

***Heterorhabditis heliothidis*: A Potential Biological Control Agent of House Flies in Caged-Layer Poultry Barns**

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House flies (*Musca domestica*) develop in the fecal droppings and associated detritus that accumulates as manure in caged-layer poultry barns. Large populations of flies are a nuisance to workers in the barns and to neighbors and are pests to the chickens as vectors of fowl mites and bacteria. Flies can be partially controlled with insecticidal surface sprays, fogging, and sugar-based baits (3), but continuous use of these pesticides induces resistance in house flies, sometimes to all registered compounds (5). Moreover, there is increasing producer and consumer interest in decreasing pesticide use in food production areas. Hymenopterous parasites of fly pupae, such as *Spalangia endius*, are available commercially to control fly populations but their success is limited (3). Two research groups (4,9) recently tested nematodes of the genera *Heterorhabditis* and *Steinernema* (= *Neoplectana*) under laboratory conditions as potential control agents of *M. domestica* maggots in poultry manure. They noted that under their experimental conditions nematodes survived only a few days in moist manure and, therefore, had little potential for fly control.

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Preliminary studies in our laboratory indicated that, locally, *Heterorhabditis* spp. are more effective than *Steinernema* spp. in killing fly maggots in moist poultry manure. This paper describes subsequent tests to evaluate *Heterorhabditis heliothidis*, under laboratory and barn conditions, as a potential control agent for fly maggots in chicken manure.

Laboratory test: Twelve 0.25-m² sections of chicken manure in a poultry barn infested with fly maggots were excavated to a depth of 10 cm, and each was placed in a plastic bag (66 × 91 cm). No flies were added to the bags. Each bag was fitted with a plastic mesh cage at the top to allow for air circulation and to facilitate removal of adult flies. All bags were kept outdoors under shelter at air temperatures ranging from 15 to 29 C. Three bags were kept as controls and the remaining nine were divided into three groups. Each of the groups of bags was treated with a different number of *H. heliothidis* North Carolina strain (NC1) infective juveniles as follows: Group 1, 0.4 × 10⁶; group 2, 2 × 10⁶; group 3, 4 × 10⁶ infective stage nematodes/m². Each nematode inoculum, in 250 ml water, was sprinkled over the surface of the manure in a bag; the controls were sprinkled with only water. Adult flies in each bag were removed and counted daily for 24 days starting from 3 days after nematode treatment.

Barn test: Two identical poultry barns, each 100 m long by 10 m wide, were the site of a test from September to November. The treated and control barns were cleaned out 2 weeks and 3 weeks, respectively, before treatment. When the experiment was initiated in mid-September the manure was

TABLE 1. Mean number (\pm SD) of house flies emerging from chicken manure in plastic bags treated with three different doses of *Heterorhabditis heliothidis* NC1 strain ($n = 3/\text{treatment}$).

Nematode dose/m ²	No. flies emerging
0 (control)	1,570 \pm 767 a
0.4 $\times 10^6$	589 \pm 641 ab
2.0 $\times 10^6$	310 \pm 21 b
4.0 $\times 10^6$	227 \pm 140 b

Means followed by the same letter are not significantly different from each other ($P < 0.05$).

about 8 cm deep in both barns and covered about 80% of the floor area. An aqueous solution (40 liters) containing 4×10^8 infective juveniles ($1 \times 10^4/\text{ml}$) of *H. heliothidis* NC1 was applied evenly over the surface of the manure in the treated barn using a motorized pesticide sprayer (Dobbins Power Sprayer) at 125 pounds per square inch. The effective nematode inoculum level was $5 \times 10^5/\text{m}^2$ of manure. The control barn was similarly sprayed with only water.

Adult fly populations were monitored indirectly by counting fly specks deposited on 10 white cards (13×7.5 cm) pinned on vertical posts about 10 m apart throughout the length of each barn. The cards were placed in each barn immediately following cleanout and were changed weekly for the initial 10-week period and monthly for 9 months thereafter. The fly specks, or a portion of them if their number was more than $10^3/\text{card}$, were counted to estimate the fly population (1). Week 0 for each barn was 2 weeks after barn cleanout (see Fig. 1a).

Three 500-g samples of the manure, to a depth of about 5 cm, were taken weekly from each barn starting 1 week after treatment. These samples were examined in the laboratory for dead fly larvae and for the presence of other arthropods. The barn air temperature and the temperature at 3 cm below the surface of the manure were measured at weekly intervals.

The data were analysed using analysis of variance and Duncan's multiple-range test.

The two highest rates of *H. heliothidis*

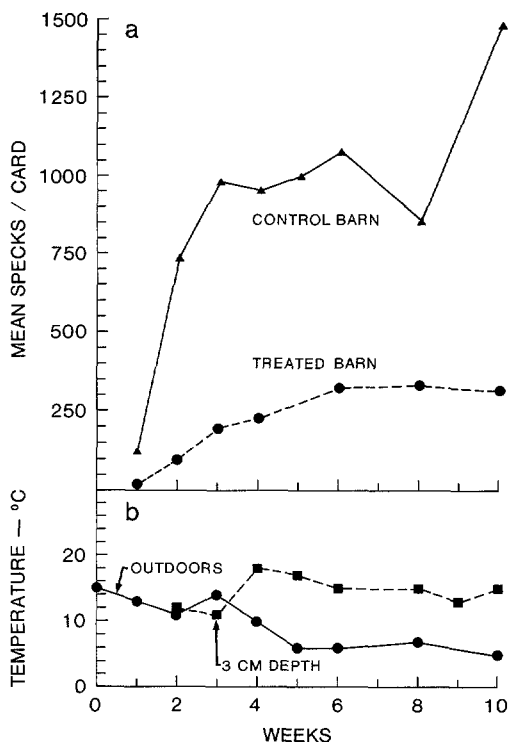


FIG. 1. a) Comparison of the average number of fly specks/card ($n = 10/\text{barn}$) over a 10-week period after treatment of the manure in two barns with either *Heterorhabditis heliothidis* (treated barn) or water (control barn) at week 0. b) Outside air and manure temperature (3 cm below the manure surface) during the treatment period.

applied to the bagged manure significantly ($P < 0.05$) decreased fly emergence over 24 days compared with controls (Table 1). The lowest inoculum level was not significantly different from controls because of high within treatment variability (Table 1) despite an apparent decrease in fly emergence of about 60%.

In an earlier study during the 18 months before treatment, flies in the barns were marked, released, and recaptured and a mean weekly fly speck count of 150/card was shown to represent an average population of about 10^6 adult flies per barn. Fly populations were about equal at the time of cleanout and dropped to a low level immediately after barn cleanout. This is attributed to the natural mortality of the adults and to the lower recruitment from the fresh manure. Fly populations then be-

gan to build up again, especially in the control barn (Fig. 1a). One week after nematode treatment (3 weeks after barn cleanout) the fly populations of both treated and control barns were low (means of 19 specks/card and 125 specks/card, respectively; $n = 10$ cards/barn). By week 2 after treatment, however, the fly population of the control barn increased much more rapidly than that of the treated barn, the fly population of which subsequently leveled off at about 20% that of the control barn (Fig. 1a). Ten weeks after treatment, the mean fly speck counts were 1,487 and 317 per card in the control barn and treated barn, respectively, representing populations of about 10×10^6 and 2×10^6 flies/barn.

Air temperatures in the barns remained relatively constant at 21 ± 2 C. Temperatures 3 cm below the surface of the manure, where most of the maggots were found, ranged from 11 to 19 C during the treatment period (Fig. 1b). Few maggots were found on the surface, which is dry, or deeper in the manure, which is warmer and anaerobic. In previous reports (4,9), the nematodes were mixed into the manure to provide a uniform distribution of fly maggots and nematodes. In this experiment, the nematode suspension was applied to the undisturbed manure surface. Mixing of nematodes with the manure is impractical in commercial nematode applications and may expose the nematodes to toxic or anaerobic conditions. Maggots killed by *H. heliothidis* were pink in color and were readily recognized in manure samples where they were recovered for up to 10 weeks after treatment. No fly pupae parasitized by *H. heliothidis* were found at any time after treatment. Histerid beetles and macrocheles mites, both of which prey on fly eggs, were not counted, but these predators appeared to increase in number throughout the observation period in both the treated and control barns. None of these predators from treated or control manure was found to be parasitized by nematodes.

The apparent success of *H. heliothidis* in

killing a large percentage of fly maggots contrasts with the results of others (4,9). The greater efficacy of our treatment could be due to differences in the manure environment, especially temperature and moisture content. The previous reports (4,9) described laboratory experiments in which the manure was maintained at 25 C which was 6–14 C warmer than the fly-infested manure in the barn in our experiment. The higher manure temperatures may have been a limiting factor, possibly through increased bacterial activity. Manure moisture levels were not quantified in our tests or in the earlier accounts (4,9), but laboratory observations suggest that nematodes survive best in moist, as distinct from wet, manure. Another factor that may have contributed to the difference between reported results is the varying susceptibility of the fly populations to different nematode strains (2).

Fly populations in the treated barn continued to drop after 10 weeks until there were fewer than 40 specks/card each month 3 months after treatment. The control barn fly populations also dropped to a very low level but not until 6 months after the populations in the treated barn. This was most probably the result of lower ambient winter temperatures.

Many arthropod species that inhabit accumulated poultry manure are predators and (or) competitors of fly maggots and can cause up to 97% reduction in fly emergence (8). Consequently, appropriate habitat management to conserve this rich biota is an effective way to control fly populations and is preferable to broad spectrum pesticide applications to the manure (6). The value of nematode treatment is that it supplements the effects of these natural control agents by selectively killing fly maggots. Since fly pupae are not parasitized by nematodes, the pupae are available as hosts for parasitic wasps. No nematode-killed maggots were found after week 10, which is consistent with the observations of others (4,9) that suggest nematodes die off quickly in manure when there are few or no suitable hosts.

In the following year, the fly population in the treated barn was very low immediately before barn cleanout and, as in the previous year, the population fell at cleanout and then rebounded slightly. At the grower's request the nematode treatment was reapplied in this barn 2 weeks after cleanout. Subsequent observations showed that about 1 month after nematode treatment the fly population had returned to the pre-cleanout level.

These experiments indicate that, under our conditions, application of *H. heliothidis* to manure can significantly decrease the number of adult flies. This degree of success, together with the lack of pathogenicity of these nematodes to mammals and birds (7), makes *H. heliothidis* an attractive alternative as a control agent of flies under these conditions. Heterorhabditid nematodes are being produced and marketed by a local biological control company as a treatment for fly maggot control in caged-layer poultry barns.

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