Short communication

Temperature and soil moisture content effects on the growth of *Lumbricus terrestris* (Oligochaeta: Lumbricidae) under laboratory conditions

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Abstract

The growth of *Lumbricus terrestris* was determined under varying temperatures and soil moisture contents under laboratory conditions. Our objective was to identify the optimum soil moisture or temperature that would aid in the reproduction of *L. terrestris* for inoculations in areas devoid of this species. A laboratory experiment using different growth chambers for temperature control with varying soil moisture contents was conducted at the National Soil Tilth Laboratory in Ames, IA. Five temperatures, three different soil moisture contents, and horse manure as a food source were selected and replicated 10 times. It was found that 30°C were fatal to *L. terrestris* after 14 d and 25°C after 182 d. Regardless of temperature, worms reared under high soil moisture content (30%) developed faster and increased in mass than those reared at 20 or 25% soil moisture content over 266 d. Optimum temperature and soil moisture content for mass rearing of *L. terrestris* was identified at 20°C and at 30% soil moisture content for these Iowa soils.

Keywords: *Lumbricus terrestris*; Soil moisture; Temperature; Earthworms; Mass rearing

*Lumbricus terrestris* (L.) is one of the more important earthworm species that contribute to soil processes (Curry and Cotton, 1983) because they incorporate organic matter, increase soil aggregation, influence soil aeration and water infiltration, etc. (Darwin, 1881; Satchell, 1983; Edwards et al., 1990; Kladivko and Timmenga, 1990; Alban and Berry, 1994). Burrows formed by this species are permanent, usually vertically oriented and may extend to depths of 2 m or more (Ehlers, 1975). *L. terrestris* feeds on plant and animal litter that may be pulled down into the burrow or used in forming middens in undisturbed land (Binet and Curmi, 1992). Natural colonization by *L. terrestris* is slow and thought to result from low reproductive and dispersal rates (Curry, 1988). Experimental releases of *L. terrestris* in coal mine spoils (Vimmerstedt and Finney, 1973; Hamilton and Vimmerstedt, 1980) and in reclaimed peat (Curry and Bolger, 1984) resulted in increased fertility and productivity. Interest has increased for developing techniques for mass rearing and inoculating earthworms in reduced or no-tillage agricultural systems. Werner’s study (1996) on inoculative release of *L. terrestris* in a California orchard showed that apple leaf litter incorporation was significantly increased by this earthworm in the organic plot where no soil disturbance occurred. Currently, inoculation projects are limited because techniques for mass rearing of earthworms are not fully developed (Curry, 1988) and because of limited information on the optimum environmental conditions for different species of earthworms.

Temperature and soil moisture are two key environmental factors that would significantly influence any techniques used in developing an appropriate procedure for mass rearing of earthworms, especially *L. terrestris*. In a series of studies Butt (1991) and Butt et al. (1992) investigated the effects of factors, such as soil temperature, soil moisture and food source on the production of *L. terrestris*. They compared the effects of temperature and nutrition on cocoon production, incubation and hatchling growth. They found cocoon development and hatchling growth were most rapid at 20°C but the greatest annual production was recorded at 15°C. Some of these findings are also echoed in the studies by Daniels et al. (1996) who found that with an adequate food source available (e.g. dandelion leaves), *L. terrestris* increased in weight at an optimal temperature of 15–17.5°C. Soil moisture obviously has an effect on the growth and activity of *L. terrestris*. When soil moisture is optimum, earthworms increase in mass and in their activity if food sources are available (Lee, 1985; Edwards and Bohlen, 1996). Our objective for this laboratory study was to determine optimum soil moisture and temperature for *L. terrestris*.
Lumbricus terrestris and its potential for mass rearing and inoculation in Iowa soil using manure as a food source.

To obtain similar earthworms, both in mass and genetic uniformity, hatchlings of *L. terrestris* were obtained from colonies that had been reared for two generations at the National Soil Tilth Laboratory in Ames, IA (USA). During rearing, colonies were maintained on a mixture of two soils (a Clarion loam, fine-loamy, mixed, mesic Typic Hapludolls and a Webster silty clay loam, fine-loamy, mixed, mesic Typic Hapludolls) and fed partially decomposed horse manure. Rearing conditions for *L. terrestris* consisted of containers held in continuous darkness with a constant supply of horse manure with a 30% constant soil moisture held at 20°C for two generations. Cocoons were collected from the rearing containers by wet sieving the soil (2 mm). The cocoons were placed on moistened paper towels in 27 cm (dia.) plastic dishes, incubated in a growth chamber at 20°C and kept in constant darkness until the hatchlings emerged from the cocoons.

The mixture of the Clarion and Webster soil was used for the test study (described under the Rearing Experiment). Each test container was prepared by weighing 750 g of air dried soil and 10 g of air-dried ground horse manure into $17 \times 12 \times 6$ cm$^3$ plastic dishes. Three soil moisture contents were maintained gravimetrically for 266 d or until the worms died. Selected soil moisture contents of 20, 25, or 30% were established in the dishes and allowed to equilibrate for approximately 24 h prior to introducing the worms. Temperature was maintained in separate computer controlled bioclimatic chambers at 10, 15, 20, 25 and 30°C for 266 d.

*L. terrestris* hatchlings weighing between 60 and 80 mg were selected for test purposes. Each container was inoculated with a single *L. terrestris* hatchling. At 14-d intervals, the worms were extracted by handsorting, weighed and examined for their general condition and sexual maturity. Development of sexual maturity was determined by observing the first appearance of tubercula pubertalis, glandular ridges in or near the ventral margin of the clitellum, and the presence of the clitellum, which is a ring of fleshy glandular tissue that is most noticeable on a mature earthworm (Fender, 1985). The earthworms were then placed into freshly prepared containers as described above. This procedure continued for 266 d or until the earthworms died.

The experiment was designed as a randomized complete block arranged in split plots and each treatment was replicated 10 times. Whole plots consisted of five temperatures and subplots consisted of three soil moistures as described above. This resulted in 30 (3 x 10) moisture containers in each (five) bioclimatic chamber for a total of 150 experimental units. Tukey’s test was used as a mean separation procedure (SAS Institute, 1989).

Soil moisture and temperature significantly affected the growth in terms of live weight of *L. terrestris* (Figs. 1 and 2). To obtain information on the optimal temperature and moisture ranges for this earthworm, we choose to average the data for temperature and soil moisture. If there were critical differences for these two factors, we separated this data by both temperature and soil moisture content (Fig. 3). When averaged across all temperatures, the most significant increase in mass for *L. terrestris* was at 20°C. In about the first 40 d, no difference was observed in the weight gain of the worms. Some of the worms that were exposed to...
temperatures greater than 20°C did not survive the test. Worms that were exposed to 25°C survived for about 182 d and survival at 30°C was reduced to 14 d (Fig. 1). When worms were held at a constant temperature of 10°C they weighed less than those held at 15 or 20°C. For example, on day 98 the mass of worms reared at 20°C were 62.3 mg which was significantly greater (p < 0.05) than those held at 15°C (47 mg worm⁻¹) or 10°C (14 mg worm⁻¹). The effect of temperature on mean daily weight gain at 10, 15, and 20°C were 32, 46, and 49 mg worm⁻¹ d⁻¹, respectively.

Regardless of temperature, greater worm mass was seen at 25 and 30% moisture after 182 d (Fig. 2). Throughout the 266 d test, worms exposed to 20% moisture weighed significantly less than 30% soil moisture content. Towards the end of the test (182–266 d), worms at 25 or 30% soil moisture content were significantly greater in mass than at 20% soil moisture. The effect of soil moisture content on average daily weight gain at 20, 25, or 30% were 35, 43, and 49 mg worm⁻¹ d⁻¹, respectively.

We examined the effect of each temperature at each soil moisture content on *L. terrestris* growth. Our data consistently shows that at each temperature the worms mass increased over time with the greatest increase at 30% soil moisture content. Furthermore, soil moisture becomes critical at higher temperatures (25 and 30°C) (Fig. 3). We present data only at 25°C because at 30°C the worms were dead after 14 d. Significant differences were observed between soil moisture contents at 30% versus 20 or 25% (Fig. 3). A decline in worm mass was seen at 126 d at 20% soil moisture and at about 182 d at 25% soil moisture content. At 25°C significantly greater mass was seen for the worms at 30% soil moisture.

Regardless of soil moisture content, worms exposed to low temperature (10°C) took longer to develop the tubercula pubertalis and clitellum (physical observation, data not presented). The duration for these developments was different for each soil moisture content. For example, worms at 10°C with a soil moisture of 20%, developed tubercula pubertalis in 166 d and acclitellum in 216 d. At the same temperature when the soil moisture content was increased to 30%, tubercula pubertalis were first noticed in 122 d and the worms became fully clitellated in 166 d. In contrast, tubercula pubertalis were first observed and the clitellum developed in 48 and 73 d, respectively, for worms at 20°C and at 30% soil moisture content.

Soil moisture content and temperature are key factors in rearing *L. terrestris* for inoculative purposes. Our study does suggest some optimal temperature and moisture conditions that should be consider for these midwestern soils. In our study, temperatures equal to or greater than 25°C were detrimental to *L. terrestris* which is consistent with other research. However, under controlled conditions these worms did survive for 182 d or about six months if the temperature was 25°C (Fig. 3), which is considerably longer than other similar studies (Butt, 1993). In some environmental conditions 20°C is the upper limit for survival of *L. terrestris* under controlled and field conditions and most of these species seem to do better at about 15°C or below (Butt et al., 1992; Daniels et al., 1996; Whalen and Parmelee, 1999). At 30% soil moisture at 25°C, worms can be reared for up to 182 d. Whalen and Parmelee (1999) observed that *L. terrestris* had the greatest mass in soil maintained at 10°C and 20% soil moisture content under laboratory conditions. An optimal temperature of 20°C with about 30% soil moisture would be recommended in a silt loam or loam Iowa soil. Our worms were at a comparable mass (weight gain) as other studies have shown (Butt, 1993). We were able to maintain our worms for considerably longer than other researchers with the primary difference in the studies being the food source. We fed our worms a steady supply of partially decomposed horse manure as compared to dried cattle manure in the experiment by Butt (1993). Further experiments using different food sources need to be conducted to determine more about the effect of this factor.

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