ORIGINAL ARTICLE

Influence of crop residues, matric potential and temperature on growth of *Exserohilum turcicum* an emerging maize pathogen in Argentina

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Significance and Impact of the Study: *Exserohilum turcicum* is an emerging pathogen in Argentina that has caused significant economic losses in different maize growing areas. However, at present there is a lack of information about the life cycle of this fungal pathogen. The present study shows the influence of abiotic factors such as temperature and water potential on the growth of *E. turcicum* on different crop residues used in rotations with maize under no-till system cultivation. According to our results, soybean residues allowed the pathogen growth in a wider range of conditions compared to wheat and maize residue, providing an inoculum source for maize infections in subsequent season.

Abstract

Northern corn leaf blight (NCLB) caused by *Exserohilum turcicum* is an endemic foliar disease in the Argentinean maize production area. Before applying a control method, it is essential to focus on factors that lead to the survival and conservation of *E. turcicum* inoculum. However, there is little information about the life cycle of this emerging pathogen in our country. The objective of the present work was to analyse the growth ability of three *E. turcicum* isolates under different type and conditions of maize, wheat and soybean residues, matric potential, temperature and their interactions. Statistical analysis demonstrated a significant effect of three factors on growth rate: residues-based media, matric potential and temperature. Among them the major effect was produced by matric potential followed by temperature. Although there were no significant differences in the growth rate among different residues types, the range of conditions in which growth occurred was different. According to these results, soybean residues would allow a better survival of *E. turcicum* inoculum under no-tillage system and providing an inoculum source for maize infections in subsequent year.

Introduction

Maize (*Zea mays* L.) is one of the main crops grown in Argentina, with an average production of 38 million tons in a planting area of 5·8 million ha (BCR, 2017). Northern corn leaf blight (NCLB) caused by *Exserohilum turcicum* (Pass.) Leonard and Suggs (Syn. *Helminthosporium turcicum* Pass.) is an endemic foliar disease in the maize production area of Argentina (Carmona *et al.* 2006), reaching severities higher than 50% in early growth stages and yield losses >40% (De Rossi *et al.* 2010). Severe

disease causes a reduction in plant photosynthetic potential and increases the incidence of stalk rots and lodging (Raymundo and Hooker 1981; Pedersen and Oldham 1992). The increase in lesion size can cause necrosis of the complete leaf. Necrosis and premature leaf death diminishes the capture of solar energy and consequently the translocation of photosynthates necessary for grain filling (Formento 2010). In susceptible hybrids, the fungus destroys foliage causing a decrease in yield that varies from 28 to 91% in the grain production (Rajeshwar *et al.* 2013).

Several factors have influenced the increase in NCLB disease in Argentina, such as late planting dates, intense and frequent rainfall during the summer months or lots irrigated by sprinkling and expansion of the area under reduced tillage or no tillage (Couretot 2010; Formento 2010). These conserving technologies offer the possibility to reduce cost of production and diminish erosion problems. However, these practices may increase the propagules of soil-borne pathogens by protecting the pathogen's refuge in the residue from negative microbial interactions or maintaining soil temperature appropriate to the spread of structures of pathogen population. *Exserohilum turcicum* overwinters as mycelia and conidia on residues left on the soil surface. Conidia may also be transformed into thick-walled resting spores called chlamydospores. As temperatures begin to rise in the spring the fungus will produce spores on the surface of the residue. These spores are then dispersed by wind, rain, or irrigation to the lower leaves of a new maize crop where they may germinate and infect a susceptible hybrid (Lipps and Mills 2002).

Plant pathogens of above ground parts may survive associated to crop debris and remain viable in the soil during the period they explore host residues nutrients (Reis *et al.* 2011). The presence of substrates such as cereal and no cereal crops residues or other nutrients results in a transformation of spores to an active phase characterized by adsorption of water, increase in respiration and biosynthesis of cell components. Morphological changes including germ tube formation and elongation occur and ultimately active vegetative mycelium is formed. This process is influenced by stress imposed by water availability and temperature. Spores of fungal species able to overcome such stress would have maximum ecological advantage, resulting in preferential colonization and exploitation of substrata. Interaction between these environmental factors is particularly important in determining the development of fungal communities colonizing substrata in soil (Griffin 1972; Magan and Lynch 1986).

Fungal growth and survival are markedly affected by water availability, which is one limiting factors in the functioning of ecosystems. In soil and crop residues, matric potential is the major component of the total water potential (Magan and Lynch 1986). Matric potential includes both adsorption and capillary effects and it is the most important factor affecting fungal growth in soil or on root surfaces (Tan 2011). Although much is known about the effect of environmental and biotic factors on sporulation and spore dispersal of *E. turcicum* (Leach *et al.* 1977; Levy and Cohen 1980, 1981, 1983) no information is available about the effects of matric potential on the survival of *E. turcicum* on residues. Therefore, understanding of survival of pathogen on residues is

fundamental step to the development of management practices in the field. Several management strategies can be used to control NCLB. Tillage and rotation are effective at limiting initial inoculum present on residue (Wise and Mueller 2011). Before applying a control method, it is essential to focus on the cycle of life and factors that lead to the survival and conservation of the pathogen inoculum. Thus, the objective of the present work was to analyse the growth ability of *E. turcicum* under different type and conditions of residues, matric potential, temperature and their interactions.

Results and discussion

During the 1990s, agriculture changed significantly in Argentina through the adoption of transgenic crops such as soybeans, maize and cotton under no-tillage system (Pengue 2005). The adoption of this type of conservation tillage was a major change affecting the pathogen populations that can colonize easily crop residues of wheat, maize and soybean (Pereyra and Dill-Macky 2008; Chiotta *et al.* 2015). Taken into account that residues crop is considered the main inoculum source of *E. turcicum*, little information is available about the survival of this emerging maize pathogen on residues in Argentina.

Analyses of variance demonstrated a significant effect of three factors on growth rate: residues-based media, matric potential and temperature. Among them the major effect was produced by matric potential ($F = 61.36$; $P < 0.0001$) followed by temperature (Table 1). This is a relevant data because *E. turcicum* survives and grows effectively in residue that resides on or near the soil surface where matric stress is the major component of total water stress. According to Adebayo and Harris (1971) the lower tolerance to matric stress relates to the greater difficulty involved in extracting water from soil pores and the consequent limited solute transport. For three *E. turcicum* isolates, growth occurred over a matric potential range

Table 1 Analyses of variance of the growth rate according residue type, isolate, residues-based media, temperature and matric potential

	df	MS	F value	P > F
Model	14	10·88	33·20	<0·0001
Residue type	2	2·41	7·36	0·0007
Isolates	2	1·13	3·44	0·0328
Residues-based media	1	5·85	17·86**	<0·0001
Temperature	3	6·25	19·09**	<0·0001
Matric potential	6	20·11	61·36**	<0·0001
Error	579	0·33		
Total	579			

DF, degrees of freedom; MS, mean square.
**Significant at $P < 0.0001$.

−0·7 to −10 MPa at temperature between 15 and 30°C, while limits for growth was −14 MPa. The maximum growth rate varied between −0·7 and −3 MPa at 25° depending of the residue type (Fig. 1). This data show ecological significance since maximum growth is observed under conditions of matric potentials and temperatures that can occur in soil during summer when the maize plant is susceptible to be infected by the pathogen. Although the three *E. turcicum* isolates were able to grow at matric potentials of −7 and −10 MPa at different temperatures, under these conditions the lag phase was greater and the growth rate was much lower compared with −0·7 and −3 MPa conditions. Nesci *et al.* (2004) evaluated the effect of matric stress on aflatoxicogenic *Aspergillus flavus* isolated from maize in Argentina. This study showed that *A. flavus* strains have a growth profile (matric potential and temperature) similar to *E. turcicum*, but growth rates of *A. flavus* were twice high than *E. turcicum* in the present work. However, in that work the

growth rate was evaluated on maize-based agar where a greater amount of nutrients is available compared with the residues evaluated in this study. Moreover, residues colonization occurs over a narrower range of water availability when compared to that which occurs in maturity maize ears as it was suggested by Giorni *et al.* (2008) or above maize leaf where solute potential is more important.

Rotation wheat/maize or maize/soybean under no-tillage system is a common practice in the centre and south of Córdoba province. Although wheat, maize and soybean residues had different chemical composition (data no show), EtRC-3, EtRC-4 and EtRC-38 isolates were able to growth on the three residues type and no significant differences in growth rate among the different crop residues was observed. By the other hand, all isolates showed lower growth rates on the residue extract medium compare to residues in a monolayer. Residues in no-tillage systems decompose more slowly than residues under
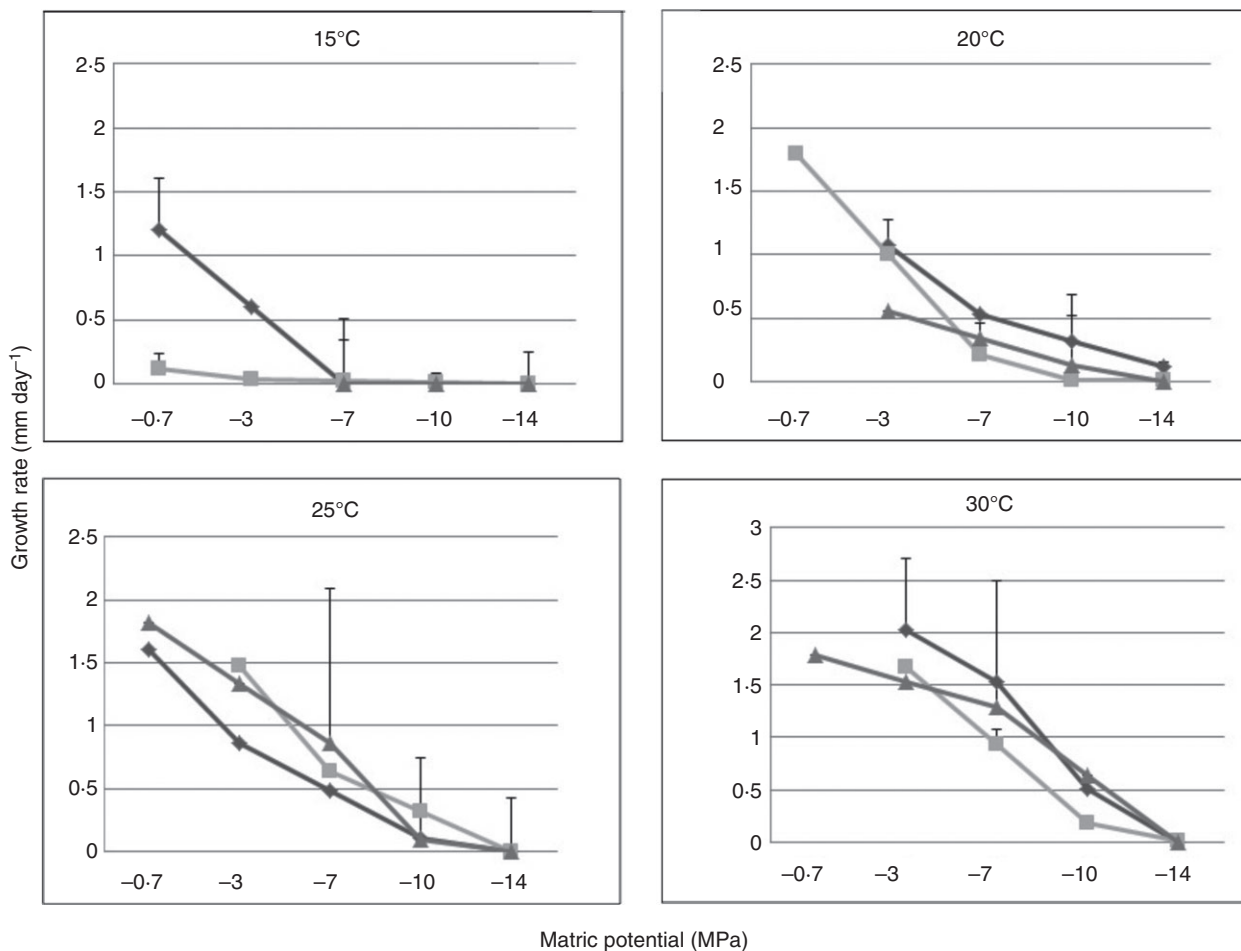

Figure 1 Comparison of the effect of matric potential modified with PEG 8000 on growth rates at 15, 20, 25 and 30°C after seven incubation days. Values are the mean extension rate of the three isolates evaluated and bars represent the SD. ♦ Soybean; ▪ Maize; ▲ Wheat.

reduced-tillage systems (Pereyra and Dill-Macky 2008), thus residues in no-till agronomic practices would support the survival of *E. turcicum* for longer periods of time. Studies on artificial substrates may not accurately reflect capabilities for growth on natural substrates (Magan and Lacey 1984). For this reason, this study was carried out on residues in layers simulating the size and physical condition that can be found in the field.

The soil-borne pathogens took advantage of the organic matter incorporated into the soil for survival (Pearson *et al.* 1984). Factors such as the age of residues, amount and layering of residues, herbicide treatment and environmental conditions during and between growing seasons will determine the importance of residues as sources of inoculum for the next season (Rodrigues Almeida *et al.* 2001). In our study, we analysed the growth of *E. turcicum* on three types of residues: maize, soybean and wheat. Although there were no significant differences in the growth rate among different residues types, the range of conditions in which growth occurred was different and growth rates were higher on soybean residues over all conditions. In addition, soybean residues allowed the growth of the pathogen in a wider range of conditions compared with wheat and maize residues. Thus, growth was observed from 15°C to matric potential of −0·3 and −7 MPa on soybean residues. Some growth of *E. turciucum* still occurred on soybean residues under the driest (−14 MPa) condition at 20°C, while the pathogen never grew in those matric potential on wheat and maize residues (Fig. 1). It has been suggested that nutrient source may also influence the minimum water potential for growth (Griffin 1972). In this study, differences in chemical composition among residues type, mainly C : N ratio and lignin concentration could influence the range of conditions in which fungal growth occurred. According to these results, soybean residues would allow a longer survival of *E. turcicum* inoculum under no-tillage system and providing better inoculum source for maize infections in subsequent year. Thus, avoid planting maize after soybean, placing a winter crop or no-host crop between them, or moving from no-tillage to reduced tillage by increasing the burial rate of the residues, could prevent the buildup of *E. turcicum* inoculum in the maize agroecosystem.

Materials and methods

Fungal isolates

Three *E. turcicum* isolates (EtRC-3, EtRC-4 and EtRC-38) recovered from symptomatic maize leaves during the 2015/2016 growing season in a field at Río Cuarto (Córdoba, Argentina) were used. These isolates were further identified based on macromorphology and micromorphology according to Barnett and Hunter (1998) and deposited in the culture collection of the Department of Microbiology and Immunology, Universidad Nacional de Río Cuarto. The cultures were maintained on potato dextrose agar medium (PDA: dextrose 20 g, potato extract 4 g, agar 15 g, distilled water 1000 ml, pH 5·6 ± 0·2) at 4°C.

Residue media preparation

Maize, soybean and wheat residues were obtained immediately before harvest during 2016/2017 season in three fields located in the maize-growing region of Córdoba Province (Argentina). Each type of residues sample consisted of 10 subsamples (100 g) combined to obtain a final sample representative of 1 kg. Residues were dried in a forced air draft oven at 30°C and finely milled using CT 193 Cyclotec SamMill, mixed and stored at room temperature until analysis. The medium used was a residue extract medium with 3% of maize, soybean and wheat residues separately. The residues extract was prepared boiling 30 g of each dry ground residues in 1 l water for 60 min and filtering through a double layer of muslin. The volume was made up to 1 l with distilled water. The matric potential of the residue extract medium was modified using Polyethylene glycol 8000 (PEG 8000) known to act predominantly by matric forces (Steuter *et al.* 1981). Known amounts of PEG 8000 were added according to the equation of Michel and Kaufmann (1973) to obtain matric potentials of −0·7; −3; −7; −10 and −14 Mpa (=0·99; 0·98; 0·95; 0·93; 0·90 a_w respectively). Water activity of representative media samples were checked using an AquaLab (Model series 4TE; Aqua-Lab Technologies, Riverside, CA) and converted to matric potential. Sterile polyester discs were placed in sterile 90 mm Petri dishes containing 20 ml of cooled residue extract medium. By the other hand, residues of 2-cm-long previously sterilized in autoclave twice (121°C- 1 atm-15 min) were incorporated into sterile 90 mm Petri dishes to form a monolayer of substrate. Then, different concentrations of PEG 8000 were added onto residues monolayer to achieve the same matric potentials described above. Petri dishes were subsequently refrigerated at 4°C for 48 h to allow absorption and equilibration. Finally, the matric potential was measured as previously described.

Inoculation, incubation and growth assessment

Plates of residues media under different matric potential were inoculated centrally with a PDA agar plug from 7-day-old fungal cultures using a 5 mm surface-sterilized cork-borer. Plates of the same matric potential were

placed into polyethylene bags and incubated in a range of 15 to 30°C, since it is the range of temperatures that can be observed in soil during the maize development. The colony ratios were measured daily or as required, in two directions at right angles to each other over periods of up to 15 days, or when the colony reached a ratio of 40 mm. The growth rates (millimetre per day) were determined by plotting the linear radial mycelial extension rates against time and obtaining the slope of the regression lines. For all experiments, three replicates per treatment were included. All experiments were carried out twice and data from the two independent experiments were combined and analysed as one.

Data analysis

Data of growth rates were subjected to ANOVA. When significant differences were observed, mean values were compared by the Scheffe′ test ($P < 0.05$). For growth rate combined analysis across isolates, type and conditions of residues, matric potential, temperatures and their interactions were subjected to an ANOVA using the program InfoStat version 2012 (Di Rienzo *et al.* 2013).

Acknowledgements

Conflict of Interest

No conflict of interest to declare.

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