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Artificial infection with *Fusarium solani* f.sp. *passiflorae* in plants of passion fruit under controlled conditions

Infección artificial con *Fusarium solani* f.sp. *passiflorae* en plantas de pasifloras bajo condiciones controladas

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ABSTRACT

Controlled infection systems constitute a valuable tool in the study of pathosystems, as they enable the observation and measurement of specific characteristics with greater precision and ease compared to natural conditions. Additionally, they are useful for exploring and identifying sources of resistance in host populations. However, the plant's response to infection can vary depending on the inoculation method; invasive methods can damage plant parts, thus facilitating pathogen entry even in the presence of certain resistance mechanisms. The purpose of this study was to evaluate the reproducibility of an artificial infection protocol using an assessment scale for primary symptoms at the stem base/root collar, as well as secondary symptoms in leaves and roots of two Passiflora species. We evaluated the effects of inoculating planting substrates of sweet granadilla and purple passion fruit plants with three concentrations of *F. solani* f. sp. *passiflorae* (0.5×10^6, 1×10^6 and 2×10^6 conidia $gram^{-1}$ of substrate), using non-inoculated plants as controls under controlled conditions. These treatments were tested in two separate experiments performed in 2017 and 2020. The study included symptom development readings integrated in

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a disease severity index (SI) for collar rot. Both experiments showed severity indices greater than 56%, indicating a high level of efficacy of the inoculation system in replicating disease symptoms under greenhouse settings. Our inoculation method reflects the conditions of natural infection of *F. solani* in sweet granadilla and purple passion fruit plants; it could be suitable for epidemiological and disease control studies, as well as for routine germplasm screening to identify sources of resistance.

Key words: collar rot; greenhouse; *Passiflora edulis* f. *edulis* Sims; *Passiflora ligularis Juss*.; severity Scale.

RESUMEN

Los sistemas de infección controlada constituyen una herramienta valiosa en el estudio de patosistemas, ya que permiten observar y medir características específicas con mayor precisión y facilidad que bajo condiciones naturales. También son útiles para explorar e identificar fuentes de resistencia en las poblaciones de hospederos. Sin embargo, la respuesta de la planta a la infección puede variar según el método de inoculación. Métodos invasivos pueden dañar la planta, facilitando la entrada del patógeno incluso en presencia de ciertos mecanismos de resistencia. El objetivo de este estudio fue evaluar la reproducibilidad de un protocolo de infección artificial utilizando una escala de evaluación para los síntomas primarios en la base del tallo/cuello de la raíz, así como los síntomas secundarios en hojas y raíces de dos especies de Passiflora. Se evaluaron los efectos de la inoculación de los sustratos de siembra de plantas de granadilla y gulupa con tres concentraciones de *F. solani* f. sp. *passiflorae* (0.5×10^6, 1×10^6 y 2×10^6 conidios · gramo^{-1} de sustrato) usando plantas no inoculadas como control en condiciones controladas. Los tratamientos se evaluaron a través de dos experimentos distintos realizados en 2017 y 2020. El estudio incluyó lecturas del desarrollo de síntomas integradas en un índice de gravedad de la enfermedad (IS) para la pudrición del cuello de la raiz. Las dos evaluaciones arrojaron índices de severidad superiores al 56%, confirmando la eficacia del sistema de inoculación en la replicación de los síntomas de la enfermedad bajo condiciones de invernadero. Nuestro método de inoculación refleja las condiciones naturales del proceso infeccioso de *F. solani* en plantas de granadilla y gulupa; podría ser adecuado para estudios epidemiológicos y de control de enfermedades, así como para el rastreo rutinario de germoplasma con el fin de identificar fuentes de resistencia.

Palabras clave: pudrición del cuello; greenhouse; *Passiflora edulis* f. e*dulis* Sims; *Passiflora ligularis Juss*.; escala de severidad.

INTRODUCTION

In Colombia, purple passion fruit (*Passiflora edulis f. edulis* Sims) and sweet granadilla fruit (*Passiflora ligularis* Juss) are economically significant crops, alongside passion fruit (*P. edulis f. flavicarpa* Degener), as they are exported as fresh and processed fruits. These crops face technological, social, and environmental challenges, along with an increase in foliar and soil-related diseases. In particular, the collar rot disease has amplified the extent of damage within cultivation areas, resulting in crop losses and even crop abandonment. This phenomenon was evidenced by the decline in sweet granadilla production in the Antioquia department during the 1990s. The national contribution of the department dropped from 44.7% to 2.6% due to collar root prevalence (Hoyos-Carvajal & Castillo, 2015).

Collar rot, caused by the fungus *Fusarium solani* f. sp. *passiflorae* (*Fsp*) (*Haematonectria haematococca* Berk. & Broome) Samuels & Nirenberg (Bueno *et al*., 2014), is the most

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significant disease affecting productive passion fruit plants. As plants mature, specific conditions linked to fungal extracellular enzymatic activity (Robledo *et al*., 2017) facilitate the initiation of colonization and infection in the root system and/or stem base. This triggers the development of characteristic aerial symptoms of the disease, ultimately leading to plant death (Ploetz, 1991; Ploetz, 2005; Porter *et al*., 2015).

In Colombia, there are few studies conducted on infection systems in passion fruits. Among them, Londoño (2012) evaluated 28 accessions from the passion fruit germplasm bank maintained by AGROSAVIA - Colombian Agricultural Research Corporation, testing two inoculation methods: 1. stem incision and 2. naked root immersion, using a strain of *Fusarium oxysporum*. Similarly, Ortiz & Hoyos (2016) aimed to standardize several inoculation methodologies for *F. solani* and *F. oxysporum*, comparing root immersion techniques with wounded and unwounded roots in four-month-old purple passion fruit plants. They found a high feasibility of disease development with the first technique and associated this phenomenon with the presence of wounds that increased the plant's susceptibility to pathogen invasion.

Ángel *et al*. (2018) compared several invasive methods, including stem incision, root immersion, stem injection, and screening in modified test tubes. The incision and screening treatments yielded 100% of disease incidence.

On an international level, in Brazil, Fischer *et al*. (2005) evaluated inoculation methods that included wounds and subsequent inoculation of *N. haematococca* on passion fruit plants of varying ages. They found that inoculations at the plant's collar led to elevated disease levels, in contrast to root system inoculations. This indicated that the pathogen's infections are more successful if the plant has injuries and in younger plants. Preisigke *et al*. (2017) assessed disease development in 14 passiflora species, which were inoculated with *F. oxysporum* through root immersion in a conidial suspension, leading to symptom development and plant death. Other studies evaluated the resistance of *P. mucronata* Lam against *F*. *oxysporum* f., sp. *passiflorae* and *F. solani* using the root immersion method, which was effective for selecting resistant rootstocks (Correia *et al*., 2022). Similar results were reported by Bueno *et al*. (2014), CEPASS & ASOHOFRUCOL (2010), and Ortiz & Hoyos (2016).

Artificial infection systems constitute a valuable tool in plant disease studies, as they allow researchers to observe and measure pathosystem-specific characteristics with greater precision and ease than under natural conditions. This type of infection systems is needed to better understand the passiflora-*F. solani* pathosystem, and to develop adecquate experimental tools to test disease control practices or biocontrol agents. They also are necessary for sound epidemiological studies and to develop routine germplasm screening protocols in the search for sources of resistance. Collar rot continues to be a limiting disease affecting passifloras worldwide, and causes severe losses wherever purple passion fruit and sweet granadilla orchards are grown in Colombia. The present

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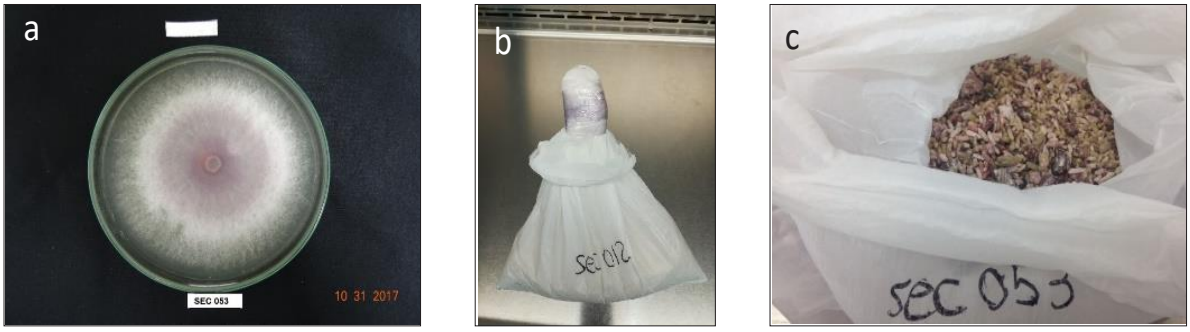
study highlights a comprehensive infection-evaluation system which will foster epidemiological and disease control studies of collar rot in the future

MATERIAL AND METHODS

Location. The experiments were conducted from January to March 2017 and from December 2019 to February 2020 at the Laboratorio de Microbiología Agrícola and the greenhouses of AGROSAVIA - Colombian Agricultural Research Corporation, Centro de Investigación Tibaitatá (Mosquera, Cundinamarca), in the Sabana de Occidente province, Cundinamarca department, Colombia; coordinates 4°41'45"N 74°12'12"W, 2516 meters above sea level. During both assessments, the average temperature was 23°C, with a relative humidity of 70%.

Strain of *Fusarium solani* f. sp. *Passiflorae*. We used strains of Fusarium strains SEC-053 (sweet granadilla) and SEC-173 (purple passion fruit) that had been previously characterized morphologically and microscopically on culture media: carnation leaf agar (CLA) and potato dextrose agar (PDA) (Bueno *et al*., 2014; Porter *et al*., 2015; Vargas, 1992), and through molecular methods for Fusarium species using ITS-Fu2, ITS-Fs5, and TEF-Fs4 markers (Arif *et al*., 2012; Bueno *et al*., 2014). The strains, which had been purified and stored in a working microorganism collection, were reactivated on a synthetic culture medium (PDA) in Petri dishes at a temperature of 25 ± 2°C in darkness for 7 to 10 days (Figure 1a).



a) Growth appearance of F. solani. Strain SEC-053, reactivated on PDA culture medium after 7 days of incubation at 25°C. b) Inoculated and sealed incubation bag. c) Substrate colonized with F. solani after 15 days of incubation at 25°C ± 2°C under continuous light. Note the pinkish coloring that results from substrate colonization by the fungus.

Figura 1. Mass multiplication process of *F. solani* inoculum in rice grains.

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Starting from the pure colonies obtained during the strain reactivation, we extracted mycelium from the colony periphery using 5 mm-diameter cork borers (as an alternative method, the colonies can be scrapped with a glass rod by adding 4 mL of sterile water). The culture medium discs with *F. solani* (or the mycelium and conidial suspension obtained from plate scraping) were used to inoculate sterilized rice substrate, facilitating the extensive propagation of the fungus.

Mass Multiplication of *F. solani* Inoculum in Rice Grains. The fungus enhancement substrate was prepared using the modified Porter method (Porter *et al*., 2015). For this, 250 g of rice was weighed and mixed with 120 mL of potato dextrose broth (PDB) in a polypropylene bag resistant to autoclaving to facilitate rapid and uniform fungal growth and colonization. To allow gas exchange and prevent excessive moisture accumulation and subsequent fermentation, the bag was sealed with a cotton plug covered with gauze and sealed with tape.

The bag containing the substrate mixture was autoclaved at 121°C and 15 pounds of pressure for 15 minutes and allowed to rest at room temperature under aseptic conditions for 6 to 12 hours before inoculation with *F. solani*. The mycelium discs, or fungal suspension, were inoculated into each 250-gram bag of substrate. The bags were then resealed with the plug and tape. This process was carried out in a laminar flow cabinet.

After the inoculation, the bags (Figure 1b) were incubated at 25°C ± 2°C for 12 to 15 days under continuous light, with manual homogenization of the contents every three days. This facilitated the uniform growth of the fungus across the substrate's surface (Figure 1c).

At the end of the incubation period, a conidial count was performed to determine the concentration. For this count, a 1:10 w/v dilution was prepared using 1 g of colonized substrate (Figure 1c) suspended in 9 mL of sterile, distilled water and homogenized using a vortex mixer. An aliquot was collected to perform *F. solani* conidial counting with a Neubauer chamber.

Plant material. We used commercial ecotype seedlings of sweet granadilla and purple passion in the vegetative state: 12 to 16 weeks old, grown in peat. Planting material for the experiments was provided by Cepass nurseries located at the department of Huila in 2017, and from the Biosystems Center of the Universidad Jorge Tadeo Lozano in Bogotá, Colombia, in 2020. In both cases, seedlings exhibited well developed root and leaf systems. Before inoculation, seedlings of both species were acclimated for a period of 15 to 20 days in a greenhouse with an average temperature of 23°C and a relative humidity of 70%. Plants were watered every week to mitigate stress and deterioration

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before transplanting them into bags. Plants were closely monitored to minimize losses and maintain uniformity among them.

Inoculation and experimental design. We chose moistened Promix® extra fine peat (1.5L of water per 10L) as the substrate for the inoculation for its aseptic characteristics and the presence of fine, loose particles that offer ease of management. We deemed this substrate optimal for fostering root system development in sweet granadilla plants.

The inoculation protocol was implemented as follows: 150 g of moistened peat was blended with different amounts of inoculum to create three distinct treatments, resulting in final concentrations of $0.5x10^6$, $1x10^6$, and $2x10^6$ conidia per gram. An uninoculated control group (plants without *Fsp*) was also included for comparative analysis. The experimental design adhered to a randomized complete block pattern with three replications and eight plants per experimental unit for the first trial conducted in 2017. Subsequent evaluations in 2020 followed a similar randomized complete block design, comprising four replications with six plants per experimental unit.

For transplanting, we filled each bag up to 2/3 of its capacity with moistened peat. An orifice was made at the center of the substrate to introduce the seedling. The bag was then filled completely with the mixture of moistened peat and colonized rice substrate. We made sure that the inoculum was evenly distributed and in contact with the base of the seedling stem. Subsequently, a nutrition regimen was implemented twice a week by applying Hoagland and Arnon solutions (Hoadgland & Arnon, 1950). The health status of the plants was continuously monitored.

Assessment of Sweet Granadilla and Purple Passion Fruit plant responses to *F. solani* Infection. Around 10 days after inoculating the sweet granadilla and purple passion fruit plants with *F. solani*, we started daily monitoring to detect any symptoms. Once initial symptoms appeared, the onset date was recorded, and assessments were conducted every three days to document damage progression in each plant. The description of disease-related damage was carried out using descriptors specifically generated for this purpose (Table 1 and Table 2).

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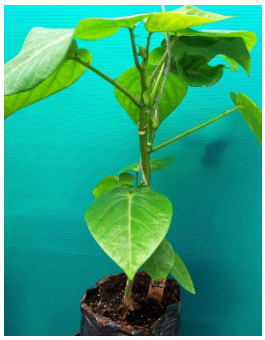
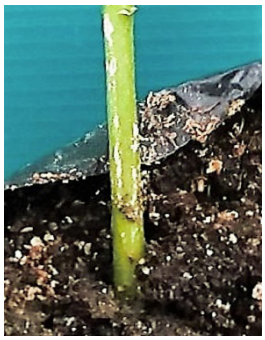
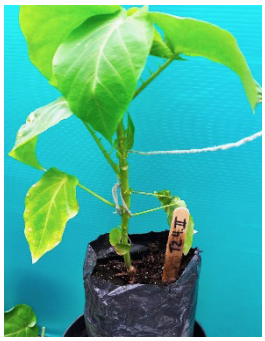
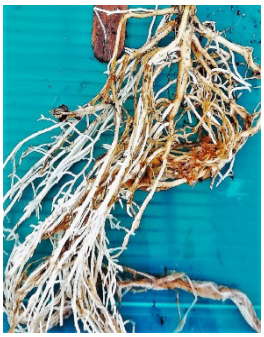
Table 1. Disease severity grades used to evaluate collar rot symptoms in sweet granadilla.

Intensity level	Leaf symptoms	Stem base/root collar symptoms	Root symptoms
0			
	Completely healthy leaves	Healthy stem base	Totally healthy roots
1			
	Slightly visible chlorosis on lower leaves	Superficial reddish discoloration, covering less than 50% of the stem base circumference. Small lesion of less than 1 cm in length on the root-stem axis.	Necrosis in less than 10% of the root volume or loss of less than 10% of roots
2			
	Initial wilting visible in young leaves; pronounced chlorosis in lower leaves	Deep reddish lesion, covering more than 50% of the stem base circumference. Onset of necrosis/canker spreading between 1 cm and 2.5 cm along the root-stem axis.	Necrosis in 20-30% of the root volume or loss of 20-30% of roots

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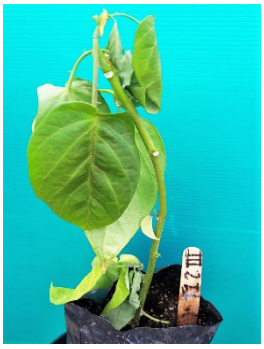
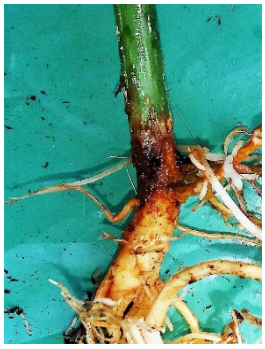
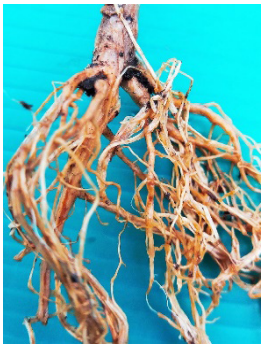
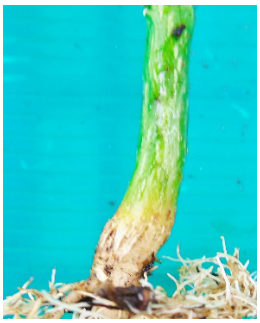
Intensity level	Leaf symptoms	Stem base/root collar symptoms	Root symptoms
3			
	Pronounced wilting and/or onset of defoliation	Necrosis/canker in 50% or more of the stem base circumference. Large lesion spreading between 3 cm and 4.5 cm along the root-stem axis.	Necrosis in 50-75% of the root volume or loss of 50-75% of roots
4			
	Complete wilting and/or total plant defoliation	Deep necrosis/canker around the entire stem base. Presence of perithecia may occur.	Complete wilting and/or total plant defoliation

Table 2. Disease severity grades used to evaluate collar rot symptoms in purple passion fruit.

Intensity level	Leaf symptoms	Stem base/root collar symptoms	Root symptoms
0			
	Completely healthy leaves	Healthy stem base	Totally healthy roots

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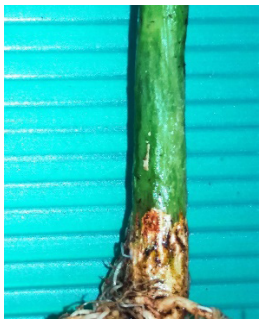
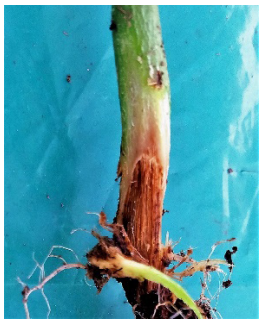
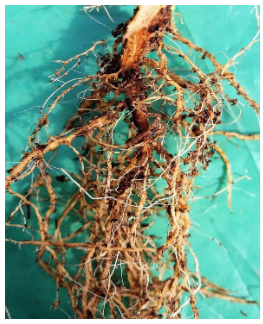
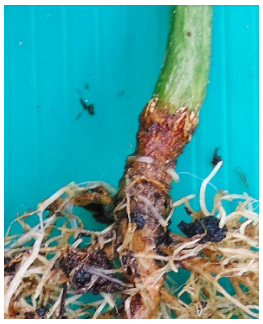
Intensity level	Leaf symptoms	Stem base/root collar symptoms	Root symptoms
1			
	Slightly visible chlorosis on lower leaves	Superficial reddish discoloration, covering less than 50% of the stem base circumference. Small lesion of less than 1 cm in length on the root-stem axis.	Necrosis in less than 10% of the root volume or loss of less than 10% of roots
2			
	Initial wilting visible in young leaves; pronounced chlorosis in lower leaves	Deep reddish lesion, covering more than 50% of the stem base circumference. Onset of necrosis/canker spreading between 1 cm and 2.5 cm along the root-stem axis.	Necrosis in 20-30% of the root volume or loss of 20-30% of roots
3			
	Pronounced wilting and/or onset of defoliation	Necrosis/canker in 50% or more of the stem base circumference. Large lesion spreading between 3 cm and 4.5 cm along the root-stem axis.	Necrosis in 50-75% of the root volume or loss of 50-75% of roots
4			
	Complete wilting and/or total plant defoliation	Deep necrosis/canker around the entire stem base. Presence of perithecia may occur.	Complete wilting and/or total plant defoliation

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Symptom assessments on foliage (SH) were conducted from the moment of inoculation until plant death. Symptoms on the stem base/root collar (SBT-CR) and roots (SR) were performed at the end of the process (or earlier in the case of plant mortality). Using the collected data, we calculated the disease severity index (DSI) using the following equation (Chiang *et al*., 2017b):

$$\boldsymbol{IS}(\%) = \frac{\sum_{y}^{i}(sh+sbt\ cr+sr)}{(\#\ observations)*(SH+SBN\ CR+SR)} \times 100$$

Where:
sh: value assigned to foliage symptoms for each plant
sbt cr: value assigned to stem base and root collar symptoms for each plant
sr: value assigned to root symptoms for each plant
SH: maximum value on the scale for foliage symptoms
SBT CR: maximum value on the scale for stem base and root collar symptoms
SR: maximum value on the scale for root symptoms.

The plants that reached intensity level 4 in SH underwent a symptom evaluation in the stem base and roots (SBT-CR and SR) (Londoño, 2012). For the assessment of symptoms in the stem base and root collar (SBT-CR), the size of the lesion was estimated based on the coverage achieved. The substrate and roots from different treatments were microbiologically analyzed using PDA and Komada agar (Komada, 1975) at the end of each experiment to isolate the inoculated strains and confirm the presence of the pathogen.

Statistical analysis

To perform the data analysis, we tested the assumptions of normality, independence of errors, and homogeneity of variance using the Shapiro-Wilk, Levene, and Durbin-Watson tests. We employed generalized linear mixed-effects models, treating the blocks and plants as random variables and the treatments as fixed factors. To determine mean differences, we performed a Fisher's LSD comparison ($p<0.05$) with Bonferroni correction to ensure rigor in the comparison of means. The analyses were conducted using the statistical software R, version 3.6.0.

RESULTS AND DISCUSION

Identifying an efficient and reproducible inoculation method that closely mimics the natural conditions of soil-borne pathogen infection is a crucial factor in studying pathosystems in the search for control methods and breeding processes (Ferreira *et al*., 2019).

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The controlled inoculations in sweet granadilla and purple passion fruit plants resulted in the expected outcomes regarding infection levels at the evaluated concentrations of *F. solani* inoculum. In the year 2017, initial symptoms appeared at 33 days post-inoculation for both sweet granadilla and purple passion fruit across all concentrations.

For the year 2020, the onset of initial symptoms varied based on species and concentrations. In sweet granadilla plants, those inoculated with $2x10^6$ conidia·g $^{-1}$ showed symptoms after 28 days. The plants inoculated with $1x10^6$ conidia·g $^{-1}$ exhibited symptoms after 31 days, and those inoculated with $0.5x10^6$ conidia·g $^{-1}$ showed symptoms after 35 days.

In the case of purple passion fruit plants, symptoms became visible at 35 days for the higher concentrations ($1x10^6$, $2x10^6$ conidia·gram^{-1}), while the lower concentration ($0.5x10^6$ conidia·gram^{-1}) led to symptoms appearing at 42 days.

Both species exhibited various stages of wilting, followed by chlorosis and subsequent defoliation associated with the pathogen infection process. We also observed symptoms at the base of the stem/root collar (canker), in some plants, and stem browning. At the root level, the most common symptoms were necrosis and growth reduction.

The experiments described in this study (2017 and 2020) demonstrated validity and reproducibility in establishing *F. solani* pathogenicity tests in sweet granadilla and purple passion fruit plants in the vegetative state under greenhouse conditions. Both plant species exhibited a higher infection pattern when inoculated with a greater concentration of pathogen conidia. The control group (without inoculation) did not exhibit symptoms related to the disease during the evaluation (data not showed).

Microbiological analyses of the substrate on PDA and Komada agar allowed for the isolation of *Fusarium* spp. colonies with morphological characteristics similar to those of the inoculated strain, confirming the presence of the pathogen in the substrate and roots of each treatment.

The three inoculum concentrations were significantly different ($F = 5.21$, $p = 0.0235$) according to the analysis of variance for the IS variable in sweet granadilla, while in purple passion fruit, no differences were observed among the three concentrations ($F= 0.86$, $p=0.4481$) (Table 3). These results suggest that using inoculum concentrations in the range of 0.5 to 2.0×10^6 *F. solani* conidia per gram of substrate is feasible. However, higher concentrations caused more damage to the plants. We observed significant differences among treatments when comparing the two experiments (2017 and 2020). In both experiments, there was a trend for higher disease severity index (SI) in response to higher concentrations of pathogen conidia for both Passiflora species (Figure 2).

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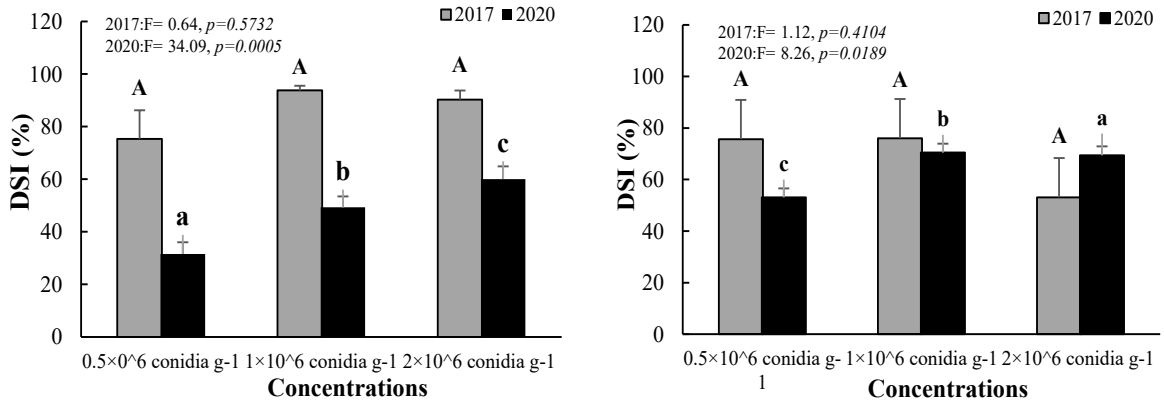
Table 3. Severity indices of collar rot, based on visual symptom estimation in sweet granadilla inoculated with different concentrations of *F. solani* strains in the two combined experiments.

Species	Treatment[1] (conidia g $^{-1}$)	SI (%)[2]	LSD Fisher (Alfa = 0.05)
Sweet Granadilla	0.5×10^6	50.35 ± 31.39	b
Sweet Granadilla	1×10^6	68.35 ± 24.54	a
Sweet Granadilla	2×10^6	73.01 ± 18.45	a
Purple Passion Fruit	0.5×10^6	62.80 ± 20.31	a
Purple Passion Fruit	1×10^6	72.87 ± 15.69	a
Purple Passion Fruit	2×10^6	62.45 ± 18.34	a

[1] The inoculum of *F. solani* strains was produced in rice and mixed with moistened peat at field capacity before its application to sweet granadilla and purple passion fruit plants. 2 SI: Severity index: mean values from the two experiments (2017 and 2020). Means ± standard deviation with the same letter do not indicate significant differences in Fisher's LSD grouping test for means at a significance level of 5%.



Means with the same letter do not indicate significant differences in the Fisher's LSD grouping test for means at a significance level of 5%.

Figure 2. Comparison of severity indices, based on visual symptom estimation in sweet granadilla (left) and purple passion fruit (right) inoculated with different concentrations of *F. solani* strains in the two experiments (2017 and 2020).

When comparing the outcomes achieved in each species across the two experiments, we notice that the inoculations carried out in 2017 yielded higher severity indices and an earlier manifestation of symptoms. This could be attributed to the fact that these plants exhibited less developed root systems and smaller stem diameters. Fischer *et al*. (2005) reported similar findings. They evaluated plants at different phenological stages and noticed that younger plants were more susceptible because they had a narrower stem base or root neck. Plants inoculated in the 2020 experiment, developed characteristic

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disease symptoms over a longer period, with less impact on roots. These results could be explained by their more advanced phenological growth stages, compared to plants used in the 2017 experiment. However, symptoms on leaves and the base of the stem/root neck were very similar across experiments.

Studies conducted by Pereira *et al*. (2019) demonstrated the effectiveness of this type of field inoculation directly into the soil. They chose concentrations similar to those evaluated in this study and used maize flour colonized with *F. oxysporum* f.sp. *passiflorae* to assess resistance in passion fruit genotypes in the field. Thangavel *et al*. (2021) applied a similar inoculation method for the identification and initial report of *F*. *oxysporum* f. sp. *passiflorae* (Fop) in passion fruit plants in Northland, New Zealand. In this study, they emphasized the importance of not affecting the roots to achieve a natural disease development. Nonetheless, there is a need to establish standardized protocols based on an understanding of the pathogen's behavior. This constitutes an innovative and easily reproducible method, considering that most inoculation systems are carried out through invasive methods. These methods show greater success in inducing symptom development in plants under controlled conditions, but they fail to mimic the natural infection process triggered by F.sp. in plants (Ángel *et al*., 2018; Forero *et al*., 2015).

Additionally, in this research, we effectively implemented the scale designed by our research team in 2017, accompanied by a pictorial guide for assessing collar rot symptoms. This scale offers a higher level of detail compared to others reported in the literature, and it is specific to sweet granadilla and purple passion fruit collar rot (Tables 1 and 2).

In this study we also incorporate the measurement of the three types of indicative symptoms of collar rot: two types of primary or direct symptoms occurring at the base of the stem and the root collar (SBTCR), as well as in the roots (SR), and a set of above-ground symptoms (SH). These above-ground symptoms, while considered indirect or secondary in nature, are crucial for disease detection in practice. The two types of symptoms are causally related and, when used together, allow for an accurate diagnosis of collar rot in sweet granadilla. Consequently, they can be integrated, and transformed into a collar rot severity index (IS). This type of approach, as discussed by Fang *et al*. (2012), represents a necessary step to support epidemiological studies.

In line with the above, the method that we propose for inoculation and evaluation of collar rot response can be easily implemented as a protocol, through a set of activities depicted on a simple chronological format covering the distinct phases of the inoculation and response evaluation process. The plant material used for inoculations must be pathogen-free, possess a well-developed root system, exhibit genetic identity, and high physiological quality. Suitable plant material can be obtained from a commercial nursery

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or produced by the researcher and must be in a vegetative state (12 - 16 weeks). The inoculum should originate from a physiologically active strain previously characterized using conventional and molecular methods. Its viability must be confirmed in advance (Marostega *et al*., 2019), and it should be applied at a concentration of 1×10^6 *F. solani* conidia g^{-1} of peat, making sure to place it in contact with an intact plant stem base (it should have no prior damage).

Taking into consideration the analyses from the two experiments, the established inoculum concentrations, along with the developed protocol for its laboratory production and application to passion fruit seedlings, constitute a standardized system of controlled infection with *F. solani* that can be replicated for research purposes in passion fruits. These purposes include germplasm evaluation, epidemiological studies, and assessments of methods and products for collar rot control (Ferreira *et al*., 2019).

Likewise, it is necessary to verify through microbiological analyses that the irrigation water, substrate, and tools used for managing the plant material in the greenhouse are free from pathogens that could interfere with the proper development of the plant material or with the various processes of the plant-pathogen interaction resulting from the inoculation of Fsp. into sweet granadilla and purple passion fruit plants.

CONCLUSIONS

Our findings support the use of the established inoculum concentrations and the laboratory production protocol for infecting sweet granadilla and purple passion fruit plants with *F. solani* f.sp. *passiflorae* under controlled conditions. Additionally, we designed a scale for assessing primary symptoms at the base of the stem/root collar, as well as secondary symptoms on leaves and roots. This scale can be used for monitoring the disease in future research, including identifying sources of genetic resistance, understanding disease epidemiology, and evaluating methods for collar rot control.

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