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Nematode dissemination by water leached in soil: Case study of *Radopholus similis* (Cobb) Thorne on nitisol under simulated rainfall

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ABSTRACT

New crop systems have been developed in the French West Indies that combine fallow or rotation crops with nematode-free vitro-plants to avoid the massive application of nematicides in banana fields. In these new banana fields, recontamination by the burrowing nematode *Radopholus similis* can then happen either by run-off or irrigation water. To understand these phenomena, we studied the vertical dissemination of *R. similis* by water leaching using soil cylinders and rainfall simulations. Steel drums were used to collect cylinders of soil (around 14.3 dm^3) with a backhoe. The soil cylinders were placed in an aspersion chamber, saturated with water, and a *R. similis* suspension was placed on the soil surface. Afterwards, rainfalls ranging from 12 to 540 mm were simulated. Nematodes were then extracted from different soil layers (0–5 to 20–25 cm depth) using either (i) a Seinhorst elutriator followed by a Baermann funnel or (ii) centrifugation–flotation combined with Meldola blue staining. Results showed that dissemination of *R. similis* at the scale of the decimetre in nitisoil is limited: less than 8% of the applied nematodes reached layers deeper than 10 cm after exceptional rainfalls that represent several times the poral volume of the soil. Dissemination below 25 cm depth seemed to be limited to very few individuals, from 0.1 to 0.2%. Among leached nematodes, the percentage of non-active and dead nematodes increased significantly with depth. It seems that *R. similis* have developed a behaviour to escape leaching. These findings question the generally accepted idea that water dissemination of plant-parasitic nematodes is mainly a passive phenomenon.

1. Introduction

The burrowing nematode *Radopholus similis* (Cobb) Thorne is a major pest of banana worldwide (Gowen et al., 2005). In Martinique (French West Indies), a cropping system has been developed based on disinfestation of lands contaminated by plant-parasitic nematodes (Sarah et al., 1983; Mateille et al., 1992). Cleanup is achieved through either a fallow period or an appropriate crop rotation, after which nematode-free, *in vitro* produced banana plants are planted. Growers can then cultivate banana for 2–4 years without a nematicide (Chabrier et al., 2005) before the banana fields are gradually reinfestated with the burrowing nematode *R. similis*. As a result, without nematicide applications, the reduction in yield due to toppled

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plants is usually high enough to lead banana growers to destroy their fields (Blake, 1972).

The rationale for this study was to know if *R. similis* individuals could be passively transported down into deeper soil layers after accidental contaminations (irrigation, run-off and leaching water, contaminated soil particles carried by workers, etc.). Numerous authors propose that water dissemination of nematodes is a major contamination process (Croll and Mathews, 1977; Bur and Robinson, 2004). Several studies have dealt with dissemination in surface water (Faulkner and Bolander, 1970; Cadet et al., 2002). However, active dissemination of plant parasitic nematodes over longer distances has long been considered as a minor phenomenon (Norton, 1978) but DuCharme (1955) reported that, in a hilly Citrus orchard, *R.similis* spread at a rate of 66 m/year downhill while the uphill rate was less than 8 m/year. Although little is known about dissemination in ground water, DuCharme (1955) concluded that ''movement of water through the soil facilitates the passage of the nematodes'' and that some living *R. similis* may be present in groundwater.

Several studies in soil columns have shown that active dissemination of nematodes may occur at the scale of meters (Prot, 1980). Thus, we studied how rainfall can influence the vertical dissemination of *R. similis* in soil. The answer to this question may influence management techniques such as nematode monitoring. Indeed, Sorribas et al. (2000) suggest that rainfall may carry nematodes below sampling depth.

2. Materials and methods

Two successive studies were performed. During the first one, ''common'' rainfalls were simulated. They were strong enough to generate run-off and leaching which happen in nitisol when rainfall exceeds 60 mm/h. These rainfalls are likely to occur a few times each month in the wet season.

One may consider that, in soil, nematodes inhabit pore spaces filled up by water and air (Quénéhervé and Chotte, 1997). In the second study, simulated rainfalls corresponding to multiples of the pore volumes were applied to evaluate the percentage of nematodes that were likely to be leached through the soil pores. These simulated rainfalls, ranging from 90 to 540 mm, corresponded to exceptional rainfalls that can occur from less than once a year to once every 20 years.

2.1. *Study 1*

Soil samples were collected in a former banana field. This land was previously contaminated with about 2000 *R. similis* per 100 g of roots before a bare-fallow period which has lasted since December 1999. Since then, this land has been used for erosion studies and was consequently hydraulically isolated from upstream with iron sheet 40 cm wide and hand weeded bimonthly to maintain the bare fallow. Before the present study, in February 2006, the absence of *R. similis* was checked using Seinhorst's (1962) elutriation method.

The soil type was a nitisol, derived from volcanic ashes (andesitic basalt). Such soil is characteristically found in the Caribbean, including in the lowlands in the central part of Martinique and on the Atlantic coast of Guadeloupe. The clay portion is predominant, but the major clay mineral is halloysite, which forms sand-size aggregates with organic compounds (Table 1). In such soil, the diffusion of water is similar to that in sandy soil. Khamsouk (2001) showed that, at field capacity, water conductivity varied between 50 and 60 mm/h. Median conductivity, measured with a membrane infiltrometer, was 52 mm/h at the surface of the bare soil. Previous studies showed that this soil contains numerous pores 30–300 μm in diameter. These results were obtained by ultrafiltration under controlled pneumatic pressure (Teissier, 1984). As the body diameter of *R. similis* varies between 12 μm for youngest juveniles to 27 μm for largest females (Van Weerdt, 1958), these pores are suitable for both active and passive displacement of *R. similis* inside the soil.

Soil cylinders were collected using steel drums with an internal diameter of 26 cm and a height of 30 cm. The base of the drums was beveled to facilitate soil penetration; two rings were welded to the upper part, permitting the use of chains to extract the drum from the soil. The drums were first coated with silicone grease to ensure watertightness between the drum and the soil sample. The drums were pushed into the soil with a backhoe. This action was performed uniformly to avoid formation of fissures in the soil that could modify macroporosity and increase the flow of water and nematodes. After reaching the −25 cm level, the backhoe extracted the drum and the soil sample by pulling gently on a chain; and the soil samples were then brought to the laboratory.

Nematodes were extracted from banana roots removed from a commercial plantation. In this plantation, more than

Table 1 – Granulometry of nitisol in Martinique obtained (i) according to standard procedures after sonification and dispersion in sodium hexametaphosphate (Standard granulometry) and (ii) by sieving without dispersion (apparent granulometry)

	Standard granulometry		Apparent granulometry	
	Particle size (μm)	Weight of soil fraction (g/100 g)	Particle size (μm)	Weight of soil fraction (g/100 g)
Sand	50–2000	11.3	500–2000	1.91
			312–500	6.71
			200–312	26.96
			100–200	29.65
			50–100	29.43
Loam	2–50	14.6	0–50	5.34
Clay	0–2	73.4		

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Table 2 – Numbers of *R. similis* applied during rainfall simulations in Study 1

Rainfall intensity (mm/h)	Rainfall duration (h:min)	Well-drained soil			Wetted soil		
		Motile	Immotile	Total	Motile	Immotile	Total
60	0:12	27600	20760	48360	16475	23400	39875
	0:32	10425	14300	24725	6210	21315	27525
	0:52	8552	25738	34290	32780	25855	58635
	1:12	10660	11235	21895	15160	12 155	27315
Average		14309	18008	32318	17656	20681	38338

Nematodes extracted from infested banana roots by mist extraction.

90% of the nematodes in roots were *R. similis*, although some *Helicotylenchus multicinctus* and *Meloidogyne* spp. were also present. Banana roots were placed in a mist chamber for 1 week to obtain fresh nematode suspensions following Seinhorst's (1950) method. The suspensions, containing both motile and immotile nematodes, were applied 5 min before the beginning of each rainfall simulation experiment (Table 2).

We simulated a 60 mm/h rainfall for this study. Such rainfall intensity is consistently observed 2 days per month during the wet season, but only 1 day every 3 months during the dry season (Meteo-France, unpublished data). Four different rainfall durations were tested: 12, 32, 52, and 72 min. Under the conditions of these events, we measured the effect of violent showers that occur around 20 times per year. We studied two initial soil moistures: (i) well-drained soils, with a water potential varying from −100 to −1 000 kPa (pF 3–4), and (ii) wetted soils, after a simulated rain, with an initial water potential of −1 kPa.

Simulated rainfalls of 60 mm/h were performed in an aspersion chamber. The ceiling of this blind room was equipped with sprinklers. The sprinklers received tap water at a pressure of three bars, and thus supplied continuous rain. The cylinder was placed in a 15 L bucket that collected all the water leaching from the soil sample. Wedges were placed under the bucket so that the upper soil surface was horizontal. Six pluviometers were placed around the soil cylinder to ensure that the intended quantity of rainfall was applied. Each experiment was replicated three times on three separate soil cylinders. Cylinders of the same replication were tested separately for each rainfall amount, with new soil collected each time.

R. similis suspensions (from 22,000 to 59,000 individuals in 200 mL of water, Table 2) were applied uniformly across the soil surface. Simulated rainfall was then applied in one sequence followed by 1 h of leaching. Next, five soil samples were collected with a steel coring probe 5 cm in depth and 5 cm in diameter (Fig. 1). This operation was reiterated five times, to collect five samples per depth, from 5 to 25 cm depth. In addition, we collected pairs of soil samples at each depth to evaluate soil moisture and water potential by ultrafiltration in a pneumatic pressure chamber (Teissier, 1984). We also collected the leached water and the soil particles that fell from the base of the cylinder into the bucket.

For the first experiment, nematodes were extracted from soil by elutriation (Seinhorst, 1962) and separated from remaining particles using Baermann funnels (Hooper, 1986). Nematodes from leached water and soil particles that had fallen into the bucket were extracted by sieving using a column of four sieves (80–25 μm) to collect nematode-size particles, followed by centrifugation–flotation extraction (Coolen and d'Herde, 1972). Then, nematodes were counted under a light-inversed microscope. At the same time, motility of nematodes was checked by nervous stimulation with a nylon needle.

As the nematodes extracted from 25 coring probes represented about 19.1% of the total volume of the soil cylinder, the percentage of recovered nematodes was calculated using the formula: % recovery = cumulated number of observed nematodes/(number of inoculated nematodes × 0.191).

To ensure the representativeness of the observed population of *R. similis*, the coefficient of variation among the five collected samples at each depth was calculated for each cylinder. Then, only the mean of five samples collected on each layer after each rainfall simulation was retained. Variance analyses were performed to compare the different rainfalls; when the Fisher test was significant for $P < 5\%$, mean values per treatment were classified using Duncan's multi-range test. To compare distribution among different layers, percentages of recovered *R. similis* were transformed using the formula $\log(x + 10^{-5})$. Linear regression was used to establish the relationship between depth and this variable, after verifying that residues were normally distributed using Shapiro–Wilk's test.

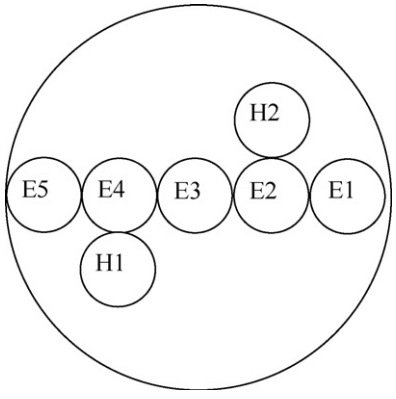

Fig. 1 – Sampling scheme of soil, at the surface of the cylinder after simulated rainfall in Study 1. E1 to E5: samples for elutriation. H1 and H2: samples for soil water contents.

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Table 3 – Numbers of *R. similis* (total and % of dead) applied during rainfall simulations in Study 2 (rainfalls that drained multiples of the pore volume of the soil)

Rainfall Intensity (mm/h)	Rainfall duration (h:mn)	Total number of *R. similis*			% *R. similis* colored by Meldola's Blue		
		Females	Males	Juveniles	Females	Males	Juveniles
30	3:00	22870	4083	13627	4.8	9.1	8.5
	6:00	7051	1296	2841	5.1	16.0	11.9
	12:00	19044	4412	8470	7.8	6.3	9.9
	18:00	7618	1179	3090	13.7	4.5	5.9
60	3:00	9373	1596	5399	10.5	7.2	7.2
Average		13191	2513	6685	8.4	8.6	8.7

Nematodes extracted from infested banana roots by mist extraction.

The conclusion of this first study led us to set up a second study to determine whether *R. similis* could be disseminated during exceptional rainfalls.

2.2. *Study 2*

Soil samples were collected in the same field as for Study 1. Nematode suspensions (Table 3) were obtained following the same procedure described previously, except that we stained an aliquot of each nematode suspension with Meldola blue (Ogiga and Estey, 1974) to discriminate living and dead nematodes among the non-motile nematodes.

We planned rainfalls to correspond to multiples of a calculated pore volume of 8.9 dm^3 within the soil cylinder. This theoretical volume was calculated as follows. If V is the total volume, Vs the cumulated volume of soil solid particles, Vv the volume of pores, and Md the dry mass of soil, we have: $V = Vv + Vs$ and also:

$$\frac{Vv}{V} = \frac{Vv}{Ms} \times \frac{Ms}{V} = \frac{(V - Vs)}{Ms} \times \frac{Ms}{V} = \left(\frac{V}{Ms} - \frac{Vs}{Ms}\right) \times \frac{Ms}{V}$$

so that:

$$\frac{Vv}{V} = \left(\frac{1}{Da} - \frac{1}{Ds}\right) \times Da = 1 - \frac{Da}{Ds} = 1 - \left(\frac{1.1}{2.68}\right) = 0.59$$

As the soil volume in the cylinders is 15 dm^3, the pore volume is estimated at 8.9 dm^3.

We simulated five rainfall types (Table 4), which represented 0.6–3.7 times the pore volume, considering that after such water flushes, all nematodes that were likely to be leached would have been displaced. These rainfalls are quite exceptional. In the past 12 years, Martinique and Guadeloupe islands have both received two rainfalls of more than 400 mm/24 h. The highest event, 690 mm in 8 h, occurred in Guadeloupe during the storm called ''Luis'' in September 1995.

Rainfalls were applied using the same procedure as in Study 1. But, like during the first study, only 21% of the applied nematodes were recovered, we doubled the number of samples we collected in each soil layer of the first replication. Half the samples were extracted by elutriation, and the other half by centrifugation–flotation (as described by Coolen and d'Herde, 1972). The sampling scheme is presented in Fig. 2. For the two other replications, we extracted *R. similis* from the soil by centrifugation–flotation only (Fig. 3). Like in Study 1, nematodes in leached water and in the soil in the bucket were extracted by centrifugation–flotation. To discriminate dead and living nematodes, we stained the samples extracted by centrifugation–flotation with Meldola blue between sieving and centrifugation according to the procedure of Ogiga and Estey (1974).

The methods of nematode extraction (elutriation followed by Baermann *vs.* centrifugation–flotation) were compared on pairs of samples. The results were compared using a bilateral Fisher's test. Other variables were compared using the statistical methods previously described for Study 1.

Table 4 – Characteristic rainfalls in Study 2

Intensity (mm/h)	Duration (h)	Total (mm)	Proportion of pore volume of the soil cylinder
30	3	90	0.6
30	6	180	1.2
30	12	360	2.4
30	18	540	3.7
60	3	180	1.2

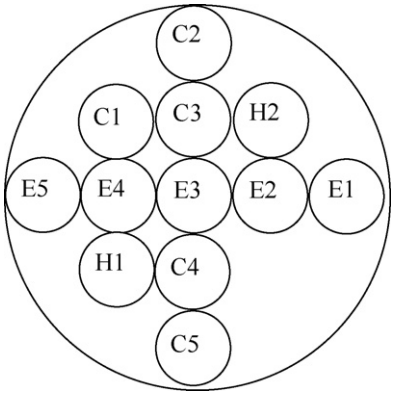

Fig. 2 – Sampling scheme of soil, at the surface of the cylinder after simulated rainfall in Study 2, repetition 1. C1 to C5: sample for centrifugation–flotation. E1 to E5: samples for elutriation. H1 and H2: samples for soil water contents.

3. Results

3.1. Study 1

No *R. similis* were found in the soil from the field before extraction of the cylinder. However, these samples comprised only low populations of both the spiral nematode *H. dihystera* and the reniform nematode *Rotylenchulus reniformis* with a few free-living nematodes.

During the first study, only 21% of the applied *R. similis* were recovered. For each depth among the five replicates of each cylinder, the coefficient of variation of the percentage of recovered *R. similis* ranged from 4 to 10%. In addition, after transformation log $(x + 10^{-5})$, residues were distributed according to Normal Law.

On well-drained soils, more than 95% of *R. similis* were recovered in the top 5 cm of the soil, only 21 specimens out of 13,855 counted were extracted between a depth of 15 and 25 cm. Among them, only six individuals were motile after mechanical stimulation. However, in these soils, population displacements increased with an increase in the duration of the rainfall: under 72 mm rainfall, a significantly higher proportion of *R. similis* reached the second layer (Table 5).

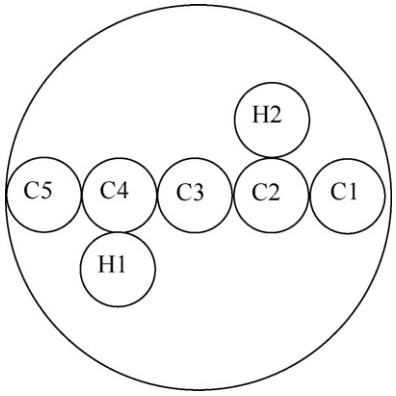

Fig. 3 – Sampling scheme of soil, at the surface of the cylinder after simulated rainfall in Study 2, repetition 2 and 3. C1 to C5: sample for centrifugation–flotation. H1 and H2: samples for soil water contents.

Similarly, on wetted soils, 96% of counted *R. similis* were recovered in the top 5 cm. Only 0.27% of recovered *R. similis* were found outside the −10 cm level. Fifteen specimens (among which four were immotile) were observed in the soil

Table 5 – Vertical distribution of recovered *R. similis* after simulated rainfalls on well-drained and wetted soils: comparison of the different rainfalls in Study 1

Depth (cm)	Rainfall (mm)	Well-drained soil		Wetted soil	
		% Recovered	% Motile	% Recovered	% Motile
2.5	12	99.82a	74.92	95.48a	79.75a
	32	98.27a	73.03	98.15a	79.38a
	52	95.27a	75.28	97.90a	78.73a
	72	86.10b	66.82	87.45b	55.63b
Mean		94.87 S	72.51 ns	94.74 S	73.37 S
7.5	12	0.18b	72.92	4.26b	66.71ab
	32	0.92b	61.67	1.66b	53.90b
	52	4.65ab	67.00	1.89b	85.51a
	72	12.52a	36.69	10.07a	72.70a
Mean		4.57 S	59.57 ns	4.47 S	69.70 S
12.5	12	0.00		0.12	72.22
	32	0.59	52.08	0.16	91.67
	52	0.00		0.20	82.48
	72	1.32	66.66	1.05	88.89
Mean		0.48 ns	59.37 ns	0.38 ns	83.81 ns
17.5	12	0.00		0.05	
	32	0.22		0.03	
	52	0.08		0.01	
	72	0.00		0.27	
Mean		0.07 ns		0.09 ns	
22.5	12	0.00		0.00	
	32	0.00		0.00	
	52	0.00		0.00	
	72	0.06		0.01	
Mean		0.02 ns		0.00 ns	

ns = Not significant, S = significant ($P = 0.05$), HS = highly significant ($P = 0.01$). Variances of percentages of recovered *R. similis* were analyzed after $\log(x + 10^{-5})$ transformation. Means in a row followed by the same letter are not different ($P = 0.05$) according to Duncan's multi-range test.

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Table 6 – Vertical distribution of recovered *R. similis* after simulated rainfalls on well-drained and wetted soils: regression comparison of the different depths (mean of three replicates) in Study 1

Rainfall (mn)	Well-drained soil			Wetted soil		
	Linear regression	R^2	F-test	Linear regression	R^2	F-test
12	$Y = 3.423 - 0.561 \times D$	0.76	HS	$Y = 4.562 - 0.472 \times D$	0.82	HS
32	$Y = 3.848 - 0.519 \times D$	0.79	HS	$Y = 4.883 - 0.572 \times D$	0.84	HS
52	$Y = 4.167 - 0.568 \times D$	0.70	HS	$Y = 5.331 - 0.591 \times D$	0.93	HS
72	$Y = 3.163 - 0.476 \times D$	0.74	HS	$Y = 3.600 - 0.358 \times D$	0.65	HS

$Y = \log(x + 10^{-5})$, x designates the percentage of recovered *R. similis* in a layer, D: depth of the layer, HS: highly significant ($P = 0.01$).

Table 7 – Vertical distribution of recovered *R. similis* after simulated rainfalls on well-drained and wetted soils: comparison of the different depths in Study 1

Depth	Well-drained soil		Wetted soil	
	%Recovered	%Motile	%Recovered	%Motile
2.5	94.87a	72.51a	94.74a	73.37
7.5	4.57b	59.57b	4.47b	69.70
12.5	0.48c	59.37b	0.38c	83.81
17.5	0.07d		0.09d	
22.5	0.02d		0.00d	
	HS	HS	HS	ns

ns = Not significant, S = significant ($P = 0.05$), HS = highly significant ($P = 0.01$). Variances of percentages of recovered *R. similis* were analyzed after $\log(x + 10^{-5})$ transformation. Means in a row followed by the same letter are not different ($P = 0.05$) according to Duncan's multi-range test.

column below 15 cm, and 32 cumulated individuals (among which 28 were immotile) were found in buckets (0.015% of the applied *R. similis*).

After each rainfall, distribution of *R. similis* followed an inverse exponential law (Table 6). Indeed, if "y" designates the percentage of recovered nematodes, "x" the depth, and ε a constant (10^{-5}) that enables calculation when y = 0, we have:

$$\log(y + \varepsilon) = b - ax, \quad \text{then} \quad y + \varepsilon = e^{b-ax} = e^{b}e^{ax}$$

These regressions were significant for both well-drained soils and wetted soils, at field capacity. No significant differences were observed among regression coefficients of both soils. Results of the first study show that there was little passive leaching of *R. similis* (Table 7).

3.2. *Study 2*

Centrifugation–flotation was a more efficient method to extract females and juveniles of *R. similis* but both methods gave similar results for males (Table 8). In the second and third repetitions, we thus extracted nematodes only by centrifugation–flotation.

Few adult *R. similis* were dragged downward, less than 4% of females were found below 10 cm (Tables 9 and 10). A few more juveniles were found in the 10–25 cm layer (7.7%). Very few nematodes (fewer than 0.15% of the applied nematodes) were found below the −25 cm soil level, and collected in the buckets (Table 11).

No significant difference was observed in the recovery percentages of *R. similis* at each depth level among the five different simulated rainfalls (Table 9). Furthermore, at each depth level, no difference was observed between the percentages of *R. similis* not stained with Meldola blue (considered as living individuals).

However, among depth levels, the percentages of *R. similis* not stained with Meldola blue differed significantly; the proportion of "living" individuals increased with depth, following a linear relation (Table 10). This was especially true for juveniles, which presented the highest multiplicative constant ($a = 2.0$).

4. Discussion

Several authors (McSorley and Frederick, 1991; Viglierchio and Schmitt, 1983) obtained better results on nematode extraction with elutriation even if only motile nematodes were extracted when elutriation was followed by Baermann filtration (Hooper, 1986). Considering that in our dissemination study, active forms are more pertinent indicators than inactive or dead specimens, we first only extracted *R. similis* using the elutriation-Baermann method. As in Study 1, we retrieved only 21% of the applied specimens of *R. similis*, we tried to extract nematodes from the soil using the centrifugation–flotation method, which extracted more efficiently (49%). This result is consistent with those of Yen et al. (1998) who obtained better extraction outputs with

Table 8 – Efficiency of the two methods of extraction of *R. similis* from soil: elutriation-Baermann compared to centrifugation–flotation. Results after five rainfall simulations, statistical tests conducted on 125 pairs of samples (5 cylinders × 5 depths × 5 replicates)

Method	Females	Males	Juveniles	Total
Elutriation + Baermann	23.0 ± 7.1	74.9 ± 33.6	8.1 ± 3.7	20.8 ± 6.5
Centrifugation–flotation	62.2 ± 18.9	65.4 ± 25.0	21.4 ± 9.6	49.1 ± 15.6
	HS	ns	HS	HS

Data were compared using bilateral Fisher's test. ns = Not significant, HS = highly significant ($P = 0.01$).

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Table 9 – Vertical distribution of recovered *R. similis* after simulated rainfalls: comparison of the different rainfalls in Study 2

Depth (cm)	Rainfall (h × mm/h)	Females		Males		Juveniles	
		%Recovered	%Alive	%Recovered	%Alive	%Recovered	%Alive
2.5	3 × 30	85.6	95.2	70.8	95.2	66.9	93.7
	6 × 30	78.7	59.2	77.7	59.9	74.4	43.8
	12 × 30	93.0	74.7	93.6	71.7	93.4	64.2
	18 × 30	76.6	84.2	77.2	85.0	74.2	64.1
	3 × 60	87.4	77.8	88.9	72.5	73.9	67.4
Mean		84.3 ns	78.2 ns	81.6 ns	76.9 ns	76.6 ns	66.7 ns
7.5	3 × 30	11.1	94.2	18.6	90.0	24.4	100.0
	6 × 30	16.1	64.2	10.4	53.6	18.1	53.6
	12 × 30	4.9	60.1	4.7	64.6	4.0	64.4
	18 × 30	19.9	72.0	13.1	80.6	13.3	31.0
	3 × 60	10.9	68.4	9.7	64.4	19.2	63.5
Mean		12.6 ns	71.8 ns	11.3 ns	70.6 ns	15.8 ns	62.5 ns
12.5	3 × 30	2.1	90.5	3.2	100.0	6.8 a	35.0
	6 × 30	3.2	52.2	3.3	50.0	4.0 a	64.3
	12 × 30	1.0	33.4	0.7	50.0	1.1 a	33.3
	18 × 30	1.2	60.6	5.5	33.3	0.8 b	0.0
	3 × 60	1.2	57.7	0.3	66.7	4.6 a	54.3
Mean		1.7 ns	58.9 ns	2.6 ns	60.0 ns	3.5 S	37.4 ns
17.5	3 × 30	1.0	37.3	6.2	100.0	1.8	0.0
	6 × 30	1.7	33.3	8.1	50.0	1.4	0.0
	12 × 30	0.9	59.7	1.0	33.3	1.4	25.0
	18 × 30	2.1	83.3	2.8	0.0	10.6	16.7
	3 × 60	0.3	55.6	0.2	100.0	0.7	66.7
Mean		1.2 ns	53.9 ns	3.6 ns	56.7 ns	3.2 ns	21.7 ns
22.5	3 × 30	0.3		1.3		0.1	
	6 × 30	0.5		0.9		2.2	
	12 × 30	0.2		0.1		0.1	
	18 × 30	0.2		1.4		1.1	
	3 × 60	0.3		0.9		1.5	
Mean		0.3 ns		0.9 ns		1.0 ns	

ns = Not significant, S = significant ($P = 0.05$), HS = highly significant ($P = 0.01$). Variances of percentages of recovered *R. similis* were analyzed after $\log(x + 10^{-5})$ transformation. Means in a row followed by the same letter are not different ($P = 0.05$) according to Duncan's multi-range test.

Table 10 – Vertical distribution of recovered *R. similis* after simulated rainfalls: comparison of the different depths in Study 2

Depth (cm)	Females		Males		Juveniles	
	%Recovered	%Alive	%Recovered	%Alive	%Recovered	%Alive
2.5	84.3 a	78.2 a	81.6 a	76.9	76.6 a	66.7 a
7.5	12.6 b	71.8 ab	11.3 b	70.6	15.8 b	62.5 a
12.5	1.7 c	58.9 b	2.6 c	60.0	3.5 c	37.4 ab
17.5	1.2 c	53.9 b	3.6 c	56.7	3.2 c	21.7 b
22.5	0.3 d		0.9 d		1.0 d	
	HS	S	HS	ns	HS	S
Regression	$L(F) = 4.71 - 0.31xD$	$F = 78.6 - 1.3xD$	$L(M) = 5.05 - 0.40xD$	$M = 77.6 - 1.4xD$	$L(J) = 4.76 - 0.35xD$	$J = 71.0 - 2.0xD$
R^2	0.71/HS	0.72/HS	0.50/HS	0.40/S	0.44/HS	0.53/HS

ns = Not significant, S = significant ($P = 0.05$), HS = highly significant ($P = 0.01$). Variances of percentages of recovered *R. similis* were analyzed after $\log(x + 10^{-5})$ transformation. Means in a row followed by the same letter are not different ($P = 0.05$) according to Duncan's multi-range test. $L(F)$, $L(M)$ and $L(J)$: % of recovered females, males and juveniles after transformation $\log(x + 10^{-5})$; D: depth (in cm), and F, M, J: % of alive females, males and juveniles

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Table 11 – Dissemination of *R. similis* in groundwater: number of specimens isolated in the bucket under each soil cylinder in Study 2

Rainfall (h × mm/h)	Females	Males	Juveniles
3 × 30	150	49	180
	7	3	3
	77	3	0
6 × 30	1	0	6
	0	0	1
	0	0	0
12 × 30	0	3	10
	0	0	0
	0	0	0
18 × 30	3	3	1
	1	0	1
	2	0	3
3 × 60	5	3	2
	0	0	0
	1	0	2
%Alive	75	100	56.5

centrifugation–flotation (up to 52%) than with the method of Baermann (never more than 15%) with *Pratylenchus coffeae* and *Meloidogyne incognita*. In addition, Rodríguez-Kábana and King (1975) found good correlations between the two methods; they considered that the differences in extraction efficiency were due to dead nematodes, which were extracted by centrifugation–flotation and not by elutriation. During Study 2, we observed 62% of living *R. similis* (not stained with Meldola blue). Our study suggests that extraction efficiency depends not only on the proportion of dead individuals, but also on the proportion of immotile individuals (dead or alive) during the extraction processes.

Extraction losses depended to a great extent on the developmental stage of *R. similis* and they were three times higher for juveniles than for adult females. This result is consistent with observations of Verschor and De Goede (2000) who reported that they mostly lost nematodes smaller than 400 μm (the average size of juvenile stages 2 and 3 of *R. similis*). Reversat (1980) showed that motility and infectivity of juveniles (*Heteroder oryzae*) was closely linked with aging and physiological factors such as their lipid content. In our studies, we applied mixtures of *R. similis* differing in sex, age and lipid reserves; these physiological differences may have influenced the motility of individuals. Thus, for one nematode species, extraction efficiency may depend on many different features like the size and the physiological status of individuals.

Few publications have dealt with passive dissemination by rainfall of nematodes below the soil surface. Dennis et al. (1999, 2000) studied the ''leaching'' of *Steinernema carpocapsae* (Weiser) in different soils and tried to explain their results using models designed for pesticide leaching. As we did, they used soil cylinders coupled with a rainfall simulator. They simulated rainfalls of almost 200 cumulated millimetres, close to the lowest amount in our Study 2 (180 mm). They observed (i) low dissemination rates: 46–75% of the recovered population remained in the top 5 cm of the soil, (ii) 0.9–3.5% below −25 cm, and (iii) paradoxically, an even lower dissemination rate in sandy soil, which is more favourable to nematode movement (Wallace, 1958).

The nematode dissemination rate observed by Dennis et al. (1999, 2000) is nevertheless much higher than the one we observed with *R. similis* (Tables 5 and 9). These differences may be due to (i) to the characteristics of simulated rainfall applied and (ii) the characteristics of the nematodes. In the studies by Dennis et al. (1999, 2000) nematodes were extracted after 48 h, and these nematodes thus had three to eighteen times as long to move and disseminate actively than during our study. In addition, in our studies, rainfall intensities were 7 or 15 times higher (30 or 60 mm/h *vs*. 4 mm/h) and thus much more likely to generate greater leaching of the nematodes in the soil.

The size of infective juveniles of *S. carpocapsae* is quite similar to *R. similis* size: 438–650 μm in length and 20–30 μm in width for *S. carpocapsae* (Adams and Nguyen, 2002) *vs*. 458–720 μm in length and 17–27 μm in width for females of *R. similis* (Van Weerdt, 1958). As our simulated rainfalls generated water flows that were one to six times greater than those of Dennis et al. (1999, 2000), purely passive dissemination should have led to a much wider distribution in our cylinder. This was not the case, as deep displacement of *S. carpocapsae* was much higher in a Tennessee soil whose granulometry is close to the apparent granulometry of the nitisol in Martinique.

Dennis et al. (2000) tried to explain their results by sorption, using models designed to study sorption and leaching of pollutants (Banerjee et al., 1995; Baham and Sposito, 1994). But as nematodes can not enter soil aggregates (Wallace, 1968; Quénéhervé and Chotte, 1997; Bur and Robinson, 2004) and as the surface of nematodes is covered by a glycocalix (Bird, 2004) preventing their sorption by soil, this interpretation is not satisfying.

In contrast, the behaviour of nematodes could provide a better explanation: a nematode can escape leaching by entering closed capillaries or by adopting a shape that enables it to stick to a soil particle. After rainfall, nematodes may also try to flee from areas where soil moisture conditions are not optimal. Furthermore, *S. carpocapsae* is an entomopathogenic nematode which is much more active than the plant-parasitic *R. similis*. Thus, in the experiment of Dennis et al. (1999, 2000), not only was more time available for nematodes to disseminate actively, but this entomopathogenic nematode also seems to be more likely to explore its close environment. As a result, the behaviour of *S. carpocapsae* could explain both its wider dissemination in sandy loam soil than in horticultural sand (Dennis et al., 1999) and also its much wider dissemination in their soil cylinder under gentle leaching than the *R. similis* dissemination we observed with severe leaching.

This interpretation may also explain why, in our studies, the proportion of motile or living nematode decreased progressively with depth in the well-drained soil column in Study 1 (Table 7). A similar decrease in the percentage of living individuals was also observed during Study 2 in females and juveniles (Table 10). This effect was statistically significant; non-motile or dead nematodes are more likely to be carried downward than active or living ones. The fact that they could not adapt their behaviour to enable them to flee from leaching could explain these observations.

Few differences appeared among the distribution of *R. similis* after the different simulated rainfalls (Tables 5 and 9). This finding is not consistent with the hypothesis of main passive movement, which should have increased leaching

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with an increase in rainfall intensity or duration. However, the finding is consistent with the hypothesis of behaviour that leads the nematode to escape leaching: for a nematode which has entered a capillary that is narrow enough to avoid the risk of it being carried away, it does not make any difference whether the rainfall lasts 12 min or 12 h.

Very few nematodes were found in the buckets in the two studies, even after the heavy rainfalls in Study 2. During this study, 2.73% of the total number of *R. similis* collected came from one cylinder (Table 11). We may have measured an artefact (imperfect waterproofness of the silicone joint along the steel drum), but it is also possible that some nematodes fell through natural small fissures that already existed in fields. Anyway, in this case, 1.3% of the applied *R. similis* reached the bottom of the cylinder. Curiously, this cylinder was one of the three that was subjected to the smallest simulated rainfall; this observation, which is in agreement with the hypothesis of active fleeing from leaching, disagrees with that of purely passive leaching.

The hypothesis that a nematode like *R. similis* is able to escape leaching is completely new for a plant parasitic nematode. Such behaviour may result from a general adaptation process that favoured their survival and dissemination. The rooting system of banana plants is usually concentrated near the corm close to the soil surface: in the French West Indies, more than 80% of the root system is in the top 30 cm of the soil, very few roots are found below 50 cm (Delvaux and Guyot, 1989; Lecompte, 2002). Moreover, in recent plantations, newly emitted roots develop in the top soil layer. If individual *R. similis* passively carried down after accidental contamination (irrigation, run-off water) are not leached down into deeper layers of the soil, they will be more likely to infest banana roots. Thus, fleeing behaviour should have given *R. similis* a considerable advantage over other species likely to colonize the same niche.

5. Conclusion

The generally accepted idea that active dissemination of plant-parasitic nematodes is mainly passive is likely to be true at a large scale (above the scale of 1 km), but not at the scale of a decimetre. At such a small scale, plant-parasitic nematode behaviour may modify their dissemination and, if well adapted to their environment and host characteristics, may increase their foraging ability.

Passive dissemination of *R. similis* in lower layers of soils appears to be a marginal phenomenon, probably limited to ''disaster'' events at the nematode scale, such as falling down a soil macropore.

In the case of infestation of banana fields, studies should emphasise surface dissemination by run-off water and active dissemination from root to root. Even shallow ditches (30–40 cm depth) may be very helpful by channelling run-off water and thus preventing this method of dissemination (Chabrier, 2008). As far as active dissemination is concerned, more knowledge of nematode behaviour is needed to elucidate the various tactisms that could incite *R. similis* to migrate.

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