



# Survival of *Diaporthe phaseolorum* var. *caulivora* (causal agent of soybean stem canker) artificially inoculated in different crop residues

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## ABSTRACT

Stem canker caused by *Diaporthe phaseolorum* var. *caulivora* is an important disease of soybean in Argentina. The objective of this study was to determine its survival ability in artificially infested straw under laboratory and field conditions. In laboratory, stem pieces of soybean, maize, sorghum, sunflower, potato and wheat were autoclaved, placed in petri dishes on Potato Dextrose Agar and Water Agar, and inoculated with a 7-day-old pathogen culture. All crop residues were colonized and produced perithecia. Debris artificially infested with *D. phaseolorum* var. *caulivora* were placed in plastic net bags and transferred to an un-cropped area in a field plot at the University of Buenos Aires. Straws were left on the ground from winter to spring season in both 2007 and 2008 years. After 6 months abundant perithecia were developed in all straws. However, a higher number of perithecia on soybean and sunflower compared to maize, sorghum and potato was determined. These findings suggest that other crops, besides soybean, could maintain alive the inoculum of *Diaporthe phaseolorum* var. *caulivora* from soybean for at least 6-7 months.

**Key words:** *Diaporthe phaseolorum* var. *caulivora*, crop residues, soybean, stem canker, survival ability.

*Diaporthe phaseolorum* var. *meridionalis* (*Dpm*) Fernández and Hanlin, and *D. phaseolorum* var. *caulivora* (*Dpc*) Athow and Caldwell, are cited as causal agents of the soybean stem canker (SSC) (Pioli et al., 2003; Hartman et al., 1999). SSC caused by *Dpm* was found for the first time in Argentina in 1983 in Tucumán Province (EEAOC, 1984) and became a serious disease problem in the 1996/97 growing season in almost all the Argentinian soybean-producing areas, leading to yield losses approaching 100% in some fields. This coincides with the increase of agricultural surface under no-tillage (NT) in Argentina (2,440,000 ha), which took place in the 94/95 growing season (AAPRESID, 2009). There have been outbreaks of the disease with epidemic characteristics in Buenos Aires Province since the 2004/05 growing season, having varied incidence records. (Grijalba & Guillin 2005; Ridao et al., 2005).

In Brazil from 1993 to 1996 SSC was considered one of the main limiting factors in soybean production in the Cerrado, when the farmers adopted NT cropping practices (Freitas et al., 2002). The use of *Dpm* resistant soybean cultivars led to an effective control of this disease.

*Dpc* was first reported in Argentina in 2001 (Pioli et al., 2003), and at present it is the main causal agent of SSC in the southernmost area of the core soybean region in the country (Lago et al., 2007; Ridao and Lago, 2007; Lago, 2010; Grijalba, 2011; Grijalba et al., 2011). One of the characteristics of high-incidence outbreaks is the cropping

history of the impacted fields. In some cases, the field in which a high-incidence outbreak has occurred has not had soybeans as a crop the previous year. A high incidence of the disease has been observed in two NT fields in Buenos Aires Province in which maize (San Antonio de Areco) and potato (Necochea) had been previously cultivated (Grijalba et al., unpublished). In the US, Chase (2011) cited outbreaks in fields which had been in a corn/alfalfa rotation. In Argentina the massive adoption of NT planting (more than 85%) and the monoculture situation (more than 50%) on the soybean fields (Rossi, 2004) might favor the conditions in which SSC becomes more frequent.

To date there is no evidence of major resistance genes to *Dpc* stem canker. Thus, other measures, such as cultural practices, production of healthy seed, chemical control and seed treatment are necessary. Most inoculum for the disease development is thought to be ascospores from perithecia or conidia from picnidia developing on overwintered infested crop residue, which are dispersed by splashing raindrops and windborne rain (Backman et al., 1985; Freitas et al., 1998). Regarding the presumed reduction of inoculum by cropping practices, and the putative enhancement of perithecial formation in NT, it must be acknowledged that field evidence is very controversial: while some studies have detected more stem canker in NT (Rothrock et al., 1985; Tyler et al., 1987), others, like Freitas et al. (2002) in Brazil found significantly less SSC in NT fields. Residues derived from NT provide soil cover and are a potential survival and

inoculum of plant pathogens. Baird et al. (1997) found that NT soybean debris harbors numerous fungi pathogenic to soybean.

The aim of this study was to determine the survival ability of *D. phaseolorum* var. *caulivora* in artificially infested crop residues of different crops, under laboratory and field conditions.

One isolate from a soybean stem plant with typical symptoms of stem canker of *D. phaseolorum* var. *caulivora* (GenBank, ITS accession number HM625752), maintained in the FAUBA fungal collection (Buenos Aires University) was used. Before use, pathogenicity tests were carried out using soybean plants. Crops for survival tissues were chosen among those most commonly used in rotation (*Zea mays*, *Sorghum bicolor*, *Helianthus annuus*, *Solanum* sp.) or succession (*Triticum aestivum*) with soybeans in Argentina. Nearly 95% of the Pampeana region is devoted to the crops selected (Ministerio de Agricultura, Ganadería y Pesca, 2009).

Stem pieces (4-5 cm long) of each selected crop were autoclaved, placed in Petri dishes on Potato Dextrose Agar, acidified with lactic acid (pH 4.5, APDA) and Water Agar (WA), inoculated with a 7-day-old mycelium of the fungus, and incubated at 20-22°C under 12 h near ultraviolet light/12 h darkness to induce reproductive stages. There were four replicates for each substrate and medium combination. Daily observations of the presence of reproductive structures were registered.

The survival test of the pathogen was conducted in the field plot at the University of Buenos Aires, Argentina. Stem pieces (25 g) of each crop were placed in individual 500-mL flasks, without culture medium, autoclaved and inoculated with a 7-day-old colony of the fungus grown in APDA (four pieces of 1 cm diameter per flask). Inoculated straws were kept in darkness at  $25 \pm 2^\circ\text{C}$  for 15 days to favor colonization. There were four replicates for each survival substrate. The artificially infested stems were placed in individual plastic net bags (20 x 15 cm) and then distributed at random on the ground of NT soil (previous crop: soybean). The experiment was conducted from winter to spring seasons, which corresponded from June to December 2007 and 2008. This period represents the approximate time without soybean in the field, within two summer crops. The 2007 test was an exploratory analysis, because well developed potato and sunflower stems were not available and so pieces of potato tuber and sunflower seedlings stem were used instead. In 2008 all residues were available. Controls for both experiments (laboratory and field) consisted of stem pieces seeded with sterilized APDA.

The evaluations of laboratory and 2007 field tests were made through the observation of the fungus reproductive structures (presence and type). For the 2008 field test, the amount of perithecia was registered using a 0.5 x 0.5 cm hole cut on a paper which was placed on the surface of the top and bottom half of 22 pieces of each

randomly selected crop residue. The number of perithecia in the hole was counted and the average of each stem was calculated. Afterwards all the content of the plastic net bags was covered with plastic bags to maintain high relative humidity and placed in a growth chamber at  $22^\circ\text{C} \pm 2^\circ\text{C}$  for 48 hs, to permit maturity of the reproductive structures formed. The presence or absence of asci and ascospores was then registered and plated in APDA in Petri dishes which were incubated in the dark at  $22 \pm 2^\circ\text{C}$ . The re-identification of the fungus was based on its morphology and cultural characteristics. In both field and laboratory tests, twenty measurements were taken for perithecia and ascospores and 10 for ascus in all the samples, randomly selected. The mean values and the standard deviation of the number of perithecia for the 2008 field experiment were calculated. The statistical significance of each treatment was evaluated by Kruskal-Wallis (KW) test and Post-hoc comparison tests under consideration of the non-homoscedasticity of samples (Conover, 1980). Infostat software (2009) was used for the calculations.

*D. phaseolorum* var. *caulivora* developed a vigorous mycelial growth on all the stems in both tests, but in neither natural nor artificial conditions the anamorphic state was present. This has been a very controversial issue. Fernandez & Hanlin (1996) mentioned the presence of pycnidia with  $\alpha$  and  $\beta$  conidia. Also, Kmetz et al. (1978) reported that the presence of pycnidia was infrequent and produced only  $\beta$  conidia. In Argentina no *caulivora* isolates presented pycnidia (Pioli et al., 2002; Lago, 2010; Grijalba, 2011). In all cases the pathogen produced perithecia mainly grouped but also isolated. On APDA abundant perithecia developed on both stem pieces and medium; on WA structures developed only on stems, and not on the medium, indicating the fungi require certain substances not available on WA.

Fitt et al. (1989) pointed out that from cover crops of a non host of *D. phaseolorum* var. *caulivora* species between two soybean crops constitutes mulch for the following crop. It can reduce the spread of the pathogen propagules, reducing the dissemination of SSC in the next season. Taking into account that apparently a secondary cycle does not occur in nature, crop rotation and reduction of the inoculum for the following crop might have a significant effect on the control of the disease, reducing the number of *Diaporthe* foci of liberation. However, if a mulch is colonized by the fungus as saprophyte, it may constitute a new source of inoculum. Our results suggest that crop residue left on the surface of the soil constitutes a potential source for *D. phaseolorum* var. *caulivora* inoculum and corroborated its high saprophytic ability found by Frosheiser (1957) who demonstrated that, in laboratory conditions, a number of crops debris serve as an adequate nutrient source for growth and reproduction of *Dpc*, and the homothallic nature of this fungus has also been reported (Ploetz & Shokes 1985; Lee & Subbarao 1993). *Dpc* formed perithecia in the field even though a severe drought occurred in 2007 and 2008. In 2007 after six months under field conditions, abundant

perithecia developed on debris of soybean, maize, wheat and sorghum, whereas pieces of potato and sunflower had disintegrated and no perithecia were observed. In 2008, perithecia developed in all the stem pieces which presented at least one perithecium. The perithecia were most numerous and nearly equal in number in soybean and in sunflower (not statistically significant) and fewer in other crops (Figure 1). More research is needed to learn about the kinetics of pathogen survival.

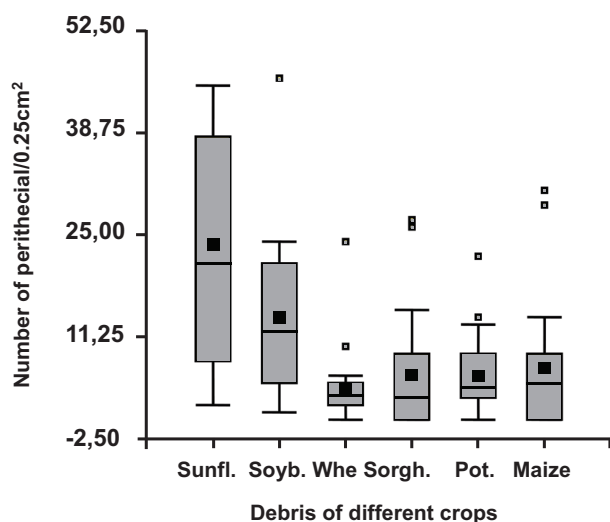
Under given soil and climatic conditions, soil organic matter level is largely controlled by the quantity and nature of organic matter inputs, of which in agricultural systems, crop residues represent an important part (Parton et al., 1987). The influence of crop residue type including the quality (C, N, or lignin content, and C/N and lignin/N ratios) on residue decomposition has been well documented (Reinertsen et al., 1984; Christensen, 1986; Ernst et al. 2002). In Argentina, Forjan (2002) informed that corn and wheat residues, which are poor in N, and with a high C:N ratio (77 and 82 respectively), decompose and release the nutrients slowly, whereas soybean and sunflower residues, rich in N and with a low C:N ratio (46 and 60 respectively), decompose quickly. Our results have shown that sunflower presented a nearly equal number of perithecia as soybean. Sunflower stem canker is produced by *D. helianthi*, and Falico de Alcaraz et al. (1998) demonstrated cross infection of *D. helianthi* and *D. phaseolorum* var. *meridionalis* in soybean and sunflower in Argentina, showing the relation between them. On the other hand, wheat presented the lowest perithecia, which coincides with the report of Lee & Subarao (1993). However, Rothrock et al. (1985) found a

greater number of severely diseased soybean plants infected by SSC in double cropped soybean/wheat compared to soybean monoculture.

The straw from the plastic net bags incubated in humid chamber showed mature perithecia, the length of which varied considerably, presenting greater values in the laboratory with an average of  $268.3 \pm 35.7 \mu\text{m}$ , whereas in the field the values in all the stem pieces were smaller with an average of  $134.2 \pm 10.07 \mu\text{m}$ . Besides, in both tests, the measurements of asci and ascospores were similar, with an average of  $23.4 \times 5.1 \mu\text{M}$  y  $8.9 \times 2.5 \mu\text{M}$  respectively. These data coincide with the findings of Fernández & Hanlin (1996): the variation in perithecia depends on substrate and environmental conditions, and the measurements of asci and ascospores are sufficiently stable across substrates. The isolates produced white colonies with the same characteristics as the original isolate. *Dpc* was consistently isolated from all the perithecia analyzed.

The fungus can colonize both resistant and susceptible cultivars infected late in the season without symptoms. In both cases, debris from these plants supports the development of perithecia for the next season (Backman et al., 1985) According to our research, soybean residues might also be colonized after the plant is dead.

These findings suggest that different types of straw may serve as an alternative source of inoculum for *D. phaseolorum* var. *caulivora* for at least 6 months under field conditions. This could be epidemiologically significant in places where both alternative crops and soybean are grown extensively and frequently in rotation.



**FIGURE 1** - Boxplot distributions of 132 data from different crop residues (2008 field test). Data are based on the respective number of perithecia in each crop residue/0.25cm<sup>2</sup>. Boxes indicate the mean (solid line) and dots refer to outlying data points (Sunf= Sunflower, Soyb= Soybean, Whe= Wheat, Sorgh= Sorghum, Pot= Potato and Maize).

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