INTERACTION OF *PRATYLENCHUS BRACHYURUS* AND *HELICOTYLENCHUS* SP. WITH MEALYBUG WILT OF PINEAPPLE IN MICROPLOTS

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ABSTRACT

Ferreira, T. D. F., R. M. Souza, W. S. S. Idalino, K. D. dos Santos Ferreira, and P. S. T. Brioso. 2014. Interaction of *Pratylenchus brachyurus* and *Helicotylenchus* sp. with mealybug wilt of pineapple in microplots. Nematropica 44:181-189.

In Brazil, phytonematodes and mealybug wilt of pineapple (MWP) are major phytosanitary problems in pineapple plantations. Phytonematodes and MWP incidence are often concomitant, and the shoot symptoms are nonspecific. 'Vitória' pineapples were cultivated in microplots for 22 months, either uninoculated or inoculated with: i) *Pratylenchus brachyurus*; ii) *Helicotylenchus* sp.; iii) the mealybug *Dysmicoccus brevipes* carrying the pineapple mealybug wilt-associated virus-1, -2, and -3; iv) *P. brachyurus* and *D. brevipes*; v) *Helicotylenchus* sp. and *D. brevipes*. The symptoms and damage were assessed at 8, 19, and 21 months after inoculation (MAI) to estimate the effect of these pests on vegetative and fruit development. The pest effect was most pronounced at 19 MAI *P. brachyurus* suppressed ($P<0.05$) root and D-leaf fresh weight and decreased the width of D-leaves and fruit. *Pratylenchus brachyurus*, *Helicotylenchus* sp., and MWP reduced the fruit length and delayed fruit maturity, while both nematodes, but not MWP, reduced the fruit weight. These results suggest that the concomitant incidence of *Helicotylenchus* sp. or *P. brachyurus* with MWP results in additive damages to pineapple. Shoot symptoms varied from none, in plants parasitized by *Helicotylenchus* sp., to wilt and chlorosis of leaves, followed by necrosis at the tip of newer leaves, and death of the older leaves in the plants affected by MWP and parasitized by *P. brachyurus*. These results indicate the need for a better understanding of the symptoms and damages induced by nematodes and MWP in commercial plantations as a necessary step towards better field diagnosis and management.

Key words: Ananas comosus, *Dysmicoccus brevipes*, pineapple mealybug wilt-associated virus, root-lesion nematode, spiral nematode

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RESUMO

Ferreira, T. D. F., R. M. Souza, W. S. S. Idalino, K. D. dos Santos Ferreira, and P. S. T. Brioso. 2014. Interação de *Pratylenchus brachyurus* e *Helicotylenchus* sp. com a murcha do abacaxizeiro, em microparcelas. Nematropica 44:181-189.

No Brasil, fitonematoides e a murcha do abacaxizeiro (MA) são sérios problemas fitossanitários da cultura do abacaxi. Com frequência a incidência desses agentes é concomitante e os sintomas induzidos na parte aérea das plantas são inespecíficos. Abacaxizeiros 'Vitória' foram cultivados em microparcelas a céu aberto por 22 meses, sadios ou inoculados com: i) *Pratylenchus brachyurus*; ii) *Helicotylenchus* sp.; iii) cochonilhas *Dysmicoccus brevipes* infectadas com o *Pineapple mealybug wilt-associated virus*-1, -2, e -3; iv) *P. brachyurus* e *D. brevipes*; v) *Helicotylenchus* sp. e *D. brevipes*. O efeito desses agentes sobre os sintomas e danos ao desenvolvimento das plantas e dos frutos foi avaliado aos 8, 19 e 21 meses após a inoculação (MAI). O efeito dos agentes foi mais pronunciado aos 19 MAI. *Pratylenchus brachyurus* reduziu ($P <0,05$) o peso fresco das raízes e da folha D e a largura da folha D e dos frutos. *P. brachyurus*, *Helicotylenchus* sp. e a MA reduziram o comprimento do fruto e retardaram a sua maturação, enquanto que ambos os nematóides, mas não a MA, reduziram o peso fresco do fruto. Esses resultados sugerem que a incidência concomitante de *Helicotylenchus* sp. ou *P. brachyurus* com a MA resulta em danos aditivos na cultura do abacaxizeiro. Na parte aérea, os sintomas variaram de nenhum, em plantas parasitadas por *Helicotylenchus* sp., à murcha e clorose das folhas, seguida de necrose na ponta das folhas mais novas e morte das folhas mais velhas nas plantas afetadas pela MA associada ao parasitismo por *P. brachyurus*. Esses resultados indicam a necessidade de uma melhor compreensão dos sintomas e danos induzidos por fitonematoides e pela MA em cultivos comerciais, como um passo essencial para a melhoria da diagnose dos problemas fitossanitários das lavouras e seu manejo.

Palavras-chaves: Ananas comosus, *Dysmicoccus brevipes*, murcha do abacaxizeiro, nematoide espiralado, nematoide das lesões radiculares.

INTRODUCTION

Pineapple (*Ananas comosus* L. Merril) is cultivated in many tropical countries and consumed all over the world. Brazil has increased production in recent years and is responsible for 53% of world production (Anonymous, 2011). In Brazil, pineapple is typically grown by smallholders and is economically vital to many regions and families (Souza *et al*., 1999; Anonymous, 2010).

Production and sustainability of quality propagative material to maintain high yields are limited by technical and phytopathological challenges (Cunha and Reinhardt, 2004; Ponciano *et al*., 2006). Among the phytopathological problems associated with pineapple, parasitism by nematodes and mealybug wilt of pineapple (MWP) stand out (Lacerda *et al*., 2009). *Pratylenchus brachyurus* Filipjev and Stekhoven, 1941 is a migratory root endoparasite that causes necrotic lesions through intra- and intercellular migration in the cortex. Yield losses in pineapple due to *P. brachyurus* vary from 47% to 80% (Lacoevilhe and Guérout, 1976; Raski and Krusberg, 1984). *Helicotylenchus* spp. are ectoparasites that induce necrotic lesions on the root surface. *Helicotylenchus cavenesse* Sher, 1966, *H. dihystera* (Cobb, 1893) Sher, 1961, *H. multicinctus* Cobb, 1893, *H. pseudorobustus* (Steiner, 1914) Golden, 1956, and *H. talonus* Siddiqi, 1972 are associated with pineapple (Hutton, 1974; Sharma, 1977; Moreira and Huang; 1980; Nath *et al*., 1998; Jiménes *et al*., 2001). A reduction in root, shoot, and D-leaf weights has been reported in pineapple parasitized by *H. dihystera* (Ko and Schmitt, 1996). Mealybug wilt of pineapple is caused by the association of pineapple mealybug wilt-associated virus-1, -2 and -3 (PMWaV-1, -2 and -3) and the mealybug *Dysmicoccus brevipes* (Cockerell, 1983). This disease complex reportedly causes yield losses of up to 70% (Agranovsky, 1996).

Pineapple parasitized by *P. brachyurus* or affected by MWP has yellow or reddish leaves with wilted tips, symptoms of hydric or nutritional deficiency, or lack of response to fertilizer. Fruits have reduced quality and value when sold (Caswell *et al*., 1990; Sipes *et al*., 2005; Lacerda *et al*., 2009). These reports suggest that, in the field, the similarity in symptoms can lead to diagnostic errors and reduce the efficiency of control measures.

The incidence and severity of plant diseases are related to a series of factors, especially to the host plant, the environmental conditions and the pathogen-host and pathogen-pathogen interactions (Back *et al*., 2002). Interactions between phytonematodes and other pathogens are not uncommon and can result in additive damages or disease complexes with distinct symptoms and greater damages. Disease complexes involving *Pratylenchus* spp. and *Helicotylenchus* spp. were summarized by Back *et al*. (2002). In pineapple, Costa and Matos (2000) suggested that *P. brachyurus* and *D. brevipes* act synergistically to reduce plant growth and fruit production, but their experimental design and results do not support their claim. Sipes *et al*. (2002) investigated the interaction between PMWaV-1 and *Rotylenchulus semipenetrans* Linford and Oliveira 1940, which has a semi-endoparasitic feeding biology that is distinct from *Pratylenchus*. Neither paper referred to MWP.

Considering the frequent occurrence of *P. brachyurus*, *Helicotylenchus* spp. and MWP in Brazilian plantations, this work was conducted in microplots aiming to: i) characterize the symptoms induced by these agents as individuals or together, ii) estimate the damage caused to vegetative growth and fruit production, and iii) assess the effects on production and quality of planting material.

MATERIALS AND METHODS

In vitro-propagated seedlings of pineapple cultivar ‘Vitória’ measuring 15 cm in height were transplanted individually into plastic pots containing 20 L of washed riverbed sand. The seedlings were grown in a greenhouse for 16 wk, receiving foliar fertilization every 2 wk (Ramos *et al*., 2010). When the seedlings reached 30 cm in height, the pots were set in the field as microplots in double or single rows with rows 40 cm or 1.20 m apart, respectively. At this time, each pot received 25 g of Osmocote® fertilizer (Fort Green, Paissandú, PR, Brazil) (15% N, 10% P, 10% K, 3.8% Ca, 1.5% Mg, 3.0% S, 0.02% B, 0.05% Cu, 0.5% Fe, 1% Mn, 0.004% Mo, and 005% Zn). This fertilization was repeated every 5 mon until the assay was concluded 21 mon later. During periods of low rainfall (July 2011 and October 2012), the plants were irrigated after fertilization. Weeds were manually removed from the pots and cut with a scythe in the rows of the experimental area. Rainfall frequency and volume (mm), temperature range, and average maximum and minimum temperatures were monitored with sensors and recorded with datalogger Watchdog® (Spectrum Technologies, Aurora, IL, USA).

Thirty days after establishing the microplots, the plants were divided into treatments: T1: control with plants free of nematodes and mealybugs, T2: plants inoculated with *D. brevipes*, T3: plants inoculated with *P. brachyurus*, T4: plants co-inoculated with *P. brachyurus* and *D. brevipes*, T5: plants inoculated with *Helicotylenchus* sp., T6: plants co-inoculated with *Helicotylenchus* sp. and *D. brevipes*. The experiment was in randomized blocks, with eight replicates (one plant per pot) of each treatment for a total of 48 plants. To avoid cross-contamination and movement of *D. brevipes*, blocks inside blocks were established with a 500 m buffer between blocks with and without *D. brevipes*. In addition, the pots inoculated with *D. brevipes* were surrounded with the formicide Mirex-s® (Atta Kill Ind. Com. de Defensivos Agrícolas, Rio Claro, SP, Brazil) to prevent ants from spreading the mealybugs.

Dysmicoccus brevipes was obtained from pineapple plantations with high incidence of MWP in the municipality of São Francisco do Itabapoana, and maintained on pineapple plants grown in a greenhouse. To confirm the incidence of PMWaV in the mealybugs and, later, in the experimental plants, a RT-PCR test according to Sether *et al*. (2005) was conducted, which indicated the co-existence of PMWaV-1, -2, and -3 in the mealybugs and in the experimental plants. For inoculation of experimental pineapple plants, 3 x 2 cm leaf fragments containing *D. brevipes* were cut and inserted into the leaf axils so that 25 mealybugs were inoculated per plant.

Pratylenchus brachyurus and *Helicotylenchus* sp. were obtained from commercial pineapple plantations situated in São Francisco do Itabapoana, and extracted from pineapple roots in a Baermann funnel. *Pratylenchus brachyurus* was multiplied on carrot (Mudiope *et al*., 2004; Gonzaga and Santos, 2010). *Helicotylenchus* sp. was multiplied on pineapple plants grown in a greenhouse. This population of *Helicotylenchus* sp. could not be positively identified (Siddiqi, 1972; Fotedar and Kaul, 1985), but resembles *H. glissus* Thorne and Malek, 1968. For inoculation, the nematodes were extracted from the pineapple roots or from the carrot in a Baermann funnel and individually transferred to glass tubes containing 10 ml water, remaining there for 1 hr at most. With a micropipette, 50 nematodes were inoculated into the root system of each pineapple plant.

Eight months after inoculation (MAI), 150 cm^3 of sand and root fragments were collected from three holes in each pot with a probe. The nematodes were extracted from the sand using the centrifugal-flotation method in a sucrose solution (Jenkins, 1964). The root fragments were weighed, and 10 g were placed in a Baermann funnel for 48 h to obtain nematodes. The following variables were evaluated: root fresh weight per sampling time, nematode population density per 150 cc of sand, and nematode population density per 10 g of root. D-leaves were collected from each plant during the morning, weighed, and measured for length and width.

The plants were induced to flower at 9 MAI, when the D-leaves had reached an average of 60 cm in length, with the application of 50 ml per plant of an aqueous solution of 0.1% Etrel®, 2% urea, and 0.035% CaCl (Veloso *et al.*, 2001).

At 19 MAI, the same nematode and plant variables were evaluated. Fruits were harvested, measured for length and width, and weighed. At 21 MAI, slips were collected, counted, and weighed. The number of slips per plant was calculated. The fresh weight of shoots and roots was measured.

For statistical analysis, the variables were tested for homogeneity of variance (Cochran and Bartlett tests) and normality of errors (Lilliefors test), at 5% probability. When these conditions were not met, the data were transformed by log (x+1) and the analysis of variance conducted. The means were compared by Tukey test ($P<0.05$). The entire experiment was repeated once and the data combined for analysis.

RESULTS

The climatic conditions were appropriate for the crop during the assays (Fig. 1) (Reinhardt *et al*., 2000; Carvalho *et al*., 2005; Azevedo *et al*., 2007). The experimental conditions were also favorable to the parasites. As for *D. brevipes*, the presence of colonies beyond the root system and leaf axils, as observed at 19 MAI, is an indication of a high population (Sanches and Matos, 1999). *Pratylenchus brachyurus* presented slightly lower population density in the soil than that reported by Sipes *et al.* (2005) causing a delay in leaf emergence, a reduction in fruit weight, and decreased ratoon growth. *Helicotylenchus dihystera* reached a soil population density slightly below 180 nematodes per 150 cm^3 soil, which suppressed the development of pineapple plants (Ko and Schmitt, 1996).

At 8 MAI the populations of *P. brachyurus*, *Helicotylenchus* sp. (Table 1), and *D. brevipes* had increased in relation to inoculum. *Dysmicoccus brevipes* established numerous powdery colonies with eggs, nymphs, and adults on the root system, leaf axils and at the junction between stem and slips. Plants parasitized by *P. brachyurus* had chlorotic leaves (Fig. 2A), while those affected by MWP and parasitized by *P. brachyurus*, were chlorotic with reduced leaf turgor during the hottest hours of the day. Chlorosis increased soon after flowering was induced (Fig. 2B). Fresh root weight was reduced in plants parasitized by *P. brachyurus* and the width of the D-leaf was lower in the plants affected by MWP and parasitized by *P.*

Table 1. Fresh root weight; length, fresh weight, and width of D-leaf; and population density of nematodes in the soil and in the roots of pineapple plants affected by mealybug wilt of pineapple (MWP) and(or) parasitized by *Helicotylenchus* sp. (H) or *Pratylenchus brachyurus* (Pb), in microplots. Evaluations were conducted 8 mon after inoculation with nematodes and (or) mealybugs carrying pineapple mealybug wilt-associated virus-1, -2, and -3.

Treatments	Fresh root weight (g)	D-leaf Length (cm)	D-leaf Fresh weight (g)	D-leaf Width (cm)	Density of nematodes Roots (10 g)	Density of nematodes Soil (150 cc)
Control	1.7[y,z] a	61.4 a	24.1 a	1.6 a	-	-
MWP	1.4 ab	61.1 a	24.1 a	1.6 ab	-	-
Pb	1.1 b	59.1 a	22.4 a	1.6 ab	64.5 A	27.5 A
MWP + Pb	1.4 ab	59.9 a	21.3 a	1.5 b	192.5 A	15.5 A
H	1.7 a	59.0 a	22.4 a	1.6 a	70.0 a	103.3 a
MWP + H	1.4 ab	61.7 a	22.7 a	1.6 ab	42.0 a	121.0 a
CV (%)	36.5	15.4	22.8 a	4.8	99.5/127.7	64.7/98.1

[y]Values are means of two experiments, each with 8 plants per treatment.
[z]Values followed by same letters in the columns are not statistically different according to Tukey test ($P<0.05$). For density of nematodes, the test compared the averages of Pb vs MWP + Pb (upper case) and of H vs MWP + H (lower case).

Table 2. Fresh root weight; length, fresh weight and width of the D-leaf; and population density of nematodes in the soil and in the roots of pineapple plants affected by mealybug wilt of pineapple (MWP) and(or) parasitized by *Helicotylenchus* sp. (H) or *Pratylenchus brachyurus* (Pb), in microplots. Evaluations were conducted 19 mon after inoculation with nematodes and (or) mealybugs carrying pineapple mealybug wilt-associated virus-1, -2, and -3.

Treatments	Fresh root weight (g)	D-leaf Length (cm)	D-leaf Fresh weight (g)	D-leaf Width (cm)	Density of nematodes Roots (10 g)	Density of nematodes Soil (150 cc)
Control	9.5[y,z] a	73.2 a	33.4 a	6.1 a	-	-
MWP	7.8 ab	70.4 a	31.5 ab	5.9 ab	-	-
Pb	2.2 b	69.5 a	27.3 bc	5.3 b	51.6 A	28.5 A
MWP + Pb	1.9 b	69.8 a	26.5 c	5.3 b	105.0 A	7.6 B
H	8.3 a	71.1 a	30.8 ab	5.5 ab	67.7 a	130.0 a
MWP + H	7.5 ab	68.4 a	27.6 bc	6.0 ab	57.0 a	88.3 a
CV (%)	78.3	7.6	12.1	10.7	99.3/109.1	87.6/86.6

[y]Values are means of two experiments, each with 8 plants per treatment.
[z]Values followed by same letters in the columns are not statistically different according to Tukey test ($P<0.05$). For density of nematodes, the test compared the averages of Pb vs MWP + Pb (upper case) and of H vs MWP + H (lower case)

Table 3. Fresh fruit weight, percentage reduction in relation to control, and length and width of fruit on pineapple plants affected by mealybug wilt of pineapple (MWP) and (or) parasitized by *Helicotylenchus* sp. (H) or *Pratylenchus brachyurus* (Pb), in microplots. Evaluations were conducted 19 mon after inoculation with nematodes and (or) mealybugs carrying pineapple mealybug wilt-associated virus-1, -2, and -3.

	Fruits			
Treatments	Fresh weight (g)	Reduction (%)	Length (cm)	Width (cm)
Control	947.8[y,z] a	-	11.7 a	10.4 a
MWP	866.1 ab	8.7	10.6 b	10.5 a
Pb	635.2 c	32.9	10.6 b	9.4 bc
MWP + Pb	619.9 c	34.6	9.6 b	8.8 c
H	742.7 bc	21.7	10.5 b	9.7 ab
MWP + H	706.9 bc	25.5	10.1 b	9.4 abc
CV (%)	24.2	-	8.1	7.1

[y]Values are means of two experiments, each with 8 plants per treatment.
[z]Values followed by same letters in the columns are not statistically different according to Tukey test ($P < 0.05$).

Table 4. Fresh root system weight (FRSW), fresh weight of slips and of the crown, and number of slips produced by pineapple plants affected by mealybug wilt of pineapple (MWP) and(or) parasitized by *Helicotylenchus* sp. (H) or *Pratylenchus brachyurus* (Pb), in microplots. Evaluations were conducted 21 mon after inoculation with nematodes and (or) mealybugs carrying pineapple mealybug wilt-associated virus-1, -2, and -3.

	Propagules			
		Weight (g)		Mean number of slips/plant
Treatments	FRSW (g)	Slips	Crown	
Control	3.8[y,z] a	2.9 a	99.0 a	5.3 a
MWP	3.6 b	2.8 a	106.3 a	3.6 ab
Pb	3.5 b	2.5 ab	83.1	4.8 ab
MWP + Pb	3.2 c	2.2 b	83.9 a	2.5 b
H	3.8 a	3.0 a	94.8 a	4.7 ab
MWP + H	3.5 b	2.9 a	92.9 a	3.2 ab
CV (%)	4.6	13.4	30.1	48.4

[y]Values are means of two experiments, each with 8 plants per treatment.
[z]Values followed by same letters in the columns are not statistically different according to Tukey test ($P < 0.05$).

brachyurus (Table 1).

At harvest at 19 MAI, the plants affected by MWP had adult insects on the upper surface of the leaves, fruit, crowns and slips. The nematode population remained high in all treatments, but in those plants affected with MWP, *P. brachyurus* population density was lower in the soil (Table 2). Plants parasitized by *P. brachyurus* had chlorotic lesions (Fig. 2C), and plants affected by MWP had leaves with reduced turgor and chlorosis in the older leaves. Plants affected by MWP and parasitized by *P. brachyurus* had leaves with reduced turgor, that were chlorotic. Necrosis at the tips of the new leaves and death of the older leaves were also seen in this treatment (Fig. 2D). Plants parasitized by *Helicotylenchus* sp. exhibited no symptoms above ground, while those affected by MWP and parasitized by *Helicotylenchus* sp. showed reduced leaf turgor and chlorosis in the older leaves.

Plants parasitized by *P. brachyurus*, with or without MWP, had necrotic roots, reduced fresh root and D-leaf weights, and reduced D-leaf width. Plants affected by MWP and parasitized by *Helicotylenchus* sp. had a reduction in D-leaf fresh weight. Fruit length was reduced in all treatments (Table 3). All the plants, except those affected by MWP, had decreased fruit weight. Fruit width was reduced by *P. brachyurus*, alone or in association with MWP. All treatments delayed fruit maturity compared to the control (Fig. 3).

At 21 MAI, all treatments except for *Helicotylenchus* sp. had a decrease in the fresh root system weight (Table 4). The number of slips per plant and the slips mean weight were reduced in plants affected by MWP and parasitized by *P. brachyurus*.

DISCUSSION

Pratylenchus brachyurus damages the cells of the epidermis and the central cylinder of pineapple plants (Costa and Matos, 2000; Ritzinger and Costa, 2004; Sipes *et al*., 2005), which explains the necrosis found in the roots and the reduced weight of the root system observed in this study. Plants with a reduced root system have a lower capacity to absorb water and nutrients and have a compromised photosynthetic capacity. Consequently, the plants may become chlorotic, have reduced development and lower fruit weight, and a delay in fruit maturity. The fertilization supplied in this experiment likely abated leaf chlorosis observed from 8 to 19 MAI in plants parasitized by *P. brachyurus*.

According to Sether *et al.* (2005) PMWaV causes vascular disturbances, explaining the reduced leaf turgor and contributing to loss in productivity. Necrosis and reduced fresh root weight was attributed to MWP by Gambley *et al.* (2008) and Sether and Hu (2002). However, under the conditions of this experiment, MWP did not cause a sharp decrease in the fresh root weight, but only a delay in fruit maturity and a marginal yet significant reduction in the length of fruit. Pineapple plants parasitized by PMWaV-1 had no significant reduction in the average weight of crowns, D-leaf, roots, or shoot in the greenhouse, and no significant reduction in length and weight of D-leaf, plant height, fruit weight, or number of slips/plant in the field (Sipes *et al.*, 2002).

Intriguingly, at 19 MAI, the association of parasitism by *P. brachyurus* with MWP caused aboveground symptoms that were much more severe

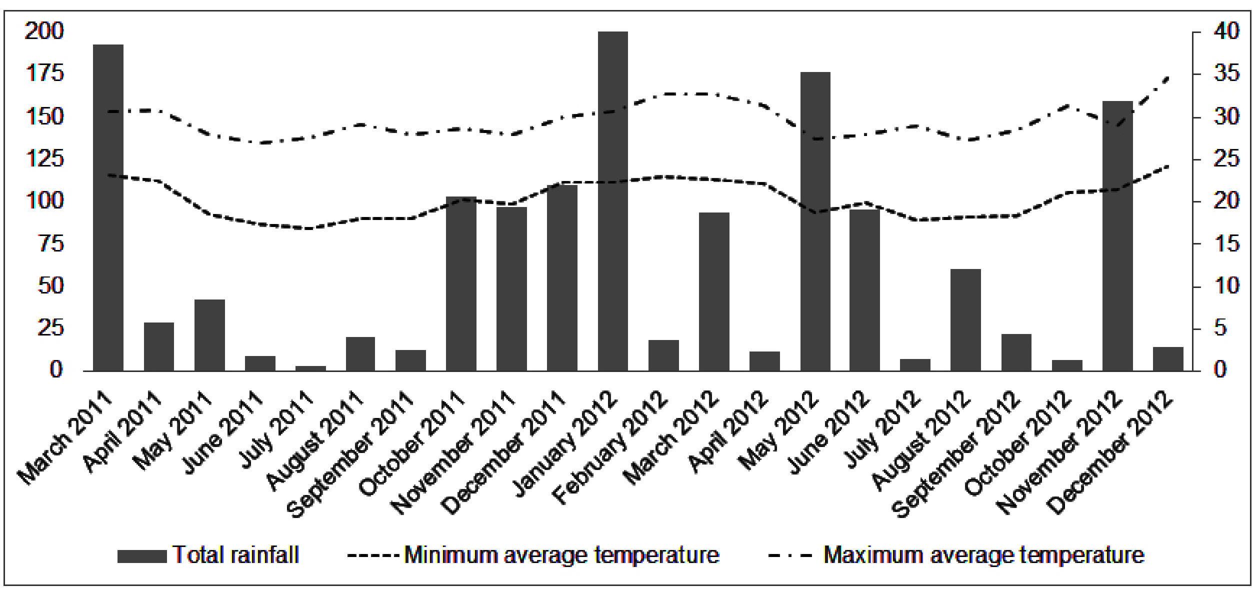

Fig. 1. Monthly rainfall and average minimum and maximum temperatures during the period in which the experiment ran in Campos dos Goytacazes, Rio de Janeiro, Brazil.

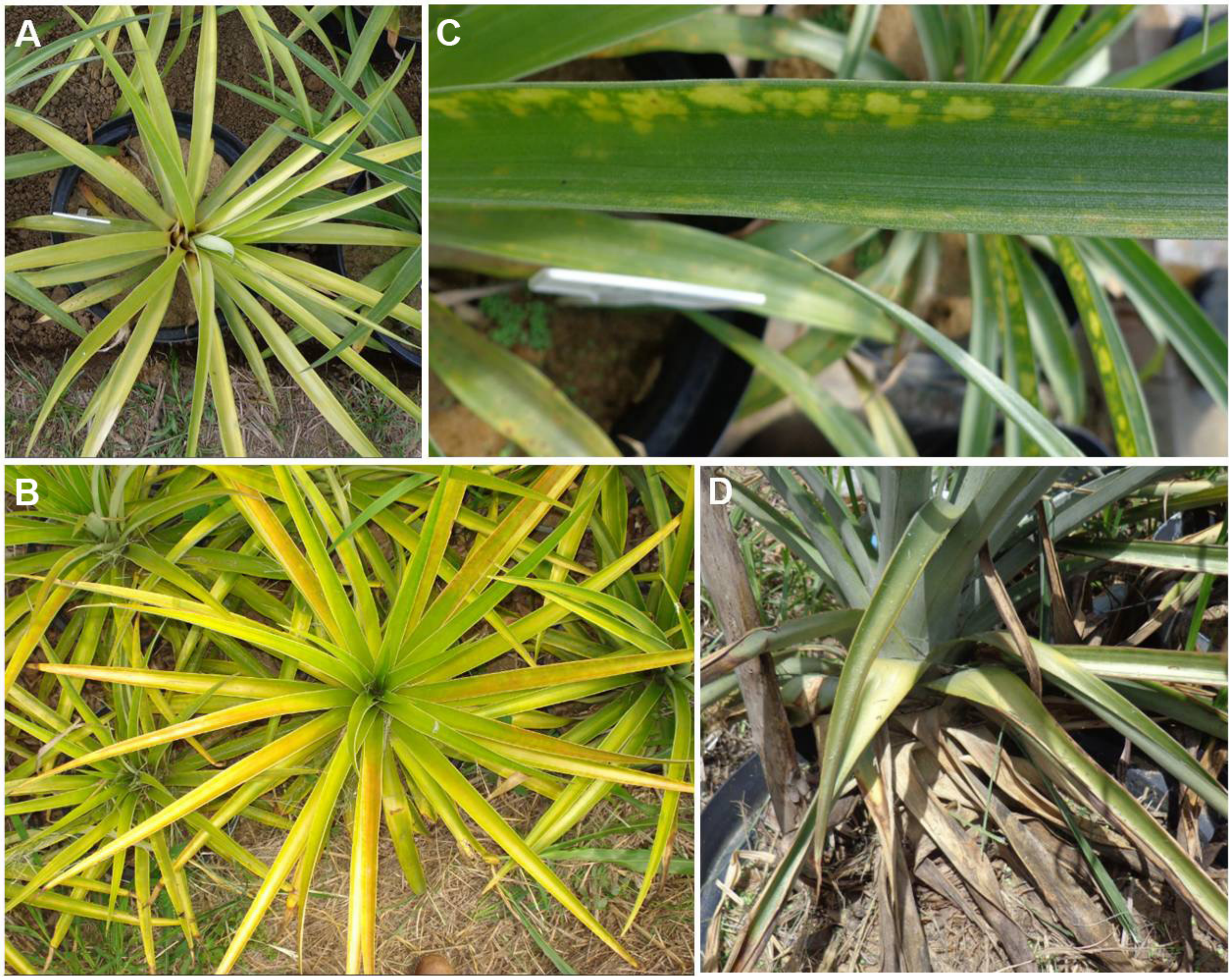

Fig. 2. A. Chlorosis in pineapple plant 8 mon after inoculation (MAI) with *Pratylenchus brachyurus*. B. Chlorosis in pineapple plant affected by mealybug wilt of pineapple (MWP) 9 MAI with *P. brachyurus* and mealybugs carrying pineapple mealybug wilt-associated virus (PMWaV) -1, -2, and -3. C. Chlorotic lesions in pineapple plant 19 MAI with *P. brachyurus.* D. Reduction in leaf turgor and death of lower leaves in pineapple plant affected by MWP 19 MAI with *P. brachyurus* and mealybugs carrying PMWaV-1, -2, and -3.

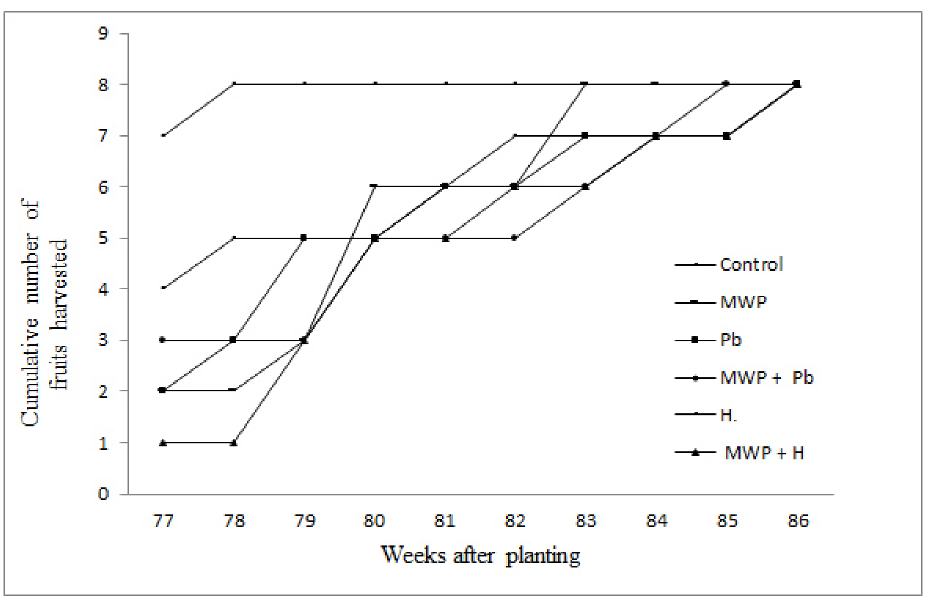

Fig. 3. Number of fruits harvested (cumulative data) per week in eight pineapple plants affected by mealybug wilt of pineapple (MWP) and(or) parasitized by *Helicotylenchus* sp. (H) or *Pratylenchus brachyurus* (Pb), in microplots. Data are means of two experiments, each with 8 plants per treatment.

than those caused by these pests individually. This set of symptoms is normally attributed to MWP alone. As far as the plant and fruit variables assessed, the interaction between these pests was roughly additive.

Helicotylenchus sp. did not cause measurable damage to the pineapple root system. However, the parasitism resulted in a significant reduction in fruit weight and led to delayed maturity. For induction and maintenance of feeding sites *Helicotylenchus* spp. causes physiological changes at the cellular level (Sijmons *et al.*, 1994). On the other hand, plants often activate post-infection resistance mechanisms, even in cases where there is an increase in nematode population over time and the nematode-plant interaction is considered compatible. Together these processes probably represent a high expenditure of energy for the plants, and they may interfere with fruit production. The interaction between *Helicotylenchus* spp. and MWP seems to be additive.

In conclusion, this work highlights the need for detailed studies in commercial pineapple plantations to understand the interaction between parasitism by *P. brachyurus*, *Helicotylenchus* spp., and MWP, individually and concomitantly, and the symptoms observed below- and above-ground. Indeed, the array of symptoms observed in this study is often reported in the phytopathological, nematological, and entomological literature as being caused by MWP, *P. brachyurus* or the mealybug *D. brevipes* alone (Sether and Hu, 2002; Sipes *et al.*, 2005; Lacerda *et al.*, 2009). When pineapple plants are fertilized and irrigated, symptoms such as chlorosis are abated, further complicating field diagnosis and management of phytosanitary problems affecting the crop.

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