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Root development of pineapple seedlings cultivars inoculated with *Fusarium guttiforme* isolates

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Abstract

Fusariosis caused by *Fusarium guttiforme* is responsible for losses in pineapple production in tropical regions. Information on potential damage to the root system at the early stages of plant development and the magnitude of damage caused by different *F. guttiforme* isolates is scarce. This study aimed to evaluate the genotype x isolate interaction in pineapple root development. The experimental trial was set up in a randomized block design with four replications in a 4 x 5 factorial arrangement (pineapple cultivars x *F. guttiforme* isolates + control). The following variables were evaluated at 90 days after inoculation: number of branches, root length, mean root diameter, total root perimeter, root surface area, shoot fresh mass, root fresh mass, shoot dry mass, and root dry mass. The data were subjected to analysis of variance and the means compared by Tukey's test ($p > 0.05$). The cultivar Pérola showed a higher reduction in root development when inoculated with *F. guttiforme*. The cultivars Smooth Cayenne and BRS Vitória showed no changes in root development when inoculated, while 'IAC Fantástico' presented damage in the root system when inoculated with the isolates CF/UENF 512, CF/UENF 516, and CF/UENF 528. Pineapple plants may experience reductions in root length, number of branches, and, consequently, root volume when infected by *F. guttiforme* at the initial stage of development.

Keywords: *Ananas comosus,* fusariosis, genetic resistance, plant breeding.

Introduction

Pineapple is a fruit important to agricultural economy of several tropical and subtropical countries. Brazil ranks fourth among pineapple-producing countries, with an estimated production of 1,59 billions of fruits in 2023 (IBGE, 2023). The brazillian pineapple production corresponded to 7.9% of world production in 2022 (FaoStat 2022). In 2023, the higher production value were estimated in the states of Pará, Tocantins, Minas Gerais, and Rio de Janeiro (IBGE 2023). In addition to the economic impacts, pineapple cultivation provides positive social impacts related to the direct generation of jobs, mainly on small farms.

The occurrence of diseases is a limiting factor for increasing pineapple productivity. Fusariosis, caused by a fungus of the species *Fusarium guttiforme* (Syn.: *Fusarium subglutinans* f. sp. *ananas*) Nirenberg & O'Donnell (1998), is the disease of greatest concern in Brazil for the production of pineapple fruits intended for the fresh market. This fungus can infect plants and develop in all their organs, causing rot (Verzignassi et al. 2009). Losses can reach 100% in fruit production and over 50% in seedling viability (Nogueira et al. 2014; Souza et al. 2018).

The exchange among pineapple producers of young seedlings (propagules) contaminated with *F. guttiforme* is the main form of dispersion of the pathogen between properties (Ventura et al., 2019). The use of resistant cultivars represents an effective strategie to avoid losses due to fusariosis. Studies have been developed in Brazil aiming to obtain cultivars resistant to fusariosis, as the registered cultivars BRS Imperial, BRS Ajubá, BRS Vitória (Ventura et al. 2009), and IAC Fantástico. However, these cultivars are still not widespread, and producers have preferred the planting of the cultivars Pérola or Smooth Cayenne, which are susceptible to fusariosis.

In this sense, the pineapple breeding programs

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have been worked to develop new pineapple cultivars resistant to fusariosis, with plants and fruits that meet the interests of producers and consumers (Lira Júnior et al. 2023).

The development of resistant cultivars is a dynamic process, in which genetic aspects of both the pathogen and the host crop need to be considered. An important aspect to considerer in the resistance evaluations is that different isolates or species of *Fusarium* can cause different resistance responses in pineapple (Souza et al. 2018).

There is a lack of studies on the impacts of *Fusarium* infection on the root system of plants when considering the initial stages of crop development from seedlings. Damage to the root system at the initial stage of development can compromise plant development and, consequently, prevent or reduce fruit production. This study aimed to evaluate whether there is an effect of the genotype x isolate interaction on the root development of pineapple.

Material and methods

The study evaluated root development traits in four pineapple cultivars inoculated with four different *F. guttiforme* isolates. The evaluated cultivars were Smooth Cayenne, Pérola, and the first generation hybrids IAC Fantástico and BRS Vitória, the first developed by the Agronomic Institute of Campinas (São Paulo, Brazil), while the second was developed by the Brazilian Agricultural Research Corporation (Embrapa) in partnership with the Institute for Research, Technical Assistance, and Rural Extension of the State of Espírito Santo (Incaper). The plants were obtained from micropropagated seedlings acquired from certified companies.

Four *F. guttiforme* isolates: CF/UENF 512, CF/UENF 516, CF/UENF 526, and CF/UENF 528 (GenBank codes) were used to test the resistance of cultivars to fusariosis and the virulence of different isolates. These isolates were collected from pineapple fruits in different pineapple plantations in the North Mesoregion of the State of Rio de Janeiro, Brazil. The species of the isolates was confirmed through sequencing of the tef-1a and β-tubulin regions and comparison with similar sequences present in the NCBI database (Ribeiro et al., 2024). The isolates are stored at the Phytosanitary Clinic of the Darcy Ribeiro State University of Northern Rio de Janeiro.

The experimental trial was set up in a randomized block design with four replications in a 4 x 5 factorial arrangement (cultivars x isolates). The factor isolate was composed of four different *F. guttiforme* isolates and a control treatment (distilled water). The experimental unit consisted of a plant grown in a pot with a capacity of 5 liters. A substrate based on sand and bovine manure (1:1) previously sterilized at a temperature of 105 °C (1 atm) for 1 hour was used. The sterilization process was repeated three times and the soil was used seven days after sterilization to stabilize compounds capable of generating phytotoxicity in plants.

Seedlings acquired from micropropagation companies were cultivated in 3-liter volume pots filled with sterilized substrate based on sand and bovine manure (1:1) before the experiment was set up. The plants were transplanted into 5-liter capacity pots in the respective plots at 30 days of development. The seedlings were injured with sterile metal blades at the time of transplanting, consisting of three equidistant points around the rhizome. The wounded seedlings were immersed in a suspension of $1{,}10^6$ conidia mL^{-1} of the *F. guttiforme* isolate, corresponding to each plot. Plants in the control treatment were injured and immersed in distilled water.

The experimental trial lasted 90 days. During this period, the plants were irrigated periodically with 300 mL of water. No chemicals or fertilizers were applied to the plants.

After uprooting, the roots of the plants were separated and washed to remove excess soil and then photographed. The images were obtained by a 12-megapixel camera fixed at a 50-cm height in a glass vat filled with 1 L of water, where the roots were deposited for photography.

The traits number of branches from the main roots (NB), total root length (LEN, m), mean root diameter (DIAM, mm), total root perimeter (PER, m), and root surface area (SA, cm^2) were obtained from the images of each plot. Estimates for root variables were obtained using the program RhizoVision Explorer (Seethepalli et al., 2021).

The shoot fresh mass (SFM, g) and root fresh mass (RFM, g) were obtained using a precision analytical balance with four decimal places. The shoots and roots of each plot were placed in paper bags and dried at 45 °C in a forced-air ventilation oven until constant mass, allowing to obtain the shoot dry mass (SDM, g) and root dry mass (RDM, g).

The data were submitted to the Shapiro-Wilk residual normality test at a 5% significance level. Subsequently, the data were submitted to the F-test, following the 4 x 5 factorial model (cultivars x inoculation) and subsequent comparison of means by Tukey's test at a 5% probability level. The analyses were conducted using the R program (R Core Team, 2021) based on the functions available in the package ExpDes.pt.

Results and Discussion

Modification of the pineapple genotype (cultivar) or *F. guttiforme* isolates caused significant changes in root traits (**Table 1**).

Furthermore, a significant interaction was observed between cultivars and isolates for all studied traits. The cultivar Pérola showed a significant reduction in root dry mass (RDM) when inoculated with *F. guttiforme*, with no significant differences between isolates (**Figure 1**–A).

Only the isolate CF/UENF 528 caused a significant reduction in the root dry mass of the cultivars Smooth Cayenne and IAC Fantástico, and the other isolates did not differ from the control. The pathogen inoculation did not result in significant effects on the root dry mass of the cultivar BRS Vitória despite the lowest root mass among all cultivars.

Root length (RL) was also reduced when any of the four isolates were inoculated in the cultivar Pérola (Figure 1–B). No reductions in root length due to the presence of the pathogen were observed for the cultivars Smooth Cayenne and BRS Vitória. The isolates CF/UENF 512 and CF/UENF 516 significantly reduced the root length in the cultivar IAC Fantástico. A result similar to the root length was observed for the number of branches (NB). However, in addition to the isolates CF/UENF 512 and CF/UENF 516, the isolate CF/UENF 526 also caused reductions in the number of branches for the cultivar IAC Fantástico (Figure 1–C).

In contrast to the traits presented above, the mean root diameter (DIAM) showed an increase when inoculated with *F. guttiforme* in some cultivars. The isolates CF/UENF 516, CF/UENF 526, and CF/UENF 528 caused a significant increase in the root diameter of the cultivar Pérola (Figure 1–D). The cultivars Smooth Cayenne and BRS Vitória presented no significant differences compared to the control although there was an increased trend, especially when inoculated with the isolate CF/UENF 512. The cultivar IAC Fantástico showed roots with higher diameter when inoculated with the *F. guttiforme* isolates CF/UENF 512, CF/UENF 516, and CF/UENF 526.

Changes in the total root perimeter and root surface area were related to a reduction in root length and the number of branches and an increase in the mean root diameter in some cultivars (**Figure 2**). Root perimeter was severely reduced when any one of the four *F. guttiforme* isolates was inoculated in the cultivar Pérola (Figure 2–A).

Significant reductions were also observed for the cultivar IAC Fantástico when the isolates CF/UENF 512, CF/UENF 516, and CF/UENF 528 were present. The cultivars Smooth Cayenne and BRS Vitória showed no significant effect of isolates on the root perimeter.

Root surface area showed similar responses to root perimeter for the cultivars Pérola and BRS Vitória in terms of differences between isolates (Figure 2–B).

However, the isolate CF/UENF 512 promoted an increase in the superficial root area of the cultivars Smooth Cayenne and IAC Fantástico when compared to the control.

In general, the four *F. guttiforme* isolates caused reductions in the root development of the cultivar Pérola. The isolate CF/UENF 512 promoted an increase in root diameter and, consequently, the root surface area of the cultivar Smooth Cayenne, but the isolates were not

Table 1. Analysis of variance for root development traits in different pineapple genotypes inoculated with different *Fusarium guttiforme* isolates under controlled conditions.

S.V.	d.f.	RDM		RL		RN	
		MS	p	MS	p	MS	p
Block	3	0.008	0.059	13.92	0.565	10.06	0.330
Cultivar (C)	3	0.226	< 0.001	979.00	< 0.001	522.81	< 0.001
Isolates (I)	4	0.142	< 0.001	143.98	< 0.001	674.15	< 0.001
C x I	12	0.117	< 0.001	1145.50	< 0.001	617.07	< 0.001
Error	57	0.050		20.31		8.61	
Mean		182 g		133.4 cm		883 und.	
		RD		RP		RSA	
		MS	*p*	MS	*p*	MS	*p*
Block	3	8.26	0.082	85.11	0.164	6.85	0.976
Cultivar (C)	3	113.55	< 0.001	1939.96	< 0.001	4976.89	< 0.001
Isolates (I)	4	28.94	< 0.001	3962.07	< 0.001	2952.59	< 0.001
C x I	12	23.36	< 0.001	2752.92	< 0.001	3779.40	< 0.001
Error	57	3.52		48.29		98.73	
Mean		0.6 mm		203.2 m		2958.1 cm²	

S.V.= source of variation; d.f. = degrees of freedom; RDM = root dry mass; RL = root length; RN = ramifications number (used RN . 10^{-3} to variance analysis); RD = rood diameter; RP = root perimeter; RSA = root superficial area; MS = mean square. p = probability value to null hypothesis (p-value).

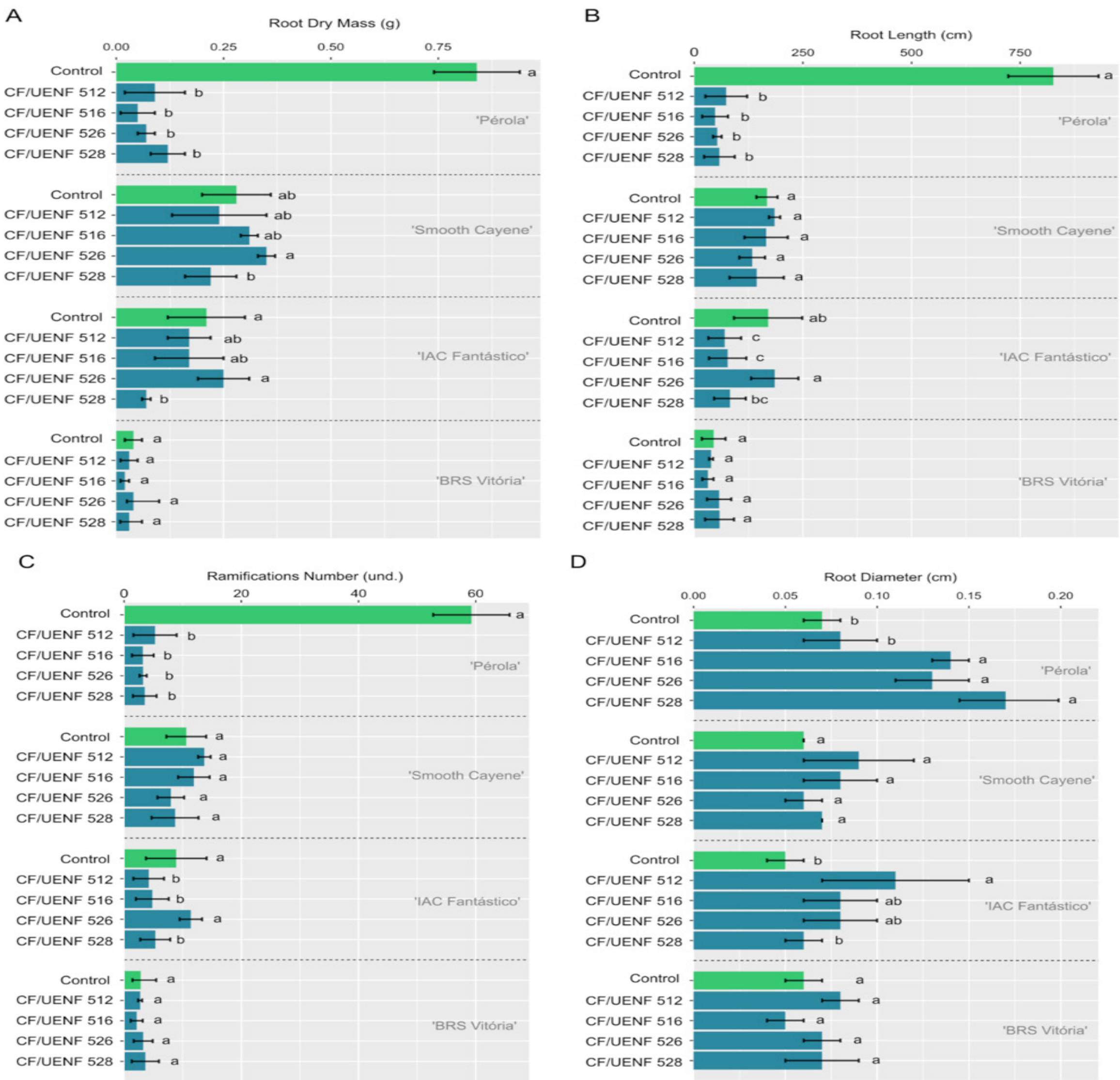

Figure 1. Effect of fusariosis on dry mass, root length, number of branches, and mean root diameter in seedlings of four pineapple cultivars (Pérola, Smooth Cayenne, IAC Fantástico and BRS Vitória) inoculated with different *Fusarium guttiforme* isolates (CF/UENF 512, CF/UENF 516, CF/UENF 526, and CF/UENF 528). Different letters indicate significant differences in the isolates means according the Tukey's test ($p < 0.05$).

effective in reducing root length, root perimeter, or root dry mass for this cultivar.

The isolate CF/UENF 512 promoted reductions in the root length and number of branches in the roots of the cultivar IAC Fantástico, with an increase in root diameter. It resulted in losses in root perimeter and an increase in surface area for this cultivar. Still, the isolates CF/UENF 516 and CF/UENF 528 caused reductions in root perimeter and root surface area for the cultivar IAC Fantástico so that the isolate CF/UENF 528 was the most effective in reducing root dry mass.

The isolate CF/UENF 512 also promoted an increase in root diameter and root surface area for the cultivar Smooth Cayenne. However, none of the four isolates significantly affected the other root traits for this cultivar. Furthermore, the four isolates caused no increase or decrease for any root trait in the cultivar BRS Vitória.

Plants with more numerous roots and well distributed in the soil can absorb nutrients more efficiently and produce more. This study showed that both the occurrence of fusariosis and the studied cultivar result in variations in root measurements. This makes it difficult to compare the root development reported by other authors, as there are few studies on the subject and they usually use few cultivars, which are different among the studies.

Research on pineapple root development as a result of disease is scarce in the literature. Some papers have reported differences in root development as a result of the application of different nutrient compounds (Liu et al. 2013), different irrigation systems (Santos et al. 2022), or even different forms of young seedling preparation (Paull

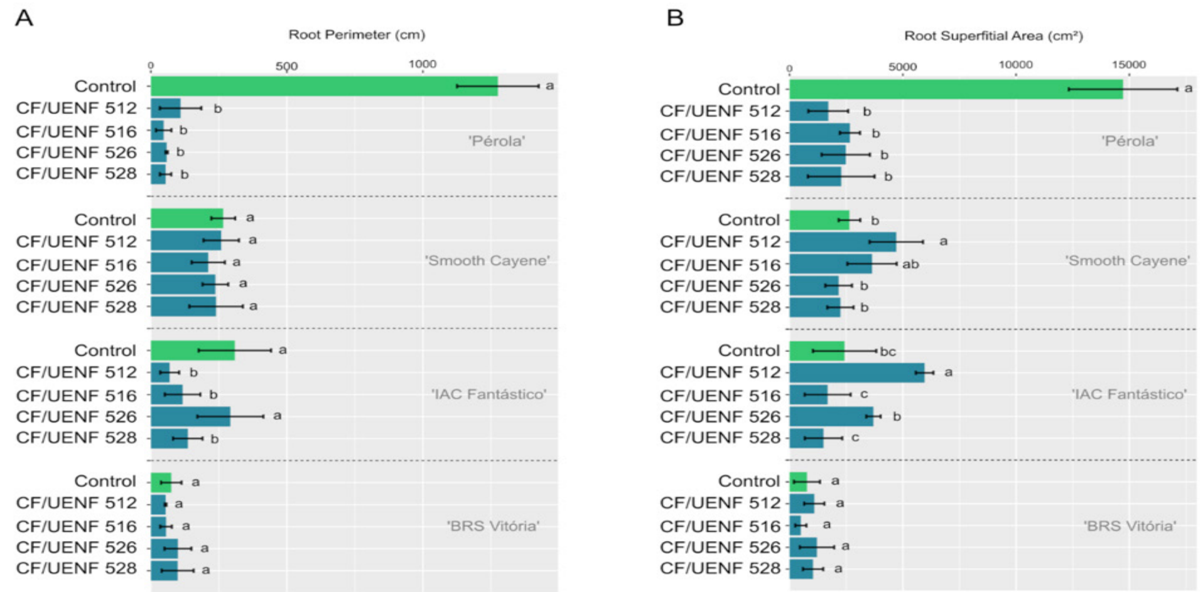

Figure 2. Effects of fusariosis on perimeter and root area of seedlings of four pineapple cultivars (Pérola, Smooth Cayenne, IAC Fantástico and BRS Vitória) inoculated with different *Fusarium guttiforme* isolates (CF/UENF 512, CF/UENF 516, CF/UENF 526, and CF/UENF 528). Different letters indicate significant differences between means of treatments according the Tukeu's test ($p < 0.05$).

et al. 2022). However, finding studies about the effects of fusariosis on the root system is difficult.

Nevertheless, the potential of *F. guttiforme* to cause root damage is nothing new. Pissarra et al. (1979) described that a reduction in root development associated with lesions that appear at the base of the stalk is common among the fusariosis symptoms in pineapples. The present study showed reductions of up to 90% in root length and the number of branches, with consequent loss of more than 90% of the root dry mass when considering the cultivar Pérola.

The cultivar Pérola was notably the most affected among the tested cultivars. The susceptibility of this cultivar to *F. guttiforme* is known among researchers and producers (Aquije et al. 2010). It makes fusariosis one of the main problems for pineapple production in Brazil, as the cultivar Pérola is predominant in cultivation areas, mainly due to the good acceptance of this type of fruit in the market for fresh consumption. Reinhardt & Medina (1992) reported this information and the scenario of preference for the cultivar Pérola remains in Brazil more than 30 years later, with few properties still producing the variety Smooth Cayenne (Hawaiian) for export (Conab, 2022).

The Brazilian Agricultural Research Corporation (Embrapa) in partnership with the Institute for Research, Technical Assistance, and Rural Extension of the State of Espírito Santo (Incaper) developed the cultivar BRS Vitória as a way of overcoming the losses caused by fusariosis (Ventura et al. 2009). However, some obstacles in the cultivation and acceptance of its fruits seem to have prevented the successful replacement of the cultivar Pérola. In this context, improvement work for the development of cultivars resistant to fusariosis and with fruit patterns desirable by the consumer market continues in development (Embrapa, 2022). In the present study, the cultivar BRS Vitória did not undergo reductions in the root structure due to fusariosis, which confirms the resistance of this cultivar. However, the cultivar BRS Vitória showed low development, both in root structures and in the shoot (**Figure 3**).

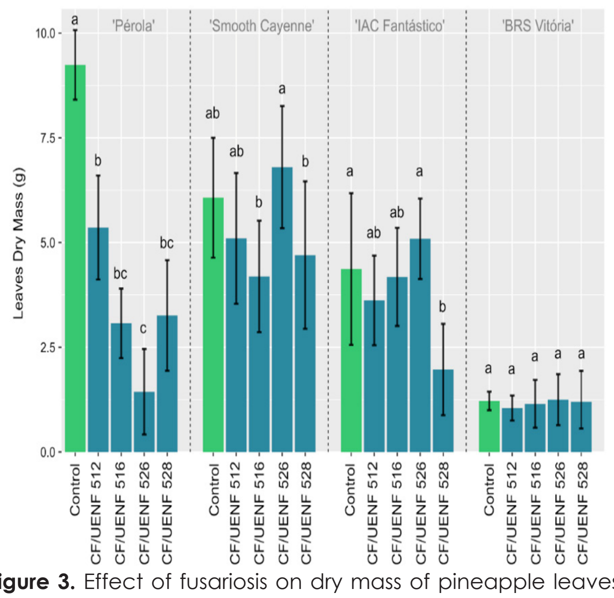

Figure 3. Effect of fusariosis on dry mass of pineapple leaves of four cultivars (Pérola, Smooth Cayenne, IAC Fantástico and BRS Vitória) inoculated with different *Fusarium guttiforme* isolates (CF/UENF 512, CF/UENF 516, CF/UENF 526, and CF/UENF 528). Different letters indicate significant differences between means of treatments according the Tukeu's test ($p < 0.05$).

The fact that the plants did not develop well may raise questions about the accuracy of estimates of fusariosis effects on plants at this stage of development. Further studies may elucidate the potential damage caused by fusariosis in 'BRS Vitória' seedlings. The low development of this cultivar is known, especially when its seedlings are developed by micropropagation techniques (Silva et al. 2020). However, the growth of 'BRS Vitória' seedlings can be accelerated with higher investment in nutrition (Santos et al. 2018).

Larger shoot structures were observed in the cultivars Pérola and Smooth Cayenne. Symptoms of fusariosis include reduced leaves, with the possibility of chlorosis and wilting in some cases (Pissarra et al. 1979). Chlorosis of some leaves was observed in the cultivar Pérola when inoculated with the studied isolates and in the cultivars Smooth Cayenne and IAC Fantástico when inoculated with *F. guttiforme* isolate 1 (**Figure 4**).

Reduced leaves and, consequently, plant diameter were observed in the cultivars Pérola, Smooth Cayenne, and IAC Fantástico when inoculated with *F. guttiforme*. The reduction of leaves in 'Smooth Cayenne' was not as pronounced as in 'Pérola' and 'IAC Fantástico.' Plants of the cultivar Smooth Cayenne are known to be susceptible to fusariosis (Hidalgo et al. 1998). Nevertheless, in agreement with the results observed in the present study, Matos & Souto (1985) had already reported that the cultivar Smooth Cayenne is less susceptible to fusariosis than 'Pérola.' It is also reported by Aquije et al. (2011) regarding the density of scales in the fruit epidermis, showing that 'Smooth Cayenne' has higher structural protection in the epidermis against *Fusarium* infection.

The cultivar IAC Fantástico is reported to be resistant to fusariosis (Freitas et al., 2024). Despite this, the results of the present study showed that some isolates could reduce plant root length and volume (Figure 1). Furthermore, significant (Figure 3) and visible (Figure 4) reductions in shoot dry mass were caused when *F. guttiforme* isolate 4 was inoculated. Garcia (2013) studied the resistance to fusariosis of different pineapple genotypes and reported that the accession referring to 'IAC Fantástico' showed no resistance to the pathogen. The author associates this result with the possibility of resistance degeneration due to in vitro multiplications. The seedlings used in this study also came from in vitro propagation. Thus, the observed results support the possibility of loss of resistance due to degeneration during propagation.

Considering that the evaluations were carried out at the initial stage of plant development is also necessary to understand the inconsistencies found between the results observed in the cultivars Smooth Cayenne and IAC Fantástico. Genetic resistance mechanisms in plants are usually mediated by hormones, which can condition different levels of plant resistance throughout the development stages (Denancé et al. 2013). Aquije et al. (2010) showed that resistance to fusariosis in the cultivar

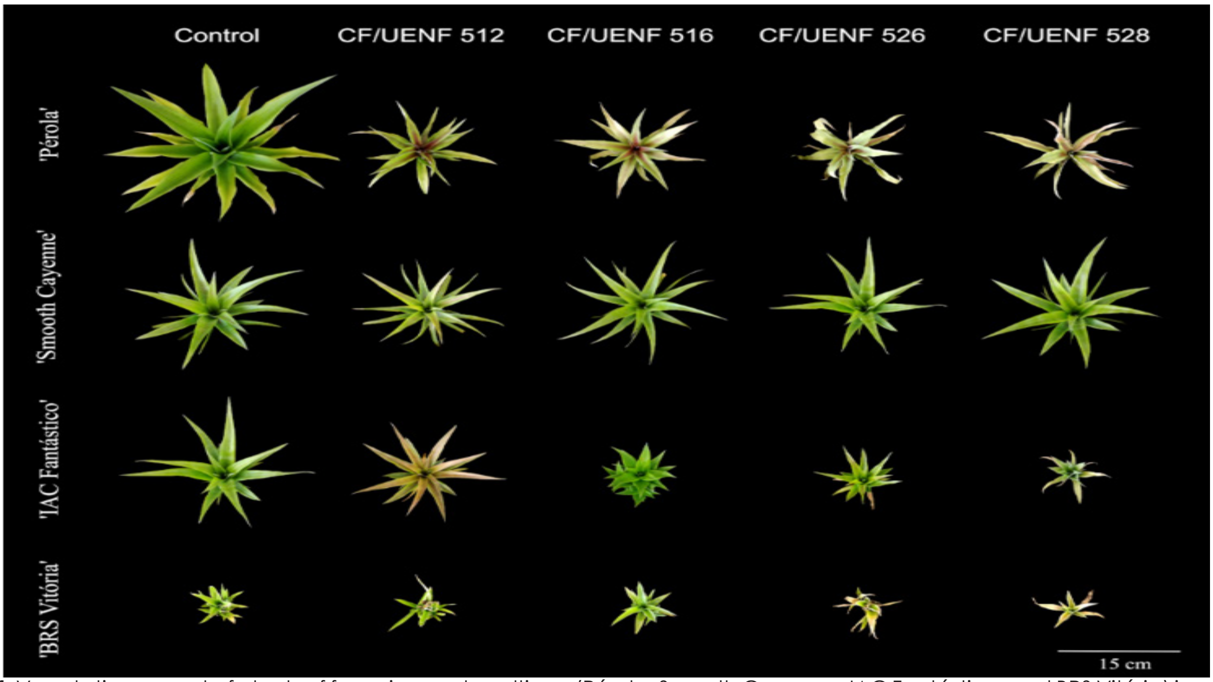

Figure 4. Vegetative aspect of plants of four pineapple cultivars (Pérola, Smooth Cayenne, IAC Fantástico, and BRS Vitória) inoculated with different isolates of *Fusarium guttiforme* (CF/UENF 512, CF/UENF 516, CF/UENF 526, and CF/UENF 528) compared to control plants (inoculation with distilled water).

BRS Vitória is associated with the capacity of the plants to produce polyphenols and lignin to contain the lesions caused by the pathogen inoculation. The importance of polyphenols in plant pathogen resistance has been reported in crops such as tomato (Setoguchi et al. 2023) and wheat (Rempelos et al. 2018). Some studies have revealed that polyphenol molecules are expressed in different amounts throughout the developmental stages of different crops (Guodong et al. 2019; Zuk et al. 2019). Marfil et al. (2019) also showed that environmental factors can interfere with the expression of polyphenol molecules by epigenetic mechanisms.

Thus, possible divergences in resistance levels indicated for some cultivars used in this study could be further investigated. Moreover, studies of root development in different pineapple genotypes, as well as the effects of fusariosis on root development, are scarce. In this sense, new studies may contribute to a better understanding of these effects, thus helping in the development of more effective disease control strategies, with a consequent increase in pineapple crop productivity.

Conclusions

Fusariosis at the pineapple early growth stages can cause damage to root development in susceptible cultivars, mainly the cultivar Pérola. This damage consists of a reduction in root length and the number of branches, with a trend towards higher mean root diameter. The use of contaminated seedlings or growing in contaminated fields can compromises production from the initial stages of crop root development.

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