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Factors influencing the survivorship of the burrowing nematode, *Radopholus similis* (Cobb.) Thorne in two types of soil from banana plantations in Martinique

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ABSTRACT

The burrowing nematode, *Radopholus similis* (Cobb.) Thorne, causes the most damage to bananas. To minimize nematicide applications, cropping systems that use fallow, crop rotation and clean planting material have been developed in the French West Indies. In order to optimize the benefit of the intercropping period, we studied the survivorship of *R. similis* in different soil types and conditions. We monitored the survivorship of calibrated populations of *R. similis* in the laboratory on a Nitisol and on an Andosol, two soils derived from volcanic ashes and pumices. We studied water potentials ranging from 0 to −700 kPa on undisturbed soil and on soil previously frozen to get rid of living nematodes. Mortality of adult *R. similis* decreased regularly, and was fairly well described by Teissier's model. In the previously frozen soils, *R. similis* survived longer in wet soils (half-life of 21–46 days at 0 to −5 kPa) than in dry soils (half-life of less than 10 days between −80 and −250 kPa). In contrast, in undisturbed soils, *R. similis* survived longer in dry soils: half-lives ranged from 57 days at −273 kPa to 17 days at water saturation in the Andosol, and 36 days at −660 kPa to 14 days at water saturation in the Nitisol. These results are consistent with the absence of anhydrobiosis in *R. similis*, unlike *Pratylenchus coffeae*. *P. coffeae* survivorship curves over time do not follow a model derived from exponential decrease like Teissier's model. These results also show that the recommended one year host-free period required to sanitize soils cannot be shortened without risk, even if flooding the soil could improve it.

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1. Introduction

The burrowing nematode *Radopholus similis* (Cobb) Thorne is a major pest of banana worldwide (Gowen et al., 2005; Quénéhervé, 2008). In large commercial banana plantations, nematode control is still based on two to four nematicide treatments per year. An alternative cropping system has been developed in Martinique and Guadeloupe (French West Indies) over the last 15 years. It is based on the cleanup of lands contaminated by plant-parasitic nematodes prior to planting. The land is cleared of nematodes either by a fallow period (Chabrier and Quénéhervé, 2003) or by an appropriate crop rotation, as *R. similis* populations may be sustained by other species including some weed species (Duyck et al., 2009). Fields are then planted with nematode-free *in vitro* banana plants. As a result, growers are able to cultivate bananas for two–three years without nematicide treatments in banana fields that are free of the burrowing nematode (Chabrier et al., 2005). However, fallow is costly for growers since during this period they derive no income from the land and because the majority of non-host rotation crops (sugarcane or dasheen, for example) are less profitable than banana. It is thus difficult for some growers to adopt the new system, especially small-scale farmers who need to optimize the cleaning periods. Knowledge of nematode survivorship should help them reduce the length or change the conditions of fallow.

The survivorship capacities of *R. similis* in soil have been studied by DuCharme (1955), Birchfield (1957), Feldmesser et al. (1960) and, above all, Tarjan (1961). These authors concluded that *R. similis* can survive without food for from three to six months. As a result, *R. similis* is generally considered as a species with poor survival abilities. However, these results were obtained from observations made on the "citrus race" of *R. similis* (Kaplan et al., 1996). "Citrus race" differs from banana strains and is found in soil and climate conditions that are difficult to compare with those of banana production areas in Central America and the Caribbean.

The "banana race" was studied by Loos (1961) in Panama and Jamaica under a humid climate, but this author limited his observations to survival time in water and flooded soils. He

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concluded that *R. similis* can survive for up to five weeks in water. Sarah et al. (1983) observed a 90% reduction in the *R. similis* population in banana roots after five weeks of submersion. These results suggest that *R. similis* is sensitive to anoxia.

The absence of food resources, temperature, humidity and soil oxygenation is considered to be the main limiting factor of nematode survivorship (McSorley, 2003). In Martinique, under a humid tropical climate, there is little variation in soil temperature, which always ranges between 20 and 30 °C; the lowest temperature is thus not limiting for the survivorship of *R. similis* (Fallas and Sarah, 1994), and the highest temperature is far lower than the maximum temperature that *R. similis* can withstand (Fallas and Sarah, 1995; Arcinas et al., 2005). We therefore studied the effect of soil humidity on *R. similis* survivorship in the absence of a host.

The absence of a resting stage in *R. similis* could explain the low survivorship of this nematode (Gowen et al., 2005). We consequently compared the survivorship of *R. similis* with that of *Pratylenchus coffeae* (Zimm.). Like the majority of *Pratylenchus, P. coffeae* can enter a state of anhydrobiosis (Glazer and Orion, 1983; Townshend, 1984). *P. coffeae* is the same size (length and diameter of the same order of magnitude) and has a similar lifestyle to that of *R. similis.* We assumed that by comparing populations of the two nematodes, we would be able to check the hypothesis of the absence of anhydrobiosis. Anhydrobiosis is a state of dormancy induced by desiccation. Anhydrobiosis facilitates survival in some tardigrades, rotifers and nematode species, and is accompanied by cessation of movement and feeding (Evans and Perry, 1976).

In addition, the biotic environment may modify nematode survivorship. Several microorganisms can affect nematode populations (Kerry, 2000). Several fungal species, such as *Paecilomyces lilacinus* (Thom.) Samson or *Fusarium oxysporum* Schltdl. (Khan et al., 2006; Athman et al., 2007) are antagonists of *R. similis*. We consequently compared the survivorship of *R. similis* on undisturbed soils and on soils in which microorganisms had been at least partially destroyed. As sterilization by heat or steam can modify the chemical composition of soils and release compounds that may modify *R. similis* survivorship, freezing was used to get rid of living nematodes that may have been previously present in soil.

The objective of the present study was to model the survival of *R. similis* at different moisture levels in soils. The absence of anhydrobiosis of *R. similis* was tested by comparing the survivorship of this species with that of *P. coffeae* in frozen soil. This study aims to help banana growers optimize their cropping system by improving the intercrop period, and by evaluating if it can be reduced in length.

2. Materials and methods

2.1. Influence of soil moisture and soil type on survivorship of *R. similis* and *P. coffeae* in sieved and frozen soils

Survivorship of *R. similis* and *P. coffeae* was assessed in the two main types of soil in which bananas are grown, an Andosol on pumice (sampled at an altitude of 460 m in a field of chayote, *Sechium edule* Sw.), and a Nitisol derived from volcanic ashes, sampled at an altitude of 65 m in a *Radopholus* free lime *Citrus latifolia* Tan. orchard. In both cases, the samples were taken in the surface horizon at a depth of between 5 and 20 cm. The texture of these soils is described in Table 1. *R. similis* and *P. coffeae* survivorship was tested at three different water potentials for each soil type: 0, −4, and −80 kPa for the Andosol and 0, −5, and −250 kPa for the Nitisol, corresponding to a gravimetric water content W of 152, 71, 53 and 72, 50 and 39 g of water/100 g of soil desiccated at 105 °C, respectively.

The soils were first air-dried for one week and then sieved through a 2-mm sieve to remove gravel, stones and plant debris.

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

	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 Clay	0–50	5

The soils were saturated with water and frozen for 24 h at −15 °C three times to kill nematodes that may have been present. After the third freezing, the soil was saturated with distilled water.

For each type of soil, five saturated soil aliquots were weighed and placed in a drying oven for one week at 105 °C. They were then weighed to determine water content at saturation. This value was used to calculate the weight required to reach given water content. Forty aliquots were used to establish an "abacus" relating moisture content to water potential. The latter values were obtained by ultrafiltration in a pneumatic pressure chamber (Teissier, 1984).

For each soil, 180 50-cm^3 polystyrene boxes were filled with 40-g aliquots. The boxes were left open in a temperature-controlled room at 28 ± 1 °C so that the soil could dry. Boxes were weighed daily until the soil weight corresponded to the desired moisture content.

Two suspensions of nematodes were extracted using a Seinhorst's mist chamber (Hooper et al., 2005) for four days; one of *R. similis* from banana plants and the other of *P. coffeae* from sorghum roots. These plants were previously grown in growth chambers and the nematode suspensions were monospecific; 200 mm^3 of suspension containing approximately 500 nematodes was subsequently deposited in each box. The boxes were then closed. They were opened for several minutes each week to: (i) renew the air in the space above the soil sample (from 2 to 3 cm in height, i.e. between 30 and 40% of the height of the box), and (ii) monitor the humidity of the soil samples by weighing them, and by adding distilled water to compensate for possible water loss through evaporation.

The boxes were kept in the dark at a temperature of 28 ± 1 °C (close to the optimum temperature for *R. similis*). Each week, one box from each series was used to extract nematodes. Nematodes were extracted the day the nematode suspension was deposited, then from the seventh to the 70th day.

To extract the nematodes from a box, its contents were suspended in 200 cm^3 of water and then 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).

Apart from *R. similis*, very few other nematodes (some *Rotylenchulus reniformis* Linford and Oliveira and bacteriophagous nematodes) were observed in the suspensions extracted from the sieved and frozen soils.

2.2. Influence of moisture and soil type on survivorship of *R. similis* in undisturbed soils

One year later, *R. similis* survivorship was tested at five different water potentials for each soil type: 0, −40, −104, −273 and −440 kPa for the Andosol and 0, −0.1, −5, −165 and −630 kPa for the Nitisol corresponding to W = 100, 60, 50, 40, 35 and 74, 60, 50, 40 and 35% (w/w), respectively.

Cores were removed at a depth between 5 and 20 cm and placed in large trays. The trays were taken to the laboratory and the cores saturated with distilled water. Like in the preceding experiment, five soil aliquots were placed in a drying oven at 105 °C for two days to assess their moisture content at saturation and to calculate the weight required to reach given moisture content.

For both types of soil, 30 series of 15 boxes were filled with 40-g soil aliquots; this operation was performed carefully to avoid modifying the aggregates. Like in the preceding experiment, the boxes then remained open so that the soil could dry at 27 °C until the soil weight corresponded to the desired moisture content.

We then proceeded in the same way as in the first experiment: 200 mm^3 of suspension containing approximately 250 *R. similis* from banana plant roots was placed in each box. The boxes were then closed and kept in the dark at a temperature of 28 ± 1 °C. We monitored their weight and renewed the air space every week. We removed one box from each series to extract its nematodes within 24 h after the nematodes were deposited, and then every seven days from the eighth to the 71st day. However, since we had observed that 16% of *R. similis* survived after 70 days in wet Nitisol at the end of the preceding experiment, additional observation dates were added to determine survivorship after 92, 120, 148 and 177 days.

As previously described, the nematodes were extracted using a sieve column of 250, 80, 50 and 32 μm. After 48 h of clarification in Baermann funnels, all the nematodes remaining in the clarified suspension were counted.

Aside from *R. similis*, bacteriophagous and phytophagous nematodes were found in the undisturbed soils: many *R. reniformis*, several *Hoplolaimus* and *Helicotylenchus*, and a few *Meloidogyne* and *Criconema*; non-herbivore nematodes were also present, above all bacteriophagous nematodes.

2.3. Statistical analyses

Survivorship of *R. similis* and *P. coffeae* in sieved and frozen soils was analyzed using a logistic Generalized Linear Model (GLM) with binomial error (McCullagh and Nelder, 1989) as a function of species, soil type, duration, water potential and interactions. Survivorship of *R. similis* in undisturbed soils was analyzed in the Nitisol and Andosol using GLM with binomial error as a function of water potential, 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).

Using analysis of deviance, the logistic model can compare the effect of the different factors: species, soil type, duration, water potential and interactions between the factors (species × soil type, species × duration, species × water potential, soil type × duration, soil type × water potential, duration × water potential, species × soil type × duration, species × soil type × water potential, species × duration × water potential, soil type × water potential × duration, and species × soil type × duration × water potential).

However, the logistic model cannot describe the decrease function precisely. Several models of survivorship decrease were thus compared with the observed data. Several classical models were tested, two of which are discussed here. The first is the exponential decrease model, for which life expectancy is a constant. In this model, the decrease in survivorship is thus expressed as follows:

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

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

Such models do not take into account the effects of aging or starvation which are likely to increase over time. This effect may cumulate and life expectancy may thus decrease steeply. Teissier's model (1933) adequately describes the survivorship curves of several plant-parasitic nematodes, including *Hirschmaniella spinicaudata* (Schuur. Stekhoven), a migratory endoparasitic nematode which belongs to the same family as *R. similis* (Reversat et al., 1997). The model is based on the hypothesis that the effects of ageing and starving increase constantly, and life expectancy thus decreases exponentially over time:

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

where E_0 is initial life expectancy, E_t is 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):

$$S_t = S_0 \times \exp\left(at - \frac{1}{a \times E_0} \times (\exp(at) - 1)\right)$$

S_0 was set to 1, and the values of '*a*' fitted to minimize the sum of square of differences between observed and calculated values.

Table 2
Statistical test of deviance analysis of study of survivorship of *Radopholus similis* and *Pratylenchus coffeae* in sieved and frozen soils, using the logistic Generalized Linear Model (GLM) with binomial error.

	Name	d.f.	Residual deviance	$P > X^2$	
Factors	Species	1	24.8	$<10^{-5}$	HHS
	Soil type	1	16.3	$5.4 \cdot 10^{-5}$	HHS
	Duration	1	5428.4	$<10^{-5}$	HHS
	Water potential	2	4682.4	$<10^{-5}$	HHS
Interactions between factors	Species × soil type	1	0.1	0.8	NS
	Species × duration	1	431.6	$<10^{-5}$	HHS
	Soil type × duration	1	3.6	0.1	NS
	Species × water potential	2	289.8	$<10^{-5}$	HHS
	Soil type × water potential	2	209.7	$<10^{-5}$	HHS
	Duration × water potential	2	1735.7	$<10^{-5}$	HHS
	Species × soil type × duration	1	186.0	$<10^{-5}$	HHS
	Species × soil type × water potential	2	239.4	$<10^{-5}$	HHS
	Species × duration × water potential	2	560.3	$<10^{-5}$	HHS
	Soil type × duration × water potential	2	218.7	$<10^{-5}$	HHS
	Species × soil type × duration × water potential	2	19.7	$5.2 \cdot 10^{-5}$	HHS
Residual deviance		372	9723.3		

d.f.: number of degrees of freedom; HHS: significant for $P = 0.0001$; NS: non-significant for $P = 0.05$.

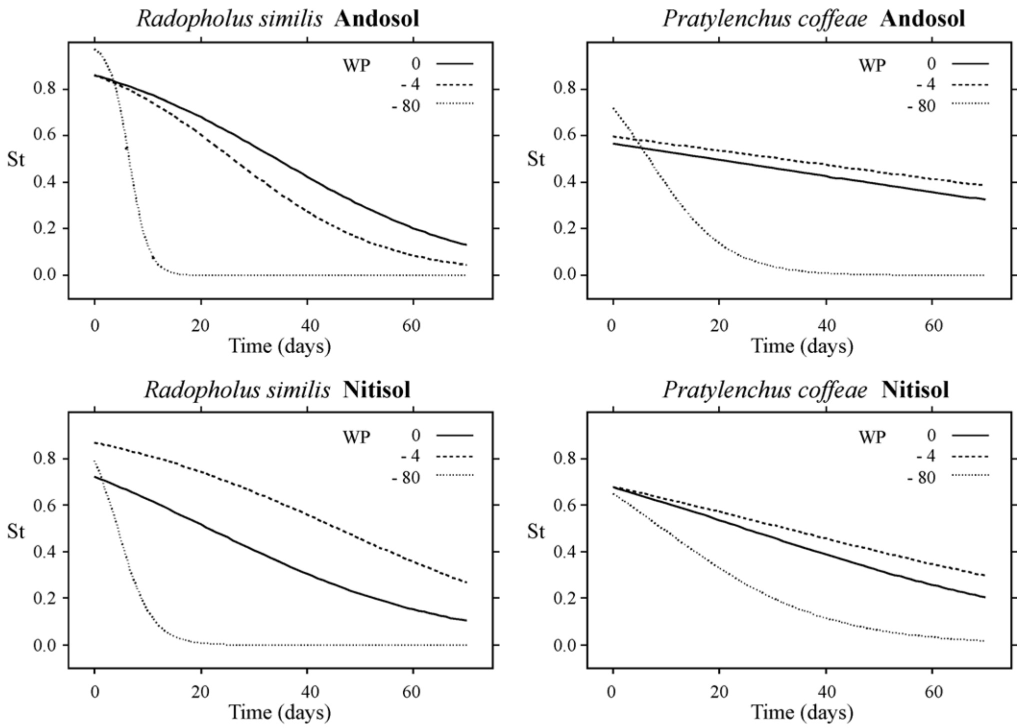

Fig. 1. Survivorship of *Radopholus similis* and *Pratylenchus coffeae* in sieved and frozen soils. Fitted curves were estimated by analysis of deviance, using the logistic Generalized Linear Model (GLM) with binomial error. S_t: proportion of survivors (survivorship). WP: water potential in kPa.
Statistical tests: for all factors (species, soil type, duration, water potential) and interactions between factors: highly significant differences ($P < 0.0001$) except species × soil ($P = 0.8$) and soil type × duration ($P = 0.1$).

Finally, to compare the evolution of survivorship of nematodes in the frozen and in the undisturbed soils, the P_t indicator was used; it corresponds to:

$$P_t = 1 - \frac{S_{1t}}{S_{2t}}$$

where S_{1t} is the average survivorship at t days in the sieved and frozen soil, and S_{2t} the average survivorship in the undisturbed soil. These rates were calculated for a given soil and a comparable potential. The coefficient of decrease in life expectancy '*a*' fitted for all the treatments represents the speed of decline of the nematode population. The higher the value of '*a*', the faster the decline (doubling '*a*' divides the half-life by two).

3. Results

3.1. Effect of soil humidity on nematode survivorship in sieved and frozen soils

The GLM logistic model revealed a significant effect of all the variables we tested (species, soil type, duration, and water potential) and of most of their interactions on the survivorship of nematodes (Table 2 and Fig. 1).

In *R. similis*, we observed a rapid decrease in populations in the drier treatment in both the Andosol and Nitisol. Table 3 shows the coefficient of decrease in life expectancy '*a*' fitted for all the treatments. In the Andosol, the '*a*' parameter varied from −0.015 per day in saturated soil to −0.153 per day in dry soil, and was almost twice as high in the Nitisol (−0.028 and −0.320 per day respectively). The '*a*' parameter was ten times higher in the saturated than in the dry soil, and was similar in the Andosol and the Nitisol. The half-life of *R. similis* in these treatments was six and four days respectively. In the two treatments in wet soils (−4 and 0 kPa in the Andosol; −5 and 0 kPa in the Nitisol), the decrease in the population of *R. similis* was slower, with a half-life of 34 and 26 days in the Andosol and of 21 and 46 days in the Nitisol. *R. similis* survived longest at −5 kPa in the Nitisol and at 0 kPa in the Andosol (Fig. 1). After 70 days of treatment in the wet and saturated soil, we recovered between 31% and 5% of the initial nematode population, whereas recovery was nearly nil in the dry soil.

In general, Teissier's model fitted rather well for *R. similis* (Fig. 2), whereas it described the survivorship of *P. coffeae* much less accurately (Table 3 and Fig. 2). We observed a rapid decrease in the population of *P. coffeae* in the drier treatment in both the Andosol and the Nitisol. In the Andosol, the range of values of the '*a*' coefficient of decrease in life expectancy fitted for *P. coffeae* was similar to that of *R. similis* (from −0.015 per day in wet soil to −0.156 per day in dry soil, Table 3). Conversely, there was no difference in '*a*' between the wet

Table 3
Fitting Teissier's model to the survivorship of *Radopholus similis* and *Pratylenchus coffeae* at different water potentials measured in sieved and frozen soils.

Water potential (kPa)	Parameters	*Radopholus similis*	*Pratylenchus coffeae*
Andosol			
0	R^2	0.710	0.021
	a	−0.015	−0.021
−4	R^2	0.892	0.229
	a	−0.028	−0.015
−80	R^2	0.979	0.975
	a	−0.153	−0.156
Nitisol			
0	R^2	0.693	0.579
	a	−0.028	−0.023
−5	R^2	0.838	0.243
	a	−0.008	−0.015
−250	R^2	0.976	0.651
	a	−0.320	−0.074

(*a*, coefficient of decrease in life expectancy, in days^{-1}; R^2, determination coefficient).

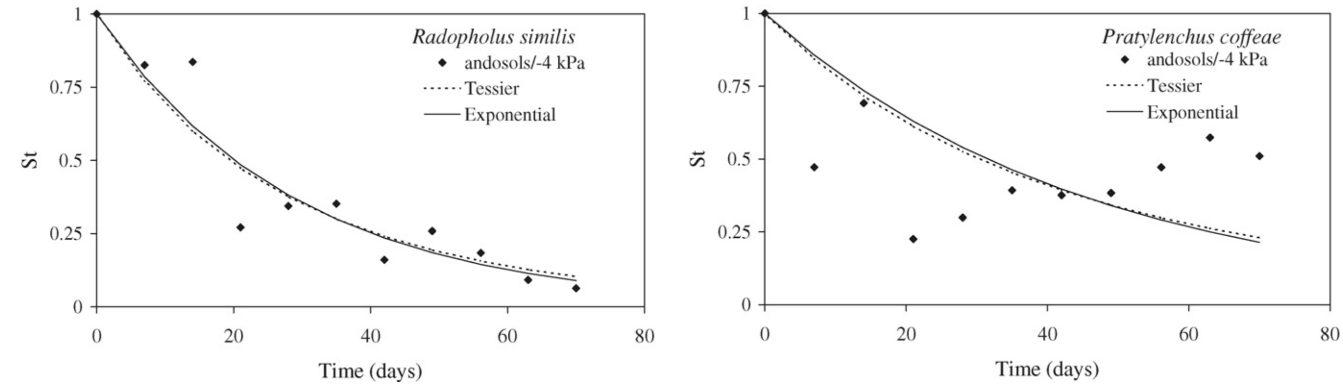

Fig. 2. Adjustment of two models, Teissier's model and the exponential decrease model, to the observed points. *Radopholus similis* (left) and *Pratylenchus coffeae* (right) in sieved and frozen Andosol at −4 kPa. Black squares: observed average points (11 dates), discontinuous line: Teissier's model, continuous line: exponential decrease.

Table 4
Statistical test of deviance analysis of study of survivorship of *Radopholus similis* undisturbed soils, using the logistic Generalized Linear Model (GLM) with binomial error.

	Name	d.f.	Residual deviance	$P > X^2$	
Factors	Soil type	1	804	$<10^{-4}$	HHS
	Duration	1	16,723	$<10^{-4}$	HHS
	Water potential	4	2,419	$<10^{-4}$	HHS
Interactions between factors	Soil type × duration	1	1,331	$<10^{-4}$	HHS
	Soil type × water potential	4	630	0.0189	S
	Duration × water potential	4	1,438	$<10^{-4}$	HHS
	Soil type × duration × water potential	4	443	0.0812	NS
Residual deviance		880	27,350		

d.f.: number of degrees of freedom; HHS: significant for $P = 0.0001$; S: significant for $P = 0.05$; NS: non-significant for $P = 0.05$.

Nitisol and the wet Andosol, and '*a*' was only −0.074 per day in the dry Nitisol. There was only a five-fold difference between '*a*' in the dry and wet Nitisol for *P. coffeae* compared with an eleven-fold difference for *R. similis*. The half-life of *P. coffeae* in the drier treatments was seven and nine days respectively, which is slightly over the half-life measured for *R. similis*. In the two treatments corresponding to wet soil, the decrease in the population of *P. coffeae* was slower: the half-life was 19 and 32 days in the Andosol and 25 and 33 days in the Nitisol. After 70 days of treatment in the wet and saturated soil, we recovered between 51% and 24% of the initial nematodes population, whereas we recovered less than 3% in the dry soil.

In conclusion, in sieved and frozen soil, we observed that: (i) nematode survivorship increased when the soil was wet (close to field capacity); (ii) *R. similis* survived significantly longer in the Nitisol than in the Andosol, which was not the case for *P. coffeae* in wet soils; (iii) *P. coffeae* survived longer than *R. similis* in both types of soil.

3.2. Effect of soil humidity on R. similis survivorship in undisturbed soils

The GLM logistic model revealed a significant effect of all tested variables (soil type, time after nematode deposit, and water potential) and of most of their interactions on the survivorship of nematodes (Table 4 and Fig. 3).

In undisturbed soils, we observed a rapid decrease in the *R. similis* population, with the maximum '*a*' coefficient of decrease in life expectancy in the saturated Nitisol at a value of −0.038 per day. In all cases, saturated soil represented the level of humidity that led to the fastest decline in the population. Fig. 3 and Table 5 show that in all cases, *R. similis* survived better in dry soil, i.e. maximum survivorship was at a value of −273 kPa in the Andosol and of −630 kPa in the Nitisol. Teissier's model generally fitted the measured data well, except for the Andosol at −40 kPa; in this case, survivorship was low during the first weeks (Fig. 4). Although Teissier's model was less accurate for the Andosol than for the Nitisol (R^2 of between 0.182 and 0.688 for the Andosol compared to 0.962 and 0.992 for the Nitisol), Table 5 shows that the decline of *R. similis* was faster in the Andosol, i.e. a mean value of '*a*' (for the data set that comprised all soil humidity) of −0.022 per day for the Andosol and of −0.024 per day for the Nitisol.

The experiments on the survivorship of *R. similis* in undisturbed soils revealed two main trends: (i) in undisturbed soils, the survivorship of *R. similis* was longest in the dry soil; (ii) the undisturbed Andosol was less favorable to the survivorship of *R. similis* than the undisturbed Nitisol.

3.3. Comparison of survivorship of nematodes in frozen and in undisturbed soils

In both soil types, the ratio P_t decreased with decreasing water potential (Table 6). It decreased with an increase in the water potential. The highest value (>13) was obtained in the saturated Nitisol. This index was close to 0 in well-drained soils where the pores capable of containing *R. similis* were completely dry. Changes in P_t with wetting were much more pronounced in the Nitisol than in the Andosol. The values calculated for P_t reached maximum after 42 days in the Nitisol and after 28 days in the Andosol. This time span is close to half-life on drained wet soils.

4. Discussion

Our results demonstrate that *R. similis* is able to starve for more than six months. Except in water-saturated soils, 1.7–9.3% of the initial population remained in the undisturbed Nitisol and 9.5–11.9% in the undisturbed Andosol after six months. The measured half-lives ranged from 5 to 17 days at 0 kPa (water saturation) to 57

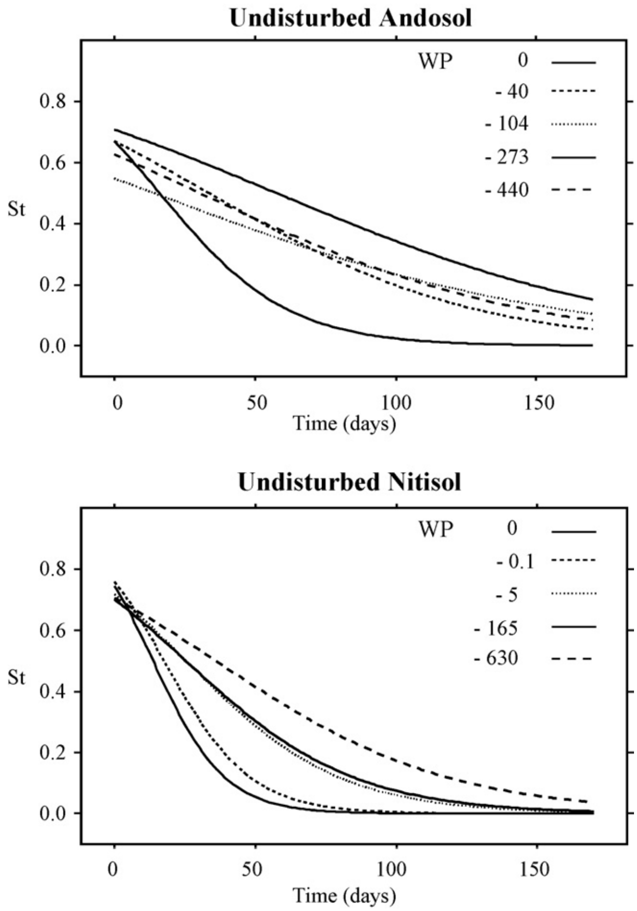

Fig. 3. Survivorship of *Radopholus similis* in undisturbed soils. Fitted curves were estimated by analysis of deviance, using the logistic Generalized Linear Model (GLM) with binomial error. S_t: proportion of survivors (survivorship). WP: water potential in kPa.
Statistical tests: for all factors (soil type, duration, water potential) and interactions between soil type × duration and duration × water potential: highly significant differences ($P < 0.0001$); interactions between soil type × water potential: significant differences ($P < 0.05$); interactions between soil type × duration × water potential: no significant ($P > 0.05$).

Table 5
Fitting Teissier's model to the survivorship of *Radopholus similis* in the Andosol and Nitisol at different water potentials measured in undisturbed soils (*a*, coefficient of decrease of life expectancy, in days^{-1}; R^2, determination coefficient).

Water potential (kPa)	Parameters	Teissier's model
Andosol		
0	R^2	0.688
	a	−0.014
−40	R^2	0.182
	a	−0.019
−104	R^2	0.562
	a	−0.006
−273	R^2	0.641
	a	−0.015
−440	R^2	0.894
	a	−0.056
Nitisol		
0	R^2	0.992
	a	−0.043
−0.1	R^2	0.954
	a	−0.025
−5	R^2	0.963
	a	−0.023
−165	R^2	0.962
	a	−0.015
−630	R^2	0.962
	a	−0.015

days at −273 kPa (and 30 days at −440 kPa) in the Andosol, and from 14 days at 0 kPa to 36 days at −630 kPa in the Nitisol. These values are higher than those reported in the literature. In experiments conducted by Birchfield (1957) and Feldmesser et al. (1960), *R. similis* populations were no longer able to infest a plant after having spent more than four months in a soil at 23 °C without food. Tarjan (1961) could not find any *R. similis* in soil samples after the fifth month. The latter study, which was fairly similar to ours (Tarjan looked for survivors in the soil and not in the roots of trap plants), was conducted in a former citrus field in Florida at much lower temperatures than those to which our boxes were exposed. We worked at temperatures ranging from 25 to 29 °C, which are very close to the optimum thermal conditions of *R. similis* (Fallas and Sarah, 1995; Pinochet et al., 1995). However, *R. similis* is quite sensitive to cool temperatures, which explains the distribution of this nematode in Cameroon and in Sri Lanka. In Sri Lanka, *R. similis* is frequently found at altitudes over 200 m and disappears at 1000 m (Gnanapragrasam and Mohotti, 2005).

Teissier's model satisfactorily described trends of *R. similis* populations in sieved and frozen soils, and to a lesser extent, in undisturbed soils. These experiments did not enable us to separate the effects of mortality linked to starvation from those of aging. Teissier's model takes both the depletion of reserves and aging into account (Reversat et al., 1997), whereas the exponential model only takes depletion of reserves into account. Two other factors could disrupt these models and mean they cannot be applied: the ability of the nematode to remain in a resting state (such as diapause or quiescence) with suspended motility, and the birth of new individuals.

Therefore, according to Teissier's model, during our first experiment, population trends of *P. coffeae* followed neither an

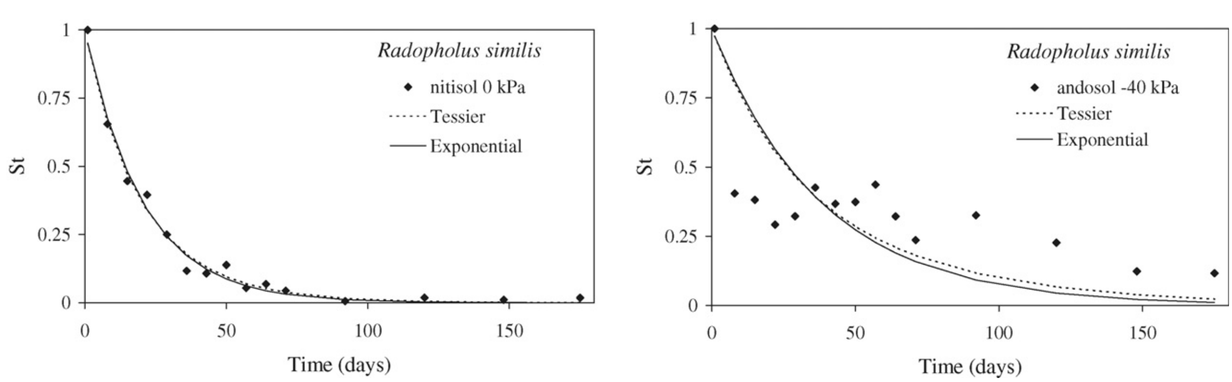

Fig. 4. Adjustment of the two models, Teissier's model and the exponential decrease model, to the observed points. Case of *Radopholus similis* at 0 kPa in undisturbed Nitisol (left) and −40 kPa in undisturbed Andosol (right). Black squares: observed average points (15 dates), discontinuous line: Teissier's model, continuous line: exponential decrease.

Table 6
Comparison of the measured survivorship of *Radopholus similis* in sieved and frozen soils and in undisturbed soils, using P_t indicator $P_t = 1 - S_{1t}/S_{2t}$ (with S_{1t} survivorship ratio after *t* days in sieved and frozen soils and S_{2t} survivorship ratio after *t* days in undisturbed soils).

Soil	Water potential (kPa)	Time after deposit (days)										Mean
		7	14	21	28	35	42	49	56	63	70	
Andosol	0	1.15	1.38	1.80	5.04	1.24	0.97	1.71	1.42	1.21	0.78	1.7
	−80/−104	1.18	0.02	0.00	0.00	0.00	0.01	0.11	0.00	0.00	0.00	0.1
Nitisol	0	1.02	2.78	0.74	7.24	4.18	13.6	7.24	6.21	8.19	3.88	5.51
	−5	1.06	2.10	1.19	1.10	1.66	2.82	1.16	3.00	4.98	2.41	2.15
	−250/−165	0.21	0.03	0.00	0.00	0.00	0.00	0.04	0.01	0.04	0.10	0.04

exponential decrease nor a decrease according to Teissier's model. According to Tobar et al. (1995), respectively six and 12 days were required for 68% and 95% of a *Pratylenchus thornei* Sher. and Allen population to emerge from anhydrobiosis. It is thus likely that the incubation time in the Baermann funnel was too short for the majority of the *Pratylenchus* spp. in the resting stage that may have been present in the dry soil to rehydrate. In the case of *P. coffeae*, we did not measure the proportion of living individuals but rather the proportion of active individuals. In contrast, the good fit obtained with *R. similis* in frozen soils is probably related to the absence of an effective survival state (quiescence or diapause).

During the two successive experiments, soil moisture conditions had a highly significant impact on the survivorship of *R. similis*. However, the conclusions of our successive experiments were contradictory: in the two undisturbed soils, life expectancy increased when the soil was dry, whereas in sieved and frozen soils, survivorship was optimal in lightly drained wet soils, and much better in saturated environments than in drier ones. To explain the differences observed between these series of experiments, we propose the following hypotheses:

(i) The toxicity of an element present in the soil that was released after freezing: however, although heat sterilization of soil can release some substances that are toxic to nematodes (manganese for example), this phenomenon has never been reported after freezing. Moreover, this hypothesis does not agree with the presence of *R. reniformis*.
(ii) The toxicity of an element in the polystyrene boxes: however, a preliminary experiment (data not shown) showed there was no effect on nematode mortality of the polystyrene compared to glass.
(iii) A change in soil porosity: sieving and freezing did considerably modify the soil structure. In these disturbed soils, the living environment was likely to be less favourable for movement by *R. similis*. It is nevertheless unlikely that these modifications alone could explain the differences we observed.
(iv) The presence of antagonists of *R. similis* and associated toxins in soils before sieving and freezing: some antagonists can act efficiently against this nematode (Paparu et al., 2006; Khan et al., 2006; Athman et al., 2007; Zum Felde et al., 2006; Mendoza et al., 2007) without being detectable with the Baermann funnel extraction technique, which we used in this study. Sieving and freezing could considerably modify the microbial community and partially eliminate microorganisms.

In our opinion, the presence of an antagonist is the most likely hypothesis to explain the observed differences in survivorship of *R. similis* between disturbed (sieved and frozen) and undisturbed soils. However, many more elements are necessary before concluding on *R. similis*–microbe interaction. A study of naturally occurring parasites and antagonists would certainly be a major undertaking, but would be a major step towards biological control of *R. similis* (Sikora and Pocasangre, 2004).

Furthermore, survivorship is not optimal in water-saturated environments. These environments represent conditions of anoxia that require specific adaptations by the nematodes that live there, i.e. the ability to ensure basic metabolism in anoxia or even to slow it down, to excrete fermentation products and to adapt behaviour (Reversat, 1975). However, *R. similis* survivorship is long enough to enable it to survive submersion lasting several days.

5. Conclusion

The burrowing nematode *R. similis* is a plant-parasitic species that is well adapted to cropping systems traditionally used in commercial banana plantations: banana monoculture with the planting of vegetative organs that provide shelter for the nematode. This system does not favour the selection of endoparasites on the basis of their resistance to starvation or adverse soil conditions. Nevertheless, for *R. similis*, this resistance is sufficient to ensure the survivorship of the species for several months in the absence of hosts.

The development of new banana cropping systems combining efficient fallow and nematode-free vitro-plants is limited by the cost of the fallow. Results of the present study show that the current recommendation of one year without plant host of *R. similis* cannot be shortened without risk. Comparison of results between frozen and undisturbed soils also suggests that microorganisms may decrease *R. similis* survivorship.

However, our results show that *R. similis* survivorship is shorter in water-saturated soils. Flooding shortly after the beginning of the fallow period could thus increase the efficiency of the fallow even though the reduction of *R. similis* survivorship disappears rapidly with a decrease in the water potential. What is more, the dissemination of *R. similis* can be facilitated by flowing water (Chabrier and Quénéhervé, 2008).

The next step will be to implement these new data concerning the soil phase of plant-parasitic nematodes in population dynamics models. For instance, the SIMBA-NEM model (Tixier et al., 2006), which was specifically designed for banana plant-parasitic nematodes, should help design improved banana-fallow cropping systems by searching for the best trade-off between length of fallow and the time before nematode populations begin to damage plants.

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References

Arcinas, A., Sipes, B.S., Hara, A.H., Tsang, M.M.C., 2005. Effect of conditioning treatments of *Radopholus similis* at high temperatures. J. Nematol. 37, 250–253.
Athman, S.Y., Dubois, T., Coyne, D., Gold, C.S., Labuschagne, N., Viljoen, A., 2007. Effect of endophytic *Fusarium oxysporum* on root penetration and reproduction of *Radopholus similis* in tissue culture-derived banana (*Musa* spp.) plants. Nematology 9, 599–607.

Birchfield, W., 1957. Observation on the longevity without food of the burrowing nematode. Phytopathology 47, 161–162.
Chabrier, C., Mauléon, H., Bertrand, P., Lassoudière, A., Quénéhervé, P., 2005. Banane antillaise, les systèmes de culture évoluent. Phytoma-L.D.V., 584, 12–16.
Chabrier, C., Quénéhervé, P., 2003. Control of the burrowing nematode *Radopholus similis* (Cobb) on banana: impact of the banana field destruction method on the efficiency of the following fallow. Crop Protect. 22, 121–127.
Chabrier, C., Quénéhervé, P., 2008. Preventing nematodes from spreading: a case study with *Radopholus similis* (Cobb) thorne in a banana field. Crop Protect. 1237–1243.
Crawley, M.J., 2005. Statistics. An Introduction using R. John Wiley & Sons edit, New York, p. 323.
DuCharme, E.P., 1955. Sub-soil drainage as a factor in the spread of the burrowing nematode. P. Fl. St. Hortic. Soc. 68, 29–31.
Duyck, P.-F., Pavoine, S., Tixier, P., Chabrier, C., Quénéhervé, P., 2009. Host range as an axis of niche partitioning in the plant-feeding nematode community of banana agroecosystems. Soil Biol. Biochem. 41, 1139–1145.
Evans, A.A.F., Perry, R.N., 1976. Survival strategies in nematodes. In: Croll, N.A. (Ed.), The Organisation of Nematodes. Academic Press, New York, pp. 383–424.
Fallas, G.A., Sarah, J.L., 1994. Effect of storage temperature on the in vitro reproduction of *Radopholus similis*. Nematropica 24, 175–177.
Fallas, G., Sarah, J.-L., 1995. Effect of temperature on the in vitro multiplication of seven *Radopholus similis* isolates from different banana producing zones of the world. Fund. Appl. Nematol. 18, 445–449.
Feldmesser, J., Feder, W.A., Rebois, R.V., Hutchins, P.C., 1960. Longevity of *Radopholus similis* and *Pratylenchus brachyurus* in fallow soil in the greenhouse. Anat. Rec. 137, 355.
Glazer, I., Orion, D., 1983. Studies on anhydrobiosis of *Pratylenchus thornei*. J. Nematol. 15, 333–338.
Gnanapragrasam, N.C.C., Mohotti, K.M., 2005. Nematode parasites of tea. In: Luc, M., Sikora, R.A., Bridge, J. (Eds.), Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. second ed. CABI Publishing, Wallingford, pp. 581–609.
Gowen, S.R., Quénéhervé, P., Fogain, R., 2005. Nematodes parasites of bananas and plantains. In: Luc, M., Sikora, R.A., Bridge, J. (Eds.), Plant parasitic nematodes in subtropical and tropical agriculture. second ed. CABI Publishing, Wallingford, pp. 611–643.
Hooper, D.J., 1986. Extraction of free-living stages from soil. In: Southey, J.F. (Ed.), Laboratory Methods for Work with Plant and Soil Nematodes. sixth ed. Ministry of Agriculture, Fisheries and Food, London, pp. 5–30.
Hooper, D.J., Hallmann, J., Subbotin, S.A., 2005. Methods for extraction, processing and detection of plant and soil nematodes. In: Luc, M., Sikora, R.A., Bridge, J. (Eds.), Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. second ed. CABI Publishing, Wallingford, pp. 53–86.
Kaplan, D.T., Vanderspool, M.C., Garett, C., Chang, S., Opperman, H., 1996. Molecular polymorphisms associated with host range in the highly conserved genomes of burrowing nematodes, *Radopholus* spp. Mol. Plant Microbe Interact. 9, 32–38.
Kerry, B.R., 2000. Rhizosphere interactions and the exploitation of microbial agents for the biological control of nematodes. Annu. Rev. Phytopathol. 38, 423–441.
Khan, A., Williams, K.L., Nevalainen, H.K.M., 2006. Control of plant-parasitic nematodes by *Paecilomyces lilacinus* and *Monacrosporium lysipagum* in pot trials. BioControl 51, 643–658.
Loos, C.A., 1961. Eradication of the burrowing nematode, *Radopholus similis*, from bananas. Plant Dis. Rep. 45, 457–461.
McCullagh, P., Nelder, J.A., 1989. Generalized Linear Models, second ed. Chapman and Hall, London, p. 511.
McSorley, R., 2003. Adaptation of nematodes to environmental extremes. Fla. Entomol. 88, 138–142.
Mendoza, A.R., Sikora, R.A., Kiewnick, S., 2007. Influence of *Paecilomyces lilacinus* strain 251 on the biological control of the burrowing nematode *Radopholus similis* in Banana. Nematropica 37, 203–213.
Paparu, P., Dubois, T., Gold, C.S., Niere, B., Adipala, E., Coyne, D., 2006. Colonisation pattern of nonpathogenic *Fusarium oxysporum*, a potential biological control agent, in roots and rhizomes of tissue cultured *Musa* plantlets. Ann. Appl. Biol. 149, 1–8.
Pinochet, J., Fernandez, C., Sarah, J.L., 1995. Influence of temperature on in vitro reproduction of *Pratylenchus coffeae*, *P. goodeyi* and *Radopholus similis*. Fund. Appl. Nematol. 18, 391–392.
Quénéhervé, P., 2008. Integrated management of banana nematodes. In: Ciancio, A., Mukerji, K.G. (Eds.), Integrated Management of Fruit Crops and Forest Nematodes. Springer, The Netherlands, pp. 3–61.
Reversat, G., 1975. Etude préliminaire de la survie en anaérobiose des juveniles du nematode *Heterodera oryzae* (Tylenchida: Heteroderidae). C.R. Acad. Sci. Paris 280, 2865–2868.
Reversat, G., Rossi, J.-P., Bernhard, P., 1997. Analyse des courbes de survie de nématodes phytoparasites selon le modèle de Teissier. C.R. Acad. Sci. Paris, Sc. de la vie 320, 259–266.
Sarah, J.L., Lassoudière, A., Guéroult, R., 1983. La jachère nue et l'immersion du sol: deux méthodes intéressantes de lutte intégrée contre *Radopholus similis* Cobb dans les bananeraies des sols tourbeux de Côte d'Ivoire. Fruits 38, 35–42.
Sikora, R.A., Pocasangre, L.E., 2004. New technologies to increase root health and crop production. InfoMusa 13 (2), 25–29.
Tarjan, A.C., 1961. Longevity of *Radopholus similis* (Cobb) in host free soil. Nematologica 6, 170–175.
Teissier, D., 1984. Etude expérimentale de l'organisation des matériaux argileux. Hydratation, gonflement et structuration au cours de la dessiccation et de la réhumectation. PhD Thesis, Univ. Paris VII, Paris, France, 361 pp.
Teissier, G., 1933. Recherches sur le vieillissement et sur les lois de la mortalité. C.R. Acad. Sci. Paris, Physiol., Physico-Chimie Biol. 10, 237–284.
Tixier, P., Malézieux, E., Risède, J.-M., Dorel, M., 2006. Modelling populations of banana phytoparasitic nematodes: a contribution to the design of sustainable cropping systems. Ecol. Model. 198, 321–331.
Tobar, A., Valor, H., Talavera, M., 1995. Kinetics of recovery from anhydrobiosis in *Pratylenchus thornei*, *Merlinius brevidens* and *Heterodera avenae* from field soils and dry roots of the host plant. Fund. Appl. Nematol. 18, 21–24.
Townshend, J.L., 1984. Anhydrobiosis in *Pratylenchus penetrans*. J. Nematol. 16, 282–289.
Zum Felde, A., Pocasangre, L.E., Carñizares Monteros, C.A., Sikora, R.A., Rosales, F.E., Rivero, A.S., 2006. Effect of combined inoculations of endophytic fungi on the biocontrol of *Radopholus similis*. InfoMusa 15, 12–18.