COMPARATIVE STUDY OF MORPHOLOGY, MORPHOMETRICS, REPRODUCTIVE FITNESS ON CARROT DISCS AND PATHOGENICITY ON *MUSA* GENOTYPES OF *RADOPHOLUS SIMILIS* PHILIPPINE POPULATIONS

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ABSTRACT

Comparative studies were conducted on three *Radopholus similis* populations collected in Quezon, Laguna and Davao, Philippines from 2008 to 2010. For the first time, morphological and morphometrical characteristics of the Philippine *R. similis* populations were studied and showed differences for the major body regions. Consequently, reproductive fitness of the three populations on a single carrot disc at 28°C was elucidated and found different from each other. The Davao population showed high nematode density (Pf = 8,447) and reproduction ratio (Rf = 422.4) 8 weeks after inoculation (WAI) indicating greater percentage of reproductive females and found most pathogenic. Ten *Musa* genotypes including the resistant reference cultivars Pisang Jari Buaya and Yangambi Km5, and susceptible Grande Naine showed various degrees of response to *R. similis* populations. Cuarenta Dias and Latundan showed high to moderate resistance and tolerance to *R. similis* based on nematode counts and root damage, respectively. The most pathogenic Davao population caused significant reduction in roots weight and necrosis on the susceptible genotypes, Bungulan, Lakatan-Davao, Morado and *M. balbisiana*. Although nematode morphology and morphometrics failed to correlate the pathogenicity of *R. similis*, the reproductive fitness could help underpin selection and breeding of *Musa* for nematode resistance.

Key words: Cuarenta Dias, Davao, Laguna, Latundan, Morado, reproduction rate

INTRODUCTION

The burrowing nematode *Radopholus similis* (Cobb) Thorne is recognized as one of the most important and widespread nematode species attacking banana and plantain (*Musa* spp.). It also attacks many other crops and weeds (Davide 1992, Sarah et al. 1996, O'Bannon 1997). It is considered the main nematode problem in vast commercial plantations of Cavendish bananas (AAA) in Central and South America, and causes damage on plantains and cooking bananas in the lowlands of Central and Eastern Africa, and in the Caribbean (Sarah 2000). In the Philippines, the burrowing nematode became a serious problem in the early 1970s, when large volumes of infected Giant Cavendish planting materials from Central America were introduced for commercial production (Davide 1992). In Davao, *R. similis* was the most destructive nematode species of Cavendish banana followed by *Helicotylenchus multicinctus* and *Meloidogyne* spp. (Boncato and Davide 1980). Davide and

Marasigan (1985) showed that *R. similis* (1000 to 4000 nematodes/plant) can cause 14 to 61% yield loss under lowland condition. Cumulative losses due to bunch weight reduction and uprooting may reach 75% in three production cycles, while root density of susceptible cultivars may be reduced by up to 70% following *R. similis* infection (Blomme 2000, Sarah et al. 1996). The frequency of occurrence of *R. similis* in the Philippines differed in the banana-growing areas that were surveyed so far. *R. similis* was abundant in Davao with 95.1% occurrence followed by Cebu (91.3%), Quirino (85.3%) and Quezon (65.0%) (Anon. 2008). These survey data indicate that the Philippine populations of *R. similis* may show diversity in terms of morphology, morphometrics and reproductive fitness, and in pathogenicity. Damage caused by *R. similis* depends on the pathogenicity of the population, which may vary greatly among production zones and appears to be linked to their reproductive fitness in the plant tissues (Boncato and Davide 1980, Fallas et al. 1995, Hahn et al. 1996, Sarah et al. 1996). Such differences in reproductive fitness and pathogenicity among populations of *R. similis* may complicate the effort in selecting and breeding improved *R. similis*-resistant *Musa* varieties. Therefore, knowledge of the geographical and biological variations of local *R. similis* populations is very important for the implementation of an efficient management strategy based on the deployment of resistant cultivars that have a broad resistance to *R. similis* and can be successfully used in a wide range of fields that may be infested with different local populations of *R. similis* (Sarah et al. 1996).

This study sought to characterize selected Philippine *R. similis* populations by comparing their morphological features and morphometrics, to determine their reproductive fitness on *in vitro* carrot discs and their pathogenicity by studying the host response of selected banana genotypes locally grown in the Philippines. Morphology and morphometrics, and reproductive fitness of the three *R. similis* populations were correlated with their pathogenicity on the selected banana genotypes.

MATERIALS AND METHODS

Three populations of *R. similis* were isolated from banana roots (*Musa* sp.) in Quezon, Laguna and Davao provinces in the Philippines where the frequency of occurrence of *R. similis* is high resulting in significant damage (Boncato and Davide 1980, Anon. 2008). Each population was established and maintained on carrot (*Daucus carota* var. Chantenay) disc cultures following the procedures described by O'Bannon and Taylor (1968).

Morphology and morphometrics of *Radopholus similis.* The nematodes were heat -killed and fixed by adding hot double - strength FG fixative containing 8% formalin and 2% glycerin in distilled water (Seinhorst 1959). Females and males were separated using a picker (coconut midrib). Semi-permanent slides were prepared by adding glycerin as the mounting medium (Seinhorst 1959). For each population, 15 adult females and 15 adult males were measured. All measurements and drawings were made using a light compound microscope with a camera lucida (Reichert Microstar IV, Austria). For the females the following body measurements were recorded: total body length, maximum body width, stylet length, head width and height, oesophagus length, body width at anus, tail length, and anterior and posterior ovary lengths. For the males the latter measurements as well as testis length, spicule length, and gubernaculum length were recorded (Elbadri et al. 1999). Tail ends of males were examined for morphological characterization of the tail shape and cuticular structures.

In vitro* reproductive fitness of *Radopholus similis. Nematode inoculum was extracted from the stock carrot disc cultures by the maceration-sieving method (Speijer and De Waele 1997). Carrot discs were rinsed with distilled water and the suspension passed through a 25-µm-mesh sieve. The nematodes retained on the sieve were collected in a beaker (fraction 1). The remaining carrot discs were macerated in a kitchen blender with distilled water (three 10-s periods separated by 5-s intervals). Macerated tissues were poured through a 100-µm-pore sieve nested on a 25-µm-pore sieve. The nematodes retained on the 25-µm-pore sieve were collected in a beaker (fraction 2). Fractions 1

and 2 were then poured on a 1-mm sieve covered with tissue paper placed on a dish with distilled water, and left overnight at room temperature. The suspension was then passed through a 25-µm-pore sieve and the retained nematodes collected. The nematodes were sterilized with a freshly prepared streptomycin sulfate solution (2000 ppm in 10 ml sterile distilled water) and left overnight in a laminar flow cabinet. The streptomycin sulfate was removed with a sterile glass Pasteur pipette from the suspension and the suspension washed three times with sterile distilled water. One ml of the suspension was poured into a sterile counting dish. Using a dissecting stereo microscope 20 individual gravid adult females were picked using a coconut midrib and inoculated aseptically on single sterilized carrot discs in a small drop of sterile distilled water.

Sterilized carrot discs were prepared from medium-sized carrots (free from any bacterial soft rot) washed with tap water, blotted dry, surface-sterilized with 70% ethanol, flamed and peeled three times. Carrots discs (5 mm thick) were placed in sterile glass Petri dishes (90 mm diam. x 15 mm high). Each Petri dish contained one medium-sized carrot disc (25 mm diam.) and was incubated at room temperature overnight prior to nematode inoculation. After inoculation, the Petri dishes were sealed with parafilm and incubated at 28ºC in an incubator.

The experiment was terminated at 4, 5, 6, 7 8 and 9 weeks after inoculation (WAI) by extracting nematodes following the previous described method. Fifteen replicates were used per observation time for each *R. similis* population. The number of nematodes was counted using a dissecting stereo microscope.

Pathogenicity of *Radopholus similis* and host plant response of *Musa* spp. Ten *Musa* genotypes were micropropagated following the standard tissue culture technique of Murashige and Skoog (1962). Ten genotypes were tested including six banana cultivars widely grown in small-scale cropping systems in the Philippines; one wild *M. balbisiana* and three reference genotypes with known host response to *R. similis* (Table 1).

Table 1. *Musa* genotypes evaluated under greenhouse conditions for their host response to three *Radopholus similis* populations from the Philippines.

Genotype	Genome	Selected synonyms	Use
Cuarenta Dias	AA	Inarnibal, Arnibal, Monkoy, Señorita, Sarat-sut, Cariñosa, Lungsuranon, Pisang Empat Puluh Hari, Pisang Lampung	Dessert
Lakatan - Davao	AA/AAA	Mapang, Pisang Berangan, Pisang Barangan, Kluai Hom Maew	Dessert
Bungulan	AAA (Cavendish)	Bunguran, Bulungan, Balangon, Pisang Masak Hijau, Pisang Ambon Lumut, Kluai Hom Khieo, Chuoi Tieu Cao #1, Tall Cavendish, Lacatan	Dessert
Morado	AAA	Raines na Pula, Gloria,Tadiao, Tinumbaga, Pisang Raja Udang Merah, Pisang udang, Kluai Nak, Chuoi Com Lua, Red, Rojo	Dessert
Latundan	AAB	Tundan, Turdan, Cantong, Pisang Rastali, Pisang Raja Sereh, Kluai Nam, Chuoi Goong, Silk Fig, Silk, Manzana	Dessert
Cardaba	BBB	Cadisnon, Pisang Chematu, Pisang Kepok Besar, Chuoi Mat	Cooking
Musa balbisiana (98-617)	BB	Pik-iw, Pacol, Butuhan	Wild
Pisang Jari Buaya[R]	AA	Tudló Datu, Morong Datu, Tudlong Dalaga, Saing Tudlo, Chuoi Tieu, Jari Buaya	Dessert
Yangambi Km5[R]	AAA	Ibota Bota, Kluai Khom Bao, Kluai Khai Thong Ruang, Pisang Saripipi	Dessert
Grande Naine[S]	AAA (Cavendish)	Pisang Ambon Jepang, Chuoi Va Huong	Dessert

[R]Resistant reference *Musa* genotype (Valmayor et al. 2000).
[S]Susceptible reference *Musa* genotype (Valmayor et al. 2000).

The selection of these genotypes was also based on root damage assessments made during surveys on various sites in Quezon, Oriental Mindoro, Davao and Cebu provinces, in which the genotypes Señorita and Cardaba had the lowest percentage root necrosis (Zorilla et al. 2005). All cultivars were maintained at the Plant Cell and Tissue Culture Laboratory in the Institute of Plant Breeding, University of the Philippines - Los Baños.

Aggregates of plants from the proliferation medium were separated and the leaves and shoots were excised. Plantlets were transferred in Murashige and Skoog (MS) coconut water medium for regeneration and finally to MS rooting medium with charcoal (Murashige and Skoog 1962). At each subculture stage of plant proliferation, an incubation condition was maintained at 28ºC with 16 h photoperiod for 4 weeks. Acclimatized plantlets (1 week prior to planting) were transferred to plastic pots (30 cm^3 capacity) containing sterilized river sand (40%), garden soil (40%), and coir dust (20%) and drenched with fungicide, Dithane M-45 (80% a.i. mancozeb, Dow Agro Sciences) to ensure that the medium was nematode and fungus free. Established plants were watered as needed and fertilized (Complete 14-14-14) every 2 weeks until inoculation. After 4 weeks, *Musa* genotypes were inoculated with 1000 *R. similis* obtained from each population.

For the host response and nematode pathogenicity (*i.e.*, the capacity to multiply on known susceptible and resistant hosts and its potential damage) tests, two separate pot experiments were conducted. Seven genotypes were used in the first batch namely: Bungulan (AAA), Cardaba (BBB), Cuarenta Dias (AA), Lakatan-Davao (AA/AAA), Latundan (AAB) and the reference genotypes Yangambi Km5 (AAA, resistant) (YKm5) and Grande Naine (AAA, susceptible). In the second batch, 10 genotypes were evaluated including the above mentioned cultivars, Morado (AAA), wild *M. balbisiana*, and another resistant reference genotype Pisang Jari Buaya (AA) (PJB). All pot experiments were laid out in a simple completely randomised design (CRD), with two factors (genotypes and *R. similis* populations) with five replications. Uninoculated plants were included as negative controls with the same number of replications.

Each plant was carefully uprooted and evaluated for the presence of and damage caused by *R. similis* at 8 WAI. Plants were washed in running water to remove the adhering soil. Roots were blotted dry using tissue paper or newspaper before cutting. Plant growth variables such as plant height, girth width (measured as diam. using a caliper), shoot weight, number of functional leaves and root weight were measured. Root damage caused by *R. similis* was determined following the protocol of Speijer and De Waele (1997). Percentage of dead roots was calculated while root health was assessed using the following scale: 1 all roots healthy, 2 most roots healthy, 3 most roots dead and 4 all roots dead. To assess root necrosis, five pieces of 10-cm root samples from each cultivar were cut lengthwise and examined for the presence of lesions. The maximum necrosis per root half is 20%, giving a total root necrosis ranging from 0 to 100%. Nematode density was assessed by counting the number of eggs, juveniles, males and females. Five g of roots were macerated in a kitchen blender three times for 10 s with 5 s intervals. The nematode suspension was sieved using a nested set of 250-, 106-, 40- and 25-µm sieves. Eggs and vermiform nematodes were collected from the 25-µm sieve. The suspension was standardized into 50 ml aliquots. Two ml of the suspension was poured into a counting dish for counting.

Data analysis

The homogeneity and normality of the morphometrics of *R. similis* populations were tested using Levene's and Kolmogorov-Smirnov tests, respectively. The differences in plant growth variables between inoculated and uninoculated plants were computed and analyzed using simple *t – test* and two-way ANOVA analysis, followed by Tukey's Honestly Significant Difference (HSD) at $P<0.05$ for comparisons of nematode pathogenicity. Nematode counts were $\log_{10}(x+1)$ transformed before analyses, while percentages dead roots and root necrosis square root transformations were used. For normal and homogeneous populations, one-way ANOVA was used to analyze the data and

means were separated with Tukey's HSD in comparing the effects of genotypes and nematode populations, respectively. All paired tests used at least 0.95 combined confidence levels with combined confidence coefficient, $\alpha = 0.5$. SPSS v13.0 for Windows Software was used.

RESULTS AND DISCUSSION

Morphometrics and morphology of *Radopholus similis*

Female morphometrics. Differences in total female body length were observed among the three populations. Considering the overlapping values of the total body lengths of the Quezon (611.9 µm) and Davao (625.1 µm) populations, adult females of the Laguna population were longer than the former two populations with a total body length of 631.7 µm (Table 2). Females of the Laguna population also had a maximum body width of 26 µm and body width at anus of 20 µm, *i.e.* wider than the females of the other two populations. The stylet length of the females of each of the three populations averaged 19.5 µm *i.e.* about two head-widths long with basal knobs rounded or anteriorly pointed. The terminal lobe of the oesophagus overlapped the intestine dorsally. Gonads were paired and outstretched, didelphic and ampidelphic with the vulval opening located near the mid-region along the ventral side of the body. Ovaries of the three populations extended anteriorly and posteriorly. The lengths of the posterior and anterior ovaries differed among the three populations. On average, the length of the posterior ovary was 127.5, 153 and 124.2 µm for the Quezon, Laguna and Davao populations, respectively. The length of the anterior ovary of the Quezon population was on average 178.7 µm and for the Laguna and Davao populations 160 and 195 µm, respectively. Tail lengths also differed among the different populations, being 77, 69.8 and 65 µm for the Laguna, Davao and Quezon populations, respectively.

Table 2. Morphometrics of females of three *Radopholus similis* populations from the Philippines (Quezon, Laguna and Davao; measurements in *µm*).

	***Radopholus similis* population**		
	Quezon	**Laguna**	**Davao**
Total body length	611.9 ± 27.9 (592.2 – 631.7)	631.7 ± 5.7 (632.0 – 640.0)	625.1 ± 46.5 (592.2 – 658.0)
Max. body width	16.2 ± 0.03 (16.2 – 16.3)	26.0 ± 2.5 (22.4 – 26.0)	19.5 ± 0.28 (18.7 – 19.8)
Stylet length	19.3 ± 0.27 (18.7 – 19.5)	19.5 ± 1.1 (18.0 – 19.5)	19.4 ± 0.27 (18.7 – 19.5)
Oesphagus length	61.7 ± 0.44 (60.2 – 61.7)	68.2 ± 1.3 (68.2 – 70.0)	61.7 ± 6.9 (52.0 – 61.7)
Tail length	65.1 ± 0.1 (65.0 – 65.1)	77.0 ± 0.4 (77.0 – 77.6)	69.8 ± 2.3 (68.2 – 71.5)
Body width at anus	13.0 ± 1.4 (12.0 – 13.8)	20.0 ± 2.0 (17.2 – 20.0)	16.2 ± 0.14 (16.2 – 16.4)
Head height	3.2 ± 0.04 (3.2 – 3.3)	3.2 ± 0.04 (3.2 – 3.3)	3.2 ± 0.4 (3.2 – 3.3)
Head width	9.4 ± 0.13 (9.0 – 9.4)	11.4 ± 1.4 (9.4 – 11.4)	9.7 ± 2.3 (6.5 – 9.7)
Anterior ovary length	178.7 ± 0.68 (178.0 – 180.5)	160.0 ± 0.33 (160.0 – 161.0)	195.0 ± 0.23 (195.0 – 195.5)
Posterior ovary length	127.5 ± 0.42 (127.0 – 128.2)	153.0 ± 0.35 (152.0 – 153.5)	124.2 ± 27.6 (104.7 – 143.7)

Data are the average values, followed by the standard variation. The minimum and maximum values are between parentheses.

Male morphometrics. Sexual dimorphism marked all three populations. Males had spherical heads which were smaller than that of the females head width (9.5 µm). Males also had a shorter stylet, on average 13 µm long, with slight basal knobs, a degenerated oesophagus, and a valveless and reduced median bulb (Table 3). On average, the total body length of the males was shorter than the females with on average 473.8 µm (Quezon), 539.6 µm (Davao) and 596.6 µm (Laguna population)

and a maximum body width of 13 µm (Quezon), 16.2 µm (Davao) and 20 µm (Laguna population). Based on these body measurements, males of the Laguna population were the largest. Measurements for the tail length and body width at anus for the Laguna population also showed differences among populations with 76.4 µm and 19.5 µm, respectively. The Davao population had 74.0 µm and 14.3 µm tail length and body width at anus, respectively; whereas the Quezon population had the shortest tail length with on average 52.0 µm and 17.7 µm body width at anus. Spicule and testis lengths and gubernacula length also differed among the populations. However, no apparent differences in stylet length and head width and height were recorded among male specimens of the three populations.

Table 3. Morphometrics of the males of three *Radopholus similis* populations from the Philippines (Quezon, Laguna and Davao; measurements in *µm*).

	***Radopholus similis* population**		
	Quezon	**Laguna**	**Davao**
Total body length	473.8 ± 1.5 (472.0 – 478.0)	596.6 ± 9.3 (592.2 – 605.4)	539.6 ± 7.2 (530.0 – 540.0)
Max. body width	13.1 ± 0.17 (13.0 – 13.5)	20.0 ± 1.1 (19.5 – 21.1)	16.2 ± 0.23 (16.0 – 16.5)
Stylet length	13.0 ± 0.4 (13.0 – 13.6)	13.4 ± 0.36 (13.0 – 13.9)	13.0 ± 0.22 (13.0 – 13.5)
Oesphagus length	62.3 ± 0.42 (61.7 – 62.3)	65.0 ± 1.3 (68.2 – 70.0)	64.0 ± 6.9 (52.0 – 61.75)
Tail length	52.0 ± 0.79 (51.0 – 53.5)	76.4 ± 6.9 (71.5 – 81.25)	74.0 ± 0.90 (73.5 – 74.0)
Body width at anus	17.7 ± 2.4 (14.3 – 17.7)	19.5 ± 0.37 (19.0 – 20.0)	14.3 ± 0.21 (14.0 – 14.5)
Head height	6.5 ± 0.39 (6.0 – 7.0)	6.5 ± 0.30 (6.5 – 7.1)	6.5 ± 0.15 (6.1 – 6.7)
Head width	9.5 ± 0.0 (9.5 – 9.5)	9.5 ± 0.09 (9.5 – 9.7)	9.5 ± 0.06 (9.5 – 9.7)
Testis length	216.0 ± 0.5 (215.0 – 216.5)	263.2 ± 15.4 (131.6 – 294.8)	216.0 ± 0.07 (216.0 – 216.1)
Spicule length	17.7 ± 0.7 (16.7 – 17.7)	18.4 ± 2.3 (16.2 – 19.4)	16.2 ± 0.13 (16.0 – 16.3)
Gubernaculum	11.8 ± 0.35 (11.5 – 12.0)	10.8 ± 2.3 (9.7 – 13.0)	6.5 ± 0.06 (6.5 – 6.6)

Data are the average values, followed by the standard variation. The minimum and maximum values are between parentheses.

Female and male morphology. Both males and females had divergent tail ends (Fig. 1). Both tail shape and length differed within and among the three populations. Females from the Davao and Quezon populations had pointed to tapering tails with smooth termini. By contrast, broad tails with smooth termini were observed in 87% of the females of the Laguna population. Tails of adult females from Davao had pointed to truncate tails with annulated to smooth termini. Sixty percent of the females from Davao had truncated, smooth tail ends, whereas 63% of the females from Quezon had a pointed terminus with evident annulations.

The form of the male tail ends also showed variations within and among the populations. However, the majority of the males had a pointed to tapering tail with smooth termini. In the Davao population, 60% of the males had broad tail ends but with annulations, while 50% of the males of the Quezon population had truncated to pointed tails with some annulations. All males from the Laguna population had truncated, annulated tails.

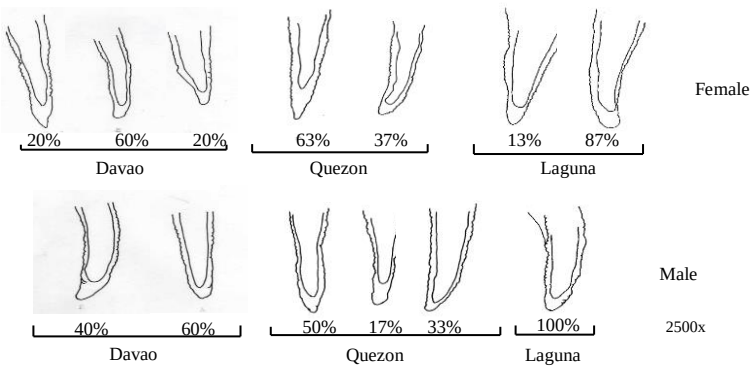

Fig. 1. Tail shapes (and their frequency of occurrence) of females and males of *Radopholus similis*.

In vitro* reproductive fitness of *Radopholus similis

All three *R. similis* populations completed their life cycle in 28 to 30 days. Nematode multiplication was relatively low with a reproductive factor (Rf) ranging from 1.7 to 3 (Table 4). These increased over time for all three populations with significant differences ($P \leq 0.05$) in Rf. The density of the Quezon population increased 1.7, 55 and 128 times at 4, 5 and 6 WAI, respectively. However, a 50% decrease was observed for this population at 7 WAI. At 5 WAI, the densities of the Laguna and Davao populations were statistically similar to each other but significantly ($P \leq 0.05$) lower than that of the Quezon population. The highest Rf (422.4) was observed for the Davao population with a density of 8,447 nematodes at 8 WAI (data not shown). The population density declined afterwards to 376 nematodes 9 WAI. The Laguna population attained its highest density of 3,577 at 7 WAI (Rf =178.9) but declined with a high number of dead nematodes observed. The Quezon population reached its highest density (2,284) of reproductive females at 6 WAI, while the Laguna and Davao populations reached their highest population density at 7 and 8 WAI, respectively. The increase in reproductive females resulted in high numbers of eggs and juvenile nematodes.

Table 4. Comparative reproductive fitness of three *Radopholus similis* populations from the Philippines on carrot discs at 4, 5, 6 and 7 weeks after inoculation (WAI)

Population	Time (week)[1]	Eggs	Juveniles	Males	Females	Pf[2]	Rf[3]	n[4]
Quezon	4	2	1	2	28	33 a[5]	1.7 a	15
	5	190	174	116	628	1108 b	55.4 ab	15
	6	109	70	90	2284	2553 b	127.6 b	10
	7	290	55	85	679	1109 b	55.4 ab	8
Laguna	4	6	17	18	19	60 a	3.0 a	10
	5	200	80	25	300	605 b	30.4 ab	10
	6	780	315	80	1500	2675 c	133.8 c	10
	7	830	463	213	2901	4404 c	178.9 c	9
Davao	4	4	4	8	45	61 a	3.0 a	15
	5	24	60	34	483	601 a	30.1 a	13
	6	112	97	126	1188	1523ab	76.2 ab	11
	7	79	448	153	2452	3132 c	157.6 b	9

[1]Observations were made up to 10 weeks after nematode inoculation to determine the stationary phase of nematode reproduction. However, due to high bacterial contamination of the carrot discs and an insufficient number of replicates at 8, 9 and 10 WAI for the three *R. similis* populations, nematode counting were done up to 7 WAI only.

[2]Final nematode population density (living nematodes only).

[3]Reproduction factor = final nematode population density/initial inoculum level (= 20).

[4]Number of replicates.

[5]Means in column per population followed by the same letter do not differ significantly according to Tukey's HSD at $P \leq 0.05$. Data were $\log_{10}(x+1)$ transformed prior to statistical analysis, however untransformed data are presented.

The growth of the three *R. similis* populations was plotted as a function of time (Fig. 2) where the total nematode population density was first $\log_{10}(x+1)$ transformed. The Quezon population had the highest slope (1.7) compared with the other two populations (both 0.6). Due to the high growth rate of the Quezon population, its stationary growth phase was reached at 6 WAI, which is 1 and 2 weeks earlier than the Laguna and Davao populations, respectively.

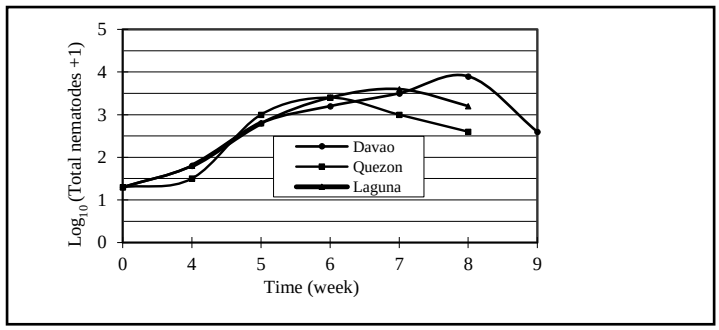

Fig. 2. Growth of three *Radopholus similis* populations from the Philippines as function of time (week) after inoculation with single female per carrot disc and incubated at 28°C.

The increase in nematode population as a function of time can be described by the Gompertz equation (Zwietering et al. 1990). The Gompertz equation; *Log Pt* = A + C*exp* (- *exp* (*exp* [B (M-*t*)]), wherein, *Pt* is the nematode population at incubation time *t* (*t* expressed in week), and A, B, C and M are model parameters, is widely used for the growth of biological organisms. This model describes three phases; (i) lag phase in which nematodes adapt to the new environment; (ii) the exponential growth phase and; (iii) stationary growth phase due to exhaustion of nutrients. Based on the growth curve, Quezon population had the highest slope between 4th and 5th week (exponential growth phase) and early stationary growth phase towards 6 WAI, followed by the Laguna and Davao populations.

Pathogenicity of *Radopholus similis* and host response of *Musa*

The reduction in plant growth of selected *Musa* genotypes infected with the *R. similis* populations observed in two separate pot experiments conducted under greenhouse conditions is shown in Table 5. In the first experiment, Bungulan and Cardaba were severely infected by *R. similis*. The percentage reductions in plant height, pseudostem girth, shoot and root weights were comparable to cv. Grand Naine. In the cv. Latundan the percentage reduction of all measured variables were statistically higher after inoculation with either the Laguna or Davao population compared with cv. Cuarenta Dias and with that of Quezon *R. similis* – inoculated Latundan. Cuarentas Dias was the least affected cultivar following infection with the Laguna and Davao populations with percentage differences of -9.1 and -4.2 on plant heights, respectively. The growth parameter data of cv. Cuarenta Dias were comparable to cv. YKm5 with less than -6.7% difference in shoot and root weights. The reduction in root weight was evident on cvs Bungulan, Cardaba, Latundan, and Grand Naine. Root growth of Grand Naine was poor after nematode infection which severely damaged both root and shoots leading to lower pseudostem girth and plant height. The Davao and Laguna populations showed significant ($P \leq 0.05$) percentage reduction in plant growth on susceptible genotypes such as Bungulan, Cardaba and Grande Naine. However, the Davao population alone significantly ($P \leq 0.05$) affected pseudostem girth, shoot and root weights with percentage reductions of -42.8, -68.5 and -81.3, respectively. The Laguna population significantly ($P \leq 0.05$) affected plant growth variables of cv. Lakatan-Davao compared to the other two populations. The Quezon population on the other hand, induced significant ($P \leq 0.05$) reductions in all growth variables of cv. Cardaba.

Table 5. Effect of three *Radopholus similis* populations from the Philippines on the growth of *Musa* genotypes 8 weeks after inoculation (WAI) with 1,000 nematodes per plant compared with uninoculated (UI) control plants.

Genotype	N[1]	Plant height (%)				Pseudostem girth (%)				Shoot weight (%)				Root weight (%)			
		UI (cm)	Quezon	Laguna	Davao	UI (cm)	Quezon	Laguna	Davao	UI (g)	Quezon	Laguna	Davao	UI (g)	Quezon	Laguna	Davao
Pot experiment 1																	
Bungulan	5	30.2	-17.6*[2]A[3]	-43.3***B	-50.7***B	2.8	-13.4*A	-46.0***B	-44.0***B	114.4	-28.6*A	-83.7***B	-81.7***B	17.6	-54.9***A	-94.3***B	-93.0***B
Cardaba	5	24.1	-53.3**AB	-15.2 A	-62.9***B	2.4	-56.6*B	-14.5 A	-67.5** B	85.9	-86.8**B	-37.4 A	-95.4** B	17.2	-79.9**A	39.9 A	-88.3**B
Cuarenta Dias	5	23.6	0.6 A	-9.1 A	-4.2 A	1.8	32.3A	22.2 A	37.3 A	44.9	59.3 A	20.0 A	50.5 A	7.2	95.3 A	39.2 A	177.9 A
Lakatan-Davao	5	24.0	6.7 A	-50.9** B	-45.3* B	2.1	13.1A	-44.0* B	-1.5 AB	67.8	9.7 A	-76.1**B	-26.4 AB	10.8	8.7 A	-76.2**B	-6.3 A
Latundan	5	31.6	-16.6 A	-48.3* B	-44.7* B	2.2	8.2A	86.1 A	-42.8* B	73.4	-12.8 A	-46.4 A	-68.5* B	11.6	-19.1 A	-33.7 A	-81.3* B
Yangambi Km 5 (R)	5	25.0	5.0 A	4.2 A	4.2 A	2.8	-4.7A	1.2 A	-0.6 A	76.0	-3.0 A	-5.1 A	-5.3 A	25.2	-4.5 A	-4.9 A	-6.7 A
Grande Naine (S)	5	17.7	-32.1**A	-46.0** B	-42.8**B	3.6	-61.3*A	-72.2*A	-76.1* A	44.8	-65.2**A	-79.3**A	-87.9**A	6.8	-72.6**A	-92.3**A	-77.9**A
Pot experiment 2																	
Bungulan	5	24.9	28.0 A	26.3 A	-0.6 A	2.1	-16.4 A	-18.6 A	-37.1 A	65.1	6.3 A	-0.2 A	-43.1 B	15.9	-34.0 A	-47.2 A	-73.3*B
Cardaba	5	25.8	2.2 A	-3.6 A	-24.3 A	2.1	3.8 A	-0.8 A	-25.6 A	71.4	17.1 A	1.1 A	-46.4 B	17.0	-17.7 A	-33.3 A	-65.3*B
Cuarenta Dias	5	21.8	57.2**A	40.8*A	40.8*A	1.9	17.5 A	15.4 A	9.1 A	61.3	56.7 B	44.1 A	17.4 A	8.3	75.3 B	46.5 B	7.3A
Lakatan-Davao[a]	5	25.8	24.6***A	20.2*A	-11.8 B	1.9	0.6 A	-0.7 A	-13.2 A	69.7	-3.0 A	-4.2 A	-40.5 B	14.3	-51.6*A	-57.3*A	-78.3*A
Latundan	5	35.7	-17.5 A	-55.1* B	1.5 A	1.9	-7.9 A	-29.4 A	-0.8 A	54.2	-4.3 A	-74.3**B	26.1 A	5.1	7.5 A	-70.8* B	6.7 A
Morado	5	44.9	-1.6 A	-10.8*A	-17.1**A	2.4	6.2 A	12.3 A	-8.6 A	118.4	17.5* A	18.2 A	-23.9*A	15.8	23.0 A	31.0 B	48.1*B
Musa balbisiana	5	20.1	-21.6 B	34.2 A	-36.7 B	1.4	-7.9 B	48.7* B	-19.6 A	32.2	-28.8 B	123.7*A	-59.1 B	3.4	-39.8 A	146.6* B	-71.8*B
Pisang Jari Buaya (R)[a]	5	45.7	-3.1 A	-17.3*A	-5.3 A	3.2	-29.4**B	-20.7*A	-26.6***AB	142.2	-34.3 A	-19.3 A	-32.5*A	14.9	-28.8 A	26.8 A	-27.9**A
Yangambi Km 5 (R)	5	17.6	26.1 A	32.5 A	10.7 A	1.0	40.0*A	75.9***A	42.7*A	15.9	118.2*A	212.1*A	81.2 A	3.3	100.0* A	185.3**A	89.6*A
Grande Naine (S)	5	24.9	-25.7* A	-28.7*A	-39.8**A	2.2	-15.2 A	-21.3*A	-30.9*A	79.7	-22.6 A	-23.8* A	-47.8* B	13.0	-13.8 A	-37.2 B	-48.5* B

UI – means of uninoculated plants.

[1]Number of replicates per treatment; [a]treatment with four replicates in the uninoculated plants.

[2]Data expressed as percent mean difference = Uninoculated minus inoculated divided by uninoculated times 100, evaluated at * ($P \leq 0.05$), ** ($P \leq 0.01$) or ***($P \leq 0.001$) according to the t-test.

[3]Means in the same rows per parameter followed by the same letter do not differ significantly ($P \leq 0.05$) according to One-way ANOVA followed by Tukey's HSD.

Table 6. Reproduction of three *Radopholus similis* populations from the Philippines and root damage on *Musa* genotypes 8 weeks after inoculation (WAI) with 1,000 nematodes per plant.

Genotype	n[1]	Nematodes/g roots[2]			Nematodes per root system[2]			Percentage dead roots[3]			Percentage root necrosis[3]		
		Quezon	Laguna	Davao	Quezon	Laguna	Davao	Quezon	Laguna	Davao	Quezon	Laguna	Davao
Pot experiment 1													
Bungulan	5	1,325 c[4]A[5]	7,638 b B	2,024 abcAB	11,124 b B	10,090 b B	3,832 abA	10.8 a A	66.5 b B	65.5 b B	42.2 b A	73.2 cAB	89.0 b B
Cardaba	5	196 abcA	289 abA	1,385 bcA	260 a A	1,448 abB	2,073 ab B	34.4 b B	6.9 abA	100 c C	49.3 b B	6.8 a A	100.0 b C
Cuarenta Dias	5	32 ab A	34 a A	61 ab A	466 a A	292 a A	1,152 ab A	3.6 a A	2.0 a A	3.1 a A	20.2 ab A	25.6 abcA	27.2 a A
Lakatan - Davao	5	228 abcA	2,068 abB	872 bc B	2,334 abA	3,095 abA	2,196 ab A	1.3 a A	51.2 b B	43.8 ab B	33.8 ab A	69.8 bcA	78.0 b A
Latundan	5	2,924 cA	4,411 b B	5,693 c B	8,912 bA	14,835 bA	23,412 b B	5.7 a B	4.0 a A	56.2 ab B	36.6 ab A	48.2 b AB	76.2 b B
Yangambi Km 5 (R)	5	8 a A	10 a A	18 a A	272 a A	339 a A	537 a A	1.2 a A	3.7 a A	3.1 a A	7.4 a A	16.0 ab A	15.6 a A
Grande Naine (S)	5	3,303 c A	2,047 abA	9,669 c B	3,780abA	7,675 b B	11,310 b B	13.7 abA	76.4 b B	100 c B	27.0 ab A	80.0 c B	100.0 b B
Pot experiment 2													
Bungulan	5	275 cA	1,421 cd B	10,435 c C	3,419 cA	10,662 c B	41,983c C	4.2bcA	2.4 aA	16.1 bA	7.6 aA	24.8 aA	70.9 bcB
Cardaba	5	8 a A	1,423 cd B	2,468 bc B	69 ab A	3,158 b B	11,232b B	5.8cA	1.2 aA	21.7 bA	13.6 aA	20.2 aA	87.8 cd B
Cuarenta Dias	5	19 ab A	483 cd B	4,236 bc C	260 bcA	4,228 b B	34,692c C	0 aA	0 aA	0 aA	5.2 aA	19.2 a B	51.4 ab C
Lakatan - Davao	5	70 bcA	2,649 d B	3,794 bc B	530 bcA	4,396 b B	11,267b B	0 aA	1.5 aA	0 aA	3.8 aA	34.0 a B	51.0 abB
Latundan	5	5 a A	636 cd B	2,175 bc B	47 ab A	1,293 b B	9,712ab C	0 aA	0 aA	0 aA	4.0 aA	11.2 aA	39.0 a B
Morado	5	2 a A	104 b B	2,406 bc C	27 ab A	1,180 b B	14,273b C	0 aA	1.3 aA	2.4 abA	3.0 aA	10.1 a B	66.0abc C
Musa balbisiana	5	13 ab A	5 a A	2,056 bc B	18 a A	27 a A	2,365a B	0 aA	5.9 aA	6.3 abA	7.0 aA	12.9 aA	90.9 dB
Pisang Jari Buaya (R)	5	13 ab A	357 cdA	1,120 ab B	151ab A	7,653 bc B	3,604ab B	0 aA	0 aA	0 aA	1.9 aA	16.0 a B	55.8abc C
Yangambi Km 5 (R)	5	3 a A	6 a A	606 a B	25 a A	42 a A	2,380a B	0 aA	0 aA	0 aA	4.0 aA	9.0 aA	39.8 a B
Grande Naine (S)	5	94 bcA	232 cdA	2,709 bc B	863 cA	3,362 b B	32,425c C	1.1 abA	0 aA	0 aA	5.2 aA	13.2 aA	52.0 ab B

[1]Number of replicates per inoculated plants.
[2]Data were log (x+1) transformed prior to statistical analyses; [3]Data were square root transformed prior to statistical analyses. Untransformed data are presented.
[4]Means in columns followed by the same small letters do not differ significantly according to One-way ANOVA followed by Tukey's HSD at 0.05 level.
[5]Means in rows per parameter followed by the same capital letter do not differ significantly according to One- ANOVA followed by Tukey's HSD at 0.05 level.

In the first experiment, significant ($P \leq 0.05$) differences in the susceptibility to *R. similis* populations were observed. The number of nematodes per root system and per root unit on cv. YKm5 was significantly ($P \leq 0.05$) lower compared to cv. Grande Naine (Table 6). Cuarenta Dias had low number of nematodes per root system in Quezon and Laguna populations with 466 and 292 mean nematode counts/plant, respectively. However, in Davao population a total of 1,152 nematodes were recovered per plant. On the other hand, Bungulan and Latundan were statistically ($P \leq 0.05$) as susceptible as Grande Naine to *R. similis*. The highest nematode count per root system and per root unit was recovered from Latundan with 5,693 and 23,412 nematodes, respectively. The number was significantly ($P \leq 0.05$) higher compared with all genotypes. Significant ($P \leq 0.05$) differences on percentage dead roots and percentage root necrosis were observed from Bungulan, Cardaba, Latundan, Lakatan-Davao and Grande Naine. The abovementioned cultivars also had root health ratings of 3 to 4 however data on RH are not shown. Using Davao population Cuarenta Dias and YKm5 showed most healthy roots (RH = 2) and lowest percentage root necrosis of 27.2 and 15.6, respectively.

In the second experiment Latundan was significantly ($P \leq 0.05$) affected in terms of plant growth and root damage variables. Plant height, shoot weight and roots weight showed percentage differences of -55.1, -74.8 and -70.8, respectively, when inoculated with the Laguna population. These data in reference to the uninoculated control were higher than the percentage difference of the susceptible check. Plant height of Morado also showed significant ($P \leq 0.05$) percentage reduction, however the level was far lower than Latundan. Lakatan-Davao on the other hand had significantly($P \leq 0.05$) higher percentage reductions on root weight with -51.6, -57.3 and -78.3 when inoculated with Quezon, Laguna and Davao populations, respectively. Bungulan and Cardaba which had the highest percentage reductions during the first evaluation, only showed significant ($P \leq 0.05$) reductions on root weight when inoculated with Davao population. Wild *M. balbisiana* (98-617) had significant ($P \leq 0.05$) reduction on root weight (-71.8%) when inoculated with the Davao population. However, *M. balbisiana* did not exihibit significant reductions on plant height, pseudostem girth, and shoot weight. The reference genotype YKm5 once again showed resistant reaction against *R. similis* unlike PJB. PJB showed significant ($P \leq 0.05$) percentage difference on pseudostem girth.

Pathogenicity tests of the three *R. similis* populations showed that Davao population contributes the highest percentage reduction on root weight on Bungulan (-73.3%) and Lakatan-Davao (-78.3%). This was followed by Laguna and the least was obtained from Quezon population. However, apparent reaction and nematode pathogenicity were exhibited on *M. balbisiana. M. balbisiana* (98-617) resulted to lowest significant ($P \leq 0.05$) reduction when inoculated with the Laguna population. Davao population caused the highest percentage reduction in all growth variables.

Differential reactions of genotypes to *R. similis* were also observed. Bungulan, Cuarenta Dias, Morado, Lakatan-Davao, Cardaba and Latundan (in decreasing order) had the highest nematode density per g and per root system. The lowest density was observed from *M. balbisiana,* PJB and YKm5. The lowest root damage was obtained from Cuarenta Dias, Lakatan–Davao and Latundan having 51.4%, 51.0% and 39.0% necrosis, respectively. The percent necrosis of Latundan was comparable to YKm5 with 39.2% RN. *M. balbisina* with the lowest nematode density of 2,365 individuals per plant, however, resulted in highest necrosis (90.9%). This low nematode count of *M. balbisiana* was attributed to the fewer roots evaluated due to high degree of root necrosis. The same level of pathogenicity of *R. similis* populations was observed under pot experiment two. Results showed that Davao population resulted to significant number of nematodes per plant and induced root damage on test genotypes. This was followed by Laguna and Quezon populations. All genotypes except Bungulan were found resistant to Quezon isolate.

This study proved that morphometrical and morphological differences occur among the Quezon, Laguna and Davao *R. similis* populations. This is the first attempt to analyze the basic

morphological characteristics of *R. similis* in the Philippines. These findings confirmed the results found by Elbadri et al. (1999) that morphological and morphometrical variations exist among African *Radopholus* populations. However, the morphological characteristics observed from the Philippine populations showed no similarities with that of the African populations but falls within the range of its morphological measurements (Appendix Tables 1 and 2) (Huettel et al. 1986, Elbadri et al. 1999). Diversity in and among populations of *R. similis* has been confirmed through morphology and morphometrics (Huettel et al. 1986, Huettel and Yaegashi 1988, Elbadri et al. 1999). However, most morphological studies done used the scanning electron microscopy (SEM). The SEM observations on *R. similis* populations have revealed several differences on the external morphology of the nematode (Huettel and Yaegashi 1988, Valette et al. 1998). Thus, in support of the morphometrical analysis conducted in this study the SEM of the cuticular structures such as shape of oral disc and lateral lip section of females and shape of the head of males is of equal importance (Valette et al. 1998). Molecular analysis of the highly conserved region related to specific phenotype of *Radopholus* species (Kaplan et al. 1996) should also be done on the Philippine populations. Although limited genetic variation had been observed among burrowing nematode populations including *R. citrophilus* and *R. similis* infecting different host plants, the molecular analysis may support the possible existence of genetic variability or homology among the described *R. similis* populations in the Philippines in relation to their morphological and morphometrical differences and may partly explain the possible differences in pathogenicity among Philippine populations. On the other hand, the general pattern of population build-up among Quezon, Laguna and Davao populations at 28°C was not uniform wherein the Davao population had the longest time reproductively fit on a single carrot disc at 28°C and produced more reproductive females as an indicator of Rf. Differences in the reproductive fitness of *R. similis* populations on carrot discs as a function of time, temperature and inoculum density have been reported (Boncato and Davide 1980, Fallas and Sarah 1995, Stoffelen et al. 1999, Elbadri et al. 2001). The time experiment showed increasing nematode reproduction at longer time interval. Moreover, populations with higher growth rate reached the stationary growth phase earlier after inoculation (Stoffelen et al. 1999). Still according to the findings of Stoffelen et al. (1999), *R. similis* populations (Cuba and Costa Rican populations) showed significant increase in nematode density with an increasing inoculum levels from 5 to 25 females whilst, inoculum of 50 to 100 females showed no further increase in density. This rapid decline of nematode population also correlated with the available food nutrients. Nematodes migrated from carrot tissues after 9 WAI and died due to lack of food and competition in space resulting to rapid stationary growth phase and decline of nematode density.

Philippine *R. similis* populations also showed varying degree of pathogenicity towards different *Musa* genotypes. The Davao population which consistently showed high degree of damage on susceptible genotypes, Bungulan, Lakatan-Davao, Morado and *M. balbisiana* indicates aggressiveness thus more pathogenic than Laguna and Quezon populations. This aggressiveness of Davao population can produce more prolific female individuals at higher rate and eventually generates more J_2 that actively feeds on roots.

CONCLUSION

Specific pathogenicity is apparently related to reproductive fitness. The higher the reproductive fitness partly explained the higher degree of root damage of *R. similis* in the field. Although, morphology and morphometrics failed to correlate with the pathogenicity of *R. similis*, possible genetic variability study in relation to nematode parasitism must be explored among Philippine isolates. For the first time, this study proved the existence of *R. similis* diversity in the country and will serve as benchmark data in selecting and breeding *Musa* genotypes for nematode resistance or tolerance.

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