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Pathogenicity and transmission of fungi detected on *Passiflora alata* seeds

Patogenicidade e transmissão de fungos detectados em sementes de **Passiflora alata**

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ABSTRACT: Passion fruit is usually propagated by seeds because of the ease and lower cost in seedling production. However, the seed is the most efficient agent for the spread of pathogens. The damages from seed-borne diseases occur mainly during the germination stages or at the formation of seedlings in nurseries. Considering the need for knowledge on the pathology of sweet passion fruit seeds, the objective was to evaluate the transmission and pathogenicity of the fungi *Alternaria* sp., *Botrytis fabae*, *Cladosporium cladosporioides*, *Fusarium* spp. and *Lasiodiplodia theobromae*, known as potentially pathogenic to this crop, and isolated from sweet passion fruit seeds. Therefore, tests on seed health, germination and seedling emergence in a sterilized commercial substrate were conducted using seeds from this species, inoculated with those fungal isolates. Leaves, stems and fruit from this plant were also inoculated with the same fungi. *Alternaria* sp., *Fusarium* spp. and *L. theobromae* were identified in seedlings obtained from inoculated seeds, confirming the transmission of these fungi by seeds. *L. theobromae* was also considered the most harmful fungus to passion fruit crop, as it causes seed rot and other disease symptoms on the leaves, stem and fruit. These findings inferred that healthy seeds of sweet passion fruit are essential for producing seedlings and to prevent the spread of the diseases caused by these fungi to exempt areas.

KEYWORDS: seeds; pathology; health; fungi.

RESUMO: O maracujazeiro geralmente é propagado por meio de sementes em virtude da facilidade e do menor custo na produção de mudas. No entanto, a semente é o agente mais eficiente de disseminação de patógenos, sendo que os danos decorrentes das doenças transmitidas por elas ocorrem principalmente durante os estágios de germinação ou na formação de mudas nos viveiros. Considerando a necessidade de informações acerca da patologia de sementes de maracujá-doce nesse contexto, objetivou-se obter informações sobre a transmissão e a patogenicidade dos fungos *Alternaria* sp., *Botrytis fabae*, *Cladosporium cladosporioides*, *Fusarium* spp. e *Lasiodiplodia theobromae*, isolados de sementes de maracujá-doce e potencialmente patogênicos à cultura. Para tanto, testes de sanidade, germinação e emergência de plântulas em substrato comercial esterilizado foram conduzidos com sementes dessa espécie, inoculadas com esses isolados. Folhas, colo e frutos dessa planta também foram inoculados com os mesmos fungos. *Alternaria* sp., *Fusarium* spp. e *L. theobromae* foram identificados em plântulas obtidas de sementes inoculadas, confirmando a transmissão por sementes. *L. theobromae* foi considerado o mais agressivo à cultura do maracujá, por ter causado podridão nas sementes, além de maiores lesões nas folhas, no colo da planta e nos frutos. Dessa forma, infere-se que a obtenção de sementes de maracujá-doce sadias é imprescindível para a produção de mudas, evitando-se assim a disseminação desses patógenos em áreas isentas.

PALAVRAS-CHAVE: sementes; patologia; sanidade; fungos.

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INTRODUCTION

Sweet passion fruit (*Passiflora alata* Curtis) is a species native to South America, especially Brazil, whose cultivation has been expanding because of the price reached by the fruit (BERNACCI et al., 2015). Nevertheless, the expansion of the cultivation areas has been favoring the emergence of new diseases and the worsening of a great number of others, which have started to cause serious damages, and might also economically preclude this culture in some regions (VIANA et al., 2003).

Passion fruit can be attacked by several fungi, viruses and bacteria, which cause diseases as bacterial spot, root and stem rot, *Fusarium* wilt, anthracnose and scab or cladosporiosis (FISCHER; REZENDE, 2008).

Among the various diseases identified in passion fruit post-harvest, the most important is anthracnose, caused by *Colletotrichum gloeosporioides*. FISCHER et al. (2007) verified the incidence of this disease in 100% of the fruit of yellow passion fruit (*Passiflora edulis* f. *flavicarpa*), in organic and conventional orchards, followed by the incidence of *Fusarium* rot.

Other microorganisms that cause post-harvest rot in passion fruit are *Alternaria alternata*, *A. passiflorae*, *Septoria passiflorae*, *Aspergillus niger*, *Cladosporium* sp., *Penicillium expansum*, *Phytophthora nicotianae* var. *parasitica*, *Rhizopus stolonifer*, *Pseudomonas syringa* pv. *passiflorae*, *Xanthomonas axonopodi* pv. *Passiflorae* (KRETZSCHMAR, 1991; LOMBARDI, 2001), *Phomopsis* spp., *Lasiodiplodia theobromae*, *Pestalotiopsis* spp., *Botrytis cinereae*, *Sclerotinia sclerotiorum* (10) and *Trichoderma harzianum* (FISCHER et al., 2005).

Passion fruit is usually propagated by seeds, given the ease and lower cost of seedling production (LEONEL; PEDROSO, 2005); therefore, among the measures for the control of the cited diseases is the use of healthy seedlings, produced in seedbeds formed in disease-free areas (FISCHER et al., 2007).

Thus, the adaptation of sanitary management techniques for this culture, according to ROLIM et al. (2002), requires the knowledge of the seed microflora, its action on their germination and vigor, and the transmissibility of phytopathogens.

Several genera known as pathogenic to the culture, with emphasis for *Cladosporium* and *Fusarium*, have been detected in passion fruit seeds (FISCHER; REZENDE, 2008). ROLIM et al. (2002) identified in the seeds of commercial fruit of yellow passion fruit in the region of Marília, SP, fungi of the genera *Alternaria*, *Aspergillus*, *Colletotrichum*, *Fusarium*, *Nigrospora*, *Penicillium*, *Phoma* and *Pestalotia*. These same authors detected *Cephalosporium*, *Fusarium*, *Penicillium* and *Phoma* in seeds of commercial fruit of sweet passion fruit.

It is also known that the presence of aryl, regardless of the cutting the of tip of the seed emergence, and in the absence of fungicide, favors the growth of *Penicillium* sp., *Aspergillus* sp. and *Fusarium* sp. in yellow passion fruit seeds, and of *Fusarium* sp. in sweet passion fruit seeds (MARTINS et al., 2006).

Nevertheless, most of the researches on pathogenicity in passion fruit were performed from isolates removed from the fruit, directed to diseases such as anthracnose, caused by *Colletotrichum* species, which can affect more than one culture, such as passion fruit, avocado and peach (TOZZE JÚNIOR, 2011).

Pathogenicity studies from seed isolates have been occurring even in forest species (PARISI; OLIVEIRA, 2006; BOTELHO et al., 2008; BENETTI et al., 2009; LISBÔA-PADULLA et al., 2010; LAZAROTTO et al., 2010; REGO et al., 2012), verifying that the damages, derived from the diseases transmitted by seeds in these species, occur mainly during the stages of germination and/or in the formation of seedlings in the nurseries (CARNEIRO, 1987). Nonetheless, there is no information of this nature available, associated to passion fruit culture.

Considering the lack of literature on this subject, the aim of this research consisted in obtaining information on the transmission and pathogenicity of fungi isolated from *P. alata* seeds.

MATERIAL AND METHODS

The experiments were conducted simultaneously at the Laboratories of Seeds of the Agronomic Institute, in Campinas, and in the Laboratory of Phytopathology of the Center West Regional Hub, in Bauru, SP.

Ripe *P. alata* fruit were collected in the production fields of the Agronomic Institute of Campinas, SP, on the month of May of 2014. The seeds were manually removed from the fruit and mucilage at the Laboratory of Seeds, and, subsequently, maintained moist (average degree of humidity of 20%), at 5ºC, inside plastic boxes (11 × 11 × 3 cm) capped and sealed with adhesive tape, to prevent them from entering in dormancy when dry.

Fungi, considered pathogenic to the culture, of the genera *Alternaria*, *Botrytis*, *Fusarium* (two different species), *Cladosporium* and *Lasiodiplodia*, were detected in the healthy test, performed in one sample of these seeds. These fungi were isolated and maintained in pure cultures at the Laboratory of Seeds of the Agronomic Institute, in Petri dishes, containing potato-dextrose-agar broth (PDA) and cultivated, for seven days, at 20 ± 2ºC, under a photoperiod of 12 hours of dark and 12 hours of light, provided by "daylight"-type lamps of 40 Watts, placed at a distance of 40 cm from the dishes. Because of the low incidence of these fungi, detected in the healthy test, the artificial inoculation of these seeds was performed with the six isolates obtained.

Firstly, the seeds collected from the *P. alata* fruit were homogenized and subjected to surface asepsis with commercial 2.5% sodium hypochlorite for 15 minutes, washed in running water to remove the excess of the product and dried for 12 hours in blotting paper, at 5ºC.

The artificial inoculation of these seeds was then performed with the six isolates mentioned, by the method of contact with the fungal colony, as described by PARISI et al. (1999). For this, the seeds were divided into seven samples and six were arranged in dishes, previously prepared with colonies characteristic of the fungi already mentioned. Subsequently, the dishes were manually shaken for five minutes, allowing a higher contact of the seeds with the inoculum and, then, maintained in incubation chamber, at 20 ± 2ºC and photoperiod of 12 hours dark and 12 hours light for 24 hours.

The seventh sample consisted of the uninoculated control, represented by seeds placed in Petri dishes, containing only PDA, and subjected to the same procedures described for the seeds inoculated with fungi.

The inoculated seeds and those of the control were analyzed regarding health, germination and emergence of seedlings on a sterilized substrate. The amount of seeds used and informed in the description of each test was defined in relation to the availability of ripe fruit, obtained from a field for the production of genetic seeds. As sweet passion fruit maturation is not homogeneous, the use of the available amount of seeds with the same degree of maturation was chosen. The experimental design used was the completely randomized, with seven treatments (six isolates and the control). The data from these determinations were informed in percentage and for normality and heterogeneity correction, they were transformed into root (x + 0.5) and subjected to the analysis of variance, comparing the means by the Tukey test (p = 0.05).

The healthy test was performed following the filter paper method (NEERGAARD, 1979), and consisted of the incubation of the seeds in Petri dishes, of 9 cm in diameter, containing 3 sheets of filter paper with 80 g/m^2, moistened with distilled water. The dishes, containing 10 seeds equidistant from each other, were maintained for seven days at 20 ± 2ºC and photoperiod of 12 hours of light, provided by "daylight"-type lamps of 40 Watts, at a distance of 40 cm. Each treatment was composed of four repetitions of 10 seeds (40 seeds/treatment). The evaluation was performed seven days after incubation, by observations, in a stereoscopic microscope, of fungal structures in the seeds. The identification of the fungal genera was performed by their morphology in an optical microscope (BARNETT; HUNTER, 1998) and molecular analysis of a part of the DNA from the fungi.

The molecular analysis was performed from DNA extraction, according to the modified methodology described by MURRAY; THOMPSON (1980), followed by the amplification of the regions ITS-5.8SrDNA, for all the fungi isolated and of a part of the gene for β-tubulin, only for the fungi of the genera *Alternaria* and *Fusarium*. The ITS-5.8SrDNA region is one of the most used for the initial identification of the fungi, for being highly conserved in fungal species, but variable among species and, for this reason, it has helped in the studies of phylogenetic relationships among species of great economic importance, such as those from the genus Fusarium (O'BRIEN et al., 2005; KVAS et al., 2009; KACHUEI et al., 2015; KUMAR et al., 2016). The gene for β-tubulin codes for the structural proteins of microtubules and other structural components in eukaryotes and has been widely used in fungal phylogenetic analysis, since it contains variable and highly conserved region*s,* presenting more stability and reliability in the differentiation of species such as those of *Fusarium* in comparison to the gene ITS (NOSRATABADI et al., 2018).

The amplification of DNA fragments was performed, using the primer sets ITS1/ITS4 and Bt2-F/Bt2-R, following the methodology described by TOZZE JÚNIOR et al. (2015). The sequences obtained from the amplified fragments were edited using the program Bio Edit Sequence Alignment Editor (1997-2005). After edition, these sequences were used to search for similar sequences by using the software Blastn of the NCBI.

The germination test was performed with four repetitions of 20 seeds per treatment, sown in rolls of germination paper moistened with water, at the proportion of 2.75 times their weight and maintained in a germinator of the type Mangelsdorf, at 27ºC. The rolls were opened weekly until completing the 42 days after test installation, registering every week the number of normal and abnormal seedlings and of dead seeds, and computing the total value of these variables, in percentage, at the end of the test.

The test of seedling emergence was conducted at the Laboratory of Phytopathology of the Center West Regional Hub of Bauru, by sowing in plastic pots of 3.5 L, containing a commercial Biomix® substrate, previously autoclaved for two consecutive days. In each pot, ten seeds were equidistantly distributed, remaining from the healthy test, with fungal development, artificially inoculated as previously described, with *Alternaria*, *Botrytis*, *Cladosporium* and *Lasiodiplodia* and two *Fusarium* species. The pots were maintained in an incubating oven at 25ºC, photoperiod of 12 hours and were daily irrigated to maintain the substrate always moist.

Seedling emergence was evaluated in weekly intervals until 42 days. With the data obtained, the emergence speed index (ESI) was calculated, using the formula proposed by MAGUIRE (1962), and the seedling emergence curve was built, informing, in percentage, the mean results of seedlings obtained from inoculated seeds. Pathogen reisolation was performed from the parts of the seedlings with symptoms. The completely randomized design was adopted, with two repetitions per treatment, with the portion represented by one pot with ten seeds. At 42 and 70 days after sowing, the treatments were compared by a non-parametric test of multiple proportions, at a level of 5% of probability (ZAR, 1999).

In another experiment conducted in this same laboratory, the inoculations in the leaves and stem of the plants were performed with the same pathogens used in seed inoculation, 45 days after sowing *P. alata* in 3 L pots, containing Biomix® commercial substrate. Initially, two leaves from each plant (fourth and fifth leaves) had the central abaxial region disinfected with

cotton soaked in 70% alcohol; subsequently, a disk of mycelium was fixed with adhesive tape, 5 mm in diameter, grown in PDA for seven days on the disinfected region, with a wound made in one of the leaves from each plant by perforation with a histological needle. In the first 24 hours after inoculation, the plants remained in a wet chamber, composed of a plastic bag and a cotton ball moistened in distilled water.

For plant stem inoculation, a disc of mycelium, 5 mm in diameter, was fixed with adhesive tape, grown in PDA on plant stem, previously wounded or not with a 3 mm circular punch, at 2 cm from the soil. As control in the inoculation of the plant leaves and stem, only the PDA disc without the pathogens was employed. The plants remained in a vegetation house and the evaluations consisted of measuring, in two perpendicular directions, leaf wound diameter (WD) four days after inoculation; the length of the wound in plant stem at weekly intervals and the incidence of withered/dead plants at weekly intervals.

Healthy fruit of sweet passion fruit, from a commercial orchard in Presidente Prudente (SP) and in an intermediary ripening stage, were washed in running water and neutral detergent and received wounds on the equatorial region with a set of three flamed histological needles (2 mm deep). The inoculum, a disc of mycelium cultivated in PDA (5 mm in diameter) of the isolates (two of *Fusarium*, one of *Cladosporium*, one of *Alternaria*, one of *Lasiodiplodia* and one of *Botrytis*) and a PDA disc (control) were deposited on the wounds. The fruit were individualized in plastic trays and stored at 25ºC, remaining in a wet chamber in the first 24 hours. The evaluation was performed four days after inoculation, measuring, in two perpendicular directions, wound diameter (WD), expressed in cm. Pathogen reisolation was performed to compare the colonies obtained with those used as inoculum.

In the plant inoculation experiments, the experimental design was completely randomized, with seven treatments (six isolates and one control) with four repetitions, with the portion represented by one leaf, stem or fruit.

The results of inoculation on sweet passion fruit leaf, stem and fruit were subjected to the analysis of variance at the level of 5% of probability and the means were compared to each other by the Tukey test, at the level of 5%. The statistical analyses were performed by the program SISVAR (FERREIRA, 2003) and when necessary, for the correction of normality and heterogeneity, the data were transformed for (x + 0.5) ^ 0,5 (SANTANA; RANAL, 2004).

RESULTS AND DISCUSSION

In the sweet passion fruit seeds, by the healthy test, the fungi from the genera *Alternaria* (4%), *Botrytis* (12%), *Cladosporium* (16%), *Fusarium* (9%) and *Lasiodiploidia* (4%), known as pathogenic to the culture, were originally detected. After the isolation and inoculation of the seeds, the incidence of these fungi increased to 100%.

By the DNA sequencing of the ITS regions, the following fungal species were identified: *Botrytis fabae*, *Cladosporium cladosporioides* and *Lasiodiplodia theobromae*. Even with the sequencing of two regions in the DNA, it was not possible to identify the species of the fungi belonging to the genera *Alternaria* and *Fusarium*. In this case, it would be interesting to perform a new characterization work, considering the sequencing of other regions, such as the elongation gene-1α (EF1α), calmodulin (CAM), RNA polymerase II (RPB2), gene 28S rRNA and the genes of the mycotoxins biosynthetic pathway, proposed as targets to identify *Fusarium* and *Alternaria* at the species level, undertaking PCR amplification (MIRHENDI et al., 2010).

The germination of the sweet passion fruit seeds was low, because of the high percentage of dead seeds. Isolates *Fusarium* sp. (isolate 1) and *L. theobromae* were prominent as the most aggressive, since they increased seed mortality in comparison to the control, resulting in a lower percentage of normal seedlings (Table 1). According to SANTOS et al. (2001), seeds germination is also affected by the presence of fungi that can cause seedling death or transmit diseases to adult plants.

Table 1. Mean values (%) of normal and abnormal seedlings and of dead seeds, determined in the germination test of *Passiflora alata* seeds inoculated or not with fungi obtained from fruit harvested in May 2014 in Campinas, SP.

Treatments	Percentage (%)		
	Normal seedlings	Abnormal seedlings	Dead seeds
Control with PDA	27 a	5 a	68 cd
Alternaria sp.	25 a	1ab	74 bcd
Botrytis fabae	25 a	2ab	73cd
Cladosporium cladosporioides	40 a	0 b	60 d
Fusarium sp. (isolate 1)	7 b	1ab	92 ab
Fusarium sp. (isolate 2)	21 a	0 b	79 bc
Lasiodiplodia theobromae	0 b	0 b	100 a
C.V. (%)	20.38	55.16	5.35

Means transformed into (x + 0.5) ^ 0,5.
Means followed by the same lowercase letter in the column do not differ from each other by the Tukey test (p ≤ 0.05).

In the test of seedling emergence on a commercial substrate (Fig. 1), there was no statistical difference among the treatments, regarding both the emergence speed index and the final value of seedlings obtained from the inoculated seeds ($p > 0.05$); nevertheless, there was a delay in the beginning of the emergence of the seedlings originated from the seeds inoculated with the fungi in comparison to the control. The fungi that caused the lowest numerical values of final emergence percentage and emergence speed index were *Fusarium* spp., *B. fabae*, *L. theobromae* and *Alternaria* sp.

Although less than 10% of the seedlings obtained from inoculated seeds presented wounds caused by the fungi inoculated in the seeds, by reisolation of the parts of the seedlings with symptoms, the presence of *Alternaria* sp., *Fusarium* spp. and *Lasiodiplodia theobromae* was identified, confirming the transmission of these fungi by the seeds.

The fungal isolates were pathogenic when inoculated in leaf, plant stem or fruit of sweet passion fruit (Table 2), with the exception of *C. cladosporioides*, which did not cause symptoms

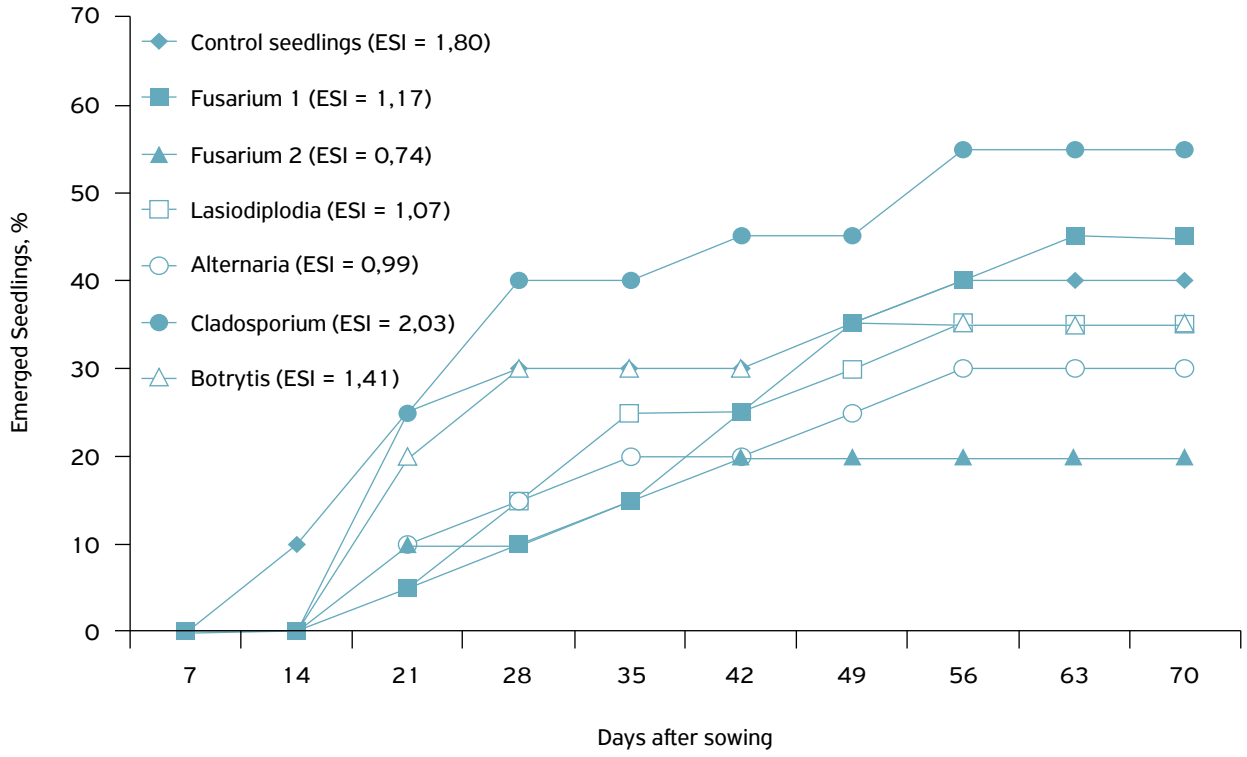

Figure 1. Emergence curve (%) and emergence speed indexes (ESI) of normal *Passiflora alata* seedlings on Biomix® commercial substrate, originated from seeds inoculated or not with fungi and obtained from fruit harvested in May 2014 in Campinas, SP.

Table 2. Diameter (cm) of the wounds in leaves and fruit of sweet passion fruit, four days after inoculation, and wound length in the plant stem, seven days after inoculation, with or without wound (only on the leaf), of different fungal pathogens, isolated from *Passiflora alata* seeds.

Pathogens	Leaf[2]		Stem	Fruit
	Without wound	With wound	With wound	With wound
Fusarium sp. (isolate 1)	0.63 bc	0.83 bc	0.53 cd	0.40 ab[1]
Fusarium sp. (isolate 2)	0.48 ab	0.45 ab	0.35 bc	0.65 ab
Lasiodiplodia theobromae	2.13 d	1.95 d	1.95 e[3]	6.93 d
Alternaria sp.	0.58 b	0.68 b	0.20 ab	0.95 bc
Botrytis fabae	0.55 b	0.70 b	0.08 a	0.70 ab
Cladosporium cladosporioides	0.00 a	0.00 a	0.00 a	0.00 a
Control	0.00 a	0.00 a	0.00 a	0.00 a
CV (%)	11.23	13.76	14.83	28.78

[1]Means followed by the same lowercase letter in the column do not differ from each other by the Tukey test ($p \leq 0.05$).
[2]Statistical analysis with the data transformed into $(x + 0.5)^{0,5}$.
[3]Plants died at the end of the experiment.

in the inoculations with or without wounds. *C. cladosporioides* is one of the causal agents of the passion fruit scab, along with *C. herbarum* (FISCHER; REZENDE, 2008) and, as verified in the present work, is one of the fungi most frequently found as saprophyte, air and food contaminant, with an important biological function in the decomposition of organic matter, being also a strong competitor with other microorganisms. Possibly, the intraspecific genetic variability, coupled to the selection pressure in populations, must have allowed the emergence and establishment of pathogenic populations inside the species *C. cladosporioides.* Differences in the severity of the wounds caused by the pathogens inoculated were observed. The isolate of *L. theobromae* was the most aggressive (Table 2), since it resulted in the death of the four plants (repetitions), two weeks after having been inoculated in their stem. According to KAGIWATA (1990), *L. theobromae* is one of the main microorganisms that cause post-harvest rots in passion fruit. The pathogens were successfully reisolated from the leaves inoculated without wound. On the leaves, the wounds, in general, were numerically higher four days after inoculation, when they were performed with wounds (Table 2). The leaves inoculated with *L. theobromae* (Fig. 2A) fell seven days after inoculation and, for the other pathogens, presented an increase of 18.3% in the wound, on the mean of the pathogens, from the fourth to the 14th day, and in the inoculation with wound, the lesions were, on average, 10.9% higher in relation to the absence of wound. *Alternaria* sp. also caused lesions on the stem and fruit, being less aggressive than *L. theobromae*. At least nine *Alternaria* species have already been reported as passion fruit pathogens, responsible for necrotic spots in leaves, branches and fruit. Abscission of the affected leaves can occur rapidly, causing intense defoliation (FISCHER; REZENDE, 2008).

On the stem of the plants, only the isolates of *L. theobromae* and *Fusarium* (isolates 1 and 2) caused characteristic wounds, differing from the control treatment, and only *L. theobromae* caused plant death (Fig. 2B), with wilt symptoms observed from the first week of inoculation. The pathogen *L. theobromae* can infect the passion fruit branches, resulting in wilt and drought of the part above the wound (FISCHER; REZENDE, 2008). For the other pathogens, there was an average increase of 15.7% in wound length from the seventh to the 21st day, without increase of the wounds in the subsequent weeks. For wound increase calculation in relation to time, the following equation was adopted: $y = a/b * 100$, in which y = is wound increase (%) in relation to time; a = length (cm) of the wound at time 2 (21st day); b = wound length (cm) at time 1 (seventh day). On the inoculation without wounds on the plant stem, with the exception of *L. theobromae,* that also caused plant death, the other pathogens did not cause characteristic necrotic wounds, with isolates *Fusarium* 1 and *Alternaria* sp. causing only superficial wounds and inferior to 0.2 cm in length, evidencing the need of wounds for the infection to occur.

On the fruit, the largest wounds were those from *L. theobromae* (Fig. 2C), followed by those of *Alternaria* sp., *Fusarium* (isolates 1 and 2) and *Alternaria* sp., four days after inoculation (Table 2). On the sixth day, *L. theobromae* colonized the whole fruit, making it impossible to measure wound diameter, but for the other pathogens an average increase of 86.7% was observed in wound diameter in relation to the fourth day. For the inoculations in the absence of wounds, they were observed for isolates *L. theobromae* and *Alternaria* sp., with a mean wound diameter of 4.6 and 0.8 cm, respectively. *L. theobromae* rot in the fruit is characterized by round light-brown lesions, which darken and get covered by fungal mycelium and pycnidia, with a further soft rot. *Alternaria* wounds are round and depressed, reaching from 1 to 3 cm in diameter, depreciating the commercial value (FISCHER; REZENDE, 2008). *Fusarium* rot reached 25.5% of incidence in fruit of yellow passion fruit on a survey of the post-harvest diseases in São Paulo's Central West, with the rots by *Lasiodiplodia* and *Alternaria* verified in lower incidences (FISCHER et al., 2007). *Botrytis fabae* is an important pathogen in fava beans (TOLESSA et al., 2015), with no reports of its occurrence in passion fruit.

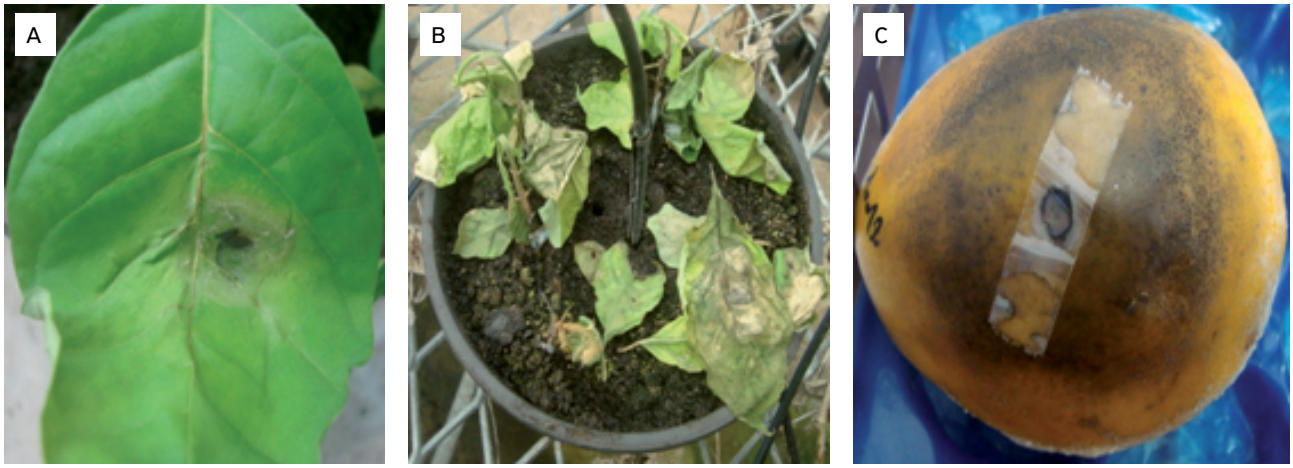

Figure 2. Symptoms of *Lasiodiplodia theobromae* rot, after pathogen inoculation in leaf (A), plant stem (B) and fruit (C) of sweet passion fruit.

Nevertheless, *B. cinerea* has already been reported causing rot in fruit of passion fruit in Japan (KAGIWATA, 1990).

In passion fruit seeds, several genera which are known as pathogenic to the culture were detected, such as: *Cladosporium* spp. and *Fusarium* spp. (MANICA, 1981; RUGGIERO, 1987), but this was the first work performed that could prove the transmission of *Alternaria* sp., *Fusarium* spp. and *L. theobromae* by seeds and *L. theobromae* pathogenicity in the culture of sweet passion fruit.

This information broadens the knowledge on the action of the microflora present in these seeds, a factor which, according to ROLIM et al. (2002), is essential for the establishment of sanitary management techniques for the culture, inferring that the acquisition of healthy sweet passion fruit seeds is mandatory for seedling production and for avoiding the dissemination of these pathogens in exempt areas.

CONCLUSIONS

The symptoms manifested in seeds, seedlings, leaves, plant stem and fruit of sweet passion fruit, resulting from the inoculation of *L. theobromae*, isolated from seeds of this culture, reveal that this fungus can compromise the establishment and production of sweet passion fruit culture.

Alternaria sp., *Fusarium* spp. and *L. theobromae* are transmissible by *P. alata* seeds.

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