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Pathogenicity and reproductive fitness of *Pratylenchus coffeae* and *Radopholus arabocoffeae* on Arabica coffee seedlings (*Coffea arabica* cv. Catimor) in Vietnam

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Abstract The pathogenicity and reproductive fitness of *Pratylenchus coffeae* and *Radopholus arabocoffeae* from Vietnam on coffee (*Coffea arabica*) seedlings cv. Catimor were evaluated in greenhouse experiments. The effect of initial population densities (Pi=0, 1, 2, 4, 8, 16, 32, 64, 128, and 256 nematodes per cm^3 soil) was studied for both species at different days after inoculation (dai). The data were adjusted to the Seinhorst damage model $Y=m+(1-m).z^{Pi-T}$. Tolerance limit (T) for *P. coffeae* was zero for the height and the diameter of the coffee plants. For the diameter, the T-value for *R. arabocoffeae* was 25.6 for 30 and 60 dai and 12.8 for 90 and 120 dai. After 4 months T was zero. The low tolerance limits indicate that Arabica coffee is highly intolerant to both nematode species. At the end of the experiment (180 dai), all plants were infected and most were dead when inoculated with *R. arabocoffeae* at initial densities of 32, 64, 128 and 256 nematodes/cm^3 soil. For *P. coffeae* plant death was already observed at the lowest inoculation densities. Growth of coffee was reduced at all inoculation levels for both species. *Pratylenchus coffeae* and *R. arabocoffeae* caused intense darkening of the roots, leaf chlorosis and a strong reduction of root and shoot growth. It was observed that *P. coffeae* mainly destroyed lateral roots rather than tap roots, whereas *R. arabocoffeae* reduced tap root length rather than the lateral roots. At the lowest inoculum densities, the reproduction factor of *P. coffeae* was 2.38 and 2.01 for *R. arabocoffeae*, indicating that arabica coffee is a host for both species. Plant growth as expressed by shoot height and shoot and root weight measured 60 dai was negatively correlated with nematode (both species) density as expressed by the geometric mean of nematode numbers at 30 and 60 dai.

Keywords Isolates · Pathogenicity · Reproduction factor · Seinhorst model · Tolerance limit

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Introduction

In Vietnam, two species of migratory plant-parasitic nematodes are important on coffee (*Coffea arabica* and *C. canephora*), *viz. Pratylenchus coffeae* and *Radopholus arabocoffeae* (Phan 1976; Doan et al. 2000; Wiryadiputra and Tran 2008; Trinh et al. 2009). The first species is distributed worldwide and a major coffee pest in a number of countries in the Caribbean, Central America, Africa and Asia (Campos and Villain 2005; Souza 2008). The latter species was described from Vietnam (Trinh et al. 2004) and has, up to now, not been detected elsewhere.

Information on the damage potential of *P. coffeae* is mainly available for banana. On coffee, damage studies were only reported from Brazil (Silva and Inomoto 2002; Kubo et al. 2003; Inomoto et al. 2007); they demonstrated Arabica coffee (cvs Mundo Novo and Catuai) to be intolerant for *P. coffeae*. Data on damage caused by *R. arabocoffeae* are even more sparse and limited to small seedlings grown in small pots and field observations (Trinh et al. 2004). Arabica coffee was found to be more susceptible for *R. arabocoffeae* than for *P. coffeae*. Plants parasitized by *P. coffeae* are stunted and exhibit pronounced leaf chlorosis and root shedding (Inomoto et al. 1998; Kubo et al. 2003; Inomoto and Oliveira 2008). Several authors reported differences in pathogenicity between isolates of different geographical origin (Fallas et al. 1995; Mizukubo 1995; Hafez et al. 1999). There is no information available on the qualitative aspects of damage caused by *R. arabocoffeae* nor on differences in pathogenicity between isolates of this species.

Data on the reproduction fitness of *P. coffeae* on coffee are also rare. Silva and Inomoto (2002) reported the influence of cultivars on the reproduction of two populations from Brazil. Tomazini et al. (2005) demonstrated that the reproduction rate of *P. coffeae* was high in some genotypes of Robusta coffee but low in Arabica coffee cv. Mundo Novo. Sometimes reproduction of the isolates is low, suggesting that coffee is a poor host for some *P. coffeae* isolates (Kubo et al. 2003).

Yet, detailed information on the damage potential and reproductive fitness of plant-parasitic nematodes is of major importance for a sound decision on nematode control at planting or replanting of a crop, or when organising screening experiments for resistance. It was demonstrated that under controlled conditions for the majority of the nematode-host combinations, the pre-plant density of plant-parasitic nematodes is a good predictor of the yield reduction (Schomaker and Been 2006). Variability in reproductive fitness can influence the interpretation of screening for resistance experiments. Therefore, the reproductive fitness of the population used should be determined, eventually compared with other populations (De Waele and Elsen 2002).

In view of the lack of this information on the interaction between *P. coffeae* and *R. arabocoffeae* and *C. arabica* cv. Catimor we set up a series of pot experiments. In this paper we report on: (i) the effect of initial densities of both *P. coffeae* and *R. arabocoffeae* on the growth of coffee seedlings, and (ii) differences in pathogenicity and reproductive fitness of nematode isolates of *P. coffeae* and *R. arabocoffeae* on *C. arabica* cv. Catimor seedlings.

Materials and methods

Nematodes

Seven isolates of *P. coffeae* were collected from roots of coffee (*C. arabica* cv. Catimor or *C. canephora*) or wild bananas in the Centre and the Western Highland of Vietnam; the sites were located at a distance between 80 and 1,500 km. Four isolates of *R. arabocoffeae* were obtained from roots of coffee (*C. arabica* cv. Catimor or *C. canephora*) in the Western Highland; they originated from sites with a distance ranging between 80 to 300 km (Table 1). The coffee plants were between three and 10 years old; there was no information available on the age of the wild banana. All isolates were maintained on carrot discs (O'Bannon and Taylor 1968). When needed, nematodes were extracted from the carrot disks in a mistifier chamber (Seinhorst 1950).

Greenhouse experiments

Three greenhouse experiments were conducted from August 2008 to February 2009 at Western Highlands Agriculture and Forestry Science Institute (WASI), Buon Ma Thuot in Dak Lak province. During the experiments, daily minimum and maximum temperatures in the glasshouse ranged from 16.2 to 34.5°C;

Table 1 Locality of sampling for *Pratylenchus coffeae* and *Radopholus arabocoffeae* isolates in Vietnam

Nematode	Code	Host	Age of host (years)	Location (District/Province)
P. coffeae	P29	*C. arabica* cv. Catimor	3	Tay Hieu Plantation (Nghia Dan/Nghe An)
	P41	*C. canephora*	9	19/5 Plantation (Nghia Dan/Nghe An)
	P103	*C. arabica* cv. Catimor	5	D'lie Ya Plantation (Krong Nang/Dak Lak)
	P187	*C. canephora*	9	Dak Krong (Dak Doa/Gia Lai)
	P196	*C. canephora*	10	Viet Duc Plantation (Cu Kuin/Dak Lak)
	P2391	*C. canephora*	9	Chu Se Plantation (Chu Se/Gia Lai)
	P2399	Wild bananas	N/A	Ea Rang (M'drak/Dak Lak)
R. arabocoffeae	R89	*C. arabica* cv. Catimor	5	Kien Duc (Dak R'lap/DakNong)
	R103	*C. arabica* cv. Catimor	4	D'lie Ya Plantation (Krong Nang/Dak Lak)
	R187	*C. canephora*	9	Dak Krong (Dak Doa/Gia Lai)
	R2383	*C. arabica* cv. Catimor	5	715 Plantation (Md'rak/Dak Lak)

air humidity varied between 50 and 90%. Coffee (*C. arabica* cv. Catimor) seedlings were grown from seeds obtained from WASI. Seeds were surface sterilised (10% NaOCl for 3 min, followed by 4 washes in water) and germinated in sterile vermiculite. Just before two cotyledons had fully developed, seedlings were transferred individually to a 2,000 cm^3 black plastic bag filled with autoclaved (121°C, 1 h) soil (9.25% sand, 57.11% silt, 33.64% clay; 6.12% organic matter content; pH 4.4). The plants were kept until full development of two pairs of true leafs (2 months) before they were inoculated. Throughout the experiments the plants were fully randomised and kept under greenhouse conditions.

Experiment 1. Damage potential

One isolate of each *P. coffeae* (P103) and *R. arabocoffeae* (R103), both originating from Krong Nang, were used. For each nematode species, mobile stages (mix of juvenile and adult stages) were inoculated in three holes (3 cm from stem, 4 cm deep) with 10 ml water at densities of 0, 1, 2, 4, 8, 16, 32, 64, 128 and 256 nematodes/cm^3 soil. After inoculation the holes were covered with autoclaved soil. Each combination of nematode species-density was repeated five times. The plants were watered upon requirements. At monthly intervals, each plant was watered with 10 ml of a liquid fertiliser (NPK 7-4-6/1,000 ml).

Thirty, 60, 90, 120, 150 and 180 days after inoculation (dai), stem diameter and shoot height were measured non-destructively. The fresh root weight, shoot weight and final nematode densities (Pf) in both roots and soil were determined 6 months after inoculation. Nematodes were extracted from 250 cm^3 soil subsamples by sieving and decanting (Cobb 1918). Nematodes were extracted from the whole root system, using the maceration and sugar centrifugal-flotation method (Coolen and D'Herde 1972). The Pf were obtained after counting the nematodes with the aid of a dissecting microscope and adding up the nematode numbers obtained from both soil and roots.

Experiment 2. Pathogenicity of nematode isolates on Coffea arabica cv. Catimor

The pathogenicity of the seven *P. coffeae* and four *R. arabocoffeae* isolates was examined on *C. arabica* cv. Catimor grown in 2,000 cm^3 black plastic bags. Each coffee seedling was inoculated with 1 or 2 nematodes/cm^3 soil of mixed mobile stages of the isolates of one of both nematode species in 10 ml water (as described earlier). Control plants were treated similarly with 10 ml water. Seven, 14, 30 and 60 days after inoculation, five pots of each combination of nematode species-density were broken up and nematodes were extracted from root and soil (as described earlier). The nematode numbers were obtained after counting with the aid of a dissecting microscope and adding up the numbers obtained from both soil and roots. Each combination of nematode species-density was repeated twenty times.

Data analysis

Experiment 1 The damage potential, i.e. the influence of initial inoculum density on the plant growth was examined using Seinfit, a computer program (Viaene et al. 1997) estimating the equation developed by Seinhorst (1965). Multiplication factors (Rf) for the different inoculum densities were subjected to ANOVA; the *t*-test was used to compare differences between the two species for each inoculation density. A factorial ANOVA was used to examine main and interaction effects on plant growth (diameter and height) with nematode species, inoculation densities and observation times.

Experiment 2 The relationship between nematode population density and plant growth was analysed using the geometric mean of the nematode numbers at 30 and 60 dai. The interaction between growing parameters of coffee seedlings and the reproduction factor of nematode populations in both species was examined with multivariate analysis. The data were log-transformed. The effect of nematode populations for both species on plant growth and multiplication (Rf) was analysed with ANOVA. Differences of multiplication between two inoculum densities in the same population were analysed with a *t*-test. For all analyses Statistica 7 was used.

Results

Experiment 1. Damage potential

The relationship between the initial densities of *P. coffeae* and *R. arabocoffeae* and the growth parameters of *C. arabica* cv. Catimor plants (height and diameter) at a series of observations between 30 and 180 days after inoculation (dai) fitted the Seinhorst model (Table 2). For *P. coffeae*, the tolerance limit (T) equalled zero for both height and diameter at all observations. For *R. arabocoffeae* the T-value for plant height was 25.6 nematodes/cm³ one month after inoculation and equalled zero in the period between 60 and 180 dai. The T-value for plant diameter was 25.6 for 30 and 60 dai and 12.8 for 90 and 120 dai; it equalled 0 at 150 and 180 dai.

The values for *z* of the models fitted to the *P. coffeae* data were all lower than those of the models fitted to the *R. arabocoffeae* data for both growth parameters and at all observations. In the model obtained for *P. coffeae*, the *m* value varied from 0.41 to 0.55 and from 0.43 to 0.53 for height and diameter of coffee, respectively. For *R. arabocoffeae*, the *m* values ranged between 0.48 and 0.61 for height and 0.46 and 0.62 for diameter. For each of the species and for both diameter and height of plant, the lowest *m* values were observed at 180 dai. *Pratylenchus coffeae* caused the highest reduction of plant height (y_m.m) at all but one (30 dai) observations; the effect on plant diameter was less species linked.

The influence of the inoculum densities of both species on the number of green leaves was not significant. However, the size of the leaves was smaller with inoculated plants compared with nematode-free seedlings (data not shown). The multivariate analysis of the plant parameters (height and diameter) showed there were interactions between inoculation density, nematode species and the time after inoculation (Table 3). For both plant height and plant diameter, the species had the greatest effect. Plant diameter was equally influenced by observation time and inoculation density; plant height was importantly affected by the interaction species×observation time.

The relationship between the initial densities of *P. coffeae* and *R. arabocoffeae* with the fresh root, shoot and total weight of *C. arabica* cv. Catimor plants observed 180 dai fitted the Seinhorst model very well ($R^2 \geq 0.86$) (Table 4). The T-values of the models for both species equalled 0. The *z*-values of the model obtained for *P. coffeae* weights at 180 dai were lower than those calculated for *R. arabocoffeae*. The *m* value for all observed plant parameters was always lower for *P. coffeae* than for *R. arabocoffeae*. As a consequence, *P. coffeae* caused the highest reduction of plant weight (y_m.m).

Irrespective the inoculum density, *R. arabocoffeae* destroyed the tap root of the coffee seedlings more than *P. coffeae*. *Radopholus arabocoffeae* was less destructive on lateral roots then *P. coffeae* (Figs. 1, 2). The destruction increased in severity with increasing inoculum density. At inoculations of 16 nematodes/cm³ soil or fewer, *R. arabocoffeae* did not result in plant death; inoculations with *P. coffeae* were so

Table 2 Parameter estimates of the Seinhorst model of plant growth of *Coffea arabica* cv. Catimor grown in 2,000 cm^3 pots, at 30 to 180 days after inoculation with *Pratylenchus coffeae* and *Radopholus arabocoffeae* at ten densities from 0 to 256 nematodes per cm^3 soil

Species	Growing parameters	Days after inoculation	Parameter[a]				$y_m.m$	R^2
			m	T	z	y_m		
P. coffeae	Height	30	0.55	0	0.76	40	22.0	0.79
		60	0.46	0	0.82	50.8	23.4	0.84
		90	0.46	0	0.78	54.4	25.0	0.81
		120	0.45	0	0.72	59.2	26.6	0.81
		150	0.44	0	0.69	60.7	26.7	0.80
		180	0.41	0	0.62	66	27.1	0.81
	Diameter	30	0.53	0	0.52	4.1	2.2	0.83
		60	0.53	0	0.31	5.3	2.8	0.77
		90	0.50	0	0.51	6.3	3.2	0.83
		120	0.49	0	0.45	7.1	3.5	0.72
		150	0.46	0	0.53	7.8	3.6	0.78
		180	0.43	0	0.75	8.5	3.7	0.81
R. arabocoffeae	Height	30	0.61	25.6	0.89	34.7	21.2	0.74
		60	0.55	0	0.83	50.8	27.9	0.76
		90	0.53	0	0.83	54.4	28.8	0.81
		120	0.51	0	0.83	59.2	30.2	0.82
		150	0.50	0	0.86	60.7	30.4	0.83
		180	0.48	0	0.84	66	31.7	0.79
	Diameter	30	0.62	25.6	0.93	3.7	2.3	0.64
		60	0.57	25.6	0.94	4.6	2.6	0.70
		90	0.49	12.8	0.95	5.9	2.9	0.82
		120	0.47	12.8	0.94	6.6	3.1	0.83
		150	0.46	0	0.94	7.8	3.6	0.83
		180	0.46	0	0.95	8.5	3.9	0.88

[a] The Seinhorst model is of the form: $Y=y_m$ for $Pi \leq T$ and $Y=y_m.m+y_m.(1-m).z^{Pi-T}$ for $Pi>T$; y_m=yield without nematode damage, m=a constant so that $y_m.m$ equals the minimum yield, z=parameter determining the slope of the curve, T=damage threshold density (nematodes per plant at inoculation)

Table 3 Significance of main and interaction effects of variables for growth parameters of *Coffea arabica cv. Catimor* as influenced by inoculations with increasing densities (1–256 nematodes/cm^3) of *Pratylenchus coffeae* and *Radopholus arabocoffeae*

Source of variation	Growing parameters			
	Diameter		Height	
	F test	*P*	*F test*	*P*
Species	607.17	0.000000	91101.04	0.000000
Time	420.61	0.000000	33.96	0.000000
Inoculation	443.16	0.000000	118.32	0.000000
Species * Time	4.07	0.001263	1144.30	0.000000
Species * Inoculation	38.72	0.000000	2.21	0.051996
Time * Inoculation	12.01	0.000000	15.48	0.000000
Species * Time * Inoculation	0.88	0.699571	11.87	0.000000

Table 4 Parameter estimates of the Seinhorst model of plant growth of *Coffea arabica* cv. Catimor, grown in 2,000 cm^3, 180 days after inoculation with *Pratylenchus coffeae* or *Radopholus arabocoffeae* at ten densities from 0 to 256 nematodes per cm^3 soil

Species	Weight	Parameters [a]				$y_m.m$	R^2
		m	T	z	y_m		
P. coffeae	Shoot	0.07	0	0.53	66.6	4.66	0.96
	Root	0.03	0	0.23	34.48	1.03	0.99
	Total	0.06	0	0.42	101.08	6.06	0.97
R. arabocoffeae	Shoot	0.20	0	0.86	66.6	13.32	0.86
	Root	0.11	0	0.49	34.48	3.79	0.90
	Total	0.19	0	0.75	101.08	19.20	0.86

[a] The Seinhorst model is of the form: $Y=y_m$ for $Pi \leq T$ and $Y=y_m.m+y_m.(1-m).z^{Pi-T}$ for $Pi > T$; y_m=yield without nematode damage, m=a constant so that $y_m.m$ equals the minimum yield, z=parameter determining the slope of the curve, T=damage threshold density (nematodes per plant at inoculation)

destructive that plant death was observed even at the lowest nematode density (1 nematode/cm^3 soil).

The reproduction for both species was highest at the lowest initial densities. At initial densities≥2 for *R. arabocoffeae* and 4 nematodes/cm^3 soil for *P. coffeae*, the reproduction factor dropped below zero. The reproduction factor decreased further with increasing initial density (Table 5). Except for the initial densities 64, 128 and 256 nematodes/cm^3 there was a significant difference (F=353.6, P=0.041*) in Rf between both species. The Rf of *P. coffeae* was greater than the one obtained for *R. arabocoffeae* at initial densities of 1 and 2 nematodes/cm^3. The opposite was observed for *R. arabocoffeae* at higher densities (4 to 32 nematodes/cm^3).

Experiment 2. Pathogenicity and reproduction of nematode isolates

The multivariate analysis for both inoculum densities, considering all plant growth parameters (height of shoot, weight of root and shoot) and the reproductive factors of *R. arabocoffeae* and *P. coffeae* isolates, indicated significant influences by isolates, moment of observation and interaction between both (Table 6). There were no significant differences in plant growth parameters and Rf between the results registered at 7 and 14 dai; differences started at 30 dai (P<0.05) for the Rf.

Fig. 1 Pot experiment showing the effect of increasing pre-plant densities of *Pratylenchus coffeae* on coffee seedlings. Densities from right: 0, 1, 2, 4, 8, 16, 32, 64, 128, and 256 individuals per cm^3 soil

Fig 2 Pot experiment showing the effect of increasing pre-plant densities of *Radopholus arabocoffeae* on coffee seedlings. Densities from right: 0, 1, 2, 4, 8, 16, 32, 64, 128, and 256 individuals per cm^3 soil

Table 5 Reproduction of *Pratylenchus coffeae* and *Radopholus arabocoffeae* on *Coffea arabica* cv. Catimor seedlings in 2,000 cm^3 pots under greenhouse conditions in the Western Highland in Vietnam

Inoculation densities	Reproduction factor (Rf)	
Nematodes/cm^3 soil	*P. coffeae*	*R. arabocoffeae*
0	–	–
1	2.380 a*	2.013 a[a]
2	1.010 b*	0.894 b
4	0.369 c*	0.399 c
8	0.181 d*	0.211 d
16	0.089 e*	0.108 e
32	0.027 ef*	0.034 f
64	0.014 f	0.009 f
128	0.009 f	0.005 f
256	0.005 f	0.004 f

Data are arithmetic mean of 5 replications. [a] Means in the same column followed by the same letter do not differ according to Duncan test ($P \leq 0.05$). *Indicates significant differences between the two species at the same inoculum density ($P < 0.05$)

Intra-specific differences in plant growth parameters and Rf were observed between isolates of *P. coffeae* and *R. arabocoffeae* 60 dai (Table 7). For *P. coffeae*, there were also significant differences in plant growth parameters and Rf between the isolates and those of non-inoculated plants for both inoculum densities (2,000 nematodes/pot: shoot height: F=30.4, df=7, P=0.00; shoot weight: F=12.3, df=7, P=0.00; root weight: F=4, df=7, P=0.003; Rf: F=56, df=6, P=0.000; 4,000 nematodes/pot: shoot height: F=33.5, df=7, P=0.00; shoot weight: F=59.8, df=7, P=0.00; root weight: F=17.4, df=7, P=0.00; Rf: F=21.8, df=6, P=0.000). The plant growth parameters for the non-inoculated plants were always significantly higher than those of plants inoculated with *P. coffeae.* Isolate P2399 from wild banana caused the lowest damage; the greatest damage was observed for isolate P41 from Robusta coffee.

As for *P. coffeae* there were also differences for *R. arabocoffeae* in plant growth parameters and Rf between the isolates and non-inoculated plants for both inoculum densities (2,000 nematodes: shoot height: F=45.256, df=4, P=0.00; shoot weight: F=12.532, df=4, P=0.00; root weight: F=34.291, df=4,

Table 6 Significance of main and interaction effects of variables for shoot height, weight (shoot and root) and reproduction factor of *Coffea arabica* cv. Catimor caused by *Radopholus arabocoffeae* and *Pratylenchus coffeae*

Species	Source of variation	Inoculation					
		2,000/cm^3 soil			4,000/cm^3 soil		
		F	df	P	F	df	P
P. coffeae	Nematodes population	139.8	28	0.00*	160.9	28	0.00*
	Date	141.2	12	0.00*	131.2	12	0.00*
	Nematodes population*Date	5.9	84	0.00*	5	84	0.00*
R. arabocoffeae	Nematodes population	137.4	20	0.00*	205.4	20	0.00*
	Date	101.8	12	0.00*	126.4	12	0.00*
	Nematodes population*Date	4.5	60	0.00*	5.8	60	0.00*

P=0.00; Rf: F=13.408, df=3, P=0.00; 4,000 nematodes/pot: shoot height: F=31.614, df=4, P=0.00; shoot weight: F=29.831, df=4, P=0.00; root weight: F=46.933, df=4, p=0.00; Rf: F=4.609, df=4, P=0.00. The plant growth parameters for the non-inoculated plants were always significantly higher than those of plants inoculated with *R. arabocoffeae*. The reproduction factor of *R. arabocoffeae* isolate R103, which is the type population for the species, was the greatest of all *R. arabocoffeae* isolates at both inoculation levels. The plant growth parameters obtained for this isolate are the lowest among the *R. arabocoffeae* isolates.

There was a negative correlation between the density of the nematode isolates (as expressed by the geometric mean of nematode numbers at 30 and 60 dai) and the shoot height and shoot and root weight measured at 60 dai (Fig. 3, R^2=0.89–0.98 for *P.*

Table 7 Effect of seven *Pratylenchus coffeae* and four *Radopholus arabocoffeae* isolates on fresh root and shoot weight, shoot height of *Coffea arabica* cv. Catimor and their reproduction factor at 60 days after inoculation

	Populations	Nematode inoculation number							
		2,000 nematodes/pot				4,000 nematodes/pot			
		Shoot height (cm)	Shoot weight (g)	Root weight (g)	Reproductive factor	Shoot height (cm)	Shoot weight (g)	Root weight (g)	Reproductive factor
Control		49.7 aA	18.4 aA	5.56 aA	–	49.7 aA	18.4 aA	5.56 dA	–
P. coffeae	P29	39.7 cd	14.4 cd	3.86 bc	1.34 b	37.9 d	11.9 cd	3.38 cde*	1.02 bc*
	P41	36.7 e	13.7 d	3.62 c	1.47 a	36.2 d	10.5 e*	3.14 e*	1.10 a*
	P103	38.24 de	13.9 d	3.88 bc	1.42 a	36.8 d	11.6 d*	3.24 bc*	1.05 b*
	P187	41.6 bc	15.3 bc	4.26 bc	1.18 c	38.4 cd*	12.7 bc*	3.62 bcde*	0.98 cd*
	P196	41.9 bc	15.3 bc	4.12 bc	1.19 c	40.4 bc	13.3 b*	3.74 bcd*	0.971 d*
	P2391	41.1 bc	15.7 bc	4.22 bc	1.07 d	40.7 b	13.4 b*	3.86 bc*	0.94 d*
	P2399	42.6 b	16.3 b	4.46 b	1.08 d	41.3 b	13.6 b*	4.08 b	0.89 e*
R. arabocoffeae	R89	42.1 C	14.4 C	4.1 C	1.16 B	40.5 B	12.9 BC*	3.48 BC*	0.98 AB*
	R103	39.4 D	14.3 C	3.94 C	1.37 A	37.7 C	11.9 C*	3.22 C*	1.05 A*
	R187	44.6 B	16.1 B	4.62 B	0.98 C	41.6 B*	14.2 B*	3.82 B*	0.90 B*
	R2383	43.1 BC	15.1 BC	4.24 C	1.04 BC	40.6 B*	12.9 BC*	3.6 BC*	0.95 B

[A] Data are arithmetic mean of 5 replications. Means in the same column followed by the same letter do not differ according to Duncan test ($P \leq 0.05$). *Show significant differences between reproduction factors of the same isolate in different inoculum densities following t-test ($P \leq 0.05$)

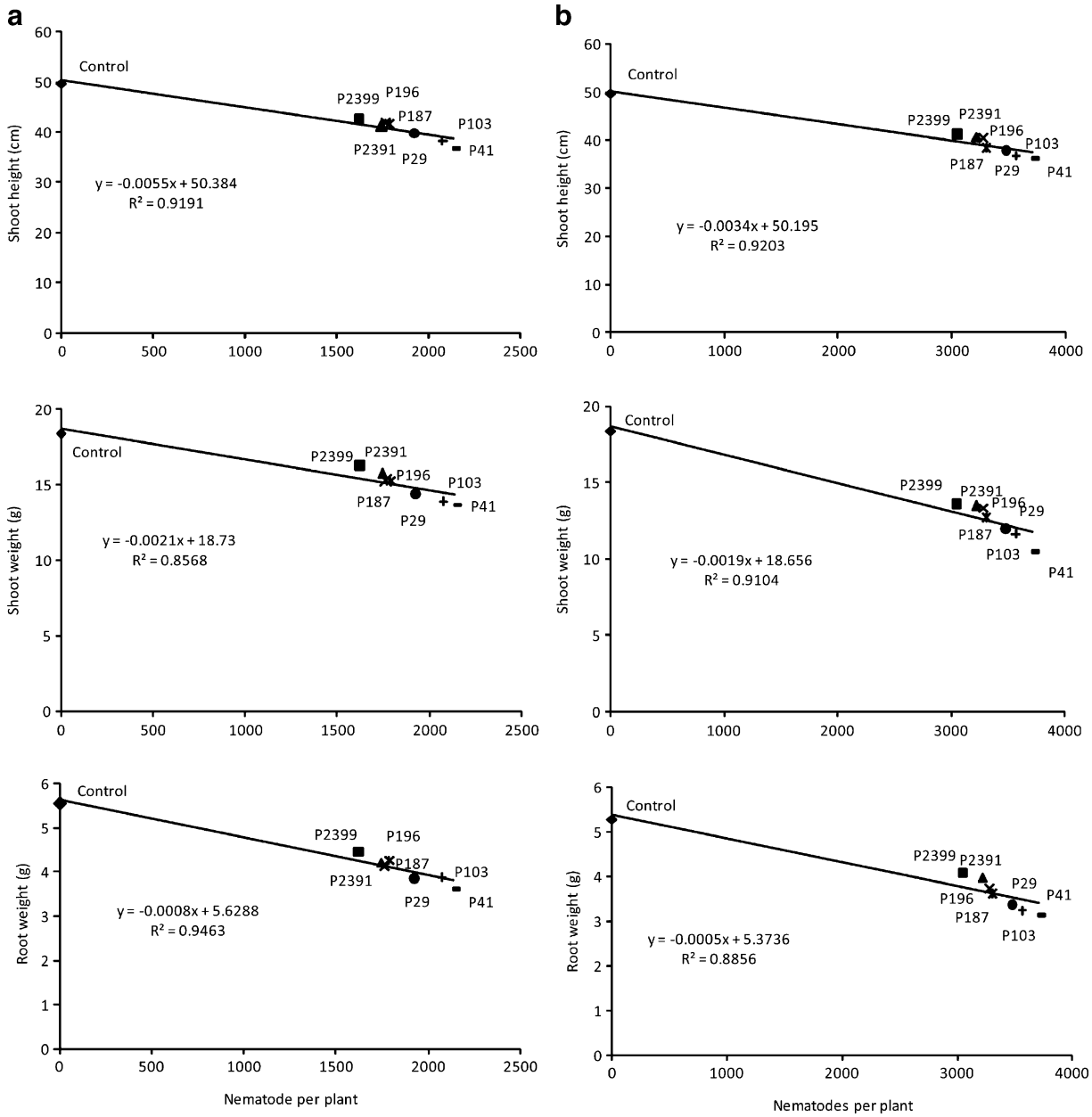

Fig. 3 Relationship between the geometric mean of nematode numbers per plant at 30 and 60 days after inoculation with 1 (**a**) and 2 (**b**) *Pratylenchus coffeae* per cm^3 and plant growth (shoot height, shoot and root weight) at 60 dai of *C. arabica* cv. Catimor. Each dot represents an isolate of which the code and full details are mentioned in Table 1

coffeae; Fig. 4, R^2 = 0.85–0.94 for *R. arabocoffeae*). For both inoculation densities, the slope of the regression model obtained for the shoot weight and root weight is similar for both species. For shoot height the slope is slightly steeper for the 2,000 nematodes/pot than for the 4,000/pot inoculation for both *P. coffeae* and *R. arabocoffeae*.

Discussion

The results of our experiments clearly confirm that both *P. coffeae* and *R. arabocoffeae* do multiply and cause damage on *C. arabica* cv. Catimor seedlings. However, these two characteristics of the interaction between the nematodes and the host differ between

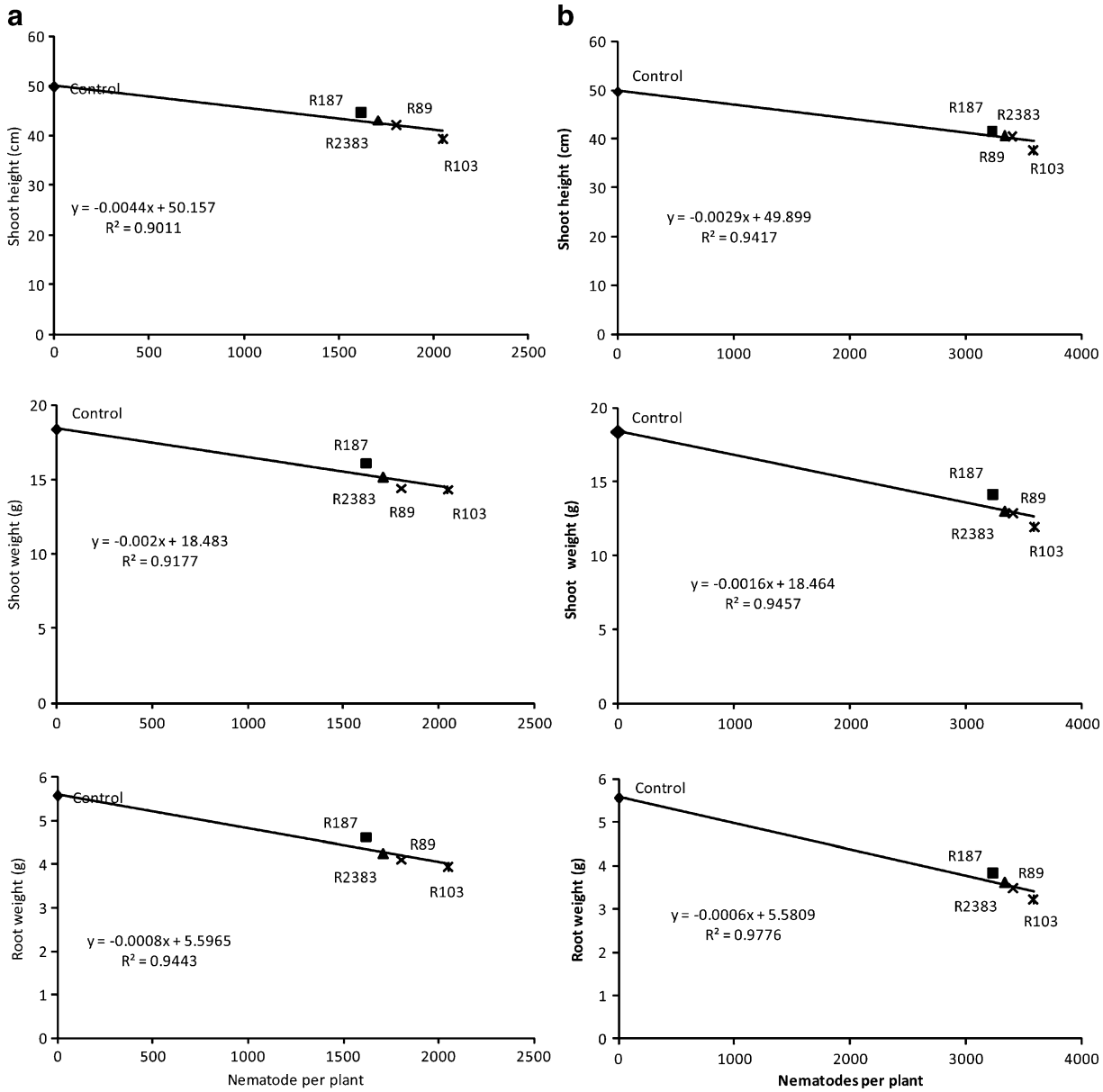

Fig. 4 Relationship between the geometric mean of nematode numbers per plant at 30 and 60 days after inoculation with 1 (**a**) and 2 (**b**) *Radopholus arabocoffeae* per cm^3 and plant growth (shoot height, shoot and root weight) at 60 dai of *C. arabica* cv. Catimor. Each dot represents an isolate of which the code and full details are mentioned in Table 1

the nematode species and their isolates. Generally isolates of *P. coffeae* were more pathogenic than isolates of *R. arabocoffeae*. The damage caused by the nematodes showed remarkable differences between the species. *Pratylenchus coffeae* mainly destroyed lateral roots rather than tap roots, whereas *R. arabocoffeae* reduced tap root length rather than the lateral roots.

The tolerance limit T of the Seinhorst damage equation we obtained for *R. arabocoffeae* varied in time. The T-value varied between 25.6 nematodes/cm^3 and zero depending on the time of observation. T equalled zero for *P. coffeae* at all observations. Obviously, *P. coffeae* affects the plant growth very rapidly, whereas the damage caused by *R. arabocoffeae* is visible only after some time. This difference in

dynamics of damage might be explained by the differences in site of destruction between the two species. Apparently, early reduction of the lateral roots has a greater impact on plant growth than does the reduction of the tap root. Lateral roots of coffee plants play an important role for nutrient uptake and subsequent growth of the plant (Charrier and Eskes 2004). Previous studies quantifying the damage caused by *P. coffeae* on coffee seedlings, showed the T-value to be zero for the usual growing parameters (weight and length of below and above ground plant parts) (Kubo et al. 2003; Trinh 2003). T-values equal to zero indicate that the lowest nematode densities already affect plant growth. The *z*-value of the Seinhorst's model is an indicator of the activity of the nematodes; the smaller the *z*, the smaller the yield (Schomaker and Been 2006). The models we obtained for all the different growth parameters all had smaller *z*-values for *P. coffeae* than for *R. arabocoffeae*.

In their experiments comparing the pathogenicity of both species, Trinh et al. (2004) obtained data suggesting a greater pathogenicity and reproduction potential for *R. arabocoffeae* than for *P. coffeae*. This is the contrary of the observation we report here. This kind of difference can be explained by differences in pathogenicity and reproduction of nematodes used, differences in temperature and soil type (Moens and Perry 2009) and the effect of growing medium, inoculum densities, exposure time and pot volume (Moens et al. 2003). The pot size and age of seedling markedly differed between the two experiments (bigger pot size and older coffee seedlings). This demonstrates the importance of the use of appropriate methods when quantifying the effect of plant-parasitic nematodes on the host.

Several studies demonstrated the variability in pathogenicity and reproductive fitness of *P. coffeae* on coffee. Since the first report of a species complex in Japan (Mizukubo 1992), the *P. coffeae* species complex problem has been a worldwide topic of research (Orui 1996; Mizukubo and Sano 1997; Duncan et al. 1999; Waeyenberge et al. 2000). Comparing the pathogenicity and reproduction ability of two Brazilian populations on coffee, Inomoto et al. (2007) obtained differences in pathogenicity and reproductive fitness that were so important that the populations might be distinct species. These populations also had different host ranges (Silva and Inomoto 2002). The reproduction rate of *P. coffeae* depends on the host plant; it was high in some genotypes of Robusta coffee and low in Arabica coffee cv. Mundo Novo (Tomazini et al. 2005). Based on their morphology and morphometrics but also on molecular data (Trinh et al., in prep.) all the isolates used in our study belong to *P. coffeae sensu stricto*. Hence, the variation on which we report in this study is intra-specific. Intra-specific variation in *R. arabocoffeae* was observed for the first time. All populations used in this study fitted the original description of the species.

As the inoculation densities of both *P. coffeae* and *R. arabocoffeae* increased, the Rf gradually decreased and the lowest Rf was observed after the highest inoculation density. The decline of the two species at high Pi most likely resulted from reduced food supply for the nematodes because of poor plant growth (Di Vito et al. 2002). Species belonging to the Pratylenchidae reduce the root mass, rendering plants incapable of supporting large nematode populations (Loof 1991).

Plant growth as expressed by shoot height and shoot and root weight measured 60 dai was negatively correlated with nematode (both species) density as expressed by the geometric mean of nematode numbers at 30 and 60 dai. The growth of the different isolates was not extremely different. Probably, the time between inoculation and observation (60 days) was too short to allow for the observation of larger differences between isolates. Nevertheless, the data suggest that differences in pathogenicity in both nematode species are caused by differences in the numbers of nematodes parasitizing the host. Fallas et al. (1995) had come to a similar conclusion for *R. similis* on banana. Our isolates originated from different coffee species and fields. Both factors probably have influenced the differences in pathogenicity and reproduction. Previous research has demonstrated differences in pathogenicity between *Pratylenchus* populations of different origin, e.g. *P. vulnus* on peach almond hybrids and apple rootstocks (Pinochet et al. 1993), *P. coffeae* on sweet potato (Mizukubo and Sano 1997) and *P. neglectus* on potato (Hafez et al. 1999). Similar results were obtained for populations originating from different hosts, e.g. *R. similis* on banana (Hahn et al. 1996).

We can conclude that Vietnamese isolates of *P. coffeae* and *R. arabocoffeae* are highly pathogenic and able to multiply on Arabica coffee cv. Catimor. Their effect on plant growth is prevalent even at low

initial densities. Isolates of *P. coffeae* and *R. arabocoffeae* differ in their reproductive fitness, which is linked to their pathogenicity. Therefore we suggest that sampling for nematodes should be carried out carefully before planting or re-planting of *C. arabica* cv. Catimor.

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