Effects of *Stenocarpella maydis* in seeds and in the initial development of corn[1]

Carolina da Silva Siqueira[2*], Ellen Noly Barrocas[3], José da Cruz Machado[2], Ursula Abreu da Silva[2], Iara Eleutéria Dias[2]

ABSTRACT – The association of the fungus *Stenocarpella maydis* with corn seed may cause a reduction of seed germination and vigor of the emerged seedlings. This work was carried out in order to evaluate the effects of *S. maydis* on corn seed quality as well as on its early development. To evaluate such effects, seeds of cultivars RB9308YG (C1) and RB9108 (C2) were inoculated by the osmotic conditioner technique with two *S. maydis* isolates for 24(P1), 48(P2), 72(P3) and 96 hours (P4). Plants were grown in a room chamber at 20 °C and 25 °C and daily assessed until 28 days after emergence. Seed germination, incidence of *S maydis*, electrical conductivity, speed of emergence index (SEI), initial and final seedling population and dry weight of emerged plants, were assessed. The longer the exposition times of the seeds to the fungal colony, the more severe negative effects of the pathogens on seed vigor were observed. *S. maydis* caused reduced seed vigor in the speed of seedling emergence in the final stand and early development of corn seedlings.

Index terms: seed pathology, seed quality, ear and stalk rot, vigor test.

Efeitos de *Stenocarpella maydis* em sementes e na fase inicial de desenvolvimento do milho

RESUMO – A associação do fungo *Stenocarpella maydis* com sementes de milho pode comprometer a germinação de sementes e o vigor das plântulas. Este trabalho foi realizado com o objetivo de avaliar os efeitos causados por *S. maydis* sobre a qualidade das sementes de milho bem como no seu desenvolvimento inicial. Para avaliar tais efeitos, sementes de milho cultivares RB9308YG (C1) e RB9108 (C2), foram inoculadas com dois isolados de *S. maydis*, pelo método de condicionamento osmótico por 24(P1), 48(P2), 72(P3) e 96 horas (P4). O cultivo foi realizado em câmara de crescimento vegetal, sob temperaturas de 20 °C e 25 °C e avaliadas diariamente até 28 dias após a emergência. Avaliou-se a germinação, incidência do fungo, condutividade elétrica, índice de velocidade de emergência (IVE), estande inicial e final e peso de matéria seca. Foi observado que, quanto maior o tempo de exposição das sementes à colônia fúngica, maiores foram os efeitos negativos destes organismos em sementes e plântulas. *S. maydis* causou redução no vigor das sementes, na velocidade de emergência das plântulas, no estande final e no desenvolvimento inicial das plântulas de milho.

Termos para indexação: patologia de sementes, qualidade fisiológica de sementes, podridão do colmo e da espiga, testes de vigor.

Introduction

Economically important crops such as corn, achieve high yields by means of the optimization of yield factors such as soil fertility, water availability, erosion control, planting date, cultivar, plant population, crop rotation and pest and diseases management, among others.

The disease caused by the fungus *Stenocarpella maydis* (Berk.) Sutton can kill the embryo of corn seeds, affect germination and seedling vigor, besides causing rot in the stem and in the ears. The stem rot interferes with normal development of the plant, affecting its functions, causing breakage of the stem base, lodging and, consequently, premature plant death. The rot of ears can cause reduction in yield and quality of harvested grains. It is also known that this fungus produces toxin, which affects the economic and nutritional value of the product (Krout-Greenberg et al., 2013; Snyman et al., 2011; Casa et al., 2006).

The use of infected corn seeds can cause the introduction of *S. maydis* in new growing areas and is an important source

[1]Submitted on 08/10/2013. Accepted for publication on 02/11/2014.
[2]Departamento de Fitopatologia, UFLA, Caixa Postal 3037, 37200-000 – Lavras, MG, Brasil.
[3]Department of Plant and Environmental Sciences, 2360. Taastrup, Denmark.
*Corresponding author <kerolpet@gmail.com>

of primary inoculum, besides being one of the main vehicles for the spread of this pathogen, even far from its place of production (Casa et al., 2006). Due to the impacts which it can cause when combined with corn seeds, it was framed in the list of propositions as Regulated Non-Quarantine Pest (RNQP) in Brazil, with a tolerance level of 2% proposed by the Ministry of Agriculture (MAPA), for all classes of certified seeds (Administrative Regulation No. 47 of February 19, 2009).

The objective of this study was to assess the effects caused by the fungus *S. maydis* in association with corn seeds on its performance and the initial stage of corn cultivation.

Materials and Methods

Origin, multiplication of isolates of S. maydis and profile of seeds used

Two isolates of *S. maydis* were used (CML698 and MY2), obtained from the Mycological Collection of Lavras (Universidade Federal de Lavras – UFLA) and from Empresa Brasileira de Pesquisa Agropecuária em Milho e Sorgo (Brazilian Agricultural Research in Maize and Sorghum), respectively. All isolates were initially cultured on PDA culture medium (20 g of agar, 20 g of dextrose and 200 g of potatoes / liter) in Petri dishes and kept in BOD chamber at 25 ± 2 °C and a photoperiod of 12 hours. Fragments of the PDA medium containing mycelium with 5 days of growth were deposited on the leaves of corn seedlings with 10 days. From the lesions formed, the isolates were reisolated to confirm their identity and pathogenicity and kept in PDA in the conditions mentioned above. Cultivar seeds RB9308YG (C1) were used, susceptible to *S. maydis* and RB9108 (C2), moderately resistant to *S. maydis*, with germination of 98% and 96%, respectively, and average incidences of 26.5% of *Fusarium verticillioides* and of 12% of *Penicillium* sp. in both lots, assessed according to Brasil (2009a, 2009b).

Inoculation of the seeds

For obtaining the corn seeds with different inoculum potential, osmotic conditioning methodology was used, according to Machado et al. (2012). The procedure consisted of initial seed disinfestation using 1% sodium hypochlorite for 1 minute followed by rapid washing in autoclaved water and dried in a laboratory environment. Colonies of fungi with five days of age were obtained from Petri dishes containing PDA culture medium, plus solute mannitol, with water potential adjusted at -1.4 MPa (Michel and Radcliffe, 1995). On fungal colonies were distributed the seeds in single layers, always in the same position. The Petri dishes were kept in BOD chamber at 25 ± 2 °C and a photoperiod of 12 hours. The seeds were exposed to the fungal colony for 24, 48, 72 and 96 hours, here called treatments P1, P2, P3 and P4, respectively. After each period of exposure the seeds were removed from the Petri dishes and dried in a laminar flow chamber for 24 hours.

To assess the effects of *S. maydis* in the performance of the seeds the following tests were conducted:

Germination: was conducted with four replications of 50 seeds distributed on paper towel moistened with distilled water in an amount equal to 2.5 times the weight of the dry paper and placed in germination at 25 ± 2 °C. Evaluations were performed at four and seven days after sowing, according to criteria established by the Rules for Seed Testing (Brasil, 2009a). Results were expressed as percentage of normal seedlings.

Cold test: the methodology described by Miguel et al. (2001) was used for cold test on roll paper with soil. Four replications of 50 seeds of each lot were distributed on two sheets of paper towel and covered with a thin layer of soil (about 1 cm) from the area cultivated with corn; then the whole was covered with a third sheet and coiled. The paper sheets were previously wet with an amount of water equivalent to three times their dry weight. The rolls were placed in plastic sealed boxes and then kept for three, five and seven days, at 10 °C. After the respective periods, the rolls were transferred to germination chamber, at 25 °C, for three days. Then the counts of normal seedlings were made. Results were expressed as mean percentage by lot.

Seed Health test: seeds were sowed on distributed on paper substrate moistened in OA medium (20 g of agar and 30 g Oatmeal. L^{-1}), which is a medium favoring the formation of pycnidia (Silva and Juliatti, 2005), in Petri dishes with 15 cm diameter, with 8 replications of 25 seeds per plate. Then, the seeds were placed in a "freezer", at -20 °C for 24 hours and subsequently incubated for 15 days in a growth chamber at a temperature of 20 ± 2 °C and photoperiod of 12 hours. After this period, the *S. maydi's* incidence was checked by stereoscopic microscope.

Electrical conductivity (EC): four replications of 50 seeds were used, previously weighed (accuracy of 0.0001 g), immersed in 75 mL of distilled water for 24 hours in BOD chamber at 25 ºC. The electrical conductivity of the immersion solution was determined by conductivity meter, DIGIMED, model 21. The results were expressed in $\mu S. cm^{-1}. g^{-1}$ of seeds (Krzyzanowski et al., 1999).

The experimental designs used for the three tests described above were completely randomized (DIC), in a triple factorial design 2 x 2 x 4 (2 isolates, 2 cultivars and 4 times of exposure to the pathogen).

To assess the effect of *S. maydis* in the initial stage of

cultivation of corn, the following tests were used:

Speed of emergence index (SEI): one hundred seeds of each cultivar of corn (C1 and C2) and for each isolate of *S. maydis* were individually distributed in plastic cups of 200 mL, containing commercial substrate (Multiplanta trop®), arranged in a number of 25 per tray, each tray corresponding to a replication. The experiment was conducted in two chambers with temperatures adjusted in 20 ± 2 °C and 25 ± 2 °C and photoperiod of 12 hours light (daylight NSK T10 40W 6500K FL40T10-6 60Hz)/12 hours dark. Daily counts of emergence of the plants were carried from the first seedling emergence until stabilization of the stand for three consecutive days. The issue of the primary leaves was considered as a reference for corn seedling emerged. The seedlings were kept in a growth chamber for 28 days after sowing (d.a.s.). The speed of emergence index (SEI) was calculated according to Maguire (1962).

Initial stand and final stand: the stands were recorded at five and 28 days after sowing, respectively, and the absolute value was converted into percentage.

Plant dry weight: emerged plants at 28 days were cut in the base stem region, taken to circulating air oven at 70 °C / 7 days and subsequently weighed. The results were expressed in $g.plant^{-1}$.

For the evaluation of the speed of emergence index (SEI), initial stand, final stand and dry weight, the experimental design in randomized block (RBD) was used, in a triple factorial design 2 x 2 x 4 (2 temperatures, 2 cultivars and 4 days of exposure to the pathogen) with four replication per treatment. In the evaluations described above, each isolate was used as reference for the analysis of other parameters. Statistical analyzes were performed with the aid of the software Sisvar® version 5.3 (Ferreira, 2011). All analyzes in this study were conducted in the triple factorial design. Means between treatments were compared by regression by Tukey test or Student t test ($P \leq 0.05$).

Results and Discussion

Analyses of variance of the effects caused by *S. maydis* in seed performance assessed by germination tests, cold test, healthy test and electrical conductivity tests for both cultivars and isolates generated no significant triple interaction ($p \leq 0.05$). For EVI, initial and final stands and dry weight under the same conditions, the analysis of variance revealed no significant triple interaction ($p \leq 0.05$).

Effect of incidence of S. maydis in the physiological quality of corn seed

According to the results observed in germinated normal seedlings in the germination test (Figure 1A) and cold test (Figure 1B), it was observed that there was little variation between isolates combinations / cultivars assessed in both assays. The highest percentage of germination occurred at the lower inoculum potential in seeds of cultivar C2, which were inoculated with isolate MY2 and the lowest percentage for all combinations was observed at the higher inoculum potential that showed death or malformation of all seeds, in some cases. For all isolate / cultivar combinations, there was a decrease in seedling percentage when there was an increase in the inoculum potential of the seeds, which was observed in both tests. These results agree with the ones from Boaro et al. (2013) and Costa et al. (2003), who, although they had assessed the pathosystems *Sclerotinia sclerotiorum* and *Fusarium oxysporum* f. sp. *phaseoli* in bean seeds, respectively, also observed a decrease in germination with increasing inoculum potential.

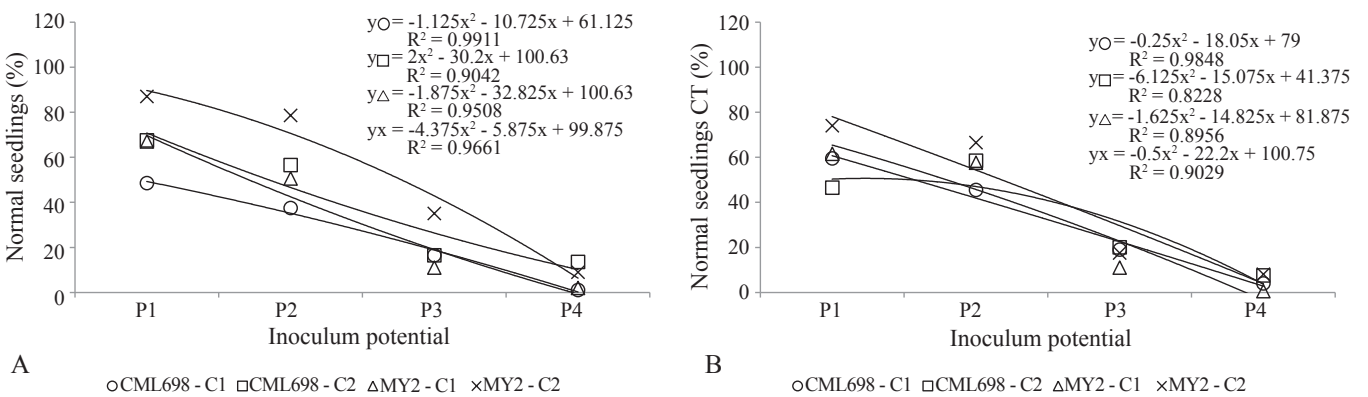

Figure 1. Percentages of normal seedlings obtained by germination test (A) and cold test (B) for seeds of cultivars C1 (RB9308YG) and C2 (RB9108) inoculated with isolates of *S. maydis* – CML698 and MY2 in the inoculum potentials, P1 (24 h), P2 (48 h), P3 (72 h), P4 (96 h).

One of the most commonly used tests to assess the effect of corn seeds has been the cold test, since it favors the development of organisms harmful to germination provided by the combination of low temperature and high humidity of the substrate (Coimbra et al., 2009). Thus, it is possible to differentiate seed lots that are more tolerant of these conditions and, therefore, more vigorous. Based on the results of this test, it was revealed that *S. maydis* fungus caused reduction in seed performance, because, as there was an increase in the inoculum potential, there was a decrease in normal seedlings in all isolates / cultivars combinations (Figures 1A and 1B) and consequent loss of vigor.

The proportional increase in the percentage of infected cells in relation to seed inoculum potential can be realized by assessing the incidence of the pathogen on inoculated seeds (Figure 2). This correlation was evident in all isolate / cultivar combinations. For isolate CML698 minimal average incidences occurred of 57.5% for P1, 60% for P2, 71.5% for P3 and 73.5% for P4. For isolate MY2 were observed the incidences of 55.5%, 68.5%, 57.5% and 79%, respectively for the same potentials. The incidence observed for this species is a reflection of the fact that *S. maydis* is the pathogen with high infective and destructive capacity in corn seeds.

Among the several purposes of the technique of osmotic conditioning, well discussed by Machado et al. (2012), stands out for the possibility of genetic materials assessment for resistance to a particular pathogen, since such a method allows the fungal colony contact with the seeds for different times without the radicle protrusion. In this study, the small difference between the cultivars in relation to the incidence of *S. maydis*, observed in the healthy test, showed that despite the commercial corn hybrids being rated as to their resistance to stalk and ear rot (Casa et al., 2006; Denti et al., 2002), in practice little difference was observed between the strength of the materials considered susceptible (C1) and moderately resistant (C2), which in fact, according to Tembo et al. (2013), is still a challenge for breeders. Based on the results of the assessment of incidence, it was also possible to infer that 24 hours of exposure to fungal colony would be enough for 50% of the seeds are infected, which can be useful not only for assessment of materials, but also to assess seed treatment, among other purposes.

The results observed in the electrical conductivity tests, also an indicator of vigor, demonstrated the impairment of the structures of the seeds when exposed to *S. maydis* (Figure 3). With the increase in seed inoculum potential, there is the loss of integrity of the cell membranes system and the reduction of the respiratory and biosynthetic activities, being these some of the early events in the process of deterioration. The high values observed in P4 reflect the negative effect caused by the fungus in the seed membrane system, which allowed greater electrolyte leaching. This fact was also observed from the first inoculum potential, but less intense. Moreover, the decrease in germination and vigor is directly proportional to the increased release of solutes, indicating that the evaluation of the conductivity by the weight method was also efficient for determine the vigor (Oliveira et al., 2012).

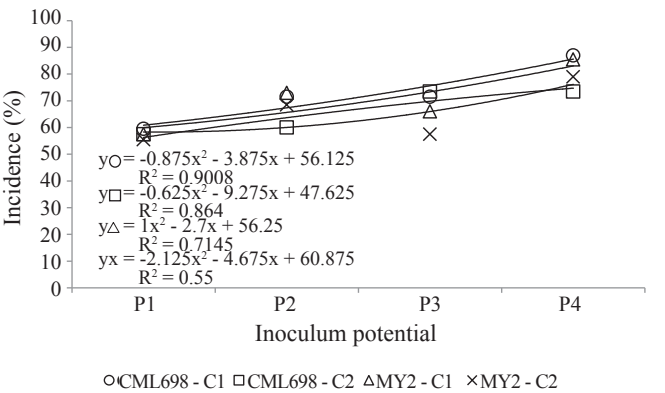

Figure 2. Incidence of *S. maydis* (isolates CML698 and MY2) in corn seeds of the cultivars C1 (RB9308YG) and C2 (RB9108) in the inoculum potentials P1 (24 h), P2 (48 h), P3 (72 h), P4 (96 h).

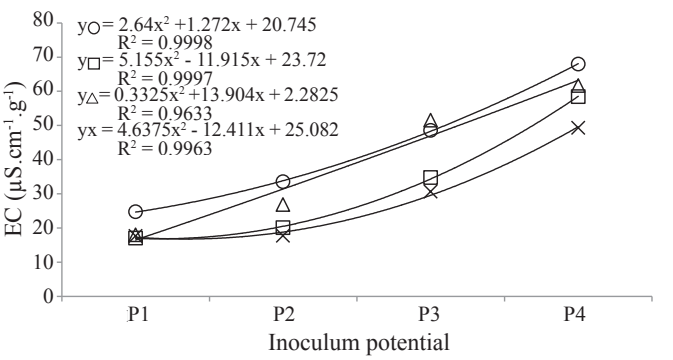

Figure 3. Average values of electrical conductivity obtained from corns seeds of cultivars C1 (RB9308YG) and C2 (RB9108), inoculated with *S. maydis* (isolates CML698 and MY2) at different inoculum potentials (P1-24 h, P2-48 h, P3-72 h, P4-96 h).

Effects of S. maydis in initial performance of corn seedlings

In assessing the speed of emergence index (Figure 4), initial stand (Figures 5A and B), and final stand (Figures 5C and D) and dry weight (Figures 6A and B), there was a direct correlation among results obtained with the inoculum

potential in seeds, where minor damage to the plants was observed at the lowest potentials, P1 and P2, and the opposite was true at the higher potential, P3 and P4. However, among isolates, cultivars and temperatures, differences were observed. The speed of emergence index decreased as it increased the inoculum potential in each treatment with the isolates of *S. maydis*. The highest rates observed were of 2.26 in combination C1/CML698/P1/25 °C, and of 3.58 in combination C1/MY2/P1/25 °C. The lowest rates were 0 for combination C1/CML698/P4/20 °C and 0.04 for combination C1/MY2/P4/20 °C. Although this study has shown that lower temperatures impaired more the seed vigor of corn infected with *S. maydis*, regardless of cultivar or isolate, it is important to consider other ideal temperature ranges for the development of *S. maydis*. According to the literature, 23 and 28 °C are ideal for faster growth of mycelium, 28 and 33 °C are ideal for spore germination (Casa et al., 2007) and 28 °C ideal to produce pycnidia (Kuhnem et al., 2012).

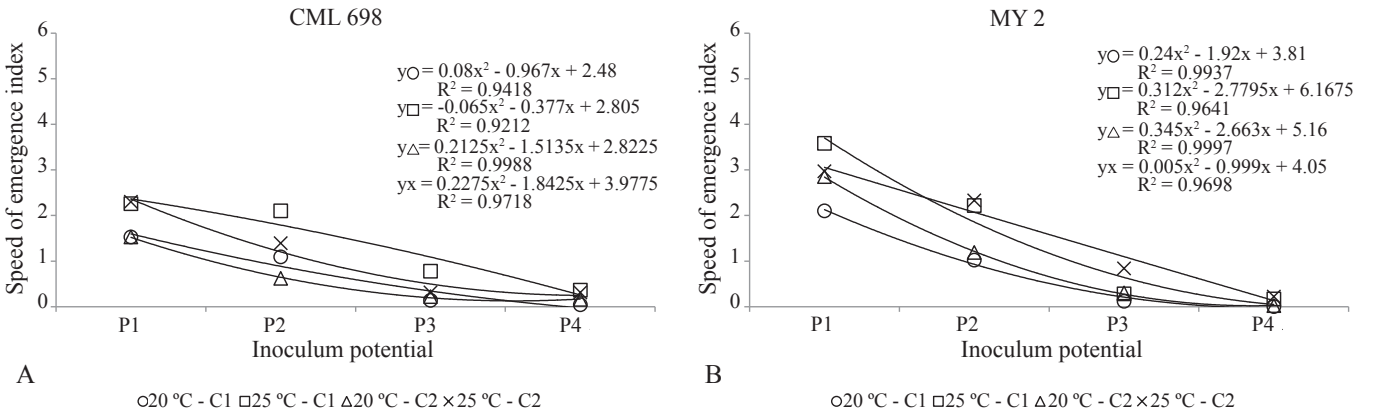

Figure 4. Speed of emergence index of corn plants, cultivars C1 (RB9308YG) and C2 (RB9108) from seeds inoculated with isolates of *Stenocarpella maydis* (A-CML698 and B-MY2) in the inoculum potentials P1 (24 h), P2 (48 h), P3 (72 h), P4 (96 h) cultivated at 20 °C and 25 °C.

Seeds with low SEI, when taken to the field, may have slower emergence, allowing the action of other factors and impairment of stand establishment. Therefore, such seeds are considered less vigorous than those with high levels of SEI (Matos et al., 2013).

According to the results of the initial stand (Figures 5A and B), cultivars / temperature combinations had the same tendency, considering each isolate assessed. However, statistical differences were observed between the potentials of inoculum for each isolate. In seeds inoculated with CML698 and MY2, in the lower potential (P1) in cultivar C1, kept in a temperature of 20 °C, the highest emergence was found (40% to 50%), having occurred, in the higher potential (P4), the lowest emergences (0 to 1%).

It was observed that the final stand (Figures 5 C and D) followed a similar pattern to the initial stand, as to the different effects caused by the inoculum potentials. By results obtained with the isolates of *S. maydis,* it is possible to notice a difference between CML698 and MY2 for potential P1 and cultivar C2, under a temperature of 20 °C, being 46% and 81%, respectively. However, the lowest values of final stand were similar among isolates, ranging 0-1%, in the potential P4, cultivar C1, grown at 20 °C.

In similar studies with other pathosystems, decreases in the SEI and in the initial and final plant stands were observed. In the three variables occurred inversely proportional results to the inoculum potentials imposed by the different contact times of the seeds with the pathogens (Araújo et al., 2006; Botelho et al., 2013; Moraes and Menten, 2006; Sartori et al., 2004).

According to information from the literature, the association fungi and seeds can reduce germination, plant population and can causes epidemic (Solorzano and Malvick, 2011; Machado, 2000).

When assessing the effect of inoculum potentials in the dry matter of inoculated plants from seeds, the results also followed the same trend as the other variables considered (Figure 6). In the treatments with lower potentials, plants of cultivar C2 were observed with at most 16 g of dry matter. The lower results were noticed for cultivar C1 in potential P4, recording plants with 0.16 g of dry matter on average.

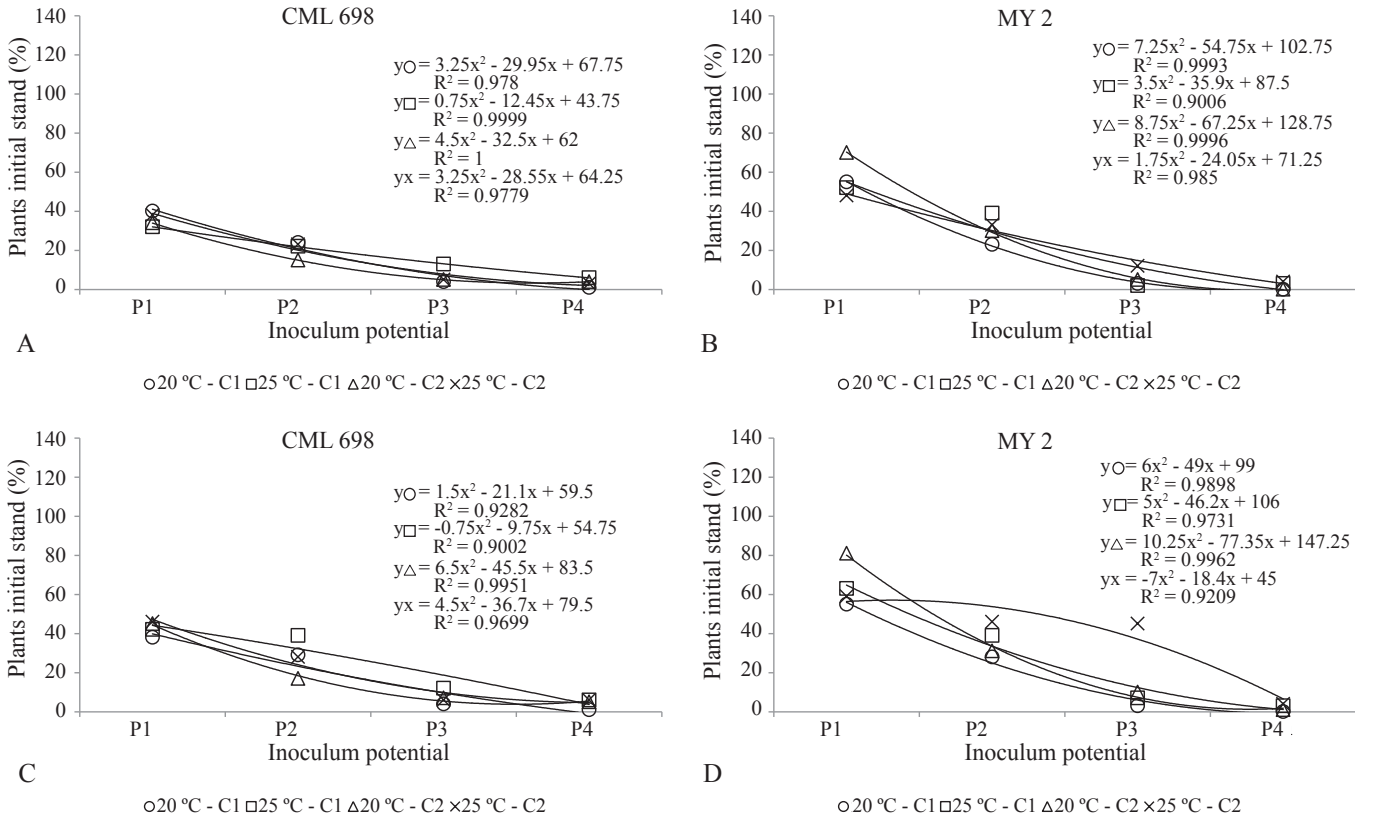

Figure 5. Initial stand (A and B) and final stand (C and D) of corn plants from seeds inoculated with the isolates of *Stenocarpella maydis*, CML698 (A) and MY2(B), with inoculum potentials P1(24 h), P2(48 h), P3(72 h) and P4(96), in cultivars C1 (RB9308YG) and C2 (RB9108) cultivated at 20 °C and 25 °C.

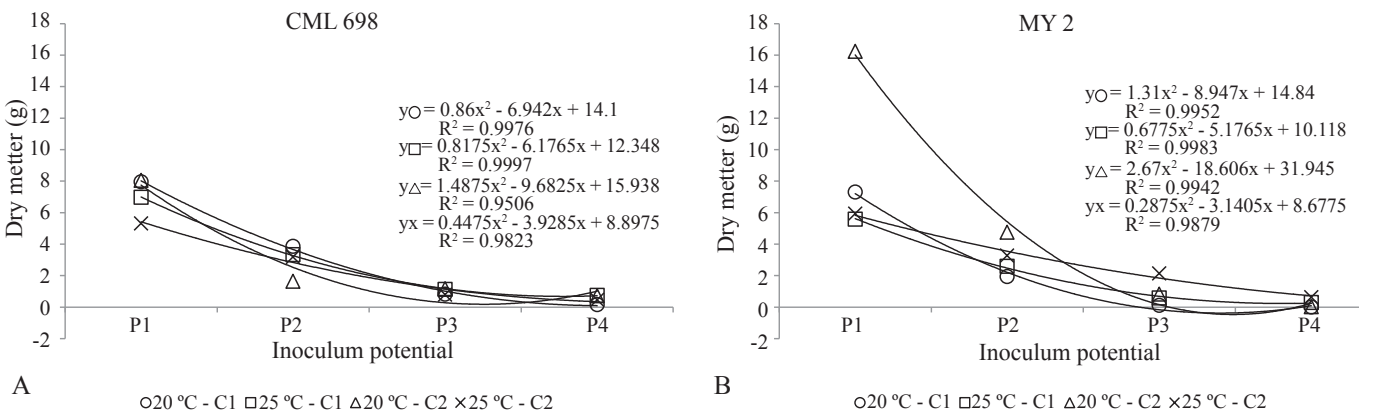

Figure 6. Dry weight (A and B) of corn plants from seeds inoculated with the isolates of *Stenocarpella maydis* CML698 (A) and MY2(B) with the inoculum potentials P1(24 h), P2(48 h), P3(72 h) and P4(96 h), in cultivars C1 (RB9308YG) and C2 (RB9108) cultivated at 20 °C and 25 °C.

It is known that *S. maydis* fungus is responsible for field losses caused by decay of the stem, death of seeds / seedlings and can produce toxins harmful to human and animal consumption (Odriozola et al., 2005). By the results obtained in this study, it was also evident that the association of this pathogen with corn seeds can impair the quality of seeds and also the early development of seedlings from seeds infected by the fungus, with increasing and proportional effects to the values of potential of initial inoculum in seeds (Barrocas et al., 2012; Casa et al., 2006). It was possible to confirm the significant

harmful effects caused by this pathogen on corn seeds. These effects are directly related to the level of infection, triggering damage to the infected seeds, as observed in the treatments with potentials P3 and P4. But it is important to consider that isolate, genotypes and environmental conditions such as light and temperature can influence all variables studied (Kuhnem et al., 2012; Casa et al., 2007). According to Araújo et al. (2006), the inoculum potential should not be used as one factor to determine the healthy quality of the seed and one should take into consideration factors related to the environment and the intrinsic factors to biology of host and pathogen.

Conclusions

The presence of *S. maydis* in corn seeds under different inoculum potentials negatively and progressively influenced the seeds germination and integrity of their membranes.

The incidence of *S. maydis* in the seeds increased gradually with increasing the inoculum potential.

S. maydis caused reduction in seed vigour speed of emergence, final stand and in early development of the corn seedlings.

Acknowledgments

To CNPq (National Council for Scientific and Technological Development), FAPEMIG (Foundation for Research Support of the Brazilian State of Minas Gerais) and CAPES (Coordination for the Improvement of High Level) for funding this work.

References

ARAÚJO, D. V.; POZZA, E. A.; MACHADO, J. C.; ZAMBENEDETTI, E. B.; CELANO, F. A. O.; CARVALHO, E. M.; CAMARGOS, V. N. Influência da temperatura e do tempo de inoculação das sementes de algodão na transmissibilidade de *Colletotrichum gossypii* var. *cephalosporioides*. *Fitopatologia Brasileira*, v.31, n. 1, p. 35-40, 2006. http://www.scielo.br/pdf/fb/v31n1/a06v31n1.pdf

BARROCAS, E. N. ; MACHADO, J.C., ALMEIDA, M. F.; BOTELHO, L. S.; VON PINHO, E.V. R. Sensibility of the PCR technique in the detection of *Stenocarpella* sp. associated with maize seeds. *Revista Brasileira de Sementes*, v.34, n.2, p. 218-224, 2012. http://www.scielo.br/pdf/rbs/v34n2/05.pdf

BOTELHO, L. S.; ZANCAN, W.L.A.; MACHADO, J. C.; BARROCAS, E.N. Performance of common bean seeds infected by the fungus *Sclerotinia sclerotiorum*. *Journal of Seed Science*. v.35, n.2, p.153-160, 2013. http://www.scielo.br/pdf/jss/v35n2/03.pdf

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. *Regras para Análise de Sementes*. Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. Brasília: MAPA/ACS. 2009 a. 395p. http://www.agricultura.gov.br/arq_editor/file/2946_regras_analise__sementes.pdf

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. *Manual de Análise Sanitária de Sementes*. Secretaria de Defesa Agropecuária. Ministério da Agricultura, Pecuária e Abastecimento Brasília: MAPA/ACS. 2009b. 200p. http://www.bs.cca.ufsc.br/publicacoes/manual_analises_sanitarias.pdf

CASA, R.T.; REIS, E.M.; ZAMBOLIM, L. MOREIRA, E.N. Efeito da temperatura e de regimes de luz no crescimento do micélio, germinação de conídios e esporulação de *Stenocarpella macrospora* e *Stenocarpella maydis*. *Fitopatologia Brasileira*, v. 32, n.2, p.137-142, 2007. http://www.scielo.br/pdf/fb/v32n2/07.pdf

CASA, R. T.; REIS, E. M.; ZAMBOLIM, L. Doenças do milho causadas por fungos do gênero *Stenocarpella*. *Fitopatologia Brasileira*, v. 31, n. 5, p. 427-439, 2006. http://www.scielo.br/pdf/fb/v31n5/01.pdf

COIMBRA, R.A.; MARTINS, C.C.; TOMAZ, C.A.; NAKAGAWA, J. Testes de vigor utilizados na avaliação da qualidade fisiológica de lotes de sementes de milho-doce (sh2). *Ciência Rural*, v.39, p.2402-2408, 2009. http://www.scielo.br/pdf/cr/v39n9/a353cr1586.pdf

COSTA, M. L. N.; MACHADO, J. C.; GUIMARÃES, R.M; POZZA, E.A; ORIDE, D. Inoculação de *Fusarium oxysporum* f.sp. *phaseoli* em sementes de feijoeiro através de restrição hídrica. *Ciência e Agrotecnologia*, Lavras, v.27, n.5, p.1023-1030, 2003. http://www.scielo.br/pdf/cagro/v27n5/a08v27n5.pdf

DENTI, E.; REIS, E. M.; FORCELINI, C. A. Reação de genótipos de milho às podridões da base do colmo. *Summa Phytopathologica***,** v.28, p.286-288, 2002. http://www.scielo.br/pdf/sp/v36n2/a03v36n2.pdf

FERREIRA, D. F. SISVAR: A computer statistical analysis system. *Ciência e Agrotecnologia*, v.35, n.6, p. 1039-1042, 2011. http://www.scielo.br/pdf/cagro/v35n6/a01v35n6.pdf

KROUT-GREENBERG, N. D.; PUSCHNER, B.; DAVIDSON, M. G.; DEPETERS, E. J. Preliminary study to assess mycotoxin concentrations in whole corn in the California feed supply. *Journal of Dairy Science*, v.96, n.4, p.2705-2712, 2013. http://download.journals.elsevierhealth.com/pdfs/journals/0022-0302/PIIS0022030213001318.pdf

KRZYZANOWSKI, F. C.; VIEIRA, R. D.; FRANCA-NETO, J. B. *Vigor de sementes*: conceitos e testes**.** Londrina: ABRATES, 1999. 218 p.

KUHNEM JÚNIOR, P.R.; CASA, R.T.; BOGO, A.; AGOSTINETO, L.;BOLZAN, J.M.; MIQUELUTI, D.J. Effects of temperature, light regime and substrates on the production and germination of *Stenocarpella maydis* pycnidiospores. *Acta Scientiarum Agronomy,* v. 34, n. 1, p. 11-16, 2012. http://www.scielo.br/pdf/asagr/v34n1/a02v34n1.pdf

MACHADO, J. C. *Tratamento de sementes no controle de doenças*. Editora UFLA, Lavras-MG, 2000. 134 p.

MACHADO, J.C.; BARROCAS, E.N.; COSTA, L.N.; GUIMARÃES, R.M; MACHADO, C. Uso da técnica de restrição hídrica ou condicionamento osmótico em patologia de sementes. *Revisão Anual de Patologia de Plantas*, v. 20, p. 37-63, 2012.

MAGUIRE, J. D. Speed of germination-aid in selection and evaluation for seedling emergence and vigour. *Crop Science*, v.2, n.2, p. 176-177, 1962.

MATOS, C.S.M.; BARROCAS, E.N.; MACHADO, J. C.; CARDOSO ALVES, F.C. Health and physiological quality of corn seeds treated with fungicides and assessed during storage. *Journal of Seed Science*, v.35, n.1, p.10-16, 2013. http://www.scielo.br/pdf/jss/v35n1/01.pdf

MICHEL, B. E.; RADCLIFFE, D. A computer program relating solute potential to solution composition for five solutes. *Agronomy Journal*, v. 87, n. 1, p. 131-136, 1995. http://www.scielo.br/scielo.php?script=sci_nlinks&ref=000161&pid=S2317-15372013000200003001 13&lng=pt

MIGUEL, M. H.; CARVALHO, M. V.; BECKERT, O. P.; MARCOS-FILHO, J. Teste de frio para avaliação do potencial fisiológico de sementes de algodão. *Scientia Agricola*, v.58, n.4, p.741-746, 2001. http://www.scielo.br/pdf/sa/v58n4/6293.pdf

MORAES, M. H. D.; MENTEN, J. O. M. Transmissão de *Alternaria* spp. através de sementes de feijão e seu efeito sobre a qualidade fisiológica das sementes. *Summa Phytopathologica*, v.32, n.4, p.381-383, 2006. http://www.scielo.br/pdf/sp/v32n4/a12v32n4.pdf

ODRIOZOLA, E.; ODEON, A.;CANTON, G.; CLEMENTE, G.; ESCANDE, A. *Diplodia maydis*: a cause of death of cattle in Argentina. *Veterinarian Journal*, v.52, p.160-161, 2005. http://www.tandfonline.com/doi/pdf/10.1080/00480169.2005.36496

OLIVEIRA, F.N.; TORRES, S.B.; VIEIRA, F.E.R.; PAIVA, E.P.; DUTRA, A.S. Qualidade fisiológica de sementes de girassol avaliadas por condutividade elétrica. *Pesquisa Agropecuaria Tropical*, v.42, n.3, p.279-287, 2012. http://www.revistas.ufg.br/index.php/pat/article/view/17793/11614

SARTORI, A.F.; REIS, E.M.; CASA, R.T. Quantificação da transmissão de *Fusarium moniliforme* de sementes para plântulas de milho. *Fitopatologia Brasileira,* v.29, p.456-458, 2004. http://www.scielo.br/pdf/fb/v29n4/a18v29n4.pdf

SILVA, A. R.; JULIATTI, F. C. Esporulação de *Diplodia maydis* e *Diplodia macrospora* em diferentes meios de cultura. *Bioscience Journal*, v. 21, n.3, p.127-131, 2005. http://www.seer.ufu.br/index.php/biosciencejournal/article/view/6616/4349

SNYMAN, L.D.; KELLERMAN, T. S.; VIEGAARR, R.; FLETT, B.C.; BASSON, K. M.; SCHULTZ, R. A. Diplonine, a neurotoxin isolates from cultures of the fungus S*tenocarpella maydis* (Berk.) Sacc. that induces diplodiosis. *Journal of Agricultural and Food Chemistry*, v.59,n.16, p.9039-9044, 2011.

SOLORZANO, C.D.; MALVICK, D.K. Effects of fungicide seed treatments on germination, population, and yield of maize grown from seed infected with fungal pathogens. *Field Crops Research*, v.122, n.3, p.173-178, 2011. http://ac.els-cdn.com/S0378429011000608/1-s2.0-S0378429011000608-main.pdf?_tid=8bbc2a5a-828f-11e3-8420-00000aab0f6b&acdnat=1390304058_43409dae462c071ee4affc4333d5503a

TEMBO, L.; SEA, G.A.; GIBSON, P.T.; OKORI, P. Resistenace breeding strategy for *Stenocarpella maydis* and *Fusarium graminearum* cob rots in tropical maize. *Plant Breeding*, v.132, p.83-89, 2013. http://onlinelibrary.wiley.com/doi/10.1111/pbr.12013/pdf