EFFECT OF *NOSEMA ALGERAE* ON THE HOUSE FLY *MUSCA DOMESTICA* (DIPTERA: MUSCIDAE)[1]

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ABSTRACT. Larvae of *Musca domestica* were exposed to spores of *Nosema algerae* on the surface of their diet. Infective concentrations (IC_{50} and IC_{90}) for the larvae were 3.6×10^4 and 1.6×10^6 spores/cm^2, respectively. The disease appeared to cause no larval mortality, but the longevity of adult females was reduced. At 30 days post-infection, there were at least 1×10^7 spores per fly in all dosage groups. At lower dosages, the development of spores was delayed and fewer spores were produced.

INTRODUCTION

Nosema algerae Vávra and Undeen is a microsporidian pathogen of mosquitoes (Vávra and Undeen 1970). It has an essentially unlimited arthropod host range when its spores are administered by intrahemocoelic inoculation but a much more restricted range by the normal *per os* route (Undeen and Maddox 1973). Savage (1975)[3] found that the house fly, *Musca domestica* Linn., was susceptible to *N. algerae* by ingestion of spores. The infections were reported to be light, and nothing was said about the fate of the infected flies.

Spores of *N. algerae* have been mass produced in an alternate host, *Heliothis zea* Boddie, for mosquito biological control tests and basic studies on microsporidian spore germination (Anthony et al. 1978b, Undeen 1978). Although an excellent host for production of *N. algerae*, *H. zea* larvae are aggressive and cannibalistic, requiring individual handling at considerable cost in labor and materials. A host that can be mass reared would be preferable for future production.

The objectives of this study were to determine the effect of *N. algerae* on the house fly and evaluate *M. domestica* as a production host for *N. algerae* spores.

MATERIALS AND METHODS

Spores of *N. algerae* were produced in *H. zea* as described by Anthony et al. (1978b). Infected adults were triturated in a blender and vacuum filtered through cotton. The filtrate was processed through a continuous flow centrifuge into Ludox density gradients (Undeen and Avery 1983). Spores from the gradients were rinsed 3 times and stored in deionized water at 5 ± 2°C.

Eggs of organophosphate- and permethrin-resistant *M. domestica* were obtained from a colony maintained at the USDA, ARS, MAVERL, Gainesville, Florida. Twenty-five grams of diet (5 parts wheat bran: 3 parts alfalfa meal: 2 parts corn meal) were mixed with 50 ml of deionized water in 100-ml plastic cups, yielding 75 g of diet with a surface area of 44 cm^2. Batches of 100 eggs were counted and transferred to pieces of black cloth (ca. 16 cm^2) with a small brush.

Starting with spore concentrations of 3.0–5.4 $\times 10^8$/ml, four serial 10-fold dilutions were made and 0.9-ml aliquots of each dilution were dripped from a pipette tip over the surface of the diet. Each replicate had 1 cup each of 5 treatment dosages with maxima of 6.2×10^6–1.1×10^7 spores per cm^2 of diet surface area and one untreated control. A black cloth with its 100 eggs was then placed, egg side down, on the surface of the diet. The cups were covered with a single layer of white organdy cloth and incubated at 27 ± 1°C. The black cloth was removed 24 h later, and incubation was continued until pupation was complete (9–10 days post-hatch). This test was replicated 7 times.

Pupae were removed from the diet and placed in cages made from 2-liter beverage bottles (Fig. 1). Food for the adults (6 parts powdered milk: 6 parts granulated sugar: 1 part powdered egg yolk) was placed between a single layer of organdy cloth and a cotton ball and inserted in a hole on the side of the bottle (Fig. 1). Water was provided in a diet cup filled with wet cotton, inverted on the organdy covering the top of the cage (Fig. 1). Another piece of organdy was hung inside the bottle to provide additional resting surface. The empty pupal cases were removed

[1] Mention of a commercial or proprietary product in this paper does not constitute an endorsement of this product by the United States Department of Agriculture.

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[3] Savage, K. E. 1975. *Nosema algerae* Vávra and Undeen, 1970 (Protozoa: Microsporida): its bionomics and development for use as a biological control agent of mosquitoes. Master's Thesis, University of Florida, Gainesville, FL.

from the cages after adult emergence was complete.

The number of pupae was scored in all 7 replicates in order to evaluate the effect of *N. algerae* on the survival of larvae. In replicates 4–7, dead adults were collected from the cap at the bottom of the cage (Fig. 1) at 1–4 day intervals and examined for infection by crushing them under coverslips and searching for spores at 400× magnification under phase contrast lighting. This longevity evaluation was not begun until after 15 days from the start of the test, when spores first began to appear in the treated flies. The rate of spore production was evaluated in test 4 and in a separate test consisting of a single treatment of 2×10^6 spores/cm^2. The daily mean number of spores per infected fly was measured by grinding each day's collection of infected adults, pooled by dosage, in a small amount of water and counting the spores in a hemocytometer.

The infective concentration (IC_{50} and IC_{90}), in terms of spores/cm^2 of the diet surface area, and the longevity of flies were estimated from the combined results of tests no. 4–7, using probit analysis. A General Linear Model (GLM) test ($P < 0.05$) was used to evaluate the effects of sex, dosage and time. The increase in spores/fly was analyzed using a SAS GLM test (SAS, version 6.04; SAS Institute, Cary, NC).

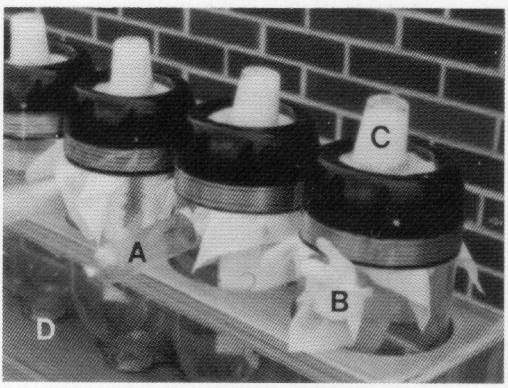

Fig. 1. Fly cages showing the tubes for pupal emergence (A); feeding station (B); water station (C). Dead adults are removed from the caps at the bottom (D). Organdy cloth hanging inside is for additional resting surface.

RESULTS

Pupation occurred between 7–9 days, with no difference between control and treated groups. According to the number of pupae produced, there was no significant larval mortality attributable to *N. algerae* (R = 0.13, PR > F: 0.48; Duncan-Waller means test). Both sexes were equally susceptible to *N. algerae* with a mean IC_{50} of 3.6×10^4 and IC_{90} of 1.6×10^6 spores/cm^2 (Table 1).

The untreated females lived longer than males, and the longevity of only the females was affected by the spore dosage (Table 2). Flies from the treated groups that were scored as "uninfected" appeared to die earlier than either the infected flies or the uninfected flies from the untreated control groups (Table 2).

Spores were not easily found in adults until about 2 wk post-egghatch. Beyond 30 days, regardless of treatment dosage, there were more than 1×10^7 spores/fly and the numbers increased until all of the flies were dead (Fig. 2). In flies treated with dosages below 1×10^5 spores per cm^2, spore production started later and significantly fewer spores were produced (Fig. 2).

DISCUSSION

Cooper et al. (1983) reported transmitting a natural fly pathogen, *Octosporea muscaedomesticae* Flu. to the larval stage of a flesh fly, *Lucilia cuprina* (Wiedemann). Other than this, there seems to be no literature on dosage-response relationships between pathogens and muscoid fly larvae. At the highest dosages used here, 100% of the flies were infected, and there was a clear dosage-response relationship.

The IC_{50} of *N. algerae* in house flies was an order of magnitude higher than in *Anopheles stephensi* Liston exposed to spores in petri dishes with a sand or soil substrate (Undeen and Alger 1975). Dosages of *N. algerae* spores sufficient to cause 100% infection had no significant effect on house fly larval mortality, a phenomenon similar to that observed in *An. stephensi*

Table 1. Infectious dosage of *Nosema algerae*, in spores/cm^2 of the diet surface area (fiducial limits below), for first instar *Musca domestica* larvae

	Female	Male	Total
IC_{50}	3.8×10^4	3.8×10^4	3.6×10^4
	2.5×10^4, 5.6×10^4	2.6×10^4, 5.6×10^4	2.7×10^4, 4.7×10^4
IC_{90}	1.5×10^6	2.5×10^6	1.6×10^6
	8.7×10^5, 3.1×10^6	1.3×10^6, 5.3×10^6	1.1×10^6, 2.6×10^6
R	0.80	0.83	0.87

(Undeen and Alger 1975) and *H. zea* (Anthony et al. 1978b).

The longevity of female flies was variable between tests, with LT_{90}s ranging between about 22 and 39 days for the most heavily infected groups compared with a range of 26–60 days for the controls. Significant differences between infected and untreated females were obvious only in those replicates with the highest control longevity. The longevity difference between *O. muscaedomesticae* infected and uninfected house flies was also masked by the short life span of uninfected controls (Kramer 1966). This was not the case for the longer-lived *Phormia regina* (Meigen) (Kramer 1966, 1968).

Depending upon the age of the larvae when exposed to *N. algerae* spores, the LT_{90} of infected *Anopheles albimanus* Wiedemann adults ranged between 19–24 days; the LT_{90} of uninfected controls was about 40 days (Undeen and Alger 1975, Anthony et al. 1978a). The life expectancy of infected and uninfected *An. stephensi* was 13.3 and 28.5 days, respectively (Undeen and Alger 1975). The effect of *N. algerae* on longevity of both male and female mosquitoes was always obvious.

Table 2. Lethal times$_{50}$ (LT_{50} with 95% confidence limits in parentheses) in days for *Nosema algerae*-infected *Musca domestica* by status of treatment

Status	Lethal time$_{50}$ (confidence limits)	
	Females	Males
Untreated	25.6A* (24.2, 27.2)	20.2A (19.6, 20.8)
Treated	20.1B (17.8, 22.7)	17.7B (16.6, 19.0)
Uninfected	18.7a (13.6, 25.0)	17.7a (15.7, 19.6)
Infected	24.2b (24.2, 25.0)	20.4 (19.5, 21.3)

* Compared vertically, the same letters indicate no significant difference (upper and lower cases compared separately).

In contrast with mosquito longevity, which was generally independent of *N. algerae* spore dosage (Undeen and Alger 1975), infected house flies from low spore dosage groups lived longer than those from high dosage groups. Another difference was the unexpectedly early death of uninfected flies from the treatment groups. A comparison of the LT_{50}s of the uninfected flies (Table 2) with the spore production displayed on Fig. 2 shows that, had they lived longer, flies scored as uninfected might have fallen into the infected category. This is particularly obvious for the low dosage groups, where spore production began about day 23, whereas the IC_{50} of the exposed, uninfected flies was 18 days. These observations suggest pathology in house flies apart from spore production. Mortality without significant spore production was also observed in laboratory infections of mosquitoes with a *Helicosporidium* sp. and was suggested to be due to an abnormal pathogen-host relationship (Avery and Undeen 1987).

Because *N. algerae* spores are unable to withstand desiccation, it has never been considered for application in a terrestrial habitat. However, the spores survived on the wet house fly larval

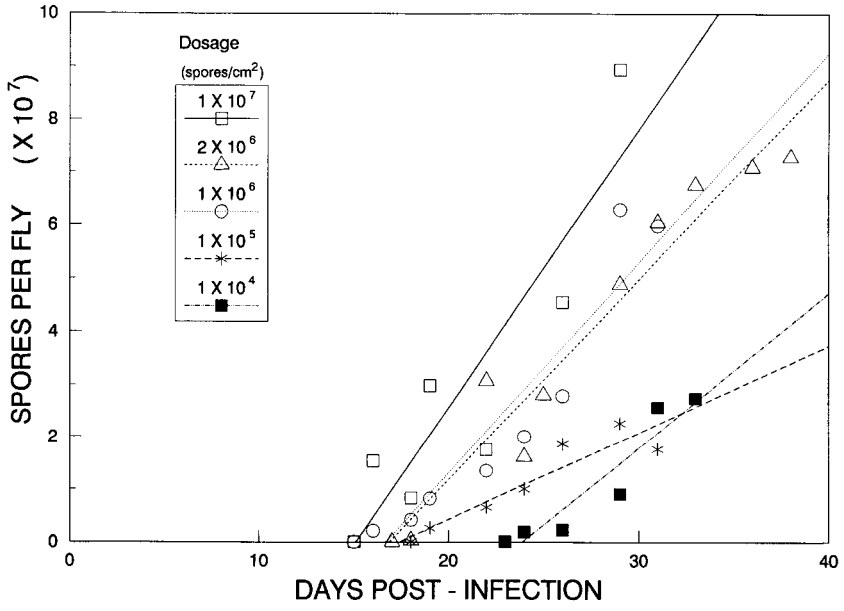

Fig. 2. *Nosema algerae* spore production in adult house flies by time and spore dosage.

medium and the larvae were susceptible enough that significant levels of infections might be achieved in the field. Even with high rates of infection, mortality rates for the infected flies are not likely to be much different from the uninfected ones in their natural habitat. In the case of mosquitoes, *Nosema* infections further reduced the reproductive potential by reducing the numbers and viability of eggs (Reynolds 1970, Anthony et al. 1978a, Haq et al. 1981). Effects on house fly populations by *N. algerae* would have to depend upon these, as yet unevaluated, factors. Alone, the reduced life expectancy observed here seems unlikely to have much impact.

The house fly is a suitable host for spore production in terms of the number of spores/fly. A single house fly produces about 1/20th as many *N. algerae* spores as a corn earworm but about 100× as many as a mosquito (Undeen and Maddox 1973). Spores produced in *M. domestica* (Savage 1975)[3] as well as all other alternate hosts tested (Undeen and Maddox 1973, Undeen and Alger 1975), were infectious to mosquitoes. The fly is easier to mass rear than either mosquitoes or *H. zea*. However, longevity of the flies must be maximized to achieve good spore production.

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