Host range characterisation, *in vivo* reproduction and damage potential of *Pratylenchus coffeae* populations from Vietnam

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Summary. The *in vivo* reproduction of ten *Pratylenchus coffeae* populations, collected in different agro-ecological regions in Vietnam, on 13 agricultural crops was very similar. Of the 13 varieties of the 13 crops included in our study (one variety per crop), the varieties of banana, sugarcane, maize and upland rice were good hosts of *P. coffeae*. The soybean variety was a poor host and the varieties of groundnut, tomato, sweet potato, ginger, sesame, pineapple and citrus were very poor hosts or nonhosts of *P. coffeae*. The *in vivo* damage potential on the banana, coffee, sugarcane and maize varieties was very similar for all ten *P. coffeae* populations. All the *P. coffeae* populations were able to cause considerable damage to the vegetative growth of banana and coffee but not to sugarcane and maize. In view of the low reproduction on coffee, the extensive damage the *P. coffeae* populations caused on this agricultural crop is surprising and illustrates the high damage potential of *P. coffeae* on coffee. In general, there was similar *in vivo* reproduction on the 13 agricultural crops examined and in general, similar *in vivo* damage potential on banana, coffee, sugarcane and maize, indicating that the ten *P. coffeae* populations from Vietnam examined belong to the same pathotype.

Key words: biodiversity, damage, host range, plant growth, reproduction factor, root-lesion nematode, root necrosis.

Pratylenchus coffeae is one of the root-lesion nematodes that are considered important plant pathogens. This migratory endoparasite has a pantropical distribution and a wide host plant range (Siddiqi, 1972; Castillo & Vovlas, 2007). Over the years, when more and more *P. coffeae* populations were studied, differences in host range, *in vivo* reproduction and damage potential on agricultural crops among some of these populations were observed.

Populations of this species were found as an important pathogen of yams in Uganda and the Pacific but did not invade the surrounding banana plants. By contrast, in Ghana, a population of *P. coffeae* was found that damaged both yams and plantains (Bridge *et al*., 1997). Edwards & Wehunt (1973) demonstrated that *P. coffeae* populations from Panama can infect maize but those from Honduras did not. Silva & Inomoto (2002) reported that different populations *of P. coffeae* can have different host ranges and suggested the existence of biotypes of *P. coffeae* based on differences in *in vivo* reproduction on coffee and citrus between two *P. coffeae* populations. Other studies on bananas (Bridge *et al*., 1997), on sweet potato (Mizukubo, 1995; Mizukubo & Sano, 1997) and on coffee (Kubo *et al*., 2003; Villain *et al*., 2002 cited by Campos & Villain, 2005; Inomoto *et al*., 2007) showed differences in damage potential among *P. coffeae* populations originating from different geographical regions. Inoculation of seven different

host plants with a *P. coffeae* population originally isolated from coffee revealed differences in *in vivo* reproduction and damage potential (Kumar & Viswanathan, 1972). Based on the *in vivo* reproduction of *P. coffeae* populations on different agricultural crops and on susceptible and resistant sweet potato cultivars, Mizukubo (1995) suggested the presence of physiological races among Japanese *P. coffeae* populations.

In Vietnam, *P. coffeae* has been reported as the most common and widespread *Pratylenchus* species (Chau & Thanh, 2000). In this country, it is found on many crops including banana, coffee, ginger, sugarcane, pineapple, *etc*. (Chau *et al*., 1997). Although it is known that *P. coffeae* can cause considerable damage to several agricultural crops worldwide (Bridge *et al*., 1997), its impact on agricultural crops in Vietnam is largely unknown. In fact, only damage caused by *P. coffeae* on bananas (van den Bergh *et al*., 2006) and coffee, in relation to yellow-leaf disease (Nghi *et al*., 1996; Trung *et al*., 2000; Sung *et al*., 2001), has been studied.

The characterisation of intraspecific differences with respect to the host range, *in vivo* reproduction and damage potential on agricultural crops among populations of the same nematode species is very important for the development of efficient and sustainable nematode management strategies such as crop rotation (Bakker *et al*., 1993). In addition, this characterisation provides additional information on the biodiversity of this nematode species. Therefore, the objectives of our study were: *i*) to establish the host range of ten *P. coffeae* populations collected from different agro-ecological regions in Vietnam; and *ii*) to compare the *in vivo* reproduction and damage potential of these *P. coffeae* populations on selected agricultural crops. The description of these agro-ecological regions (Tuyet *et al*., 2008), the morphological, morphometrical and molecular characterisation of the *P. coffeae* populations (Tuyet *et al*., 2012, 2014), and the effect of temperature on their *in vitro* reproduction (Tuyet *et al*., 2013) have been reported previously.

MATERIALS AND METHODS

Host range experimental set-up. To study the host range of the ten *P. coffeae* populations from Vietnam (Table 1), 13 agricultural crops commonly grown in Vietnam were included in four glasshouse experiments: soybean (*Glycine max* (L.) Merr. var. VX93), groundnut (*Arachis hypogea* L. var. V79), tomato (*Solanum lycopersicum* L.), banana (*Musa* cv. Ngop Dui Duc BBB), sweet potato (*Ipomoea batatas* (L.) Poir. cv. Hoang Long), coffee (*Coffea arabica* L. var. Catimor), ginger (*Zingiber officinali* Rosc. cv. Rose), pinapple (*Ananas comosus* (L.) Merr. cv. Cayen), sesame (*Sesamum orientale* L. var. V67), upland rice (*Oryza sativa* L. var. CIRAD141), sugarcane (*Saccharum officinarum* L. var. ROC20), maize (*Zea mays* L. var. LVN10) and citrus (*Citrus nobilis* Lour. var. nobilis).

Seedlings were grown in plastic pots, each of which contained 2,000 ml of a sterilised compost mixture of a sandy alluvial soil: composted manure: rice chaff (6:1:2). For coffee and citrus, seeds were first sown in trays containing sterilised sand and seedlings with two fully expanded cotyledons were individually transplanted to the plastic pots. For banana, sugarcane and pineapple, *in vitro* produced plantlets were first transplanted to trays containing sterilised sand. After 4 weeks (for banana and sugarcane) and 8 weeks (for pineapple), the plantlets were individually transplanted to the plastic pots. For ginger, ginger tubers were first planted into trays containing sterilised sand. After 1 month, uniformly sized plantlets were individually transplanted to the plastic pots. For sweet potato, three-node stem cuttings were individually planted in the plastic pots. For maize, sesame, tomato, upland rice, groundnut and soybean, seeds were directly sown in plastic pots: one seed for maize, tomato, groundnut and soybean, two seeds for sesame and five seeds for upland rice. The plants were watered as needed.

The banana, pineapple and sugarcane plantlets were inoculated 4 weeks after transplanting. The sweet potato plantlets were inoculated 10 days after planting of the nodal cuttings. Maize, sesame, tomato and rice seedlings were inoculated 2 weeks after emergence of the seeds. The groundnut and soybean seedlings were inoculated 10 days after the emergence of the seeds. The coffee plants were inoculated 8 months after sowing when the seedlings had 8-10 leaves (1st experiment) and 2 months after sowing when two leaves were expanded (2nd experiment). The citrus plantlets were inoculated 6 weeks after emergence of the seeds.

During the duration of the experiments, the soil temperature was measured. A thermometer was put 8 cm deep in the soil in the pots and the temperature recorded at the hottest time of the day, *i.e.*, between 14:00 and 16:00. The monthly minimum and maximum soil temperature in the glasshouse ranged from 20.0 to 32.5°C.

The experiments were designed as completely randomised blocks with nine replicates for experiment 1 and six replicates for the experiments 2, 3 and 4. Each pot was considered a replicate. In the experiments 2 and 3, the banana plants were

used as the reference (control) host plant to confirm the viability of the inoculum and the effectiveness of the inoculation.

Damage potential experimental set-up. To study the damage potential of the *P. coffeae* populations from Vietnam, four agricultural crops commonly grown in Vietnam were included in four experiments: banana (*Musa* cv. Ngop Dui Duc BBB), coffee (*C. arabica* var. Catimor), sugarcane (*S. officinarum* var. ROC20) and maize (*Z. mays* var. LVN10). The preparation of the seedlings as well as the time of inoculation and the experimental conditions were similar to those described for the host range experiments.

The experiments were designed as completely randomised blocks with seven replicates for banana, five replicates for coffee and six replicates for sugarcane and maize.

Preparation of nematode inoculum and inoculation. The nematode inoculum was obtained from *in vitro* carrot disc cultures (Moody *et al*., 1973; Speijer & De Waele, 1997; Tuyet *et al*., 2012) with vigorous developing nematode populations (many active nematodes in the Petri dish). Nematodes were extracted from the carrot discs by the maceration-sieving technique as described by Speijer & De Waele (1997). Suspensions of all vermiform developmental stages were used as inoculum for the experiments. Each pot was inoculated with 1,000 vermiforms. Three holes were made in the soil around every plant, 5 ml of the nematode suspension was added with a pipette and the holes were recovered with soil.

Assessment of the nematode reproduction. Fourteen weeks after inoculation, the plants were removed from the pots, and the entire root systems and 200 ml of soil sampled. The nematode population densities were assessed both in the soil and roots. For the extraction of the nematodes from the plant roots, roots were cut into 1-cm-long pieces and macerated in a kitchen blender for 30 s (10-s periods separated by 5-s intervals). The suspension was passed through 260, 106 and 40 µm-pore sieves, rinsed with tap water and the nematodes from the 40 µm-pore sieve collected and counted using a stereomicroscope. For the extraction of the nematodes from the soil, the modified Baermann dish method was used (Hooper *et al*., 2005). Two hundred ml of soil was placed on a sieve in a dish containing 300 ml of distilled water and left at room temperature for 48 h. Then the suspension in the dish was collected and the nematodes counted using a stereomicroscope. The number of nematodes in the soil in the pots was determined based on the number of nematodes in 200 ml of soil.

Assessment of the host plant range. The final nematode population density (Pf) was calculated as the number of vermiform nematodes in both root and soil. The reproduction factor (RF = Pf/Pi) was used to determine the host plant response to *P. coffeae*. When RF > 1 the host plant was considered a good host. When RF < 1 but > 0.5 the host plant was considered a poor host. When RF < 0.5 the host plant was considered a nonhost (Pinochet & Duarte, 1986; Robinson & Percival, 1997; Bell & Watson, 2001).

Assessment of the damage potential. To assess the damage potential of the *P. coffeae* populations from Vietnam on banana, coffee, sugarcane and maize, plant height, shoot and root fresh weights of plants inoculated with the *P. coffeae* populations and uninoculated plants were recorded at 14 weeks after inoculation. For banana, the root necrosis was determined by following the methodology of Speijer & De Waele (1997). Five 10 cm-long pieces of roots were collected randomly and cut lengthwise. The percentage of necrosis was scored for one half of each of the five roots. The maximum root necrosis per root half was 20% giving a maximum root necrosis of 100% for the five root halves together. For sugarcane, ten roots were collected randomly to assess the damage caused by *P. coffeae*. The percentage of necrosis on the surface of each root was scored. The maximum root necrosis per root was 10% giving a maximum root necrosis of 100% for the ten roots together.

Data analysis. For the statistical analysis of the results, the STATISTICA® package (Anonymous, 1997) was used. The Shapiro-Wilk's test was applied to evaluate whether the dependent variable was normally distributed within groups. The homogeneity of the variances of the groups was tested with the Levene's test. The nematode population densities were $\log_{10}(x+1)$ and the root necrosis data were arcsin(x/100) transformed before analysis. When less than ten replicates per group were available, the outliers were determined by calculating the standardised residuals. Outliers were defined as data with a standardised residual falling outside the range from –2 to 2. One-way ANOVA was used to analyse the data. The means were separated using Tukey's Honestly Significant Difference test ($P \leq 0.05$).

RESULTS

Host range of *Pratylenchus coffeae* populations from Vietnam. The results of the four host range experiments are presented in Table 2. In the three experiments in which banana was included as one of the host plants, all the *P. coffeae* populations

Table 1. Origin and population codes of the ten *Pratylenchus coffeae* populations from Vietnam used in our study

Host plant	Province	Agro-ecological region	Population code
Banana	Dien Bien	Northwest	NW
Coffee	Yen Bai	Northeast	NE1
Banana	Yen Bai	Northeast	NE2
Banana	Phu Tho	Northeast	NE3
Banana	Bac Kan	Northeast	NE4
Banana	Ha Tay	Red River Delta	RRD1
Ornamental tree	Hung Yen	Red River Delta	RRD2
Banana	Thanh Hoa	North Central Coast	NCC1
Coffee	Nghe An	North Central Coast	NCC2
Coffee	Dak Lak	Central Highlands	CH

examined reproduced very well on banana: in the 1st experiment, the reproduction factor (RF) on banana ranged on average from 16.0 to 27.2, in the 2nd experiment from 28.9 to 37.7 and in the 3rd experiment from 29.4 to 42.5. The RF on banana among the *P. coffeae* populations examined was similar. All *P. coffeae* populations examined reproduced on rice (with the exception of the Red River Delta 1 population), sugarcane and maize. On these three crops, RF between 2 and 5 were observed. On soybean, the RF of all the *P. coffeae* populations examined fluctuated on average around 1 (0.7 to 2.5) while on the other eight crops included in the experiments the RF was < 1. No nematodes were extracted from the roots of groundnut, ginger and pineapple.

***In vivo* reproduction and damage potential on banana.** The results of the *in vivo* reproduction experiment on banana cv. Ngop Dui Duc are presented in Table 3. Differences were observed in *in vivo* reproduction on this banana cultivar among the ten *P. coffeae* populations from Vietnam examined. The highest average nematode population density observed per root system (32,209, North Central Coast 1 population, originally isolated from banana) was seven times higher ($P \leq 0.05$) than the lowest average nematode population density observed per root system (4,457, North Central Coast 2 population, originally isolated from coffee). The RF of the Northwest, Northeast 4 and North Central Coast 1 populations were on average significantly ($P \leq 0.05$) higher than the RF of the Northeast 2, Red River Delta 2, North Central Coast 2 and Central Highlands populations. The highest average nematode population density observed per 10 g fresh roots (13,793, North Central Coast 1 population) was 15.4 times higher ($P \leq 0.05$) than the lowest average nematode population density observed per 10 g fresh roots (893, North Central Coast 2 population). The average nematode population densities of the population originally isolated from the roots of an ornamental tree (Red River Delta 2) per root system and per 10 g fresh roots were 7,520 and 1,512, respectively, which was not significantly different from the Northeast 2, Red River Delta 1, North Central Coast 2 and Central Highlands populations which were originally isolated from banana and coffee.

The relationship between the average nematode population densities per 10 g fresh roots and the percentage root necrosis is illustrated in Fig. 1. In general, the nematode populations with the highest root population densities also caused the highest percentage of root necrosis. The North Central Coast 2 population, which had the lowest nematode population densities per root system and per 10 g fresh roots, still caused about 10% root necrosis, a percentage that was not significantly different from the root necrosis caused by several other populations such as the Northeast 2, Northeast 3 and Red River Delta 2 populations.

The plant height, shoot and root fresh weights of this banana cultivar were reduced by all the ten *P. coffeae* populations from Vietnam examined with 13.4-38.7%, 7.4-50.7% and 3.4-42.4%, respectively, compared with the uninoculated control plants (Table 3). Significant ($P \leq 0.05$) differences were observed in reduction in plant height and shoot fresh weight among the populations examined. For instance, the Northwest and Northeast 4 populations decreased these two plant growth variables significantly ($P \leq 0.05$) more than the Northeast 3, North Central Coast 2 and Central Highlands populations. No differences were observed in damage potential among the populations originally isolated from banana and coffee. Also the Red River Delta 2 population originally isolated from the roots of an ornamental tree caused a reduction in plant

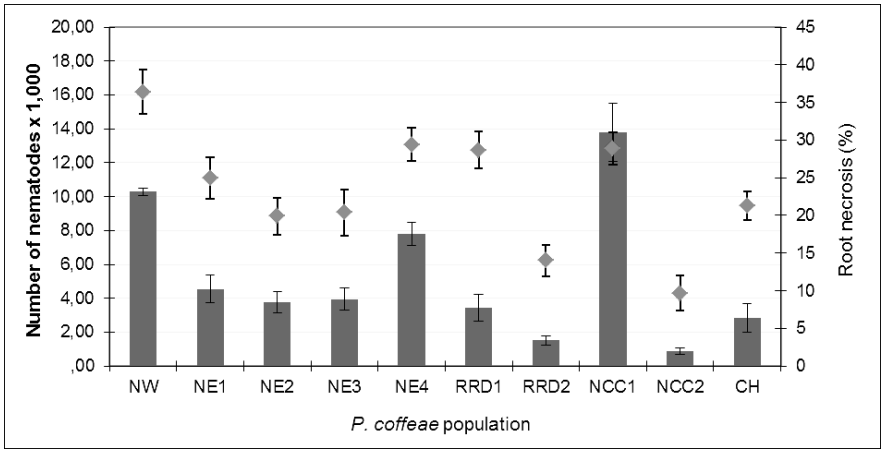

Fig. 1. Nematode population density per 10 g fresh roots and percentage root necrosis caused by ten *Pratylenchus coffeae* populations from Vietnam on banana cv. Ngop Dui Duc, 14 weeks after inoculation with 1,000 vermiforms per plant. ■: number of nematodes per 10 g fresh roots; ♦: percentage root necrosis. Each point and bar is the mean ± standard error of seven replicates. For the list with the nematode population codes see Table 1.

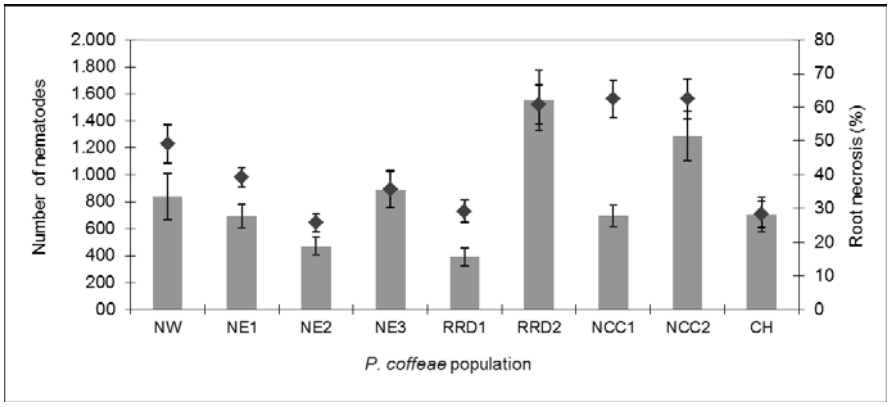

Fig. 2. Nematode population density per 10 g fresh roots and percentage root necrosis caused by ten *Pratylenchus coffeae* populations from Vietnam on sugarcane var. ROC20, 14 weeks after inoculation with 1,000 vermiforms per plant. ■: number of nematodes per 10 g fresh roots; ♦: percentage root necrosis. Each point and bar is the mean ± standard error of seven replicates. For the list with the nematode population codes see Table 1.

growth similar to most of the other populations examined. Only the North Central Coast 1 population significantly ($P \leq 0.05$) reduced root fresh weight compared with the uninoculated control plants. The lowest reduction (3.4%) was observed in the banana plants inoculated with the Red River Delta 2 population. By contrast, this population reduced the plant height and shoot fresh weight with 30.8 and 32.6%, respectively.

***In vivo* reproduction and damage potential coffee.** The results of the *in vivo* reproduction experiment on coffee var. Catimor are presented in Table 4. The RF of all ten *P. coffeae* populations examined was on average < 1. The highest average nematode population density observed per root system (212, Northeast 1 population, originally isolated from coffee) was 5.9 times higher ($P \leq 0.05$) than the lowest average nematode population density observed per root system (36, North Central Coast 1 population, originally isolated from banana). The RF of the Northeast 1 and North Central Coast 2 populations were on average significantly ($P \leq 0.05$) higher than the RF of the Northwest, Red River Delta 1, and North Central Coast 1 populations. The highest average nematode population density observed per 1 g fresh roots (330, North Central Coast 2 population, originally isolated from coffee) was 7.7 times higher ($P \leq 0.05$) than the lowest average nematode population density observed per 1 g fresh roots (43, North Central Coast 1 population, originally isolated from banana). The average nematode population densities of the population originally isolated from

the roots of an ornamental tree per root system and per 1 g fresh roots were 44 and 98, respectively, which was not significantly different from several other populations which were originally isolated from banana and coffee.

At 14 weeks after inoculation, the root fresh weight of the uninoculated control plants was on average 3.1 g while the root fresh weight of the plants inoculated with the *P. coffeae* populations was on average < 1 g (Table 4). The plant height, shoot and root fresh weights of this coffee variety were reduced by all the ten *P. coffeae* from Vietnam examined with 31.1-35.4%, 53.8-61.5% and 71-90.3%, respectively, compared to the uninoculated control plants. No significant differences were observed in reduction of these three plant growth variables among the populations examined. Also, the Red River Delta 2 population originally isolated from the roots of an ornamental tree caused a reduction in plant growth similar to most of the other populations examined.

***In vivo* reproduction and damage potential on sugarcane.** The results of the *in vivo* reproduction experiment on sugarcane var. ROC20 are presented in Table 5. Few differences were observed in *in vivo* reproduction on this sugarcane variety among the ten *P. coffeae* populations from Vietnam examined. The highest average nematode population density observed per root system (3,513, Red River Delta 2 population, originally isolated from the roots of an ornamental tree) was 2.4 times higher ($P \leq 0.05$) than the lowest average nematode population density observed per root system (1,448, Red River Delta 1 population, originally isolated from banana). The RF of the Red River Delta 2 population was significantly ($P \leq 0.05$) higher than the RF of the Central Highlands population. The highest average nematode population density observed per 1 g fresh roots (1,552, Red River Delta 2 population) was four times higher ($P \leq 0.05$) than the lowest average nematode population density observed per 1 g fresh roots (391, Red River Delta 1 population).

The relationship between the average nematode population densities per 1 g fresh roots and the percentage root necrosis on sugarcane is illustrated in Fig. 2. In general, the nematode populations with the highest root population densities also caused the highest percentage of root necrosis. However, the North Central Coast 1 population, which had one of the lowest nematode population densities per 1 g fresh roots, caused 62.5% root necrosis, similar to the percentage root necrosis caused by the North Central Coast 2 population from which almost twice as many nematodes per 1 g roots were extracted.

All three plant growth variables measured were not significantly decreased by the ten *P. coffeae* populations from Vietnam examined, compared with the uninoculated control plants (Table 5). A few differences in damage potential were observed among the *P. coffeae* populations examined. The North Central Coast 2 and Central Highlands populations caused a decrease in plant height of 16 and 18.1%, respectively, while the North Central Coast 1 population caused an increase in plant height of 7.1% ($P \leq 0.05$). The Central Highlands population caused a decrease in shoot fresh weight of 22.3% while the Red River Delta 2 population caused an increase in shoot fresh weight of 13.3% ($P \leq 0.05$). The Central Highlands population caused a decrease in root fresh weight of 30% while the Northeast 2 and Red River Delta 1 populations caused an increase in root fresh weight of 10% ($P \leq 0.05$).

***In vivo* reproduction and damage potential on maize.** The results of the *in vivo* reproduction experiment on maize var. LVN10 are presented in Table 6. Few differences were observed in *in vivo* reproduction on this maize cultivar among the ten *P. coffeae* populations from Vietnam examined. For seven out of the nine *P. coffeae* populations examined, the average nematode population density per root system ranged from about 1,000 to 1,800 while for eight out of the nine *P. coffeae* populations examined, the average nematode population density per 10 g fresh roots ranged from about 500 and 1,000. The highest average nematode population density observed per root system (1,806, Red River Delta 2 population, originally isolated from the roots of an ornamental tree) was 5.2 times higher ($P \leq 0.05$) than the lowest average nematode population density observed per root system (346, Red River Delta 1 population, originally isolated from banana). There was no significant difference in RF among the populations examined. The highest average nematode population density observed per 10 g fresh roots (1,074, Central Highlands population, originally isolated from coffee) was 5.2 times higher ($P \leq 0.05$) than the lowest average nematode population density observed per 10 g fresh roots (205, Red River Delta 1 population).

All three plant growth variables measured were not significantly decreased by the ten *P. coffeae* populations from Vietnam examined, compared with the uninoculated control plants. A few differences in damage potential were observed among the *P. coffeae* populations examined. The Red River Delta 2 population caused a decrease in plant height of 16.6% while the Northwest and Northeast 3 populations caused an increase in plant height of 9.7 and 10.2%, respectively ($P \leq 0.05$).

DISCUSSION

Soil temperature is an important environmental factor affecting the reproduction of plant-parasitic nematodes including *Pratylenchus* spp. According to Radewald *et al*. (1971), Acosta & Malek (1979) and Gowen (2000), the optimum temperature for development and reproduction of *P. coffeae* is 25 to 30°C. This is precisely the temperature at which our experiments were carried out.

Out of the 13 agricultural crops included in our study, the reproduction factor of all the *P. coffeae* populations from Vietnam examined was always > 1 on banana, rice (with the exception of the Red River Delta 1 population), sugarcane and maize and these crops can be considered as good hosts of this nematode species. Compared to the latter three crops, banana is a much better host. The host response of rice, sugarcane and maize was similar. No reproduction was observed on the other eight crops.

Table 2. Reproduction factors (RF: final nematode population density/initial nematode population density) of ten *Pratylenchus coffeae* populations from Vietnam on 13 selected agricultural crops, 14 weeks after inoculation with 1,000 vermiforms per plant

Crop		*Pratylenchus coffeae* population								
		NW	NE1	NE2	NE3	RRD1	RRD2	NCC1	NCC2	CH
Exp. 1	Soybean	–	0.9	–	1.0	1.1	0.7	–	–	2.5
	Groundnut	–	0	–	0	0	0	0	–	0
	Tomato	–	0.1	–	< 0.1	< 0.1	0.1	0.1	–	< 0.1
	Banana	–	27.2	–	16.0	20.8	17.9	27.2	–	25.2
	Sweet potato	–	0.1	–	0.1	0.1	0.3	0.1	–	0.1
	Coffee	–	< 0.1	–	< 0.1	0	< 0.1	< 0.1	–	< 0.1
Exp. 2	Coffee	0.1	< 0.1	0	0.1	0	< 0.1	< 0.1	0.2	< 0.1
	Ginger	0	0	0	0	0	0	0	0	0
	Pinapple	0	0	0	0	0	0	0	0	0
	Sesame	0.1	< 0.1	< 0.1	< 0.1	0	0.5	0.2	< 0.1	0
	Banana	29.7	37.2	30.5	28.9	31.6	36.9	37.7	–	29.6
	Rice	3.0	2.9	–	3.2	0.2	5.4	5.3	–	2.3
Exp. 3	Sugarcane	4.5	3.5	2.8	3.6	3.3	5.1	3.6	4.7	2.6
	Maize	2.8	3.7	3.3	2.2	2.0	4.1	3.5	3.4	4.5
	Banana	33.9	29.4	32.7	33.7	34.2	35.2	38.4	37.7	42.5
Exp. 4	Citrus	0	0	0	0	< 0.1	0	0.4	0.1	0

'–' indicates missing data. For the list with the nematode population codes see Table 1.

Table 3. *In vivo* reproduction, percentage root lesions and effect on plant growth caused by ten *Pratylenchus coffeae* populations from Vietnam on banana cv. Ngop Dui Duc, 14 weeks after inoculation with 1,000 vermiforms per plant (n = 7)

P. coffeae population	Mean number of nematodes			RF	Root lesion (%)	Plant height		Shoot weight		Root weight	
	per 10 g fresh roots	per root system	in soil			(cm)	% change	(g)	% change	(g)	% change
NW	10,280e	31,561c	3,745	35.3d	36.4d	21.9a	(-38.7)	63.7a	(-49.9)	30.7ab	(-35.5)
NE1	4,547cd	13,731abc	3,782	17.5bcd	25.0bc	24.0ab	(-32.8)	81.6abc	(-35.8)	37.2ab	(-21.8)
NE2	3,760bcd	12,000abc	2,837	14.8abc	19.9abc	24.1ab	(-32.5)	73.6ab	(-42.1)	35.5ab	(-25.4)
NE3	3,940cd	17,817bc	3,622	21.4bcd	20.4abc	29.3bcd	(-17.9)	112.5bcd	(-11.5)	41.6ab	(-12.6)
NE4	7,794de	29,894c	8,042	37.9d	29.4cd	22.7a	(-36.4)	71.6a	(-43.7)	39.1ab	(-17.9)
RRD1	3,453bc	9,518ab	14,211	23.8bcd	28.7cd	23.7ab	(-33.6)	62.7a	(-50.7)	29.7ab	(-37.6)
RRD2	1,512ab	7,520ab	702	8.2a	14.0ab	24.7abc	(-30.8)	85.7abc	(-32.6)	46.0b	(-3.4)
NCC1	13,793e	32,209c	3,620	35.8d	28.9cd	26.4abcd	(-26.1)	71.0a	(-44.1)	27.4a	(-42.4)
NCC2	893a	4,457a	5,140	9.7ab	9.7a	30.9de	(-13.4)	116.7cd	(-8.2)	41.5ab	(-12.8)
CH	2,824bc	14,939abc	1,454	16.4ab	21.3bc	30.3cde	(-15.1)	117.7cd	(-7.4)	41.0ab	(-13.9)
Control						35.7e	(0.0)	127.1d	(0.0)	47.6b	(0.0)

RF: final nematode population density/initial nematode population density (= inoculum).
Means in the same column followed by the same letter are not significantly different according to Tukey's test ($P \leq 0.05$). % change compared with the uninoculated (control) plants.
For the list with the nematode population codes see Table 1.

Table 4. *In vivo* reproduction and effect on plant growth caused by ten *Pratylenchus coffeae* populations from Vietnam on coffee cv. Catimor, 14 weeks after inoculation with 1,000 vermiforms per plant (n = 5)

P. coffeae population	Mean number of nematodes			RF	Plant height		Shoot weight		Root weight	
	per 10 g fresh roots	per root system	in soil		(cm)	% change	(g)	% change	(g)	% change
NW	65 ab	52 ab	10	0.1 ab	14.0 a	(-33.0)	2 a	(-61.5)	0.9 a	(-71.0)
NE1	307 bc	212 b	80	0.3 c	14.1 a	(-32.5)	2.4 a	(-53.8)	0.9 a	(-71.0)
NE2	109 abc	84 ab	80	0.1 abc	14.4 a	(-31.1)	2.3 a	(-55.8)	0.8 a	(-74.2)
NE3	58 a	40 a	50	0.1 abc	14.3 a	(-31.6)	2.1 a	(-59.6)	0.8 a	(-74.2)
NE4	150 abc	48 ab	50	0.1 abc	13.5 a	(-35.4)	2.0 a	(-61.5)	0.5 a	(-83.9)
RRD1	78 ab	48 a	30	0.1 ab	13.6 a	(-34.9)	2.0 a	(-61.5)	0.7 a	(-77.4)
RRD2	98 abc	44 a	70	0.1 abc	14.2 a	(-32.1)	2.2 a	(-57.7)	0.6 a	(-80.6)
NCC1	43 a	36 a	10	< 0.1 ab	13.9 a	(-33.5)	2.1 a	(-59.6)	0.9 a	(-71.0)
NCC2	330 c	100 ab	130	0.2 c	13.7 a	(-34.4)	2.2 a	(-57.7)	0.3 a	(-90.3)
CH	107 abc	88 ab	70	0.2 bc	14.0 a	(-33.0)	2.1 a	(-59.6)	0.8 a	(-74.2)
Control					20.9 b	(0.0)	5.2 b	(0.0)	3.1 b	(0.0)

RF: final nematode population density/initial nematode population density (= inoculum).
Means in the same column followed by the same letter are not significantly different according to Tukey's test ($P \leq 0.05$). % change compared with the uninoculated (control) plants.
For the list with the nematode population codes see Table 1.

On soybean, the reproduction factor fluctuated around 1 (0.7 to 2.5) and therefore we consider this crop as a poor host. Groundnut, tomato, sweet potato, coffee, ginger, sesame, pineapple and citrus can be considered as very poor hosts or nonhosts of *P. coffeae*.

Our results confirm that banana is a good host of *P. coffeae* (Gowen *et al*., 2005). The banana variety used in our experiments belongs to the BBB genome group, which was previously demonstrated as one of the most susceptible banana varieties in Vietnam to *P. coffeae* (van den Bergh, 2002). The good reproduction of all the *P. coffeae* populations from Vietnam examined on this crop also confirms the viability of the *P. coffeae* populations and effectiveness of the inoculation method used in our experiments.

Table 5. *In vivo* reproduction, percentage root lesions and effect on plant growth caused by ten *Pratylenchus coffeae* populations from Vietnam on sugarcane var. ROC20, 14 weeks after inoculation with 1,000 vermiforms per plant (n = 6)

P. coffeae population	Mean number of nematodes			RF	Root lesion (%)	Plant height		Shoot weight		Root weight	
	per 10 g fresh roots	per root system	in soil			(cm)	% change	(g)	% change	(g)	% change
NW	839 bcd	2,226 b	3,500	4.5 ab	49.2abc	37.3 ab	(-14.6)	38.7 ab	(-17.1)	2.6 abc	(-13.3)
NE1	692 abc	2,166ab	1,393	3.6 ab	39.2 ab	39.7 ab	(-9.2)	39.2 ab	(-16.1)	3.2 bc	(6.7)
NE2	473 ab	1,650 a	1,350	2.8 ab	25.8 a	38.0 ab	(-13.0)	38.9 ab	(-16.7)	3.3 c	(10.0)
NE3	889 bcd	2,326ab	2,960	3.7 ab	35.8 a	37.5 ab	(-14.2)	42.2 ab	(-9.6)	2.6 abc	(-13.3)
NE4	391 a	1,448 a	2,060	3.3 ab	29.2 a	41.2 ab	(-5.7)	39.7 ab	(-15.0)	3.3 c	(10.0)
RRD1	1,552 d	3,513 b	1,915	5.1 b	60.8 bc	38.6 ab	(-11.7)	52.9 b	(13.3)	2.4 abc	(-20.0)
RRD2	695 abc	1,986ab	1,591	3.6 ab	62.5 c	46.8 b	(7.1)	47.8 ab	(2.4)	2.8 abc	(-6.7)
NCC1	1,289 cd	2,873 ab	2,387	4.7 ab	62.5 c	36.7 a	(-16.0)	44.3 ab	(-5.1)	2.3 ab	(-23.3)
NCC2	704 abc	1,530 a	1,086	2.6 a	28.3 a	35.8 a	(-18.1)	36.3 a	(-22.3)	2.1 a	(-30.0)
CH	839 bcd	2,226 ab	3,500	4.5 ab	49.2 abc	43.7 ab	(0.0)	46.7 ab	(0.0)	3.0 abc	(0.0)
Control						37.3 ab	(-14.6)	38.7 ab	(-17.1)	2.6 abc	(-13.3)

RF: final nematode population density/initial nematode population density (= inoculum).
Means in the same column followed by the same letter are not significantly different according to Tukey's test ($P \leq 0.05$). % change compared with the uninoculated (control) plants.

Table 6. Reproduction and effect on plant growth caused by ten *Pratylenchus coffeae* populations from Vietnam on maize var. LVN10, 14 weeks after inoculation with 1,000 vermiforms per plant (n = 6)

P. coffeae population	Mean number of nematodes			RF	Plant height		Shoot weight	Root weight
	per 10 g fresh roots	per root system	in soil		(cm)	% change	(g)	(g)
NW	535 ab	846 ab	2,000	2.8	117.7 b	(9.7)	40.8	19.7
NE1	616 ab	1,093 b	2,430	3.7	106.2 ab	(-1.0)	46.0	18.6
NE2	558 ab	1,186 b	2,086	3.3	112.3 ab	(4.7)	54.1	22.2
NE3	507 ab	1,150 b	1,312	2.2	118.2 b	(10.2)	45.7	21.6
RRD1	205 a	346 a	2,300	2.0	103.2 ab	(-3.8)	43.1	18.0
RRD2	970 b	1,806 b	2,313	4.1	89.5 a	(-16.6)	37.4	19.3
NCC1	689 ab	1,532 b	2,193	3.5	110.8 ab	(3.3)	37.8	21.2
NCC2	848 b	1,410 b	2,392	3.4	105.8 ab	(-1.4)	41.9	16.1
CH	1,074 b	1,793 b	2,800	4.5	111.8 ab	(4.2)	41.3	16.1
Control					107.3 ab	(0.0)	46.3	21.1
				n.s.			n.s.	n.s.

RF: final nematode population density/initial nematode population density (= inoculum).
Means in the same column followed by the same letter do not differ significantly according to Tukey's test ($P \leq 0.05$). % change compared with the uninoculated (control) plants.
n.s. indicates no significant diference according to the analysis of variance (ANOVA; $P \leq 0.05$).
For the list with the nematode population codes see Table 1.

According to our results, rice, sugarcane and maize are good hosts for all the examined *P. coffeae* populations from Vietnam, while sweet potato, coffee and citrus are very poor hosts. This observation is, in general, in contrast with the nematological literature in which rice, sugarcane and maize are usually not reported as good hosts of *P. coffeae* in contrast to sweet potato, coffee and citrus which are usually reported as (very) good hosts of *P. coffeae* (Castillo & Vovlas, 2007). Interestingly, Silva & Inomoto (2002) made a more or less similar observation when they characterised the host range of two *P. coffeae* populations originally isolated from coffee in Brazil and observed that coffee, citrus (*Citrus limonia* Osbeck) and also banana were not among the better host plants of these two populations but rather rice and maize. As mentioned in the introduction, also differences in *in vivo* reproduction on *Musa* spp. (Bridge *et al*., 1997), maize (Edwards & Wehunt, 1973; Silva & Inomoto, 2002), rice and sesame (Silva & Inomoto, 2002) among *P. coffeae* populations have been reported before.

The contradictions among these studies suggest that the host status classification of an agricultural crop based on the study of one cultivar or variety of this crop cannot and should not be generalised. As emphasised by Jacobsen *et al*. (2009), this classification might be influenced by several factors including host plant response differences among cultivars and varieties, differences in damage potential among nematode populations and methodological differences among studies. As mentioned in the introduction, differences in damage potential among *P. coffeae* populations originating from different geographical regions have been reported. In the experiments conducted by Silva & Inomoto (2002), one of the two *P. coffeae* populations from Brazil used was originally isolated from coffee plants in the field but maintained on alfalfa callus before the host range experiments were carried out. Culturing plant-parasitic nematodes *in vitro* on monoxenic plant tissue cultures (such as alfalfa callus or carrot discs) might influence the reproduction fitness, virulence and/or damage potential of the cultured nematodes, although we are not aware of any report in this respect. Finally, methodological differences such as the time of sampling and the extraction method used may also have contributed to the observed differences in host status among the published studies.

Although three out of the ten *P. coffeae* populations from Vietnam used in our experiments were originally isolated from coffee plants in the field, none of the nematode populations examined reproduced well on the coffee variety used in our experiments. This indicates that coffee var. Catimor is a poor host of *P. coffeae*. In Brazil, Silva & Inomoto (2002) observed low reproduction (RF < 2.5) on coffee 10 weeks after inoculation with 1,000 nematodes of a *P. coffeae* population originally isolated from coffee roots in the field.

In their experiments with the two *P. coffeae* populations from Brazil, Silva & Inomoto (2002) also observed that peanut was a poor host (RF < 1) which

is in agreement with our results while soybean was a good host (RF ranging from 2 to 3). In our study, RF of soybean fluctuated around 1 (RF = 0.7 to 2.5).

The *in vivo* reproduction of all the *P. coffeae* populations from Vietnam examined on the 13 agricultural crops was, in general, very similar. In some rare cases, differences were observed. On banana, reproduction of the Northwest, Northeast 4 and North Central Coast 1 populations was about 4.5 times higher than reproduction of the Red River Delta 2 population in one experiment but in another experiment no differences in reproduction on banana among these populations were observed. On sugarcane, only the reproduction of two *P. coffeae* populations (Red River Delta 2 and Central Highlands populations) was significantly different from each other but the difference was small (RF = 5.1 *vs* 2.6). On rice, the Red River Delta 1 population was the only population out of seven populations included in the experiment that did not reproduce on this crop (RF = 0.2) but the reproduction factor of the other six populations was also not very high ranging from 2.3 to 5.4. On maize, no differences in reproduction were observed among the populations examined.

The results of the damage potential experiments carried out in our study indicate that the *P. coffeae* populations from Vietnam examined were able to cause considerable damage to the vegetative growth of banana and coffee but not of sugarcane and maize. Our results confirm many earlier observations on the percentage root lesion and damage *P. coffeae* can cause to banana (Gowen *et al*., 2005), whilst there are, to our knowledge, no reports on damage caused by *P. coffeae* to sugarcane (Cadet & Spaull, 1985, 2005) and maize (Mc Donald & Nicol, 2005).

Remarkable is the damage caused by all *P. coffeae* populations included in the experiment on coffee in spite of the very low reproduction of these populations (RF < 1). Compared with the uninoculated plants, infection with *P. coffeae* caused about 33% (31.1-35.4%) decrease in plant height; about 60% (53.8-61.5%) decrease in shoot fresh weight and about 70 to 90% decrease in root fresh weight. The root fresh weight of the uninoculated plants at the termination of the experiment was only 3.1 g and this suggests that the fragility of the roots may have resulted in a high sensitivity to damage by *P. coffeae*. Our results are similar to those of Inomoto *et al*. (2007) who inoculated two coffee cultivars (Mundo Novo and Catuai) with 8,000 vermiform nematodes of a *P. coffeae* population originally isolated from coffee roots in the field and maintained on alfalfa callus prior to inoculation. About 37 weeks after inoculation and in spite of a low reproduction (RF < 1.5) they observed a 72 and 61% reduction in plant weight, 95 and 89% reduction in shoot fresh weight and 93 and 86% reduction in root fresh weight, respectively. The average root fresh weight at 37 weeks after inoculation of the uninoculated control plants was around 10 g. Our results thus confirm the highly destructive nature of *P. coffeae* on coffee, especially coffee seedlings. *Pratylenchus coffeae* has been reported as a very destructive nematode to coffee in South America and Asia (Campos & Villain, 2005). This nematode species may cause the destruction of the whole root system resulting in production losses up to 80%, decay of coffee seedlings and trees leading to their death and even the abandonment of coffee fields.

The *in vivo* damage potential of all the *P. coffeae* populations from Vietnam examined on banana, coffee, sugarcane and maize was, in general, very similar. As was the case for the *in vivo* reproduction, differences were only observed in some very rare cases. Exceptionally, the effect on vegetative plant growth of one or a few *P. coffeae* populations were significantly different either compared with the uninoculated control plants or compared with the other *P. coffeae* populations.

Finally, our results did not indicate any relationship between host plant range, *in vivo* reproduction and damage potential on the one hand and geographical origin and host plant from which the *P. coffeae* populations were originally isolated on the other hand. In conclusion, the *in vivo* reproduction of all the ten *P. coffeae* populations from Vietnam on the 13 agricultural crops included in our experiments was, in general, very similar. This supports our previous finding based on the *in vitro* reproductive fitness on carrot discs experiments that the ten *P. coffeae* populations from Vietnam examined belong to the same biotype (Agrios, 1997). Based on the results of our *in vivo* glasshouse experiments, we can confirm that banana is a good host of the *P. coffeae* populations from Vietnam examined. Surprisingly, rice, sugarcane and maize appeared also to be good hosts of *P. coffeae* while sweet potato, coffee and citrus are very poor hosts. This observation is, in general, in contrast with the nematological literature in which rice, sugarcane and maize are usually not reported as good hosts of *P. coffeae* in contrast to sweet potato, coffee and citrus which are usually reported as (very) good hosts of this nematode species. Additional experiments should be carried out to clarify these contradictory findings. It is possible that the host plant specificity is (plant) genotype-dependent. In general, the *in vivo* damage potential on banana, coffee, sugarcane and maize of all the

ten *P. coffeae* populations from Vietnam examined was very similar. These populations were able to cause considerable damage to the vegetative growth of banana and coffee but not of sugarcane and maize. In view of the low reproduction on coffee, the extensive damage the *P. coffeae* populations from Vietnam caused on this agricultural crop is surprising and illustrates the high damage potential of *P. coffeae* on coffee.

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Nguyen Thi Tuyet, A. Elsen, Ho Huu Nhi and D. De Waele. Оценка круга хозяев, размножение *in vivo* и потенциал вредоносности популяций *Pratylenchus coffeae* Вьетнама.

Резюме. Параметры размножения *in vivo* (на 13 видах растениях-хозяевах) десяти популяций *Pratylenchus coffeae*, собранных в различных агро-экологических зонах Вьетнама, оказались сходными. Было отобрано по одному сорту от каждого вида сельскохозяйственных культур. Из 13 испытанных культур только бананы, сахарный тростник, кукуруза и суходольный рис являлись подходящими хозяевами для *P. coffeae*. Соевые бобы плохо поддерживали размножение пратиленхусов, тогда как арахис, томаты, бататы, имбирь, сезам, ананас и цитрусы оказались еще худшими хозяевами для *P. coffeae*, или не поддерживали их развитие вообще. Степень вызываемых *P. coffeae* повреждений на бананах, кофе, сахарном тростнике и кукурузе была сходной для всех 10 исследованных популяций. Все популяции *P. coffeae* вызывали существенное угнетение вегетативного роста у бананов и кофе, но не у сахарного тростника и кукурузы. Учитывая слабое размножение этих нематод на кофе, значительный уровень вреда, причиняемый ими данной культуре, представляется довольно неожиданным. Выявленные сходные темпы размножения *in vivo* на 13-и сельскохозяйственных культурах, и сходный уровень угнетения при размножении на бананах, кофе, сахарном тростнике и кукурузе, свидетельствуют о принадлежности всех 10 вьетнамских популяций *P. coffeae* к одному патотипу.