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RESEARCH ARTICLE / *ARTIGO*

Inoculum potential of *Fusarium verticillioides* and performance of maize seeds

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ABSTRACT

This work was conducted to evaluate the effect of *Fusarium verticillioides* on the early stage development of maize seedling/plants, considering different inoculum potentials of this fungus in seeds, under controlled conditions. To obtain seeds with different inoculum potentials of that fungus, the osmo-priming technique as referred to in literature was used. By this technique, the non-germinated seeds of the same lot, exhibiting typical structures of *F. verticillioides*, were grouped into five categories according to a grading system that was established based on the visible area of the seeds covered by the fungal structures. Seeds of each category were sowed in soil substrate and plants were grown under controlled conditions, 25ºC and 16 h light/day, for 25 days. All variables used, plant population (stands), speed of seedling emergence, and height/weight of emerged plants, were negatively affected by the increase of the inoculum intensity of the fungus initially present in the seeds. The regression equation showed high values of correlation coefficient (>0.85) for all variables, except for plant height which presented correlation coefficient of 0.77. The most severe effect of the fungus in this study occurred on the root system of the emerged plants as determined by the highest inoculum intensity of the pathogen on the planted seeds.
Key words: *Fusarium moniliforme*, *Zea mays*, mannitol, osmo-priming technique.

INTRODUCTION

The interaction between *Fusarium verticillioides* (syn. *F. moniliforme*) and maize seeds is an old and conflicting matter under discussion by plant pathologists and seed producers. To some researchers, that fungus is a weak pathogen of maize causing little damage to seed germination, and to others, it is responsible for significant losses to farmers by causing stalk and root rot in the field and ear rot after harvest (De Leon, 1984; Fernandes and Oliveira, 2000; Yates et al., 2005).

That species of *Fusarium* is also reported as part of the fungal complex producing toxins, such as fumonisin, when associated with kernels and that becomes a big concern for the quality control of maize food stuff used by humans and animals (Neergaard, 1979; Singh et al., 1991; Lazzari, 1993; Rheeder et al., 2002; Dall'Asta et al., 2012). According to literature, *F. verticillioides* is commonly found in association with seeds and kernels of maize at variable levels, mostly at high incidence (Neergaard, 1979; Richardson, 1979; Wilke et al., 2007). Under the Brazilian conditions, *F. verticillioides* is associated mostly with pre-emergence seed rot and seedling damping-off in maize (Fernandes and Oliveira, 2000), being detected in commercial seed lots at high incidences. Results of seed health analysis conducted in the Seed Pathology Laboratory of the Federal University of Lavras over the last ten years reveal that such *Fusarium* species is quite frequent and occurs at high percentages in certified seed samples of maize.

According to reports in literature *Fusarium veriticilllioides* is able to reduce seed germination of maize as well as vigor at variable levels, but no precise data are provided to support that effect. According to Machado (2000) different variables can be used to evaluate the effect of a pathogen on seed germination and vigor of which inoculum potential is quite important in addition to incidence of the organism in the seed lot. The conflicting information found in literature about the relationship between *Fusarium veriticillioides* and maize seeds may be the result of the use of seed lots presenting different incidence levels of the organism, without any indication on inoculum potential in the seeds. In the present work the objective was to evaluate the effect of *Fusarium verticillioides* on maize seed quality by using the osmo-priming technique which is the only mean to provide different inoculum potentials of a pathogen from a same seed lot as demonstrated for other pathosystems (Machado et al, 2012).

MATERIAL AND METHODS

Seed origin and selection

The selection of the seed lot was made basing on the results of seed health and standard germination tests

(Neergaard, 1979; ISTA, 1996; Machado et al., 2002) run at the Seed Pathology Laboratory of the Federal University of Lavras. From those analysis, seeds of maize, Hybrid 30 F80, presenting a germination percentage of 97% and an incidence of 60.5% of *Fusarium verticillioides*, were selected. That seed lot presented also *Penicillium oxalicum* at incidence of 1%.

Procedure to obtain seeds with different inoculum potentials of *Fusarium verticillioides*

Following previous reports (Carvalho, 1999; Machado et al., 2012), the selected seeds naturally contaminated with *F. verticillioides* were submitted to standard blotter method modified by the addition of mannitol to the water used to moisten the filter paper pad substrate. The concentration of mannitol (water restrictor) was based on the software SPPM (Michel and Radcliffe, 1995) so as to provide a solution with osmotic potential of –1.2 MPa.

Seeds were evenly distributed on wet blotters in 15-cm-diameter Petri dishes (30 seeds/dish) and kept in BOD incubator at 20 ± 2ºC with photoperiod of 12 hours for 6 days. One hundred dishes were used in that phase meaning three thousand seeds plated out.

Non-germinated seeds with typical frutifications of *F. verticillioides* were grouped into five categories: 1- seeds with no visible fungal mycelium; 2- mycelium covering up to 25% of the seed surface; 3- mycelium on 25 to 50% of the seed; 4- mycelium covering 50-75% of the seeds; and 5- mycelium present on over 75% of the seed surface (Figure 1).

Seed sowing and seedling/plant performance evaluation

One hundred seeds of each category (inoculum intensity) were sown in plastic trays (45 × 38 × 12 cm) containing soil substrate composed by an autoclaved mixture of soil, sand, and manure in the proportion of 1:1:1. Twenty five seeds were evenly sowed per tray, all trays being kept in a growth room at 25ºC under 16 h light/8 h dark for 25 days, with daily controlled irrigation during the experiment period.

The effects of *F. verticillioides* on the performance of maize seeds were evaluated by making records of the following variables: 1- plant population (stands) at 15 and 25 days after sowing; 2- speed of emergence index according to Maguire (1962); 3- plant height on the 15th day, and fresh and dry weights of shoot and root systems of plants on the 25th day after sowing. Dry weights were determined by using a circulating air incubator at 40ºC for 72 h.

Experimental design and statistical analysis

The experiment was set up in a completely randomized design with four replicates, each replicate represented by one seeding tray with 25 seeds. Data were submitted to regression analysis to determine correlations

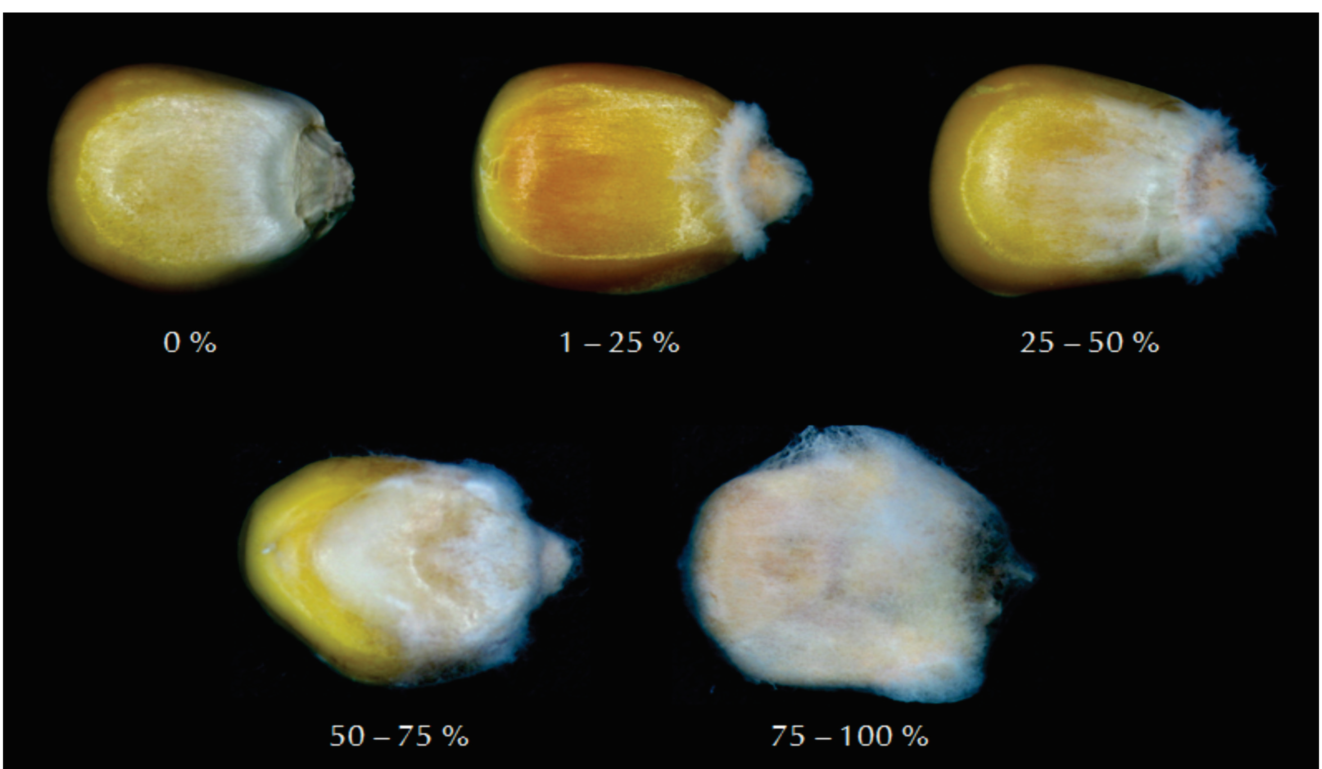

FIGURE 1 - Grading scale established for *Fusarium verticillioides* inoculum potential, ranging from one to five, based on the percentage area of maize seeds covered by the fungal structures as seen in stereo-microscope.

between classes of inoculum intensity (potential) and all variables considered in this trial.

RESULTS

The results shown in Figures 2 and 3 provide evidence that *Fusarium verticillioides* may be quite damaging to maize at the initial stage of the crop development depending on its inoculum potential present in the seeds. Plant population (stands) declined gradually in the proportion of the inoculum potential evaluated at 15 and 25 days after sowing (Figure 2); a reduction of about 60% in such variable occurred when inoculum intensity of the fungus was higher than 75% according to the grading system used in this work. For both features initial and final stands, the correlation coefficients (R^2) were high, then explaining the exclusive effect of that pathogen on seed and seedling performance of maize under the circumstances of this experiment.

For the other variables like emergence speed index, and plant heights and weights (Figures 2 and 3), the effects

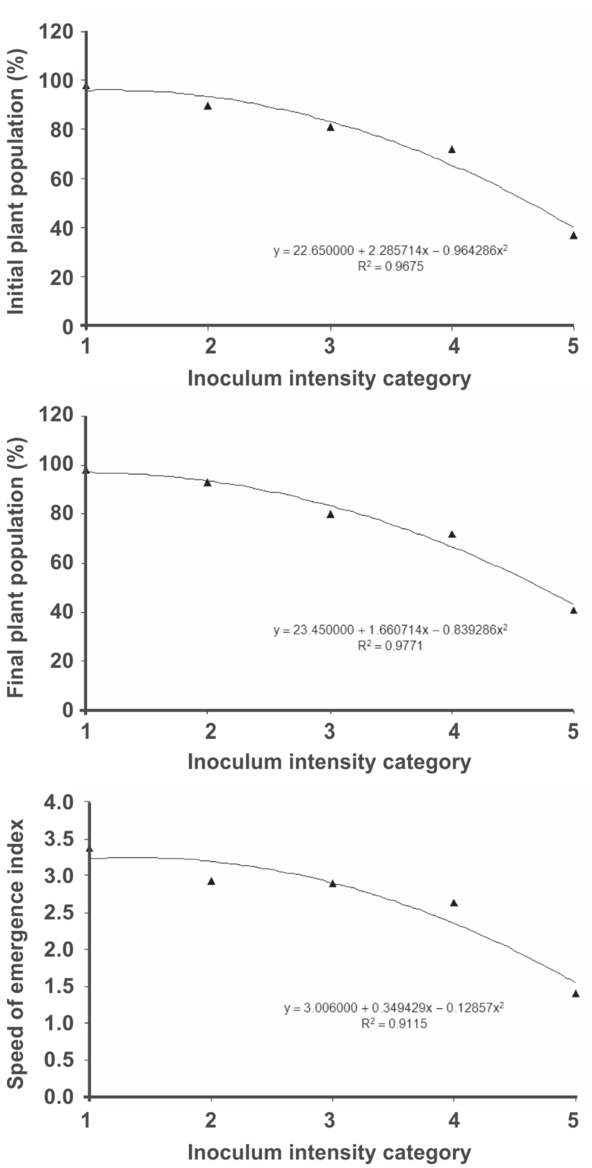

FIGURE 2 - Correlation between plant stands and speed of emergence index and inoculum intensity (potential) of *Fusarium verticillioides* in maize seeds. Inoculum intensity categories as defined by the percentage area of the seeds covered by the fungal structures.

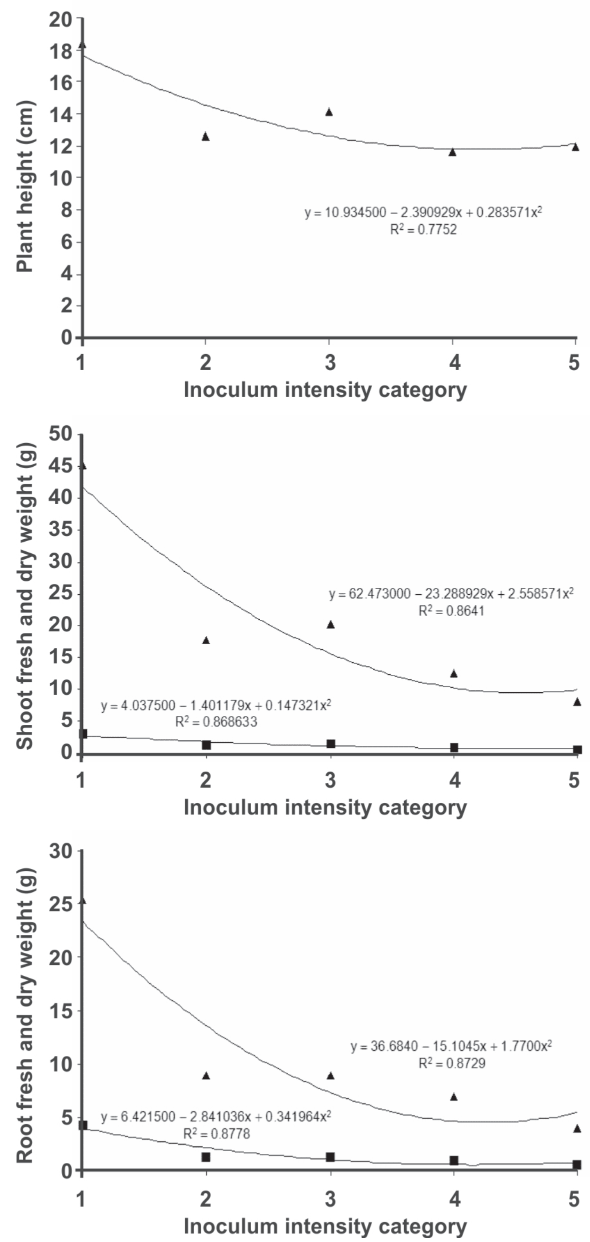

FIGURE 3 - Correlation between plant height and fresh (▲) and dry (■) weights of shoot and root systems and inoculum intensity (potential) of *Fusarium verticillioides* in maize seeds. Inoculum intensity categories as defined by the percentage area of the seeds covered by the fungal structures.

of *F. verticillioides* on maize performance were similar as recorded for plant stands, with little variation between averages. Regarding the effects of that fungus on the root system, it was clear that the damages were also quite high with the increased inoculum potential in the seeds. The dry weight in this case was reduced to almost nil when seeds, used for planting, were covered by the fungal mycelium at proportions higher than 75% (Figure 3). It is also timely to register that, in any case, necrotic symptoms on the roots caused by *F. verticillioides* were seen. Except for the emergence speed index, the effects of *F. verticillioides* on the initial development of plants of maize for all variables considered were inversely proportional to the initial inoculum intensity (potential) in the seeds.

DISCUSSION

The seed lot used in this study exhibited initially a high incidence of *F. verticillioides* and a high percentage of germination in the laboratory. To the Brazilian quality standards that seed lot can be commercialized for planting by growers. For that fungus no health standard has been settled in the certification programs and then no restriction exists about the occurrence of *F. verticillioides* in seed lots. In general, a high proportion of seed lots in Brazil presents variable levels of such fungal species. Under normal weather conditions, crop damage caused by that organism is hardly observed, neither on seed germination nor on the developing crop in the field. However, under stress conditions, in which high and prolonged relative humidity occur in some seasons damages caused by that species can be expressive, as part of a complex of other organisms causing seed deterioration (Williams et al., 2006; Wilke et al., 2007). Information on the single effects of *F. verticillioides* on seed performance and on the crop is hardly found in literature, except in cases mycotoxins are concerned (Williams et al., 2006).

From these results, it becomes clear that the presence of *F. verticillioides* in seed lots of maize may affect the performance of the emerged plants at variable intensity depending on the proportion of infected seeds with high inoculum potential. The highest inoculum potential in seeds, the most severe are the effects of the fungus on the development of the seeds and seedlings/plants.

In this work the osmo-priming technique used to screen seeds with different inoculum potentials of *F. verticillioides* proved to be useful and reliable as it has been demonstrated for other pathosystems (Machado et al ,2012) . It is worth to register that other techniques, as discussed by Machado and Carvalho (1975), Colhoun (1983), Cunfer (1983), Machado et al. (2002, 2012) in studies focused on the relationship between seed infection and disease development in the field, do not take into consideration the position and inoculum potentials of the pathogens in seeds. They consider only the levels of incidence (percentage) by taking different seed lots. In that case, several sources of variation, such as fungal races, origin of the lot, genetic nature of the host, may lead to different interpretation of the results. In addition, the inoculation of seeds by the soaking process, in which seeds are imbibed in conidial suspensions or by dry mixing seeds with inoculum, deals with the contamination process rather than infection (Machado and Langerak, 1993; Silva et al., 1999; Salustiano et al., 2005; Yates et al., 2005; Williams et al., 2006). It has been also demonstrated that seeds of some species submitted to soaking treatment may be physically injured.

The results of this work provide therefore an overview of the risk run by farmers on using maize seed lots with high proportion of seeds with high inoculum potential of *Fusarium verticillioides.*. Under stress conditions that fungus may cause both quantitative and qualitative losses at large scale.

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