# Development of the DD-136 Strain of Neoaplectana carpocapsae at Constant Temperatures

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*Abstract:* The development of the DD-136 strain of *Neoaplectana carpocapsae* was studied on three food sources at 10, 15, 20, 25, 30, 33, 35, and 37 C. No growth occurred at 10 or above 33 C. At 15, 20, and 25 C, growth and reproduction occurred. The most favorable growth occurred at 25 C. At 30 C, *N. carpocapsae* developed to adults but did not reproduce. *Key Words:* temperature-growth effects, DD-136 strain.

*Neoaplectana carpocapsae,* along with its associated bacterium *Achromobacter nematophilus,* is a promising biological control agent of insect pests. [The taxonomic status of *A. nematophilus* is uncertain (9), but for the purpose of this paper *A. nematophilus* will be used.] This nematode has been tested against a number of pests in the field and laboratory with some encouraging results (7).

The importance of moisture for the survival of neoaplectanids has been well documented (7, 11). In contrast, there are few studies on the effects of temperature on

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the development of neoaplectanids. Survival of the dauer larvae of the DD-136 and the Czechoslovakian strains of *N. carpocapsae* at different temperatures was studied by Schmiege (10) and Weiser (12), respectively, whereas Dutky et al. (2) demonstrated the infectivity of the dauer larvae of the DD-136 strain to its insect host at various temperatures. Jackson (5) reported on the survival, growth, and reproduction of *Neoaplectana glaseri* in a chemically defined medium at different constant temperatures. The purpose of this study was to determine the growth and development of *N. carpocapsae* under different temperature regimes. Knowledge of *N. carpocapsae*'s temperature tolerance would indicate its potential as a biological control agent under various temperature conditions and its pathogenicity to homoiothermal animals.

## MATERIALS AND METHODS

*Neoaplectana carpocapsae,* along with its associated bacterium *A. nematophilus,* was added to two kinds of media or to last instar larvae of *Galleria mellonella.* Approximately 50-75 dauer larvae were placed asceptically on a modified, sterile, dog food medium (4) in a petri dish or on a pork kidney-peptone agar medium (PKPA) (2) in test tubes. The dog food medium consisted of 10 g of crushed Gaines Gravy Train (General Foods Corp., White Plains, New York) added to 25 ml of 1% agar in a petri dish. Immediately after the media were inoculated, the nematodes were placed at different constant temperatures (Table 1). Last instar *Galleria* larvae were infected with *N. carpocapsae* by placing ca. 2,500 dauer larvae onto filter paper in a petri dish. Twenty *Galleria* were placed into each dish and left for 48 h at 25 C. After this period, dead *Galleria* were placed in test tubes (150 x 16 mm) containing 10 ml of sterilized distilled water. Each *Galleria* larva was supported above the water level by a 4 x 4-cm piece of filter paper placed right at the water level. The top of the test tube was covered with parafilm. The tubes were then placed at different constant temperatures.

Data on reproduction were obtained by taking a 5-mm diameter (19.6 $mm^2$) sample of dog food with a number 2 corkborer 14 days after placement at the various temperatures. The sample was placed in 10 ml of distilled water in a test tube, gently agitated, and the number of live nematodes per ml counted with a 1-ml Peters counting slide (Hawksley and Sons Ltd., Sussex, England). Three samples were taken from each dish. The total number of live nematodes/19.6 $mm^2$ was calculated. There were 3-10 replicates/temperature.

For the tests with the PKPA medium, 10 ml of Ringer's solution (1/4 strength)

TABLE 1. Development of the DD-136 strain of *Neoaplectana carpocapsae* when it is reared at different temperatures for 14 days on dog food medium, pork kidney-peptone agar medium, and in *Galleria mellonella* larvae.

| Temperature (C) | Dog food | | Pork kidney-peptone | | *Galleria* | |
|---|---|---|---|---|---|---|
| | Replicates | No. ± SE live nematodes per 19.6 $mm^2$ | Replicates | No. ± SE live nematodes per test tube | Replicates | No. ± SE dauer larvae emerged per host |
| 10 | 8 | 2 ± 1 | 5 | 40 ± 13 | 3 | 0[b] |
| 15 | 8 | 81 ± 29 | 5 | 300 ± 131 | 3 | 0[c] |
| 20 | 5 | 97 ± 29 | 5 | 60,400 ± 15,600 | 3 | 103,500 ± 24,000 |
| 25 | 10 | 1,255 ± 102 | 5 | 298,000 ± 82,680 | 5 | 184,000 ± 49,100 |
| 27 | 3 | 852 ± 92 | — | — | — | — |
| 30 | 10 | 0[a] | 5 | 20 ± 20 | 5 | 0[d] |
| 33 | 8 | 0 | 5 | 6 ± 4 | 5 | 0[e] |
| 35 | 5 | 0 | 5 | 0 | 3 | 0 |
| 37 | 5 | 0 | — | — | 3 | 0 |

[a]Live adults found on medium but not recovered in sampling.
[b]Upon dissection, live fourth-stage larvae found.
[c]Upon dissection, all stages of nematode development found; no dauer larvae emerged from host.
[d]Upon dissection, only adults found.
[e]Two out of 25 larvae contained dead adults.

were placed in each test tube, agitated gently, and the number of live nematodes counted. These counts were made 14 days after placement at the various temperatures. There were five replicates for each temperature. Data on reproduction of *N. carpocapsae* in *Galleria* larvae were obtained by determining the total number of dauer larvae that emerged/insect larva 14 days after placement at the various temperatures. *Galleria* larvae which did not produce dauer larvae were dissected and examined with a dissecting microscope to determine the fate of the nematode and the stage of development. There were three to five replicates containing five larvae each.

Growth of the associated bacterium, *A. nematophilus,* at the different temperature regimes was also determined. The bacterium was grown in a 1% peptone broth with 0.5% NaCl (pH 7.0) (9). Growth of the bacterium was determined by measuring turbidity at 600 nm in a Bausch & Lomb Spectronic 20 colorimeter-spectrophometer.

## RESULTS

At temperatures above 35 C, *N. carpocapsae* did not develop and live nematodes were not recovered at the end of the 14-day period on the three food sources (Table 1). At 33 C, no live nematodes were recovered from the dog food medium or in *Galleria* larvae, although a few dauer larvae were found on the pork kidney-peptone agar (PKPA) medium. However, 2 out of 25 *Galleria* larvae contained dead adults.

At 30 C, *N. carpocapsae* developed to adult males and females but did not reproduce on the three food sources. Although no live nematodes were recovered from the dog food medium by the sampling method utilized, adults were observed on the medium. The ovaries of 10-day-old females were atrophied. Dissection of *Galleria* larvae held at 30 C for 14 days revealed dead and live adult nematodes but no progeny. Only live adults were recovered from the PKPA medium.

Between 15 and 27 C, the nematodes developed and reproduced. Maximal growth and reproduction occurred at 25 C on the three food sources. Dauer larvae began to emerge 7 days at 25 C and 10 days at 20 C after placement of *Galleria* larvae at their respective temperatures. Although no dauer larvae emerged from *Galleria* larvae at 15 C at the end of the 14-day period, dissections revealed the presence of nematodes in all stages of development. In one replicate, some of the *Galleria* larvae were held for 21 days at 15 C, but no dauer larvae emerged. The nematode did not develop on the three food sources at 10 C. Dauer larvae were recovered from dog food and PKPA media. Dauer and fourth-stage larvae were recovered from *Galleria* larvae. In one replicate, the *Galleria* larvae were held for 14 days at 10 C and then held at 25 C for 10 additional days. All stages of the nematodes were found in the *Galleria* larvae, but no dauer larvae emerged during the 10-day period.

*Achromobacter nematophilus* showed maximal growth between 30 and 33 C. No detectable growth was observed at the end of the 48-h period at 10, 15, or 37 C (Table 2).

TABLE 2. Growth of the DD-136 strain of *Achromobacter nematophilus* after a 48-h period at different temperatures.

| Temperature C | Mean % absorbance[a] | |
|---|---|---|
| | *A. nematophilus* | Control |
| 10 | 0 | 0 |
| 15 | 0 | 0 |
| 20 | 14 | 0 |
| 25 | 18 | 0 |
| 30 | 21 | 0 |
| 33 | 22 | 0 |
| 37 | 0 | 0 |

[a]Growth expressed as % absorbance measured in a Bausch & Lomb Spectronic colorimeter-spectrophometer at a wavelength of 600 nm; mean of 3 replicates.

## DISCUSSION

The present study has confirmed Dutky's observation (1) that the most favorable temperature for the growth and reproduction of the DD-136 strain of *N. carpocapsae* is between 23-28 C. I observed no development at 10 C or above 33 C. Interestingly, this nematode develops to the adult stage at 30 C but it cannot reproduce. Although the reason for the infertility is not known, it may result from the lack of viable sperms or ova or from the mating behavior of the nematode.

The possibility of development occurring with the DD-136 strain in situations where temperatures exceed 30 C for any length of time is unlikely even though adequate moisture is available. For example, in bee nests, temperatures constantly above 30 Care common (6). Each neoaplectanid's temperature tolerance must be determined because of differences in their responses to high temperatures. Hackett and Poinar (3) reported infection and development of the "agriotos" strain of *N. carpocapsae* in honey bee (*Apis mellifera*) adults at 34 C. Reproduction of this strain at 34 C was not checked. Similarly, *N. glaseri* developed at higher temperatures than the DD-136 strain (5).

Very little has been published on the effects of neoaplectanids on homoiothermal animals. Schmiege (10) and Poinar (8) reported that mice fed the DD-136 strain of *N. carpocapsae* and its associated bacterium showed no ill effects. On the basis of the temperature requirements of the DD-136 strain, the likelihood of this neoaplectanid developing in birds and mammals is remote. However, the safety of *N. carpocapsae* should be determined by directly challenging homoiothermal animals. Additionally, its development in poikilothermal vertebrates and other invertebrates needs further study.

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