NOTE

Maintenance of an Ectoparasitic Nematode, *Noctuidonema guyanense* (Nematoda: Aphelenchoididae), on *Spodoptera frugiperda* (Lepidoptera: Noctuidae) Moths[1]

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J. Entomol. Sci. 28(4):335-337 (October 1993)

KEY WORDS Fall armyworm, *Spodoptera,* Nematoda, *Noctuidonema.*

The ectoparasitic nematode, *Noctuidonema guyanense* Remillet and Silvain, is one of a few metazoan parasites attacking the adult stage of the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Marti et al., 1990, Ann. Entomol. Soc. Am. 83: 956-960). No parasitic Hymenoptera or Diptera are known to attack adult *S. frugiperda* (Ashley, 1979, Fla. Entomol. 62: 114-123), and no biological control programs are directed against the moth stage, despite the economic importance of this species as a crop pest (Luginbill, 1928, USDA Tech. Bull. No. 34; Sparks, 1979, Fla. Entomol. 62: 82-87).

The fall armyworm lacks a diapause. Populations, therefore, must be re-established in temperate regions each spring by moths migrating northward from the subtropics or tropics (Mitchell et al., 1991, J. Entomol. Sci. 26: 39-50). These migrants include individuals infested with *N. guyanense* (Simmons and Rogers, 1991, J. Entomol. Sci. 26: 136-148). Local populations of the nematode are thus re-established and maintained each year by interbreeding of overlapping generations of *S. frugiperda.*

Because *N. guyanese* occurs only on adult Lepidoptera (Remillet and Silvain, 1988, Rev. Nematol. 11: 21-24; Rogers et al., 1990, J. Agric. Entomol. 7: 241-245), live nematodes are not available for controlled studies unless wild moths are collected and colonized in the laboratory. To avoid this problem, we established an *in vivo* colony of *N. guyanense* on laboratory-reared *S. frugiperda* adults. This note describes our procedure for establishing and maintaining a colony of *N. guyanense.*

[1] Accepted for publication 07 July 1993.

Feral males of *S. frugiperda* collected in Universal moth traps (International Pheromone Systems, Merseyside, England) baited with a commercially prepared sex pheromone (Scentry, Inc., Billings, MT) are examined for nematodes under a stereoscopic microscope at 30X. By gently restraining the moth in one hand, the posterior edge of the 8th abdominal segment is grasped with blunt forceps to expose the dorsal surface of the 8th segment, which is the preferred infestation site on the male (Rogers and Marti, 1992, J. Entomol. Sci. 27: 354-359; Rogers and Marti, 1992, Environ. Entomol. 21: 417-421). With care, moths can be examined without harm and selected for mating with laboratory-reared females.

One infested male and two to three 2-d-old laboratory-reared female moths are placed in a 0.4-liter cardboard cage in which a cotton wick soaked in 10% honey solution and a water-soaked wad of tissue are provided. The lower half of a 9-cm plastic petri dish is used as the bottom of the cage and is fastened in place with tape; the lid of the dish serves as the top of the cage. Moths may be introduced into cages via a 0.8-cm hole which is then sealed with a size 0 cork. The cork may be removed to replenish water and honey solutions in the cage.

Cages are placed overnight in an incubator at 27°C, 70-80% relative humidity and a 14:10 L:D cycle. To prolong the life of the moths, the cages are moved the following day to another incubator and maintained at 20°C. Maintenance of a high relative humidity in both incubators is critical; survival of the moths and nematodes and growth of the nematode population is favored by a high RH (Simmons and Rogers, 1990, Ann. Entomol. Soc. Am. 83: 1084-1087). Although the growth rate of *N. guyanense* is higher at 27°C than at 20°C, maintenance of the colonies at the higher temperature reduces moth longevity considerably, both by depletion of stored fat and by growth of nematode populations.

Four days after confinement, female moths to be examined for nematodes are released in a small, screened, wooden cage (30 × 30 × 16 cm) fitted with a cloth sleeve. Moths are then captured by hand and examined individually under a stereoscopic microscope. In early infestations of female moths, nematodes are usually confined to the membrane between the 8th and 9th abdominal segments where their detection is difficult without harming the moth. To avoid injury to female moths, they are examined on the ventral side. A moth is restrained in one hand while the posterior margin of the 8th segment is grasped with a blunt forceps and gently stretched to expose the intersegmental membrane between the 7th and 8th segments. Moths that are negative upon initial examination are placed in a fresh cage, incubated as described above, and examined again after 3-4 d. Moths negative after the second examination are discarded. Positive females are placed in individual cages with two to three 2-d-old laboratory-reared male moths and incubated as described above.

Male moths are re-examined for nematodes by the procedure described above for males. Negative males are placed in a cage, incubated an additional 3-4 d, and re-examined. Males negative on re-examination are discarded. Positive males are placed in a cage with 2-3 young, unmated females and incubated as described above.

No more than 5 moths are placed in each cage. These may be 1-2 infested moths plus 2-3 laboratory-reared moths of the opposite sex. Virgin moths 2-5 d of age are used for mating with infested individuals of the opposite sex. Older

moths are not used because they have a lifespan of only about 14 d, and the nematode populations may not have sufficient time to develop and infest new moths before death of the host.

Cages, humidifiers, and incubator conditions are checked daily. Water and honey are replenished daily. After a nematode colony is established, it is examined twice weekly and fresh cages are prepared. Frequently, infested moths survive to mate with a second group of younger adults. The time required to care for the colony depends on the number of moths that are maintained. For a colony of 15 cages, 2 h twice weekly plus 10 min daily are sufficient.

If necessary to prevent loss of the colony, nematodes are manually transferred from infested moths to uninfested moths. Several nematodes may be removed from a host on the point of a pin or dissecting needle and placed beneath scales at the posterior end of female moths or in the genital chamber of males. With practice, *N. guyanense* can be manually transferred with a high rate of success.

Nematodes on wild moths are typically bright yellow or orange. After several generations in an *in vivo* colony, *N. guyanense* is blue on female moths and colorless or pale blue on males. The color of the nematodes is produced by ingestion of host hemolymph, which in turn is influenced by the diet of the moth while in the larval stage. Occasionally blue or green nematodes are observed on wild moths, particularly on *S. latifascia* (Walker).

Nematodes which transfer from wild to laboratory-reared hosts retain their yellow color. Even after several days on a lab-reared moth, the original colonizers may be identifiable by their yellow coloration in the posterior intestine; the anterior intestine will contain the blue or colorless hemolymph typical of lab-reared moths. The anus of *N. guyanense* is apparently imperforate; gut contents are retained throughout life.

Using the procedure described here, we have successfully maintained *N. guyanense in vivo* on adult *S. frugiperda* moths for 5 months, during which only a single manual transfer was necessary to maintain the colony. An *in vitro* rearing procedure for this nematode is not yet available. In the absence of wild infested moths during the winter months in temperate climates, the procedure described above is satisfactory for providing live *N. guyanense* for laboratory studies.