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INFLUENCE OF TEMPERATURE AND FOOD SOURCE ON THE LIFE CYCLE OF THE EARTHWORM *DENDROBAENA VENETA* (OLIGOCHAETA)

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Summary—The life cycle of *Dendrobaena veneta* was studied to assess the potential of breeding this species for the fish-bait market. Development, growth and reproduction were investigated by rearing worms at 10°, 15°, 20° or 25°C on horse manure or on aerobic paper sludge for 6 months. At 10°C, the maturity was slow with a long period of development (104 d) and a low cocoon production. Productivity at 25°C was better than at 20°C or 15°C. The effect of temperature on cocoon production differed according to the source of food; on paper sludge, cocoons were produced at the same rate at 20 or 25°C while on horse manure cocoon production was better at 25°C than at 20°C. Whatever temperature, cocoon production was significantly greater on paper sludge than on horse manure. On paper sludge, maturation of juveniles was faster and adult worms larger. Adults started to produce cocoons at a younger age and more cocoons were produced worm^{-1} and d^{-1}. These results showed that the life cycle of *D. veneta* may be influenced by the type of food and that horse manure is not the best substrate for breeding this species. © 1997 Elsevier Science Ltd

INTRODUCTION

In vermiculture, experience with the growing of *Eisenia fetida* and other species showed that litter-dwelling earthworms could be easily bred (Loehr *et al*., 1985). On the other hand, production of large and deep burrowing earthworms such as *Lumbricus terrestris* is more difficult. Aside from the traditional fish bait market, waste processing and production of earthworm biomass, the application of vermiculture may be useful for the production of large numbers of selected species of earthworms for land restoration (Butt *et al*., 1992).

Among the different variables necessary for raising earthworms it seems that the type of food is most important. Unfortunately, little is known about earthworm nutrition for mass-rearing but micro-organisms, C and N availability appear to play a major role. In practice, *E. fetida* can utilize a variety of food sources such as horse manure, municipal sludge or household refuse with good results (Fayolle, 1985; Hartenstein, 1986). Among these different organic materials, Neuhauser *et al*. (1980) observed that activated sludge was superior to either horse manure or municipal and household sludge as food for *E. fetida*. In a comparative study on the growth and cocoon production of *Lumbricus rubellus*, *Eisenia andrei and Dendrobaena rubida*, Cluzeau and Fayolle (1989) showed that the smaller species, *D. rubida*, can be bred on trophic resources that are degraded. In contrast, *L. rubellus*, a bigger species, can only be grown and produce cocoons on fresh and easily degradable organic matter such as horse manure or household refuse.

In the European bait market, growers are searching for species such as *D. veneta* to produce large earthworms of the same size as *L. terrestris*. Studies on *D. veneta* show that municipal digested sludge is a good source food for growth and cocoon production (Loehr *et al*., 1985; Lofs-Holmin, 1986). If we consider the results obtained with *L. terrestris* and other deep-burrowing species (Butt *et al*., 1992; Butt, 1993), it appears that a synthetic medium of paper pulp and yeast extract is a better food than separated cattle slurry.

Preliminary results showed us that unamended paper sludge from a French factory produced good results in the breeding of *D. veneta*. Our objective was to determine the life cycle of *D. veneta* at four temperatures and with two kinds of food; horse manure as a reference and an aerobic paper sludge.

MATERIALS AND METHODS

Experimental cocoons came from a French grower who raises *D. veneta* indoors. The cultures were set up with newly-hatched worms. Earthworms were raised in cylindrical plastic containers, ventilated at the top of 0.5 l for 42 d and 0.9 l from day 42 to

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Table 1. Characteristics of the two feeds given to *D. veneta*

		% of total solids						
Food	Total solids %	Ash	Total C	Total N	C-to-N	P_2O_5	K_2O	CaO
Paper sludge	21.7	10.1	45.9	1.01	45.4	0.16	0.07	1.8
Mixture of horse droppings and brown peat	22.0	39.2	31.2	1.21	25.4	0.71	1.21	3.4

189. The habitat consisted of a 4–5 cm layer of brown peat with 20% solids (wet wt) was placed on the container bottom. The food supply consisted of a mixture of horse manure and brown peat (1: 1, v/v) or aerobic paper sludge each with 22% solids (wet wt). Food, freely available on the soil, was supplied on the basis of 50 mg dry matter (paper sludge) or 100 mg dry matter (mixture of horse droppings and peat) g^{-1} fresh worm d^{-1}.

Growth and cocoon production were determined for each trophic resource studied at the following temperatures of 10°, 15°, 20° or 25°C (± 1°C). Seven containers, each initially stocked with five hatchlings were used for each treatment. For the first 6 weeks, the containers were monitored every 10–11 d (simple manual sorting) and the culture medium and food completely changed at the end, the worms and cocoons being recovered by wet-sieving. From wk 6 to 27, the containers were at the time of medium renewal monitored every 21 d. The containers were placed in dark air-conditioned rooms at the chosen temperature with relative humidity near saturation to prevent the media from drying out.

To complete the demographic studies, cocoons produced on paper sludge and 0–2 d old were incubated in one of two ways. In the first, 50 cocoons for each temperature were placed in an individual incubator on moist plaster. In the second, groups of 10 cocoons with five replicates for each temperature were placed in incubators containing brown peat. Incubation period, hatching rate and number of hatchlings per cocoon were determined for each treatment.

RESULTS

Food

The composition of foods given to *D. veneta* showed that amount of carbon is higher in paper sludge than in the mixture of horse manure and brown peat (Table 1). We also observe differences in C-to-N ratio and mineral concentrations.

Survival, growth and maturation

The rate of mortality for each treatment was equal to or less than 6% during 27 wk of growth and cocoon production. This survival rate was independent of food type and temperature; we observed a higher mortality at the beginning of the experiment with young animals.

Figure 1 shows curves of mean growth on paper sludge at four temperatures during 189 d. At 10°C, we observed a slower weight gain compared to growth rate at other temperatures. Sexual maturity was attained after 104 d with a mean biomass of 1.6 g. Growth rate was higher at 15, 20 and 25°C, and the worms required only 62 d to reach full maturity determined with clitellum apparition. The highest mean biomass of 2.7 g was attained at 126 d in the range 20–25°C and 166 d at 15°C. On horse manure, growth curves for the four temperatures are given in Fig. 2. The effect of temperature on growth rates was similar to those on paper sludge. The growth rate of worms at 25°C was initially greater and 80% of animals attained maturity after 62 d. At 10°, 15° and 20°C sexual maturity was reached after 145, 104 and 83 d respectively. The

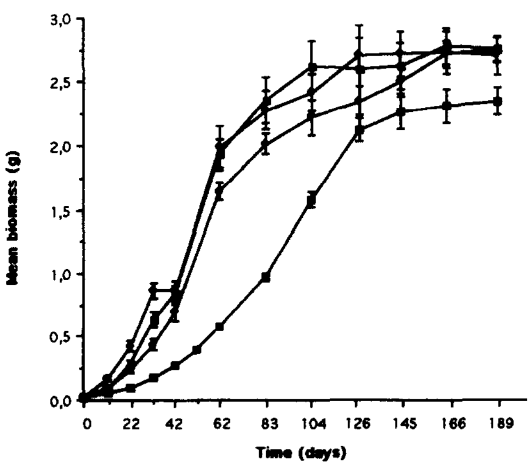

Fig. 1. Individual growth (mean biomass ± se) over time (days) on paper sludge at 10°C (—□—), 15°C (—●—), 20°C (—■—) and 25°C (—○—).

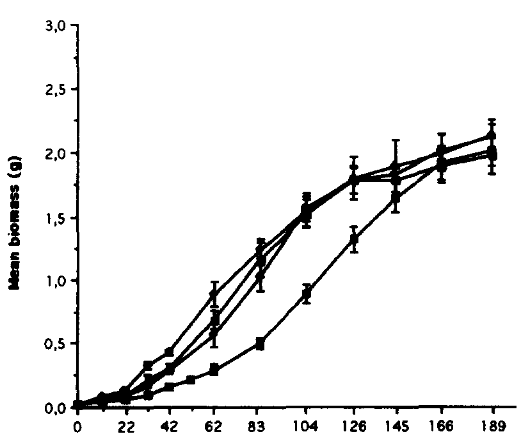

Fig. 2. Individual growth (mean biomass ± se) over time (days) on horse droppings at 10°C (—□—), 15°C (—●—) 20°C (—■—) and 25°C (—○—).

Table 2. Biomass and cocoon production (with mean and standard error) from hatchlings on horse manure and paper sludge at three temperatures. Means within rows compared by a Newman and Keuls test ($P = 0.05$), results with the same letter were not significantly different

	Time interval (days)	Horse manure			Paper sludge			Test F
		15°C	20°C	25°C	15°C	20°C	25°C	
Biomass	0–32	148 ± 14^{f}	198 ± 28^{e}	309 ± 29^{d}	413 ± 45^{c}	619 ± 60^{b}	838 ± 63^{a}	<1%
production	0–83	997 ± 103^{d}	1142 ± 140^{c}	1220 ± 79^{c}	1991 ± 83^{b}	2336 ± 180^{a}	2256 ± 151^{a}	<1%
mg worm^{-1}	0–126	1756 ± 108^{c}	1758 ± 105^{c}	1773 ± 169^{c}	2329 ± 109^{b}	2571 ± 242^{a}	2679 ± 174^{a}	<1%
Cocoon	62–83	0	0.02 ± 0.02^{d}	0.16 ± 0.05^{c}	0.30 ± 0.05^{b}	0.68 ± 0.06^{a}	0.67 ± 0.04^{a}	<1%
production worm^{-1} d^{-1}	126–145	0.37 ± 0.05^{c}	0.35 ± 0.07^{c}	0.51 ± 0.09^{b}	0.57 ± 0.03^{b}	0.68 ± 0.05^{a}	0.74 ± 0.05^{a}	<1%

final mean biomass of 2.1 g was the same for worms grown at temperatures in the range 10–25°C. According to the growth curves for each food (Fig. 1 and Fig. 2), growth rate was better on paper sludge than on horse manure throughout the trial. These results were confirmed by variance analysis of the animal's biomass (Table 2). The difference in weight gained was important during the first period of growth, biomass production at 15, 20 and 25°C was about three times higher on paper sludge after 32 d (Table 2). The superiority of paper sludge for biomass production was observed after 83 and 126 d (Table 2). Final mean biomass was also greater on paper sludge than on horse droppings (Fig. 1 and Fig. 2).

Cocoon production

Cocoon production on paper sludge began between 42–62 days at 25°C and 62–83 d at 15 and 20°C (Fig. 3). At 10°C, cocoon production began after 126 d and only 9 cocoons worm^{-1} were produced at 189 d. At the other temperatures, cocoon production was considerably increased and reached 58, 82 and 85 cocoons worm^{-1} at 15, 20 and 25°C respectively. At 20°C, a mean of 4.5 cocoons worm^{-1} d^{-1} was produced during the reproductive phase.

On horse manure, cocoon production began at an older age for each temperature (Fig. 4). At 10°C, only 4 cocoons were produced worm^{-1} of 189 d old against a mean of 30 to 45 cocoons at the other temperatures. Compared to paper sludge, the mean reproductive rate on horse droppings was 2 cocoons worm^{-1} d^{-1}.

Cocoon production was influenced by temperature and significantly higher on paper sludge compared with horse manure ($P < 0.01$—Table 2). On paper sludge, cocoons were produced at the same rate at 20 or 25°C while cocoon production was higher at 25°C than at 20°C on horse droppings. On paper sludge cocoon production for 20 week old worms was about 1.8 times higher than on horse droppings. Our data showed that age did not correspond to a decrease in size and that at 189 d cocoon production continued at a steady rate.

Cocoon incubation

The mean biomass of incubated cocoons varied between 27.2 and 29.6 mg (Table 3). The duration of cocoon incubation defined as time for 50% of hatchings was more than 90 d at 10°C. Hatching of cocoons was faster in the range of 20–25°C with a mean incubation time of 36 d in brown peat and a longer incubation time of 44 d on moist plaster which can be explained by the greater dessication of these incubators. At 15°C, incubation duration was 51–52 d on moist plaster or in brown peat. The hatching success was in the range of 84–100% and 1.02 living worms were produced per cocoon.

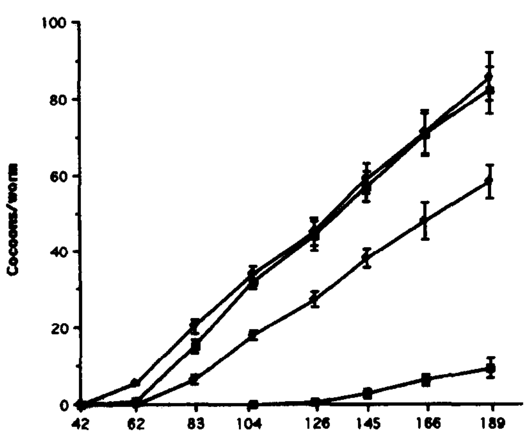

Fig. 3. Cumulative cocoon production (mean ± se) over time (days) on paper sludge at 10°C (—□—), 15°C (—●—) 20°C (—■—) and 25°C (—○—).

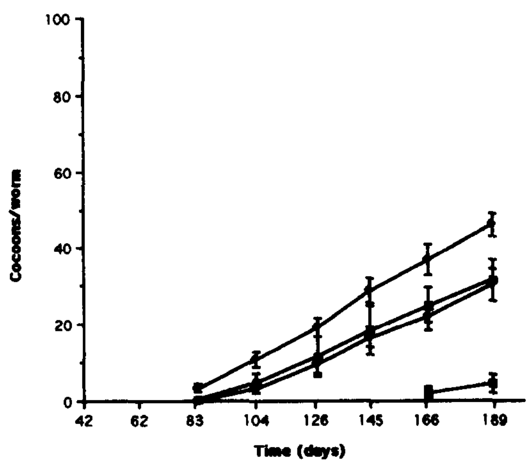

Fig. 4. Cumulative cocoon production (mean ± se) over time (days) on horse droppings at 10°C (—□—), 15°C (—●—) 20°C (—■—) and 25°C (—○—).

Table 3. Mean incubation time and hatching success of 50 cocoons carried out on moist plaster (a) or in brown peat (b) at four temperatures

		Temperatures °C			
		10	15	20	25
Cocoon mass (mg)		28.1 ± 2.1	29.6 ± 2.7	27.2 ± 1.9	28.3 ± 2.4
Incubation time (days)	a	>90	51	45	43
	b	>90	52	36	35
Hatching success (%)	a	0	100	84	90
	b	0	96	92	88

DISCUSSION

From the above data, it is clear that *D. veneta* has a very long life cycle at 10°C. Development time was reduced considerably when temperatures were increased from 10–15°C to 20–25°C. At 15°C and 25°C, growth rate and cocoon production on horse manure (Table 2) were better than on cattle manure reported by Viljoen *et al.* (1992). The different types of food and the fact that substrate was not renewed or worms weighed every 5 d by these authors can explain the differences. Mean incubation period of cocoons observed at 25°C or 15°C by Viljoen *et al.* (1992) were also longer than in our experiment. A hatching success of more than 80% for cocoons (Table 3) was similar to results observed by Loehr *et al.* (1985).

On paper sludge, growth rate at 15, 20 or 25°C was about twice as fast than on horse manure. Moreover, adults started to produce cocoons at a younger age and up to twice as many cocoons were produced during 189 d on paper sludge. These results on paper sludge are similar to those obtained by Loehr *et al.* (1985) with an aerobically-digested sludge as food. They showed that *D. veneta* began to produce cocoons after 60–70 d and needed 16 wk to achieve a maximum weight of 3.2 g. These authors observed that *D. veneta* can produce 5 cocoons $worm^{-1}$ wk^{-1} at 25°C, however total worm numbers produced from a hatchling in 20 wk was 19. We showed that on paper sludge hatchlings can produce more than 40 worms during 20 wk at 25°C.

Cluzeau and Fayolle (1989) showed that fresh and readily-decomposable organic matter such as heathland litter or horse manure enables rapid development for large epigeous species such as *L. rubellus*. Growth and cocoon production of an epi-anecic species such as *L. terrestris* were possible with a separated cattle slurry (Butt *et al.*, 1992). They also obtained a better growth without mortality on a synthetic food composed of paper pulp and yeast extract with a C-to-N ratio of 40. Our results showed that *D. veneta* grew better and produce more cocoons on paper sludge (C-to-N = 45) than on horse manure (C-to-N = 25) and are similar to those obtained by Butt *et al.* (1992) on the synthetic food source containing paper sludge. Although Neuhauser *et al.* (1980) showed that *E. fetida* can gain weight at low C-to-N ratio in the range 15–35, our knowledge about feeding is poor. It is known that micro-organisms, such as bacteria, fungi and protozoa, play an important role in the nutrition of earthworms (Rouelle, 1982). Rapid growth rate and better cocoon production of large earthworm species on paper or aerobically-digested sludge can be explained by their microbial biomass content.

Many factors such as temperature, soil moisture, worm density have been studied as attempts to understand earthworm ecology. Our data demonstrated that food and *temperature* can greatly influence the life cycle of *D. veneta*. The breeding of *D. veneta* for the bait market may be optimised by using a food source such as paper sludge and rearing worms at temperature between 20 to 25°C.

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