Journal of Nematology 18(2):221–224. 1986.

Respiration Rate of *Steinernema feltiae* Infective Juveniles at Several Constant Temperatures

J. E. LINDEGREN, R. E. RIJ, S. R. ROSS, AND D. C. FOUSE[1]

Abstract: Respiration was measured in dauer stages of the insect-parasitic nematode *Steinernema feltiae* (= *Neoaplectana carpocapsae*) at 7, 17, and 27 C. Respiration, Q_{10}, and nematode viability were temperature dependent. Mean O_2 consumption for 5×10^5 nematodes the first 24 hours was 0.27 ml at 7 C, 0.83 ml at 17 C, and 2.68 ml at 27 C. The Q_{10} was 3.10 for 7–17 C and 3.24 for 17–27 C. Some nematodes died during 2, 14, and 21 days at 27, 17, and 7 C, respectively. The respiratory quotient was below 1 at all temperatures tested. A standard asymptotic model is expressed as oxygen consumed = 2.77 * {1 − exponent[−time * exponent(−B + C * temperature)]}; where 2.77 is the maximum response at 27 C. This model estimates nematode O_2 consumption and viability at storage temperatures between 7 and 27 C. The nematodes died when the O_2 concentration reached 0.5 ml/5×10^5 nematodes. This model may be used to predict O_2 requirements of *S. feltiae* infective juveniles when stored as a waterless concentrate.

Key words: carbon dioxide, infective juveniles, mortality, *Neoaplectana carpocapsae*, oxygen consumption, physiology, Q_{10}, respiration, standard asymptotic model, *Steinernema feltiae*, temperature, viability, biocontrol.

A wide range of insects is susceptible to steinernematid nematodes under laboratory conditions (20). Commercial use of these nematodes has been restricted primarily to cost effective, noninundative applications for control of insects inhabiting confined environments (5,11,12,14). Reduction in the cost of steinernematid production reported by Bedding (1–3) may now make inundative application (4,13,16,18,22) economically feasible. An important step for commercial use is the development of methods for storing the nematodes and transferring them to fields or orchards. Nematodes have been stored and (or) transported in oxygenated water (7), aerated water (1), sterilized water or 0.1% formalin in flasks (19), 0.1% formalin in tissue culture flasks, in plastic petri dishes, on moist polyurethane sponges, or on water-saturated filter paper (3,8,9,15). These methods are too bulky for inundative commercial application and may be unwieldy, especially if refrigeration is required or if an additional extraction is necessary.

Ideal commercial storage of steinernematid juveniles would probably be a waterless concentrate that could be transported easily to the application site. The required concentration could then be used without additional processing. The varied temperatures encountered in storing and transporting infective juvenile nematodes to a field site require a model for predicting nematode viability and respiration at several temperatures.

Burman and Pye (6) determined the oxygen consumption of *Steinernema feltiae* infective juveniles grown at 20 or 25 C and then stored at five temperatures (13–30 C) in low-density aerated, oxygenated, and buffered water. We wanted to avoid this need for aeration and the limitations of O_2 availability inherent when large numbers of nematodes are stored at various depths in unaerated water; therefore, we modified the moist filter technique of Howell (9) and sampled the atmosphere around nematodes stored at 100% relative humidity in the absence of free water. We also developed a model for predicting the respiratory activity and O_2 demand and its effect on the viability of concentrated nematodes stored with limited O_2 at various constant temperatures.

MATERIALS AND METHODS

Infective *Steinernema feltiae* (Mexican strain) dauer juveniles were packaged as a concentrate in water-saturated polyurethane foam in a polyethylene bag by a commercial laboratory in northern California 5 days after harvest and transported to

Received for publication 8 May 1985.

[1] Horticultural Crops Research Laboratory, U.S. Department of Agriculture, Agricultural Research Service, Fresno, CA 93727.

We thank Bruce Mackey, Mathematical Statistician, Biometrical and Statistical Service Staff, USDA ARS, Western Regional Research Center, Albany, Calif., for assistance in analysis of the data.

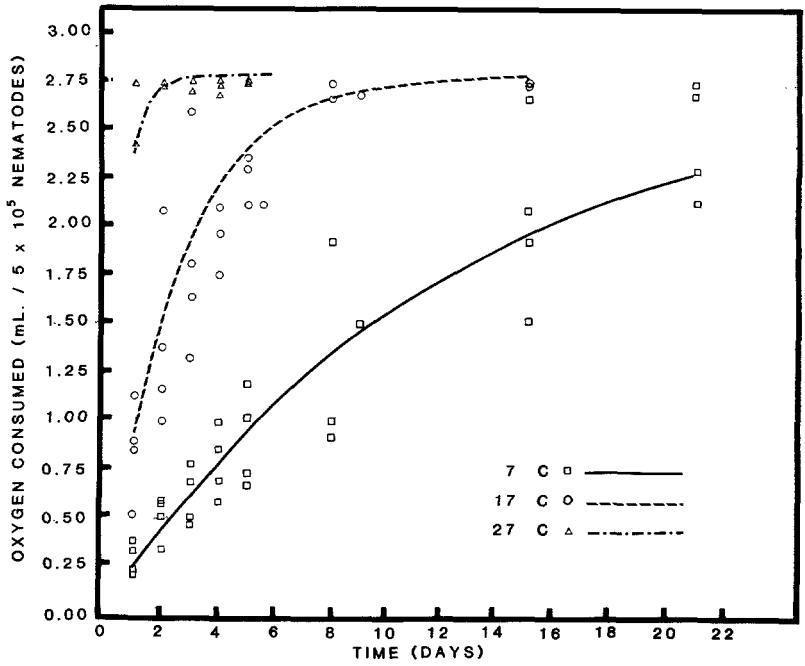

FIG. 1. Cumulative O_2 consumption of *Steinernema feltiae* infective juveniles stored at 7, 17, or 27 C. Each point represents one replicate of three samples each. Solid line is fitted using a standard asymptotic model.

Fresno in an insulated cooler. No mortality was observed in a sample of 50 nematodes as determined by activity in response to mechanical probing. The nematodes were washed from the foam with deionized water into a stirred stock suspension from which five 0.5-ml samples were taken with a calibrated syringe. Each sample was diluted with 499.5 ml deionized water to make a 1:1,000 dilution. Five 0.5-ml samples from this 1:1,000 suspension were placed in a 100-mm plastic petri dish top and used for counting. The stock suspension was adjusted to obtain a count of 50,000 ± 5,000/ml.

The corrected nematode suspension was then transferred with a 10-ml graduated pipet to a moistened 9.0-cm grade 131 glass-fiber filter paper previously placed on a Leramie-Buchner funnel. The pipet was then flushed with an additional 5 ml deionized water onto the filter paper. The pressure was held continuously at −50.7 kPa for 1 minute. The filter paper was removed and folded so that the edges were to the outside and the nematodes were contained inside. The folded filter paper with the nematodes was then inserted into a gas-tight 13.5-ml serum vial. The sample vials were capped loosely with a butal-type septum cap and held for 48 hours at 7 C to inactivate the nematodes. The vials were capped with an air-tight aluminum seal and held in chambers at 7, 17, or 27 ± 0.5 C for 21, 14, or 5 days, respectively, until the available O_2 was consumed. A single 0.5-ml gas sample was taken from the head space of each unsampled vial at 24-hour intervals for the first 5 days for each temperature and then at 7-day intervals for 7 and 17 C for a total of 21 days and 14 days, respectively. The gas samples were analyzed for O_2 and CO_2 content with a Fisher Model 1200 gas partitioner equipped with a thermoconductivity detector. Nematode survival was determined by mechanical probing within 2 hours of each gas sampling. Nematodes were not checked for infectivity.

Data from four replications of each statistic per temperature were analyzed by means of a standard asymptotic model using Marquardt's nonlinear least square procedure (17).

RESULTS

Respiration rate of *S. feltiae* infective juveniles was temperature dependent. Oxygen consumption averaged 0.267, 0.827, and 2.679 ml $O_2/5 \times 10^5$ nematodes after

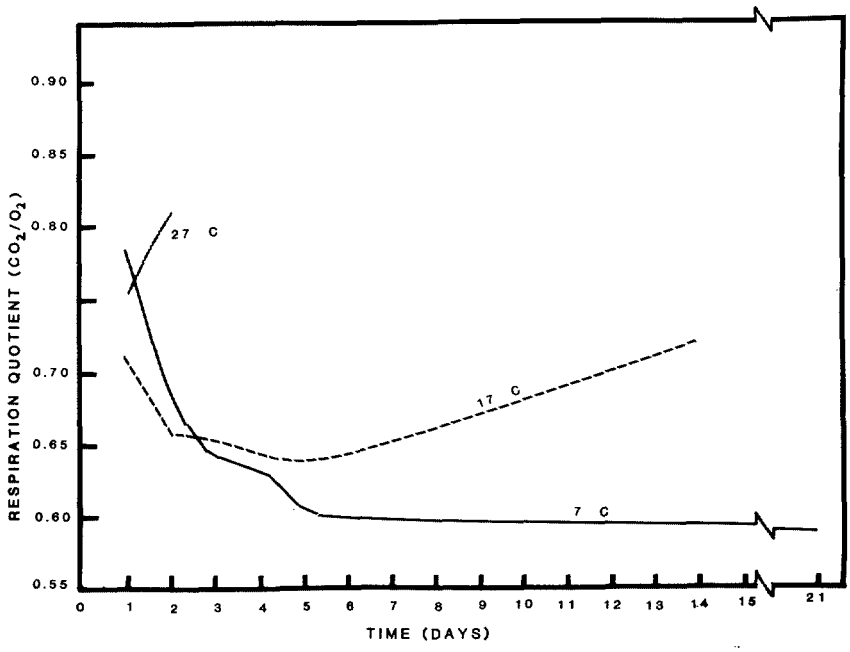

FIG. 2. Respiratory quotient of *Steinernema feltiae* infective juveniles stored at 7, 17, or 27 C.

24 hours at 7, 17, and 27 C, respectively. Cumulative O_2 consumption was 2.25 ml at 7 C, 2.72 ml at 17 C, and 2.72 ml at 27 C for 5×10^5 nematodes at 21, 14, and 2 days, respectively (Fig. 1).

Figure 1 shows the fitted lines for the relationships between O_2 consumption and time at each selected temperature. The line follows a standard asymptotic model forced through the origin. The model is expressed as oxygen consumed = 2.77 * {1 − exponent[−time * exponent(−B + C * temperature)]}; where 2.77 is an estimate of the maximum response at 27 C.

The amount of O_2 consumed per day was highest the first day and then decreased at all three temperatures. At 27 C consumption was three times greater than at 17 C and 10 times greater than at 7 C; it also was three times greater at 17 C than at 7 C. The Q_{10} values, 3.10 and 3.24, were nearly the same for the temperature intervals of 7–17 C and 17–27 C, respectively.

When available O_2 reached less than 0.5 ml $O_2/5 \times 10^5$ nematodes in a vial, no activity upon probing was detected. This level was reached in 2 days at 27 C, 14 days at 17 C, and 21 days at 7 C.

The respiratory quotient (RQ) was below 1 for all temperatures (Fig. 2). The RQ for nematodes held at 7 C decreased during the first 5 days and then remained constant for the remainder of the 21-day storage period. The RQ for nematodes held at 17 C decreased from 0.71 to 0.66 the first 2 days, then changed little for 3 days before increasing to 0.73 by 14 days. The RQ for nematodes held at 27 C increased during the 2 days of storage.

DISCUSSION

The respiratory rate of *S. feltiae* infective juveniles is highly temperature dependent under the conditions described. We also observed, contrary to published reports (6), that the nematodes died at very low O_2 concentrations. Burman and Pye (6) observed nematode movement even after 43 days of storage at very low (0.5% of saturation) O_2 tension, probably because they bubbled O_2 into the water samples before measuring dissolved O_2 consumption, whereas we measured O_2 concentration in the head space.

In our study the respiratory quotient was below 1. An RQ below 1 indicates that the nematodes were reacting to anaerobic conditions or were utilizing fats (21). Imbriani and Platzer (10) pointed out that *Romanomermis culicivorax* infective juveniles functioned at less than 1% O_2.

Commercial shipping of *S. feltiae* dauer juveniles may be possible at the magnitude needed for large scale field applications.

Our standard asymptotic model may be used to predict the O_2 requirements of the nematodes over likely storage times and temperatures. Further investigations should concern additional methods for prolonging nematode viability in storage by providing adequate O_2 concentrations and (or) reducing nematode O_2 consumption.

Literature Cited

1. Bedding, R. A. 1976. New methods increase the feasibility of using *Neoaplectana* spp. (Nematoda) for the control of insect pests. Proceedings of the First International Colloquium on Invertebrate Pathology, Kingston, Canada. Pp. 250–254.

2. Bedding, R. A. 1981. Low cost in vitro mass production of *Neoaplectana* and *Heterorhabditis* species (Nematoda) for field control of insect pests. Nematologica 27:109–114.

3. Bedding, R. A. 1984. Large scale production, storage and transport of the insect parasitic nematodes *Neoaplectana* spp. and *Heterorhabditis* spp. Annuals of Applied Biology 104:117–120.

4. Bedding, R. A., and L. A. Miller. 1981. Disinfesting black current cuttings of *Synanthedon tipuliformis,* using the insect parasitic nematode, *Neoaplectana bibionis.* Environmental Entomology 10:449–453.

5. Bedding, R. A., and L. A. Miller. 1981. Use of a nematode, *Heterorhabditis heliothidis,* to control black vine weevil, *Otiorhynchus sulcatus,* in potted plants. Annuals of Applied Biology 99:211–216.

6. Burman, M., and A. E. Pye. 1980. *Neoaplectana carpocapsae*: Respiration of infective juveniles. Nematologica 26:214–219.

7. Dutky, S. R., J. V. Thompson, and G. E. Cantwell. 1964. A technique for the mass propagation of the DD-136 nematode. Journal of Insect Pathology 6:417–422.

8. Hara, A. H., J. E. Lindegren, and H. K. Kaya. 1981. Monoxenic mass production of the entomogenous nematode, *Neoaplectana carpocapsae* Weiser, on dog food/agar medium. Advances in Agricultural Technology, USDA Western Series, Number 16.

9. Howell, J. F. 1979. New storage methods and improved trapping techniques for the parasitic nematode *Neoaplectana carpocapsae.* Journal of Invertebrate Pathology 33:155–158.

10. Imbriani, J. L., and E. G. Platzer. 1981. Gaseous requirements for postparasitic development of *Romanomermis culicivorax.* Journal of Nematology 13: 470–476.

11. Kovacs, I. A., K. V. Deseö, G. O. Poinar, Jr., and A. DéLeonardis. 1980. Prove di lotta contro insetti con applicazione di nematodi entomogeni. Atti Giornate Fitopathologiche 1:449–456 (English summary).

12. Lindegren, J. E., and W. W. Barnett. 1982. Applying parasitic nematodes to control carpenterworms in fig orchards. California Agriculture 36(11&12):7–8.

13. Lindegren, J. E., C. E. Curtis, and G. O. Poinar, Jr. 1978. Parasitic nematode seeks out navel orangeworm in almond orchards. California Agriculture 32(6):10–11.

14. Lindgren, J. E., T. T. Yamashita, and W. W. Barnett. 1981. Parasitic nematode may control carpenterworm in fig trees. California Agriculture 35(1&2):25–26.

15. Lindegren, J. E., D. F. Hoffmann, S. S. Collier, and R. D. Fries. 1979. Propagation and storage of *Neoaplectana carpocapsae* Weiser using *Amyelois transitella* (Walker) adults. Advances in Agricultural Technology, USDA Western Series, Number 3.

16. Lindegren, J. E., J. E. Dibble, C. E. Curtis, T. T. Yamashita, and E. Romero. 1981. Compatibility of NOW parasite with commercial sprayers. California Agriculture 35(3&4):16–17.

17. Marquardt, D. W. 1963. An algorithm for least-squares estimation of non-linear parameters. Journal of the Society for Industrial Applied Mathematics 2: 431–441.

18. Miller, L. A., and R. A. Bedding. 1982. Field testing of the insect parasitic nematode, *Neoaplectana bibionis* (Nematoda: Steinernematidae) against currant borer moth *Synanthedon tipuliformis* (Lep: Sesiidae) in black currants. Entomophaga 27:109–114.

19. Poinar, G. O., Jr. 1975. Entomogenous nematodes. Leiden: E. J. Brill.

20. Poinar, G. O., Jr. 1979. Nematodes for the biological control of insects. Boca Raton, Florida: CRC Press.

21. Precht, H., J. Christophersen, H. Hansel, and W. Lorden. 1973. Temperature and life. NewYork: Springer-Verlag.

22. Toba, H. H., J. E. Lindegren, J. E. Turner, and P. V. Vail. 1983. Susceptibility of the Colorado potato beetle and the sugarbeet wireworm to *Steinernema feltiae* and *S. glaseri.* Journal of Nematology 15: 597–601.