected, the degree of infection in the non-anopheline mosquitoes was markedly less.

Savage and Lowe (1970) also reported extensive infections in A. quadrimaculatus in laboratory studies. In some cases the infection was so intense that there were no recognizable organs in the mosquito.

Nosema stegomyiae has also been reported in nature from Aedes detritus (Haliday) and Anopheles maculipennis Meigen (Tour et al., 1971). In laboratory studies, Reynolds (1971) was able to infect Culex pipiens fatigans by hatching mosquito eggs in water to which a known concentration of spores of N. stegomyiae had been added. He concluded that N. stegomyiae would be of little value as a biological control agent for C. p. fatigans because of the limited pathological effects produced. In laboratory studies, Savage (1975) was able to produce infections per os in the following species of mosquitoes: Anopheles crucians Wiedemann, A. triannulatus (Neiva and Pinto), Aedes triseriatus Say, A. taeniorhynchus Wiedemann, Culex restuans Theobald, C. tarsalis Coquillett, C. nigripalpus Theobald, C. territans Walker, C. tritaeniorhynchus Giles and Wyeomyia medioalbipes Lutz. Although these mosquitoes were infected, only C. tarsalis and C. nigripalpus were highly susceptible. Little pathology was noted in the other species tested. Attempts to infect Psorophora

ciliata Fabricius and Toxorhynchites rutilus septentrionalis Dyar and Knab were unsuccessful.

Effect of Microsporidian Parasites on Vectored Disease

Several workers have observed that the developmental cycle of the malaria parasite in adult mosquitoes is impaired by simultaneous microsporidian infection. Garnham (1956) reported that Plistophora culicis influenced the normal development of the oocysts and sporozoites in laboratory colonies of Anopheles gambiae and A. stephensi. Bano (1958) observed an inhibitory effect on the development of Plasmodium cynomolgi Mayer in A. stephensi, reflected primarily in low oocyst counts and a retardation in the growth of the oocysts. Similarly, Fox and Weiser (1959) fed A. gambiae on patients with gametocytes of P. falciparum (Welch) and found that only about 4% of mosquitoes infected with Nosema stegomyiae developed oocysts, while 46% of the Nosema-free mosquitoes developed oocysts. In a study of A. stephensi infected with N. algerae, it was discovered that control mosquitoes produced 10,000 sporozoites of P. berghei Vincke and Lips per female salivary gland, while those infected with the microsporidan contained only 1621 sporozoites per female (Hulls, 1971). Studies by Savage et al. (1971) indicated that when A. quadrimaculatus was infected

with Nosema algerae, fewer of the adults subsequently developed sporozoites of P. gallinaceum Brumpt. Concurrent infection of A. stephensi with N. algerae and P. cynomolgi produced a reduction in the mean number of oocysts which developed in the adult female mosquito midgut possibly because the midgut wall was so disintegrated that suitable sites were not available (Ward and Savage, 1972).

Host Specificity of Other Microsporida

Although Microsporida as a group are sometimes considered to be rather host-specific, often this is not the case. As pointed out by Weiser (1961), the basic principle of old taxonomy that a new host species automatically means a new parasite species can no longer be considered valid.

Stempellia magna (Kudo) has been recorded in nature from a number of mosquitoes, including Culex pipiens Linn., C. territans Walker (Kudo, 1925b), C. restuans Theobald (Kudo, 1962; Bailey et al., 1967; Simmers, 1974), Anopheles punctipennis (Say) (Simmers, 1974), Aedes detritus (Tour et al., 1971), A. sierrensis Ludlow (Clark and Fukuda, 1967) and Culiseta inornata Williston (Simmers, 1974). Plistophora collessi Laird was reported from Culex tritaeniorhynchus and C. gelidus

Theobald in Singapore (Laird, 1959). Species of Thelohania have been reported from at least 37 species of mosquitoes in 5 genera: Aedes, Culex, Anopheles, Culiseta and Psorophora (Kellen et al., 1965; Wills and Beaudoin, 1965; Chapman et al., 1966; Anderson, 1968; Chapman et al., 1973). In Europe, Weiser (1963) reported Thelohania opacita Kudo from Aedes vexans Meigen, A. sticticus Meigen, A. annulipes (Meigen) and A. communis DeGeer and found T. legeri Hesse in Anopheles maculipennis and A. bifurcatus Linn.

This non-host-specificity is not restricted to mosquitoes by any means. Octosporea muscaedomesticae Flu has been reported in nature from 2 species of Drosophila, 2 species of Musca and 4 species of other muscoid flies (Kramer, 1964a). Nosema kingi Kramer, a parasite of Drosophila willistoni Sturtevant was found to be infective to Phormia regina (Meigen), Phaenicia cuprina (Weidemann) and Musca domestica Linn., although attempts to infect the lepidopterous larvae Bombyx mori (Linn.), Galleria mellonella (Linn.), Pseudaletia unipuncta (Haworth) and a beetle, Tribolium confusum Duval, were unsuccessful (Kramer, 1964b).

Thelohania pristiphorae Smirnoff, originally described from Pristiphora erichsonii (Hartig) was shown

to infect 10 other species of sawflies (Hymenoptera), including 6 genera and 2 families, in laboratory feeding tests performed by Smirnoff (1974). This same species also infected 2 species of tent caterpillars (Smirnoff, 1968). Fantham and Porter (1913) reported Nosema apis Zander as infective to a wide variety of insects, including bumble bees, mason bees, wasps, 4 lepidopterans, and 3 dipterans. A species of Nosema, originally isolated from Tribolium, infected Tenebrio molitor Linn., Galleria mellonella and Bombyx mori per os (Fisher and Sanborn, 1962). These authors also found that 3 species of cockroaches, 3 species of Lepidoptera and Tribolium were infected when the microsporidan was introduced surgically.

Lipa (1968) found Nosema coccinellae Lipa in 3 of 16 species of coccinellid beetles examined and Milner (1972) found Nosema whitei Weiser infective to 3 species of Tribolium and to Oryzaephilus surinamensis Linn.

Henry (1967) observed Nosema acridophagus Henry infecting 4 species of grasshoppers in nature and reported Nosema cuneatum Henry as experimentally transmissible to 6 species of grasshoppers (Henry, 1971a). In studies with Nosema locustae Canning to control grasshoppers, the same author found that 17 species had become infected in the field in tests conducted in 1969 (Henry, 1971b)

and 20 species became infected in later tests (Henry et al., 1973). This microsporidan is known to infect a total of 59 species of Orthoptera including 3 families (Henry, 1969).

In a study of Nosema oryzaephili Burges, Canning and Hurst, Burges et al. (1971) found 8 species of granivorous insects, including 5 beetles and 3 moths, to be susceptible to the infection.

Perhaps the microsporida of Lepidoptera are the best known as having wide host ranges. Nosema infesta Hall occurs naturally in 3 species of Lepidoptera and experimentally infected per os 11 other species, all in different genera (Hall, 1952, 1954). Brooks (1970) reported Nosema sphingidis Brooks of Manduca sexta (Linn.) to be experimentally transmissible to 5 species of sphingids and 3 noctuids. Thirteen lepidopteran species in 5 families were susceptible to N. trichoplusiae Tanabe and Tamashiro but 4 species of Diptera tested were not susceptible (Tanabe and Tamashiro, 1967). Finlayson and Walters (1957) were successful in infecting 7 lepidopterans and 1 dipteran with a Nosema species found in Hyalophora cecropia (Linn.). N. bombycis Nageli is known to infect experimentally at least 5 species of lepidopterans (Kudo and DeCoursey, 1940). N. destructor

Steinhaus and Hughes is experimentally infective to 9 species of moths, and Plistophora californica is infective to 9 species of Lepidoptera, 2 species of hymenopterous parasites and Chrysopa californica Coquillett (Steinhaus and Hughes, 1949). Of 5 moths tested for susceptibility to N. phryganidiae Lipa and Martignoni, only 2 became infected in tests conducted by Lipa and Martignoni (1960). Kellen and Lindegren (1969) were able to infect the naval orangeworm and the greater waxmoth with 2 species of Microsporida found in the Indian-meal moth, but could not infect 3 other moths and 2 beetles tested. These same authors, working with N. invadans Kellen and Lindegren, were able to infect 6 lepidopterous hosts (Kellen and Lindegren, 1973a). Pleistophora schubergi Zwolfer, a microsporidan of the orange-striped oakworm, also infected 10 other forest lepidopterous insects in laboratory studies in Connecticut (Kava, 1973).

Several cases have been reported of a microsporidian infection in an insect also occurring in its hymenopterous parasite. Allen (1954) found a Nosema of the potato tuberworm also present in one of its parasites, Macrocentrus ancylivorus Rohwer. Tanada (1955) reported Perezia mesnili Paillot as infecting both Pieris rapae (Linn.) and its parasite Apanteles glomeratus Linn. York (1961) reported Perezia pyraustae Paillot as

infecting 3 parasites of the European corn borer. Smirnoff (1971) found Thelohania pristiphorae parasitizing a eulophid parasite of 4 species of sawflies in Canada.

Collections by Cort et al. (1960) revealed a Nosema sp. infecting 12 species of strigeoid trematodes of snails. This species was subsequently named Nosema strigeoideae by Hussey (1971). Lie and Nasemary (1973) found that Nosema eurytremae Canning could easily be transmitted from one trematode species to another by feeding spores to snails. They were also able to infect Aedes aegypti, A. albopictus (Skuse) and A. togoi (Theobald) by feeding them spores. Canning et al. (1974) also found that microsporidian parasites of trematodes were not host-specific. Dissanaike (1958) experimentally infected 2 species of tapeworms and 2 oribatid mites with Nosema helminthorum Moniez, a parasite of Moniezia cestodes.

Wide host ranges are also known from other insect parasites. For example, Helicosporidium parasiticum Keilin was infective to 15 species of insects in 3 orders, including Culex pipiens, as well as 3 species of mites (Kellen and Lindegren, 1973b). Mattesia is infective to 12 species of Lepidoptera, 3 Coleoptera, 1

Siphonaptera and 1 Hymenoptera. The fungus Metarrhizium anisopliae (Metschnikoff) is known to attack over 50 species of insects in 7 orders and Beauvaria bassiana (Balsamo) may have an even wider host range (Bailey, 1971).

The Predators Tested

The following predators were used as test animals in this study: Gambusia affinis (Baird and Girard), Anax junius (Drury), Hydrophilus sp., Coptotomus interrogatus (Fabricius), Notonecta undulata Say, Belostoma fluminea Say, Ranatra australis Hungerford, Chauliodes rastricornis Rambur, and Procambarus sp.

Gambusia affinis is a prolific live-bearer known as the mosquito fish. Because of its widespread use in mosquito control programs (Bay, 1967; Gerberich and Laird, 1968; Hoy et al., 1971), it is now considered to be the most widely distributed fresh-water fish in the world (Bay, 1967). Females can begin to produce young at 6 weeks of age, although more often they overwinter before beginning to breed at 8-10 months. In their lifetime, 3-4 broods are produced, each containing up to 300 young. Given favorable conditions, several thousand specimens can be obtained by September or October from as few as 50-100 stocked in a pond in June (Bay, 1967).

Anax junius is a large species of dragonfly in the family Aeshnidae. The nymph passes through 13 instars. In laboratory rearing studies conducted by Calvert (1934), the minimum time from egg to adult was 177 days. A reported development time of 4 months in nature has not been verified. Calvert (1934) states that the age of a given nymph can be determined only within an approximation of 1 or 2 instars because different individuals and different parts of the same individual grow at different rates. Odonata are often reported as predators of mosquitoes. Sailer and Lienk (1954) reported Odonata as consuming Culiseta, Anopheles, and Culex larvae in Alaska. Pritchard (1964) reported Aeshna, Leucorrhinia and Sympetrum as feeding upon Culex territans and Anopheles earlei Vargas. Brooke and Proske (1946) reported Anax junius as a predator of A. quadrimaculatus using radioactive tracers. In laboratory studies, Lee (1967) found Anax junius to feed readily upon Culiseta incidens Thomson.

Arnett (1960) reported 206 species of hydrophilids occurring in the United States. Eggs are laid in silken cases, all larvae pass through 3 instars and all except Enochrus pupate out of water in earthen cells. The larval stage of Hydrophilus triangularis (Say) is said to be 15

days while H. obtusatus Say became full grown in about 30 days, depending on temperature. A species of Enochrus was reported to have a larval period of 2 months (Balduf, 1935). H. obtusatus was an important predator of Aedes stimulans Walker and A. trichurus Dyar in radioactive tracer studies conducted by Baldwin et al. (1955) and James (1961). James (1965) reported Enochrus and Berosus as important predators of Aedes atropalpus (Coquillett). Two species of Tropisternis were also found to be mosquito predators in Canada (James, 1967). In laboratory studies, Clarke (1938) claimed that 3 larvae of H. triangularis consumed 1000 mosquitoes each in one day.

Arnett (1960) reported 329 species of Dytiscidae from the United States. All have 3 larval instars. Eggs of Coptotomus are inserted in submerged plants and pupation takes place out of water in a mud cell. The larval stage of Dytiscus semisulcatus Muller varied from 16 days at 26° C to 15 weeks at 6° C. In Cybister lateralimarginalis DeGeer the total larval stage was passed in 42-47 days in the summer in Germany. Hydroporus depressus Fabricius was reported to have a 21-day larval stage (Balduf, 1935). Service (1973) reported Agabus and Dytiscus as the most important predators of Aedes cantans (Meigen) using the precipitin test. A large number of

dytiscids, including Coptotomus interrogatus, have been reported as important predators of mosquitoes, including Aedes atropalpus, A. trichurus, A. stimulans and A. communis in radioactive tracer studies by James (1961, 1965, 1966, 1967). Two species of Laccophilus readily fed on Culex pipiens and a chironomid in laboratory studies (Roberts et al., 1967).

Notonectids, or back swimmers, have long been known as predators of mosquito larvae (Hungerford, 1917; Clark, 1928; Toth and Chew, 1972a) and recent laboratory studies indicated a preference of Notonecta undulata for mosquito larvae when other food was available (Ellis and Borden, 1970). Twinn (1931) reported a Notonecta sp. as feeding on Culex larvae. Lee (1967) reported N. undulata as an important predator of mosquito larvae. Hinman (1934) referred to N. undulata as the most voracious of all hemipterans known to him. Clark (1928) found notonectids to feed on a wide variety of organisms including Coptotomus interrogatus. In laboratory rearing studies, Toth and Chew (1972a) reported 57.3 days as the minimum generation time for N. undulata at 23° C. Eggs hatched in 14 days, nymphal stages I-IV required 22.1 days, stage V required 12.7 days, and 8.5 days was necessary from adult to the appearance of the first eggs. Nymphs and adults were cannibalistic.

Belostomatids are known as effective predators of mosquitoes (Jenkins, 1964). Severin and Severin (1911) reported Belostoma fluminea as feeding on many insects including N. undulata. Blatchley (1926) refers to this species as a predacious carnivore. Young (1921) reported a Belostoma sp. as a natural enemy of Aedes aegypti and Culex pipiens fatigans. In radioactive tracer studies, Baldwin et al. (1955) found B. fluminea preying upon A. stimulans and A. trichurus. Eggs of Belostoma are glued to the backs of the males and are carried there until they hatch. In Canada, Brooks and Kelton (1967) report that the 5 nymphal instars require about 7 weeks to develop. In laboratory studies by Torre-Bueno (1906a), 3 specimens of B. fluminea were reared from eggs. The time required from the date of oviposition to mature adults was 43, 53 and 54 days, respectively.

Ranatra australis was reported by Blatchley (1926) to be the most common Ranatra in Florida. Ranatra fusca Beauvois, a close relative, was reported to feed on Anopheles and Culex larvae by Jenkins (1964), A. quadrimaculatus by Brooks and Proske (1946) and on Aedes spp. by Baldwin et al. (1955). Several other genera of Nepidae are also reported as predators of mosquito larvae (Jenkins, 1964). Torre-Bueno (1906b) presented the

life cycle of R. quadridentata Stal. The nymph passed through 5 instars and matured in 61 days in the laboratory. In nature it is known to overwinter in the bottom mud.

Cuyler (1958) reported only 2 species of Chauliodes in North America. Usinger (1971) reported that eggs are deposited on stones, branches or bridges that overhang the water. The length of the larval period is not known but may be 3 years. In discussing Megaloptera in general, Pennak (1953) reported the larvae as passing through 10 instars in 2-3 years. They pupate on shore. Chauliodes sp. was reported as a predator of Aedes stimulans and A. trichurus by James (1961). The hellgrammite, Corydalus cornutus (Linn.) is a close relative.

Procambarus is a large genus of common crayfish, with many species occurring in Florida (Hobbs, 1942). Crayfish do not leave the parent until the third instar and individuals hatching in the spring have 6-10 molts by autumn. Copulation usually occurs in the first autumn of life although some females are not sexually mature until early the following spring. Subsequent to the first mating season, most crayfish have only 2-4 molts before death (Pennak, 1953). Barnes (1963) reports most decapods as predators or predators and scavengers. Pennak

(1953) refers to crayfish as mainly scavengers, but they will eat animal food. In the present study, mosquitoe larvae were fed upon quite readily.

Purpose of the Present Study

If an organism such as Nosema algerae is going to be released into the environment in an effort to control anopheline mosquitoes and hopefully malaria, information about its effects on organisms other than mosquitoes should be obtained. Therefore, studies were initiated at the USDA, ARS, Insects Affecting Man Research Laboratory, Gainesville, Florida, to see if various predators of mosquito larvae would become infected with Nosema after having fed upon diseased larvae.

MATERIALS AND METHODS

Maintenance of Infected Mosquitoes

A supply of Anopheles quadrimaculatus larvae infected with a strain of Nosema algerae originally obtained from the Walter Reed Army Institute of Research, Washington, D.C. (Hazard, 1970) was maintained continuously in the laboratory. First instar larvae obtained from the laboratory stock colony were exposed to a concentrated aqueous spore suspension of approximately 5 x 10^6 spores/ml in a small petri dish for 1-2 hours and then transferred to white enamel rearing pans (30 x 19 x 5 cm) containing about 700 ml of water. One hundred fifty to 200 larvae were reared in each pan on a diet consisting of a 1:1:1 mixture of liver powder, dried brewer's yeast and finely ground hog supplement (Ralston Purina Company). A slurry was made with 2 gm of this mixture per 100 ml of water and then 15 ml of the slurry was added to each pan. No additional food was added until day 4 when a small amount of hog supplement was dusted on the water surface. This was continued daily until day 12 or 13. Pupation began on day 9 or 10. Pupae were then collected with a pipette and

transferred to cages (28 x 22 x 22 cm) to await adult emergence.

Adults of the stock laboratory colony were maintained at 29°C and 70% R.H. under natural light conditions (through windows in the room) during the day and were provided with only a small light in one corner of the room during the night. Cages measured 76 x 76 x 64 cm. Rabbits with closely shaved backs were provided for 2 hours each day for the females to feed on and cotton wads soaked in 20% sugar water were provided as a supplemental food source. The cage was supplied with a white enameled pan (25 cm diam. x 7.6 cm deep) containing about 2.5 cm of distilled water for oviposition. All viable eggs float. For hatching, eggs were placed in a pan (30 cm diam. x 5 cm deep) containing distilled water mixed with 200 mg of brewer's yeast. Newly hatched larvae were transferred to the 30 x 19 x 5 cm pans and reared as mentioned above at 29°C and 70% R.H. under conditions of constant light.

All rearing of infected larvae, as well as maintenance of all predators tested and exposure of the larvae to Nosema, was carried out in one room under the following conditions: 30°C, 70% R.H. and a 16 hr photoperiod. Infected adult mosquitoes were maintained in a separate room at 26.5°C, 70% R.H. and a 12 hr photoperiod. Cotton

balls soaked in 20% sugar water were provided as a food source for the adults.

Adult mosquitoes that were about 1 week old were crushed in a Ten Broeck glass tissue grinder to produce the spore suspension that was immediately used to infect the next set of first instar larvae. The spore suspension was filtered through Kimwipes™ to remove large debris and was then centrifuged at 3000 rpm (1700 G) for 5 minutes to help eliminate bacteria and other extraneous material before being fed to the larvae. In this manner, a constant supply of infected larvae was available to be fed to the test organisms.

Collection and Maintenance of Predators

Gambusia affinis and Chauliodes rastricornis were collected from Hatchet Creek near Gainesville, Florida. The crayfish, Procambarus sp., was collected from Lake Alice on the University of Florida campus, Gainesville. All other predators studied were collected in the Gainesville area from a small temporary pond near Castle Gate Mobile Home Park.

Pregnant females of Gambusia were held in 20 ℓ aquaria and fed on uninfected mosquito larvae (any species that was available) until the young were born. These were

then fed on progressively larger larvae until fourth instar A. quadrimaculatus could be taken (at about 1 month), whereupon they were separated and kept individually in pint Mason jars for the subsequent feeding tests using infected larvae. Aeration was unnecessary when the water was changed about once a week, and mortality was negligible using this simple system.

Adults of Notonecta undulata were held in 20 ℓ aquaria until egg-laying took place. As suggested by Ellis and Borden (1969) and Toth and Chew (1972b), rubber sink matting suspended in the water provided a substrate for the eggs as well as a resting place for adults (Figs. 1-3). Mating presumably had already taken place in the field since eggs were usually seen only 2 or 3 days after the adults had been brought into the laboratory. Since the young are cannibalistic, they were separated into small glass rearing dishes of about 300 ml capacity (11.5 cm diam. x 5 cm deep) as soon as the eggs hatched. As with young Gambusia, progressively larger mosquito larvae (uninfected) were used as food until fourth instars could be taken (about 1 week), at which time infected larvae were used for the duration of the tests. Again, aeration was unnecessary when the water was changed once a week. Mortality was negligible after an initial die off due to

improper hatching or molting, cannibalism before separation, etc.

Crayfish were collected in 3 size ranges. Small and medium size specimens were maintained separately in the small glass rearing dishes. Larger individuals were kept in enamel mosquito rearing pans when the bowls appeared to be too small and confining.

All other predators studied were held separately in the small glass rearing bowls.

In all cases, except the 7 large specimens of Procambarus, it was the young, immature stages of the predators that were exposed to the Nosema.

These organisms were chosen because they are known mosquito predators, they were readily collected in nature, they were rather easy to maintain in the laboratory and they represent a fairly wide range of animals taxonomically speaking.

Standard Feeding Procedure

Each individual predator was held in a separate container, as described above, to make sure that each had eaten a known number of mosquitoes. Infected fourth instar A. quadrimaculatus that were 10 days or older were used as food and were counted out for each predator. The

number fed per individual varied according to the size of the test animal, from small notonectids eating only 2 or 3 a day to large crayfish consuming 20-25 a day (Tables 1-9). Each container was checked the following day to determine how many larvae had been ingested during the previous 24 hr period.

Each day 10 larvae were chosen at random, macerated in a tissue grinder, and spore counts were taken with the aid of a hemacytometer. Knowing the number of larvae and the amount of water they were crushed in allowed a calculation of the average number of spores of N. algerae per mosquito. Thoughout the testing, the number of spores per larva was within the range of 1.0×10^5 to 3.8×10^6. By keeping a record of the number of mosquitoes fed to each predator and of the number of spores per mosquito, it was possible to calculate the approximate number of spores ingested by each test animal.

In most cases they were fed infected larvae daily for about 3 weeks and then examined 7-10 days later (Tables 1-9) to determine if they had become infected with N. algerae.

Tests for Presence of Nosema

Several standard laboratory techniques were utilized in searching for the presence of Nosema in the predators tested. Spores of most Microsporida are highly refractive and can be easily observed using phase microscopy (Fig. 4). The usual procedure was to cut the specimen in two locations, smear some body fluid and tissue on a slide, add a cover slip and immediately look for spores. On the larger species (i.e., Anax, Gambusia), organs were dissected out and smeared, while on very small ones (i.e., Coptotomus) the whole body was smeared. Giemsa smears were also prepared in order to identify the presence of vegetative stages of the pathogen (Figs. 5-8). The specimen or tissue was crushed on a slide, allowed to air dry, fixed in methanol for 5 minutes, stained in a 9:1 mixture of pH 7.41 buffer and Giemsa for 10 minutes, washed gently with tap water and allowed to air dry (Humason, 1967).

If spores were discovered in the initial body fluid smears, then permanent wet mounts were prepared. Tissue was smeared on a cover slip and dropped immediately into aqueous Bouin's fixitive for at least 2 hrs. It was then soaked in 70% ethanol overnight, treated with iron alum mordant for 5 hours and stained overnight in Heidenhain's hematoxylin diluted 1:1 with distilled water. The pre-

paration was then destained in iron alum, dehydrated in graded ethanols, cleared in xylene and mounted (Humason, 1967).

As additional proof that the infection was indeed Nosema algerae, some infected material was retained for electron microscope preparation, using 4% gluturaldehyde and 1% osmium tetroxide as fixatives, propylene oxide as the transitional solvent and Epon-Araldite as the embedding medium (Pease, 1964; Kay, 1965) (Figs. 15-16). Also, some material was saved to be fed back to mosquitoes. In this case, infected predators were crushed in a tissue grinder and a concentrated spore suspension was produced. First instar Anopheles albimanus and A. quadrimaculatus were then exposed to the resulting spore suspension, as previously discussed, under the standard laboratory conditions and then transferred to the rearing pans until old enough to check for infection.

In order to determine what tissues became infected, paraffin sections were prepared. Infected predators were fixed in Carnoy's solution for 2-4 hrs, rinsed in 70% ethanol for 1 hour and then soaked in 70% ethanol overnight. The tissue was dehydrated in graded ethanols, rinsed in a 1:1 mixture of absolute ethanol and butanol, soaked in 3 changes of 100% butanol, transferred to a 1:1 mixture of butanol and paraffin, infiltrated with paraffin

under vacuum and finally embedded in fresh paraffin. Tissue thus embedded was sectioned and allowed to air dry on slides before being stained. Paraffin was removed in 3 changes of xylene, tissue was hydrated through graded ethanols to distilled water, soaked in iron alum for 5 hrs and stained overnight in Heidenhain's hematoxylin. The preparation was then destained in iron alum, counterstained in Eosin, dehydrated through graded ethanols back to xylene and mounted in Histoclad™ (Humason, 1967).

RESULTS

The results of the tests are presented in Tables 1-9. In most cases, more than one attempt was made to infect the test animals. This is indicated by test no. 1, 2, etc. The number of animals fed may include ones that had died only 2 or 3 days after the feeding of infected larvae had ceased. But the majority were examined on the day indicated in the last column or, where large numbers were involved, on the following day as well. As mentioned previously, the predators were exposed continuously to Nosema-infected larvae and the extent of the exposure is recorded in column 3. The maximum time available for development of the pathogen, therefore, is the sum of columns 3 and 7. Careful records were also kept of the number of larvae fed to each predator and the number of spores contained within each larva. This data is presented in columns 4 and 5. Many of the predators consumed a large number of infected mosquitoes and consequently were exposed to a high dosage of spores. For example, in 1 test with Anax, 222 larvae were consumed by each nymph over a 24-day period (Table 1). During this time they ingested over 190 million spores each. In a test using Gambusia 125 larvae were consumed,

representing about 230 million spores (Table 9). In 2 instances (Procambarus and Hydrophilus), size variation in the collected material was obvious and so they were divided into 2 or 3 groups. This is indicated in parentheses as small, medium and large under column 2.

Table 10 summarizes the data for all tests conducted, indicating the number of tests, the number of predators tested and the percentage of those which subsequently became infected. Only 1 of 9 species tested was susceptible to Nosema algerae after having fed upon diseased larvae under laboratory conditions.

Nosema infection was found during all 4 tests conducted with Notonecta. The percentage did not vary appreciably from test to test and averaged 47.9%. All adults used during these tests were collected from the same temporary pond. Eggs were laid by only 2 or 3 of the females collected for each test and it was felt that transovarial transmission from the female to the egg of a natural microsporidian infection resembling N. algerae could have accounted for the infections observed in the nymphs. Therefore, during the last test (#4), 20 controls, which were fed uninfected larvae, were set up along with the test animals. None of the control nymphs were found to be infected, while 56.2% of the test insects were. Also, many more adults than were necessary to recover enough

eggs for testing were collected. After enough eggs were laid, all 28 specimens collected were sacrificed and examined for the presence of a natural infection. All were negative.

As further proof that N. algerae was the pathogen involved, infected notonectids were ground up to produce a spore suspension to feed back to mosquitoes. Four notonectids, containing an average of 5.5×10^7 spores per individual, were used for this purpose. Both Anopheles quadrimaculatus and A. albimanus became infected after an exposure of 1 hr to a spore suspension of 5.5×10^6 spores/ml. This procedure was performed twice, once for test 3 nymphs and once for test 4 nymphs. On both occasions, 90% of A. quadrimaculatus and 100% of A. albimanus were found to be infected after 8 days.

Some material was prepared for electron microscope observations in order to compare the appearance of N. algerae in N. undulata and in A. quadrimaculatus. Fixation of the microsporidan in the notonectid was not as good as in the mosquito. Nevertheless, comparison of spore size, spore shape, spore wall thickness, number of turns of the polar filament and the binucleate nature of the spore indicate the parasite is identical in the 2 hosts (Figs. 15-16).

Microscopic examination of paraffin sections revealed the following tissues to be infected in the adult notonectids: gut, muscle, fat body, Malpighian tubules, tracheal epithelium, testes, brain, ommatidia and hypodermis (Figs. 9-14). The most heavily infected tissues were fat and thoracic musculature. The Malpighian tubules, testes and gut tissue were moderately infected. Tracheal epithelium was usually lightly infected. At times, infection was so heavy that identification of the tissue involved was quite difficult. In some specimens, the fat body was practically non-existent. The thoracic musculature was always much more heavily infected than the abdominal musculature. Spores were generally evenly dispersed throughout the tissue, although they did occur in batches in the testes. This is not surprising, as the testes are composed of small sacs or follicles.

Examination of infected A. quadrimaculatus adults revealed the most heavily infected tissues to be gut and Malpighian tubules. The ommatidia and fat body were somewhat less heavily infected. Infection of the tracheal epithelium was much heavier than that of N. undulata, while the thoracic musculature and fat body usually were not as heavily infected as in the notonectids.

Mortality records were only kept for tests 3 and 4. In test 3, 12 of 52 backswimmers tested died by the end

of the examination period (23.1%) and all 12 were infected. In test 4, 5 of 16 died (31.2%) and all 5 were infected. This compares with a mortality of 20% for the controls. It is assumed that had the tests been continued for a longer period of time, a higher rate of mortality would have been recorded for the test animals since 10 of those still alive in test 3, and 4 still alive in test 4 were subsequently found to be infected and probably would have died.

The tissue specificity of the microsporidan in N. undulata and mosquitoes, the comparison of spores of the pathogen in N. undulata and A. quadrimaculatus using electron microscopy, the positive results obtained in feeding spores from infected notonectid back to mosquitoes, and the results of the fourth test with notonectids in which controls were not infected while 56.2% of the test insects were, all demonstrate transmission of N. algerae to N. undulata.

Thus, of 9 mosquito predators tested for per os susceptibility to Nosema algerae, only Notonecta undulata became infected. This result was obtained in all 4 tests in which Notonecta was used.

Table 1. Per os susceptibility of *Anax junius* to *Nosema algerae*.

Test No.	No. animals fed	No. days fed	No. larvae ingested per animal	No. spores ingested per animal	% infected	No. days between last feeding of spores and examining animals for infection
1	5	15	240	1.2×10^7	0	22
2	5	15	116	1.5×10^8	0	13
3	8	24	222	1.9×10^8	0	10
4	7	14	90	6.7×10^7	0	9

Table 2. Per os susceptibility of *Hydrophilus* sp. to *Nosema algerae*.

Test No.	No. animals fed	No. days fed	No. larvae ingested per animal	No. spores ingested per animal	% infected	No. days between last feeding of spores and examining animals for infection
1	5	9	36	6.8×10^7	0	8
2	2 (large)	14	160	1.2×10^8	0	9
	14 (small)	14	85	6.3×10^7	0	9

Table 3. Per os susceptibility of *Coptotomus interrogatus* to *Nosema algerae*.

Test No.	No. animals fed	No. days fed	No. larvae ingested per animal	No. spores ingested per animal	% infected	No. days between last feeding of spores and examining animals for infection
1	6	15	21	2.9×10^7	0	10

Table 4. Per os susceptibility of Notonecta undulata to Nosema algerae.

Test No.	No. animals fed	No. days fed	No. larvae ingested per animal	No. spores ingested per animal	% infected	No. days between last feeding of spores and examining animals for infection
1	8	17	83	9.0×10^7	50.0	4
2	51	22	110	7.2×10^7	43.1	7
3	52	22	86	5.2×10^7	42.3	14
4	16	19	105	6.1×10^7	56.2	10

Table 5. Per os susceptibility of Belostoma fluminea to Nosema algerae.

Test No.	No. animals fed	No. days fed	No. larvae ingested per animal	No. spores ingested per animal	% infected	No. days between last feeding of spores and examining animals for infection
1	6	15	120	1.7×10^8	0	10
2	9	24	125	1.1×10^8	0	10
3	7	14	70	8.9×10^7	0	10

Table 6. Per os susceptibility of Ranatra australis to Nosema algerae.

Test No.	No. animals fed	No. days fed	No. larvae ingested per animal	No. spores ingested per animal	% infected	No. days between last feeding of spores and examining animals for infection
1	4	24	137	1.2×10^8	0	10
2	4	19	95	1.6×10^8	0	8
3	9	15	165	1.1×10^8	0	9

Table 7. Per os susceptibility of Chauliodes rastricornis to Nosema algerae.

Test No.	No. animals fed	No. days fed	No. larvae ingested per animal	No. spores ingested per animal	% infected	No. days between last feeding of spores and examining animals for infection
1	5	23	184	1.5×10^8	0	2

Table 8. Per os susceptibility of Procambarus sp. to Nosema algerae.

Test No.	No. animals fed	No. days fed	No. larvae ingested per animal	No. spores ingested per animal	% infected	No. days between last feeding of spores and examining animals for infection
1	12 (small)	17	79	3.1×10^7	0	12
	6 (medium)	17	160	6.3×10^7	0	12
	7 (large)	17	250	9.9×10^7	0	12

Table 9. Per os susceptibility of Gambusia affinis to Nosema algerae.

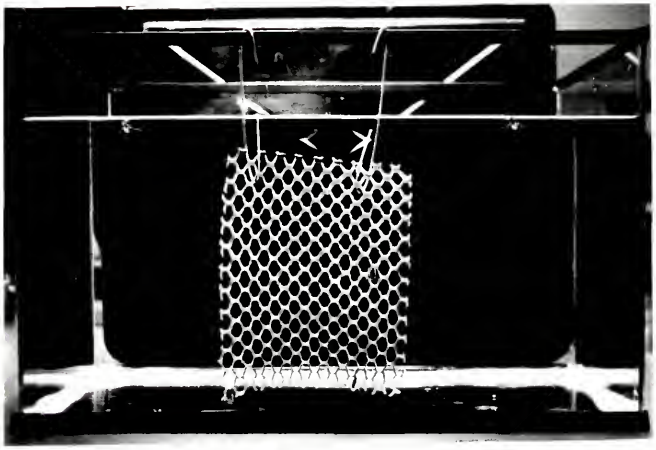
Test No.	No. animals fed	No. days fed	No. larvae ingested per animal	No. spores ingested per animal	% infected	No. days between last feeding of spores and examining animals for infection
1	8	28	125	2.3×10^8	0	14
2	7	24	120	1.3×10^8	0	2
3	18	14	70	5.4×10^7	0	6
4	12	17	85	6.8×10^7	0	4

Table 10. Summary of per os susceptibility tests for *Nosema algerae* to 9 predators of mosquitoes.

Host	No. of Tests	Total No. animals fed	% infected
Odonata			
Anax junius	4	25	0
Coleoptera			
Hydrophilus sp.	2	21	0
Coptotomus interrogatus	1	6	0
Hemiptera			
Notonecta undulata	4	127	47.9
Belostoma fluminea	3	22	0
Ranatra australis	3	17	0
Megaloptera			
Chauliodes rastricornis	1	5	0
Decapoda			
Procambarus sp.	1	25	0
Chordata			
Gambusia affinis	4	45	0

Fig. 1 Set up for rearing Notonecta undulata, showing 20ℓ aquarium and sink matting suspended in the water. Note several adults on the water surface and on the matting.

Fig. 2 Close up of Fig. 1, showing an adult N. undulata, eggs, and one newly-hatched nymph.



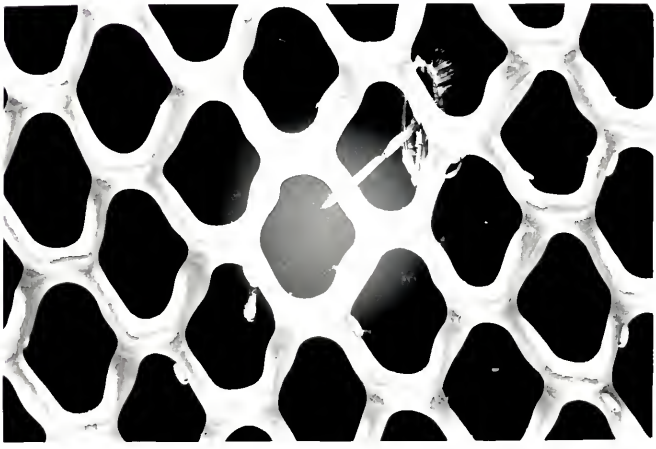


Fig. 3 Close up of N. undulata egg on a portion of sink matting.

Fig. 4 Spores of Nosema algerae from fourth instar A. quadrimaculatus as seen in an oil suspension using phase microscopy. (1000 X)

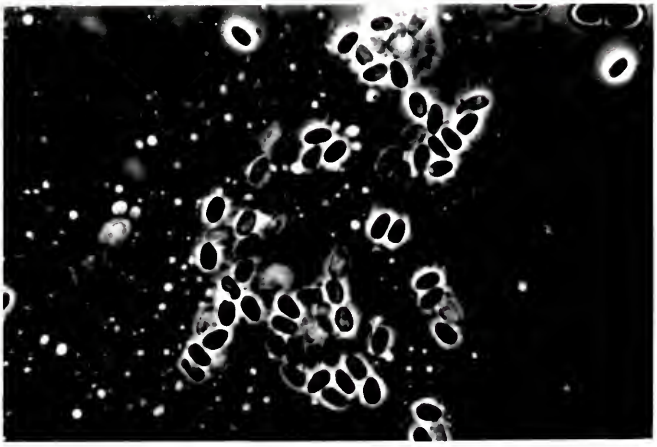
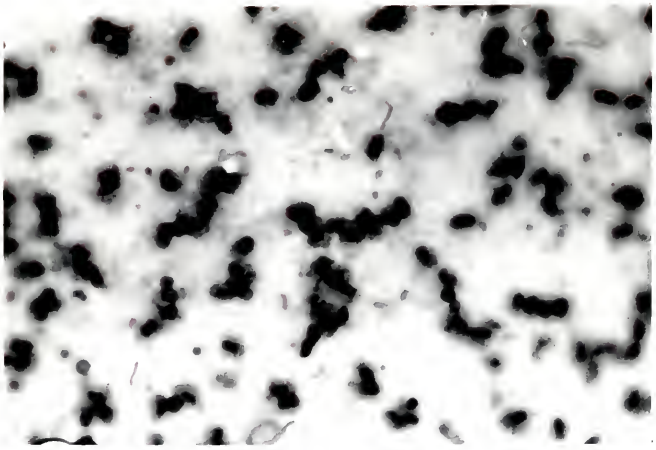


Fig. 5 Spores of N. algerae from adult N. undulata fixed in methanol and stained in Giemsa. (1000 X)

Fig. 6 Binucleate vegetative stages of N. algerae from adult N. undulata fixed in methanol and stained in Giemsa. (1000 X)



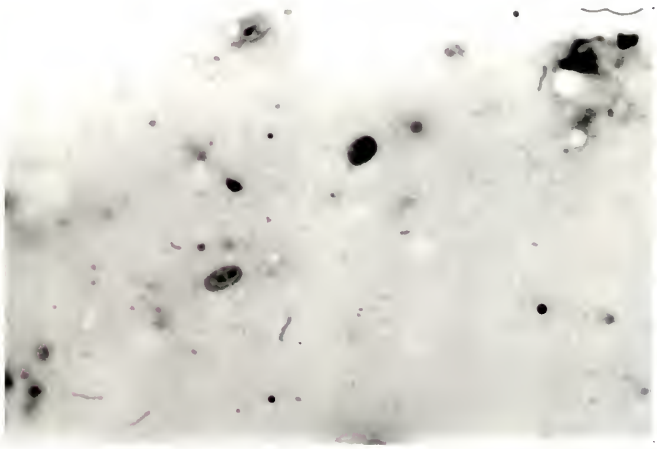
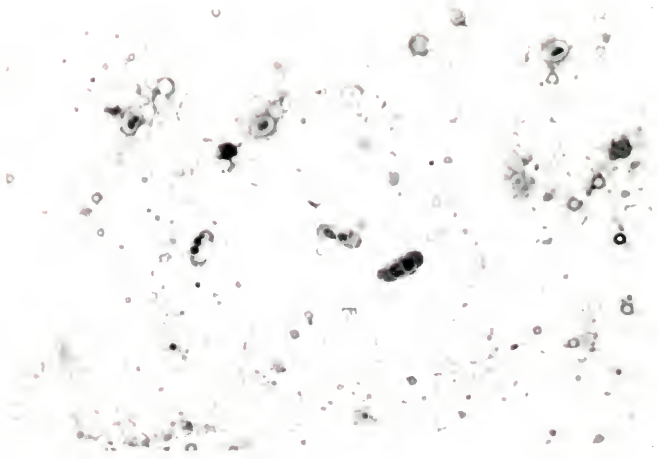


Fig. 7 Binucleate stages of N. algerae from fourth instar A. quadrimaculatus with several spores noticeable in top left portion of the photograph, Giemsa stain. (1000 X)

Fig. 8 Later binucleate stage of N. algerae from adult N. undulata, Giemsa stain. (1000 X)



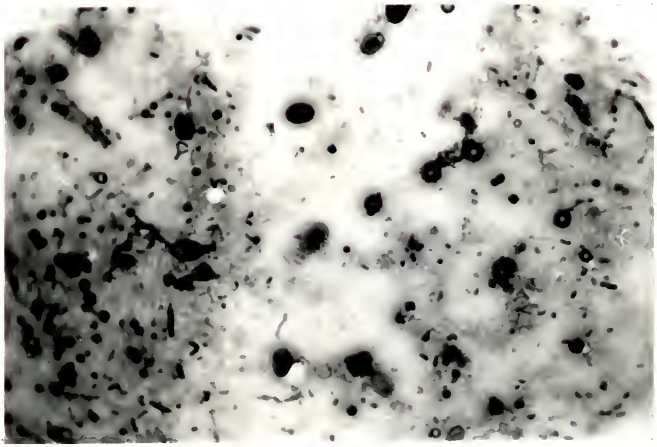


Fig. 9 Spores of N. algerae (s) in muscle tissue of adult N. undulata. (250 X)

Fig. 10 Heavily infected portion of muscle of adult N. undulata. (250 X)


S

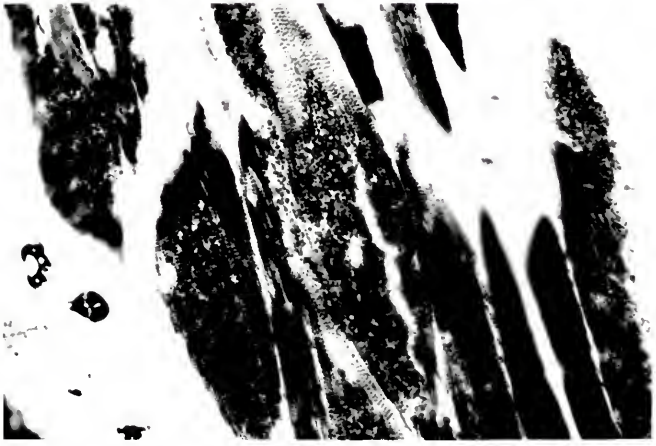
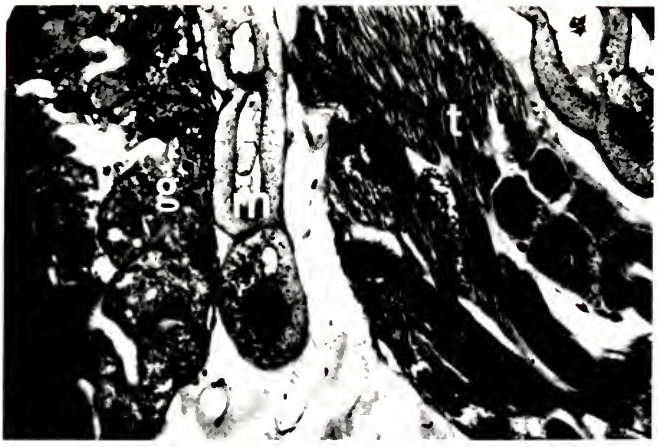


Fig. 11 Infection of gut (g), Malpighian tubule (m) and testes (t) of adult N. undulata. (250 X)

Fig. 12 Spores of N. algerae (s) in testes of adult N. undulata. (250 X)


g
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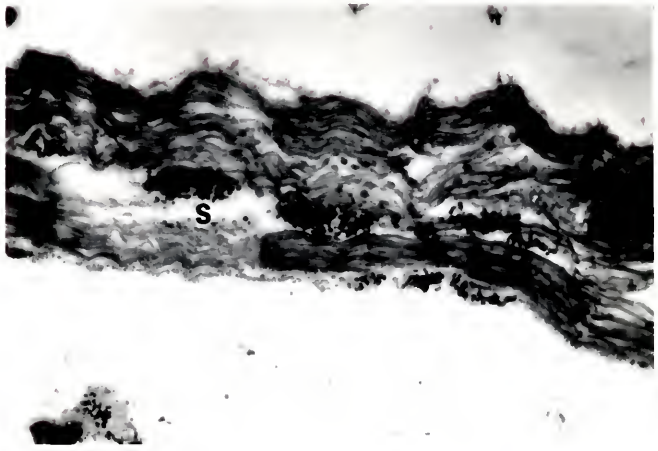
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Fig. 13 Section through brain of adult N. undulata showing spores of N. algerae (s). (250 X)

Fig. 14 Spores of N. algerae (s) in hypodermal cells of adult N. undulata. (250 X)

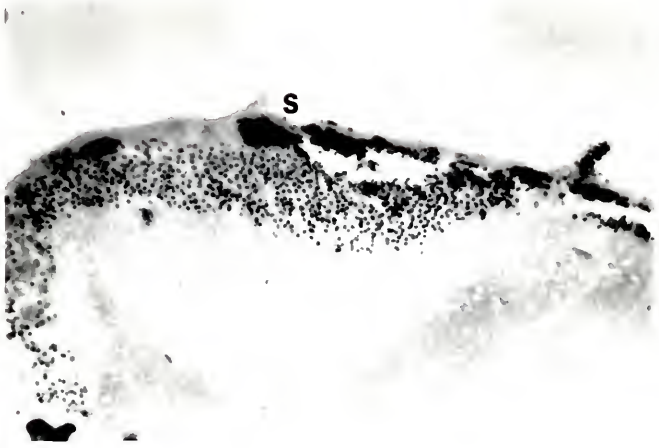
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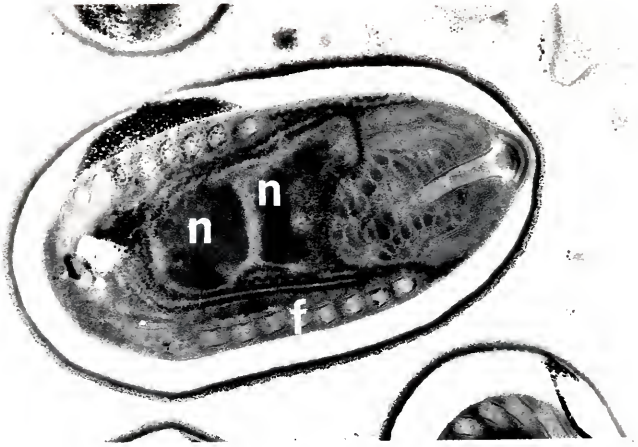
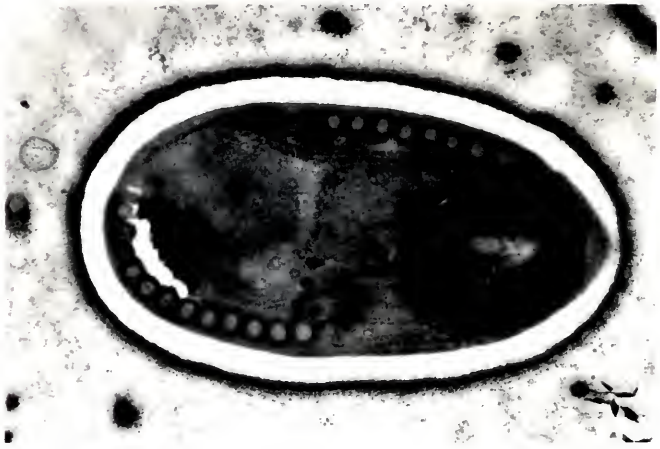
Fig. 15 Electron micrograph of spore of N. algerae from adult A. quadrimaculatus, showing the 2 nuclei (n) and coils of the polar filament (f). (20,000 X)

Fig. 16 Electron micrograph of spore of N. algerae from adult N. undulata. (20,000 X)


n
n
f



DISCUSSION

As previously indicated, although wide host ranges are known among various terrestrial insects, this phenomenon is not as widespread in aquatic species. It is therefore somewhat surprising that in the present study Nosema algerae was infective to Notonecta undulata. A species of Notonecta is also susceptible to Coelomomyces (Ignoffo and Hink, 1971) and Laird (1971) thinks it conceivable that C. notonectae Bogoyavlensky represents an infection derived from larval mosquito prey. This is the only report of fungi of the genus Coelomomyces outside of the Diptera (Roberts, 1970).

Having a wide host range is not necessarily detrimental. In the case of Nosema oryzaephili, which can infect 8 species of granivorous insects, including beetles and moths, its non-host-specificity is a distinct advantage in a situation where many pests often occur together. Although it has not been demonstrated in the field, the infectivity of N. algerae to a large number of mosquito species may also be an advantage. And, as McLaughlin (1971) points out, the larger the host range, the more persistent the pathogen is likely to be in nature.

It should be emphasized that the infection of N. undulata was produced in the laboratory and not in the field. Infectivity in the field is undoubtedly a multifactorial quality and changes of various factors in nature that would allow infection to occur in a new host are perhaps not probable. However, there is no way of knowing whether the changes that are necessary will or will not occur (Bailey, 1971). As pointed out by Laird (1973), the fungus, Beauvaria bassiana, has been shown to reproduce in bees under laboratory conditions, but no infection of bees has ever been shown in nature, despite the world-wide distribution of the fungus and the fact that bees are among the best known of insects.

Another point is that the infection was produced by continuous exposure of the animal to a large dosage of spores - a dosage that is not likely to occur in nature. And, since the tests were conducted in the laboratory, the animals involved may have been stressed, although all other animals tested would also have been stressed and yet were not susceptible.

The results also indicate that those which do become infected eventually die of the disease.

In studies with N. algerae, Undeen and Maddox (1973) were able to infect Heliothis zea (Boddie) per os but were unable to infect Anopheles atroparvus Van Thiel, a Chiro-

nomus species, Corydalus cornutus (Linn.), Blaberus discoidalis Serville and 3 other species of lepidopterans. Although not a natural infection route, injecting animals with spores produced infections in 11 species of insects and a crayfish. They were unsuccessful when injecting a triclad platyhelminth, an annelid, a frog and a mouse.

Savage (1975) was also able to infect H. zea, Musca domestica Linn. and Stomoxys calcitrans (Linn.) with N. algerae. In fact, mass production of spores has been undertaken using H. zea, although 24 hr food deprivation is required to produce a large number of spores. These hosts are not likely to come in contact with spores released for control of mosquito larvae.

Although Notonecta undulata became infected in the laboratory, another aspect to be considered is that infected mosquitoes served as the entire diet, while in nature, other prey may be available. Quantitative information as to what percentage of the natural diet is composed of mosquitoes is not known. In most cases this would vary from place to place depending on the variety of other food present. In Frink Spring, California, mosquitoes were the most available natural food for backswimmers (Toth and Chew, 1972a) and, as already mentioned, these authors reported N. undulata as preferring mosquitoes to other prey that was available to them in the laboratory. Ellis and Borden

(1970) considered N. undulata well adapted as a mosquito predator. But, in a locality where a wide variety of aquatic life is present, mosquitoes would certainly not be the sole source of food, as notonectids are known to feed on a large number of prey species (Hungerford, 1917; Clark, 1928).

The tissue specificity of N. algerae in N. undulata was very similar to that of N. algerae in A. stephensi (Vavra and Undeen, 1970). Both hosts showed infections in gut, muscle, fat body, Malpighian tubules, tracheal epithelium, testes and hypodermis. Vavra and Undeen (1970) reported the gut as the most heavily infected tissues in adult mosquitoes. Fat and muscle were the most heavily invaded tissues in N. undulata in the present study. Hazard and Lofgren (1971) reported essentially the same results with Nosema infections in A. quadrimaculatus They found the fat body, gut, muscle and Malpighian tubules were the tissues most heavily attacked. Although these authors found Culex pipiens quinquefasciatus and Culex salinarius to be less susceptible to Nosema algerae, the fat body, gut and Malpighian tubules were the most commonly invaded tissues in both species. They found the muscles rarely infected in C. p. quinquefasciatus and never found the testes or ovaries to be invaded in any of

the 3 species of mosquitoes mentioned above. In Aedes aegypti only nerve tissue was parasitized. Fox and Weiser (1959) also found gut, Malpighian tubules and fat to be the most heavily infected tissues in their studies of Anopheles gambiae. Although the trachea were not invaded, the ovaries were. Esophageal and salivary gland tissues were the first to be invaded, the infection spread along the alimentary tract, including the Malpighian tubules, and then to fatty tissues, muscle, nerve and finally to gonadal tissue. In heavy infections the parasites were so numerous that none of the midgut tissue could be seen. Similarly, Savage and Lowe (1970) found Nosema infections of A. quadrimaculatus to be so intense at times that invaded tissues could not be recognized. This was also the case with N. undulata in the present study.

Savage (1975) found a wide range of susceptibility of various mosquitoes to N. algerae. Anopheline mosquitoes, such as A. albimanus and A. stephensi were highly susceptible while Culex nigripalpus and Culex tarsalis appeared to be of intermediate susceptibility. In Aedes taeniorhynchus and A. aegypti, the infection was very limited, usually involving only nerve tissue. Psorophora ciliata and Toxorhynchites rutilus septentrionalis, 2 predacious species of mosquitoes, were not susceptible.

Vavra and Undeen (1970) found that Anopheles atroparvus was not susceptible to N. algerae, although Aedes aegypti was. They reported the same tissues infected in A. aegypti and Anopheles stephensi.

As mentioned previously, Savage (1975) was also able to infect Musca domestica, Stomoxys calcitrans and a chironomid. All 3 were lightly infected. Gut and fat tissue were invaded in the housefly and chironomid, just as they were in all previously reported susceptible hosts except A. aegypti tested by Hazard and Lofgren (1971). Muscle tissue was the most heavily invaded tissue in the housefly, as was the case with N. undulata in the present study.

It appears that the primary sites of infection of N. algerae are fat, muscle, gut and Malpighian tubules. These tissues were reported to be infected in nearly all species of insects that have been tested. While the gut was most heavily invaded in the majority of mosquitoes studied, the thoracic musculature was the most heavily attacked in at least 2 non-mosquito hosts examined - M. domestica and N. undulata.

Anopheline mosquitoes appear to be the primary hosts of N. algerae. But, Vavra and Undeen (1970) could not infect Anopheles atroparvus even though the same tissues

which were infected in Anopheles stephensi were also invaded in Aedes aegypti. Histological studies, as well as laboratory transmission experiments, indicate that N. undulata, a hemipteran, may be more susceptible to N. algerae than some species of mosquitoes. Obviously, more host susceptibility studies should be undertaken, not only with N. algerae, but with other pathogens as well.

Further studies with notonectids could involve the determination of minimum dosage levels required to produce infection, elucidation of the sequence of tissues invaded as the infection proceeds, discovery of any effects the parasite may have on adult longevity and fecundity, and determination of how the pathogen might be transmitted. Although transovarial transmission has not been reported for N. algerae, eggs could possibly be mechanically contaminated in N. undulata since the testes are known to become infected.

Why N. undulata became infected and the other predators did not, is not readily apparent, especially when one considers that other hemipterans were tested, as well as a megalopteran and several coleopterans, which are intermediate to the Diptera and Hemiptera phylogenetically speaking. Smith (1973) points out that conditions in which the microsporidan extrudes its polar filament are the main

factors in determining its host-specificity. Similarly, Fisher and Sanborn (1962) believe specificity is determined by properties of the host's gut, observing that insects are more susceptible during or immediately following a molt when the cuticular lining of the foregut and midgut is shed. In the present study, the predators were fed infected larvae for a prolonged period of time so that molting would occur during the time of exposure.

Age is also a factor. At least in some species, younger individuals are more susceptible than older ones (Canning, 1970). Therefore, as indicated previously, young immature stages of each predator were chosen for study.

As indicated by Baily (1971), tests with beneficial insects are too few in number to give a clear picture of the potential host ranges of most insect pathogens. Marshall Laird (1970, p. 578) put it this way:

> Clearly, a great deal more work lies ahead of us before the integrated control of mosquitoes becomes more than a fond hope... And, by tempering with a degree of realism our enthusiasm for the course that we all desire, let us ensure that our anxieties about preservation of the ecosystem will become the more credible to those charged with the responsibility for executive decisions.

In the final analysis, the true susceptibility of non-target organisms to various insect pathogens will

only come with detailed studies of field tests. Nevertheless, laboratory studies should be undertaken as the first line of investigation. As an extension of the present study, tests on scavenger-type organisms, such as blackflies, psychodids, annelids and other invertebrates, would also be beneficial.

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BIOGRAPHICAL SKETCH

Frank William Van Essen was born on February 6, 1947 in Pittsburgh, Pennsylvania. He attended high school in Bethel Park, a suburb of Pittsburgh, and graduated in 1965. He then attended Allegheny College in Meadville, Pennsylvania and received a B.S. in biology in 1969. During this time, one summer was spent at the Theodore Stone Laboratory of Ohio State University at Put-in-Bay, Ohio.

He was awarded a graduate assistantship in Entomology at the University of Delaware to work on mosquito biology and received his M.S. in June, 1971. He married the former Barbara Jean Ross of Norfolk, Massachusetts on August 29, 1970.

Following a summer of work on insect identification for the Department of Entomology at Delaware, he began work toward the PhD degree in September, 1971.

During his stay in Florida, a strong interest in karate was fostered and he was promoted to black belt in January, 1974 in Cuong Nhu, a Vietnamese style. He has also had an avid interest in motorcycles.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Jerry F. Butler, Chairman
Associate Professor of Entomology

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Donald E. Weidhaas, Co-Chairman
Professor of Entomology

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Dale H. Habeck
Professor of Entomology

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Minter J. Westfall
Professor of Zoology

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This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

March, 1975

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