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# Growth rates of *Aporrectodea caliginosa* (Oligochaetae: Lumbricidae) as influenced by soil temperature and moisture in disturbed and undisturbed soil columns

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#### Summary

Earthworm growth is affected by fluctuations in soil temperature and moisture and hence, may be used as an indicator of earthworm activity under field conditions. There is no standard methodology for measuring earthworm growth and results obtained in the laboratory with a variety of food sources, soil quantities and container shapes cannot easily be compared or used to estimate earthworm growth in the field. The objective of this experiment was to determine growth rates of the endogeic earthworm Aporrectodea caliginosa (Savigny) over a range of temperatures  $(5-20 \degree C)$  and soil water potentials (-5 to - 54 kPa) in disturbed and undisturbed soil columns in the laboratory. We used PVC cores (6 cm diameter, 15 cm height) containing undisturbed and disturbed soil, and 1 l cylindrical pots (11 cm diameter, 14 cm height) with disturbed soil. All containers contained about 500 g of moist soil. The growth rates of juvenile A. caliginosa were determined after 14-28 days. The instantaneous growth rate (IGR) was affected significantly by soil moisture, temperature, and the temperature  $\times$  moisture interaction, ranging from -0.092 to  $0.037 d^{-1}$ . Optimum growth conditions for A. caliginosa were at 20 °C and -5 kPawater potential, and they lost weight when the soil water potential was -54 kPa for all temperatures and also when the temperature was  $5 \,^{\circ}$ C for all water potentials. Growth rates were significantly greater in pots than in cores, but the growth rates of earthworms in cores with undisturbed or disturbed soil did not differ significantly. The feeding and burrowing habits of earthworms should be considered when

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choosing the container for growth experiments in order to improve our ability to extrapolate earthworm growth rates from the laboratory to the field. © 2005 Elsevier GmbH. All rights reserved.

# Introduction

Earthworms are known to accelerate nutrient mineralization and improve soil fertility in temperate agroecosystems (Lee, 1985; Edwards and Bohlen, 1996). The contribution of various earthworm species to nutrient mineralization is affected by their feeding habits and life-history strategies, because individuals from different ecological groups are active in different parts of the soil profile when environmental conditions are favourable (Bouché, 1977; Brown et al., 2004). Furthermore, earthworm-mediated nutrient mineralization may be related to their activity and growth (Marinissen and de Ruiter, 1993). Earthworm growth rates are very responsive to fluctuations in soil temperature and moisture, and may be used to estimate activity and dynamics of earthworm populations (Buckerfield et al., 1997). In temperate agricultural soils, earthworm growth is fastest at soil temperatures from 15-20°C when the soil moisture is close to field capacity (Daniel et al., 1996; Holmstrup, 2001; Wever et al., 2001; Baker and Whitby, 2003). However, soil temperatures range from about 0-25 °C and there may be periodic flooding and drought during the crop growing season. Researchers wishing to estimate nutrient mineralization from earthworms require detailed information on how earthworm growth rates fluctuate with changing soil temperature and moisture conditions.

There is no standard methodology for measuring earthworm growth rates. A review of the literature reveals that growth rates for the major lumbricid earthworm species have been determined using a variety of food sources, amounts of soil and containers (Butt, 1997; Fayolle et al., 1997; Whalen and Parmelee, 1999; Booth et al., 2000). When provided with abundant organic matter with a high N content, earthworms grow faster than when they receive a restricted amount of food or one with a low N content (Boström and Lofs-Holmin, 1986; Boström 1988; Daniel, 1991). Many earthworms grow faster when they consume finely ground than coarsely ground organic substrates (Boström and Lofs-Holmin, 1986; Lowe and Butt, 2003). Little is known of the relationships between the amounts of soil or the shape of the culture vessel may have on earthworm growth rates. Growth rates have been measured commonly in the laboratory in 40-2000 g of soil in containers with volumes ranging from 0.12 to 2.21 (Butt et al., 1994; Whalen and Parmelee, 1999; Baker and Whitby, 2003). In these studies, loose soil was packed or placed into the container before earthworms were added.

We hypothesize that earthworm growth rates will differ when earthworms are grown in disturbed soil than in undisturbed soil. An undisturbed soil core obtained from the field will likely contain some burrows and macropores that facilitate earthworm movement and reduce their energy expenditure in moving through soil, thereby increasing growth rates. Containers may constrain earthworm movement, reducing the energy used to burrow and increasing the energy allocated for growth. Whalen and Parmelee (1999) reported that growth rates of Aporrectodea tuberculata (Eisen) were similar in 0.121 laboratory pots and 7.91 field cores, but juvenile Lumbricus terrestris L. had slower growth rates in field cores than in laboratory cultures. The amount of soil and shape of the culture vessel used in laboratory studies should provide growth data that is representative of earthworm activity under field conditions.

The objectives of our experiment were: (1) to determine how growth rates of *Aporrectodea caliginosa* were influenced by soil temperature and moisture; and (2) to determine whether earthworm growth rates were influenced by soil disturbance and culture vessel shape.

# Materials and methods

#### Collection of earthworms and soils

Juvenile individuals of *A. caliginosa* were collected by hand sorting in September 2003 from fields under alfalfa (*Medicago sativa* L.) and soybean (*Glycine max* (L.) Merrill) production at the Macdonald Campus Farm of McGill University, Ste-Anne-de-Bellevue, Qué., Canada. Earthworms were reared for about 6 weeks at room temperature (20 °C) in soil from the field site, moistened to near field capacity. Newly emerged earthworms (<0.25g) and pre-clitellite earthworms (>0.70g) were excluded from the analysis as their growth rates may not be truly representative of juvenile earthworms. Yet, fewer than 20% of the

earthworms in this study were excluded from the analysis due to being smaller or larger than the desired size range (0.25-0.70 g).

The soil was a sandy-loam mixed, frigid Typic Endoquent of the Chicot series taken from a field under soybean production. It had a pH (H<sub>2</sub>O) of 6.3, a C content of  $30.2 \text{ g C kg}^{-1}$ , and contained  $580 \text{ g kg}^{-1}$  sand,  $300 \text{ g kg}^{-1}$  silt and  $120 \text{ g kg}^{-1}$  clay. Soils were air-dried to about 10% gravimetric moisture content (-200 kPa matric potential) before use. The earthworm food was composted cattle manure containing about  $383 \text{ g C kg}^{-1}$  and  $19.9 \text{ g N kg}^{-1}$  (Carlo Erba Flash NC Soils Analyzer, Milan, Italy).

#### Calculation of soil moisture content

Four soil gravimetric moisture contents (15%, 20%, 25%, and 30%) were used to test earthworm growth in response to moisture conditions. Since matric potential is a more meaningful way to express biological water availability, we converted the gravimetric moisture content to matric potential using the Rosetta software program (Schaap, 2000). A SSCBD (texture and bulk density) pedotransfer function was used to predict the parameters necessary for calculating matric potential using the van Genuchten function for water retention (van Genuchten, 1980; Schaap et al., 1998). The calculated matric potentials (+standard deviation, S.D.) were -5 ( $\pm 1$ ), -11 ( $\pm 2$ ),  $-23 (\pm 4), -54 (\pm 14)$  kPa, corresponding to 30%, 25%, 20%, and 15% gravimetric moisture content, respectively.

#### Pot experiment

This experiment involved a completely randomised factorial design with four temperatures (5, 10, 15, and 20 °C), and four soil water potentials (-5, -11, -23, and -54 kPa), for a total of 16 factorial treatments. Each treatment was replicated 10 times. Each replicate pot was a 1-l cylindrical plastic pot (11 cm diameter, 14 cm height) with a perforated lid containing 400–480 g of air dry soil (sieved <10 mm mesh, 500 g of moist soil), and 3 g (dry matter basis) of manure (sieved <4 mm mesh). The manure was mixed into the top 5 cm of the soil where endogeic earthworms typically consume their food. The food and soil mixture was incubated for 2–5 days before adding the earthworm.

Juvenile earthworms with a mean mass of  $0.35\pm0.11$  g (S.D.) (n = 1028) were washed and placed on moistened paper to void their guts for

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24 h. The next day the earthworms were washed, gently blotted dry with paper towels and weighed (gut-free fresh weight). One earthworm was added to each pot, which was then sprayed with approximately 3 ml water to remoisten the earthworm and soil surface. Pots were placed into controlled climate incubators at four temperatures in darkness for the duration of the experiment.

Earthworms were reared in pots for 8 weeks and were removed every 13-15 days for weight measurements. At each weighing, earthworms were washed, placed on a moistened paper to void their guts for 24h, weighed (gut-free fresh weight) and then returned to the same pot for 13-15 days. Washing and keeping the earthworms on a moistened paper for 24h insures that the earthworms from different soil moisture treatments have equal hydration status when weighed. Before returning earthworms to the pots, about 1g (dry matter basis) of manure was added to the soil surface, pots were weighed and tap water was added to replace moisture lost through evaporation. When dead earthworms were found, they were removed and a replacement earthworm of similar weight and age class was added to the pot. The growth rates for replacement earthworms were considered to be missing values in the statistical analysis.

#### Core experiment

The experiment was designed as a completely randomised factorial design with three temperatures (10, 15 and 20 °C), three soil water potentials (-5, -11 and -23 kPa), and two soil disturbance treatments (undisturbed and disturbed) with eight replicates of each treatment. Each replicate core was soil in a PVC plastic tube with an internal diameter of 6 cm, a height of 15 cm and a volume of 0.4251. Disturbed soil cores contained sieved (<10 mm mesh) soil that was packed to a bulk density of  $1.23 \pm 0.01 \text{ g cm}^{-3}$  (S.E.) (*n* = 72), equivalent to the bulk density found in the undisturbed cores. This was achieved by gently pounding the core on the lab bench until the desired bulk density was achieved. Undisturbed soil cores, taken from the same field site, were obtained by hammering the PVC tube into the ground above a visible earthworm burrow and digging out the core. Fine plastic mesh (1.5 mm) was secured with elastic bands on both ends of the core to prevent soil losses. Undisturbed soil cores were kept in a cold room at 0 °C for 6 weeks to kill any earthworms that may have been collected in the core. Each core contained between 300 and 425 g of air dry soil (400-600 g of moist soil after adding different amounts of tap water based on the moisture treatments).

Juvenile earthworms were washed and placed on moistened paper to void their guts for 24h, then removed, washed, gently blotted dry with paper towels and weighed (gut-free fresh weight). Earthworms added to the undisturbed and disturbed soil cores had a mean gut-free fresh biomass of 0.43 + 0.14g (S.D.) (n = 59), and 0.38 + 0.11g(S.D.) (n = 61), respectively. One earthworm was added per core, and 5g dry matter of manure was placed on the soil surface. The surface of the soil in each core was sprayed with approximately 3 ml water to remoisten the earthworm and soil surface. Cores were placed in controlled climate incubators in darkness for 28 days, then earthworms were removed from each core, placed on a moistened paper to void their guts for 24h, and their gut-free fresh weights determined. Replicates with dead earthworms were excluded from the statistical analysis.

#### Calculation of earthworm growth rates

Earthworm growth rates are commonly reported as either average growth rates or relative growth rates, and while these measurements may be useful for laboratory experiments in which the growth of an age-specific cohort is followed to maturity, they assume that earthworm growth through time is a continuous linear function (Whalen, 1998). It has been well established that earthworm growth through time follows a logistic curve (Phillipson and Bolton, 1977; Daniel et al., 1996). As an earthworm approaches maturity, a greater proportion of the energy from food resources is likely used in the formation of sexual organs and reproduction rather than the formation of new tissues (Daniel et al., 1996). Instantaneous growth rates (IGR,  $d^{-1}$ ), which assume that growth proceeds logistically rather than linearly, are better able to account for these factors by calculating the change in an individual's growth during an infinitely short time interval (Pertrusewicz and Macfavden, 1970; Diehl and Audo, 1995). The IGR was calculated using the equation,

$$IGR = \ln(W_f/W_i)/\Delta t, \qquad (1)$$

where  $W_i$  and  $W_f$  are initial and final earthworm mass (g), respectively, and  $\Delta t$  is the growth interval measured in days (Brafield and Llewellyn, 1982). The IGR was calculated for 14- and 28-day growth intervals in the pot study, and for a 28-day interval in the core study. The effects of container shape on earthworm growth were assessed using the IGR calculated for a 28-day growth interval.

#### Statistical analysis

The effect of temperature, moisture, container type, sampling time and the temperature  $\times$  moisture interaction on earthworm growth rates from the pot and core study were evaluated using the PROC MIXED function of SAS software (SAS Institute, 2001). The MIXED procedure uses generalized least squares to estimate and test for fixed effects in the model, which is superior to the ordinary least squares used by the GLM procedure, and is the preferred method for analysis of animal growth experiments with repeated measures data since it can handle missing data in an unbalanced design (Wang and Goonewardene, 2004; Spilke et al., 2005). The difference between least square means of significant treatment effects were evaluated at the 95% confidence level using the LSMEANS statement in SAS. Regression lines were fitted using the PROC REG function of SAS.

## Results

## Mortality

Earthworm mortality in the pot study was generally less than 8%, although in soils at -54 kPa water potential there was up to 26% mortality. In the core study, mortality ranged from 0–28.5%, and was not different in the intact and packed cores.

# Temperature and moisture effects on earthworm growth

In the pot study, soil temperature (F = 26.1, P < 0.0001), moisture (F = 23.8, P < 0.0001) and the interactions between temperature and moisture (F = 4.1, P < 0.0001) were all significant factors affecting growth. Growth rates were significantly affected (F = 4.8, P < 0.003) by the repeated weight measurements on the same individual. This indicates a change in growth rate as the individual earthworm grows. The change in growth rates as an individual changes in weight is a common relationship in many earthworm and animal growth studies (McElroy et al., 1997; Mir et al., 1998; Wang and Goonewardene, 2004). All earthworms lost weight when placed in soil with a water potential of -54 kPa, so the growth data for this treatment were excluded from Fig. 1. Growth was negative (indicating weight loss) at 5°C, regardless of the moisture content, and at 10 °C when the soil water



**Figure 1.** Influences of soil temperature and moisture on the instantaneous growth rate (IGR) of *A. caliginosa* in pots. Values are shown as mean  $\pm$  S.E. (n = 10). Columns with the same letters did not differ significantly (P < 0.05).

potential was -11 and -23 kPa (Fig. 1). The IGR was greatest at -5 and -11 kPa water potential.

#### Effects of container on growth

In the core study, soil moisture (F = 63.0, P < 0.0001) was the most significant factor affecting growth, followed by soil temperature (F = 34.3, P < 0.0001), the interactions between temperature and moisture (F = 10.7, P < 0.0001) and container type (F = 4.9, P < 0.008). A paired means comparison test showed that growth rates in the pot study were greater than in disturbed soil cores (P = 0.017) and undisturbed soil cores (P = 0.006). However, the growth rates obtained from undisturbed and disturbed soil cores were not significantly different.

In soils at 10 °C, earthworm growth rates were positive at water potentials greater than -11 kPa (Fig. 2A). In soils at 15 and 20 °C, positive growth rates were observed at dryer conditions in pots (-23 kPa) than cores (-11 to -15 kPa) (Fig. 2B and C). Logistic growth describes best earthworm growth in pots at all three temperatures, whereas earthworm growth in disturbed and undisturbed cores were described best by linear equations at 10 °C, and both linear and logistic equations at 15 °C and 20 °C (Table 1).

# Discussion

The rates of growth of *A. caliginosa* were influenced by interactions between soil temperature and moisture. Growth rates increased logisti-



Figure 2. Effects of container type, soil temperature and soil water potential on the instantaneous growth rate (IGR) of *A. caliginosa*. Values are shown as mean  $\pm$  S.E.

cally with rising water potential when the soil temperature was 10-20°C, but growth remained negative at 5°C for all water potentials. Growth rates were significantly greater at -5 kPa than at  $-11 \,\text{kPa}$  when the soil temperature was  $10-20 \,^{\circ}\text{C}$ , but were not different between water potentials of -11 and -23 kPa for temperatures between 5 and 15 °C. In other experiments soil temperature and moisture interacted significantly to influence the growth of A. tuberculata (Wever et al., 2001) and L. terrestris (Berry and Jordan, 2001). They found that earthworm growth rates were influenced more by soil moisture at higher temperatures (20 °C or higher) than at lower temperatures. In our study, earthworms lost weight when the soil water potential was lower than -11 kPa at 10°C, and -23 kPa at 15 and 20 °C, suggesting that there may be critical moisture levels for earthworm growth. Holmstrup (2001) reported a significant reduction

$IGR = -0.0042 \ln(\psi) + 0.011$	$R^2 = 0.986$
$IGR = -0.0002\psi + 0.0029$	$R^2 = 0.989$
$IGR = -0.0004\psi$ +0.004	$R^2 = 0.940$
$IGR = -0.0034Ln(\psi)+0.0118$	$R^2 = 0.991$
$IGR = -0.0001\psi$ +0.0024	$R^2 = 0.953$
$IGR = -0.0073Ln(\psi)+0.0195$	$R^2 = 0.958$
$IGR = -0.007Ln(\psi)+0.022$	$R^2 = 0.967$
$IGR = -0.0079Ln(\psi)+0.0218$	$R^2 = 0.997$
$IGR = -0.0007\psi + 0.0123$	$R^2 = 0.985$
	$IGR = -0.0042 \text{ Ln}(\psi) + 0.011$ $IGR = -0.0002\psi + 0.0029$ $IGR = -0.0004\psi + 0.004$ $IGR = -0.0034 \text{ Ln}(\psi) + 0.0118$ $IGR = -0.0001\psi + 0.0024$ $IGR = -0.0073 \text{ Ln}(\psi) + 0.0195$ $IGR = -0.0072 \text{ Ln}(\psi) + 0.022$ $IGR = -0.0079 \text{ Ln}(\psi) + 0.0218$ $IGR = -0.0007\psi + 0.0123$

**Table 1.** Regression equations describing the instantaneous growth rate (IGR) for *A. caliginosa* as a function of soil water potential ( $\psi$ ) for each container type and soil temperature conditions presented in Fig. 2

Lines were fitted through the average IGR values at each water potential.

in weight of adult and juvenile *A. caliginosa* when the water potential was lower than -12 and -19 kPa, respectively. At water potentials lower than -19 kPa, all juveniles entered diapause and lost weight. Similar results were obtained for other species in laboratory studies. *A. trapezoides* avoided soil with a water potential less than -15 kPa in sandy loam and -25 kPa in loam (Doube and Styan, 1996), and *A. longa* lost weight at water potentials lower than -40 kPa (Kretzchmar and Bruchou, 1991).

The earthworm growth rates in this experiment ranged from -0.092 to  $0.037 d^{-1}$ , and were slightly slower than those reported elsewhere (Whalen and Parmelee, 1999; Booth et al., 2000). The growth rates for A. tuberculata (Whalen and Parmelee, 1999) were 2–3 times faster  $(0.0108-0.0167 d^{-1})$ than those in this experiment at 10 °C and water potentials from -5 to -23 kPa. The growth rates for A. tuberculata (Wever et al., 2001) ranged from -0.05 to  $0.05 d^{-1}$  at 20 °C and from -0.007 to  $0.015 d^{-1}$  at 15 °C in soils with moisture contents of 10-25%. These results agree with our values obtained at similar moisture contents (water potentials from -11 to -23 kPa). Booth et al. (2000) measured growth rates for A. caliginosa over the same range of gravimetric moistures (15–30%) and temperatures (5–20  $^\circ\text{C})$  as we did, but with more variability in their experiment. In their experiment, optimal conditions for earthworm growth were at 10-15 °C in soils with 25-30% moisture content, and the IGR ranged from  $0.026-0.063 d^{-1}$ . Earthworms lost weight when the soil moisture was 15%, regardless of temperature (Booth et al., 2000). Mazantseva (1982) reported that the IGR of *Nicodrilus caliginosus* (a variant name for *A. caliginosa*, Reynolds (1977)) was  $0.019-0.028 d^{-1}$  at  $15-20 \degree C$  and optimal soil moisture, while earthworms lost weight at temperatures below  $12 \degree C$ , similar to our findings.

Differences in the IGR of *A. caliginosa* in these studies may be explained by the initial body mass of the earthworm. Earthworm growth rates are related inversely to their initial body masses, where rates of weight gain decrease as the initial body masses of earthworms increase (Daniel et al., 1996; Whalen and Parmelee, 1999). Mazantseva (1982) showed that the IGR was 50% less for 20–30-day-old earthworms than for newly emerged earthworms. The earthworms used in many previous studies were smaller than those used in this study, which may explain why they reported faster growth rates for *A. caliginosa*.

Other factors that may affect growth rates are the quantity of soil, shape of the container and fluctuating temperature regimes. Some researchers kept earthworms in 40g (Whalen and Parmelee, 1999) and 100 g of soil (Wever et al., 2001), which is 10-25 times less than the quantity used in other experiments (Booth et al., 2000). We demonstrated that growth rates of earthworms in pots were greater than those of earthworms in soil cores. It is important to consider the behaviour of earthworms when selecting a container for measuring earthworm growth rates. The soil cores had half the diameter of the pots, which may have forced the earthworms to burrow vertically, contrary to the natural habits of this endogeic species to build temporary, shallow horizontal burrows (Francis et al., 2001; Jégou et al. 2001). Uvarov (1995) showed that earthworms kept in cultures at a constant temperature ( $15 \,^{\circ}$ C) lost more weight than those kept in cultures at a fluctuating temperature regime ( $10-20 \,^{\circ}$ C). However, the effects of different fluctuating temperature regimes on weight loss were not significant until after 4 months in culture (Uvarov, 1995). Since our earthworms were kept for only 8 weeks in controlled climate incubators, we assume that there was no effect of a constant temperature regime on growth rates.

The treatment effects of container type are not entirely due to the shape of the container only. To maintain an undisturbed soil it was not possible to mix the food into the top 5 cm of the soil as in the pot study. Therefore, the pot and cores have different shapes and placement of food. However, since endogeic earthworms typically consume more humified organic matter in the mineral horizons of the soil (Edwards and Bohlen, 1996), the placement of fresh organic matter on the surface would most likely have had little effect on available food resources. The volume of soil in each container was small compared to how much soil an earthworm could burrow through, therefore regardless of where the food was placed it was still easily accessible to the earthworm. Visual observations confirmed that earthworms were active throughout the containers and came into contact with the surface applied food. We assume that the different placement of food in the two container types could be a considered a minor source of error.

Soil disturbance did not affect the growth of A. caliginosa because the IGR did not differ between disturbed and undisturbed soil cores. Since the amounts of soil were similar in both pot and core studies, we suggest that the container shape influenced earthworm growth more than soil disturbance. It appears that the presence of intact earthworm burrows and other macropores in undisturbed soil cores did not increase A. caliginosa growth. Capowiez and Belzunces (2001) reported that earthworm burrow systems are individual structures, rarely used by other earthworms. They suggest that abandoned burrows may be recolonised only by earthworms from the same ecological class. The undisturbed soil cores were obtained above a surface burrow, most likely created by an anecic earthworm, and were probably not used by the endogeic A. caliginosa species introduced into the core.

Our study confirms that temperature and moisture strongly influence earthworm growth rates and activity. Optimum environmental conditions for growth of *A. caliginosa* were 20 °C and a water potential of -5 kPa. Higher temperatures were not tested, but the upper limit for survival of many lumbricid species is around 25 °C, because many life history parameters, such as growth rates, cocoon production, and time to reach sexual maturity, decrease at temperatures above 20 °C (Butt, 1991; Daniel et al., 1996; Berry and Jordan, 2001; Baker and Whitby, 2003). Furthermore, we showed that earthworm growth rates were influenced by the shape of the container used. Further work is needed to establish standard experimental parameters (i.e., food source, growth interval, quantity of soil and shape of container) that ensure laboratory measurements of earthworm growth rates are representative of those in the field.

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