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Transmission and effects of *Fusarium oxysporum* f. sp. *vasinfectum* on cotton seeds

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This paper aimed to evaluate the transmission of *Fusarium oxysporum* f. sp. v*asinfectum* (*FOV*) and the effects of this pathogen on the initial development of cotton plants following inoculation of seeds. Two cultivars (susceptible and resistant) and two strains (most and least aggressive) of the pathogen were used in this study. The inoculation method was based on the contact between seeds and fungal colonies on substrates containing mannitol. Percentage of *FOV* in seeds and the percentage of seed germination were evaluated by blother test and germination test, after inoculation. Emergence of seedlings and speed index, initial and final stands, size and dry weight of the plants were verified in trays containing soil substrate. Disease severity, pathogen transmission and plant infection, from seed to plant, were determined in separate trial on plants. Occurrence of the pathogen was higher when inoculum potential was increased for all variables analyzed. The number of normal seedlings, determined by seed germination test, decreased when the incidence of the pathogen in the seed was increased. The same occurred to other variables, in which there was difference between cultivars where IAC 20-233 presented the best performance. No significant differences were found between strains for emergence speed index, initial and final stands variables. Transmission and infection rates were increased according to the inoculum potentials increasing and the maximum pathogen transmission rate, from seed to plant was around 50%.

Key words: Transmissibility, pathogenicity, fusarium wilt disease, cotton.

INTRODUCTION

Fusarium wilt, caused by *Fusarium oxysporum f. sp. vasinfectum* (Atk.) W.C. Snyd. & H. N. Hans, is one of the most important diseases of cotton (*Gossypium hirsutum* L.) causing severe yield losses worldwide. The disease can reduce cotton productivity to intolerable levels in countries located specially in Africa, Asia, USA, South America and Oceania (Davis et al., 1996, 2006; Wang et al., 2006). The pathogen is considered to be

soil-borne with a parasitic phase in the plant and a saprophytic phase in the soil or in plant debris after harvest (Wang et al., 2006). In addition to soil, *FOV* can be disseminated by infected seeds and plant materials and by contaminated tools (Hillocks and Kibani, 2002). The occurrence of this pathogen on seed lots of cotton is variable, ranging from 0.6 to 47% (Kulkarni, 1934; Veigas, 1935; Perry, 1962; Bennett et al., 2008). However, the mechanisms of seed infection of *FOV* and its transmission from seed to seedling and seedling to plant still not fully understood and disserve our attention.

The use of resistant cultivars has been the most effective strategy to control diseases like *FOV* wilt in several crops. However, the success of breeding programs to control wilt disease in cotton depends on the understanding of the population structure of *FOV* as well as how the pathogen is transmitted (Wang et al., 2006). In addition, other difficulties faced in this pathos system are the presence of other organisms that have been found associated to wilted cotton plants during their early stage of seedling development. Symptoms of *FOV* wilt in cotton can be identified at both early and late stages of plant development depending upon the aggressiveness of the isolate (Smith et al., 1981; Kim et al., 2005; Davis et al., 2006).

Information on specific resistance of cotton cultivars to *FOV* wilt that are transmitted through seed is presently absent. This information, in addition to others of epidemiological nature, is of fundamental importance as that pathogen has been proposed to be part of the list of non-quarantine regulated pests in Brazil (Machado, 1994). Therefore, the establishment of health standards of *FOV* in seed lots as a part of the seed certification programs in Brazil could give a good contribution to provide healthier planting material. Thus, the objectives of this study were to evaluate the transmission rate of two strains (most and less aggressive) of *FOV* from cotton seeds to emerged plants and to study the effect of this infection on the development of two cultivars of cotton (susceptible and resistant) in the initial stage of plant under controlled conditions.

MATERIALS AND METHODS

Seed

Seeds of cotton, cultivars IAC 20-233 and FM 966, considered resistant and susceptible to Fusarium wilt, respectively, were used in the present study. Both cultivars were provided by Agronomic Institute of Campinas (IAC), Sao Paulo, Brazil. All seed batches used in this research were submitted initially to germination and health tests, following descriptions in the Brazilian Rules for Seed Analysis (Brazil, 2009). The germination test was carried out on towel paper, in which 25 seeds were distributed evenly on each roll pad, with four pads per treatment. For the health test blotter method was used following description in BRASIL (2009), except that 2,4-D (Sodium dichlorophenoxyacetate) at 10 ppm concentration was incorporated to blotter substrate (Neergaard, 1979; Machado and Langerak, 1993). These preliminary evaluations were conducted at the Seed Pathology Laboratory of the Plant Pathology Department and at the Seed Analysis Laboratory of the Agronomy Department, both in the Federal University of Lavras, Minas Gerais, Brazil.

Seed inoculation

The two strains of *FOV*, used were selected according to their virulence levels presented in previously pathogenicity test carried with 49 isolates.

Seeds were inoculated using the method of osmoconditioning as described by Machado et al. (2004). According to this methodology, colonies of the selected strains were initially produced on SNA medium under incubation at 25 ± 2°C, with photoperiod of 12 h for seven days. Using a Neubauer chamber, conidial suspensions were prepared with 20 ml sterile water to obtain a final concentration of 10^6 spores/ml. One millimeter of each suspension for each strain was distributed in Petri dishes on PDA medium amended with 46.3 g of mannitol (osmotic restrictor) per liter, to provide an osmotic potential of -1.0 Mega-Pascal (MPa). The amount of mannitol was indicated and adjusted according to the software MPPS (Michel and Radcliffe, 1995). The amended medium with mannitol was used to achieve different inoculum levels on infected seeds for preventing the germination seed (Machado et al., 2004). After three days of Petri dishes incubation, the strains were grown on the agar amended medium, completely. Then, seeds of the selected cultivars, were previously immersed in sodium hypochlorite 2% solution for 1 min, washed in sterile water three times and were dried over night at room temperature. After that, disinfested seeds were distributed evenly on the developing colonies of the *Fusarium* strains, organized in a single layer on the top of the agar amended medium. All Petri dishes were then incubated at temperature of 25 ± 2°C, were kept for 48, 72 or 108 h. Agar amended medium without inoculum was used with seeds of both cultivars as controls. Following each incubation period, seeds were removed from the dishes and placed on filter paper pads to dry during two days at room conditions.

Seed health analysis and germination test after inoculation of seeds

Inoculated seeds were surface disinfected as described previously. Seeds were tested by the PCNB Agar method as described by Sousa et al. (2008), with addition of mannitol at concentration indicated by the software MPPS (Michel and Radcliffe, 1995). To achieve -1.0 Mega-Pascal (MPa), 46.3 g of mannitol were used for each liter of medium. After seven days of incubation, at temperature of 22 ± 2°C and a photoperiod of 12 h, the seeds were examined in a stereomicroscope (Coleman®, 40x) for the presence of *FOV*. Identification of pathogen was based on morphological features of the conidia (shape, size and arrangement), conidiogenous cell formation (mono- or polyphialides) as well as growth pattern, pigmentation and conlony shape (Leslie and Summerrell, 2006). The occurrence of *FOV* was registered in a

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total of 200 seeds and the incidence was determined as percentage of each *FOV* strain in the sample of seeds.

For determination of germination test, 200 seeds of each cultivar inoculated with *FOV* were used and, unlike the seed health test, seeds were not previously disinfected. At the germination test was determinated the percentage of normal seedlings. Both health and germination tests was performed according to the Brazilian Rules for Seed Analysis (BRASIL, 2009) and were conducted in completely randomized design with eight and four replicates, respectively.

Transmission of *F. oxysporum* f. sp. *vasinfectum* from inoculated seeds to emerged plants

To study of the transmission of *FOV* from seed to seedlings, seeds were sowed in polyethylene pots of 5 kg capacity and trial conducted for 45 days. Ten seeds were sown for each treatment on a substrate composed by a mixture of autoclaved soil and sand in the proportion of 1:1. The experiment was in randomized design with for replicates an conducted in growth room at 25 ± 3°C with a photoperiod of 12 h. The transmission of the pathogen was calculated based on the disease severity (DS), infection rate (IR) and transmission rate (TR). The evaluation of DS was performed by observing symptoms on the leaves and stems and basing on a grading scale varying from 0 to 3, in which, 0 = healthy plants, 1 = wilted plants 2= dead plants and 3 = plants not emerged. The data were analyzed by applying the formula described by McKinney (1923). IR and TR were determined using the methodology adapted from Teixeira and Machado (2003). To determine the IR, stem fragments of emerged plants were sampled and cut out about three centimeters above the substrate line and then sectioned longitudinally and decontaminated respectively with sodium hypochlorite (2%) for one minute. Disinfected fragments were placed on Petri dishes with PCNB medium and then incubated at 22± 2°C, with photoperiod of 12 h for seven days (Sousa et al., 2008). After this period, the plates were examined for the occurrence of mycelial growth of *FOV* on the fragments and on the culture medium, using stereomicroscope (Coleman®, 40x). The TR was determined based on the mathematic relation between infection percentage (IR) and the incidence percentage (I) of that pathogen as recorded on the tested fragments of cotton plants and on inoculated seeds by the health testing.

Effects of *F. oxysporum* f. sp. *vasinfectum* on the growth of cotton seedlings

The assays were carried out in polyethylene boxes with dimensions of 48 × 29 × 10 cm containing substrate composed of soil and sand (1:1) previously treated with methyl bromide. Two hundred seeds were sown per treatment, 50 seeds per box. The experiment was conducted in a growth room with temperature of 25±3°C and a photoperiod of 12 h for 25 days after sowing (DAS). The variables observed were: Emergence Speed Index (ESI), Initial and Final Stand (IS and FS), Seedling Height (SH), and Dry Weight (DW).

The emergence of seedlings was determined by daily counts of seedlings until the stabilization of the number of seedlings for three consecutive days. The ESI was calculated according to Maguire (1962) by equation:

$$ESI = \sum_{i=1}^{n} N_i/D_i$$

Where ESI = Emergence Speed Index; Ni = Number of emerged seedlings the 1st count, 2nd count, ... nth count, respectively; Di = Number of days after sowing the 1st count, 2nd count, ... nth count, respectively.

The values for the IS and FS were recorded at 8 and 25 days after sowing, respectively, and the number of plants recorded at these two periods was converted in percentage of emerged seedlings. Data on seedling height (cm) were obtained by measuring, with a ruler, ten emerged plants taken at random, per replicate, by cutting them at the ground level. Then all sectioned plants were submitted to drying in forced air oven (Marconi® model MA035) at temperature of 50°C for 72 h. Plant material was weighed using a semi-analytical scale (Gehaka® model AG 200) and the results were reported in grams (g).

Statistical analysis

The analysis of variance, from all the variables, were carried out in a factorial 4 × 2 × 2, where the factors were four exposure times of seeds to pathogen (0, 48, 72 and 108 h); two cultivars (IAC 20-233 and FM 966); and two strains (CML 1098 and CML 1135) using a computer statistical analysis system (SISVAR) (Ferreira, 2011). The data were processed using the $\sqrt{x}$ or $\sqrt{x + 0.5}$ transformations when necessary and the means between treatments were compared by mean comparison test (Tukey test, $P \leq 0.05$) or by regression according to the nature of the data.

RESULTS AND DISCUSSION

Initially germination and health tests, of both cultivars of cotton, IAC 20-233 (resistant) and FM 966 (susceptible), presented 85 and 72% of germination and 1.5 and 0% of incidence of *FOV*, respectively.

It was observed that percentage of germination of seeds in both cultivars was proportionally lower whereas the inoculum potentials in the seeds increased, represented by exposure times of the seeds to the pathogen (Figure 1). Comparing the effects of the isolates on the cultivars germination, it was observed that the cultivar IAC 20-233 presented better performance than the cultivar FM 966 in the presence of the two isolates whereas no difference was noticed between the isolates CML 1098 and CML 1135 for the cultivar IAC 20-233 (Table 1). This result can be directly traced not only to the cultivar resistance but also to the initial physiological quality of the cultivars used.

Analyzing the initial profile of the seeds, cultivar IAC 20-233 showed better physiological condition than the cultivar FM 966 (Figure 2). This result was observed due to the higher percentage of seed germination of this cultivar in relation to cultivar FM 966. Despite being considered 'resistant' to Fusarium, the presence of *FOV* in seeds of cultivar IAC 20-233 provided germination reduction of 21 and 22% for the strains CML 1098 and CML 1135, respectively. The occurrence of *FOV* on seeds was significantly different among the times of seed exposition to the pathogen or, in other words, according to the increasing inoculum potential. The increase in the pathogen incidence on inoculated seeds through all inoculum potentials proved the efficiency of this methodology which allowed the presence of the pathogen

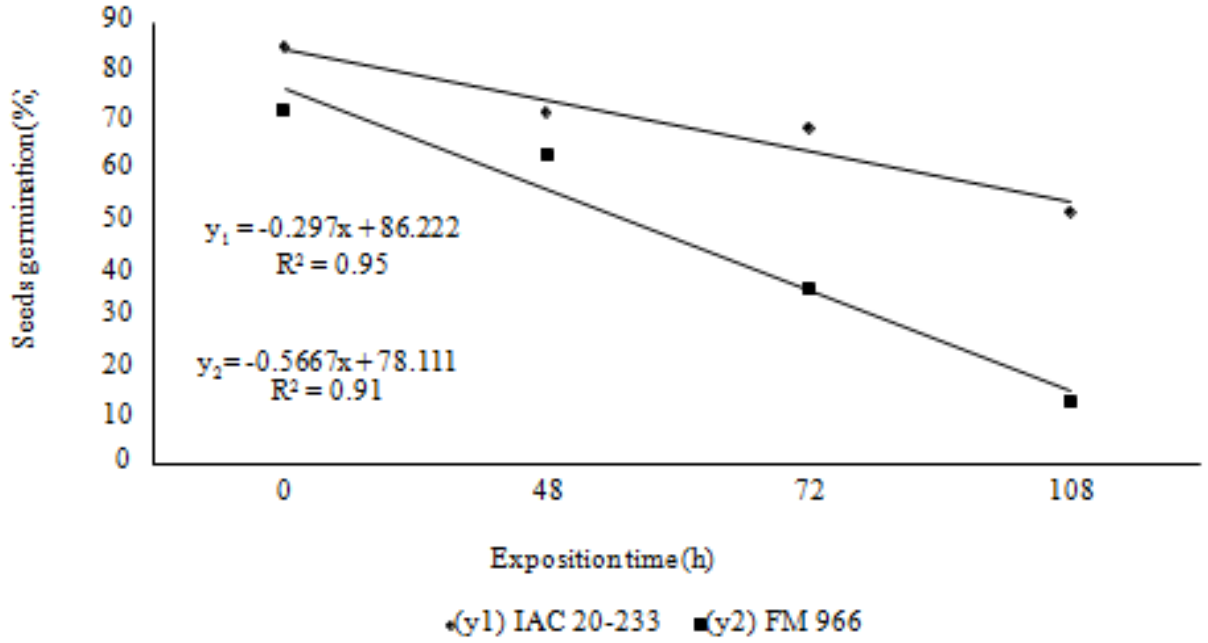

Figure 1. Cotton seeds germination from the cultivars IAC 20-233 and FM 966 inoculated with *Fusarium oxysporum* f. sp. *vasinfectum*, related to the time of exposition to the pathogen.

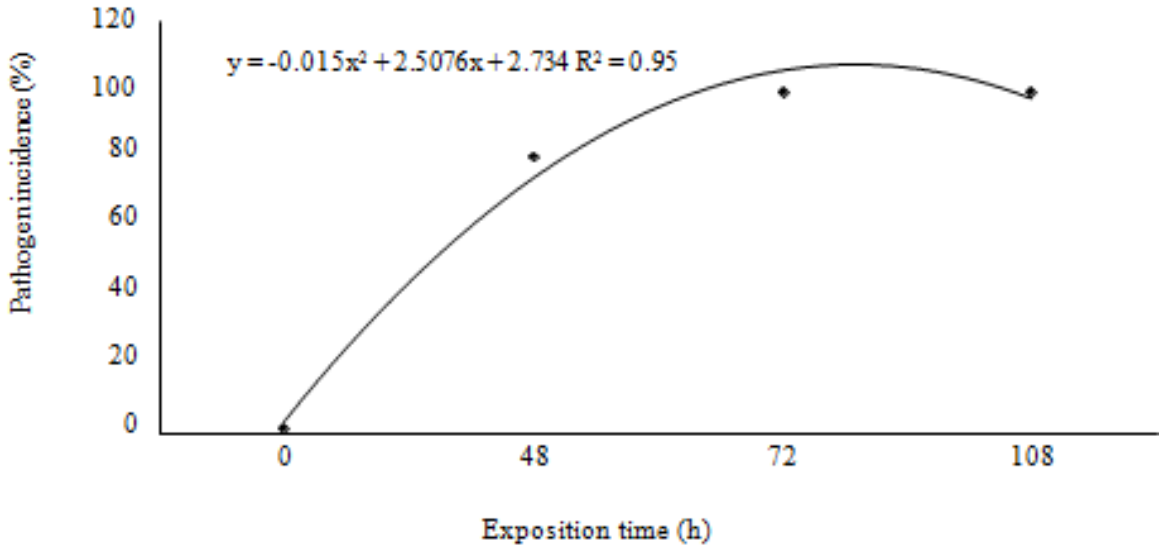

Figure 2. Incidence of *Fusarium oxysporum* f. sp. *vasinfectum* on cotton seeds according to the exposition time.

in high levels in seeds. Accordingly, similar results and observations from other papers support this as the most appropriate technique (Teixeira and Machado, 2003; Teixeira et al., 2005; Araújo et al., 2006; Sousa et al., 2008).

The effect of inoculum potential on the seeds, observed in the health test, was reflected in the DI measured in cotton plants 45 days after sowing. There was an increase of the DI while the inoculum potential obtained by the time of inoculation increased from 0 to 108 h of seed exposition to the pathogen for both cultivars studied. The highest DI was obtained for the cultivar FM 966 (89.2%) whereas for the cultivar IAC 20-233, considered susceptible was 47.5% (Figure 3).

According to DI results of the work, Sousa et al. (2008) found an increase in DI while the exposure time of seeds

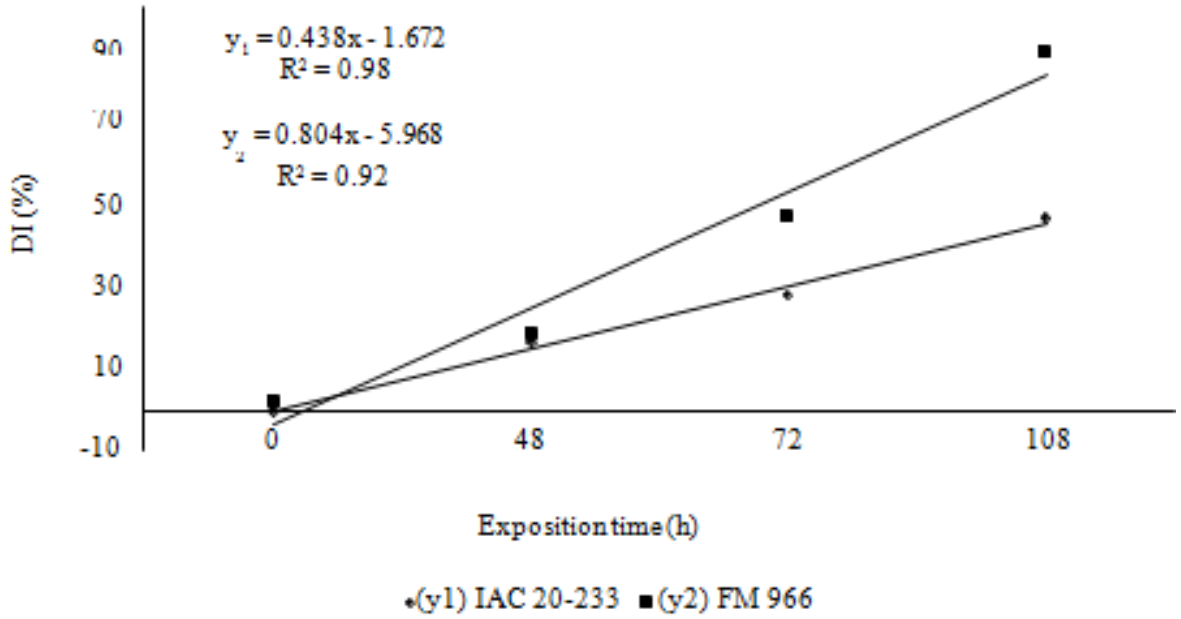

Figure 3. Disease index (DI) of cotton plants (45 days after sowing) related to the IAC 20-233 and FM 966 cultivars, from seeds inoculated with *Fusarium oxysporum* f. sp. *vasinfectum* in different exposition times.

to *FOV* was from 24 to 120 h for all three seed inoculation methods used. In the other work, Teixeira et al. (1997) also observed that the exposure time (0, 15 and 30 h) influenced the DI of cotton seedlings, grown from seeds inoculated with *Colletotrichum gossypii* South. Additionally, the authors reported the increase of the DI in both desinfected and non desinfected seeds after inoculation. On the other hand, Araújo et al. (2006), evaluating the pathosystem *C. gossypii* South. var. *cephalosporioides* A.S. Costa in cotton plants, found that the inoculum potential did not increase significantly the incidence and severity of seedling disease in cotton in all exposure time evaluated (36, 72 and 108 h) even though these rates have been high starting in the shortest period of the seeds exposure to the pathogen.

The influence of strains in severity was greater for the cultivar FM 966 than for the cultivar IAC 20-233 (Table 1). In this context, the resistance factor may be clearly noticed in the infection process and transmission of the pathogen from seed to plant.

In this study, one of the objectives was to investigate the infection rates and the transmission of the pathogen aiming to clarify this gap on the infection process. The results observed show the significant difference between the times of exposure to the pathogen reinforcing that the inoculum potential does make difference in the infection and transmission process. As the assessment of the rates was made in plants that remained alive until 45 days after sowing. The results about the rates of infection and transmission observed in this study may be related to the low percentage of emergence observed, especially in the time of 108 h. This may have occurred due to the death of seeds, caused by high infection by the pathogen at 108 h.

Thus the rates of infection and transmission, measured in stem fragments of cotton plants were higher in proportion to the increase of the inoculum potential, reaching a maximum of 51% in both cases. The point of maximum infection and transmission was observed at 78 h of contact between the seeds and the pathogen. From that point on only a small reduction in the values was observed for both the rates (Figure 4).

In this context, it was clear that the infection of the plant tissues may be directly related to the inoculum potentials reached by the seeds. From these results, it is assumed that the transmissibility of *FOV* from cotton seeds is effective and can reflect higher or lower rates of infection and transmission, depending on the level of seed infection presented.

In a similar study conducted with corn kernels inoculated with *Acremonium strictum* Gams, Teixeira and Machado (2003) observed, however, that the infection rate, as measured in the aerial parts of the plants 28 days after sowing, was higher according to the increase of the inoculum potential of the seeds (0 to 120 h). Nevertheless, no difference was reported for the transmission rate at 24, 72 and 120 h, differing only in time 0 h.

Reports of natural infection of cotton seeds from symptomatic plants have been described in Tanzania with levels around 47% (Perry, 1962). However, values below 10% of infection have been more common, particularly when seeds are originated from non-

Table 1. Seed germination, disease index (DI) in plants with 45 days, emergence speed index (ESI) and final stand for 25 days related to both strains of *Fusarium oxysporum* f. sp. *vasinfectum* (CML 1098 and CML 1135) and both cotton cultivars (IAC 20-233 and FM 966).

Strains	Seed germination (%)*		DI (%)*		ESI*		Final stand (%)*	
	IAC 20-233	FM 966	IAC 20-233	FM 966	IAC 20-233	FM 966	IAC 20-233	FM 966
CML 1098	64.0^{Aa}	33.0^{Bb}	28.9^{Ab}	52.2^{Aa}	35.01^{Aa}	25.08^{Ab}	35.0^{Aa}	26.0^{Ab}
CML 1135	63.0^{Aa}	41.0^{Ab}	33.3^{Ab}	52.5^{Aa}	31.41B^{a}	25.67^{Ab}	33.0^{Aa}	24.0^{Ab}
CV (%)	16.08		20.36		8.85		15.34	

*Means within a column and line followed by the same letter were not different (Tukey P > 0.05); CV: Coefficient of variation.

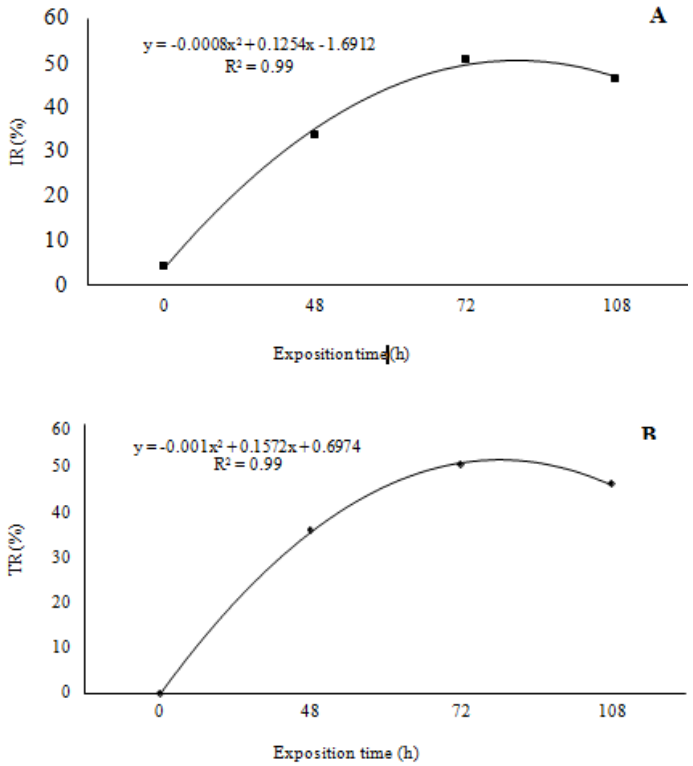

Figure 4. Infection Rate (IR) (A) and Transmission Rate (TR) (B) of *Fusarium oxysporum* f. sp. *vasinfectum* evaluated in cotton plants (45 days after sowing), depending on the exposition times to the pathogen.

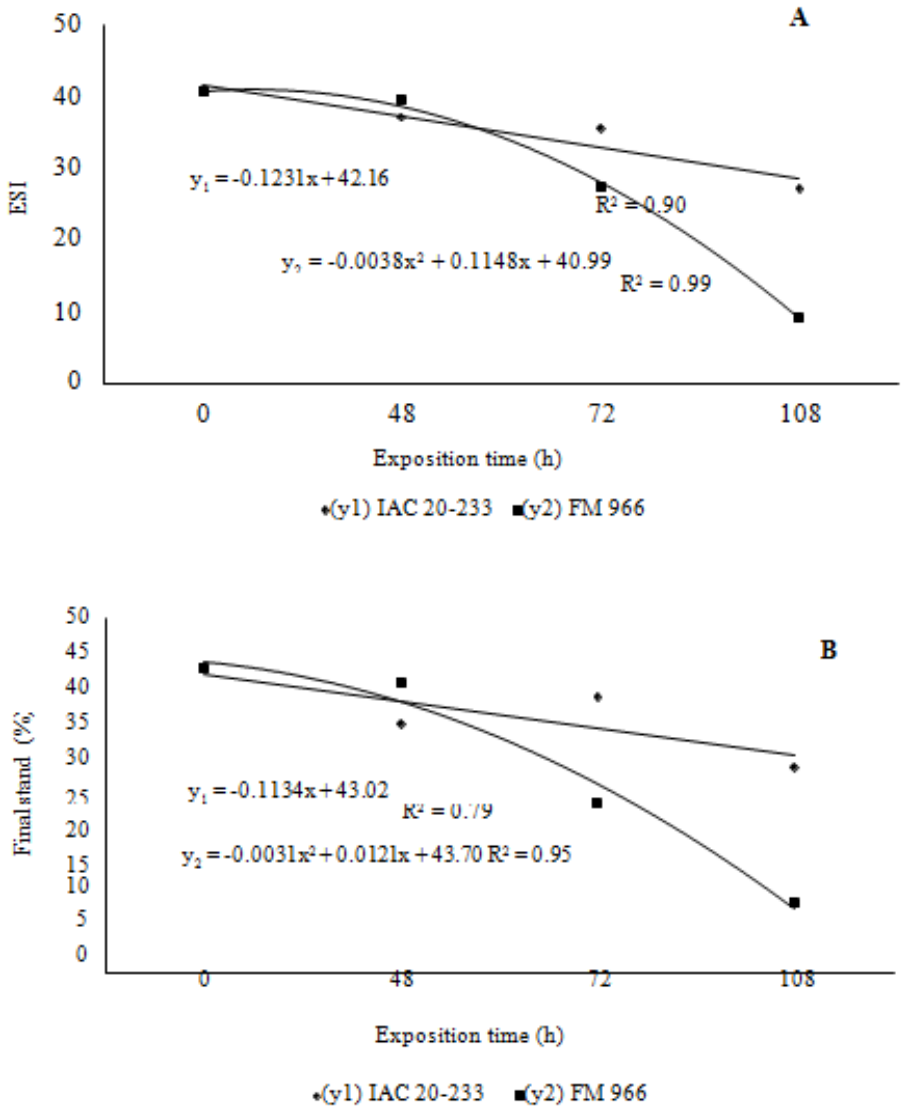

Figure 5. Emergence speed index (ESI) (A) and final stand (B) of cotton cultivars, IAC 20-233 and FM 966 related to the exposition times of seeds to the pathogen.

symptomatic cultivars that have moderate resistance (Hillocks, 1992). According to Bennett et al. (2008), low seed infection rates may influence the capacity of the pathogen to survive to acid-delinting and subsequently infect seedlings. The same authors have observed that *FOV* race 4 is able to infect the cottonseed in California field conditions confirming the pathogen seed transmission ability.

Other noticeable results were observed such as the reduction in both seed vigor, represented by the emergence speed index (ESI), as well as in the final stand (FS), according to the inoculum potential increasing for both the cultivars evaluated. As a whole, as the inoculum potential in the seeds increased the germination and vigor of it was significantly reduced and influencing the initial and final stands as well. The reduction was greater in cultivar FM 966, considered susceptible to *Fusarium* wilt and this result could be related to the health tests and germination standard (Figure 5).

Results about initial and final stands obtained here are consistent with those obtained by Sousa et al. (2008), who observed decrease in ESI and on final stand of cotton seedlings inoculated with *FOV*. In another work, studying the influence of various pathogens in cotton, among which *FOV* was tested, the variables in question were also negatively affected by the difference in osmotic potential and exposure time of seeds to the pathogens (Machado et al., 2004).

Comparing the isolates used, even though their effects on the final stand were not observed for both cultivars in the IVE for cultivar IAC 20-233, not only a difference was noticed but it was also verified that the

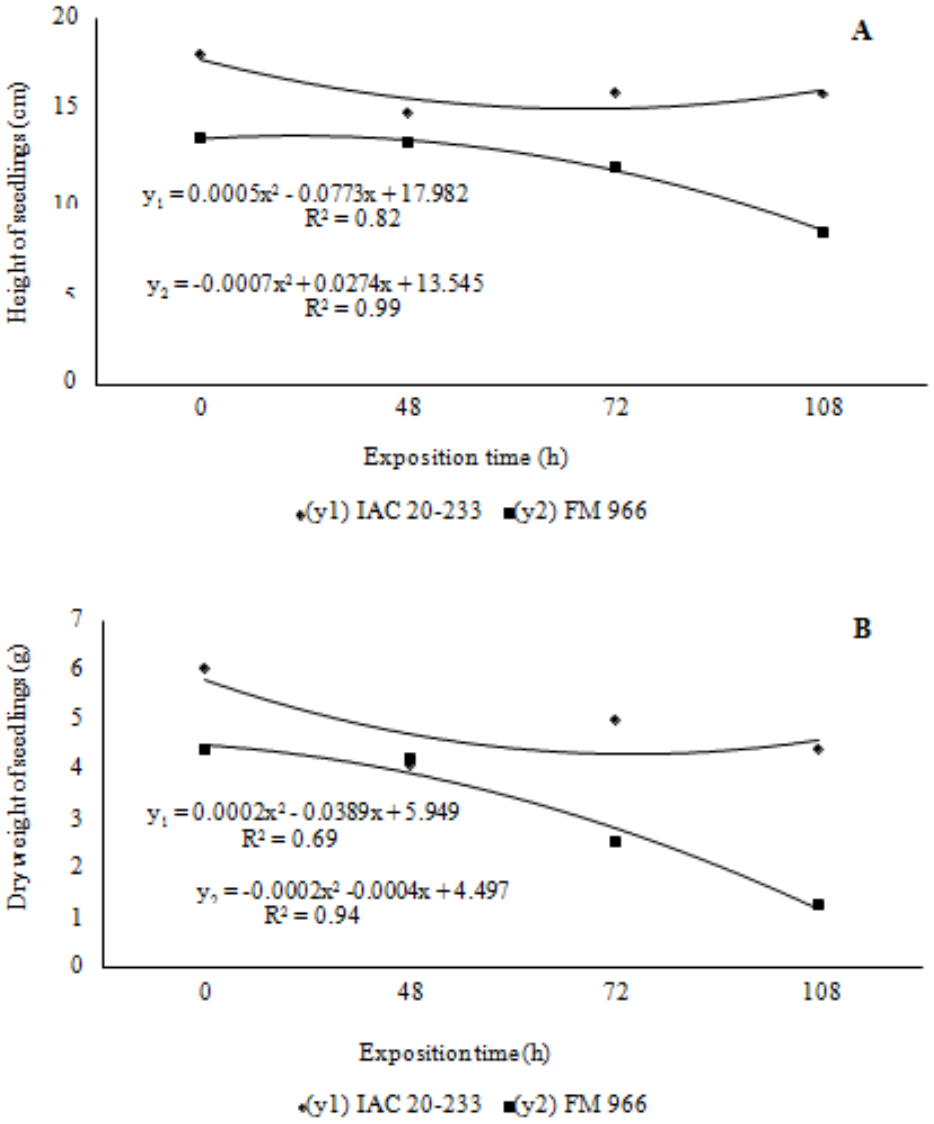

Figure 6. Height (A) and shoot dry weight (B) of seedlings of the cotton cultivars, depending on the time of exposition to the pathogen.

CML 1135 strain significantly reduced the emergence rate compared to the CML 1098 strain (Table 1).

In the same way, the inoculum potential significantly reduced the percentage of cotton seedlings, while this was increased from 36 to 108 h of seeds infected by *C. gossypii* var. *cephalosporioides* (Araújo et al., 2006). In contrast, different results were observed by Teixeira and Machado (2003), in which the effect of the priming (osmotic restriction) and exposure time increased the ESI for corn seedlings from seeds inoculated with *A. strictum*, and the highest values were observed at the points of 72 and 120 h. This stimulation at the speed of emergence was also observed by Carvalho (1999), in which the solute mannitol added to the culture medium of -1.0 Mega-Pascal (MPa) for up to 120 h, caused a priming effect, stimulating the emergence of bean seedling.

The effect of exposure time or inoculum potential on the seedling height and dry weight was gradually similar for both cultivars. However, the reduction noticed for the cultivar IAC 20-233 was lower than that observed in cultivar FM 966 (Figure 6).

Despite the amount of inoculum present in seeds, seedlings, cultivar IAC 20-233 presented more resistance, resulting in higher development and production of dry weight, even when time increased from 0 to 108 h of exposure. Likewise, the height and dry weight of maize seedlings inoculated with *A. strictum* were lower with increasing time of exposition to the pathogen (0, 24, 72 and 120 h), showing the effect of inoculation on growth of seedlings (Teixeira and Machado, 2003).

The differences between both cultivars in this work showed clearly the genetics of host resistance taking

place against the pathogen during the vegetative period. This fact may be considered with great relevance to better understanding all the events related to pathogen transmission from seeds, as well as to improve measures of diseases control on seed production fields.

Conclusion

In general, the findings of this study made it possible to see the differences between both cultivars in respect of genetics of host resistance and the inoculum potencial,where the occurrence of the pathogen were higher when inoculum potential was increased for all variables analyzed. The number of normal seedlings decreased when the incidence of the pathogen in the seed was increased. The same occurred to other variables, in which there was difference between cultivars where IAC 20-233 presented the best performance. Also, no significant differences were found between strains for emergence speed index, initial and final stands variables and the transmission and infection rates were increased according to the inoculum potentials increasing and the maximum pathogen transmission rate, from seed to plant was around 50%.

Conflict of Interests

The authors have not declared any conflict of interests.

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REFERENCES

Araújo DV, Pozza EA, Machado JC, Zambenedetti EB, Carvalho EM, Celano FAO (2006). Relação entre níveis de inóculo de Colletotrichum gossypii var. cephalosporioides nas sementes e o progresso da ramulose do algodoeiro. Fitopatol. Bras. 31:147-151.

Bennett RS, Hutmacher RB, Davis RM (2008). Seed Transmission of *Fusarium oxysporum* f. sp. vasinfectum Race 4 in California. J. Cotton Sci. 12:160-164.

BRASIL (2009). Ministério da Agricultura, Pecuária e Abastecimento. Regras para análise de sementes (Seed analysis rules) . Mapa/ACS, Brasília. P 399.

Carvalho JCB (1999). Uso da restrição hídrica na inoculação de Colletotrichum lindemuthianum em sementes de feijão (*Phaseolus vulgaris* L.). M*S*c. Tese, Universidade Federal de Lavras, Lavras.

Davis R, Colyer P, Rothrock C, Kochman J (2006). Fusarium wilt of cotton: Population diversity and implications for management. Plant Dis. 90(6):692-703.

Davis RD, Moore NY, Kochman JK (1996). Characterization of a population of Fusarium oxysporum f. sp. vasinfectum causing wilt of cotton in Australia. Aust. J. Agric. Res. 47:1143-1156.

Ferreira DF (2011). Sisvar: A computer statistical analysis system. Ciênc. Agrotecnologia 35:1039-1042.

Hillocks RJ (1992). Fusarium wilt. In: Cotton diseases. CAB International. Wallingford, UK. pp. 127-160.

Hillocks RJ, Kibani THM (2002). Factors affecting the distribution, incidence and spread of Fusarium wilt of cotton in Tanzania. Exp. Agric. 38:13-27.

Kim Y, Hutmacher R, Davis R (2005). Characterization of california isolates of *fusarium oxysporum* f. sp. vasinfectum. Plant Dis. 89(4):366-72.

Kulkarni GS (1934). Studies in the wilt disease of cotton in the Bombay Presidency. Indian J. Agric. Sci. 4:976-1045.

Leslie JF, Summerell BA (2006). The Fusarium laboratory manual 2(10). Ames, IA, USA: Blackwell Pub.

Michel BE, Radcliffe D (1995). A computer program relating solute potential to solution composition for five solutes. Agron. J. 87:131-136.

Machado JDC (1994). Padrões de tolerância de patógenos associados a sementes. Revisão Anual de Patologia de Plantas. 2:229-263.

Machado JC, Oliveira JA, Vieira MGGC, Alves MC (2004). Uso da restrição hídrica na inoculação de fungos em sementes de algodoeiro (*Gossypium hirsutum*). Rev. Bras. Sementes 26:62-67.

Mckinney HH (1923). Influence of soil temperature and moisture on infection of wheat seedings by *Helmintosporium sativum.* J. Agric. Res. 26:195-219.

Maguire JD (1962). Speed of germination-aid in selection and evaluation for seedling emergence and vigour. Crop Sci. 2:176-177.

Machado JC, Langerak CJ (1993). Improvement of a blotter method to detect economically important fungi associated with seeds of cotton. In: Ista Plant Disease Committee Symposium On Seed Health Testing. pp. 48-58.

Neergaard P (1979). Seed pathology. McMillan, London.

Smith SN, Ebbels RH, Garber H, Kappelman-Jr J (1981). Fusarium wilt of cotton. In: Nelson PE, Toussoun TA, Cook RF (Eds.). Fusarium: disease, biology and taxonomy. The Pennsylvania State University/University Park and London. pp. 29-38.

Sousa MV, Machado JC, Pfenning LH, Kawasaki VH, Araújo DV, Silva AA, Neto AM (2008). Métodos de inoculação e efeitos de *Fusarium oxysporum f. sp. vasinfectum* em sementes de algodoeiro. Trop. Plant Pathol. 33:41-48.

Teixeira H, Machado JC, Vieira MGGC (1997). Influência de *Colletotrichum gossypii South.*no desenvolvimento inicial do algodão (*Gossypium hirsutum* L.) em função da função da localização do inóculo e desinfestação das sementes. Rev. Bras. Sementes 19:9-13.

Teixeira H, Machado JC, Oride D, Alves MC, Noda A (2005). Técnica de restrição hídrica: efeito sobre *Acremonium strictum*, protrusão de sementes e obtenção de sementes de milho infectadas. Fitopatol. Bras. 30:109-114.

Teixeira H, Machado JC (2003). Transmissibilidade e efeito de *Acremonium strictum* em sementes de milho. Ciênc. Agrotecnologia 27:1045-1052.

Veigas AP (1935). A murcha do algodoeiro. Rev. Agric. 10:49-51.

Perry DA (1962). Fusarium wilt of cotton in the lake province of Tanganyika. Emp. Cotton Growing Rev. 39:22-26.

Wang B, Brubaker CL, Tate W, Woods MJ, Matheson BA, Burdon JJ (2006). Genetic variation and population structure of *Fusarium oxysporum f. sp. vasinfectum* in Australia. Plant Pathol. 55:746-755.