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## Intensity and duration of water deficit on the pathosystem sugarcane x *Meloidogyne incognita*

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### Key words:

enzyme activity  
biomass  
root-knot nematode  
*Saccharum*

### ABSTRACT

This study aimed to evaluate the interaction between intensity and duration of water deficit (90, 56 and 22% of pot capacity [PC] for 30, 60 and 90 days under continuous stress) associated to the parasitism of the nematode *Meloidogyne incognita* on the growth of the sugarcane variety RB92579 and the activity of the enzymes catalase and ascorbate peroxidase. The experiment was conducted in completely randomized design in a 7 x 2 factorial scheme (seven water deficit treatments: control [90% PC], 56% PC for 30, 60 and 90 days, 20% PC for 30, 60 and 90 days; and two densities of *M. incognita*: 0 and 20000 eggs plant<sup>-1</sup>), with four replicates. The water stress corresponding to 56% PC for 30 or 60 days did not affect RB92579 development. The evaluated water treatments increased ascorbate peroxidase activity, but it did not affect catalase activity. Nematode inoculation did not affect RB92579 responses to drought stress conditions. The higher severity of water deficit (22% PC for 90 days) reduced *M. incognita* reproduction.

### Palavras-chave:

atividade enzimática  
biomassa  
nematóide de galhas  
*Saccharum*

## Intensidade e duração do déficit hídrico no patossistema cana-de-açúcar x *Meloidogyne incognita*

### RESUMO

Neste trabalho objetivou-se estudar a interação entre a intensidade e a duração do déficit hídrico (90, 56 e 22% capacidade de pote [CP] por 30, 60 e 90 dias de estresse contínuo) associado ao parasitismo do nematoide *Meloidogyne incognita* no crescimento inicial da cana-de-açúcar variedade RB92579 e à atividade das enzimas catalase e ascorbato peroxidase. O delineamento adotado foi inteiramente casualizado em esquema fatorial 7 (tratamentos hídricos: controle [90% da CP], 56% CP com duração de 30, 60 e 90 dias, 22% CP com duração de 30, 60 e 90 dias) x 2 (densidade de *M. incognita*: 0 e 20000 ovos por planta), com quatro repetições. O estresse hídrico correspondente a 56% da CP com duração de 30 e 60 dias não afetou o desenvolvimento da variedade RB92579. Os tratamentos hídricos testados aumentaram a atividade da enzima ascorbato peroxidase, porém não afetaram a atividade da enzima catalase. O nematoide não afetou as respostas da RB92579 nas condições estudadas; enfim, o déficit hídrico com maior severidade (22% CP por 90 dias) reduziu a reprodução do *M. incognita*.

## INTRODUCTION

In Brazil, the cultivation of sugarcane (*Saccharum* spp.) is subjected to many stress-causing agents, especially water deficit and the attack of root-knot nematodes (Gonçalves et al., 2010).

Water deficit is responsible for the damages in plant physiology and metabolism, resulting in reductions of yield (Taiz & Zeiger, 2009). When water deficit occurs in the beginning of the development, it affects yield and other sugarcane parameters, such as leaf area and number, height, diameter and weight of industrializable stems (Cha-Um & Kirdmanee, 2009). Besides the effect caused by the drought intensity, the time of exposure to water deficit negatively affects shoot growth, especially leaf production, accelerating the leaf senescence of the plant (Gonçalves et al., 2010).

In the state of Pernambuco, the problems caused by water deficit become worse with the expansion of sugarcane cultivation to the coastal plains, areas with predominance of the nematode species from the genus *Meloidogyne*, in which the presence of sandy soils and the occurrence of long dry seasons are common (Barbosa et al., 2009).

Although the parasitism of *Meloidogyne* spp. in sugarcane is limited to the roots, which absorb nutrients for growth and development, the resulting effects affect the physiology of the entire plant. The plant cell selected for infection by the nematode suffers profound changes characterized by hypertrophy, increase in the number of nucleuses and organelles, absence of central vacuole, thickening of cell wall and increase in metabolic rates (Hussey & Williamson, 1998). The feeding process by the parasite causes a continuous drain of a large amount of solutes from plant shoots, increasing the metabolic activities in root segments close the infected area. As a consequence, roots become poor in rootlets and incapable of absorbing water and nutrients necessary for an adequate development of the plant, which then becomes shorter, stunted and chlorotic (Dias-Arieira, 2010).

With water deficit intensified by the attack of nematodes, plants close stomata in order to limit water loss through transpiration. However, stomata closure restricts CO<sub>2</sub> inflow to the leaves, which damages the photosynthetic apparatus, resulting in the production of reactive oxygen species (ROSs), such as hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) (Vasconcelos et al., 2009). The increase in H<sub>2</sub>O<sub>2</sub> causes peroxidation of lipids and damages on pigments, proteins and nucleic acids. In response, plant mechanisms reduce the formation of free radicals and minimize damages caused by the oxidative stress, by removing H<sub>2</sub>O<sub>2</sub> through the key detoxifying enzymes, like catalase (CAT) and ascorbate peroxidase (APX) (Willadino et al., 2011).

This study aimed to evaluate the interaction between intensity and duration of water deficit associated with the parasitism of *Meloidogyne incognita* on sugarcane growth and on the activity of the enzymes catalase and ascorbate peroxidase.

## MATERIAL AND METHODS

The experiment was carried out in a greenhouse at the Federal Rural University of Pernambuco, Recife-PE, Brazil, from May to August 2012.

The soil used in the experiment, according to the classification of Atterberg, was sand, which was sterilized in autoclave at 120 °C and pressure of 1 atm for 1 h, repeating the procedure after 24 h.

The population of *M. incognita* was obtained from sugarcane roots, identified by the isoenzyme electrophoresis technique, purified and multiplied in 'Santa Cruz Kada' tomato (*Solanum lycopersicum* L.), in a greenhouse. The inoculum was extracted from the root system of the plants using the methodology described by Hussey & Barker (1973).

The selected sugarcane variety was RB92579, a plant very efficient in water use. The micropropagated seedlings underwent an acclimation period of 15 days in a substrate in the greenhouse. Then, they were selected regarding sanity and homogeneity, and transplanted to 0.005 m<sup>3</sup> pots filled with autoclaved soil. During 15 days after transplantation, plants were kept in soil at pot capacity (PC), which was previously determined by the gravimetric method, according to Souza et al. (2000). After the first 10 days at pot capacity, plants were inoculated with 20000 eggs of *M. incognita*.

The differentiation of the treatments began 5 days after the inoculation of plants with the nematode. The experimental design was completely randomized, in a 7 x 2 factorial scheme, with 4 replicates. The treatments consisted of 7 water regimes: control (90% of PC), 56% PC with duration of 30, 60 and 90 days, 22% PC with duration of 30, 60 and 90 days; and 2 inoculation densities: 0 and 20000 eggs of *M. incognita* per plant. The plants under imposition of water deficit (56 and 22% PC) were kept under the control condition. Treatments were maintained daily by weighing the pots and replenishing the evapotranspired water, using a digital scale with capacity for 15 kg and precision of 0.005 kg.

Monthly evaluations (0, 30, 60 and 90 days after imposing the treatments) were performed for leaf area and the biochemical analysis for the activity of the enzymes catalase and ascorbate peroxidase. Leaf area (LA) was determined based on the methodology proposed by Hermann & Câmara (1999). For the biochemical analysis, the third leaf of each plant was used. The activity of APX and CAT was determined according to Nakano & Asada (1981) and Havir & Mchale (1987), respectively.

At 90 days after imposing the water treatments, the final harvest of the experiment was performed. Plants were evaluated for plant height, stem diameter, shoot and root dry matter and reproduction of *M. incognita* in the root system.

Plant height ( $h_{\text{plant}}$ ) was determined by the distance from the base to the insertion of the +1 leaf, using a tape measure. Stem diameter ( $DM_{\text{stem}}$ ) was measured using a caliper rule. Then, plants were removed from the pots, and the roots were separated from shoots (stem and leaves) and accommodated in paper bags. The root system was divided into two parts with equal weights, one was used to determine nematode reproduction and the other was taken along with shoot biomass to dry in an forced-air oven at 65 °C, until constant weight. After this period, shoot dry matter (SDM), root dry matter (RDM) and total dry matter (TDM) were determined. Based on these data, root biomass allocation and root/shoot ratio (R/S) were calculated according to the methodology described by Benincasa (2003). In order to evaluate nematode reproduction, the following

parameters were determined: number of eggs per root system (EGGS/RS), number of eggs per gram of dried root (EGGS/R) and reproduction factor, obtained by the ratio between the final and the initial nematode population ( $RF = Pf/Pi$ ).

The data were subjected to analysis of variance and, when necessary, analysis of measurements repeated over time were applied, using the software SAS – Statistical Analytical System (SAS Institute, 2009). Linear, quadratic and cubic regression models were tested in order to obtain the best description of the behavior of the data over time. When necessary, means

were compared by Tukey test at 0.05 probability level. For the statistical analysis, the data of number of eggs of *M. incognita* were transformed into  $\log_{10}(X+1)$ .

## RESULTS AND DISCUSSION

The variables leaf area and ascorbate peroxidase, evaluated over time, indicated the interaction between the evaluated time and the studied water treatments. The parasitism of *M. incognita* did not affect the studied plant variables (Figure 1).

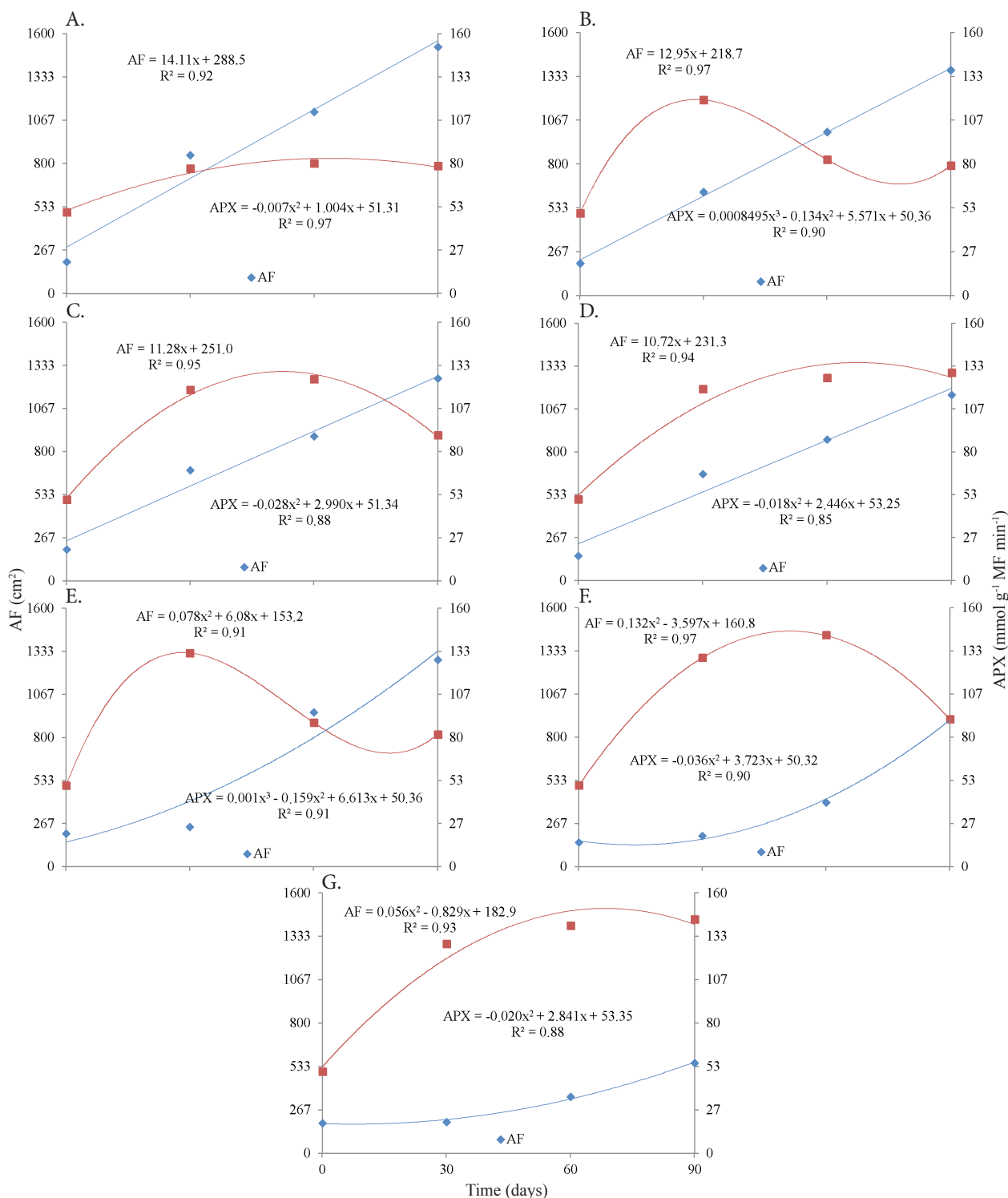


Figure 1. Activity of ascorbate peroxidase (APX) and leaf area (AF) of the sugarcane variety RB92579 under the conditions of 90% pot capacity (PC) during all the experiment (A), 56% PC during 30 days (B), 56% PC during 60 days (C), 56% PC during 90 days (D), 22% PC during 30 days (E), 22% PC during 60 days (F) and 22% PC during 90 days (G)

Regardless of the intensity of the water deficit (22 and 56% PC) and of its duration (30, 60 and 90 days), the growth in leaf area of the RB92579 was reduced (Figure 1). The reduction was more intense for 22% PC, in which plants subjected to this stress for a longer time had the lowest rates of absolute growth, 8.46 and 4.11 cm<sup>2</sup> d<sup>-1</sup> for the durations of 60 and 90 days, respectively (Figures 1 F and G), when compared with the control (14.70 cm<sup>2</sup> d<sup>-1</sup>). For the studied water deficit intensities, 22 and 56% PC, the leaf area had a maximum reduction of 65.13 and 22.70%, respectively, when the stress lasted for 90 days (Figures 1 G and D). According to Silva et al. (2008), leaf area is one of the first morphological variables to be affected, reducing the area available for transpiration and the metabolic expenditure to maintain tissue turgescence. Significant reductions in leaf area were also found by Gonçalves (2008) and Pincelli & Silva (2012) in sugarcane cultivars subjected to water deficit.

In general, comparing the water deficits of 22 and 56% PC, with durations of 30 and 60 days, it is evident that at the longest time (60 days) the recovery of the plants in the following month was slower (Figures 1B, C with E, F).

The enzyme ascorbate peroxidase showed increase in activity when plants were under 22% PC, regardless of the duration. This increase corresponded to at most 38.08%, compared with the control condition (Figure 1G). These values corroborate the ones observed by Silva (2010), where the sugarcane genotype CL002 showed increase of 30.23% in peroxidase activity compared with the control, when under maximum stress (0 to 20% of available water in the soil) for 90 days, while the activity of catalase remained constant.

An important point related to the enzyme ascorbate peroxidase is that, regardless of the water deficit intensity, 22 or 56% PC, the rate of increase in the enzyme activity reduced as the exposure time increased, tending to stabilization, as observed in Figure 1D, when the rate reduced from 2.29 to 0.23, until 0.11 μmol g<sup>-1</sup> MF min<sup>-1</sup>, as water deficit extended to 90 days. Cia et al. (2012) report that whenever the water stress became severe in sugarcane, the antioxidant defense system, especially the enzyme ascorbate peroxidase, began to collapse.

Significant changes in the catalase activity were not detected throughout the experiment. Catalase is an enzyme related to the metabolization of H<sub>2</sub>O<sub>2</sub> generated in the photorespiration (Wang et al., 2009; Foyer & Shigeoka, 2011). Since sugarcane is a C<sub>4</sub> grass, it has mechanisms to maintain photorespiration at extremely low levels by maintaining the high ratio of CO<sub>2</sub>:O<sub>2</sub> in the action sites of the enzyme ribulose 1.5 biphosphate (RuBP) carboxylase/oxygenase, favoring the reactions of carboxylation (Azevedo et al., 2011). The metabolism C<sub>4</sub> is a physiological evolution that avoids the effects caused by water stress (Jaspers & Kangasjärvi, 2010).

Stem diameter and plant height were only affected by the water treatments. Stem diameter had development compromised when the variety RB92579 remained for 60 and 90 days under 22% PC (Table 1). The maximum reduction, corresponding to 42.86% compared with the control, was observed under 22% PC for 90 days. For Gonçalves (2008), sugarcane stem diameter is affected negatively by water deficiency in the initial phase of development. Macêdo et al. (2012), studying the varieties RB835486 and RB867515 under

Table 1. Plant height (h<sub>plant</sub>) and stem diameter (DM<sub>stem</sub>) for the sugarcane variety RB92579 subjected to seven water treatments at 120 days after planting

Water treatments <sup>#</sup>	DM <sub>stem</sub>	h <sub>plant</sub>
	(cm)	
90% PC - C	1.33 a	76.38 a
56% PC - D30	1.32 a	68.94 ab
56% PC - D60	1.23 ab	68.25 ab
56% PC - D90	1.16 ab	58.63 bc
22% PC - D30	1.15 ab	46.13 cd
22% PC - D60	1.06 b	42.63 d
22% PC - D90	0.76 c	27.63 e
CV (%)	10.90	16.83

<sup>#</sup>PC – Pot capacity, C – Control, D30 – duration of 30 days

Means followed by the same letters in the column do not differ statistically by Tukey test at 0.05 probability level

different water regimes, observed that stem diameter varied according to the environment and the variety; also, it was found that the highest productive potential of the varieties was expressed in irrigated conditions.

The height of the variety RB92579, when subjected to 22% PC for 90 days, had maximum reduction (63.83%) compared with the control. Efeoğlu et al. (2009), studying water deficit in maize plants, verified that plant growth was significantly reduced as the stress was intensified. However, the observed reduction was higher than the one obtained by Silva (2010), when sugarcane genotypes were subjected to severe stress (0 to 20% of available water in the soil), with a mean reduction of 25%.

Biometric evaluations, such as plant height, are important in studies on water stress, since the growth in height continues until the occurrence of some limitation in water supply (Silva et al., 2008).

The variables shoot dry matter, root dry matter, total dry matter, root biomass allocation and root/shoot ratio were not affected by the parasitism of *M. incognita*, only by the tested water treatments. Shoot dry matter suffered significant reductions of 68.87 and 79.97% when RB92579 plants remained under water stress of 22% PC for 60 and 90 days, respectively (Table 2). Significant reductions were also observed when plants were under water stress of 56% PC for 30, 60 and 90 days and 22% PC for 30 days. The results obtained by Gonçalves (2008) show that, among the varieties tested under water

Table 2. Shoot dry matter (SDM), root dry matter (RDM), total dry matter (TDM), root biomass allocation (RBA) and root/shoot ratio (R/S) in the sugarcane variety RB92579 subjected to seven water treatments at 120 days after planting

Water treatments <sup>#</sup>	SDM	RDM	TDM	RBA	R/S
	(g)			(%)	
90% PC - C	25.06 a	4.83 d	29.89 a	16.19 e	0.19 d
56% PC - D30	21.79 b	6.24 c	28.03 ab	22.33 d	0.29 c
56% PC - D60	20.13 bc	7.34 ab	27.47 ab	26.75 c	0.37 b
56% PC - D90	19.60 bc	8.09 a	27.68 ab	29.25 b	0.41 b
22% PC - D30	17.84 c	6.64 bc	24.48 b	27.19 bc	0.37 b
22% PC - D60	7.80 d	3.73 e	11.54 c	32.31 a	0.48 a
22% PC - D90	5.02 d	2.60 f	7.62 c	33.95 a	0.52 a
CV (%)	12.42	11.10	11.52	5.50	8.17

<sup>#</sup>PC – Pot capacity, C – Control, D30 – duration of 30 days

Means followed by the same letters in the column do not differ statistically by Tukey test at 0.05 probability level

deficit, RB92579 had the highest reduction in shoot dry matter, 59.2% after 71 days under maximum stress.

The highest reductions of root dry matter (Table 2) occurred in plants subjected to 22% PC for 60 and 90 days, with values of 22.7 and 46.17% compared with the control. Gonçalves (2008) also observed reductions (more than 40%) in root dry matter in the varieties tested under severe water deficit.

For total dry matter, the water deficit of 22% PC for 60 and 90 days caused the highest reductions, respectively 61.39 and 74.51%. Negative effects for total dry matter were observed in other water treatments, as in 56% PC for 30, 60 and 90 days and 22% PC for 30 days (Table 2).

Root biomass allocation (Table 2) showed that the most extreme water treatments tested in this study, i.e., 22% PC for 60 and 90 days, were the ones allowing the highest allocation of biomass in the roots. Queiroz (2006), studying the effect of intensity (55, 40 and 25% of soil water availability - SWA) and duration (30 and 60 days) in two sugarcane cultivars (IAC91-2195 and IAC91-5155, sensitive and tolerant to water stress, respectively), observed that with severe water deficit (25% SWA) the cultivars tended to distribute the largest part of photoassimilates to the root system at the expense of the shoots.

For the root/shoot ratio (Table 2), the variety RB92579 presented morphological adaptation to the water deficit when subjected to extreme water deficit (22% PC for 60 and 90 days), developing the root system more than the shoots. This characteristic allows the plant to explore a larger soil volume in search for water, making it more tolerant to the water deficit effects (Taiz & Zeiger, 2009). As water deficit became milder, i.e., from the condition of 22% PC for 30 days to 56% PC for 30 days, the root/shoot ratio changed its behavior, reaching lower values over time.

The multiplication of *M. incognita* was affected by the tested water treatments (Table 3). In general, there was a multiplication of the nematodes, since the number of eggs at the end of the experiment was higher than the inoculated one (20000 eggs plant<sup>-1</sup>); except for the water deficit of 22% PC for 90 days, when the final number of eggs was lower than the inoculated one, differing statistically from the other water treatments tested.

For the number of eggs g<sup>-1</sup> dried root, the most severe water treatments, 56% PC for 90 days and 22% PC for 90 days, were statistically lower than the other ones imposed to

Table 3. Mean number of eggs per root system (EGGS/RS), number of eggs g<sup>-1</sup> dried root (EGG/GR) and reproduction factor (RF) of *M. incognita* in the sugarcane variety RB92579 subjected to seven water treatments at 120 days after planting

Water treatments <sup>#</sup>	EGGS/RS	EGGS/GR	RF
90% PC - C	96,340 a	19,472 a	4.82
56% PC - D30	62,660 ab	10,379 ab	3.13
56% PC - D60	50,810 ab	7,094 ab	2.54
56% PC - D90	46,945 ab	5,382 b	2.35
22% PC - D30	43,700 ab	6,515 b	2.18
22% PC - D60	29,715 ab	6,407 b	1.49
22% PC - D90	13,015 b	5,642 b	0.65
CV (%)	62.44	63.27	

<sup>#</sup>PC – Pot capacity, C – Control, D30 – duration of 30 days

Means followed by the same letter in the column do not differ by Tukey test at 0.05 probability level

the sugarcane variety RB92579. The intensity and duration of the water deficit interfered with the multiplication of *M. incognita*, explaining the absence of effects of nematodes on the other studied variables. According to Dias-Arieira et al. (2010) and Santos et al. (2013), the females deposit their eggs in masses involved by a gelatinous matrix, which acts as an indicator. Under water deficit conditions in soil, the gelatinous matrix dehydrates, favoring the interruption of the embryonic development inside the eggs. In addition, the low water supply causes roots to develop thicker layers, which make difficult the penetration of the parasite, depending on the inoculated plant species. For Santos et al. (2013), the reproduction of *M. incognita* (EGGS/RS and EGG/GR) was reduced under the condition of 40% PC compared with 100%. The reduction in reproduction under 40% PC is associated with the difficulty in movement and penetration of the parasite in the roots. The reproduction factor was inversely proportional to the intensity and duration of the water deficit, with the highest value observed for the control and the mild water deficit treatments.

## CONCLUSIONS

1. The water stress corresponding to 56% of pot capacity for 30 and 60 days does not affect the development of the variety RB92579.
2. The tested water treatments increase the activity of the enzyme ascorbate peroxidase, but do not affect catalase activity.
3. The increase in water deficit duration affects negatively the post-stress recovery.
4. The nematode does not affect the responses of the sugarcane variety RB92579 under the studied conditions.
5. With higher severity (22% PC for 90 days), the water deficit reduces the reproduction of *M. incognita*.

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