

Effect of α -Tocopherol and Culture Method on Reproduction of *Turbatrix aceti*

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Abstract: The effect of α -tocopherol on the reproductive capacity of the free-living nematode *Turbatrix aceti* was determined using three different culture methods: mass culture, pair culture, and single culture. Significant differences were observed between control and α -tocopherol cultured nematodes for all reproductive parameters measured. The reproductive period started at a significantly earlier time and the length of the reproductive period was significantly longer in α -tocopherol cultured nematodes. The average number of offspring was 34 in control cultures as compared to 75 in α -tocopherol cultures. The eggs of α -tocopherol cultured females showed a more regular outline and uniform distribution of yolk than did eggs from control females. **Key words:** physiology, reproduction, α -tocopherol, vitamin E, oocytes.

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Alpha-tocopherol (Vitamin E) enhances the growth rate of *Turbatrix aceti* (7) and significantly increases the mean lifespan of this and other nematodes species (1,3,6). It is essential for reproduction in mammalian (9) and in some invertebrate (5,8,11) species. Its effect on nematode reproduction, however, has not been described. The purpose of these studies, therefore, was to describe the effect of α -tocopherol on *T. aceti* reproduction.

MATERIALS AND METHODS

Animals used and medium: Axenic cultures of *T. aceti* were obtained from Dr. M. Rothstein, State University of New York at Buffalo, and cultured according to methods previously described (6). In this method, heated liver extract, which is essential for the maintenance of optimum growth and development (10), is added to a basal medium containing soy peptone, yeast extract, and 2% glacial acetic acid. A pH between 3.4 and 4.1 was maintained for all experiments. Individual nematodes were isolated manually with a micropipette. Single nematodes were maintained individually in each of the 24 wells of tissue culture clusters (Costar 3524). Medium in the wells was changed every other day.

Preparation of vitamin E: Pure dl- α -tocopherol in liquid form was obtained from ICN Pharmaceuticals (Cleveland, Ohio, Catalog No. 100562). It was initially diluted 1:100 in the acetic acid used in the *T. aceti* growth medium. Its final concentra-

tion in the culture medium was 0.1 μ l per ml. The oily α -tocopherol appeared to be dispersed or dissolved in the medium and did not require the addition of Tween-80, which we have used as a solubilizing agent for α -tocopherol in other experiments carried out in dilute aqueous medium at neutral pH (2). Pilot testing showed that this dosage had maximal biological effects; generation time was shorter and population increase to day 21 was more rapid than for any other dosage.

Culture methods: Three culture conditions were used to evaluate the effects of α -tocopherol on reproduction: mass culture, paired culture, and single culture. Twelve synchronized nematodes were transferred to petri dishes to ensure an adequate male-to-female ratio for optimum fertilization in mass culture. For pair culture, pairs of nematodes were isolated into single-culture chambers before the onset of their reproductive period. To establish single cultures, nematodes were left in mass cultures during their mating time. After mating occurred, females were isolated in culture-cluster chambers so that their reproductive capacities could be measured.

Criteria used to evaluate reproduction: Four criteria were used to evaluate the reproductive potential of *T. aceti*: onset of the reproductive period, length of the reproductive period, number of live progeny, and reproductive rate. The onset of the reproductive period was defined as the time when offspring first appeared in culture wells containing females of known age which had been isolated after fertilization. The length of the reproductive period was defined as the time interval between the

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appearance of the first larvae and the time at which newborn larvae were no longer present. This interval was measured by removing parents from their offspring every 12 hours and continuing to monitor reproduction. To determine the reproductive rate of the control and the α -tocopherol cultured nematodes, the number of live offspring produced in each 24-hour period was counted. The parents were transferred to new culture dishes so that counting could continue on subsequent days. Dead offspring were not counted. Data were evaluated for statistical significance using the student t-test.

Cytological preparations: To observe the effects of α -tocopherol on egg morphology, adult female nematodes were placed in a drop of glycerine and gently squashed under a coverslip. A drop of methylene blue was then added and drawn under the coverslip using absorbant tissue. After staining, the specimens were observed and photo-micrographs taken of the ovary and eggs. Twenty control and twenty α -tocopherol treated female nematodes were examined in this manner.

RESULTS

Table 1 presents a summary of data obtained for three reproductive parameters. It shows that regardless of the culture method used or the reproductive parameter

measured, nematodes cultured in α -tocopherol supplemented medium showed significantly greater reproductive capacity than did the controls. The onset of the reproductive period was at least 2 days later in control than in α -tocopherol cultured nematodes. The mean length of the reproductive period was always significantly shorter in control nematodes, as compared to α -tocopherol cultured ones, although the percentage difference varied considerably depending upon the culture method used. The mean number of live offspring was greatly reduced in control nematodes, as compared to those cultured in α -tocopherol. All the differences between control and α -tocopherol cultured nematodes were significant ($p < 0.05$).

In no case did the culture method used alter or override the stimulative effect of α -tocopherol. It is evident from the data in Table 1 that the pair cultures showed the least reproductive success. In pair cultures the mean onset of the reproductive period was retarded by about 3 days, the mean length of the reproductive period was 1-3 days shorter, and the mean number of offspring was significantly reduced, as compared to single or mass culture. There is a wide range in the total number of offspring produced, both for α -tocopherol cultured and for control nematodes. Control pair-cultured nematodes produced 1-89 offspring, with an average of 34; α -tocopherol

Table 1. The effect of dl- α -tocopherol (0.1 μ l/ml) on three parameters of reproduction of *Turbatrix aceti*.

Parameter	Culture method	Control	Tocopherol	Difference (%)
Mean onset of reproductive period (days \pm S.D.)	Mass†	10.0 \pm 1.40	*8.0 \pm 0.70	25.0
	Single‡	9.8 \pm 0.93	*7.9 \pm 0.88	24.1
	Pair†	12.4 \pm 1.20	*9.4 \pm 0.80	31.9
Mean length of reproductive period (days \pm S.D.)	Mass†	5.5 \pm 1.00	*7.3 \pm 1.00	24.6
	Single‡	7.3 \pm 0.73	*8.1 \pm 0.60	9.9
	Pair†	4.2 \pm 0.81	*7.6 \pm 0.67	47.7
Mean number of live offspring (\pm S.D.)	Single†	56.2 \pm 13.0	*73.8 \pm 2.4	23.8
	Pair†	34.6 \pm 6.8	*76.0 \pm 13.1	54.5

*Significantly different from control at $P = 0.05$.

†N = 4 \times 24

‡N = 4 \times 30

DISCUSSION

pair-cultured nematodes produced 11–110 offspring, with an average of 75.

When females were isolated from mass culture and placed in single culture on day 4 or 5, neither those from the α -tocopherol nor those from control cultures produced any offspring, so fertilization must not have occurred. However, by day 6, 40% of the α -tocopherol cultured females, but none of the control females, had started to reproduce and by day 7, 10% of the control females had reproduced (Fig. 1).

The α -tocopherol supplemented females produced a larger number of offspring on the first day of their reproductive period than did control females, regardless of the culture method used. In single cultures, control females produced an average of 12 offspring on the first day while α -tocopherol cultured females produced an average of 32 offspring. The differences were less pronounced on subsequent days.

The oocytes of control females had an irregular appearance and clumped yolk (Fig. 2B). In contrast, the oocytes of all α -tocopherol cultured females showed a regular outline and a uniform distribution of yolk (Fig. 2A).

Several invertebrates are known to have requirements for vitamin E. When raised on purified diets, the house cricket, *Acheta domestica*, requires vitamin E for spermatogenic activity and egg production (8). In a study of the nutritional requirements of the snail, *Biomphalaria glabrata*, raised under axenic conditions, Vieira (11) observed that vitamin E was required for normal egg production. The addition of α -tocopherol to the culture medium brings about significant increases in the average lifespan, the average number of offspring, and the average length of the reproductive period in the rotifer *Philodina* (2). In nematodes, α -tocopherol supplementation has been shown to extend lifespan in *T. aceti* (1,6) and in *C. elegans* (3). It also enhances growth rate in *T. aceti* (7). In the present study, our results show that α -tocopherol had a stimulatory effect on all reproductive parameters measured in *T. aceti*. This may result from the early maturation of oocytes in α -tocopherol treated nematodes. The consistent regular appearance and uniform yolk distribution char-

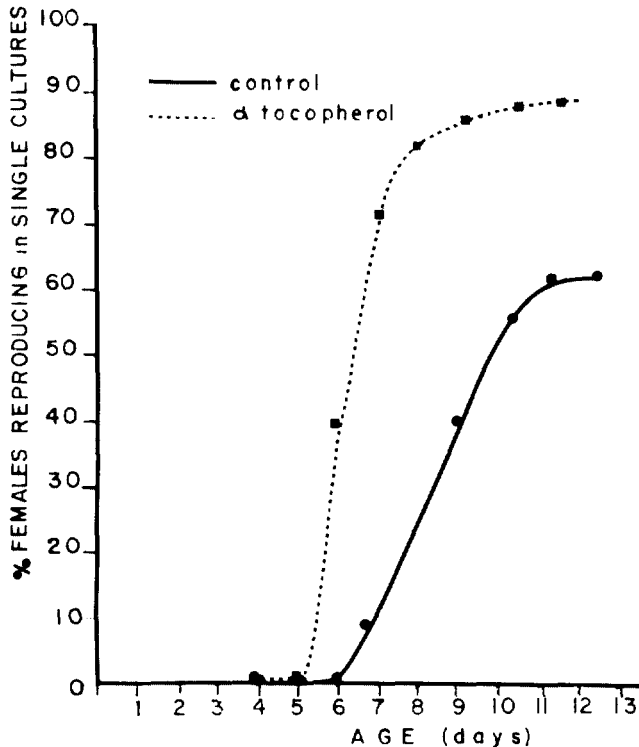


Fig. 1. Graph illustrating the difference in the percentage of *Turbatrix aceti* females which reproduced after isolation from mass cultures on different days after sexual maturation in control and α -tocopherol cultures. (n = 30)

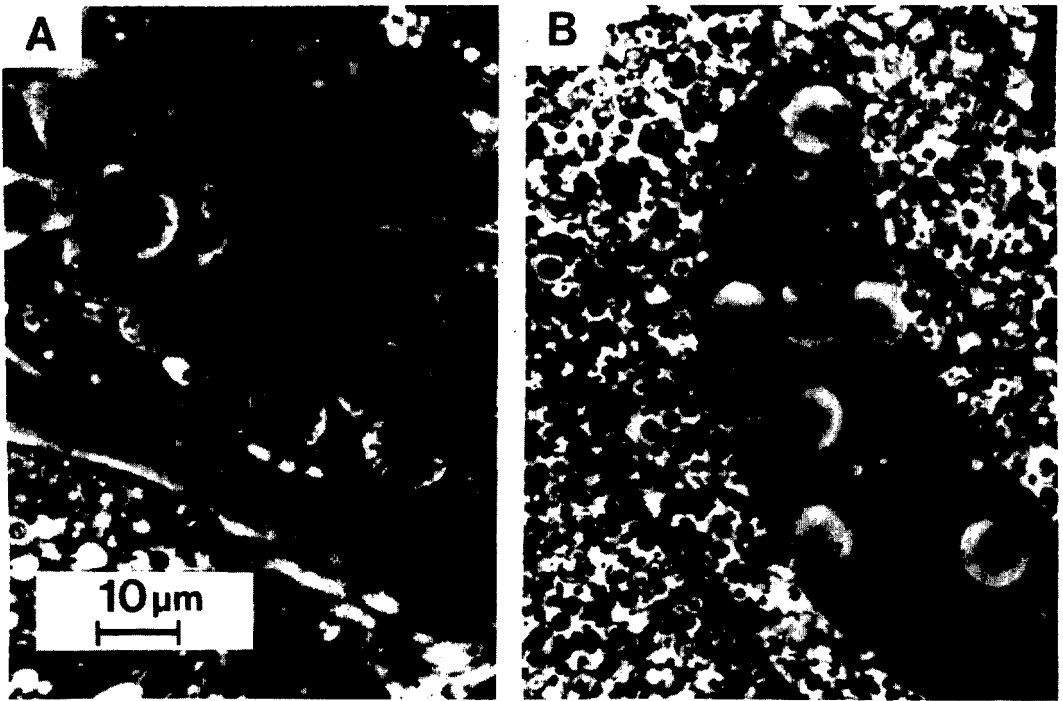


Fig. 2. Oocytes in the ovary of a *Turbatrix aceti*. A) α -tocopherol cultured female. B) Control female.

acteristic of the eggs of α -tocopherol supplemented females suggest more uniform and advanced development than in controls. Observation of occasional unfertilized but mature eggs in paired cultures, and of non-fertile females in single cultures, confirms that oogenesis was sometimes impaired in control nematodes. Other studies (1,7) suggest that α -tocopherol also ensures early growth and maturation of larvae in this species. α -Tocopherol was continuously available to the nematodes, since the medium was replenished regularly. However, we do not know about the conformation of the α -tocopherol in the medium; it may have absorbed to particulate matter in the medium and been dispersed in this manner. Gilbert (4) has established that dl- α -tocopherol-5-methyl- H^3 is taken up into rotifers, but no such studies have been carried out with nematodes.

Each of the three culture methods used in this study had certain advantages. The mass-culture method proved satisfactory for measurement of mating times and time of onset of the reproductive period, but less adequate for measurement of the length of

the reproductive period and the number of progeny. The advantage of a mass-culture experiment is that the large number of nematodes ensures the probability of mating. The pair-culture method could be used to measure most parameters of reproduction, but proved to be inaccurate in measuring time of onset of reproduction. The probability of both male and female being mature enough to reproduce is diminished in this culture method. The single-culture method ensures optimum fertilization, due to the abundance of males in the mass cultures. This procedure provides information concerning the earliest mating time and the length of the period between fertilization and birth of progeny. A stimulative effect of α -tocopherol was observed in all three culture conditions.

We conclude from our results that the effects of α -tocopherol on reproduction of *T. aceti* are probably due to accelerated maturation; α -tocopherol probably acts initially on oocyte development in females and enhances maturation under all three culture conditions methods used in this study.

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