Survival of the burrowing nematode *Radopholus similis* (Cobb) Thorne without food: Why do males survive so long?

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ABSTRACT

The burrowing nematode *Radopholus similis* is the most damaging banana nematode. To minimize nematicide applications, cropping systems based on fallow, crop rotation, and using clean planting material have been developed in the French West Indies. The survivorship of *R. similis* in water and soil was evaluated to optimize the collapse of its populations during the intercropping period and to better understand the risk of dissemination of nematodes by run-off water. In both Andosols and Nitisols, survivorship was significantly higher for males than for females. After 180 days, 21.7% of males and 9.8% of females were still alive, whereas no juveniles survived after 150 days. Survivorship was much lower in water and soil solution than in soil. The mean half-life of males was 8.8 days and only 6.2 days for females, but the difference between sexes was less after 1 month: 8% of both males and females in the initial population survived after 35 days. These results suggest that resource allocation for males was directed towards reproduction, whereas females expend energy foraging and laying eggs, and are thus disadvantaged in the absence of host plants. The relatively long survivorship of males of *R. similis* enables them to fecundate females without competing for food after becoming adults.

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1. Introduction

The burrowing nematode *Radopholus similis* (Cobb) Thorne is the nematode that causes the most damage to banana (Gowen et al., 2005; Quénéhervé, 2009). To minimize nematicide applications, cropping systems based on fallow, crop rotation and the use of clean planting material have been developed in the French West Indies. To optimize the benefit of the intercropping period and to increase the economic sustainability of banana cropping systems, we evaluated the survivorship of *R. similis* in soil.

In the absence of food, temperature, humidity and soil oxygenation are considered to be the main limiting factors of nematode survival (McSorley, 2003). Because of high mean temperatures in the French West Indies, soil temperature is not assumed to be a limiting factor for *R. similis*. Results of previous studies showed that saturated soils were less favourable than drained and even dry natural soils (Sarah et al., 1983; Chabrier et al., 2010). Evaluating the survivorship of *R. similis* survival in soil solution is crucial to determine suitable conditions to minimize nematode longevity. To this end, we evaluated the ability of the nematode to survive in water and in soil solutions that mimicked the environment of the capillaries in which they live.

The length of time banana plants can be cultivated without *R. similis* depends on the presence of alternative host plants (Duyck et al., 2009) and the ability of this species of nematode to disseminate and re-contaminate. In previous studies, we showed that *R. similis* can be spread by run-off water (Chabrier et al., 2009). The efficiency and the length of this dissemination process depend on the nematode's ability to survive in water.

In our previous study on survival in the soil (Chabrier et al., 2010), despite the fact that females were more abundant in the initial population, males were more abundant after 70 days in the soil without food. The present paper presents results of investigations of the survivorship of *R. similis* in two types of soils, at different degrees of humidity, in soil solution and in water, with particular focus on the influence of sex.

2. Materials and methods

2.1. Survivorship in soil

Survivorship of *R. similis* was measured following the method described by Chabrier et al. (2010) for the two main soil types on which bananas are grown in Martinique: Andosol on pumice (sam-

Table 1
Texture of soils used to determine nematode survivorship. These textures were obtained by sieving without dispersion (apparent soil texture).

	Particle size (μm)	Weight of soil fraction (g/100 g)
Andosol (pH: 5.8; organic mater content: 7.9%)		
Sand	50–2000	44
Loam	2–50	47
Clay	0–2	9
Nitisol (pH: 5.5; organic mater content: 2.8%)		
Sand	200–2000	36
	50–200	59
Loam	0–50	5
Clay		

Table 2
Mineral analysis of soil solution extracted by centrifugation. Solutions 1, 2 and 3: supernatant after successive centrifugation at 270 rpm, then 855 and 2700 rpm, which correspond to water present in capillaries with a diameter >300, 30–300, and 3–30 μm.

	Distilled water	Tap water	Solution 1	Solution 2	Solution 3
pH	5.33	6.93	5.74	5.68	5.84
Conductivity mS/cm	8.6	181.1	134.9	133.6	175.4
Potassium g % g	0.00	0.00	0.00	0.00	0.00
Calcium g % g	0.00	30.00	15.58	10.28	8.50
Magnesium g % g	0.00	3.30	4.05	3.25	4.00
Ammonia mg/L	0.00	0.00	0.00	0.00	0.00
Nitrate mg/L	0.00	1.00	10.70	10.45	11.88
Iron mg/L	0.00	0.00	0.02	0.04	0.06
Manganese mg/L	0.00	0.00	0.00	0.00	0.00
Zinc mg/L	0.00	0.00	0.00	0.00	0.00
Sodium mg/L	0.00	7.34	4.06	4.22	6.66
Chlorate mg/L	0	114	114	90	114

pled at an altitude of 460 m in a field of chayote, *Sechium edule*), and Nitisol derived from volcanic ashes, sampled at an altitude of 65 m in a lime orchard. In both cases, the samples were taken in the surface horizon (between 5 and 20 cm). The soil texture of these soils is described in Table 1. *R. similis* was tested at five different water potentials for each soil type: 0, −40, −104, −273 and −440 kPa for Andosol, and 0, −0.1, −5, −165 and −630 kPa for Nitisol corresponding to a gravimetric water content of 100, 60, 50, 40, 35 and 74, 60, 50, 40 and 35 g of water for 100 g of dried soil, respectively.

A total of 500 plugs were removed at a depth of 5–20 cm and placed in large trays. The soil plugs were brought to the laboratory and saturated with distilled water. Five aliquots of soil saturated with water were placed in a drying oven at 105 °C for 2 days, and then weighed to measure soil moisture content at saturation according to the formula:

$$W\% = (\text{wet weight} - \text{dry weight})/\text{dry weight} \times 100$$

To calculate the amount of water required to reach a given moisture content, we established an abacus that allowed moisture content to be linked to water potential. Five pressures corresponding to five water potentials were applied to series of eight water-saturated plugs using a pneumatic pressure chamber (Tessier, 1984); after 1 week, pores of diameters "*r*" corresponding to $r = K/P$ (with $K = 1.47$) were emptied. After immersion of soil samples in petrol, the volumes of emptied pores were measured by petrol thrust and the corresponding weight of water was calculated.

For each type of soil, 30 series of 15 boxes were filled with 40-g soil aliquots; this operation was performed with great care to avoid modifying the structure of the aggregates or soil porosity. The boxes were then left open so that the soil could dry at 27 °C until the weight corresponding to the desired moisture content was reached.

At the same time, a monospecific suspension of *R. similis* was extracted from infected banana roots using a Seinhorst mist chamber (Hooper et al., 2005) for 4 days on banana plants grown in a growth chamber. A volume of 200 mm³ of suspension containing approximately 250 *R. similis* from banana plant roots was subsequently deposited in each box. The boxes were then closed and maintained in the dark at a temperature ranging from 27 to 29 °C (close to the optimum temperature for *R. similis*). We monitored the weight of the boxes to evaluate plug water content and renewed the air space every week. One box from each series was removed to extract its nematodes within the 24 h after the sample was deposited, and then every 7 days from the 8th to the 71st day. However, since 16% of *R. similis* survived after 70 days in the wet Nitisol, further observations were made to determine survival after 92, 120, 148 and 177 days.

To extract nematodes from a box, its contents were suspended in 200 cm³ of water and poured into a sieve column (250, 80, 50 and 32 μm). The residues in the 80, 50 and 32 μm sieves were placed in a Baermann funnel for 48 h according to the technique of Whitehead and Hemming (Hooper, 1986, p. 9). All nematodes extracted were counted under an inverted light microscope.

2.2. Survivorship in water and soil solution

The decrease in population was evaluated in five liquids: distilled water, tap water, and three soil solutions corresponding to three classes of capillaries.

To obtain the soil solution, Nitisol was sampled in the same field and according to the same procedure as that used for the soil survival study. Soil samples weighing 10 kg were collected at a depth of 5–20 cm with a spade, placed in a bucket and humidified with bidistilled water until saturation. After 3 days in a dark chamber at 25 ± 1 °C, soil aliquots were placed in centrifuge tubes. Three successive centrifugations were applied; after each, we obtained a soil solution that corresponded to the water present in one of three capillary classes. To prepare solution at the expected water potential, we calculated the required centrifugation force according to the equation that links centrifugation force and capillarity forces:

$$G = 1.119 \times 10^{-5} \times r \times N^2$$

where "*G*" is the relative gravitational force, "*N*" is the rotation speed (in rpm) and "*r*" is the rotation radius of the rotor (12 cm for this experiment). We then used Jurin's law to calculate the required capillarity force.

$$F = h \times \rho \times g$$

where "*h*" is the ascension of water in a capillary of "*c*" radius, "ρ" is the density of water and "*g*" is gravity acceleration.

After 10 min of centrifugation at 270, 855 and 2700 rpm, three supernatants were obtained corresponding to water potentials >−1 kPa (Solution 1), −10 to −1 kPa (Solution 2), and −100 to −10 kPa (Solution 3), respectively. These three solutions corresponded to the water present in capillaries with a diameter >300, 30–300, and 3–30 μm, respectively. As the diameter of the body of a *R. similis* varies between 12 and 14 μm during the juvenile stage, between 14 and 21 μm for adult males, and between 17 and 27 μm for adult females (Van Weerdt, 1958), the solutions corresponded to soil solutions present in (1) large, (2) average, (3) small capillaries in which *R. similis* live.

The chemical composition was analyzed using routine methods (colorimetry for nitrates and ammonia, spectrophotometry for the other elements). These solutions differed slightly from tap water (Table 2). Solutions 1 and 2 were almost identical, while Solution 3 was richer in sodium, nitrates and iron.

Nematode populations were deposited in 100 cm³ tubes that had previously been filled with 50 cm³ of the five solutions

Table 3

Deviance analysis of the survivorship of *Radopholus similis* at different water potentials in Andosols and Nitisols, using the logistic Generalized Linear Model (GLM) with binomial error. d.f.: number of degrees of freedom; HHS: significant for $P = 0.0001$; NS: non-significant for $P = 0.05$.

Soil type	Sex	Factor	d.f.	Residual deviance	$P > Chi^2$	
Nitisol	Males	Water potential	4	1905	$<10^{-5}$	HHS
		Duration	1	7,384	$<10^{-5}$	HHS
		Water potential × duration	4	476	$<10^{-5}$	HHS
		Residual deviance	440	23,643		
	Females[a]	Water potential	4	1,438	9.9×10^{-5}	HHS
		Duration	1	11,449	$<10^{-5}$	HHS
		Water potential × duration		n.a.		
		Residual deviance	444	12,089		
Andosol	Males	Water potential	4	1,614	$<10^{-5}$	HHS
		Duration	1	4,518	$<10^{-5}$	HHS
		Water potential × duration	4	622	$<10^{-5}$	HHS
		Residual deviance	440	30,790		
	Females	Water potential	4	2414.4	$<10^{-5}$	HHS
		Duration	1	6,658.2	$<10^{-5}$	HHS
		Water potential × duration	4	978.7	$<10^{-5}$	HHS
		Residual deviance	440	14,984.3		

[a] To account for overdispersion of residues, in this case the model was adapted to quasi-binomial dispersion. Therefore, interaction between water potential and duration was not calculated (n.a.: not available).

(Table 2). After 0, 1, 2, 4, 7, 14, 21, 28 and 35 days, the suspensions were homogenised using an aquarium pump; a 5 cm^3 aliquot was sampled and coloured with Meldola blue (method of Ogiga and Estey, 1974). After 24 h, the coloured aliquots were sieved (using a 5-μm sieve) and the residues were suspended in distilled water. All the nematodes present were counted under an inverted light microscope.

2.3. Statistical analyses

Survivorship of *R. similis* in soils was analyzed using a Generalized Linear Model (GLM with binomial error, McCullagh and Nelder, 1989) as a function of sex, soil type, water potential, duration and interactions. Survivorship of *R. similis* in water and soil solution was analyzed in the Nitisol and Andosol using GLM with binomial error as a function of the stage (adult female, adult male or juvenile), the type of solution, duration and interactions. The significance of each term was assessed through the change in deviance between models with and without that term. All statistical analyses were done using R Software (Crawley, 2005).

This logistic model was used to compare the effect of the various factors on nematode survivorship in soils: sex, soil type, duration, water potential and interactions between these factors by analysis of deviance. In the same way, for the study of survivorship in the different solutions, the logistic model was used to compare the effect of the various factors: water solution, duration, sex and stage, and interactions between these factors by analysis of deviance.

However, the logistic model cannot describe the decrease function sufficiently and accurately. Several models of survivorship decrease were thus compared with observed data. The first was the exponential decrease model, for which life expectancy is a constant. In this model, survivorship decreases as follows:

$$S_t = S_0 \times \exp(ct)$$

where S_t is the survivorship at day 't', i.e. the number of living individuals at day t divided by the number of individuals present on the first day of the experiment and "c" is the coefficient of decrease in survivorship, expressed in day^{-1}.

The second was Teissier's model (1933), which adequately describes the survivorship curves of several plant parasitic nematodes, including endoparasitic nematodes in the same family as *R. similis* (Reversat et al., 1997). This model is based on the hypothesis that the effect of aging and starvation increases constantly, so that life expectancy decreases exponentially over time:

$$E_t = E_0 \times \exp(at)$$

where E_0 and E_t are the initial life expectancy and life expectancy at time t, and 'a' is the coefficient of decrease in life expectancy, expressed in day^{-1}. In this case, survivorship 'S_t' values evolve according to the following equation (Reversat et al., 1997):

$$S_t = S_0 \times \exp(at - (1/(a \times E_0) \times (\exp(at) - 1)))$$

S_0 was set at 1 and the values of 'a' were fitted in order to minimize the sum of square of differences between observed and calculated values.

3. Results

3.1. Effect of sex on R. similis survivorship in soils

The GLM logistic model shows a significant effect on the survivorship of nematodes of all tested variables (soil type, duration, sex, and water potential) and interactions between these variables (Table 3), except for interactions between water potential and duration for females in the Nitisol. Indeed, because of overdispersion of the residues, interactions between potential and duration could not be tested. Fig. 1 shows that in all cases, *R. similis* survived longer in dry soils, i.e. with a humidity that maximized survival equal to −440 and −5 kPa for males in the Andosol and Nitisol, and −273 and −630 kPa for females in the Andosol and Nitisol, respectively.

Teissier's model fitted rather well for males in the Nitisol (Table 4). In the Andosol, the same model fitted water potentials of 0, −40 and −440 kPa. For water potentials of −104 and −273 kPa, the model did not fit well, especially at the beginning of the experiment (Fig. 2.1). For females, Fig. 2.2 and 2.3 illustrates the best and worst fit of Teissier's model. It fitted well for the Andosol (R^2 varying between 0.37 and 0.90) and very well for the Nitisol (R^2 varying between 0.83 and 0.99). We observed a rapid decrease in the population of *R. similis* with the 'a' parameter of Teissier's model maximal for females in saturated Nitisol with a value of −0.073. In all cases, saturated soil led to the fastest decline in population. Even though Teissier's model was less accurate for the Andosol than for the Nitisol, especially for *R. similis* males, Table 4 shows that the decline in *R. similis* was faster in the Andosol than in the Nitisol, i.e. mean value of 'a' for all soil humidity −0.009 and −0.004 for males and −0.015 and −0.037 for females, for the Andosol and Nitisol, respectively. These results also show that the males survived sig-
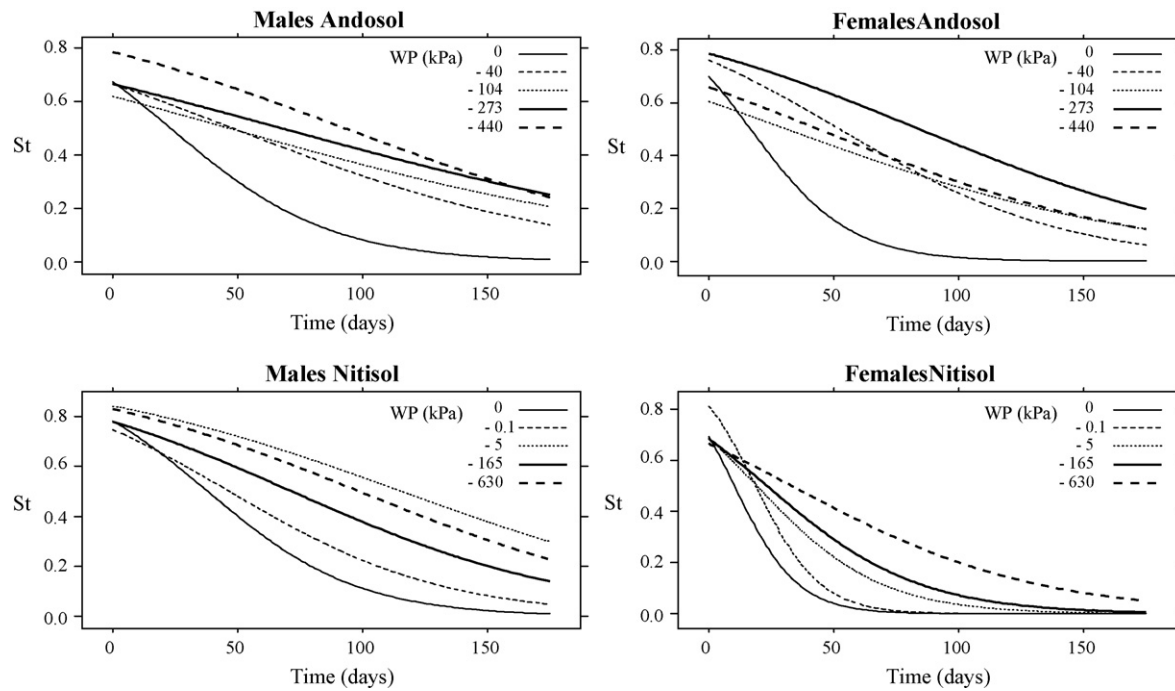
Fig. 1. Decrease in *Radopholus similis* populations in soils. Fitted curves were estimated by analysis of deviance using the logistic Generalized Linear Model (GLM) with binomial error. S_t: proportion of survivors (survivorship). WP: water potential in kPa. Statistical tests: for all factors (water potential, duration and interaction water potential × duration) highly significant differences ($P < 10^{-5}$) were found except for the interaction between water potential and duration in females in the Nitisol ($P = 0.1$).

nificantly longer than females in both the Andosol and the Nitisol. At the end of the experiment, 21.7% of males were still alive vs. 9.8% of females.

The binomial dispersion hypothesis was not validated for juveniles in soils. Juvenile populations were higher from the 7th to the 28th day of the experiment than on the first day. In this case, S_t is greater than 1 and this variable cannot follow a binomial law. In the Nitisol, the juvenile populations observed were almost zero

after 90 days; only two young individuals were observed in the 90 corresponding boxes. In the Andosol, residual populations (13 individuals found among 30 boxes after 150 days and 11 at day 180) were observed at the end of the study. These populations consisted of several small individuals, J2 juveniles or young J3 that could only have been laid by the gravid females that remained during the days before extraction. Therefore, for juveniles, the S_t curve did not correspond to a survival curve but to the evolution of a population that
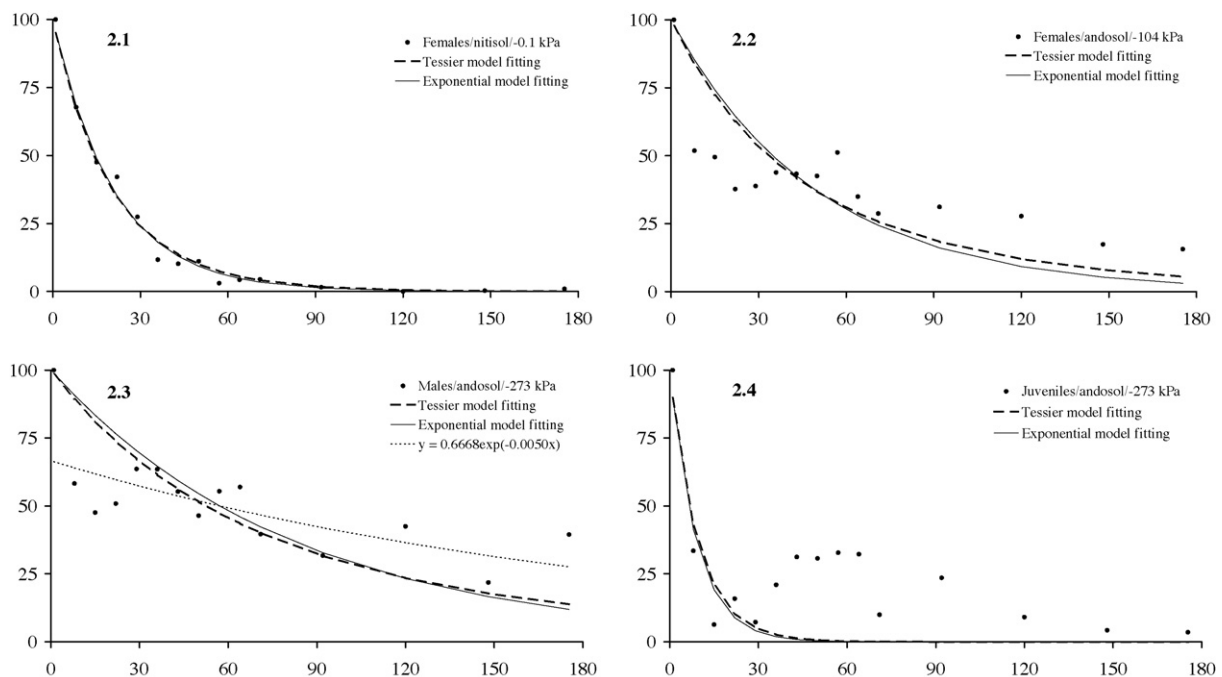

Fig. 2. Fitting of two models, Teissier's model and the exponential decrease model, to the observed survivorship of *Radopholus similis*. (2.1) (top left): females in the Nitisol at −0.1 kPa; (2.2) (top right): females in the Andosol at −104 kPa; (2.3) (bottom left): males in the Andosol at −273 kPa; (2.4) (bottom right): juveniles in the Andosol at −273 kPa. Black dots: observed average points (15 dates); dashed line: Teissier's model; solid line: exponential decrease.

Table 4
Fitting of Teissier's model to the survivorship of *Radopholus similis* measured at different water potentials in the two soil types.

Water potential (kPa)	Parameters	Males	Females
Andosol			
	R^2	0.679	0.905
0	a	−0.025	−0.043
	R^2	0.442	0.840
−40	a	−0.008	−0.008
	R^2	0.019	0.367
−104	a	−0.008	−0.013
	R^2	0.108	0.735
−273	a	−0.004	−0.001
	R^2	0.673	0.570
−440	a	−0.0001	−0.010
Nitisol			
	R^2	0.841	0.891
0	a	−0.014	−0.073
	R^2	0.778	0.986
−0.1	a	−0.009	−0.042
	R^2	0.358	0.868
−5	a	0.004	−0.033
	R^2	0.645	0.835
−165	a	−0.003	−0.024
	R^2	0.706	0.854
−630	a	−0.001	−0.015

Fig. 3. Decrease in *Radopholus similis* populations in water and soil solutions. Fitted curves were estimated by analysis of deviance using the logistic Generalized Linear Model (GLM) with binomial error. S_t: proportion of survivors (survivorship). Statistical tests: highly significant differences ($P < 10^{-5}$) were found for all factors (water solutions, duration, sex and stage) and interactions between these factors.

tends towards 0 because: (i) of the mortality of juveniles, and (ii) of the decrease in egg-laying by surviving gravid females capable of generating new young individuals in the environment. For the same reason, neither an exponential decrease nor Teissier's model fitted the evolution of the juveniles' S_t curve (Fig. 2.4).

The test of the effect of the sex on the survival of *R. similis* in undisturbed soils revealed three major trends: (i) males of *R. similis* survived significantly longer than females in both the Andosol and the Nitisol; (ii) Andosol was less favourable to the survivorship of *R. similis* than undisturbed Nitisol regardless of the sex and (iii) *R. similis* of both sexes survived longer in dry soils.

3.2. Effect of sex of R. similis survivorship in water and soil solutions

In contrast to what was observed in the soil, the binomial dispersion hypothesis was validated not only for adult males and females, but also for juveniles in water and in the soil solutions. The GLM logistic model showed a significant effect of all tested variables (water or soil solution, duration and sex) and all their interactions on the survivorship of nematodes (Table 5). Teissier's model provided a correct description of the curve shape, with R^2 ranking from 0.36 to 0.70 for juveniles, from 0.44 to 0.67 for males and from 0.42 to 0.72 for females (Table 6). We observed a very fast decrease in the *R. similis* population, faster than the decrease observed in soil, the maximum 'a' parameter of Teissier's model for males and juveniles was observed in Solution 3 with a value of −0.293 and −0.377, respectively; for females, the maximal 'a' parameter was observed in Solution 1 with a value of −0.198. Fig. 3 and Table 6 show that for males and juveniles, the survival of *R. similis* was shorter in Solution 3, which corresponded to the soil solution in 3–30 μm pores. In contrast, females of *R. similis* survived longer in this solution. The population of females decreased faster at the beginning of the experiment: the half-life of females varied from 4 to 10 days, with a mean value of 6.2 days, while the half-life of males varied from 3 to 12 days, with a mean value of 8.8 days. But the point at which only 10% of the initial population remained alive was reached after mean values of 27.0 days for males and 27.2 for females; after 35 days 8% of the initial population of both sexes were recovered alive. These results show that the difference in survivorship between males and female was much smaller in water
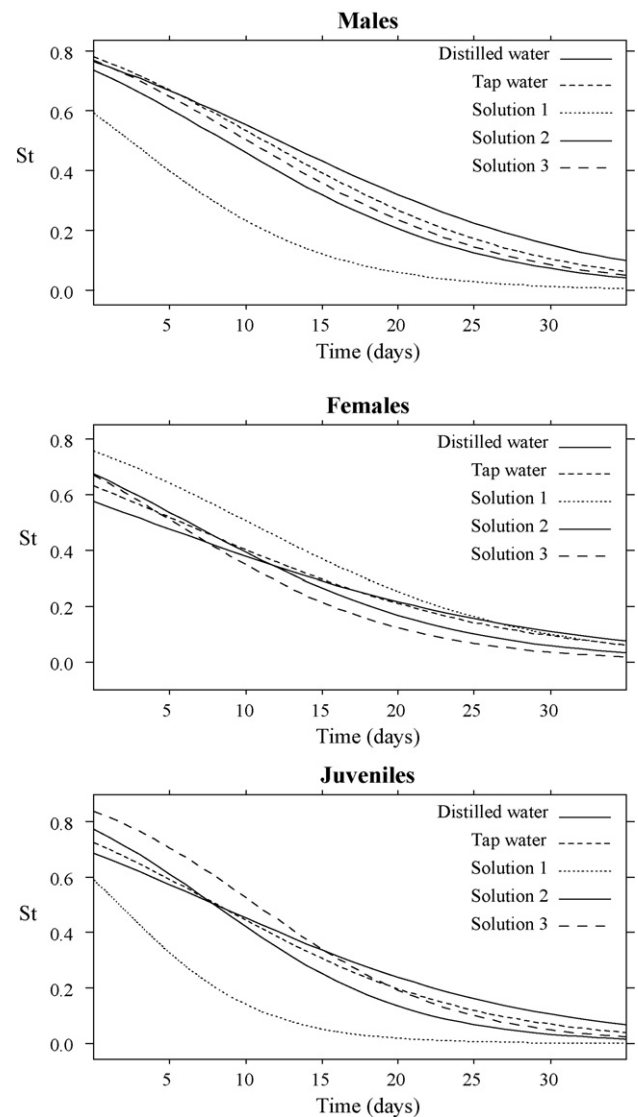
solutions than in soils, and that the life expectancy of males was not much longer than that of females.

4. Discussion

Teissier's model satisfactorily described trends of adult female populations in soil. The exponential model, according to which life expectancy is constant over time so that $S_t = S_0 \times \exp(at)$, fitted less well to measured results for females. For males, few differences appeared between Teissier's and the exponential model. These studies did not enable us to separate the effects of mortality linked to starvation and to aging. Teissier's model accounts for both the depletion of reserves and aging (Reversat et al., 1997), whereas the exponential model only accounts for depletion of reserves. Two other factors could disrupt these models and make them inapplicable: the ability to remain in a resting state (such as diapause or quiescence) with suspended motility, and the birth of new individuals.

Table 5
Deviance analysis of survivorship of *Radopholus similis* in different water and soil solutions using the logistic Generalized Linear Model (GLM) with binomial error. d.f.: number of degrees of freedom; HHS: significant for $P = 0.0001$; NS: non-significant for $P = 0.05$.

	Name	d.f.	Residual deviance	$P > X^2$	
	Solution	4	194.6	$<10^{-5}$	HHS
Factors	Duration	1	12013.5	$<10^{-5}$	HHS
	Sex/stage	2	175.0	$<10^{-5}$	HHS
	Solution × duration	4	99.2	$<10^{-5}$	HHS
Interactions between	Solution × sex/stage	8	552.0	$<10^{-5}$	HHS
factors	Duration × sex/stage	2	77.4	$<10^{-5}$	HHS
	Solution × duration × sex/stage	8	127.4	$<10^{-5}$	HHS
Residual deviance		402	16276.0		

Male survival curves in soil were described satisfactorily by both Teissier's model and the exponential model. Adult *R. similis* males do not eat because their stylet and oesophagus are degenerated or even absent (Van Weerdt, 1958; Siddiqi, 2000); the effects of aging and starvation on their mortality are nearly always confused. At the beginning of the study, the male populations were very heterogeneous, consisting of individuals with different levels of reserves. Consequently mortality linked to starvation was more variable in males than in females, as all females had reserves at the beginning of the study. Overall, the models described population trends in the Andosol less satisfactorily than in the Nitisol. However, the percentages of males in the population used for repetitions 4–6 were low (the last suspension contained only 20% males), hence, the precision of the measurements of males in the Andosol was too low to draw any conclusions beyond those of the logistic model adapted to binomial dispersions.

In soil, the trends in the juvenile population corresponded poorly to the predictions of Teissier's model. This may be due to the fact that the length of juvenile stages in *R. similis* represents only approximately twice the time step that separates observations within a same series. We did not actually observe a survival curve but, instead, the evolution of successive cohorts of individuals which are born and die, or become adults. However, the fact that few older juveniles were observed after the fourth week of the study indicates that very few juveniles had reached adulthood after 1 month. It seems likely that in the absence of food resources, juveniles cannot pursue their normal development. The appearance of new adult cohorts was therefore marginal and could not or only very slightly (except perhaps at the very beginning of the experiment) have influenced the survival curves. For adults, these curves were not disturbed by the arrival of new individuals, and decay models therefore fairly accurately described adult population trends.

Conversely, in water solutions, Teissier's model relatively accurately described (R^2 values ranking from 0.358 to 0.701) the decrease in the juvenile population. This is probably due to the shorter lifespan observed in water solutions: $S_t = 0.1$ values (the length of time after which 10% of the population was still alive) were 21–31 days for females in water and soil solutions, and 54–96

days in the Nitisol and more than 175 days in the Andosol. The average lifespan of the females was too short for hatching to have substantially influenced the population of juveniles in the water solution.

Life expectancies of *R. similis* in water and soil solution were indeed far lower than those measured in soil. In soil, these results are consistent with those of Birchfield (1957) and Tarjan (1961); in water, population decreases measured in the present study were slightly lower than those measured by Loos (1961) who found 11% of the initial populations of females and 10% of males alive after 35 days, compared with 7.7% of females and 7.3% of males in the present study. However, Loos' (1961) experiment was performed with a population of only 240 nematodes, while 2450 *R. similis* were counted on the first day of our experiment. Loos (1961) pursued his observations until the death of the last nematode, which was impossible in our case, as staining with Meldola blue may influence the life span of nematodes. Loos (1961) measured a maximum life span of nine weeks for females (but only six weeks for gravid females) and seven weeks for males. Our results revealed the same trends as those observed by Loos (1961).

The model revealed a highly significant effect of sex on *R. similis* survival in soil. The better survival capacity of male populations than of female populations is surprising. There is no mention of these phenomena in the literature. However, in publications dealing with the survival of *Pratylenchidae*, the sex of individuals is not taken into account. According to Demeure et al. (1978), Reversat (1980) and Hass et al. (2002), the survival of an active nematode depends on its lipid reserves. In the case of *R. similis*, these reserves are much more abundant in females than in males. We therefore assumed from the beginning that only females would survive for longer. Two hypotheses could explain our results:

(i) Young juveniles born female developed into males during the study. In certain nematode species, particularly in *Meloidogyne* spp., males are observed particularly when environmental conditions are unfavourable (Siddiqi, 2000). Nevertheless, according to histological observations by Van Weerdt (1960), sexual differentiation appears very early in *R. similis*. Moreover, juveniles rapidly disappeared in the first weeks of the study as they cannot develop correctly without food. Thus, even if we did in fact observe the reappearance of juveniles on the 63rd and 70th day in the Nitisol (juveniles probably hatched from eggs that were laid earlier), we only observed two individuals at the 90th day (among 60 soil samples) and none thereafter.

(ii) Females used part of their reserves to look for food, develop and lay eggs (thus explaining the reappearance of young juveniles around the 70th day and sporadically in the third and fourth months). In contrast, males, which do not feed, would not have used their reserves either to look for food or to lay eggs. Their metabolic activity was therefore lower. The latter hypothesis appears to be the most plausible.

Table 6
Fitting of Teissier's model to the survivorship of *Radopholus similis* in water and soil solution extracted from the Nitisol by centrifugation.

Water potential (kPa)	Parameters	Males	Females	Juveniles
Distilled water	R^2	0.435	0.694	0.643
	a	−0.065	−0.108	−0.106
Tap water	R^2	0.541	0.636	0.612
	a	−0.084	−0.174	−0.081
Solution 1	R^2	0.670	0.524	0.358
	a	−0.104	−0.198	−0.106
Solution 2	R^2	0.532	0.415	0.701
	a	−0.083	−0.158	−0.114
Solution 3	R^2	0.498	0.723	0.699
	a	−0.293	−0.077	−0.377

In contrast to what we observed in soils, in water solutions, males and females of *R. similis* had approximately the same life expectancy. Indeed, in water solutions, nematodes were covered with a column of water that prevented them from moving efficiently (Wallace, 1958). In test tubes, non-gravid females consequently did not expend energy foraging for food. Furthermore, Loos (1961) observed that non-gravid females survived longer than gravid females; this may be explained by the energy expended by a gravid female for egg-laying.

At the scale of the *R. similis* species, the benefit of the fact that males do not forage or feed is unclear. Moreover, males of *Pratylenchus coffeae*, a nematode species belonging to the same family whose females and juveniles have similar life history traits to those of *R. similis*, including a functional digestive system identical to that of females (Siddiqi, 2000). *R. similis* is considered as a nematode which reproduces via amphigony, with separate parents (Rivas and Roman, 1985; Kaplan and Opperman, 2000). *R. similis* has been described as a possible parthenogenic species, but in fact, females can develop into syngonic hermaphrodites, leading to alternative auto-fecundation, which requires supplementary energy expenditure (Brooks and Perry, 1962; Kaplan and Opperman, 2000). The better survivorship of males may allow them to impregnate enough females and counterbalance their absence of a functional digestive system. In addition, their longer survivorship might also facilitate their dispersal by run-off water (Chabrier et al., 2009).

5. Conclusion

Results indicate that males of *R. similis* survive longer than females in soils because they may expend less energy as they do not need to forage for food and/or to lay eggs. In tubes filled with water or soil solutions, non-gravid females are likely to expend almost the same amount of energy as males and, in this case, their survival ability was equivalent to that of males. On the whole, males can efficiently impregnate females although they do not have a functional digestive system, and thus do not compete with females for food resources after becoming adults.

Furthermore, even though *R. similis* survivorship is far lower in water than in soils, they can nevertheless survive for several weeks in water. It is thus possible that this species can be spread by run-off water not only at the field scale (Chabrier and Quénéhervé, 2008) but also over large distances. Future studies should account for the different survivorship between males and females, especially when modelling population dynamics, for example with the SIMBA-NEM model (Tixier et al., 2006).

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