# Effect of *Noctuidonema guyanense* (Nematoda: Acugutturidae) on the Longevity of Feral Male *Spodoptera frugiperda* (Lepidoptera: Noctuidae) Moths[1,2]

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**Abstract** *Noctuidonema guyanense* Remillet and Silvain is an ectoparasitic nematode of certain species of adult Lepidoptera, particularly Noctuidae, in the Western Hemisphere. It is transferred to a new host when the insects mate. Survival of infested and uninfested feral adult males of *Spodoptera frugiperda* (J. E. Smith), the fall armyworm, was compared at three different temperature regimens (20°C, 30°C, and 30/20°C) at 14:10 L/D at 80% RH. Apparent age differences were controlled by pairing moths having the same amount of scale loss. Overall mean nematode infestations of 239.3 and 0 in infested and uninfested groups produced significantly different mean longevity of 3.7 and 6.2 days, respectively. At 20°C, survival of both groups increased to 6.2 and 13.3 days, but remained significantly different. The results demonstrate a deleterious effect of *Noctuidonema* infestation on longevity of feral adult male *S. frugiperda.*

**Key Words** *Noctuidonema guyanense, Spodoptera frugiperda,* Lepidoptera, Noctuidae, Nematoda, ectoparasitic nematode, survival, longevity

*Noctuidonema guyanense* Remillet and Silvain was the first ectoparasitic nematode of adult Lepidoptera to be discovered and described (Remillet and Silvain 1988). Subsequent reports described two additional species of the genus (Anderson and Laumond 1992, Marti and Rogers 1995). More than 60 species in 6 families of Lepidoptera in the Americas and the Fiji Islands have been reported to be parasitized by *Noctuidonema* sp. (Simmons and Rogers 1996, Rogers et al. 1997, Marti et al., unpubl. data). Most of the reported host species, including *Spodoptera frugiperda* (J. E. Smith), belong to the family Noctuidae and are serious pests of agricultural crops. A related nematode, *Acugutturus parasiticus* Hunt, is an ectoparasite of the cockroach, *Periplaneta americana* L. in the West Indies (Hunt 1980).

*Noctuidonema* adults and juveniles feed on moths by inserting a long flexible stylet through the thin cuticle of the intersegmental membranes. They ingest hemolymph and acquire a color (yellow, orange, blue, green, or transparent) determined by the species of plant on which the moth fed as a larva (Simmons and Rogers 1996). Moths become infested by mating with infested individuals of the opposite sex (Remillet and

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Silvain 1988, Rogers and Marti 1994). Pathological changes associated with *Noctuidonema* infestation of *S. frugiperda* were described by Marti et al. (1990).

Simmons and Rogers (1994) collected feral male *S. frugiperda,* maintained them under laboratory conditions at 27°C, and compared the survival of infested and uninfested moths. Although they found that about 50% of uninfested moths lived for 72 h or more after collection, compared to 20% of infested moths, the two groups were not controlled for possible age differences. Older moths are more likely to be infested because they had more opportunities to mate and had time to develop larger nematode burdens. Rogers et al. (1993), in a separate survival study comparing infested and uninfested laboratory-reared male *S. frugiperda,* controlled for age differences in the two groups by mating feral infested males to laboratory-reared females and then mating the females to laboratory-reared males. They were, therefore, able to produce and use infested laboratory-reared moths of known ages. Their results showed that all the infested moths died within 8 d after mating and that uninfested males survived nearly twice as long.

In another study, Rogers et al. (1996) compared body weights of infested and uninfested feral *S. frugiperda* that had been assigned to four separate groups based on a subjective evaluation of scale wear (None, Low, Moderate, and High) to estimate relative age differences. Their results showed that the infested moths weighed less than uninfested moths. We used their description of scale wear groups as a relative estimate of moth age to compare the survival of infested and uninfested feral *S. frugiperda* in the laboratory at three different temperature regimens 20°C, 30°C, and 30/20°C D/N).

## Materials and Methods

Live feral male moths of *S. frugiperda* were collected between July and September in universal moth traps baited with a commercially available synthetic pheromone lure. Traps were placed along a fence near corn fields and were checked daily before 8:00 am. Moths from traps containing more than about 20 individuals were not used in order to minimize scale loss and debility caused by moth activity in the trap during the night. Moths from traps containing fewer than about 20 individuals were taken to the laboratory, examined under a dissecting microscope, and sorted into two groups based on the presence or absence of *N. guyanense.* Moths were then assigned to one of four scale loss groups (1 = None, 2 = Low, 3 = Moderate, 4 = High) based on a subjective evaluation of scale loss described by Rogers et al. (1996). Scale loss groups can be indicative of relative, but not absolute, moth age because the youngest moths (group 1) have little or no scale loss and the oldest (group 4) have the most scale loss, with groups 2 and 3 being intermediate and comprising most of the individuals collected. Each infested male moth was paired with an uninfested male moth most similar to it in the same scale loss group. Individual moths were not included in the study if they could not be paired with another moth collected the same day and having the same amount of scale loss.

Each moth was placed into a separate 460 cc cardboard container covered with netting and was maintained at one of three temperature regimens: 20°C (24 h), 30°C (24 h), or 30°C day (14 h) and 20°C night (10 h). All temperature regimens were maintained at 80% relative humidity and 14:10 day:night light cycle. A cotton wick saturated with 10% honey solution was placed in each container and replaced daily.

Moths used in this study were collected between 29 June 1998 and 14 September 1998 in Tifton, GA.

Moths were checked for mortality twice daily. Longevity was determined by subtracting the collection date from date of death (Julian dates), to the nearest half-day. Dead moths were stored in individual 2.0-ml vials containing 10% buffered formalin until examination. To remove nematodes for counting, the formalin was poured into a Petri dish (100 × 15 mm) and the moth abdomen was stretched and scraped with two pairs of fine forceps. Nematodes were counted with the aid of a stereo microscope and identified as male, female, or juvenile. Eggs were not counted. Nematode totals represent the number present when the moth died, not necessarily the number present when the moth was collected.

A total of 268 moths (134 pairs) were placed on test in a paired-comparison design. Of these, 44 moths (22 pairs) were deleted from the study because individuals intended to be uninfested controls were later found to be infested. A total of 224 moths (112 pairs) were therefore used in the analysis. Data were analyzed by the General Linear Models procedure of SAS (SAS Institute 1985) and graphs were prepared with Statistica (StatSoft 1997).

## Results and Discussion

Length of survival of feral male *S. frugiperda* adults was affected by incubation temperature, moth age as indicated by scale loss group (= subjective index of initial age), and by infestation with the ectoparasitic nematode, *N. guyanense*. Overall, infested moths had a mean of 239 nematodes and survived 3.7 d on test, significantly less ($F = 8.05$; df = 1, 200; $P = 0.005$) than uninfested moths, which survived 6.2 d (Table 1).

Moths maintained at 20°C survived longer than those maintained at 30°C ($F = 61.20$; df = 1, 178; $P < 0.0001$) or 30/20°C ($F = 35.30$; df = 1, 134; $P = 0.0001$). There was a significant interaction ($F = 7.47$; df = 2, 199; $P = 0.0007$) between survival due to nematode infestation and incubation temperature (Fig. 1). There were no differences between survival of infested and uninfested moths maintained at 30°C ($F = 2.68$; df = 1, 79; $P = 0.11$) or at 30/20°C ($F = 0.25$; df = 1, 36; $P = 0.62$). However, at 20°C, uninfested moths survived a mean of 13.4 d, whereas uninfested moths survived only 6.4 d ($F = 12.56$; df = 1, 84; $P = 0.0006$) with a mean of 297.0 nematodes at time of death.

Moths with no scale wear were the youngest and survived the longest, whereas those with a high amount of scale wear were the oldest and survived the least. Because of the unfavorable effects of incubation temperature regimens of 30°C and 30/25°C on moth survival, this relationship is best seen at 20°C (Fig. 2). At 20°C, there was no significant difference ($F = 2.01$; df = 1, 4; $P = 0.23$) between longevity of infested and uninfested moths in scale loss group 1, even though survival of the uninfested group was nearly twice that of the infested group (18.8 vs 9.7 d). This can be explained by the low numbers of observations in scale loss group 1 because very few positive moths with no scale wear could be collected and the means had high standard errors. However, in the remaining three scale loss groups (2, 3, and 4), which represent most of the moths in the population, the numbers of observations were higher and overall survival was significantly less ($F = 19.42$; df = 1, 84; $P = 0.00003$) in infested moths compared to uninfested moths (5.9 ± 0.60 days vs 12.9 ±

**Table 1. Least square mean days survival (±SEM) of feral male *Spodoptera frugiperda* moths positive or negative for the ectoparasitic nematode, *Noctuidonema guyanense,* across all temperatures (20, 30, and 30/20°C) and scale wear groups (None, Low, Medium, and High)**

| Factor | n | Mean survival (days) LS mean ± SEM | Number of nematodes LS mean ± SEM |
|---|---|---|---|
| Nematode infestation | | | |
| Positive moths | 112 | $3.7 \pm 0.62^a$ | $239.3 \pm 34.64^a$ |
| Negative moths | 112 | $6.2 \pm 0.62^b$ | $0^b$ |
| Scale loss group | | | |
| None | 24 | $7.4 \pm 1.40^a$ | 264.4 ± 149.3 |
| Low | 100 | $5.5 \pm 0.50^a$ | 190.5 ± 53.2 |
| Medium | 68 | $4.2 \pm 0.68^a$ | 250.0 ± 72.8 |
| High | 32 | $2.7 \pm 0.93^b$ | 195.7 ± 99.1 |
| Incubation temperature (°C) | | | |
| 20 | 92 | $9.9 \pm 0.68^a$ | 276.0 ± 72.3 |
| 30/20 | 44 | $2.34 \pm 1.11^b$ | 152.4 ± 117.6 |
| 30 | 88 | $2.64 \pm 0.56^b$ | 247.2 ± 59.7 |

Means followed by different superscripts within factors are significantly different ($P < 0.05$).

1.45 days, respectively). At 20°C, infested moths in scale loss groups 1 through 4 had mean nematode burdens of 287, 239, 400, and 178, respectively.

Although overall mean survival of uninfested moths at 20°C was 13.3 ± 1.42 d, survival varied from a low of 1.0 d to a high of 39.5 d, with 23 individuals surviving ≥10 d. Mean survival of infested moths was 6.2 ± 0.57 d and varied from a low of 0.5 d to a high of 15.0 d. Only 5 infested individuals survived more than 10 d on test. Regression of days survival on total nematodes at 20°C (Fig. 3) was significant for positive moths ($r^2 = 0.40$, $P < 0.01$) but was not significant for negative moths ($r^2 = 0.00$, $P > 0.10$).

An interesting feature of the regression lines in Fig. 3 is the positive slope exhibited by survival of infested moths compared to the nearly flat slope for uninfested moths. Survival of uninfested moths was unrelated to nematode burdens, because they had none, but was clearly related to initial age as indexed by scale loss (Figs. 2 and 4). All of the infested moths with relatively light nematode burdens (less than 200) survived less than 10 days and three of four individuals with a burden of more than 800 nematodes survived more than 10 days, producing a line with a positive slope instead of one parallel to that of the negative moths. The maximum nematode burden counted from a moth maintained at 20°C was 1,131. The largest nematode burden (2,377) in this study was from a moth which survived only 2.5 d at 30°C. Simmons and Rogers (1994) reported large mean nematode populations (2,977 and 3,339) in two groups of infested moths. These observations indicate that under favorable conditions, which include reduced activity at low temperature (20°C) and adequate initial fat reserves, some infested moths are able to survive sufficiently long to permit development of

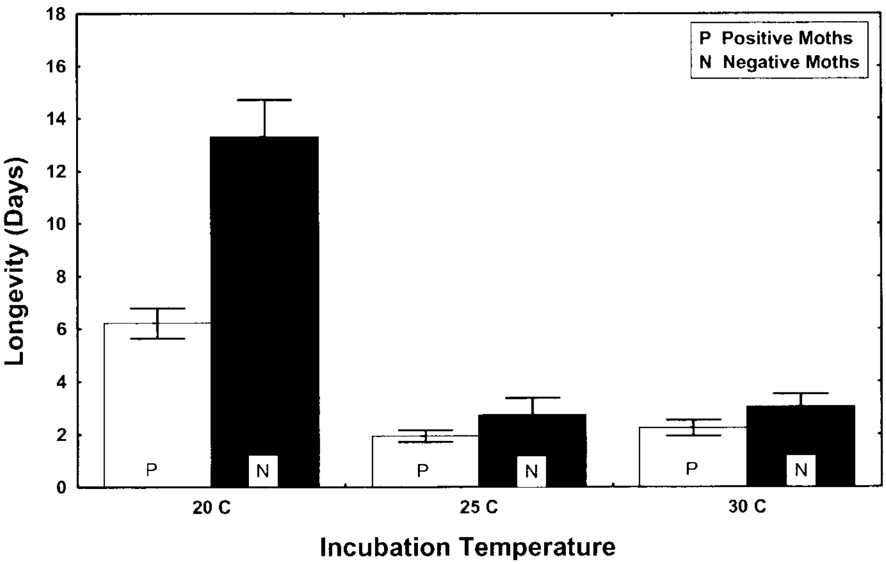


Fig. 1. Effect of incubation temperature on survival (means ± SEM) of feral male *Spodoptera frugiperda* adults positive or negative for the ectoparasitic nematode, *Noctuidonema guyanense.*

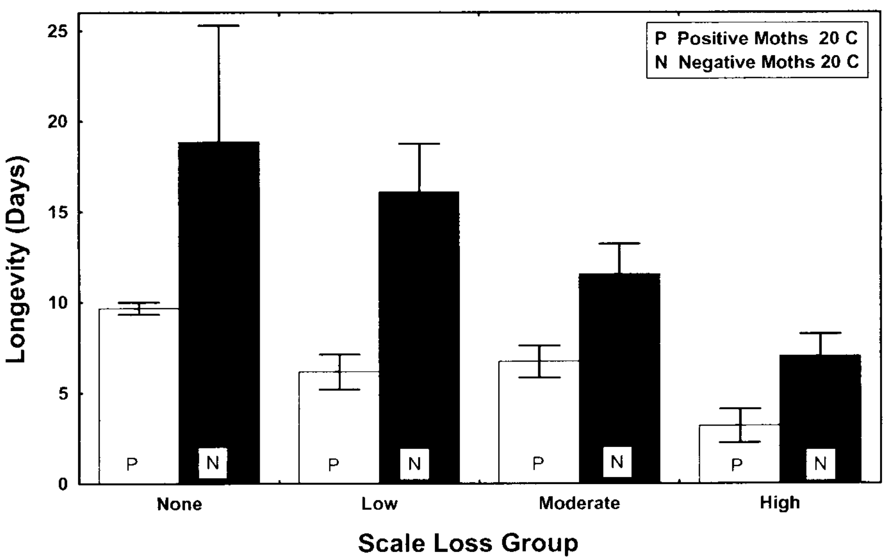


Fig. 2. Survival (Means ± SEM) of feral male adults of *Spodoptera frugiperda,* infested with the ectoparasitic nematode, *Noctuidonema guyanense,* in four scale loss groups, 20 C.

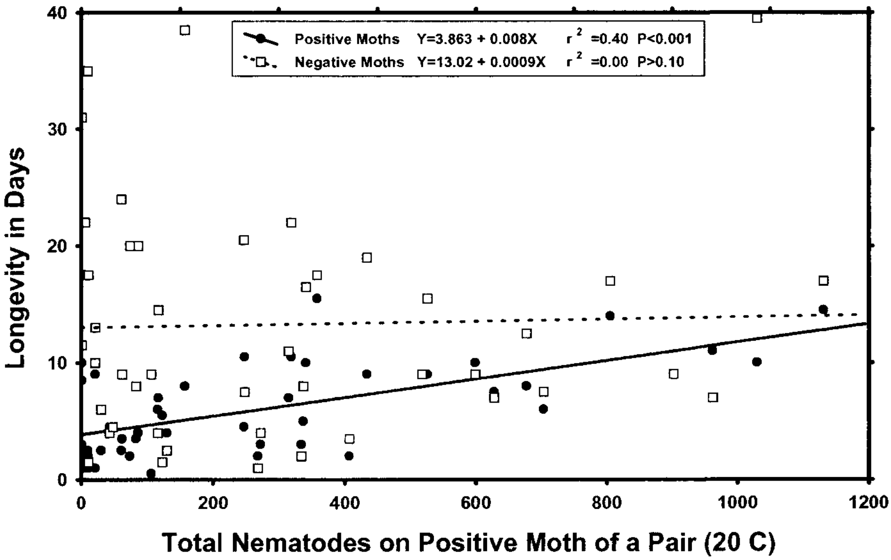


Fig. 3. Survival of feral male *Spodoptera frugiperda* adults as a function of infestation with the ectoparasitic nematode, *Noctuidonema guyanense* at 20°C. Data are plotted as pairs of points representing longevity vs. total nematodes, with one point of a pair representing the positive moth and the other representing the negative moth. Since no nematodes were present on the negative moths, a common X axis of numbers of nematodes on the positive moths was used to produce both regression lines. Several data points overlap.

relatively large numbers of nematodes. A few individuals with large initial nematode burdens may also have been occasionally collected and used in the study.

The nematodes counted on the moths in this study represented the numbers present at death of the individual insects. It was not possible to count nematodes on moths at the initiation of the study. In an investigation of the numbers of *Noctuidonema* transferring from feral male *S. frugiperda* to laboratory-reared females at mating, Rogers and Marti (1994) reported that an average of 3.7% of the nematodes on the males transferred to the females. They reported a mean of 266.5 ± 31.4 nematodes on the males in that investigation. Because of the wide variation in nematode numbers on infested males, with some individuals having considerably more than the mean, it is to be expected that during mating some individual moths will acquire large infestations of *Noctuidonema* and will nevertheless survive a few days longer than average. These often large variations in nematode burdens produce large standard errors in group means and emphasize the necessity for adequate controls in order to separate treatment effects.

In order to determine the effect of *Noctuidonema* infestation on *S. frugiperda,* adequate control for differences in age of moths placed on test is essential. Rogers et al. (1993) showed in laboratory studies that *S. frugiperda* infested with *N. guy-*

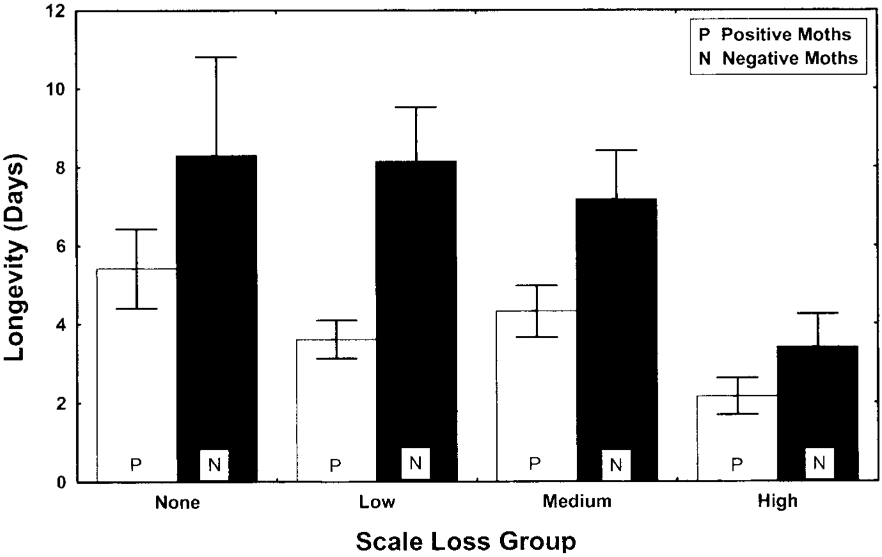


Fig. 4. Survival (means ± SEM) of feral male *Spodoptera frugiperda* adults in four scale loss groups infested with the ectoparasitic nematode, *Noctuidonema guyanense.* (All temperatures.)

*anense* survived only half as long as uninfested moths and that infested moths weighed less than uninfested moths. The results of the present study confirm the detrimental effect of *Noctuidonema* infestation on feral male *S. frugiperda.*

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